Annals of Botany **93**: 521–528, 2004
doi:10.1093/aob/mch070, available online at www.aob.oupjournals.org

Genetic Variability of the Narrow Endemic Tree *Antirhea aromatica* Castillo-Campos & Lorence, (Rubiaceae, Guettardeae) in a Tropical Forest of Mexico

JORGE GONZÁLEZ-ASTORGA[1,*] and GONZALO CASTILLO-CAMPOS[2]

[1]*Laboratorio de Genética de Poblaciones, Jardín Botánico Clavijero, Instituto de Ecología, A. C.; and*
[2]*Departamento de Sistemática Vegetal. Instituto de Ecología, A. C., Km. 2.5 Antigua Carretera a Coatepec No.351, Xalapa 91070, Veracruz, México*

Received: 25 April 2003 Returned for revision: 16 October 2003 Accepted: 23 December 2003 Published electronically: 31 March 2004

• *Background and Aims* Genetic structure and variability were examined in the only three extant populations of the narrow-endemic tree *Antirhea aromatica* (Rubiaceae, Guettardeae), an endangered species of the tropical forest of eastern Mexico. Patterns of genetic diversity within and among populations for adult plants and seedlings were obtained.
• *Methods* Allozyme electrophoresis of 15 loci was conducted and the data analysed with statistical approximation for obtaining genetic diversity, structure and gene flow.
• *Key Results* The mean expected heterozygosity (H_e) in the adult and seedling populations was 0·18 ± 0·08 and 0·20 ± 0·09, respectively. The genetic variation explained by differences among populations was 51 and 35 %, for adult and seedling populations, respectively. On average, gene flow between paired adult populations was low (Nm = 0·26 ± 0·09), compared with other trees from the tropical forest.
• *Conclusions* The results indicated that the populations evaluated have high genetic variability, compared with other endemic and geographically narrowly distributed plant species, in areas with high levels of environmental heterogeneity (e.g. tropical forests). The conservation implications of the results are discussed, and in this regard it is proposed that *A. aromatica* should be considered as an indicator species with economic potential. It is suggested that sustainable management practices should be implemented and that the areas where the species is distributed should be declared a natural reserve to ensure the species conservation.

Key words: *Antirhea aromatica*, endemic, genetic variability, conservation biology, tropical forest, Mexico.

INTRODUCTION

The genetic structure of populations refers to the distribution of genetic variation within and among populations, and is affected by demographic factors (Antonovics and Via, 1987; Loveless and Hamrick, 1984) as well as evolutionary processes (Wright, 1951). The genetic variation within a population is considered to represent its evolutionary potential (Wright, 1978), and the range of geographical distribution is one of the major factors correlated with the genetic variability of plant populations (Hamrick and Godt 1996*a*, *b*; Savolainen and Kuittien, 2000). Thus, genetic variation has implications for conservation at the species level (Holsinger *et al*., 1999; Lande, 1999), and the assessment of genetic variability is the first step in evaluating the long-term conservation status of species in natural conditions. This is particularly important in plant species with low population sizes exposed to the effects of inbreeding and genetic drift (Barrett and Kohn 1991; Frankham, 1995).

Plant species with restricted geographical distributions tend to have lower levels of genetic variation than their more widespread congeners (Gitzendanner and Soltis, 2000). However, high gene diversity has been reported for the rare ferns *Adenophorus periens* (Ranker, 1994), and *Polystichum otomasui* (Maki and Asada, 1998), the endangered tree *Caesalpinia echinata* (Cardoso *et al*., 1998), an endangered pine *Pinus rzedowskii* (Delgado *et al*., 1999), the rare Mexican pinyon pine *Pinus maximartinezii* (Ledig *et al*., 1999), the endemic *Agave victoriae-reginae* (Martínez-Palacios *et al*., 1999), three endemic plants from Florida (*Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae*; Dolan *et al*., 1999), the annual endemic *Warea carteri* (Evans *et al*., 2000), the endemics *Iris cristata* and *I. lacustris* (Hannan and Orick, 2000), the narrow and endemic species *Antirrhinum charidemi* and *A. valentinum* (Mateu-Andrés and Segarra-Moragues, 2000), the endemic monoecious shrub *Brongniartia vazquezii*, of tropical dry forests of Central Mexico (González-Astorga and Núñez-Farfán, 2001), *Viola palmensis* endemic of Canary Islands (Batista and Sosa, 2002), and the cycad *Dioon edule* of eastern Mexico (González-Astorga *et al*., 2003).

Conservation programs for long-lived tropical trees must take into account the ecological and genetic relevance of environmental conditions fluctuating over large periods of time (Alvarez-Buylla *et al*., 1996*b*; Lande, 1999; Hedrick, 2001).

