Heredity **69** (1992) 217–228

Received 7 October 1991

Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): implications for evolution and conservation

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The population genetics of the understory tropical rain forest palm *Astrocaryum mexicanum* were studied in Los Tuxtlas, Veracruz, Mexico, using enzyme electrophoresis. The percentage of polymorphic loci was 31.8 and the mean expected heterozygosity was 0.153. Segregation patterns for five polymorphic loci met Mendelian expectations. Outcrossing rates were estimated using single and multilocus methods, and in most cases were not statistically different from 1.0. An excess of heterozygotes, both for seeds and adults, was found, as shown by the fixation indices estimated in 1987 (mean F for adults $= -0.41$, mean F for seeds $= -0.19$). Low but significant levels of genetic differentiation were found, especially for adults (mean F_{st} for adults $= 0.040$, mean F_{st} for seeds $= 0.009$). There was a positive relationship for adults between trunk growth and an individual's heterozygosity. No significant correlation was found between heterozygosity and fecundity. The genetic structure of *A. mexicanum* appears to be the result of a balance between cross-pollination and long distance movement of pollen by pollinators (beetles) that reduce genetic differentiation among plots, and natural selection that could operate during the long life cycle of this palm, and may increase the genetic differentiation among sites and the proportion of heterozygotes. The relatively high level of genetic variation, low genetic spatial differentiation, excess of heterozygotes, high outcrossing rate and heterosis found in *A. mexicanum* seem to be common in tropical trees. These population genetics characteristics appear not to satisfy the conditions necessary for non-adaptive evolution, a hypothesis commonly invoked to explain high tropical tree diversity. Management and conservation strategies aimed at preserving tropical tree's high intrapopulation genetic variation will probably require the maintenance of large tree populations.

Keywords: *Astrocaryum mexicanum*, genetic structure, heterosis, mating systems, palm, tropical rain forest.

Introduction

Knowledge about the genetic structure of tropical tree populations can help to explain the evolution of high diversity in the tropics and to design adequate forest conservation and management strategies. Several authors have hypothesized that the high diversity of tropical tree species is a product of non-adaptive speciation resulting from inbreeding and genetic drift (Corner, 1954; Baker, 1959; Federov, 1966; van Steenis, 1969). This inbreeding and genetic drift could be the result of low densities of conspecifics and was based on the assumption that most of the tree species were mainly self-pollinated. This 'non-adaptive' hypothesis assumes that the genetic structure of tropical trees is characterized by high inbreeding (high fixation indices), low effective population sizes and high spatial genetic differentiation, as measured by genetic distance or Wright's F_{st}. An alternative hypothesis, the 'microniches/equilibrium', originally proposed by Dobzhansky (1950), contends that the tropical tree diversity results from adaptation of tree populations to very specific niches defined by both biotic and abiotic components (pollinators, seed dispersors, soil, light, succession, seed predators, etc.)

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(Janzen, 1970; Connell, 1978; Hubbell, 1979; Denslow, 1987; Bawa, 1990). This hypothesis requires low inbreeding and high effective population sizes.

The genetic structure of tree populations imposes constraints on management and conservation strategies. If tropical trees usually occur in small, inbred populations, as the non-adaptive hypothesis predicts, then a species could be preserved with few individuals from each population but collection from many different populations and *ex situ* preservation could be practical and feasible (Frankel & Soulé, 1981; Eguiarte & Piñero, 1990). Conversely, if tropical trees present high effective population sizes and low inbreeding (as the microniches hypothesis suggests), then the deleterious effects of maintaining low populations should be severe both in the short term, because of the inbreeding depression, and long term, because of genetic drift (Franklin, 1980; Eguiarte & Piñero, 1990). If this last case is true, viable conservation proposals should contemplate the maintenance of large ecological preserves, and *ex situ* conservation becomes impossible (Frankel & Soulé, 1981; Eguiarte & Piñero, 1990).

Population genetics information for tropical tree species is scarce and fragmentary. Some studies report data on levels of genetic variation (Gan *et al.*, 1977, 1981; Hamrick & Loveless, 1986), while others analyse the genetic structure (Buckley *et al.*, 1988; Hamrick & Loveless, 1989), or the mating system (O'Malley & Bawa, 1987; O'Malley *et al.*, 1988; Hamrick & Loveless, 1989).

The population ecology and reproductive biology of the monoecious understory tropical palm *Astrocaryum mexicanum* are relatively well known (Piñero *et al.*, 1984, 1986; Sarukhán *et al.*, 1984; Búrquez *et al.*, 1987). Here we present a comprehensive study of its genetic variation, outcrossing rates, genetic structure and heterosis using allozyme marker genes. We compare these parameters with data from other tropical tree populations and argue that available data does not provide support for the non-adaptive evolution scenario for tropical tree diversity.

