

RECENT EVOLUTION AND DIVERGENCE AMONG POPULATIONS OF A RARE MEXICAN ENDEMIC, CHIHUAHUA SPRUCE, FOLLOWING HOLOCENE CLIMATIC WARMING

F. THOMAS LEDIG,^{1,2} VIRGINIA JACOB-CERVANTES,³ PAUL D. HODGSKISS,¹
AND TEOBALDO EGUILUZ-PIEDRA³

¹Institute of Forest Genetics, Pacific Southwest Research Station, USDA Forest Service, 2480 Carson Road,
Placerville, California 95667

²E-mail: fswa/s=t.ledig/ou=r05f03d57a@mhs.attmail.com

³Centro de Genética Forestal, Universidad Autónoma Chapingo, Apartado Postal No. 37, Chapingo,
México C.P. 56230, México

Abstract.—Fragmentation and reduction in population size are expected to reduce genetic diversity. However, examples from natural populations of forest trees are scarce. The range of Chihuahua spruce retreated northward and fragmented coincident with the warming climate that marked the early Holocene. The isolated populations vary from 15 to 2441 trees, which provided an opportunity to test whether census number is a good predictor of genetic diversity. Mean expected heterozygosity, H_e , based on 24 loci in 16 enzyme systems, was 0.093 for 10 sampled populations, which is within the range reported for conifers. However, estimates varied more than twofold among populations and H_e was closely related to the logarithm of the number of mature trees in the population ($r_{H_e,N} = 0.93$). Diversity among populations, F_{ST} , was 24.8% of the total diversity, which is higher than that observed in almost all conifer species studied. Nei's genetic distance, D , was not related to geographic distance between populations, and $\bar{D}$ was 0.033, which is higher than estimates for most wide-ranging species. Most populations had excess homozygosity and the fixation index, F_{IS} , was higher than that reported for all but one species of conifer. Nm , the number of migrants per generation, was 0.43 to 0.76, depending on estimation procedure, and is the smallest observed in conifers. The data suggest that populations of Chihuahua spruce have differentiated by drift and that they are effectively isolated. The results illustrate how a combination of paleontological observation and molecular markers can be used to illuminate recent evolutionary events. Multilocus estimates of outcrossing for two small populations were zero (complete selfing) and 0.153, respectively, which are in striking contrast to the near complete outcrossing observed in most conifers. The high fixation index and a high proportion of empty seeds (45%) suggest that inbreeding may be a serious problem for conservation of Chihuahua spruce.

Key words.—Gene flow, genetic diversity, genetic drift, inbreeding, isozymes, *Picea*, population decline.

Received January 14, 1997. Accepted August 13, 1997.

*The earth was younger then,
in the deep green shine of spruce.*

Maryann Whalen (1995)

Fragmentation of habitat and isolation of populations as a result of the climate changes projected for the next century may threaten biodiversity. The effects on genetic diversity of fragmentation, reduction in population size, and isolation are known in theory, but empirical data from natural populations of forest trees are relatively scarce. Theory suggests that small populations will lose genetic variability more rapidly than large ones (Wright 1969). Simulation studies, however, suggest that variability might not be lost as rapidly as indicated by Wright's model (Lesica and Allendorf 1992); selection for heterozygotes, whether heterozygote advantage results from overdominance or inbreeding or some other cause, could slow the loss of alleles. Chihuahua spruce (*Picea chihuahuana* Martínez) provides an opportunity to test theory relating population size to diversity, to see which of two models that describe the loss of diversity (Wright 1969; Lesica and Allendorf 1992) best matches observations. We investigated genetic diversity and genetic structure in Chihuahua spruce and used the paleobotanical and paleoclimatic literature to infer its recent evolutionary responses to fragmentation and isolation.

Chihuahua spruce is an endangered species whose range retreated northward during Holocene warming. Pollen in the

ancient bed of Lake Texcoco, which is now Mexico City, and in Lake Chalco in the basin of Mexico show that spruce occurred in the surrounding uplands at the end of the Pleistocene (Clisby and Sears 1955) and at least as recently as 7000 to 8000 years before present (yr B.P.; Lozano-García et al. 1993; M. S. Lozano-García, pers. comm. 1997). The nearest Chihuahua spruce are now about 700 km northwest in the Sierra Madre Occidental. Other species of spruce occur about 500 km north of Mexico City in the Sierra Madre Oriental (Patterson 1988). All Mexican spruces may have had ranges as far south as Mexico City, but Chihuahua spruce is most likely to have occurred there. The topography of Mexico is more conducive to migration of high-elevation taxa between Mexico City and the Sierra Madre Occidental than between Mexico City and the Sierra Madre Oriental, where other relict spruce occur. In addition, the high endemism of the subalpine habitats in the Sierra Madre Oriental suggest that they were not linked during the Pleistocene with the Transverse Volcanic Belt in which Mexico City lies (McDonald 1993). In any case, the palynological observations indicate that the range of spruce retreated northward since the Pleistocene and all Mexican spruces are now characterized by small, fragmented populations.

The endemic spruces are a minor element in the flora of Mexico, yet potentially important from the standpoint of science, their unique contribution to the biodiversity of Mexico, and their value as genetic resources. Chihuahua spruce was

included on a list of endangered arboreal taxa prepared for the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP) by Vera (1990), and qualifies as threatened under the guidelines of the International Union for the Conservation of Nature and Natural Resources (IUCN). The Sierra Madre Occidental was nominated by the IUCN as a global center of plant diversity. Chihuahua spruce occupies sites with some of the richest arboreal species diversities in the Sierra Madre Occidental (e.g., Gordon 1968), or in all of temperate North America, and for that reason its habitat will certainly be a crucial focus for protection.

Spruce (*Picea* A. Dietr.) is an essentially boreal genus and, depending on taxonomist, includes 31 to 50 species (Dallimore and Jackson 1923; Wright 1955; Bobrov 1970; Everett 1981). The occurrence of spruces in the subtropical latitudes of Mexico is surprising. Only Morrison spruce (*Picea morrissonicola* Hayata) of Taiwan grows at such southerly latitudes (Wright 1955). Spruce in Mexico occurred at least as far south as the Isthmus of Tehuantepec (18°09'N) in the mid-Pliocene, five million years ago (Graham 1993). At present, the southernmost stand of Chihuahua spruce, Arroyo de la Pista, lies a few kilometers south of the Tropic of Cancer (23°30'N).

Chihuahua spruce was first reported in 1942 from a site called Talayotes (Martínez 1953), and we now know of 35 stands. The stands are scattered over a north-south range of nearly 800 km in Chihuahua and Durango, and are restricted in elevation to a relatively narrow band, usually between 2200 and 2700 m. They are almost always found on the north slopes of steep-walled arroyos, and always in a riparian strip. Stands vary from nearly pure to less than 50% spruce. Associates include pines (*Pinus* spp. L.), oaks (*Quercus* spp. L.), and occasionally firs (*Abies* spp. Mill.) and Douglas-firs (*Pseudotsuga* spp. Carr.; Gordon 1968; Narváez et al. 1983). Every spruce in Chihuahua has been counted (Narváez et al. 1983). The smallest stand in Chihuahua has a population of 15 mature trees and the largest, 2441. Only three have more than 1000 mature spruce. The stands in Durango have not yet been censused, but even allowing a generous estimate, the species cannot number over 20,000 trees in total. A related species, Martínez spruce (*Picea martínezii* T. F. Patterson) is known from two small stands in the Sierra Madre Oriental of Nuevo Leon and Coahuila (Patterson 1988).

The decline of spruces in Mexico and their retreat northward coincides with a period of global warming that ended the Pleistocene. The increase in temperature at the end of the last glacial period was about equal to that projected after a doubling of atmospheric carbon dioxide, which could occur in less than half a century. Mexican spruces may decline to extinction if current projections of global warming materialize.

It is not known whether Chihuahua spruce lost genetic diversity following population collapse in the wake of Holocene climate change, whether a reduced gene pool limited its range of adaptation, what the limits to gene flow might be, or whether inbreeding is a serious problem in conserving the species. Among the plants, conifers have the highest levels of genetic diversity, on average, and are almost completely outcrossing (Schemske and Lande 1985; Hamrick and Godt 1996). However, several authors have hypothesized that Chi-

huahua spruce is genetically depauperate; that is, that lack of genetic diversity and inbreeding were responsible for the post-Pleistocene collapse of Chihuahua spruce and that inbreeding is contributing to its continuing decline and increasing the threat of extinction (Sánchez and Narváez 1983), that Chihuahua spruce has a limited gene pool that restricts its environmental tolerances and may lead it to extinction (Gordon 1968), and that its "gene pool is undoubtedly limited" (Taylor and Patterson 1980).

We undertook a survey of the amount and structure of genetic diversity in Chihuahua spruce to determine whether the data supported a drastic range reduction congruent with the warming climate of the current interglacial. We also hoped to decide whether genetic diversity (i.e., a reduced gene pool) and inbreeding were factors in its decline as speculated by Gordon (1968), Taylor and Patterson (1980), and Sánchez and Narváez (1983). The extremely disjunct distribution of Chihuahua spruce and the variation in population size (over two orders of magnitude) provide an excellent opportunity for testing relationships between diversity on the one hand and population size or degree of isolation on the other. Genetic distances among the fragments can be used to calculate the time since their isolation (e.g., Ledig and Conkle 1983).

The distribution of genetic diversity in Chihuahua spruce will be important in setting priorities for conservation. If choices must be made, the best course is to save populations that have the greatest diversity rather than those that have retained only a depauperate sample. If inbreeding is indeed a problem, then active management is needed rather than passive preserves.