Antirhea aromatica (Rubiaceae, Guettardeae) is a monoecious tree, of 6–15 m height and a diameter at breast

* For correspondence. E-mail astorga@ecologia.edu.mx

height of 10–30 cm. It takes 7–10 years for the species to reach maturity, and the plant's lifespan is approx. 150 years. Local people of the region use the fruits and the bark of *A. aromatica* as a natural remedy for dental diseases (Castillo-Campos, 1995). Active components with antiseptic, antioxidant and antibiotic principles have been isolated from a congener, *A. acutata* (Lee *et al*., 2001). The species *A. aromatica* is endemic to central Veracruz, with a highly restricted geographical distribution. The species has been registered only in the type locality area, in Jalcomulco and Apazapan, Veracruz, from 350–500 m a.s.l. (Castillo-Campos and Lorence, 1985). *Antirhea aromatica* inhabits remnant patches of tropical lowland rainforest (*sensu* Miranda and Hernández, 1963) with a population density of approx. 200 individual adults $ha^{-1,}$ and these populations cover an area of approximately 20 ha (Castillo-Campos, 1995). This species blooms from July to September (43·4 ± 7·2 flowers per plant) during the summer. Its white aromatic flowers (6·4 ± 0·3 cm long) are visited by moths, bumblebees (*Bombus* sp.) and bats; the seeds are dispersed by bats. The aim of the present study was to determine the patterns of genetic variation and differentiation among the three existing populations of *A. aromatica*. Additionally, both adults and seedlings were analyzed and compared. Finally, the population size of the species and its fragmented distribution offers the opportunity to determine the relationship between the genetic structure, population size and geographic isolation. It is hypothesized that genetic diversity will be low and genetic differentiation high, with a subsequent decrease in gene flow of the extant populations.

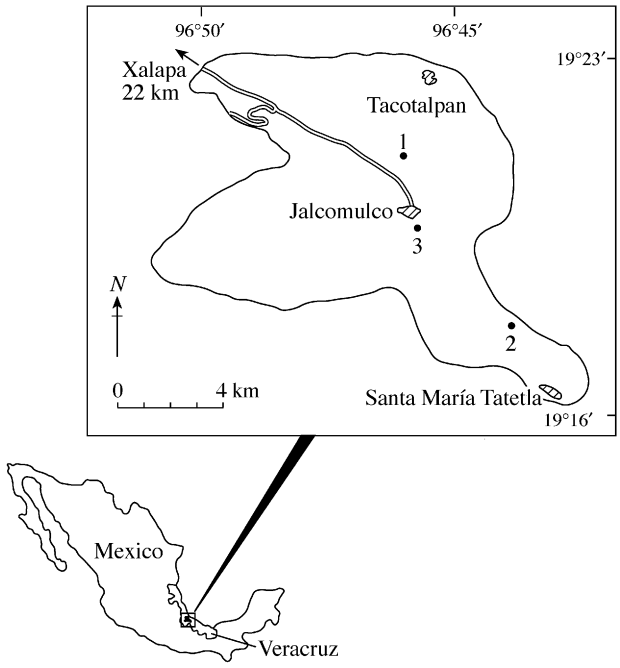

FIG. 1. Geographical distribution of populations examined of *Antirhea aromatica* in Veracruz, Mexico.

METHODS

Study sites

The study was conducted during 1998–99, in the localities of Jalcomulco and Apazapan (96°41′–99°08′W; 19°18′–19°27′N) in central Veracruz, in Eastern Mexico (Fig. 1) (Castillo-Campos, 1995). The study area is a fragmented landscape of lowland tropical rainforest (*sensu* Miranda and Hernández, 1963) surrounded by roads, cultivated fields and pasturelands. The climate is semi-warm humid [(A)C(m)] following the classification of Köeppen (1948), the mean annual precipitation ranges from 1200–1500 mm, and the mean annual temperature ranges from 22–24 °C (García, 1988).

Sample collection

Tissue sampling was done in the three extant populations of *Antirhea aromatica* Castillo-Campos & Lorence in Jalcomulco, Veracruz, Mexico. Populations are separated by distances that range from 2·7–15·5 km. The sampled populations of *A. aromatica* encompassed the total geographic range of the species (Fig. 1). Fully expanded young leaves were collected from 40 reproductive individuals in each population. Simultaneously, leaves of 40 seedlings of each population were collected. This tissue was transported in ice-filled containers, and then stored in a freezer at –70 °C until extraction for electrophoretic analysis.