Materials and methods

Study site

Field work was conducted at the Estación de Biología Tropical Los Tuxtlas, of the National University of Mexico (UNAM). It is located in the coastal side of the range of Los Tuxtlas, State of Veracruz, Mexico (long. between 95° 04′ and 95° 09′N, lat. between 18° 34′ and 18° 36′W). The vegetation and climate have been described by Piñero *et al.* (1977) and Bongers *et al.* (1988).

Seed collections

In *A. mexicanum*, the adult plants present from 0 to 5 infructescences in a given year, each containing a mean of 23 fruits, every fruit containing only one seed (Búrquez *et al.*, 1987).

Twenty seeds from a single infructescence were collected for electrophoresis from each palm reproducing in a given year inside four permanent plots (20 × 30 m) of contrasting densities of *A. mexicanum* in a virgin rain forest [A and B plots are high density and C and CC are low density plots, Table 1 (Piñero *et* al., 1977, 1984)], plus a 10-m strip on each side of every plot, producing plots of 40 × 50 m. In 1985 and 1988 seeds were collected from plot B only; in 1987 plots A, B, C and CC were collected. A larger plot (50 × 60 m) was collected for the site B in 1988, in order to have a larger sample that year. The mean distance between plots was 365 m (Table 1). Data on the number of infructescences produced from 1975 to 1986, and on the increase in height from 1975 to 1982 (M. Martínez-Ramos *et al.* unpubl., data) were available for roughly one-third of the adults from which seeds were collected (90 adults for inflorescences, 87 for growth). Collected seeds were kept alive by maintaining them in humid soil from Los Tuxtlas or in humid agrolite until used.

Table 1 Abundances of *Astrocaryum mexicanum* in permanent plots [600 m², Piñero *et al.* (1977, 1984)], distances among plots and number of collected adults for this study in Los Tuxtlas, Veracruz, Mexico

	Plots			
	A	B	C	CC
Individuals in 1975				
Seedlings	261	150	54	54
Juveniles	65	60	25	18
Adults	101	88	36	49
Distances among pairs of plots (m)				
A		250	360	200
B			450	370
C				560
Number of collected adults for this study				
1985 inside plot	—	20	—	—
1985 around plot*	—	51	—	—
1987 inside plot	32	28	20	22
1987 around plot*	40	53	22	23
1988 inside plot	—	21	—	—
1988 around plot†	—	77	—	—

*Strip of 1400 m² around plot.
†Strip of 2400 m² around plot.

Controlled crosses

Controlled crosses were performed in 1988 with the methods described in Búrquez *et al.* (1987). We crossed 11 mothers with four fathers (one father was used in seven pollinations, another in two and two others in only one pollination), to analyse the segregation patterns for five loci (*Mdh*-1, 6-*Pgd*-1, *Pgi*-1, *Adh*-1, *Lap*-2) used to evaluate genetic structure and outcrossing rates.

Electrophoretic procedures

Fresh embryos were ground in two drops of gel buffer, adsorbed onto filter paper wicks and inserted into a 12.5% starch gel (Sigma Chemical Co.) for horizontal electrophoresis. The buffers used were histidine pH 7.0, Poulik pH 8.1/8.6 (Piñero & Eguiarte, 1988) and Tris-citrate pH 8.0 (Selander *et al.*, 1986). Enzyme stain recipes were used as described by Vallejos (1983) and Piñero & Eguiarte (1988). The 11-enzyme systems studied were: acid phosphatase (ACPH), isocitric dehydrogenase (IDH), shikimic dehydrogenase (SDH), xanthine dehydrogenase (XHD), alcohol dehydrogenase (ADH), esterase (Est), leucine aminopetidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM). We used five loci (*Mdh*-1, 6*Pgd*-1, *Pgi*-1, *Adh*-1, *Lap*-2) to study the genetic structure and outcrossing rates for different plots and years.

Data analyses

A sample of seeds collected from all plots in 1987 was used to estimate the genetic variation parameters (Brown & Weir, 1983; Hedrick, 1983). Single and multilocus outcrossing rate (t) estimates, their standard errors and maternal genotypes were estimated with Ritland & Jain's (1981) mixed mating model method, using Ritland's (1990) MLT program. Violations of the mixed mating model were analysed using Ritland's (1983) approach. Allelic frequencies for both adults and seeds, Nei (1972) multilocus genetic identities (I) and distances (D) between all pairs of plots, fixation indices and the Wright's F-statistics (Wright, 1965) were obtained with Nei's (1987) formulas using a program developed by Gerardo Coello and Ana María Escalante. Confidence intervals at 95 per cent for the F-statistics were calculated by a jackknife procedure (Weir, 1990).

Parametric and non-parametric regressions were performed (Sokal & Rohlf, 1969) on the number of infructescences produced from 1975 to 1986, on increases in height from 1975 to 1982, and on height in 1975, using the number of heterozygous loci per individual as the independent variable. As growth and fecundity may be correlated with size, ANOVA were done with the residuals of the regression of growth against height in 1975, and with the residuals of the regression between fecundity against initial height in 1975, in both cases using the number of heterozygous loci as classificatory variable.