MATERIALS AND METHODS

Cones were collected from 10 stands of Chihuahua spruce (see Table 1 and Fig. 1 for locations) in September and October 1988. These 10 populations were chosen to bracket as much of the north-south range of the species as possible, include the smallest and the largest populations, and achieve a geometric distribution in the intermediate size classes. The sampled trees in each stand were widely spaced over the area occupied by the spruce. Cones were maintained separate by tree and transported to the Centro de Genética Forestal in Chapingo, Mexico. Seeds were extracted after the cones opened and were stored at 1°C until needed.

At Cerro de la Cruz ($N = 17$) and Arroyo del Infierno ($N = 36$), all cone-bearing trees were sampled. In the other eight stands, the goal was to sample 35 trees, but that was not attainable because of difficulties in access and the low frequency of trees with cones. Furthermore, seed germination was low; many trees with cones failed to yield viable seeds. In retrospect, diploid, vegetative tissue would have provided larger sample sizes. However, genetic interpretation of isozyme variants is more difficult with diploid, vegetative tissue than with haploid megagametophytes from the seeds, and mating system analysis is not possible with vegetative tissues.

In 1989, seeds were germinated for isozyme analysis, and when the radicles appeared through the seed coat, the megagametophytes and embryos were excised and separated. Because the megagametophyte of spruce is haploid, alleles at a locus can be detected by segregation among seeds from a

TABLE 1. Chihuahua spruce populations sampled and census counts, where available.

Population	Number of trees ¹	Area ¹ (ha)	Latitude N ²	Longitude W ²	Elevation ³ (m)
Arroyo de la Pista	—	—	23°19'52"	104°45'00"	2700
Arroyo del Infierno	36 ⁴	—	23°39'40"	105°26'08"	2700
Cebollitas	—	—	25°05'45"	106°26'35"	2450
Arroyo de la Quebrada	—	—	26°08'48"	106°22'53"	2600
Rio Vinihueachi	2441	45	27°44'53"	107°41'58"	2150
Talayotes	377	7.8	27°55'03"	107°49'01"	2350
Cerro de la Cruz	17 ⁵	0.7	27°57'44"	107°36'05"	2350
El Realito	587	5.4	27°55'50"	107°36'43"	2300
La Tinaja	120	1.1	27°57'24"	107°46'08"	2400
Arroyo Ancho	683	9.6	28°03'58"	107°46'41"	2250

¹ Mature trees, according to Narváez et al. (1983), unless noted.

² Coordinates may not be exact; confirmation is currently in progress using a geographic positioning system.

³ Rounded to the nearest 50 m.

⁴ Gordon (1968).

⁵ Personal count on November 23, 1995.

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 by chance the seeds sampled included only one allele. We attempted to assay six megagametophytes per tree; actual sample size varied, but averaged 6.2. The probability of misclassifying a heterozygote as a homozygote with a sample of six is 0.03. That is, the probability that all 6 megagametophytes in a sample from a heterozygous tree carry the same allele is $(1/2)^6 = 0.03$.

We used the techniques of starch gel electrophoresis described by Conkle et al. (1982) to assay 16 enzyme systems. 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* Ait.), an almost invariably homozygous species, were included on each gel to aid interpretation. Where several zones of activity were observed for a single enzyme, hyphenated numerals following the enzyme abbreviation were used for identification. Twenty-four presumptive loci were consistently scored and used in the statistical analysis.

We used electrophoresis of megagametophyte and embryo pairs to analyze the mating system in two populations, Cerro de la Cruz and Arroyo del Infierno. Knowing the contribution of the egg (the haploid genotype of the megagametophyte) to the zygote, the pollen contribution can be deduced by subtraction, which makes it possible to detect some outcrossed embryos. An average of 6.5 gametophyte-embryo pairs were assayed in the progeny of 11 trees from Cerro de la Cruz and an average of 7.3 pairs in the progeny of 10 trees from Arroyo del Infierno. Only two polymorphic loci in each population proved suitable for analysis, MDH-3 and PGM-1 in Cerro de la Cruz and ACO-1 and MDH-3 in Arroyo del Infierno.

We used BIOSYS (Swofford and Selander 1981) to estimate genetic diversity, genetic relationships among populations, and *F*-statistics, and Ritland's (1989) MLTR for mating system analysis. The various measures of genetic diversity and genetic structure and the formulas are as discussed in

Guries and Ledig (1982). Our inferences apply to mature, cone-bearing trees.

The degree of genetic isolation among populations was estimated by *N_m*, the number of migrants per generation. *N_m* was calculated by two methods, by the relationship between *F_{ST}* and *N_m* and by the method of private alleles. From Wright (1951):

$$N_m = (1 - F_{ST})/4F_{ST}, \quad (1)$$

where *F_{ST}* is the proportion of the total genetic diversity among populations.

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

$$\log_{10}[\bar{p}(1)] = a \log_{10}(N_m) + b, \quad (2)$$

where $\bar{p}(1)$ is the mean frequency of private alleles and *a* and *b* are constants determined by fitting simulated data. We used values for *a* and *b* developed for sample sizes of 10 and 25 (from Barton and Slatkin 1986) and corrected for our mean sample size.

RESULTS

Of the 24 loci, 11 (CAT, EST, FDP-4, GDH, GOT-1, IDH, LAP, MDH-1, MNR-2, 6PG-2, and SKD-2) were invariant in Chihuahua spruce. Two other loci (ADH and PGI-2) were polymorphic in only one population each. Several loci were polymorphic in only a few populations. Five of the 10 populations had a total of six private alleles (i.e., alleles found in only one population). Allele frequencies (Table 2) at polymorphic loci tended toward a uniform distribution (Fig. 2), which contrasts sharply with the situation in most conifers (e.g., Guries and Ledig 1982). Allele frequency distributions are generally U-shaped with many alleles at low (< 0.05) or high (> 0.95) frequency (Chakraborty et al. 1980).

Genetic diversity (i.e., expected heterozygosity) ranged from 0.055 to 0.131 among populations, more than a twofold difference (Table 3). The mean expected heterozygosity was 0.093. The percent polymorphic loci, *P*, also varied widely among populations, from 16.7% to 41.7%. The number of alleles per locus, *A*, varied only from 1.2 to 1.6 and averaged

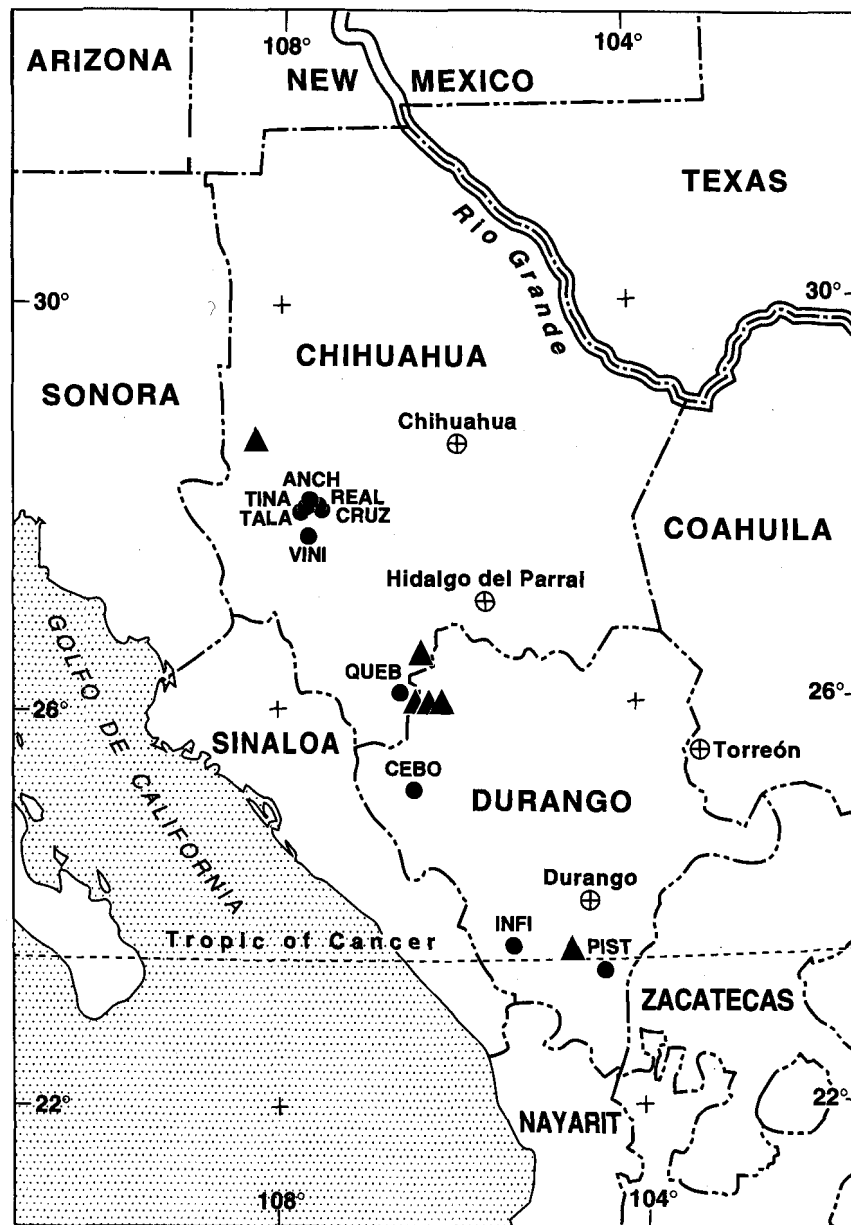


FIG. 1. The states of Chihuahua and Durango, Mexico, showing the location of Chihuahua spruce populations sampled for isozyme analysis (●) and other known populations or clusters of populations (▲). Abbreviations are: CRUZ = Cerro de la Cruz; VINI = Rio Vinihueachi; REAL = El Realito; TINA = La Tinaja; TALA = Talayotes; ANCH = Arroyo Ancho; QUEB = Arroyo de la Quebrada; CEBO = Cebollitas; INFI = Arroyo del Infierno; PIST = Arroyo de la Pista.