Electrophoresis

Multilocus genotypes of 40 mature individuals and 40 seedlings from each population were determined through horizontal starch gel electrophoresis (12 % w/v). Allozymic variation was scored at 15 loci for each individual plant, nine of which were polymorphic: malate-dehydrogenase (E.C. 1.1.1.37, loci *Mdh1* and *Mdh2*), esterase (E.C. 3.1.1, loci *Est1*, *Est2* and *Est3*), phosphoglucoisomerase (E.C. 5.3.1.9, loci *Pgi1* and *Pgi2*), glutamate oxaloacetate transaminase (E.C. 2.6.1.1, locus *Got*), and phosphoglucomutase (E.C. 5.2.2, locus *Pgm*). The remaining six were monomorphic: 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44, locus *6Pgd*), diaphorase (E.C. 1.6.99.-, loci *Dia1* and *Dia2*), isocitrate dehydrogenase (E.C. 1.1.1.41, locus *Idh*), leucine aminopeptidase (E.C. 3.4.11.1, locus *Lap*) and peroxidase anodic (E.C. 1.11.1.7, locus *Apx*). The extraction buffer (tris-HCl pH 7·5, sucrose, PVP-40, mercaptoethanol, ascorbic acid, diethyldithiocarbamate, bovine serum albumin, sodium metabisulphite and sodium tetraborate; Wendel and Weeden, 1989) was added to dissolve and stabilize the enzyme extracts, which were stored on filter paper wicks at –70 °C until used for analyses. The buffers (gel and electrode) used were histidine pH 5·7, and citric acid (Soltis *et al*., 1983). Electrophoresis was carried out at 4 °C over 6 h (constant current of 70 mA, and voltage of 200 V).

Statistical methods

The bands from each allozyme system were assigned to alleles and genotypes based on theoretical expectations and observed banding patterns. The TFPGA 1.3 package (Miller, 1997) was used to obtain the genetic estimators from the data analysis. The genotypic frequencies obtained were used to calculate observed mean heterozygosity (H_o) and allelic frequencies. Allelic frequencies at each population were used to estimate the mean number of alleles per locus (A), the average proportion of polymorphic loci (P), and expected mean heterozygosity (H_e), based on Hardy–Weinberg expectations (Hartl and Clark, 1997). The significance of estimators was obtained by Monte Carlo methods (Weir, 1990).

Partitioning of genetic variability was done by using F-statistics (Wright, 1965, 1978), which were calculated according to the formula of Weir and Cockerham (1984) that estimates genetic structure by partitioning variation in the same way as a regular analysis of variance. The θ statistic (analogous to F_{st}) estimates populations' divergence through allele frequencies, whereas f (similar to F_{is}) and F (similar to F_{it}) estimate heterozygote excess (<0) and deficit (>0) relative to Hardy–Weinberg expectations in local populations and the total set of populations, respectively. To determine whether f and F estimations for each locus were significantly different from zero, Chi-square statistics [$\chi^2 = F(2N)\,(k-1)$] were obtained, with $k(k-1)/2$ degrees of freedom, where N is the sample size and k the number of alleles (Weir, 1990). To determine the significance of the θ statistic per locus, the chi-square statistic was used: $\chi^2 = (2N)\,\theta(k-1)$, with $(k-1)\,(n-1)$ degrees of freedom, where n is the number of populations (Workman and Niswander, 1970). The confidence intervals (at 95%) of the F-statistics were obtained by bootstrapping over loci for the multilocus estimate and jackknifing over populations for the single-locus estimates (Weir and Cockerham, 1984; Weir, 1990). The average gene flow among populations (Nm) was estimated from θ-values, as $\theta = 1/(4Nm\alpha + 1)$, where $\alpha = [n/(n-1)]^2$ and n is the number of populations (Crow and Aoki, 1984). Nm is interpreted as the number of migrants per generation between two given populations (Slatkin 1993, 1994).

RESULTS

Genetic variation

The average number of alleles per locus was 1·76 ± 0·102 and 1·64 ± 0·102 for adults and seedlings of *A. aromatica*, respectively (Table 1). The t-test indicated no significant differences between averages of number of alleles per locus among adults and progeny (t = 1·7, df = 44, P = 0·09). Allelic frequencies for 15 loci were scored for each individual plant (Table 1). In the adults, the percentage of polymorphic loci per population varied from 33·3 % (population 2) to 60 % (populations 1 and 3), with an average of 51·1 %. In seedling populations, the percentage of polymorphic loci varied from 46·6 % (population 3) to 60 % (populations 1 and 2), with an average of 55·5 % (Table 2).

Observed mean heterozygosity was 0·14 ± 0·04 (range 0·08–0·17) and 0·17 ± 0·08 (range 0·07–0·23) for the adult and seedling populations, respectively. Expected mean heterozygosity was 0·18 ± 0·08 *vs* 0·20 ± 0·09, for the adult and seedling populations, respectively (Table 2).

Genetic structure

The Wright's F-statistics, F (similar to F_{it}) and f (similar to F_{is}), were positive and significantly different from zero for all polymorphic loci ($P < 0{\cdot}05$) in both adult and seedling populations, indicating inbreeding (Table 3). Similarly, all polymorphic loci showed values of θ (similar to F_{st}) significantly different from zero ($P < 0{\cdot}05$). The mean F was higher for the adult than for the seedling population (0·64 ± 0·049 *vs*.0·46 ± 0·04; $F_{(1,28)} = 1330$, $P < 0{\cdot}00001$). Similarly, the f-statistic was higher for adult than for seedling populations ($F_{(1,28)} = 101$, $P < 0{\cdot}00001$) (Table 3).