Results

Levels of genetic variation

Four of the 11 assayed enzymes were monomorphic (ACPH, and XDH with two 'loci' each, and IDH and SDH with one 'locus' each) and seven were polymorphic for at least one 'locus' (Table 2). We analysed 22 putative loci of which seven were polymorphic. We found two alleles for all but one of the polymorphic loci, *Lap*-2, for which we found three alleles. At Los Tuxtlas, *A. mexicanum* has 31.8% polymorphic loci (P), 1.36 and 2.14 effective alleles considering all loci and only the polymorphic ones respectively, and a mean expected heterozygosity (H) of 0.153 (Hedrick, 1983).

Controlled crosses

For the five loci analysed most of the crosses showed a Mendelian segregation pattern (due to the small family sizes, all the crosses for each class were pooled, Table 3), with the exception of the crosses among heterozygotes for *Pgi*-1, where there appears to be an excess of heterozygotes ($\chi^2_{[2]} = 7.47$, $P < 0.05$).

Mating system

Both single and multilocus estimates of outcrossing rate, when considered for different plots and different years, were close to one (Table 4). The mean single locus estimate for all enzymes and plots in 1987 was 1.030, and for plot B the average over 3 years was 1.098. The multilocus outcrossing rates varied less with a mean of 1.011 for the 1987 data and 0.997 for plot B over 3 years. All multilocus estimates except one (plot CC, *Mdh*-1; Table 4) were not significantly different from one. In 48% of the cases we found departures from the mixed mating model, using Ritland's (1983) Chi-square method (Table 5). These departures have little effect on the estimates when using multilocus methods to obtain the outcrossing rate (Ritland, 1983; Brown *et al.*, 1985).

Table 2 Genetic variation in *A. mexicanum* at Los Tuxtlas, Veracruz, Mexico, in 1987. Buffers in which enzymes were assayed were Poulik (P) and histidine (H) (Piñero & Eguiarte, 1988)

Enzyme (buffer system)	Sample size	Locus number	Allelic frequencies
Alcohol dehydrogenase, ADH (H)	963	1	0.529, 0.471
Esterase, EST (H)	674	1	1.0
	674	2	1.0
	674	3	0.437, 0.563
	674	4	1.0
Leucine aminopeptidase, LAP (H)	963	1	1.0
	963	2	0.5, 0.44, 0.06
Malate dehydrogenase, MDH (H)	963	1	0.678, 0.322
	963	2	1.0
	963	3	1.0
6-phosphogluconate dehydrogenase, 6-PGD (H)	963	1	0.543, 0.457
Phosphoglucoisomerase, PGI (H)	963	1	0.586, 0.414
	963	2	1.0
	963	3	1.0
Phosphoglucomutase, PGM (P)	40	1	1.0
	40	2	0.500, 0.500

Table 3 Segregation patterns for five loci in *A. mexicanum* at Los Tuxtlas, Veracruz, Mexico. Expectations assuming Mendelian proportions are shown in parentheses. Chi-squares were done with Yates continuity correction for small samples (Sokal & Rohlf, 1969)

		Progeny genotypes				
Locus	Cross	11	12	22	χ^2	d.f.
Mdh-1	12 × 12	17 (12.75)	21 (25.5)	13 (12.75)	1.74	2
Mdh-1	11 × 12	54 (46)	38 (46)	0	2.45	1
Mdh-1	11 × 22	0	6	0		
6*Pgd*-1	12 × 12	29 (23.5)	44 (47)	21 (23.5)	1.29	2
6*Pgd*-1	11 × 12	4 (4.5)	5 (4.5)	0	0	1
6*Pdg*-1	22 × 12	0	7 (5.5)	4 (5.5)	0.36	1
Pgi-1	12 × 12	28 (30.75)	76 (61.5)	19 (30.75)	7.47*	2
Pgi-1	11 × 12	27 (34)	41 (34)	0	2.49	1
Adh-1	12 × 12	43 (39.5)	76 (79)	39 (39.5)	0.31	2
Adh-1	12 × 11	10 (12)	14 (12)	0	0.38	1
Lap-2	12 × 12	6 (7)	19 (14)	3 (7)	3.23	2
Lap-2	12 × 11	45 (53.5)	62 (53.5)	0	2.39	1
Lap-2	12 × 22	0	10 (11)	12 (11)	0.05	1

*$P < 0.05$.