1.37. The correlation between expected heterozygosity and the logarithm of population size, based on the populations for which census counts were available, was high ($r_{H,N} = 0.93$, $P \approx 0.004$; Fig. 3). The correlation between number of alleles per locus and population size was also high ($r_{A,N} = 0.78$, $P \approx 0.047$), but the correlation between percent polymorphic loci and population size was not significant ($r_{P,N} = 0.61$, $P \approx 0.154$).

Observed heterozygosity averaged 0.073 and was lower than the unbiased estimate of expected heterozygosity in eight of 10 populations (in the other two populations, observed heterozygosity was identical to expected heterozygosity). Observed heterozygosity was lower, sometimes much

lower, than expected heterozygosity for some loci in some populations. For example, at Cebollitas in a sample of 24 trees, 10.89 heterozygotes were expected at the 6PG-1 locus, but none was observed. Deviations from Hardy-Weinberg equilibrium genotype frequencies were significant (chi-square, $P < 0.05$) in 14 of 65 cases. Given the number of tests and the chosen α of 0.05, we would expect only three tests to indicate a deviation (Type I error).

The heterozygote deficiency is reflected in a mean F_{IS} of 0.185. F_{IS} is a measure of the deviation of the genotypic proportions from Hardy-Weinberg equilibrium over all populations and loci. Positive values suggest inbreeding. Mean values of F_{IS} were positive (excess homozygosity) for eight

TABLE 2. Allele frequencies for 13 polymorphic loci in 10 populations of Chihuahua spruce.

Locus/allele	Population ¹ (sample size) ²									
	PIST (20.8)	INFI (14.5)	CEBO (22.9)	QUEB (18.0)	VINI (18.2)	TALA (7.7)	CRUZ (11.5)	REAL (14.0)	TINA (13.9)	ANCH (20.8)
ACO-1/1	0.395	0.933	0.040	0.737	0.156	0.687	0.833	0.286	1.000	0.500
2	0.263	0.000	0.460	0.263	0.187	0.125	0.125	0.321	0.000	0.095
3	0.342	0.067	0.500	0.000	0.656	0.187	0.042	0.393	0.000	0.405
ADH/1	1.000	1.000	1.000	1.000	1.000	1.000	0.864	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.136	0.000	0.000	0.000
FEST/1	1.000	1.000	1.000	1.000	0.763	1.000	1.000	1.000	0.679	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.321	0.000
3	0.000	0.000	0.000	0.000	0.237	0.000	0.000	0.000	0.000	0.000
FDP-2/1	0.929	1.000	0.941	0.917	0.972	1.000	1.000	1.000	1.000	1.000
2	0.071	0.000	0.059	0.083	0.028	0.000	0.000	0.000	0.000	0.000
GOT-3/1	0.978	0.900	1.000	0.947	1.000	0.937	1.000	0.929	1.000	1.000
2	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000
4	0.022	0.100	0.000	0.000	0.000	0.063	0.000	0.071	0.000	0.000
MDH-2/1	0.978	1.000	0.920	0.974	1.000	1.000	1.000	1.000	1.000	1.000
2	0.022	0.000	0.080	0.026	0.000	0.000	0.000	0.000	0.000	0.000
MDH-4/1	0.283	0.679	0.771	0.875	0.447	0.375	0.958	0.192	0.643	0.429
2	0.543	0.000	0.229	0.125	0.421	0.625	0.000	0.654	0.357	0.524
3	0.174	0.321	0.000	0.000	0.132	0.000	0.042	0.154	0.000	0.048
MNR-1/1	1.000	1.000	1.000	1.000	0.917	1.000	0.958	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.083	0.000	0.042	0.000	0.000	0.000
6PG-1/1	0.630	0.346	0.667	0.417	0.525	0.562	0.792	0.500	0.857	0.643
2	0.000	0.000	0.000	0.028	0.000	0.063	0.000	0.219	0.000	0.000
3	0.370	0.654	0.333	0.556	0.475	0.375	0.208	0.281	0.143	0.357
PGI-2/1	1.000	1.000	1.000	1.000	0.972	1.000	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000
PGM-1/1	1.000	1.000	1.000	0.944	0.921	0.937	0.542	0.923	0.536	0.524
2	0.000	0.000	0.000	0.056	0.079	0.063	0.458	0.077	0.464	0.476
PGM-2/1	0.425	0.958	0.889	0.806	0.605	0.750	0.000	0.625	0.000	0.500
2	0.250	0.042	0.000	0.167	0.053	0.187	1.000	0.000	1.000	0.235
3	0.225	0.000	0.111	0.028	0.184	0.063	0.000	0.156	0.000	0.265
4	0.100	0.000	0.000	0.000	0.158	0.000	0.000	0.219	0.000	0.000
SKD-1/1	0.978	1.000	1.000	1.000	0.944	1.000	1.000	0.808	1.000	1.000
2	0.022	0.000	0.000	0.000	0.056	0.000	0.000	0.115	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000

¹ CRUZ = Cerro de la Cruz; VINI = Rio Vinihueachi; REAL = El Realito; TINA = La Tinaja; TALA = Talayotes; ANCH = Arroyo Ancho; QUEB = Arroyo de la Quebrada; CEBO = Cebollitas; INFI = Arroyo del Infierno; PIST = Arroyo de la Pista.

² Mean number of trees per locus.

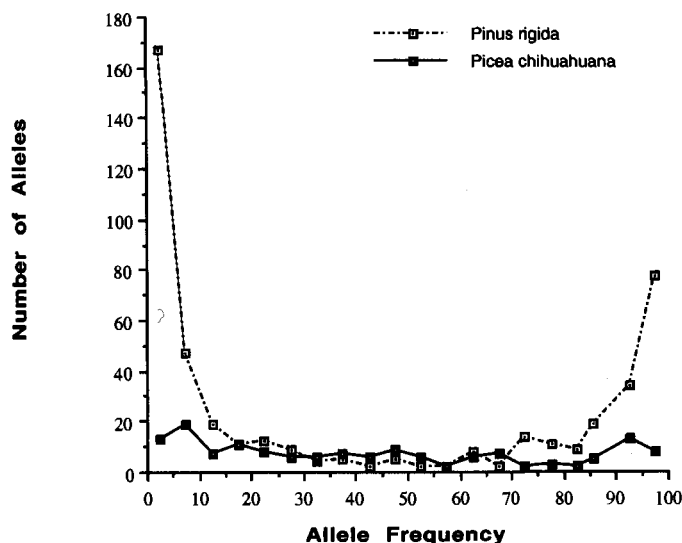


FIG. 2. The distributions of allele frequencies in Chihuahua spruce and pitch pine, a wide-ranging conifer.

of the 13 polymorphic loci (Table 4). The negative deviations (excess heterozygosity) for the other five loci were small.

The genetic structure of Chihuahua spruce can be inferred with Wright's (1931) F -statistics (Table 4). The extent of heterogeneity among populations is estimated by F_{ST} , which is essentially the same as Nei's (1973) G_{ST} . F_{ST} was 0.248, a very high value for conifers. This can be interpreted to mean that 75.2% of the total genetic variation is within populations and 24.8% among populations.

Estimates of genetic distance (Nei 1972) among populations also provides an indication of the genetic structure of Chihuahua spruce. Values range from 0.003 between Talayotes and Arroyo de la Pista to 0.090 between La Tinaja and Cebollitas (Table 5). The average is 0.033, which is high for conifers and indicates substantial differentiation among populations, in agreement with the estimate of F_{ST} . The high values for genetic distance reflect major differences in gene frequencies among populations. For example, La Tinaja is fixed for allele-2 at PGM-2, while the estimated frequency of allele-2 at Arroyo del Infierno is only 0.042 (Table 2). However, genetic distances were not correlated with the geographic distances between populations; the correlation coefficient was -0.07 ($P \approx 0.65$).

TABLE 3. Genetic diversity in Chihuahua spruce: sample size (n), mean expected heterozygosity (H_e , unbiased estimate), observed heterozygosity (H_o), percent polymorphic loci (P ; absolute and 95% criteria), number of alleles per locus (A), fixation index or mean deviation from Hardy-Weinberg equilibrium (F), population census (N), and effective population size (N_e) calculated as explained in the text. Standard errors appear in parentheses.