The average genetic differentiation among adult populations (θ = 0·51) was higher than for seedling populations (θ = 0·35). Thus 51 and 35 % of the genetic variation for adults and seedlings, respectively, is due to differences among populations of *A. aromatica* (Table 3). Also, the exact tests for population differentiation of Raymond and Rousset (1995), indicated significant differences among adult (χ^2 = 178·3; df = 18; $P < 0{\cdot}00001$), and seedling (χ^2 = 161·2; df = 18; $P < 0{\cdot}00001$) populations. The θ-values were different among loci in both populations (adults, range 0·47–0·54; and seedlings, range 0·32–0·38), and suggest that genetic drift and inbreeding have been the dominant differentiating processes. Both estimations are significantly different from zero, and there were differences between them ($F_{(1,28)}$ = 688, P = 0·00001).

Gene flow

Indirect estimates of gene flow (Nm) for *A. aromatica* indicate that an average of 0·26 ± 0·08 migrant individuals per generation between populations pairs. These are relatively low values of gene flow with respect to other plant species with similar reproductive systems. The lowest Nm value was obtained between populations 1 and 2 (Nm = 0·16) separated by 15·55 km, and the highest one between populations 2 and 3 (Nm = 0·31) separated by 2·74 km.

DISCUSSION

Tropical endemic trees are vulnerable to forest fragmentation because of their low densities, complex demographic dynamics, high genetic differentiation (θ ≈ 1), and self-incompatibility systems (Bawa *et al*., 1985; Barrett and Kohn, 1991; Alvarez-Buylla and Garay, 1994; Schemske *et al*., 1994; Alvarez-Buylla *et al*., 1996*b*). Also, tropical forest fragmentation is likely to decrease gene flow, increase inbreeding and therefore produce a high differentiation among remnant populations (Alvarez-Buylla *et al*., 1996*b*; González-Astorga and Núñez-Farfán, 2001). This would be particularly the case for *A. aromatica* where one would expect a low genetic diversity. Contrary to this expectation, we found high levels of genetic diversity for *A. aromatica*,

TABLE 1. *Allelic frequencies of 15 enzymatic loci in individuals (adults and seedlings) of three populations of* Antirhea aromatica *in Veracruz, Mexico.*

Locus	Allele	Populations 1	2	3
Mdh1				
Adults	a	0·1714	0·1471	0·7778
	b	0·8286	0·0588	0·0370
Seedlings	c	0·0000	0·7941	0·1852
	a	0·6282	0·1719	0·9375
	b	0·3718	0·1094	0·0625
	c	0·0000	0·7188	0·0000
Mdh2				
Adults	a	0·8649	0·1029	0·2115
	b	0·1351	0·0588	0·0769
	c	0·0000	0·8382	0·7115
Seedlings	a	0·6875	0·1000	1·0000
	b	0·3000	0·2333	0·0000
	c	0·0125	0·6667	0·0000
Est1				
Adults	a	0·3194	0·9559	0·8889
	b	0·0833	0·0441	0·1111
	c	0·5972	0·0000	0·0000
Seedlings	a	0·7143	0·3750	0·0667
	b	0·2857	0·6250	0·9333
Est2				
Adults	a	0·5294	0·9722	0·6800
	b	0·4706	0·0278	0·3200
Seedlings	a	0·7639	0·1452	0·8667
	b	0·2361	0·8548	0·1333
Est3				
Adults	a	0·1111	0·0714	0·5000
	b	0·8889	0·9286	0·5000
Seedlings	a	0·6447	0·3833	0·8235
	b	0·3553	0·6167	0·1765
Pgi1				
Adults	a	0·1351	0·9857	0·3000
	b	0·8649	0·0143	0·1200
	c	0·0000	0·0000	0·5800
Seedlings	a	0·5811	0·1452	0·9375
	b	0·4189	0·8548	0·0625
Pgi2				
Adults	a	0·8243	0·9286	0·2500
	b	0·1757	0·0714	0·7500
Seedlings	a	0·6389	0·3833	0·0938
	b	0·3611	0·6167	0·9063
Got				
Adults	a	0·7027	0·6250	0·3077
	b	0·2973	0·3750	0·6923
Seedlings	a	0·6410	0·6563	0·8000
	b	0·3590	0·3438	0·2000
Pgm				
Adults	a	0·0735	0·9714	0·2222
	b	0·9265	0·0286	0·2593
	c	0·0000	0·0000	0·5185
Seedlings	a	0·5921	0·2813	0·9706
	b	0·4079	0·7187	0·0294
6Pgd				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Dia 1				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Dia 2				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Idh				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Lap				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Apx				
Adults	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Seedlings	a	1·0000	1·0000	1·0000
	b	0·0000	0·0000	0·0000
Mean no. of alleles/locus (± SD)				
Adults		1·67 (0·617)	1·73 (0·704)	1·87 (0·834)
Seedlings		1·67 (0·617)	1·73 (0·704)	1·53 (0·516)

for both adults and seedlings. In fact, the values found in this species are among the highest ones recorded for tropical forest trees (Eguiarte *et al*., 1992; Alvarez-Buylla and Garay, 1994; Cardoso *et al*., 1998; Loveless *et al*., 1998; Lee *et al*., 2002; Ledig *et al*., 2002), and especially with respect to those found in fragmented environments (Hall *et al*., 1996; Cascante *et al*., 2002).