Genetic differentiation

Difference between plots for allelic frequencies were relatively small for reproductive adults and even smaller for seeds (Table 6). Workman & Niswander (1970) chi-square heterogeneity in variances test suggests that in adults the differences in allelic frequencies, although small, were significant for all loci except *Pgi*-1 (Table 6). For seeds we found significant heterogeneity in allelic frequencies only for *Mdh*-1, where allele 1 had higher frequencies in both low density plots (C and CC) (Table 7). This indicates that the pollen allelic pool was different from the adult local allelic frequencies and indicates extensive pollen movement. Genetic identities in 1987 for pairs of plots for the five selected loci were high in reproductive adults

Table 4 Single and multilocus outcrossing rates (t) estimates for *A. mexicanum*. 95% confidence intervals are shown in parentheses

	Year and plot					
Locus	1985 B	1987 A	1987 B	1987 C	1987 CC	1988 B
Mdh-1		1.105	1.040	0.833	0.558	1.145
		(0.194)	(0.276)	(0.473)	(0.263)	(0.198)
6*Pgd*-1	0.942	0.806	1.212	0.539	1.311	0.920
	(0.221)	(0.372)	(0.114)	(0.780)	(0.206)	(0.204)
Pgi-1		1.181	1.363	0.811	1.263	1.388
		(0.255)	(0.210)	(0.560)	(0.233)	(0.292)
Adh-1		1.091	0.910	1.196	0.893	0.971
		(0.198)	(0.319)	(0.192)	(0.337)	(0.171)
Lap-2	1.080	1.179	1.160	1.036	1.110	1.043
	(0.149)	(0.165)	(0.167)	(0.145)	(0.157)	(0.148)
Mean t	0.956	1.077	1.078	1.070	1.068	1.045
	(0.176)	(0.065)	(0.039)	(0.065)	(0.666)	(0.053)
Multilocus t	0.933	1.050	0.992	1.018	0.985	1.007
	(0.174)	(0.066)	(0.039)	(0.063)	(0.065)	(0.053)

Table 5 Chi-square for deviations from the expected values according to the mixed mating model (Ritland, 1983), for *A. mexicanum*. Degrees of freedom in parentheses; as they are approximate, we used an α of 0.01, as suggested by Ritland (1983)

	Year and plot					
Locus	1985 B	1987 A	1987 B	1987 C	1987 CC	1988 B
Mdh-1		4.23 (1)	7.07 (1)*	6.98 (1)*	17.53 (1)*	5.54 (1)
6*Pgd*-1	1.26 (1)	3.60 (1)	4.05 (1)	8.58 (1)*	4.66 (1)	4.09 (1)
Pgi-1		8.23 (1)*	3.19 (1)	2.31 (1)	7.41 (1)*	4.30 (1)
Adh-1		6.24 (1)	18.39 (1)*	5.84 (1)	13.15 (1)*	14.87 (1)*
Lap-2	3.90 (1)	31.48 (9)*	45.72 (9)*	17.69 (9)	45.13 (1)*	36.96 (9)*

*$P < 0.01$.

(Table 8, mean $I = 0.9764 \pm 0.0116$ s.d.) and higher in their progeny (Table 8, mean $I = 0.9944 \pm 0.0038$ s.d.)

Fixation indices and F*-statistics*

In most cases (94.4%) the fixation indices were negative, for both adults and seeds, and differed significantly from random maing expectations in 66.7% of the cases (Table 9), being significantly positive in only one (plot CC, 1987, seeds, *Mdh*-1). Both adults and seeds show an excess of heterozygous individuals (mean F 1987 adults $= -0.411$, seeds $= -0.186$), which suggests strong selection favouring heterozygotes. The excess of heterozygotes is significantly greater in adults than in seeds (Wilcoxon paired samples test $T = 346$, $P = 0.0002$). This trend indicates that increased survivorship of the heterozygous individuals from the seeds to the reproductive adult stage is likely.

F_{is} is significantly negative for all loci both in adults and seeds (Table 10), as was expected from the fixation indices. F_{st} estimates are small but significantly different from zero for all loci (except *Pgi*-1 for adults) in both adults and seeds (Table 10). F_{st} is significantly higher in the adults than in the seeds (Table 10). F_{it} estimates are significantly negative in all cases, except in *Mdh*-1 for seeds (Table 10).

Heterosis

Table 11 shows a correlation matrix of the infructescences produced in 13 years (1975–1987), trunk

Table 6 Allelic frequencies for adults of *A. mexicanum* and Chi-square tests for heterogeneity in allelic frequencies among plots (Workman & Niswander, 1970). 95% confidence intervals are shown in parentheses (Brown & Weir, 1983; Richardson *et al.*, 1986)

		Plot					
Locus	Allele	A	B	C	CC	χ^2 (d.f.)	$P <$
Mdh-1	1	0.854 (0.057)	0.771 (0.064)	0.929 (0.055)	0.833 (0.077)	10.4 (3)	0.02
Mdh-1	2	0.146 (0.057)	0.228 (0.064)	0.929 (0.055)	0.833 (0.077)		
6*Pgd*-1	1	0.694 (0.075)	0.617 (0.074)	0.452 (0.106)	0.722 (0.092)	17.33 (3)	0.005
6*Pgd*-1	2	0.306 (0.075)	0.383 (0.074)	0.548 (0.106)	0.278 (0.092)		
Pgi-1	1	0.847 (0.059)	0.815 (0.060)	0.786 (0.078)	0.767 (0.087)	2.74 (3)	0.50
Pgi-1	2	0.153 (0.059)	0.185 (0.060)	0.214 (0.078)	0.233 (0.087)		
Adh-1	1	0.743 (0.071)	0.506 (0.077)	0.654 (0.101)	0.500 (0.103)	23.05 (3)	0.005
Adh-1	2	0.257 (0.071)	0.494 (0.077)	0.345 (0.101)	0.500 (0.103)		
Lap-2	1	0.556 (0.081)	0.444 (0.076)	0.298 (0.097)	0.333 (0.097)	23.91 (6)	0.005
Lap-2	2	0.430 (0.081)	0.543 (0.076)	0.642 (0.102)	0.611 (0.100)		
Lap-2	3	0.014 (0.019)	0.012 (0.017)	0.054 (0.051)	0.055 (0.047)		