Population	n	H_e	H_o	P		A	F	N	N_e
				100	95				
Arroyo de la Pista	21.6 (0.7)	0.114 (0.048)	0.090 (0.038)	33	21	1.5	0.091	—	—
Arroyo del Infierno	14.6 (0.2)	0.055 (0.027)	0.054 (0.029)	21	17	1.2	-0.042	36	23
Cebollitas	23.5 (0.8)	0.076 (0.032)	0.045 (0.025)	25	25	1.3	0.343	—	—
Arroyo de al Quebrada	17.9 (0.5)	0.080 (0.030)	0.063 (0.029)	33	29	1.4	0.151	—	—
Rio Vinihueachi	18.3 (0.4)	0.131 (0.044)	0.103 (0.037)	42	33	1.6	0.179	2441	—
Talayotes	7.8 (0.2)	0.094 (0.039)	0.094 (0.039)	25	25	1.4	-0.077	377	59
Cerro de la Cruz	11.4 (0.3)	0.066 (0.029)	0.028 (0.029)	25	17	1.3	0.414	17	29
El Realito	14.2 (0.2)	0.127 (0.048)	0.090 (0.034)	29	29	1.5	0.197	587	518
La Tinaja	14.0 (0)	0.071 (0.034)	0.071 (0.035)	17	17	1.2	-0.059	120	33
Arroyo Ancho	20.7 (0.4)	0.115 (0.047)	0.093 (0.038)	21	21	1.3	0.164	683	154
Mean		0.093	0.073	27	23	1.37			

Nm , the number of migrants per generation, estimated from F_{ST} was 0.76. The number of private alleles was six and the average population sample, n , was 17.33. Therefore, we could estimate Nm from Barton and Slatkin's (1986) relationship for $n = 10$ and for $n = 25$ and apply correction factors. The two estimates were 0.36 and 0.51, respectively, and their average is 0.43. This estimate is close to the value of 0.76 calculated from Wright's F_{ST} . Either estimate indicates a high degree of isolation among populations.

The multilocus estimates of outcrossing (t_m) were 0.00 (± 0) for Cerro de la Cruz (i.e., complete selfing) and 0.15 ($0.00 < t_m < 0.56$; $P = 0.95$) for Arroyo del Infierno. These estimates are in agreement with an F_{IS} that suggests a high

degree of inbreeding. The mean of single locus estimates of outcrossing were 0.00 and 0.13 for Cerro de la Cruz and Arroyo del Infierno, respectively. Pollen allele frequencies varied among seed parents, as might be expected when selfing predominates.

DISCUSSION

As a species, Chihuahua spruce is not genetically depauperate. It is unlikely that its range collapsed because the species lacked genetic diversity. The largest population in our sample, Rio Vinihueachi, is polymorphic at an estimated 41.7% of its loci and has an expected heterozygosity of 0.131. This is the closest that we can come to an estimate of pre-collapse levels of diversity (but see below). Even the smallest population has an expected heterozygosity of 0.066 and is

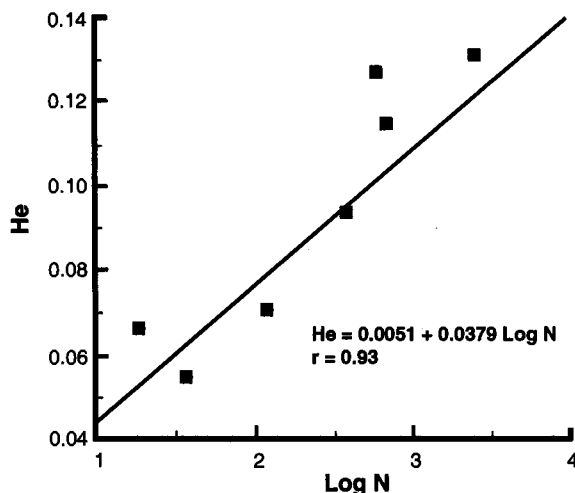


FIG. 3. The correlation between expected heterozygosity and the logarithm of population census (mature trees) for Chihuahua spruce.

TABLE 4. Estimates of Wright's (1965) F -statistics for 13 polymorphic loci in Chihuahua spruce.

Locus	F_{IS}	F_{IT}	F_{ST}
ACO1	0.045	0.316	0.284
ADH	0.614	0.662	0.124
FEST	0.354	0.518	0.254
FDP2	-0.072	-0.025	0.044
GOT3	-0.078	-0.027	0.047
MDH2	-0.063	-0.013	0.047
MDH4	0.119	0.319	0.227
MNR1	-0.075	-0.013	0.058
6PG1	0.260	0.331	0.095
PGI2	-0.029	-0.003	0.025
PGM1	0.162	0.397	0.281
PGM2	0.364	0.632	0.421
SKD1	0.424	0.480	0.098
Mean	0.185	0.387	0.248

TABLE 5. Estimates of Nei's genetic distance (D) above diagonal and geographic distance (km) below diagonal between populations of Chihuahua spruce.

Population ¹	ANCH	TINA	REAL	CRUZ	TALA	VINI	QUEB	CEBO	INFI	PIST
ANCH		0.036	0.014	0.038	0.008	0.015	0.026	0.027	0.036	0.010
TINA	14		0.075	0.009	0.046	0.075	0.056	0.090	0.073	0.051
REAL	21	16		0.079	0.006	0.007	0.030	0.018	0.037	0.003
CRUZ	22	17	3		0.052	0.072	0.044	0.075	0.064	0.053
TALA	19	6	20	21		0.014	0.010	0.020	0.016	0.003
VINI	37	24	22	23	23		0.028	0.010	0.035	0.007
QUEB	243	232	223	222	231	208		0.020	0.006	0.022
CEBO	350	338	330	330	337	314	108		0.035	0.017
INFI	550	538	529	529	537	515	307	202		0.031
PIST	599	589	578	578	589	566	360	266	93	

¹ CRUZ = Cerro de la Cruz; VINI = Rio Vinihueachi; REAL = El Realito; TINA = La Tinaja; TALA = Talayotes; ANCH = Arroyo Ancho; QUEB = Arroyo de la Quebrada; CEBO = Cebollitas; INFI = Arroyo del Infierno; PIST = Arroyo de la Pista.

polymorphic at 25% of its loci. The mean expected heterozygosity of 0.093 is lower than values reported for most species of spruce, but within the range observed in the genus and in conifers in general. Norway spruce (*Picea abies* [L.] Karst.) ranges across vast areas in Asia and Europe and estimates of mean expected heterozygosity range from 0.115 to 0.220 in studies that used large numbers of loci (M. T. Conkle, pers. comm. 1986; Lagercrantz and Ryman 1990; Goncharenko and Potenko 1991). In the most extensive study of Norway spruce (70 populations), mean expected heterozygosity was 0.115 (Lagercrantz and Ryman 1990), which is less than that reported here for the population from Rio Vinihueachi. Other species of spruce (Table 6) have expected heterozygosities that range from 0.13 for the rare Serbian spruce (*Picea omorika* [Pančić] Purk.) to 0.351 for the transcontinental black spruce (*Picea mariana* [Mill.] B.S.P.). In pine species, estimates of expected heterozygosity have ranged from zero to 0.334, although the latter value was based on only 15 loci (reviewed in Ledig, in press). However, the average for pines, 0.154 (Hamrick and Godt 1996), is higher than the mean value estimated here for Chihuahua spruce, though not much above the estimate of 0.131 for Rio Vinihueachi. Several authors hypothesized that Chihuahua spruce was genetically depauperate (Gordon 1968; Taylor and Patterson 1980; Sánchez and Narváez 1983). However, if genetic diversity at isozyme loci reflects diversity in the genome as a whole, lack of diversity per se is not the reason for the relic status of Chihuahua spruce.

However, the range in expected heterozygosity is wider than that observed for most north temperate conifers, the group most thoroughly investigated, and suggests that genetic drift has been an important factor in determining the level of diversity in Chihuahua spruce. For example, expected heterozygosities in five isolated populations of black spruce ranged only from 0.344 to 0.360 (Despots and Simon 1987). Heterozygosity in Norway spruce ranged from 0.076 to 0.174, slightly more than twofold, but that was based on a large sample of 70 populations (Lagercrantz and Ryman 1990). The range found here was also twofold, from 0.055 to 0.131, among only 10 populations of Chihuahua spruce. Heterozygosity at El Realito was 0.127 but at Cerro de la Cruz, only about 3 km distant, heterozygosity was 0.066. Because of the small sample sizes, however, the values are within two standard errors. The most convincing argument

for the action of drift on genetic diversity is the correlation between expected heterozygosity and population size (Fig. 2). Measures of genetic diversity have been related to population size in another rare conifer, bog-pine (*Halocarpus bidwillii* [Kirk] Quinn), endemic to New Zealand (Billington 1991).

The estimate of F_{ST} indicates that 24.8% of the observed diversity is among populations. Values this large are rarely measured in conifers. The largest reported value in spruce (among 13 reports) is 6% among isolated, marginal populations of black spruce (Despots and Simon 1987). Among 28 species of pines, the highest reported value is 100% for Torrey pine (*Pinus torreyana* Parry ex Carr.), which has only two extant populations (Ledig and Conkle 1983). With the exception of this extreme case, only one other study reported a larger F_{ST} (or G_{ST}) than that found here for Chihuahua spruce: G_{ST} among 11 peripheral populations of Swiss stone pine (*Pinus cembra* L.), chosen because they were isolated and widely separated, was 32% (in El-Kassaby 1991; calculated from data in Szmidt 1982). Uniform-garden and reciprocal-transplant studies have demonstrated adaptive differentiation in most wide-ranging conifers, yet conifers are characterized by low G_{ST} . According to Hamrick and Godt (1996), the mean G_{ST} for pine species is 6.5%, only one-quarter of the estimate for Chihuahua spruce.

Populations of Chihuahua spruce are also highly differentiated as judged by Nei's genetic distance, D . Mean genetic distance ($\bar{D}$) between populations was 0.033. In other spruce species, $\bar{D}$ varies from 0.005 to 0.032 (Table 6). In wide-ranging pines with large, continuous populations, like pitch pine (*Pinus rigida* Mill.), $\bar{D}$ is nearly an order of magnitude smaller ($\bar{D} = 0.005$ in Guries and Ledig 1982) than estimates presented here for Chihuahua spruce.