The mean percentage of polymorphic loci in *A. aromatica* was higher (51·1 and 55·5 %, adults and seedlings, respectively) than that reported for other long-lived perennial and endemic plant species (48·1 %; Hamrick and Godt, 1996*a*). The mean expected heterozygosity within populations for *A. aromatica* was also higher (0·185 and 0·203, adults and seedlings, respectively), than that reported for other regionally distributed (*sensu* Hamrick and Godt, 1989), tropical long-lived trees, and even higher than those for temperate long-lived trees (0·125 and 0·145, respectively; Hamrick *et al*., 1994). It is also higher than those of other long-lived perennial and endemic species (0·105; Hamrick and Godt, 1996*a*). This shows that *A. aromatica* has exceptionally high genetic diversity and variability, despite its low population density (cf. Young *et al*., 1996; González-Astorga and Núñez-Farfán, 2001). This phenomenon is likely to be associated with the reproductive system. It has been reported that other Rubiaceae have a pre-zygotic self-incompatibility crossing system (Anderson, 1973; Richards, 1997; Faivre and McDade, 2001) that reduces inbreeding, and loss of genetic variation. The heterostyly observed in *A. aromatica* (J. González-Astorga, personal observation) denotes the existence of a self-incompatibility reproductive system (Sobrevila *et al*., 1983; Richards and Kortur, 1993; Riveros *et al*., 1995), even though outcrossing rate [$t = (1 - f)/(1 + f)$; *sensu* Allard *et al*., 1968] in adult

TABLE 2. *Levels of genetic variation for adult and seedling populations of* A. aromatica. A*, mean number of alleles per locus;* P*, percentage of polymorphic loci;* N_i*, average sample size;* H_o *and* H_e *are the observed and expected mean heterozygosity, respectively.*

Population	*A*	*P*	N_i	H_o	H_e
1					
Adults	1·67	60·00	36·33	0·1549	0·1884
Seedlings	1·67	60·00	38·53	0·2044	0·2682
2					
Adults	1·73	33·33	35·73	0·0847	0·1056
Seedlings	1·73	60·00	31·46	0·2309	0·2466
3					
Adults	1·87	60·00	26·46	0·1696	0·2607
Seedlings	1·53	46·66	16·40	0·0775	0·0952
Mean (± SD)					
Adults	1·76 (0·102)	51·11 (15·40)	32·84 (5·53)	0·1364 (0·045)	0·1849 (0·078)
Seedlings	1·64 (0·102)	55·55 (7·70)	28·80 (11·30)	0·1709 (0·082)	0·2033 (0·094)

populations of *A. aromatica* indicates that 57 % of the offspring are a product of exogamy. This result contrasts with an average outcrossing rate of 88 ± 12 % for of 30 species of tree and shrubs reported by Eguiarte (1990), and 90 ± 5 % reported by Boshier (2000) for seven tropical trees. However, because the *t*-value is an indirect estimate, caution has to be taken in its interpretation (Ledig *et al*., 1997).

Pollinator efficiency allows the establishment of a stable genetic neighbourhood supporting an adequate genetic variability within populations, and is reflected in the high genetic diversity found in *A. aromatica* populations (P = 51·1 and H_e = 0·185 in adults, and P = 55·5 and H_e = 0·203 in seedlings).

The inbreeding values found in *A. aromatica* were high when compared to other tropical species (Eguiarte, 1990; Boshier, 2000). These values were significantly greater in adults that in seedlings. The relatively low inbreeding observed in seedlings suggests that the individuals sampled came from an exceptional fruit-setting year for many adult trees, which could be due to a random sampling effect from the gene pools among cohorts (Husband and Schemske, 1996), or a past cornucopia effect (cf. Sazima *et al*., 2001; Leite and da Encarnacao, 2002).

On the other hand, the high genetic differentiation found among populations (θ = 0·51, adults; and θ = 0·35, seedlings) may be due to two processes: in the immediate-term to reduced pollinator-efficiency as a result of flowering asynchrony between populations (cf. Murren, 2002), and in the longer-term to reduced spatial distribution and increased population isolation due to fragmentation (Young *et al*., 1996), which in turn could restrain gene flow and disrupt the demographic structure of formerly stable populations (Loveless and Hamrick, 1984). Alternatively, the reduced gene flow detected in *A. aromatica* adult populations may be due to low seed dispersal efficiency. Frugivore bats deposit massive amounts of seeds of the same mother tree under very few resting trees, such as *Brosinium alicastrum*, *Bursera simaruba*, *Hyperbaena mexicana*, *Manilkara zapota* and *Protium copal* (Castillo-Campos and Lorence, 1985; Castillo-Campos, 1995). Although we did not evaluate gene flow in seedlings, we would expect a similar pattern to the one observed in the adults if we assume that most of the seedlings would eventually reach maturity.