height increase in 7 years (1975–1982), height of the palms in 1975 (a measure of their size), and the number of heterozygous loci per individual. The only significant correlations are between growth and heterozygosity, and between initial height and fecundity. To control for the correlation between size and fecundity, two standardized ANOVA were done using the residuals of the regression of infructescence production and height in 1975 in one, and the residuals of the regression of trunk height increase and height in 1975 in the other. The number of heterozygous loci for both ANOVA was the classificatory variable. With the standardized data we found a significant positive relation between the number of heterozygous loci and the deviation from average growth for reproductive adults of a given size (ANOVA $F_{4,82} = 2.524$; $P = 0.047$). There was no significant relationship between the number of heterozygous enzymes with the deviation from the average number of infructescences produced in 13 (1975–1987) years for a given size plant ($F_{4,85} = 0.944$; $P = 0.443$) or with initial height ($F_{4,82} = 0.508$; $P = 0.730$).

Discussion

Given the high phenotypic variability described in the Los Tuxtlas *A. mexicanum* population (Sarukhán *et al.*, 1984), and the fact that most plants, especially trees, show high levels of genetic variation (Hamrick *et al.*, 1979; Hamrick & Godt, 1989), we expected high levels of electrophoretically detectable genetic variation. Our results confirm this expectation.

Originally we expected a high outcrossing rate (t) for *A. mexicanum*, as Búrquez *et al.* (1987) found that although individuals are self-compatible, self-pollinations produced 23% seeds/ovules, relative to cross-pollinations. The temporal and spatial separation of the female and male flowers in a given inflorescence (Piñero & Sarukhán, 1982; Búrquez *et al.*, 1987), should contribute to a high outcrossing rate. We found that for both single and multilocus estimates the outcrossing rate of *A. mexicanum* was not significantly different from one, indicating no detectable self-fertilization. The high outcrossing rate found may result from the peculiar reproductive behaviour of *A.*

Table 7 Allelic frequencies for seeds of *A. mexicanum* and Chi-square tests for heterogeneity in allelic frequencies among plots (Workman & Niswander, 1970). 95% confidence intervals are shown in parentheses (Brown & Weir, 1983; Richardson *et al.*, 1986)

		Plot					
Locus	Allele	A	B	C	CC	χ^2 (d.f.)	$P <$
Mdh-1	1	0.644 (0.051)	0.619 (0.049)	0.782 (0.063)	0.762 (0.057)	37.51 (3)	0.005
Mdh-1	2	0.355 (0.051)	0.389 (0.049)	0.218 (0.063)	0.238 (0.057)		
6*Pgd*-1	1	0.515 (0.054)	0.574 (0.050)	0.508 (0.077)	0.549 (0.066)	5.68 (3)	0.25
6*Pgd*-1	2	0.485 (0.054)	0.426 (0.050)	0.492 (0.077)	0.451 (0.066)		
Pgi-1	1	0.617 (0.039)	0.583 (0.050)	0.563 (0.076)	0.558 (0.066)	4.23 (3)	0.30
Pgi-1	2	0.383 (0.039)	0.417 (0.050)	0.437 (0.076)	0.442 (0.066)		
Adh-1	1	0.537 (0.054)	0.545 (0.050)	0.550 (0.076)	0.476 (0.066)	6.12 (3)	0.25
Adh-1	2	0.463 (0.054)	0.454 (0.050)	0.450 (0.076)	0.524 (0.066)		
Lap-2	1	0.529 (0.054)	0.494 (0.050)	0.462 (0.077)	0.490 (0.066)	9.99 (6)	0.25
Lap-2	2	0.410 (0.052)	0.464 (0.050)	0.458 (0.076)	0.439 (0.066)		
Lap-2	3	0.061 (0.025)	0.042 (0.020)	0.080 (0.042)	0.070 (0.034)		

Table 8 Unbiased genetic distances (above the diagonal) and identities (below the diagonal) for adults and seeds (Nei, 1972, 1987) between pairs of plots

	Sites			
	A	B	C	CC
A adults		0.0220	0.0373	0.0295
A seeds		0.0015	0.0067	0.0072
B adults	0.9783		0.0245	0.0028
B seeds	0.9985		0.0095	0.0086
C adults	0.9633	0.9758		0.0283
C seeds	0.9933	0.9905		0.0003
CC adults	0.9710	0.9972	0.9721	
CC seeds	0.9929	0.9914	0.9997	

mexicanum and the high mobility of beetle pollinators (Búrquez *et al.*, 1987).