Either selection or drift might explain the relatively high level of differentiation among populations of Chihuahua spruce as measured by F_{ST} and $\bar{D}$. However, the evidence suggests that drift, not selection, is the explanation. If selection were responsible, then we might expect populations in close proximity to be more similar than widely separated populations because the macroenvironment (and, therefore, some selective forces) were likely to be more similar over short distances than over long distances. The fact that Chihuahua spruce occupies similar habitats in diverse parts of its range does not negate this argument. The correlation be-

TABLE 6. Mean expected heterozygosity (H_e), observed heterozygosity (H_o), percent polymorphic loci (P), number of alleles per locus (A), Wright's fixation index (F_{IS}), proportion of total genic diversity among populations (G_{ST}), mean Nei's genetic distance ($\bar{D}$), number of migrants per generation (Nm), and rate of outcrossing (t) in natural populations of several spruce species.

Species ¹	H_e	H_o	P^2	A	F_{IS}	G_{ST}	$\bar{D}$	Nm^3	t	Citation
<i>abies</i> (26, 6)	0.252	—	62	2.8	—	0.044	0.020	5.4	—	Krutovskii and Bergmann (1995)
<i>abies</i> (18, 5, 20)	0.265	0.226	—	2.52	—	—	—	—	—	Müller-Starck (1995)
<i>abies</i> (15, 9)	0.28	—	—	—	—	0.042	—	5.7	—	Paule and Gömöry (1993)
<i>abies</i> (10, 6)	0.36	—	—	—	—	0.025	—	9.8	—	Paule and Gömöry (1993)
<i>abies</i> (21, 9)	0.165	—	46	1.83	—	0.042	0.019	5.7	—	Giannini et al. (1991)
<i>abies</i> (19, 19)	0.162	—	43 ⁴	1.78	—	—	—	—	—	Morgante and Vendramin (1991)
<i>abies</i> (5, 5, 40)	—	—	—	—	—	0.055	0.074	4.3	—	Konnert and Franke (1991)
<i>abies</i> (27, 5)	0.194	0.215	75 ⁶	—	-0.063	0.032	0.017	9.8	—	Goncharenko and Potenko (1991)
<i>abies</i> (8, 5, 3)	—	0.227	84	2.21	—	—	—	—	—	Bergmann and Ruetz (1991)
<i>abies</i> (9, 5, 12)	0.200	—	—	—	—	0.120	—	1.8	0.78	Muona et al. (1990)
<i>abies</i> (22, 70)	0.115	—	73 ⁴	1.58	0.032	0.052	0.007	4.6	—	Lagercrantz and Ryman (1990)
<i>abies</i> (34, 9)	0.22	—	82	—	—	—	—	—	—	Conkle, pers. comm. (1986)
<i>abies</i> (7, 21)	0.41	—	87	2.7	—	—	—	—	—	Bergmann and Gregorius (1979)
<i>abies</i> (4, 5, 11)	0.358	—	—	—	—	—	0.023	—	—	Lundkvist and Rudin (1977)
<i>engelmannii</i> (21, 2)	0.152	0.126	39 ⁶	1.5	0.154	0.017 ⁷	0.007	22.5	—	Shea (1990)
<i>engelmannii</i> (6, 2)	—	—	—	—	-0.034	—	—	—	0.87	Shea (1987)
<i>glauca</i> (4, 2)	—	—	—	—	—	—	—	—	0.73	Innes and Ringius (1990)
<i>glauca</i> (13, 4)	0.270	0.262	92	3.00	—	0.015 ⁸	0.015	16.4	—	Alden and Loopstra (1987)
<i>glauca</i> (18, 2)	0.174	—	83	—	—	—	0.005	—	—	Yeh and Arnott (1986)
<i>glauca</i> (12, 5, 1)	0.183	—	—	—	—	—	—	—	0.98	Cheliak et al. (1985)
<i>glauca</i> (4, 5, 1)	0.14	—	—	—	0.039	—	—	—	0.89	King et al. (1984)
<i>glehnii</i> (25, 3)	0.185	0.915	58 ⁶	1.73	-0.034	0.035	0.024	6.9	—	Goncharenko and Potenko (1992)
<i>jezoensis</i> (8, 1)	0.174	—	—	—	—	—	—	—	—	Gömöry and Paule (1990), reviewed in Krutovskii and Bergmann (1995)
<i>mariana</i> (4, 2)	—	—	—	—	0.099	—	—	—	0.62	Sproule and Dancik (1996)
<i>mariana</i> (13, 2)	0.247	0.221	—	—	0.102	0.009	0.005	27.5	—	Boyle et al. (1990)
<i>mariana</i> (25, 5)	0.351	0.466	80 ⁴	2.0	-0.340	0.060	0.032	3.9	—	Despots and Simon (1987)
<i>mariana</i> (23, 21)	0.107	0.120	38	1.44	-0.065	0.059	0.014 ⁹	4.0	—	Yeh et al. (1986)
<i>mariana</i> (15, 10)	0.22	—	52	—	-0.009	—	0.020	—	—	O'Reilly et al. (1985)
<i>mariana</i> (5, 5, 2)	—	—	—	—	—	—	0.055	—	—	Knowles (1985)
<i>obovata</i> (26, 2)	0.213	—	62	2.4	—	0.011	0.007	22.5	—	Krutovskii and Bergmann (1995)
<i>obovata</i> (27, 2)	0.186	0.199	66 ⁶	2.02	-0.044	0.017	0.009	22.5	—	Goncharenko and Potenko (1991)
<i>omorika</i> (5, 1)	—	—	—	—	—	—	—	—	0.84	Kuittinen and Savolainen (1992)
<i>omorika</i> (19, 1)	0.13	—	37	1.33	—	—	—	—	—	Kuittinen et al. (1991)
<i>schrenkiana</i> (24, 6)	0.114	0.140	46 ⁶	1.88	—	0.118	0.049	1.9	—	Goncharenko et al. (1992)
<i>shrenkiana</i> (25, 4)	0.154	0.126	44 ⁶	1.76	—	—	—	—	—	Goncharenko et al. (1991)
<i>sitchensis</i> (5, 5, 1)	—	—	—	—	—	—	—	—	0.91 ¹⁰	Chaisurisri et al. (1994)
<i>sitchensis</i> (18, 3)	0.199	—	76	—	—	—	0.018	—	—	Yeh and Arnott (1986)
<i>sitchensis</i> (24, 10)	0.150	—	51	—	—	0.079	0.014	2.9	—	Yeh and El-Kassaby (1980)

¹ Number of loci followed by number of populations (in parentheses).

² A locus was considered polymorphic if more than one allele was detected, unless otherwise noted.

³ Calculated from Wright's F_{ST} or Nei's G_{ST} .

⁴ A locus was considered polymorphic if the most common allele had a frequency less than 0.95.

⁵ Based only on polymorphic loci.

⁶ A locus was considered polymorphic if the most common allele had a frequency less than 0.99.

⁷ Among sites 100 m apart; additional variation was observed among arbitrary 20 × 50 m quadrats within sites.

⁸ Based on sum of site and site subdivision components.

⁹ Between regions.

¹⁰ Upper crown, the rate of outcrossing for the lower crown was 0.076.

tween genetic and geographic distances was weak and near zero; for illustration, D between Cerro de la Cruz and El Realito, only 3 km apart, is greater than that between Cerro de la Cruz and Arroyo de la Pista, nearly 600 km distant.

The distribution of allele frequencies also implicates the action of drift in molding the genetic structure of Chihuahua spruce. The uniform distribution of allele frequencies in Chihuahua spruce relative to the U-shaped distribution commonly observed in conifers is strong evidence for bottleneck effects and the loss of rare alleles (Chakraborty et al. 1980).

Estimated values of Nm suggest that gene flow among populations of Chihuahua spruce is highly restricted. Nm is literally the number of immigrants per generation. An Nm of 0.5 is a critical value, marking the point at which populations

will diverge as a result of drift. With an initial gene frequency of 0.5 and an Nm of 0.5, occasional fixation is expected, and for rare alleles, frequent fixation is expected (Wright 1969; p. 363). All gene frequencies become equally probable. However, many generations are required for Nm to reach equilibrium after gene flow ceases among populations, perhaps 100 to 1000 generations (Slatkin and Barton 1989). That is, estimates of Nm reflect past contact as well as present gene flow and, therefore, likely underestimate present levels of exchange. Furthermore, a given Nm may not counterbalance drift as much as expected if the migrants are related, if they come only from the neighboring population(s), or if migration rates vary in time (Levin 1988), which are all possibilities in the present case. Therefore, current exchange among these

populations almost certainly averages less than the estimates of 0.43 or 0.76 migrants per generation.

An Nm of 0.5 is approximately an order of magnitude lower than values reported for other conifers. In nine reports for other species of spruce (Table 6), Nm was never less than 2.9 and was as high as 22.5. For nine species of pine (reviewed in Ledig, in press), Nm was never less than 4.6 and averaged 12.4. However, Chihuahua spruce populations are small and widely separated in contrast to the structure of many other conifers.

