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LOW LEVELS OF GENETIC VARIATION WITHIN AND HIGH LEVELS OF GENETIC DIFFERENTIATION AMONG POPULATIONS OF SPECIES OF *ABIES* FROM SOUTHERN MEXICO AND GUATEMALA[1]

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Populations of *Abies* in southern Mexico and Guatemala (*A. flinckii, A. guatemalensis, A. hickeli,* and *A. religiosa*) have a patchy distribution. This pattern is particularly clear in *A. guatemalensis.* Genetic diversity within populations, measured by average heterozygosity at 16 isozyme loci, is lower than the range reported for most conifers (mean H_o ranging from 0.069 in *A. guatemalensis* to 0.113 in *A. flinckii*), while differentiation among populations is higher than that observed in most conifer species studied ($\theta = F_{st}$ ranging from 0.073 in *A. hickeli* to 0.271 in *A. flinckii*). Estimated levels of gene flow are low (ranging from 0.672 in *A. flinckii* to 3.17 in *A. hickeli*). Populations in most cases had an excess of homozygosity over that expected under Hardy-Weinberg equilibrium, suggesting some inbreeding (F_{is} ranging from 0.074 in *A. flinckii* to 0.235 in *A. guatemalensis*). A significant relationship between gene flow and geographic distance was observed in *A. religiosa,* but not in the other three taxa studied. The patterns of genetic variation appear to have been influenced by the distributions and histories of these species. Paleoclimatic evidence suggests that the ranges of these species retreated upwards during the Pleistocene glaciation and became fragmented during the warming period that followed. The populations could have passed through genetic bottlenecks that reduced genetic variation and led to interpopulation differentiation.

Key words: *Abies flinckii*; *Abies guatemalensis*; *Abies hickeli*; *Abies religiosa*; gene flow; genetic variation; genetic drift; inbreeding.

Most conifers studied have high levels of genetic diversity, as measured by isozymes (Hamrick, Godt, and Sherman-Broyles, 1992; Fady and Conkle, 1993; Matusova, 1995; El-Kassaby and Ritland, 1996). It has also been found that the majority of forest tree species show low levels of isozyme differentiation among populations. These studies have mostly been conducted with economically important conifers of widespread and often continuous distributions. The high within-population genetic diversity and low among-population differentiation observed in conifers have been attributed to common life-history traits, such as longevity and extensive gene flow (Hamrick, Godt, and Sherman-Broyles, 1992; Streiff et al., 1998). However, the biogeographic history of a species should also contribute significantly to current patterns of genetic variation.

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The *Abies* species of southern Mexico and Guatemala provide an excellent opportunity to study the effects of species distributions on patterns of genetic variation because the different species have contrasting geographical distributions. There are ~50 species of *Abies* (firs) in the world, distributed in the temperate regions of the Northern Hemisphere (Welch, 1991). Of these, Martínez (1948) listed eight species as occurring in Mexico, six of them endemic to the country. Liu (1971) reduced this number to six species, four of them endemic to Mexico. Farjon (1990) listed six species in Mexico, four of them endemic to the country. Rushforth (1989) has since described two additional species from western Mexico, and Debreczy and Racz (1995) have described three more.

We considered four species of *Abies* that occur in southern Mexico and Guatemala. *Abies guatemalensis* Rehder is the southernmost representative of the genus, distributed at altitudes between 2000 and 4000 m in El Salvador, Guatemala, Honduras, and the southern Mexican states of Chiapas, Oaxaca, Guerrero, Jalisco, Hidalgo, and San Luis Potosí (Martínez, 1948; Donahue et al., 1985). Although widely distributed, it has relatively few isolated populations and is considered a threatened species (FAO, 1986). *Abies religiosa* (H. B. K.) Schl. et Cham. is distributed principally above 2000 m along the Transversal Volcanic Belt, in the states of Guerrero, Jalisco, Michoacán, México, Morelos, Hidalgo, Tlaxcala, Puebla, and Veracruz and the Distrito Federal (Martínez, 1948). Its distribution is more continuous than that of *A. guatemalensis. Abies religiosa* var. *emarginata* Martínez is distributed in the states of Jalisco and Michoacán, and *A. guatemalensis* var. *jaliscana* Martínez has a very re-

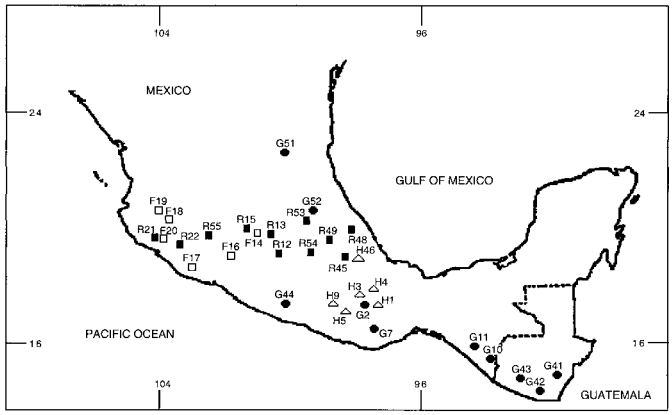

Fig. 1. Locations of populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R) sampled for isozyme analysis.

stricted distribution in western Jalisco. Martínez (1948) described these two varieties, but Rushforth (1989) subsequently combined them to form a new species, *A. flinckii* Rushforth. Our field observations suggest that these populations are indeed morphologically similar to each other and distinct from *A. religiosa* and *A. guatemalensis.* They differ even more strikingly in phenology from the latter two species, beginning vegetative growth as much as 3 mo earlier. The maintenance of very different morphologies and phenologies in trees of *A. flinckii* and *A. religiosa* intermixed at the same site in the Sierra de Manantlán strongly suggests that these are reproductively isolated separate species. *Abies hickeli* Flous et Gaussen has a limited range, with a few populations in the states of Oaxaca and Veracruz (Martínez, 1948).