In about half of the cases we found deviations from the expectations of the mixed mating model. Similar deviations have been reported in most studies (Smyth & Hamrick, 1984; O'Malley & Bawa, 1987; O'Malley *et al.* 1988; Sampson *et al.*, 1989) and are not surprising given the constraints of the mixed mating model (Brown *et al.*, 1985). In *A. mexicanum* there are five possible causes for departure from the expectations of the mixed mating model.

1 Allelic frequencies in the pollen may be biased because of a non-random sample of pollen donors. This may be important in *A. mexicanum*, considering that in a given year only a fraction (about 31%) of the total of reproductive adults produce flowers (Piñero & Sarukhán, 1982).

2 Matings may be correlated in the sense of Schoen & Clegg (1984, 1986); the pollen donors of an infructescence may be few with respect to the total set of potential fathers. Correlated mating may also occur in *A. mexicanum* because of the relatively low number of individuals that flower in a given day (Búrquez *et al.*, 1987).

3 Segregation distortion may occur at certain loci. We cannot rule out this probability because the sample size in the controlled crosses was low (Ellstrand & Devlin, 1989).

Table 9 Fixation indices for adults and seeds in *A. mexicanum*. 95% confidence intervals shown in parentheses (Rasmussen, 1964; Weir, 1990)

	Year and plot					
Locus	1985 B	1987 A	1987 B	1987 C	1987 CC	1988 B
Mdh-1 adults		−0.1707	−0.2960*	−0·0769	−0.2000	−0.2683*
		(0.1109)	(0.1285)	(0.0960)	(0.1480)	(0.1255)
		=†	=	=	<	=
Mdh-1 seeds		−0.2694*	−0.0849	−0.0827	+0.1967*	−0.0650
		(0.1037)	(0.1037)	(0.1628)	(0.1967)	(0.0940)
6*Pgd*-1 adults	−0.3793	−0.4400*	−0.6200*	−0.8261*	−0.3845*	−0.7103*
	(0.2765)	(0.1518)	(0.1427)	(0.1580)	(0.1849)	(0.1493)
	=	<	<	<	=	<
6*Pgd*-1 seeds	+0.0391	−0.0963	−0.0165	−0.1600	−0.1369*	−0.0163
	(0.1999)	(0.1220)	(0.1055)	(0.1765)	(0.1344)	(0.0940)
Pgi-1 adults		−0.1803	−0.2273*	−0.2727	−0.3043*	−0.2000*
		(0.1126)	(0.1160)	(0.1720)	(0.1720)	(0.1126)
		=	=	=	=	=
Pgi-1 seeds		−0.3031*	−0.3212*	−0.2979*	−0.3189*	−0.2963*
		(0.1030)	(0.0980)	(0.1686)	(0.1285)	(0.0898)
Adh-1 adults		−0.3458*	−0.8274*	−0.4219*	−0.7330*	−0.7027*
		(0.1427)	(0.1224)	(0.2360)	(0.1998)	(0.1543)
		=	<	=	<	<
Adh-1 seeds		−0.2998*	−0.2251*	−0.1716	−0.2262*	−0.1037*
		(0.1091)	(0.1018)	(0.1753)	(0.1329)	(0.0940)
Lap-1 adults	−0.5407*	−0.5652*	−0.6064*	−0.2515	−0.4747*	−0.4936*
	(0.3173)	(0.1870)	(0.1697)	(0.2765)	(0.2360)	(0.0620)
	<	<	=	=	=	<
Lap-1 seeds	+0.0584	−0.2092*	−0.3267*	−0.0757	−0.3397*	−0.1983*
	(0.1999)	(0.1104)	(0.1440)	(0.1786)	(0.1285)	(0.0919)
Mean adults	−0.4600	−0.3404	−0.5154	−0.3698	−0.4193	−0.4750
	(0.1582)	(0.1486)	(0.2181)	(0.1265)	(0.1775)	(0.2083)
	<	=	=	<	=	<
Mean seeds	+0.0487	−0.2356	−0.1949	−0.1576	−0.1650	−0.1359
	(0.0189)	(0.0758)	(0.1225)	(0.0786)	(0.1908)	(0.0980)

*$P < 0.05$, chi-square test (Li & Horvitz, 1953).
†Equal in adults and seeds (=), or smaller in the adults than in the seeds (<), according to the 95% confidence intervals.

4 Negative assortative mating may occur (Brown *et al.*, 1985).
5 Selection may favour heterozygote genotypes prior to the time of sampling (Brown *et al.*, 1985).

The last two processes are the only ones that can produce the observed excess of heterozygotes in the progeny and hence we consider them to be the more important ones.