The estimate of the fixation index, F_{IS} , for Chihuahua spruce was 0.185, which is also outside the bounds of most previous studies in the conifers. The largest estimate of F_{IS} previously reported in spruce was 0.102, based on 13 loci in black spruce (Boyle et al. 1990). Only four of 10 studies of spruce (Table 6) indicated a heterozygote deficiency. Based on unbiased estimates of H_e , we observed a heterozygote deficiency for eight of 10 sampled populations of Chihuahua spruce, and for the other two, H_e and H_o were equal (compare H_e with H_o in Table 3). However, the means of locus-by-locus estimates of deviations from Hardy-Weinberg expectations (F) were slightly negative (excess heterozygosity) in three of 10 populations (Table 3). Excess heterozygosity is the common observation in conifers, so these results are unusual. Among conifers, only Pacific yew (*Taxus brevifolia* Nutt.) seems to have a fixation index (0.472) higher than that of Chihuahua spruce (El-Kassaby and Yanchuk 1994). Pacific yew occurs in disjunct populations, like Chihuahua spruce, but has a wider range.

The index of fixation can be used to infer the relationship among mating trees in a population. Malecot's coefficient of relationship is twice the fixation index, or coefficient of inbreeding. Therefore, an F_{IS} of 0.185 indicates a relationship coefficient of 0.37 among the parents of trees in the present generation. This suggests that mates in the preceding generation were, on average, more closely related than half-siblings and less closely related than full-siblings because the relationship coefficient among half-siblings is 0.25 and the relationship coefficient among full-siblings is 0.50. This level of inbreeding seems remarkably high for a conifer.

The fixation index can be used to estimate the outcrossing rate under the assumption that equilibrium has been reached (Allard et al. 1968):

$$t = (1 - F_e)/(1 + F_e), \quad (3)$$

where F_e is the equilibrium fixation index. Assuming F_{IS} represents the equilibrium value (almost certainly incorrect), t is 0.69. This is much higher than the observed values but, in any case, suggests substantially more inbreeding than observed in any other spruce or pine.

The mating system analyses indicate that both Arroyo del Infierno and Cerro de la Cruz are experiencing high rates of selfing (85–100%). A predominance of selfing over outcrossing has never been reported previously in a conifer. Even the rare, self-fertile Serbian spruce has a rate of outcrossing of 84% in natural stands (Kuittinen and Savolainen 1992).

Arroyo del Infierno—the Creek of Hell—has been described in detail (Gordon 1968). The spruce population consists of only 36 mature trees (according to Gordon 1968), scattered in a mixed stand of Durango fir (*Abies durangensis*

Martínez), Douglas fir, pines (*Pinus ayacahuite* Ehrenb. and *Pinus durangensis* Martínez), Mexican cypress (*Cupressus lindleyi* Klotzsch), oak (*Quercus castanea* Nee), and cherry (*Prunus serotina* var. *rufula* Woot. et Standl.). Arroyo del Infierno is near the southern extreme of the range of Chihuahua spruce and is about 100 km from any other stand of the species. It is likely that it has been isolated longer than many of the other populations in this study, and inbreeding may be more severe. Nevertheless, its fixation index indicates a slight excess of heterozygotes, which suggests that selection must offset the high rate of selfing.

Cerro de la Cruz was the other population used for the mating system analysis. It is not surprising, perhaps, that inbreeding would be common at Cerro de la Cruz. The population has only 17 mature trees, many with dead tops, and the highest fixation index observed, 0.414. Mating system analysis indicated pollen pool heterogeneity among seed parents. Pollen pool heterogeneity could occur as a result of selfing or because crosses were restricted predominantly to those between a few neighbors. This might be expected in such extremely small populations and where the spruce were often dispersed among associated species. Furthermore, because of differences in flowering phenology, often observed among trees in conifer species (Eriksson et al. 1973; El-Kassaby et al. 1984; Griffin 1984), each seed parent is likely to sample a small array of pollen parents and violate the assumption of pollen pool homogeneity.

In most conifers, selfing and other forms of inbreeding depress seed yield and progeny growth (Franklin 1970). If Chihuahua spruce was initially diverse, as indicated by an H_e of 0.131 for the largest population in our sample, it probably carried a correlated load of recessive deleterious alleles (Ledig 1986; Lande 1995), and selfing or mating among relatives is likely to lead to high proportions of empty seeds. We did not count empty or filled seeds from every tree because fire destroyed the main building at the Centro de Genética Forestal and with it, the seeds reserved for such a study. Nevertheless, it was obvious from the seeds germinated for the electrophoretic study that germinative vigor was low (indicative of inbreeding in conifers). We dissected samples of 25 seeds from each of 20 trees that had low rates of germination and found a range in empty seeds from 4% to 84%, with a mean of 45%, a severe reduction in reproductive capacity. We suspect that inbreeding is the major present threat to Chihuahua spruce. Reduced seed production, combined with demographic stochasticity, perhaps driven by climatic warming and associated climatic variability, suggests that many of the extant populations of Chihuahua spruce are precariously balanced between survival and extinction.

The data on genetic diversity, genetic structure, and inbreeding taken together with paleontological evidence can be used to infer the recent evolutionary history of Chihuahua spruce. Spruce pollen has been found in the sediment of ancient Lake Texcoco under Mexico City and in Chalco Lake (Clisby and Sears 1955; Lozano-García et al. 1993). Although the percentage of spruce pollen was only 1–2% in published studies and about 10% in some recent cores (M. S. Lozano-García, pers. comm. 1997), this nevertheless suggests that spruce was locally abundant around the basin of Mexico. Spruce is always underrepresented in sediments, except in

extensive boreal forest (Jackson 1994; Jackson and Smith 1994), and sites in the basin of Mexico are not in a good position to record pollen from distant sites in the surrounding mountains.

In Clisby and Sears's (1955) "Bellis" core from Lake Texcoco, spruce pollen was found at depths of 13 to 20 m, sediments deposited roughly between the Cary and the Manakato maxima in Flint's (1947) reconstruction of the Wisconsin glaciation and spanning a period from about 15,000 to 10,000 yr B.P. (Sears and Clisby 1955). Based on chronologies at Chalco Lake, another location within the basin of Mexico, "spruce forest began to expand" at the end of the Pleistocene, 12,500 to 9000 yr B.P., and spruce pollen was still present in sediments deposited between approximately 8000 to 7000 yr B.P. when climate was warming (Lozano-García et al. 1993; M. S. Lozano-García, pers. comm. 1997). A date of 10,000 yr B.P. is usually taken as the start of rapid warming in the present interglacial period, but Heine and Ohngemach (1976) place the start of this epoch, the Holocene, at about 8500 to 9000 yr B.P. in Mexico. The start of the Xerothermic, a stage warmer and dryer than the present, is usually dated to 8500 yr B.P. in northern North America. The paleoclimatic and palynological evidence makes it likely that the range of Chihuahua spruce began to shrink northward and fragment after about 8000 yr B.P. Chihuahua spruce is now present only in scattered, relictual populations 700 km north of Mexico City in cool, moist refugia in the barrancas of the Sierra Madre Occidental.

Nei's (1972) genetic distance (D) can be used to estimate the time (T) since populations began to diverge and, therefore, date the start of fragmentation: $T = D/2\alpha$, where α is the mutation rate. For assumed α of 10^{-5} and 10^{-6} and a mean genetic distance of 0.033, the estimate of T is 1700 to 17,000 yr, respectively, which, although a broad range, is at least consistent with a hypothesis of isolating events dating to about 8000 years B.P. Since Chihuahua spruce can live to be at least 200-years-old (Gordon 1968), 8000 years could represent as little as 40 generations.

The genetic structure of Chihuahua spruce suggests that these populations have been at low numbers and closed for much of their recent history. The estimates of 0.43 or 0.76 for Nm are consistent with a series of closed populations. In a closed population with mating at random, including selfing and mating among relatives, the expected heterozygosity after T generations (H_T) is:

$$H_T = (1 - 1/2N_e)^T H_A, \quad (4)$$

where H_A is ancestral heterozygosity and N_e is effective population number (Crow and Kimura 1970). Because the population at Rio Vinihueachi has the highest expected heterozygosity, it may approximate the ancestral level for the species. We used $H_T = 0.131$ and $N_e = 2441$, the values observed at Rio Vinihueachi, and $T = 40$ to calculate H_A . We then used the calculated value for H_A , 0.132, and substituted our estimates of expected heterozygosity for each population for H_T and solved for N_e . The calculated N_e were generally smaller than observed N (Table 3), as would be expected, but are reasonably well correlated, supporting the argument that these populations have been closed for some time.

In summary, Chihuahua spruce has moderate levels of ge-

netic diversity, within the range observed for conifers (Hamrick and Godt 1996). However, most conifers have little genetic differentiation among populations, have low values for Wright's fixation index (i.e., show heterozygote excess), have high numbers of migrants (Nm) among populations, and are strongly outcrossing. By contrast, Chihuahua spruce populations have a wide range in levels of genetic diversity (H_e from 0.055 to 0.131) even within a small area; significant levels of total diversity among populations (F_{ST} of 0.248); and little migration among populations (estimates of Nm from 0.43 to 0.76); and are highly selfing (t_m of 0.00 and 0.15 in two small populations). The observations suggest the importance of drift and inbreeding in the recent evolution of the species.