In conclusion, our results show that the three extant populations of *A. aromatica* present a relatively high genetic diversity when compared with other plants with similar attributes. The conservation implications for the species are evident, since the species has only been found in three forest patches of a very scarce vegetation type in Mexico. In this region the human population has continuously increased since the 16th century (de la Madrid *et al*., 1988). This has resulted in the extensive cultivation of agricultural crops, initially such as sugar cane and later mango and coffee plantations, with the subsequent fragmentation of the original distribution range of *A. aromatica* (Castillo-Campos, 1995). The isolation and reduction of the species' populations have reduced intrapopulation gene flow and have generated a systematic process of genetic isolation. However, our results suggest that *A. aromatica* has genetically viable populations, and at present the main threats are primarily associated with changes in the environment due to human activities.

We suggest that this tree species should be considered an indicator species (*sensu* Noss, 1990) with unexplored economic potential (Given, 1994). The preservation of the extant populations of *A. aromatica* through the creation of a nature reserve would be ideal. In addition, we would recommend the implementation of management practices by local people in order to reinforce conservation of the species, such as is the case for the conservation of the cycad *Dioon edule* by means of sustainable utilization in other regions close to our study area (Vovides and Iglesias, 1994; Vovides *et al*., 2002).

ACKNOWLEDGMENTS

This study was supported partially by four grants: CONACyT 34077-N 902-10, CONACyT-SEMARNAT 2002-C01-0183, FMCN, A.C. B-2·00/013 to J.G.-A., and 902-14 to G. C.-C., of the Instituto de Ecología, AC. We thank Jesús Vargas García for technical assistance in the

TABLE 3. *Wright's* F-*statistics for adult and seedling populations of* Antirhea aromatica *in Veracruz, Mexico.*

Loci	*F*	*q*	*f*
Mdh1			
Adults	0·6565**	0·4867**	0·3308**
Seedlings	0·4563*	0·3261*	0·1932**
Mdh2			
Adults	0·6386**	0·4961**	0·2827*
Seedlings	0·4503*	0·3229*	0·1881*
Est1			
Adults	0·6640**	0·5089**	0·3158**
Seedlings	0·4686**	0·3489	0·1838*
Est2			
Adults	0·6397**	0·5267*	0·2387*
Seedlings	0·4477**	0·3238*	0·1832*
Est3			
Adults	0·6420**	0·5216**	0·2517**
Seedlings	0·4620**	0·3666*	0·1506*
Pgi1			
Adults	0·6213**	0·4735*	0·2807**
Seedlings	0·4531*	0·3331*	0·1800**
Pgi2			
Adults	0·6277**	0·5086**	0·2423**
Seedlings	0·4764*	0·3584*	0·1840**
Got			
Adults	0·6379*	0·5439**	0·2060**
Seedlings	0·4980*	0·3760*	0·1955*
Pgm			
Adults	0·6037**	0·4729*	0·2481**
Seedlings	0·4744*	0·3467*	0·1955*
6Pgd			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Dia 1			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Dia 2			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Idh			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Lap			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Apx			
Adults	0·6370	0·5050	0·2666
Seedlings	0·4654	0·3450	0·1838
Mean			
Adults	0·6384	0·5107	0·2690
Seedlings	0·4673	0·3477	0·1841
Standard deviation			
Adults	0·0491	0·0664	0·1085
Seedlings	0·0439	0·0534	0·0374
Confidence interval to 95%			
Adults	0·5410–0·7258	0·3496–0·6126	0·0740–0·4492
Seedlings	0·3582–0·5368	0·2319–0·4336	0·1275–0·2553

* $P < 0·05$; ** $P < 0·01$.

laboratory. Our sincere gratitude is expressed to Mikael Hedrén, Luis Eguiarte, Daniel Piñero, Andrew P. Vovides, Andrea Cruz-Angón and two anonymous reviewers for their comments to improve the manuscript. Alejandro Flores-Palacios, Miriam Ferrer, Maria Luisa Martínez, Víctor Luna, José García-Franco and Israel Acosta provided tireless assistance in the field.

LITERATURE CITED

Allard RW, Jain SK, Workman PL. 1968. The genetics of inbreeding populations. *Advances of Genetics* **14**: 55–131.

Alvarez-Buylla ER, Garay AA. 1994. Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer species. *Evolution* **48**: 437–453.

Alvarez-Buylla ER, Chaos A, Piñero D, Garay AA. 1996*a*. Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. *Evolution* **50**: 1155–1166.

Alvarez-Buylla ER, García-Barrios R, Lara-Moreno C, Martínez-Ramos M. 1996*b*. Demographic and genetic models in conservation biology: Applications and perspectives for tropical rain forest tree species. *Annual Review of Ecology and Systematics* **27**: 387–421.

Anderson WR. 1973. A morphological hypothesis for the origin of heterostyly in the Rubiaceae. *Taxon* **22**: 537–542.