Negative fixation indices and negative F_{is} were unexpected, especially for the seeds. We expected low but positive values for both, as we suspected some selfing and inbreeding because of poor seed dispersal (usually less than 3 m L. E. Eguiarte *et al.* unpublished observations). A high outcrossing rate and long distance pollen dispersal can produce low fixation indices but not negative fixation indices, even if the parents present very negative fixation indices. Only if pollen moves long distances and seeds very little can a slight local excess of heterozygotes be generated (Prout, 1981); pollen and seed dispersal in *A. mexicanum* (L. E. Eguiarte *et al.* unpublished observations) seem to conform to this pattern. Negative fixation indices can also be generated by negative assortative mating (Hedrick, 1983) but we have no data on this process. We suggest, however, that the most important cause of the negative fixation indices is selection in favour of the more heterozygous individuals (Linhart *et al.*, 1981; Waser, 1987). This hypothesis is supported by the increase in heterozygosity from seed to adults and the positive correlations between

growth and heterozygosity for adults. Negative fixation indices have also been described for other plants, mainly long lived perennials (Linhart *et al.*, 1981; Smyth & Hamrick, 1984; Shea, 1987; Sampson *et al.*, 1989) and in some animal populations (Schwartz & Armitage, 1980).

The observed increase in heterozygote frequencies from seeds to adults could be generated by increased survivorship of the more heterozygous individuals. Similar increases have been reported in other tree species (Phillips & Brown, 1977; El-Kassaby *et al.*, 1987; O'Malley & Bawa, 1987; Shea, 1987; O'Malley *et al.*, 1988; Sampson *et al.*, 1989).

Low genetic differentiation among Los Tuxtlas *A. mexicanum* plots could be expected because of the relatively short distance between them. Allelic frequency differences, genetic distances among different plots and F_{st} were relatively small but significant. We also found significantly more differentiation in adults than in seeds. For instance there were significant differences in allelic frequencies in adults for most loci, but only for one locus for seeds. The increase in differentiation in adults may be due to adaptation to local conditions, and this differentiation is lowered in seeds because of the extensive pollen movement. It also means that the pollen gene pool is different from the local adult allelic frequencies.

Piñero & Sarukhán (1982) and Sarukhán *et al.* (1984) demonstrated significant variation in growth and reproduction for *A. mexicanum* that could not be predicted from the ecological characteristics of their growing sites. We hypothesized that some of this variation could be explained by differences in heterozygosity among individuals. Accordingly, we found a positive relationship of individual heterozygosity with growth rate but not with fecundity. The relationship between growth and heterozygosity is weak. This may be expected given that the sampled loci are only a small part of the total genome and that growth is also affected by several ecological conditions such as available sunlight, soil and competition (Sarukhán *et al.*, 1984). The lack of correlation between fecundity and individual heterozygosity may be due to a greater effect of the environment on fecundity than on growth (Piñero & Sarukhán, 1982). A palm of a given genotype may grow at a more or less constant rate, using available surplus resources in reproduction. A similar pattern of positive correlations between heterozygosity

Table 10 Wright's F statistics (Wright, 1965; Nei, 1987) for *A. mexicanum* in 1987, for adults and seeds. 95% confidence intervals for the means in parentheses (Weir, 1990)

Locus	F_{is}	F_{st}	F_{it}
Mdh-1 adults	−0.2014*	0.0318*	−0.1632*
Mdh-1 seeds	−0.0713*	0.0258*	−0.0436
6*Pgd*-1 adults	−0.5756*	0.0563*	−0.4869*
6*Pgd*-1 seeds	−0.1004*	0.0048*	−0.0951*
Pgi-1 adults	−0.2432*	0.0141	−0.2257*
Pgi-1 seeds	−0.3081*	0.0046*	−0.3021*
Adh-1 adults	−0.5957*	0.0537*	−0.5100*
Adh-1 seeds	−0.2284*	0.0058*	−0.2212*
Lap-2 adults	−0.4687*	0.0422*	−0.4067*
Lap-2 seeds	−0.2341*	0.0044*	−0.2287*
Mean adults	−0.4169 (0.1623)	0.0396 (0.0152)	−0.3585 (0.1369)
	=†	>	=
Mean seeds	−0.1885 (0.0871)	0.0091 (0.0082)	−0.1781 (0.0927)

*$P < 0.05$, chi-square test, for F_{is} and F_{it} according to Li & Horvitz (1953), for F_{st} according to Workman & Niswander (1970).
†Equal in adults and seeds (=) or larger in the adults than in the seeds (>), according to the 95% confidence intervals.

Table 11 Correlation matrix between number of heterozygous enzymes per individual (heterozygosity), the number of infructescences produced in 13 years, the increase in height of the trunk in 7 years (growth) and height in 1975 in *A. mexicanum*. Above the diagonal parametric Pearson correlations, below Spearman non-parametric correlations

	Heterozygosity	*N* of infructescences	Growth	Height in 1975
Heterozygosity		0.153	0.307**	0.020
N of infructescences	0.153		0.083	0.422**
Growth	0.318**	0.087		−0.022
Initial height	0.034	0.377**	−0.066	

**$P < 0.01$.

with growth and lack of correlation with fecundity is found in many other perennial species (Mitton & Grant, 1980, 1984; Ledig *et al.*, 1983; Strauss, 1986; Govindaraju & Dancik, 1987; Shea, 1987).