In this study, we used isozyme markers to examine the levels and patterns of genetic variation in the *Abies* of southern Mexico and Guatemala. In particular, we were interested in the effects of species distribution and historical factors, such as habitat fragmentation and isolation of populations due to climatic changes.

MATERIALS AND METHODS

Branch samples were collected from 33 populations of *Abies* in southern Mexico and Guatemala. Included were ten populations of *A. guatemalensis,* 11 of *A. religiosa,* six of *A. flinckii,* and six of *A. hickeli* (Fig. 1). These populations represent most of the geographic range covered by these species. Because of our need to collect vegetative buds, the short period in which *Abies* cones are intact before disintegrating, and the large geographic area we sampled, we were unable to sample cones from these populations in the field. The populations we sampled were, however, previously described based on vegetative and reproductive characteristics by Martínez (1948), Donahue et al. (1985), and/or samples in the Herbario Nacional (MEXU). Forty individuals were sampled in each population, when possible. In the laboratory, vegetative buds were removed from the branch samples and were stored at −80°C for further use in gel electrophoresis. We maintained branch samples from each population as vouchers.

Standard methods for gel electrophoresis were followed (Conkle et al., 1982; Cheliak and Pitel, 1984). Buds from each individual were ground with extraction buffer (3:1 [v:v] mixture of buffer YO from Yeh and O'Malley [1980] and VegII from Pitel and Cheliak [1984]). The extract was absorbed in 1.2 × 1.5 mm chromatographic paper wicks. The enzyme analysis was done using two systems of electrode and gel buffers. The H buffer (Cheliak and Pitel, 1984) was used to assay shikimate dehydrogenase (SDH, Enzyme Commission number 1.1.1.25, two loci), isocitrate dehydrogenase (IDH, E.C. 1.1.1.41, one locus), phosphoglucomutase (PGM, E.C. 2.7.5.1, two loci), and menadione reductase (MNR, E.C. 1.6.99.2, one locus). The R buffer (Ridgeway, Sherburne, and Lewis, 1970) was used to assay leucine amino-peptidase (LAP, E.C. 3.4.11.1, one locus), phosphoglucoisomerase (PGI, E.C. 5.3.1.9, two loci), glutamic-oxaloacetic transaminase (GOT, E.C. 2.6.1.1, one locus), peroxidase (PER, E.C. 1.11.1.7, one locus), peptidase (PEP, E.C. 3.4.11.11, three loci), glutamate dehydrogenase (GDH, E.C. 1.4.1.3, one locus), and ribulosebiphosphate carboxylase (RUB, E.C. 4.1.1.39, one locus). Starch gels (12%) were run at 60 mA for 6–7 h on the H system and 4 h on the R system. All genotypic data for all individuals in this study are in the databases of the Comisión para el Uso y Conocimiento de la Biodiversidad (CONABIO; http://www.conabio.gob.mx) in Mexico City.

Allelic frequencies were obtained for each locus in each population. Based on these data, levels of polymorphism (P), observed (H_o) and Hardy-Weinberg expected (H_e) heterozygosity, and mean number of alleles per locus (A) were estimated with the program BIOSYS (Swofford and Selander, 1981). Wright's (1965) F statistics, total inbreeding (F_{it}), subdivision among populations (F_{st}), and inbreeding within populations (F_{is}) were estimated for each species by the method described in Weir and Cockerham (1984) ($F = F_{it}$, $\theta = F_{st}$ and $f = F_{is}$) , using the program FSTAT (version 1.2; Goudet, 1995). Partitioning of variation within and among species was estimated using the WRIGHT78 procedure of the program BIOSYS (Swofford and Selander, 1981).

An estimate of gene flow (Nm), the average effective number of migrants exchanged between populations in each generation, was calculated from θ, and also from the number and frequency of private alleles (Barton and Slatkin, 1986). Wright (1951) showed that in an island model, where equilibrium has been reached between genetic drift and migration, $Nm = (1 - \theta)/4\theta$, where θ is the proportion of total genetic diversity represented by differences among populations.

Nm can also be calculated from the number and frequency of private alleles, unique alleles found in only one population (Slatkin, 1985), as follows:

$$\log_{10}[p(1)] = a \log_{10}(Nm) + b,$$

where $p(1)$ is the mean frequency of private alleles and a and b are constants determined by simulated data. We used values for a and b obtained for a sample size of 25 and corrected for our mean sample size (Barton and Slatkin, 1986). This method of estimating gene flow is likely to be more sensitive to errors in data collection (Slatkin, 1994).

The relationship of gene flow between pairs of populations (M) to geographic distance (Slatkin, 1993) was examined. Values of M were obtained by computing θ (a measure of among-population differentiation; Weir and Cockerham, 1984) for each pair of locations using a program provided by Slatkin (isolation by distance program). A regression of $\log_{10}M$ on $\log_{10}k$ (k = geographic distance) was then constructed to see if there was a linear relationship. The significance of the relationship was evaluated by Mantel's (1967) test, since the usual statistical methods cannot be used because values of M from different pairs of populations are not independent. This was done for each taxon studied. Finally, we used PHYLIP (Felsenstein, 1995) to construct a neighbor joining tree (Saitou and Nei, 1987), based on Nei's unbiased genetic distances (Nei, 1978). One hundred bootstrap samples over loci were used to estimate the confidence of the branch points.

RESULTS

Levels of polymorphism and expected average heterozygosity were lowest for *A. guatemalensis,* intermediate for *A. hickeli,* and highest for *A. flinckii* and *A. religiosa* (Table 1). The range of variation among populations in

TABLE 1. Geographic coordinates, elevation (m), mean number of individuals analyzed per locus (*N*), mean number of alleles per locus (*A*), percentage of polymorphic loci (frequency of most common allele < 0.95) (P), observed heterozygosity (H_o), mean unbiased estimate of expected heterozygosity (H_e), and fixation index (*F*) across 16 isozyme loci for populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R). Standard errors are in parentheses.