Antonovics J, Via S. 1987. Genetic influences on the distribution and abundance of plants. In: Devy AJ, Hutchings MJ, Watkinson AR, eds. *Perspectives on plant population biology*. Sunderland, MA: Sinauer Associates, 185–203.

Barrett SCH, Kohn JR. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE, eds. *Genetic and conservation of rare plants*. New York: Oxford University Press, 3–30.

Batista F, Sosa PA. 2002. Allozyme diversity in natural populations of *Viola palmensis* Webb & Berth. (Violaceae) from La Palma (Canary Islands): implications for conservation genetics. *Annals of Botany* **90**: 725–733.

Bawa KS, Perry DR, Beach JH. 1985. Reproductive biology of tropical lowland rainforest trees. 1. Sexual systems and incompatibility mechanisms. *American Journal of Botany* **72**: 331–345.

Boshier DH. 2000. Mating systems. In: Young A, Boshier D, Boyle T, eds. *Forest conservation genetics*. Australia: CABI Publishing, 63–79.

Cardoso MA, Provan J, Powell W, Ferreira PCG, de Oliveira DE. 1998. High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminoseae-Caesalpinioideae). *Molecular Ecology* **7**: 601–608.

Cascante A, Quesada M, Lobo JJ, Fuchs EA. 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conservation Biology* **16**: 137–147.

Castillo-Campos G. 1995. *Ecología del paisaje del municipio de Jalcomulco, Veracruz*. MSc thesis. UNAM, México.

Castillo-Campos G, Lorence DH. 1985. *Antirhea aromatica* (Rubiaceae, Guettaradeae), a new species for Veracruz, México. *Annals of Missouri Botanical Garden* **72**: 268–271.

Crow JF, Aoki K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences of the United States of America* **81**: 6073–6077.

de la Madrid HM, Bartlett DM, Gutierrez BF, Olmedo RC, González AS. 1988. *Los Municipos de Veracruz*. México: Colección, Encyclopedia de los Municipios de México.

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

Dolan RW, Yahr R, Menges E S, Halfhill MD. 1999. Conservation implications of genetic variation in three rare species endemic to Florida rosemary scrub. *American Journal of Botany* **86**: 1556–1562.

Eguiarte LE. 1990. *Genética de poblaciones de* Astrocaryum mexicanum *Liebm. en los Tuxtlas, Veracruz*. Ph.D. thesis. UNAM, México.

Eguiarte LE, Perez-Nasser N, Piñero D. 1992. Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): implications for evolution and conservation. *Heredity* **69**: 217–228.

Evans MEK, Dolan RW, Menges ES, Gordon DR. 2000. Genetic diversity and reproductive biology in *Warea carteri* (Brassicaceae), a narrowly endemic Florida scrub annual. *American Journal of Botany* **87**: 372–381.

Faivre AE, McDade LA. 2001. Population-level variation in the expression of heterostyly in three species of Rubiaceae: does

reciprocal placement of anthers and stigmas characterize heterostyly. *American Journal of Botany* **88**: 841–853.

Frankham R. 1995. Conservation genetics. *Annual Review of Genetics* **29**: 305–327.

García E. 1988. *Modificaciones del sistema de clasificación climática de Köeppen*. México, DF: Instituto de Geografía, UNAM**.**

Gitzendanner MA, Soltis PS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* **87**: 783–792.

Given DR. 1994. *Principles and practice of plant conservation*. Portland Oregon, Timber Press.

González-Astorga J, Núñez-Farfán J. 2001. Effect of habitat fragmentation on the genetic structure of the narrow endemic *Brongniartia vazquezii*. *Evolutionary Ecology Research* **3**: 861–872.

González-Astorga J, Vovides AP, Ferrer MM, Iglesias C. 2003. Population genetics of *Dioon edule* Lindl. (Zamiaceae, Cycadales): biogeographical and evolutionary implications. *Biological Journal of the Linnean Society* **80**: 457–467.

Hall P, Walker S, Bawa KS. 1996. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conservation Biology* **10**: 757–768.

Hartl DL, Clark AG. 1997. *Principles of population genetics*. 3rd edn. Sunderland, MA: Sinauer Associates.

Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, MA: Sinauer, 43–63.

Hamrick JL, Godt MJW. 1996*a*. Conservation genetics of endemic plant species. In: Avise JC, Hamrick JL, eds. *Conservation genetics. Case histories from nature*. New York: Chapman & Hall, 281–304.

Hamrick JL, Godt MJW. 1996*b*. Effects of the history traits on genetic diversity in plants. *Philosophical Transactions of the Royal Society of London Biological Sciences* **351**: 1291–1298.

Hamrick JL, Schnabel A, Wells PV. 1994. Distribution of genetic diversity within and among populations of Great Basin conifers. In: Harper KT, St Clair LL, Thorne KH, Hess WW, eds. *Natural history of the Colorado Plateau and Great Basin*. Niwot, CO, USA: University of Colorado Press, 147–161.