Higher growth rate may be correlated with general vigour in a given individual [thus more heterozygous individuals have lower mortality rates (Mitton & Grant, 1984)], which explains the observed increase in heterozygous frequency from seeds to adults. In addition, rapid growth decreases the probability of death before reproduction by a falling branch or tree, one of the most important causes of death of *A. mexicanum* palms (Martínez-Ramos *et al.*, 1988).

Population genetics of trees

Most studies indicate high genetic variation in tropical tree populations (Gan *et al.*, 1977, 1981; Hamrick & Loveless, 1986). Genetic variation levels in *Astrocaryum mexicanum* are similar to the average for tropical trees. The genetic estimates of outcrossing rates described for tropical trees (O'Malley & Bawa, 1987; O'Malley *et al.*, 1988; Moran *et al.*, 1989), are high, as in our estimate for *A. mexicanum*. Fixation indices of tropical trees are either low or negative suggesting little inbreeding (Sytsma & Schaal, 1985; O' Malley & Bawa, 1987; O'Malley *et al.*, 1988). Our results on both genetic identities and F_{st} confirm the pattern of little spatial differentiation among plots that have been documented for several tropical species (Heywood & Fleming, 1986; Buckley *et al.*, 1988; Hamrick & Loveless, 1989; Moran *et al.*, 1989).

Apparently, tropical trees behave similarly to other trees, both angiosperms and gymnosperms. Most tree populations exhibit high levels of genetic variation (Hamrick *et al.*, 1979; Hamrick & Godt, 1989), high outcrossing rates (Schemske & Lande, 1985; Brown *et al.*, 1985), low fixation indices (Brown 1979; Eguiarte, 1990) and low genetic differentiation between sites (Loveless & Hamrick, 1984).

Tropical tree species diversity

This study, along with data from other papers in the literature, indicates that tropical tree populations do not present any of the characteristics assumed by the non-adaptive hypothesis of tropical tree diversity (Corner, 1954; Baker, 1959; Federov, 1966; van Steenis, 1969). Rather they exhibit exactly the opposite. *Astrocaryum mexicanum* has an effective population size of at least 400 individuals and probably much larger (L. E. Eguiarte *et al.*, unpublished observations). Data on genetic differentiation of other tropical trees (G_{st}) also suggest very large effective population sizes and/or extensive gene flow (Hamrick & Loveless, 1989). These large population sizes, coupled with more or less constant environments, would permit natural selection to be very effective and allow very fine ecological adaptation, as predicted by the micro-niches/equilibrium hypothesis of Dobzhansky (1950). However, the actual process of speciation in tropical trees remains to be explained.

Implications for conservation

The low densities usually present in tropical tree species indicate different conservation strategies from other organisms with similar population genetics (e.g. conifers). We suggest the following guidelines for the genetic conservation of tropical trees.

(a) Given the high genetic variation and low or negative fixation indices, we predict that inbreeding depression should be important in tropical trees populations. Selfing, inbreeding and small population numbers should be avoided in management strategies.

(b) Ecological preserves should be very large to maintain the high intrapopulation genetic variation and to avoid inbreeding and genetic drift.

(c) The low genetic differentiation between different sites shown by most tropical tree species suggests that for most species it will not be necessary to have a large number of natural preserves. However, smaller areas that can function as corridors and stepping-stones which may allow gene flow among sites, should also be preserved.

(d) *Ex situ* conservation strategies, based on seed and germplasm collection and preservation in gene banks, botanical gardens, etc., seem to be of little practical value for the conservation of the genetic diversity of tropical forest trees, given the high numbers of seed and parents needed to be collected to preserve at least part of the genetic variation present in a population (Brown & Clegg, 1983), the high number of different species, and the problems in preserving, maintaining the viability and germinating tropical rain forest trees seeds (see Vázquez-Yanez & Toledo, 1990). Only *in situ* conservation strategies seem to be of any real value for the conservation of tropical trees.

Acknowledgements

This work was supported by a grant from the CONACYT (México) and by doctoral scholarships from the CONACYT and the DGAPA, Universidad Nacional Autónoma de México to LEE. We thank V. Souza, A. M. Escalante, G. Coello, C. Domínguez, J. Nuñez and C. Cordero for enthusiastic field and/or laboratory assistance and critical discussions. M.

Martínez-Ramos permitted us access to data on individual growth and fecundity. M. T. Clegg, B. S. Gaut, G. Learn, E. Alvarez-Buylla, V. Souza, N. Ellstrand, S. Waller, A. Fontdevilla, A. Hoffman, A. Kamping, C. Martínez del Rio, and one anonymous reviewer, provided valuable comments on the manuscript. This paper owes much to the influence and ideas of Professor J. Sarukhán and we wish to acknowledge him.

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