Population code	Lat N/ long W	elevation	*N*	*A* (SE)	P(%)	H_o (SE)	H_e (SE)	*F* (SE)
F14	19°35′	2340	26.6	1.7	25.0	0.055	0.089	0.284
	100°45′			(0.2)		(0.024)	(0.035)	(0.444)
F16	19°20′	2250	24.4	1.6	37.5	0.184	0.158	−0.120
	101°21′			(0.2)		(0.059)	(0.049)	(0.271)
F17	18°46′	2500	19.6	1.4	12.5	0.031	0.064	0.322
	102°57′			(0.2)		(0.018)	(0.037)	(0.550)
F18	20°12′	2100	39.3	1.7	37.5	0.125	0.125	−0.006
	104°43′			(0.2)		(0.043)	(0.044)	(0.253)
F19	20°21′	2490	34.6	1.8	37.5	0.119	0.130	0.165
	104°59′			(0.2)		(0.048)	(0.044)	(0.426)
F20	19°27′	2500	29.9	1.4	31.3	0.097	0.109	0.027
	103°56′			(0.2)		(0.041)	(0.047)	(0.205)
A. flinckii mean			29.1	1.6	30.2	0.102	0.113	0.112
				(0.17)	(10.0)	(0.054)	(0.033)	(0.174)
G2	17°10′	2800	30.8	1.3	25.0	0.047	0.060	0.124
	96°30′			(0.1)		(0.022)	(0.027)	(0.378)
G7	16°11′	2500	25.4	1.3	18.8	0.033	0.044	0.139
	96°18′			(0.1)		(0.016)	(0.023)	(0.254)
G10	15°07′	3330	28.8	1.4	25.0	0.051	0.072	0.250
	92°07′			(0.1)		(0.028)	(0.032)	(0.456)
G11	15°27′	2610	36.1	1.4	18.8	0.072	0.070	0.107
	92°16′			(0.2)		(0.032)	(0.033)	(0.440)
G41	15°04′	2970	35.8	1.3	18.8	0.043	0.058	0.173
	89°55′			(0.1)		(0.020)	(0.027)	(0.280)
G42	14°31′	2610	20.5	1.3	12.5	0.046	0.058	0.083
	90°08′			(0.1)		(0.027)	(0.036)	(0.139)
G43	14°52′	2730	25.7	1.3	12.5	0.047	0.063	0.079
	91°17′			(0.1)		(0.025)	(0.036)	(0.244)
G44	17°35′	2670	32.8	1.4	18.8	0.044	0.079	0.160
	99°51′			(0.1)		(0.024)	(0.041)	(0.387)
G51	22°27′	1770	27.6	1.4	25.0	0.075	0.090	0.209
	99°27′			(0.2)		(0.038)	(0.043)	(0.285)
G52	20°21′	2310	33.3	1.7	25.0	0.070	0.094	0.331
	98°20′			(0.2)		(0.031)	(0.036)	(0.478)
A. guatemalensis mean			29.7	1.38	20.0	0.053	0.069	0.165
				(0.123)	(4.9)	(0.014)	(0.015)	(0.079)
H1	17°10′		30.8	1.6	31.3	0.041	0.090	0.394
	96°22′			(0.2)		(0.017)	(0.034)	(0.451)
H3	17°22′	2904	31.4	1.4	18.8	0.055	0.087	0.179
	96°26′			(0.2)		(0.023)	(0.039)	(0.277)
H4	17°27′	2530	10.6	1.3	12.5	0.078	0.073	−0.125
	96°24′			(0.1)		(0.055)	(0.047)	(0.551)
H5	16°44′	2600	38.9	1.6	43.8	0.145	0.135	−0.002
	97°07′			(0.2)		(0.056)	(0.051)	(0.270)
H9	17°03′	3000	39.8	1.6	18.8	0.091	0.087	−0.047
	97°45′			(0.2)		(0.045)	(0.042)	(0.062)
H46	18°58′	2910	28.6	1.7	43.8	0.115	0.125	0.108
	97°12′			(0.2)		(0.042)	(0.045)	(0.357)
A. hickeli mean			30.2	1.53	28.2	0.088	0.100	0.085
				(0.15)	(13.6)	(0.038)	(0.025)	(0.186)
R12	19°11′	3240	31.1	1.8	43.8	0.121	0.155	0.133
	99°48′			(0.2)		(0.039)	(0.048)	(0.275)
R13	19°26′	2800	29.1	1.4	31.3	0.070	0.087	0.104
	100°10′			(0.2)		(0.031)	(0.039)	(0.207)
R15	19°40′	2880	35.9	1.4	31.3	0.051	0.080	0.332
	100°49′			(0.1)		(0.031)	(0.036)	(0.512)
R21	19°27′	2500	36.3	1.1	0.0	0.009	0.008	−0.036
	103°56′			(0.1)		(0.006)	(0.006)	(0.006)
R22	19°35′	3330	31.6	1.1	12.5	0.033	0.032	−0.041
	103°35′			(0.1)		(0.023)	(0.024)	(0.089)
R45	18°58′	3060	36.5	1.8	37.5	0.105	0.128	0.136
	97°21′			(0.2)		(0.038)	(0.041)	(0.320)

TABLE 1. Continued.