We speculate that some event, most obviously the warming climate of the Holocene, resulted in the rapid decline of Chihuahua spruce beginning about 7000 to 8000 yr B.P. (palynological evidence). In a short period of time the species was restricted to widely scattered pockets of sheltered habitat. Gene flow was reduced to low levels. The small populations diverged largely because of drift (as evidenced by the relationship between population size and measures of diversity and by differences among populations uncorrelated with proximity, a surrogate of environment). Most populations are now effectively isolated (Nm below 1.0) and have persisted at reduced numbers long enough to accumulate the effects of inbreeding (high F_{IS}). Differentiation and the relation of diversity to population size correspond to expectations based on theory (Wright 1969). Selection for heterozygosity, as proposed by Lesica and Allendorf (1992) does not seem to have slowed the loss of variability significantly, at least not at the isozyme loci that we surveyed or linked segments of the genome. Genetic depauperization was probably not the initial cause of decline in Chihuahua spruce, however. Perhaps, Chihuahua spruce either has not had time to adapt to a changed climate, the change exceeded its capacity to adapt, or other species better adapted to the new conditions excluded it from much of its former range by competition or predation.

The high fixation index, prevalence of empty seeds, and the high level of selfing or mating between neighbors raises fears that inbreeding may reduce reproductive capacity and contribute to the rarity of Chihuahua spruce. Uncontrolled harvest, grazing, and fire all threaten to further reduce populations. Some populations may now be in an extinction vortex.

Conservation efforts could be focused on populations such as Rio Vinihueachi with high genetic diversity as measured by percent polymorphic loci and expected heterozygosity. However, given the frequency of private alleles and the evidence that drift has already contributed to the differentiation of populations, we expect that several are probably worthy of conservation (e.g., Lesica and Allendorf 1995).

If the goal of conservation is to prevent the extinction of Chihuahua spruce, the most obvious tactic would be to restore gene flow among populations. This could be accomplished by pollen transfer and controlled or mass pollination, relatively costly processes that require precise timing. A less expensive means would be to collect seeds, grow seedlings, and reciprocally transplant them among populations within a geographic area. If the seedlings survived and matured, this

would effectively encourage crossbreeding among populations that are likely fixed for different deleterious alleles. Representatives of the local population should be included in any such attempt at artificial regeneration to avoid merely replacing one local population with another.

ACKNOWLEDGMENTS

This study was an undertaking of the Forest Genetic Resources Study Group/North American Forestry Commission/Food and Agricultural Organization of the United Nations. It was completed with the help of National Research Initiatives Competitive Grant Program award no. 95-37101-1916 to FTL. Seed collections were funded by the USDA Office of International Cooperation and Development, project no. 190-6. We thank S. T. Jackson and S. Lozano-García for help with the palynology literature, and M. T. Conkle, G. R. Furnier, C. I. Millar, S. R. Mori, and an anonymous reviewer for helpful comments. We are grateful for the assistance of R. Benitez-Trujillo and A. Olivas-Meza in organizing the seed collections and for informative discussion; to J. Sánchez-Cordova for sharing his great knowledge of Chihuahua spruce with us; and to M. Caballero-Deloya for his support of this project. Without the aid of the foresters of Durango and Chihuahua, especially Unidad de Administracion Forestal Directors J. Ruiz-Ramirez, J. Manuel Cassian-Santos, and R. Modesto-Terrazas, and of K. E. Clausen of the Centro de Genetica Forestal, we would not have been able to locate the Chihuahua spruce—thank you, friends, for many exciting days together in the mountains of Mexico.

LITERATURE CITED

- ALDEN, J., AND C. LOOPSTRA. 1987. Genetic diversity and population structure of *Picea glauca* on an altitudinal gradient in interior Alaska. *Can. J. For. Res.* 17:1519–1526.
- ALLARD, R. W., S. K. JAIN, AND P. L. WORKMAN. 1968. The genetics of inbreeding populations. *Adv. Genet.* 14:55–131.
- BARTON, N. H., AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56:409–415.
- BERGMANN, F., AND H.-R. GREGORIUS. 1979. Comparison of the genetic diversities of various populations of Norway spruce (*Picea abies*). Pp. 99–107 in D. Rudin, ed. *Proceedings of the conference on biochemical genetics of forest trees*, Umeå, Sweden 1978. Swedish Univ. of Agricultural Sciences, Umeå, Sweden.
- BERGMANN, F., AND W. RUETZ. 1991. Isozyme genetic variation and heterozygosity in random tree samples and selected orchard clones from the same Norway spruce populations. *For. Ecol. Manag.* 46:39–47.
- BILLINGTON, H. L. 1991. Effect of population size on genetic variability in a dioecious conifer. *Conserv. Biol.* 5:115–119.
- BOBROV, E. 1970. History and systematics of the genus *Picea* A. Dietr. *Novosti Sistematiki Vysshikh Rastenii* 7:5–40 (in Russian).
- BOYLE, T., C. LIENGSI, AND C. PIEWLUANG. 1990. Genetic structure of black spruce on two contrasting sites. *Heredity* 65:393–399.
- CHAISSURISRI, K., J. B. MITTON, AND Y. A. EL-KASSABY. 1994. Variation in the mating system of Sitka spruce (*Picea sitchensis*): evidence for partial assortative mating. *Am. J. Bot.* 81:1410–1415.
- CHAKRABORTY, R., P. A. FUERST, AND M. NEL. 1980. Statistical studies on protein polymorphism in natural populations. III. Distribution of allele frequencies and the number of alleles per locus. *Genetics* 94:1039–1063.
- CHELIAK, W. M., J. A. PITEL, AND G. MURRAY. 1985. Population structure and mating system of white spruce. *Can. J. For. Res.* 15:301–308.
- CLISBY, K. H., AND P. B. SEARS. 1955. Palynology in southern North America. Part III: Microfossil profiles under Mexico City correlated with the sedimentary profiles. *Bull. Geol. Soc. Am.* 66:511–520.
- CONKLE, M. T. 1981. Isozyme variation and linkage in six conifer species. Pp. 11–17 in M. T. Conkle, tech. coord. *Proceedings of the symposium on isozymes of North American forest trees and forest insects*, July 27, 1979, Berkeley, California. U.S. Forest Service General Technical Report PSW-49.
- CONKLE, M. T., P. D. HODGSKISS, L. B. NUNNALLY, AND S. C. HUNTER. 1982. Starch gel electrophoresis of pine seed: a laboratory manual. U.S. Forest Service General Technical Report PSW-64.
- CROW, J. F., AND M. KIMURA. 1970. *An introduction to population genetics theory*. Harper and Row, New York.
- DALLIMORE, W., AND A. B. JACKSON. 1923. *A handbook of Coniferae including Ginkgoaceae*. Edward Arnold and Company, London.
- DESPONTS, M., AND J.-P. SIMON. 1987. Structure et variabilité génétique de populations d'épinette noire (*Picea mariana* (Mill.) B.S.P.) dans la zone hémiarctique du Nouveau-Québec. *Can. J. For. Res.* 17:1006–1012.
- EL-KASSABY, Y. A. 1991. Genetic variation within and among conifer populations: review and evaluation of methods. Pp. 61–76 in S. Fineschi, M. E. Malvolti, F. Cannata, and H. H. Hattemer, eds. *Biochemical markers in the population genetics of forest trees*. SPB Academic Publishing, The Hague, The Netherlands.
- EL-KASSABY, Y. A., AND A. D. YANCHUK. 1994. Genetic diversity, differentiation, and inbreeding in Pacific yew from British Columbia. *J. Hered.* 85:112–117.
- EL-KASSABY, Y. A., A. M. K. FASHLER, AND O. SZIKLAI. 1984. Reproductive phenology and its impact on genetically improved seed production in a Douglas-fir seed orchard. *Silvae Genet.* 33:120–125.
- ERIKSSON, G., A. JONSSON, AND D. LINDGREN. 1973. Flowering in a clone trial of *Picea abies* Karst. *Stud. For. Suec.* 110:1–45.
- EVERETT, T. H. 1981. *The New York Botanical Garden illustrated encyclopedia of horticulture*. Vol. 9. Par-Py. Garland Publishing, New York.
- FLINT, R. F. 1947. *Glacial geology and the Pleistocene epoch*. Wiley, New York.
- FRANKLIN, E. C. 1970. Survey of mutant forms and inbreeding depression in species of the family Pinaceae. USDA Forest Service Research Paper SE-61.
- GIANNINI, R., M. MORGANTE, AND G. G. VENDRAMIN. 1991. Allozyme variation in Italian populations of *Picea abies* (L.) Karst. *Silvae Genet.* 40:160–166.
- GONCHARENKO, G. G., AND V. V. POTENKO. 1991. The parameters of differentiation in Norway spruce (*Picea abies* (L.) Karst.) and Siberian spruce (*Picea obovata* Ledeb.) populations. *Genetika* 27:79–92 (in Russian, English summary).
- . 1992. Genetic structure, variation, and differentiation of Sakhalin spruce (*P. glehnii* Mast.) on Sakhalin. *Dokl. Akad. Nauk USSR* 321:606–611. Translated from Russian by Plenum Publishing, New York.
- GONCHARENKO, G. G., V. V. POTENKO, N. ABDYGANYEV, AND V. G. KRIVKO. 1991. The parameters of genetic diversity in natural populations of Tien-Shan spruce *Picea schrenkiana* Fisch. et Mey. *Dokl. Akad. Nauk USSR* 35:740–744 (in Russian, English summary).
- GONCHARENKO, G. G., V. V. POTENKO, AND N. ABDYGANYEV. 1992. Variation and differentiation in natural populations of Tien-Shan spruce (*Picea schrenkiana* Fisch. et Mey.) *Genetika* 28:83–96 (in Russian, English summary).
- GORDON, A. G. 1968. Ecology of *Picea chihuahuana* Martinez. *Ecology* 49:880–896.
- GRAHAM, A. 1993. Historical factors and biodiversity in Mexico. Pp. 109–127 in T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa, eds. *Biological diversity of Mexico: origins and distribution*. Oxford Univ. Press, New York.
- GRIFFIN, A. R. 1984. Clonal variation in radiata pine seed orchards. II. Flowering phenology. *Aust. For. Res.* 12:295–302.