Population code	Lat N/ long W	elevation	*N*	*A* (SE)	P(%)	H_o (SE)	H_e (SE)	*F* (SE)
R48	19°31′	3510	37.9	1.6	37.5	0.116	0.118	−0.032
	97°09′			(0.2)		(0.037)	(0.039)	(0.140)
R49	19°41′	2760	34.7	1.6	25.0	0.099	0.116	0.183
	98°05′			(0.2)		(0.044)	(0.050)	(0.247)
R53	20°09′	2940	38.1	1.4	25.0	0.065	0.088	0.143
	98°42′			(0.2)		(0.032)	(0.042)	(0.262)
R54	19°23′	3330	26.6	2.2	68.8	0.132	0.235	0.398
	98°40′			(0.2)		(0.033)	(0.048)	(0.301)
R55	19°23′	3030	25.6	1.6	37.5	0.099	0.139	0.283
	102°19′			(0.1)		(0.047)	(0.049)	(0.503)
A. religiosa mean			33.0	1.54	31.8	0.082	0.108	0.146
				(0.32)	(17.6)	(0.039)	(0.061)	(0.148)

expected heterozygosity was particularly high in *A. religiosa,* which included the population with the lowest genetic diversity (R21, $H_e = 0.008$), as well as the population with the highest value (R54, $H_e = 0.235$).

In most populations the fixation index (*F*) was near zero or positive (Table 1). The estimates were high in all species, with *A. hickeli* having the lowest value. Standard errors of *F* estimates for the populations were in general very high. The mean coefficient of inbreeding within subpopulations ($f = F_{is}$) and the total inbreeding coefficient ($F = F_{it}$) estimates are generally positive, with the highest average values for *A. guatemalensis* and *A. religiosa* and the lowest for *A. flinckii* (Table 2). There is significant differentiation among populations in all four species, with relatively high $\theta = F_{st}$ estimates for all species, particularly *A. flinckii* and *A. religiosa* (Table 2). The mean estimate of differentiation among populations ($F_{st} = \theta$) considering all species is 0.280, with 0.221 due to differences among populations within species and only 0.056 due to differences among species.

The mean estimates of *Nm* obtained from θ for each species were 0.672 (95% confidence interval = 0.46–1.43) for *A. flinckii,* 1.8 (1.34–3.72) for *A. guatemalensis,* 0.75 (0.43–1.57) for *A. religiosa,* and 3.17 (1.83–11.65) for *A. hickeli,* with an overall mean of 1.6 (obtained with the mean θ, when we analyze all species together). Estimates of *Nm* calculated from the method of private alleles were 3.42 for *A. flinckii,* 2.88 for *A. guatemalensis,* 1.67 for *A. religiosa,* and 2.70 for *A. hickeli.* Estimates of *Nm* from private alleles were higher than estimates from θ. It has been shown that both methods can provide accurate estimates of *Nm* under several circumstances (Slatkin and Barton, 1989), but the method of private alleles is more sensitive to errors in data collection and could be less accurate in practice (Slatkin, 1994). Simulations done by Slatkin (1993) show that in a stepping stone model, *M* (gene flow) and geographic distance should be inversely correlated, with a slope of −1 in linearly distributed populations. This pattern was observed in *A. religiosa,* but not in any of the other species (Fig. 2).

The mean genetic distance (Nei, 1978) between populations of *A. flinckii* was 0.051 (SD = 0.035), between populations of *A. guatemalensis* 0.011 (0.009), between populations of *A. religiosa* 0.040 (0.033), and between populations of *A. hickeli* 0.010 (0.006). These estimates of genetic distances, particularly for *A. religiosa* and *A. flinckii,* are relatively high compared to other north-temperate conifers and show high differentiation between populations. This is in agreement with the estimates of θ. Considering all populations of all species together, the mean overall genetic distance was 0.040, similar to the mean genetic distances between populations within the same species. The phenogram constructed from these distances showed a monophyletic group for *A. flinckii,* within a group containing eight of the ten *A. guatemalensis* populations and distinct from the *A. religiosa* populations (Fig. 3). *Abies hickeli* populations from Oaxaca also formed a monophyletic group. *Abies religiosa* populations formed another monophyletic group that also included two populations of *A. guatemalensis* and the one population of *A. hickeli* from Veracruz. Within this group, there were two subgroups, one consisting of populations from the Mexico City area and west (G44, R12, R13, R15, R21, R22, R49, and R53) and the other consisting of populations principally from the Mexico City area and east (G52, H46, R45, R48, and R54, with R55 being the exception). Levels of confidence in the branch points of this phenogram are relatively low.

DISCUSSION

The levels of genetic diversity we observed in these *Abies* species were lower than means reported from studies of other gymnosperms (P = 71.1, *A* = 1.83, H_e = 0.151), other temperate zone species (P = 63.5, *A* = 1.81, H_e = 0.166), and other species with outcrossing mating systems and wind-dispersed pollen (P = 53.0, *A* = 1.84, H_e = 0.173) (Hamrick, Godt, and Sherman-Broyles, 1992). The estimates were also lower than those reported for other species of *Abies* (Table 3). There are relatively few reports of natural populations of tree species with very low levels of genetic variation. Such low levels of variation usually occur in species with very narrow geographic distributions, species that are inbreeding colonizers (Brown and Marshall, 1981), or species with very small population sizes (Hartl and Clark, 1989). In many populations we observed hundreds of individuals, enough to counteract the effects of genetic drift. In some of the populations, however, we observed relatively low total sizes, particularly for *A. guatemalensis,* a species in which we found lower levels of heterozygosity. Estimates of expected heterozygosity varied from almost twofold to almost 30-fold among populations within species (0.064–

TABLE 2. Weir and Cocherham's (1984) estimates of Wright's F statistics calculated for each locus for all populations of *Abies flinckii*, *A. guatemalensis*, *A. hickeli*, and *A. religiosa*. Significance of deviations from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) were tested by using permutations. Means and standard deviations were obtained by jackknifing over loci, and confidence intervals were obtained by bootstrapping over loci.

	A. flinckii			*A. guatemalensis*			*A. hickeli*			*A. religiosa*		
Locus	F_{is}	F_{it}	θ	F_{is}	F_{it}	θ	F_{is}	F_{it}	θ	F_{is}	F_{it}	θ
Gdh1	1.000***	1.000***	−0.004	1.000***	1.000***	0.003	1.000***	1.000***	0.043***	0.866***	0.878***	0.090***
Got1	0.129	0.238***	0.125***	0.687***	0.754***	0.213***	0.120*	0.216***	0.109***	0.401***	0.590***	0.316***
Idh2	−0.187	−0.125	0.052***	−0.138	−0.009	0.114***	−0.215*	0.010	0.185***	0.253***	0.288***	0.048***
Lap1	1.000***	1.000***	0.053***	0.722***	0.748***	0.093	−0.032	−0.008	0.023*	0.110	0.200***	0.101***
Mnr1	1.000***	1.000***	0.011				0.002	−0.001	−0.003			
Pep1										0.660***	0.667***	0.020**
Pep3										0.498***	0.498***	0.001
Per3	0.067	0.310***	0.260***	0.058	0.142***	0.089***	0.005	0.023	0.018	−0.021	0.141*	0.159***
Pgi1	0.125	0.160**	0.040***	0.321*	0.373***	0.076***	−0.022	−0.019	0.003	0.171***	0.317***	0.176***
Pgi2	0.365***	0.586***	0.348***	−0.024	−0.002	0.021*	0.325***	0.325***	0.004	0.810***	0.967***	0.824***
Pgm1	0.025	0.465***	0.451***	0.035	0.099	0.035***	0.345***	0.362**	0.026**	0.258***	0.303***	0.060***
Pgm2	−0.118	0.116	0.209***	−0.066	−0.040	0.024**	−0.068	−0.020	0.045*	0.242***	0.340***	0.128***
Sdh1	−0.153	−0.006	0.128***	0.195***	0.303***	0.134***	0.200***	0.217***	0.021	0.100*	0.302***	0.225***
Sdh2	0.025	0.436***	0.421***	1.000***	1.000***	0.095***				0.219*	0.340***	0.155***
Mean	0.074**	0.325***	0.271***	0.235***	0.330***	0.122***	0.121***	0.183***	0.073***	0.216***	0.414***	0.250***
SD	(0.057)	(0.072)	(0.058)	(0.101)	(0.106)	(0.024)	(0.065)	(0.046)	(0.030)	(0.078)	(0.089)	(0.054)
95% CI	−.027–0.194	0.166–0.438	0.149–0.351	0.080–0.477	0.144–0.557	0.063–0.157	−0.025–0.243	0.079–0.275	0.021–0.120	0.106–0.370	0.263–0.577	0.137–0.366

0.158 for *A. flinckii*, 0.044–0.094 for *A. guatemalensis*, 0.073–0.135 for *A. hickeli*, and 0.008–0.235 for *A. religiosa*), providing further evidence for the possible action of genetic drift on some of these populations.

The F and F_{is} values we observed suggest inbreeding in the analyzed populations. This was strongest for *A. guatemalensis*, but significant for all species. For *A. guatemalensis* and *A. hickeli*, values of F_{is} (0.235 and 0.121, respectively) were even higher than values of θ, indicating that a high portion of the total reduction in observed heterozygosity (F_{it}) is due to inbreeding within subpopulations, although interpopulational differentiation is also considerable, especially for *A. guatemalensis*. F_{is} was also high in *A. religiosa* (0.216), but lower than θ. While most conifers studied have displayed an excess of heterozygosity over that expected under Hardy-Weinberg equilibrium, heterozygote deficits have been reported for some predominantly outcrossing species (Parker and Hamrick, 1996), including *Abies* (Shea, 1990; Fady and Conkle, 1993; Table 3).

Estimates of θ for the populations analyzed show higher amounts of genetic differentiation among populations within species than that found for most other conifers and other species of *Abies*. This should not be surprising given the general bias in the literature toward studies of widespread, economically important conifers of the Northern Hemisphere. Mean θ values were 0.271 for *A. flinckii*, 0.122 for *A. guatemalensis*, 0.073 for *A. hickeli*, and 0.250 for *A. religiosa*. The relatively lower value for *A. hickeli* may be due to its more restricted geographic distribution, with all but one of the populations occurring in central Oaxaca. Hamrick and Godt (1996a) reported a mean F_{st} of 0.101 for a variety of species with wind-dispersed pollen, and Hamrick, Godt, and Sherman-Broyles (1992) reported mean F_{st} values of 0.063 for seven species of *Abies* and 0.073 for a variety of gymnosperm species.

The relatively high values of θ for the *Abies* species of southern Mexico and Guatemala reflect spatial genetic structure and suggest that genetic drift or selection or both have been important forces in their evolution. These results also suggest restricted levels of gene flow. The estimated levels of gene flow (*Nm*) in these species are lower than those needed to counteract genetic drift ($Nm > 4$; Jorgensen and Hamrick, 1997) and are lower than the mean values reported for other outcrossing species with wind-dispersed pollen (Hamrick, 1987).

Gene flow in *A. religiosa* is somewhat geographically restricted, with long-distance dispersal not sufficiently common to prevent isolation by distance. The sampled populations have likely existed for a long time, sufficient to permit isolation by distance to become apparent (Slatkin, 1993). The failure to detect an isolation by distance pattern in the other species could be due to continuing gene flow among populations, such as in the case of *A. hickeli* ($Nm = 3.17$), or due to the populations not being at drift-migration equilibrium because of historical events, such as recent range expansion or fragmentation of populations (Comes and Abbot, 1998). Gene flow may have been so restricted between even the closest populations within *A. flinckii* and *A. guatemalensis* that the regression between M and distance was not sensitive enough to detect a pattern of isolation by distance. Also,