- GURIES, R. P., AND F. T. LEDIG. 1982. Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). *Evolution* 36: 387–402.
- HAMRICK, J. L., AND M. J. W. GODT. 1996. Conservation genetics of endemic plant species. Pp. 281–304 in J. C. Avise and J. L. Hamrick, eds. *Conservation genetics: case histories from nature*. Chapman and Hall, New York.
- HEINE, K., AND D. OHNGEMACH. 1976. Die Pleistozän/Holozän-Grenze in Mexiko. Münstersche Forschungen zur Geologie und Paläontologie 38/39:229–251.
- INNES, D. J., AND G. G. RINGIUS. 1990. Mating system and genetic structure of two populations of white spruce (*Picea glauca*) in eastern Newfoundland. *Can. J. Bot.* 68:1661–1666.
- JACKSON, S. T. 1994. Pollen and spores in Quaternary lake sediments as sensors of vegetation composition: theoretical models and empirical evidence. Pp. 253–286 in A. Traverse, ed. *Sedimentation of organic particles*. Cambridge Univ. Press, Cambridge.
- JACKSON, S. T., AND S. J. SMITH. 1994. Pollen dispersal and representation on an isolated, forested plateau. *New Phytol.* 128: 181–193.
- KING, J. N., B. P. DANCIC, AND N. K. DHIR. 1984. Genetic structure and mating system of white spruce (*Picea glauca*) in a seed production area. *Can. J. For. Res.* 14:639–643.
- KNOWLES, P. 1985. Comparison of isozyme variation among natural stands and plantations: jack pine and black spruce. *Can. J. For. Res.* 15:902–908.
- KONNERT, M., AND A. FRANKE. 1991. Die Fichte (*Picea abies* (L.) Karst.) im Schwarzwald: Genetische Differenzierung von Beständen. *Allg. Forst Jagdztg.* 162:100–106.
- KRUTOVSKII, K. V., AND F. BERGMANN. 1995. Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) Karst., and Siberian, *P. obovata* Ledeb., spruce species studied by isozyme loci. *Heredity* 74:464–480.
- KUITTINEN, H., AND O. SAVOLAINEN. 1992. *Picea omorika* is a self-fertile but outcrossing conifer. *Heredity* 68:183–187.
- KUITTINEN, H., O. MUONA, K. KÄRKKÄINEN, AND Ž. BORZAN. 1991. Serbian spruce, a narrow endemic, contains much genetic variation. *Can. J. For. Res.* 21:363–367.
- LAGERCRANTZ, U. AND N. RYMAN. 1990. Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. *Evolution* 44:38–53.
- LANDE, R. 1995. Mutation and conservation. *Conserv. Biol.* 9:782–791.
- LEDIG, F. T. 1986. Heterozygosity, heterosis, and fitness in outcrossing plants. Pp. 77–104 in M. E. Soulé, ed. *Conservation biology: the science of scarcity and diversity*. Sinauer, Sunderland, MA.
- . In press. Genetic variation in *Pinus*. In D. M. Richardson, ed. *Ecology and biogeography of Pinus*. Oxford Univ. Press, Oxford.
- LEDIG, F. T., AND M. T. CONKLE. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). *Evolution* 37:79–85.
- LESICA, P., AND F. W. ALLENDORF. 1992. Are small populations of plants worth preserving? *Conserv. Biol.* 6:135–139.
- . 1995. When are peripheral populations valuable for conservation? *Conserv. Biol.* 9:753–760.
- LEVIN, D. A. 1988. Consequences of stochastic elements in plant migration. *Am. Nat.* 132:643–651.
- LOZANO-GARCÍA, M. S., B. ORTEGA-GUERRERO, M. CABALLERO-MIRANDA, AND J. URRUTIA-FUCUGAUCHI. 1993. Late Pleistocene and Holocene paleoenvironments of Chalco Lake, central Mexico. *Quat. Res.* 40:332–342.
- LUNDKVIST, K., AND D. RUDIN. 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. *Hereditas* 85:67–74.
- MARTÍNEZ, M. 1953. Las Pináceas Mexicanas. Subsecretaría de Recursos Forestales y de Caza, Secretaría de Agricultura y Ganadería, México.
- MCDONALD, J. A. 1993. Phytogeography and history of the alpine-subalpine flora of northeastern Mexico. Pp. 681–703 in T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa, eds. *Biological diversity of Mexico: origins and distribution*. Oxford Univ. Press, New York.
- MORGANTE, M., AND G. G. VENDRAMIN. 1991. Genetic variation in Italian populations of *Picea abies* (L.) Karst. and *Pinus leucodermis* Ant. Pp. 205–227 in G. Müller-Starck and M. Ziehe, eds. *Genetic variation in European populations of forest trees*. J. D. Sauerländer's Verlag, Frankfurt, Germany.
- MÜLLER-STARCK, G. 1995. Genetic variation in high elevated populations of Norway spruce (*Picea abies* (L.) Karst.) in Switzerland. *Silvae Genet.* 44:356–362.
- MUONA, O., L. PAULE, A. E. SZMIDT, AND K. KÄRKKÄINEN. 1990. Mating system analysis in a central and northern European population of *Picea abies*. *Scand. J. For. Res.* 5:97–102.
- NARVÁEZ F., R., J. SÁNCHEZ C., AND A. OLIVAS M. 1983. Distribución y población de *Picea chihuahuana*. Secretaría de Agricultura y Recursos Hidráulicos (SARH), Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Centro de Investigaciones Forestales Norte, Nota Técnica no. 6.
- NEI, M. 1972. Genetic distance between populations. *Am. Nat.* 106:283–292.
- . 1973. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci.* 70:3321–3323.
- O'REILLY, G. J., W. M. PARKER, AND W. M. CHELIAK. 1985. Isozyme differentiation of upland and lowland *Picea mariana* stands in northern Ontario. *Silvae Genet.* 34:214–221.
- PATTERSON, T. F. 1988. A new species of *Picea* (Pinaceae) from Nuevo Leon, Mexico. *SIDA* 13:131–135.
- PAULE, L., AND D. GÖMÖRY. 1993. Genetic structure of Norway spruce (*Picea abies* Karst.) populations from mountainous areas in Slovakia. *Lesnictví-Forestry* 39:10–13.
- RITLAND, K. 1989. Correlated matings in the partial selfer *Mimulus guttatus*. *Evolution* 43:848–859.
- SÁNCHEZ C., J., AND R. NARVÁEZ F. 1983. *Picea chihuahuana* Mrtz. Una conífera en peligro de extinción. Secretaría de Agricultura y Recursos Hidráulicos (SARH), Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Centro de Investigaciones Forestales Norte, Nota Técnica no. 5.
- SCHEMSKE, D. W., AND R. LANDE. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52.
- SEARS, P. B., AND K. H. CLISBY. 1955. Palynology in southern North America. Part IV: Pleistocene climate in Mexico. *Bull. Geol. Soc. Am.* 66:521–530.
- SHEA, K. L. 1987. Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. *Evolution* 41:124–136.
- . 1990. Genetic variation between and within populations of Engelmann spruce and subalpine fir. *Genome* 33:1–8.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39:53–65.
- SLATKIN, M., AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43:1349–1368.
- SPROULE, A. T., AND B. P. DANCIC. 1996. The mating system of black spruce in north-central Alberta, Canada. *Silvae Genet.* 45: 159–164.
- SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* 72: 281–283.
- SZMIDT, A. E. 1982. Genetic variation in isolated populations of stone pine (*Pinus cembra* L.). *Silva Fenn.* 16:196–200.
- TAYLOR, R. J., AND T. F. PATTERSON. 1980. Biosystematics of Mexican spruce species and populations. *Taxon* 29:421–469.
- VERA, G. 1990. Algunas especies arbóreas raras o amenazadas en México. Secretaría de Agricultura y Recursos Hidráulicos (SARH), Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), México.
- WHALEN, M. 1995. Winter: woman looking from the window at a spruce tree. *Wilderness* 59(212):3.
- WRIGHT, J. W. 1955. Species crossability in spruce in relation to distribution and taxonomy. *For. Sci.* 1:319–349.

- WRIGHT, S. 1931. Evolution in Mendelian populations. *Genetics* 16:97-159.
- . 1951. The genetical structure of populations. *Ann. Eugen.* 15:323-354.
- . 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19:395-420.
- . 1969. Evolution and genetics of populations. Vol. 2. The theory of gene frequencies. Univ. of Chicago Press, Chicago.
- YEH, F. C., AND J. T. ARNOTT. 1986. Electrophoretic and morphological differentiation of *Picea sitchensis*, *Picea glauca*, and their hybrids. *Can. J. For. Res.* 16:791-798.
- YEH, F.C. AND Y.A. EL-KASSABY. 1980. Enzyme variation in natural populations of Sitka spruce (*Picea sitchensis*). I. Genetic variation patterns among trees from 10 IUFRO provenances. *Can. J. For. Res.* 10:415-422.
- YEH, F. C., M. A. K. KHALIL, Y. A. EL-KASSABY, AND D. C. TRUST. 1986. Allozyme variation in *Picea mariana* from Newfoundland: genetic diversity, population structure, and analysis of differentiation. *Can. J. For. Res.* 16:713-720.

Corresponding Editor: D. Piñero