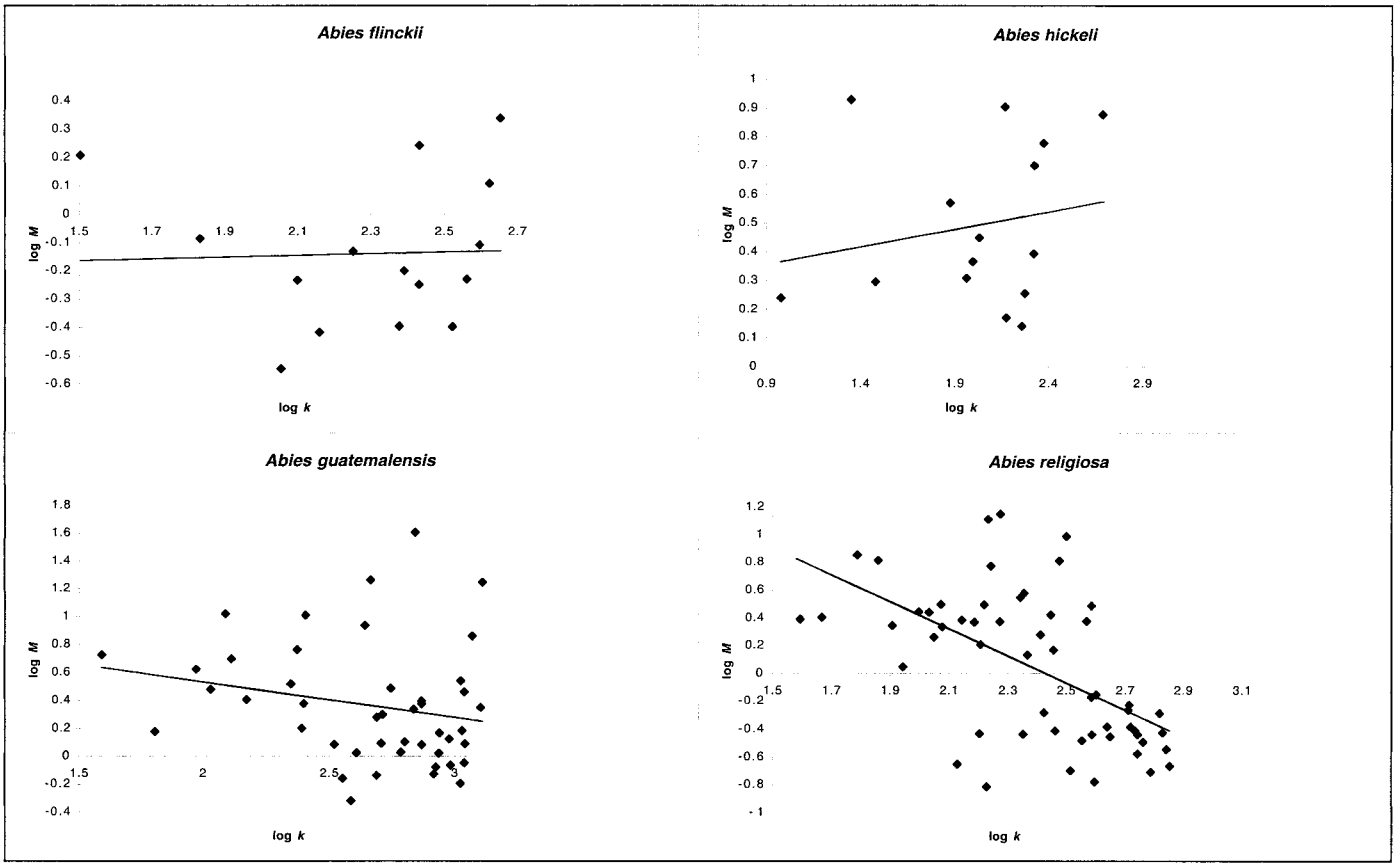

Fig. 2. Plots of all pairwise $\log_{10}M$ (M = gene flow between pairs of populations) values against $\log_{10}k$ (k = geographic distance between pairs of populations). The linear regression equations and probabilities (p) of obtaining these relationships given the null hypothesis of no relationship between $\log_{10}M$ and $\log_{10}k$ are, respectively, $\log_{10}M = 0.0298 \log_{10}k - 0.207$ and $p = 0.842$ for *Abies flinckii,* $\log_{10}M = -0.253 \log_{10}k + 1.036$ and $p = 0.537$ for *A. guatemalensis,* $\log_{10}M = 0.122 \log_{10}k + 0.246$ and $p = 0.881$ for *A. hickeli,* and $\log_{10}M = -0.974\log_{10}k + 2.366$ and $p = 0.0053$ for *A. religiosa.*

the very scattered and isolated populations of *A. guatemalensis* may not meet the assumption of the isolation by distance model.

Our phenogram provisionally suggests the existence of three major groups, *A. guatemalensis* (including *A. flinckii*), *A. hickeli,* and *A. religiosa.* We must caution, however, that the bootstrap confidence levels in this tree are low, likely due to the low levels of variation in the species, and further data from more variable markers will be necessary to draw firm phylogenetic conclusions.

While the populations of *Abies flinckii* form a monophyletic group, they are embedded within the *A. guatemalensis* group, suggesting that these may comprise a single species, as suggested by Martínez (1948) and Farjon (1990). This would not be entirely surprising, given the geographic distributions (Fig. 1) of the two species, but the great differences in phenology we observed between these species in the field suggest that they may be reproductively isolated. The clear difference between *A. flinckii* and *A. religiosa* is most strikingly apparent in populations F20 and R21, which represent trees mixed together on the same site in the Sierra de Manantlán. Although growing on the same site, their morphologies and phenologies are very different and no intermediates are found.

Branch lengths suggest that the populations of *A. flinckii* and the westernmost populations of *A. religiosa* (R21, R22, and R55) have undergone the highest degree of differentiation. These populations are quite isolated geographically and are not very large, conditions that may have permitted accelerated differentiation due to genetic drift. The relatively high θ value for *A. religiosa* may be due, in part, to the divergence between the western (e.g., R15, R21, R22, R49, and R53) and eastern (e.g., R45 and R48) populations that we observed in the tree.

All three of the populations that did not cluster with their conspecific populations (G44, G52, and H46) fall clearly within the reported geographic range of *A. religiosa.* The vegetative morphological differences between these populations and *A. religiosa* are not great and the species may have been misidentified by those who originally described these sites. In our field observations of vegetative characteristics, these three populations differed from *A. religiosa* principally in having leaves that were emarginate at the apex instead of acute, obtuse, or rounded. None of the populations showed evidence of being a mixture of species or hybrids. These populations are quite geographically isolated from their conspecific populations, and it is possible that they have undergone a very high degree of genetic drift and are now very different from the other populations in their species.

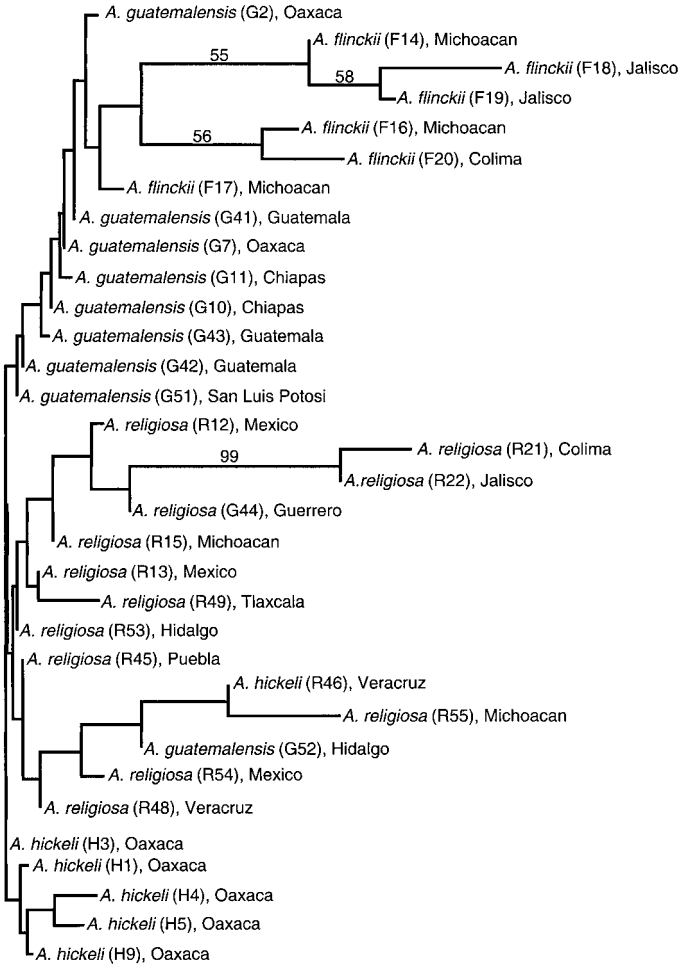

Fig. 3. Neighbor-joining dendrogram based on Nei's unbiased genetic distances between populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R). See Fig. 1 for population locations. Numbers are bootstrap values (in percentages), values below 50 are not shown.

Population H4 corresponds to the type site for *A. zapotekensis* Debreczy, Rácz, and Ramírez, a new species described by Debreczy and Racz (1995). Our data suggest that it is a population of *A. hickeli*. Population R22 corresponds to the type site of *A. colimensis* Rushforth & Narave, a new species described by Rushforth (1989). Our data suggest that it is a population of *A. religiosa*, although the branch length suggests that it is a relatively more differentiated population. Population G10 corresponds to the type site of *A. guatemalensis* var. *tacanensis* (Lundell) Martínez. Our data suggest that it is a population of *A. guatemalensis*.

The short branch lengths separating the three major groups in the phenogram are consistent with the low amount of differentiation accounted for by differences among species (0.056). This low level of interspecific differentiation may be considered somewhat surprising, but high levels of genetic drift and low levels of gene flow among populations within species could generate high levels of intraspecific differentiation without necessarily leading to high levels of interspecific differentiation.

The effect of present geographic distribution on the patterns of genetic structure is not entirely clear. We found lower levels of genetic variation in *A. guatemalensis* than in the rest of the taxa, which was expected because of its smaller and more isolated populations. We also found indications of higher levels of inbreeding in this species. In contrast to what we expected, levels of interpopulational differentiation were higher in *A. religiosa* than in *A. guatemalensis*, although in both cases they were rather high.

In temperate species, cycles of glacial and interglacial periods likely have played an important role in shaping the present genetic variation patterns of plant populations (Konnert and Bergmann, 1995). The last glacial period reached a peak between 25 000 and 18 000 yr ago (Roberts, 1998). During this period, land temperatures dropped as much as 20°C and ocean temperatures ~5°C. These climatic changes led to changes in the distributions of many species, with species of the Northern Hemisphere displaced southwards. Afterwards, when warming

TABLE 3. Percentage polymorphic loci (P), mean expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles per locus (A), Wright's fixation index (F_{is}) and total genetic diversity among populations (G_{st} or F_{st}) for different species of *Abies*.

Species	P[a] (%)	H_e	H_o	A	F_{is}	F_{st}	Reference
A. balsamea		0.274	0.266	2.05	−.008		Neale and Adams, 1985
A. fraseri	30.8	0.286[b]	0.258[b]	1.10	0.007	0.002	Diebel and Feret, 1991
A. alba			0.397		0.006	0.071	Breitenbach-Dorfer et al., 1997
A. alba	54.5	0.182	0.149	1.6			Fady and Conkle, 1993
A. cephalonica	72.7	0.221	0.161	2.0	0.234	0.048	Fady and Conkle, 1993
A. borisii regis	59.1	0.198	0.161	1.8			Fady and Conkle, 1993
A. lasiocarpa	43.4	0.124	0.081	1.6	0.341	0.017	Shea, 1990
Abies from the eastern U.S.	42.1			1.4			Jacobs, Werth, and Guttman, 1984
A. kawakamii	78	0.283	0.033	2.2			Kormutak and Yang, 1998
A. hickeli	28.2[c]	0.100	0.088	1.5	0.121	0.021	This study
A. guatemalensis	20[c]	0.069	0.053	1.38	0.235	0.122	This study
A. religiosa	31.8[c]	0.108	0.082	1.5	0.216	0.250	This study
A. flinckii	30.2[c]	0.113	0.102	1.6	0.074	0.271	This study

[a] A locus was considered polymorphic if more than one allele was detected.
[b] Based only on polymorphic loci.
[c] A locus was considered polymorphic when the frequency of the most common allele was ≤95%.

began in the current interglacial period (~11 500 yr BP), species migrated northwards (Roberts, 1998).

The genus *Abies* has a long history in Mexico, with pollen present in Mexico at least as far south as Veracruz (Paraje Solo Formation), in the middle Pliocene (five million years ago; Graham, 1993, 1999). While we can estimate the current total sizes of these populations, we do not know their effective population sizes nor their historical sizes, both factors that can influence observed levels of variation. There are no detailed climatic data for Mexico during the glacial periods, but in general terms there is consistency in the data regarding the cold and dry climates during the glacial maximum (~18 000 yr BP; Lozano-García, 1993). It is proposed that between 12 500 and 9000 yr BP, at the end of the Pleistocene, the climate was humid and colder than at present and forests reached their maximum development in Mexico (Lozano-García, 1993). This suggests that during the last glaciation temperate species had a broader distribution than at present in Mexico, and populations of species that are now isolated could have been in contact. After 9000 yr BP, the climate became warmer (Lozano-García, 1993), and populations could have been fragmented, becoming smaller and more isolated, with reduced effective population sizes (N_e).

The *Abies* species of southern Mexico and Guatemala may have experienced fragmentation of populations and range retractions with the warming trend that peaked ~6000 yr BP. Their populations could have been displaced to higher regions, possibly causing a greater degree of isolation between populations than we observe at present. These populations may have passed through a number of genetic bottlenecks that led to a loss of genetic diversity and interpopulational differentiation due to genetic drift. Small population sizes could also have led to inbreeding and the observed heterozygote deficiencies (Nei, Maruyama, and Chakraborty, 1975). A number of the populations that we sampled have more than 100 individuals, which should be large enough to counteract genetic drift if all individuals reproduce, but *Abies* species are long-lived perennials with reproductive maturity at 20 yr and an average life span of 60 yr (Jacobs, Werth, and Guttman, 1984). If new variation is accumulated in proportion to the number of generations (meiotic events) over time, then these species may not have had enough time to recover the variation that they lost due to genetic drift.

The majority of conifers studied by isozyme analysis display relatively high levels of genetic diversity and low levels of interpopulational differentiation compared to other groups of plants. They also generally display genotypic frequencies consistent with Hardy-Weinberg equilibrium or an excess of heterozygotes. These conifers are mostly species with relatively large ranges in the North Temperate zones of Asia, North America, north of Mexico, and Europe. These areas were heavily impacted by Pleistocene glaciation, with much of their areas covered by ice. The current distributions of many of these species are very recent, not giving them enough time to develop high levels of interpopulational differentiation. Many of them were able to maintain relatively large continuous populations south of the glacial front during the Pleistocene, avoiding drastic losses of variation due to genetic drift. Their current large, often continuous populations help to maintain variation and prevent interpopulational differentiation and inbreeding.

In contrast, we observed relatively low levels of genetic diversity and relatively high levels of heterozygote deficiency and interpopulational differentiation in the *Abies* species of southern Mexico and Guatemala, suggesting the action of genetic drift and relatively low levels of gene flow. These same patterns were also observed by Keiman-Freire (1997) in a study of the *Abies* species of northern Mexico. *Picea chihuahuana,* another conifer with relatively small and isolated populations in northern Mexico, displayed the same pattern, with a low level of variation (H_e = 0.093), and high levels of heterozygote deficiency (F_{is} = 0.185) and interpopulational differentiation (F_{st} = 0.248) (Ledig et al., 1997). *Pinus rzedowski,* a conifer with a small number of populations in a restricted area of west-central Mexico (western Michoacán) displays considerably higher within-population diversity (H_e = 0.219), but also has a significant deficiency of heterozygotes (F_{is} = 0.247) and a high level of interpopulational differentiation (F_{st} = 0.175; Delgado et al., 1999). These data suggest that the levels and patterns of genetic variation observed for conifers in the North Temperate zone (Hamrick, Godt, and Sherman-Broyles, 1992) are not consistent with those of the conifers of Mexico studied until now. Ge et al. (1998) also reported very high levels of interpopulation differentiation in *Cathaya argyophylla,* a conifer with a restricted distribution in southern China. Hamrick and Godt (1996b) pointed out that, not only life-history traits, such as those shared by conifers, influence the levels and distribution of genetic diversity among species, but also the phylogenetic, biogeographical, and evolutionary histories of the particular species are important.

From the standpoint of conservation, the high levels of interpopulation genetic differentiation we observed pose a real challenge. This may be the case for many Mexican conifers and much of the Mexican flora. Other isozyme studies of Mexican plant species have found relatively high levels of interpopulation differentiation (Izquierdo and Piñero, 1998; Núñez-Farfán et al., 1996; Cornejo-Romero, 1998; Martínez-Palacios, Eguiarte, and Furnier, 1999). This pattern of variation poses the challenge of preserving the many genetically distinct populations necessary to adequately capture the genetic variation of the species, within a country with strong social pressures to economically exploit the land (Carabias, Arriaga, and Cervantes, 1994).

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