Hannan GL, Orick MW. 2000. Isozyme diversity in *Iris cristata* and the threatened glacial endemic *I. lacustris* (Iridaceae). *American Journal of Botany* **87**: 293–301.

Hedrick PW. 2001. Conservation genetics: where are we now? *Trends in Ecology and Evolution* **16**: 629–636.

Holsinger KE, Mason-Gamer RJ, Whitton J. 1999. Genes, demes, and plant conservation. In: Landweber LA, Dobson AP, eds. *Genetic and extinction of species* Princeton, NJ: Princeton University Press, 23–46.

Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

Köeppen W. 1948. *Climatología*. México: Fondo de Cultura Económica.

Lande R. 1999. Extinction risks from anthropogenic, ecological, and genetic factors. In: Landweber LA, Dobson AP, eds. *Genetic and extinction of species.* Princeton, NJ: Princeton University Press, 1–22.

Lee D, Park EJ, Cuendent M, Axelrod F, Chavez PI, Fong HHS, Pezzuto JM, Kinghorn, AD. 2001. Cyclooxygenase- inhibitor and antioxidant constitutes of aerial parts of *Antirhea acutata*. *Bioorganic & Medicinal Chemistry Letters* **11**: 1565–1568.

Ledig FT. Jacob-Cervantes V, Hodgskiss PD, Eguialuz-Pineda T. 1997. Recent evolution and divergence among populations of rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. *Evolution* **51**: 91–99.

Ledig FT, Conkle MT, Bermejo-Vázquez B, Eguialuz-Pineda T, Hodgskiss PD, Johnson DR, Dvorak WS. 1999. Evidence for an extreme bottleneck in a rare Mexican pinyon: genetic diversity, disequilibrium, and the mating system in *Pinus maximartinezii*. *Evolution* **53**: 91–99.

Ledig FT, Hodgskiss PH, Jacob-Cervantes V. 2002. Genetic diversity, mating system, and conservation of a Mexican subalpine relict, *Picea mexicana* Martínez. *Conservation Genetics* **3**: 113–122.

Lee SL, Ng KKS, Saw LG, Norwati A, Salwana MHS, Lee CT, Norwati M. 2002. Population genetics of *Intsia palembanica* (Leguminoseae) and genetic conservation of Virgin Jungle in Peninsular Malaysia. *American Journal of Botany* **89**: 447–459.

Leite IRD, da Encarnacao CRF. 2002. Phenology of coconut on the Coastal Zone of Pernambuco, Brazil. *Pesquisa Agropecuaria Brasileira* **37**: 745–752.

Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of the Ecology and Systematics* **15**: 65–95.

Loveless MD, Hamrick JL, Foster RB. 1998. Population structure and mating system in *Tachigali versicolor*, a monocarpic neotropical tree. *Heredity* **81**: 134- 143.

Maki M, Asada Y. 1998. High genetic variability revealed by allozymic loci in the narrow endemic fern *Polystichum otomasui* (Dryopteridaceae). *Heredity* **80**: 604–610.

Martínez-Palacios A, Eguiarte LE, Furnier GR. 1999. Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan desert. *American Journal of Botany* **86**: 1093–1098.

Mateu-Andrés I, Segura-Moragues G. 2000. Population subdivision and genetic diversity in two narrow endemics of *Antirrhinum* L. *Molecular Ecology* **9**: 2081–2087.

Miller MP. 1997. Tools for population genetic analyses (TFPGA) 1.3: a Windows program for the genetic data. Computer software distributed by author.

Miranda F, Hernández X. 1963. Los tipos de vegetación de México y su clasificación. *Boletín de la Sociedad Botánica de México* **28**: 29–179.

Murren CJ. 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology* **90**: 100–107.

Noss RF. 1990. Indicators of monitoring biodiversity: a hierarchical approach. *Conservation Biology* **4**: 355–364.

Ranker TA. 1994. Evolution of high genetic variability in the rare Hawaiian fern *Adenophorus periens* and implications for conservation management. *Biological Conservation* **70**: 19–24.

Raymond M, Rousset F. 1995. An exact test for population differentiation. *Evolution* **49**: 1280–1283.

Richards AJ. 1997. *Plant breeding systems*. 2nd edn. London, UK: Chapman & Hall.

Richards JH, Kortup S. 1993. Floral variation and distyly in *Guettarda scabra* (Rubiaceae). *American Journal of Botany* **80**: 31–40.

Riveros GM, Barría OR, Humaña PAM. 1995. Self-compatibility in distylous *Hedyotis salzmannii*. *Plant Systematics and Evolution* **194**: 1–8.

Savolainen O, Kuittien H. 2000. Small populations processes. In: Young A, Boshier D, Boyle T, eds. *Forest conservation genetics.* Collingwood: CABI Publishing, 91–100.

Sazima M, Vogel S, do Prado AL, de Oliveira DM, Franz G, Sazima I. 2001. The sweet jelly of *Combretum lanceolatum* flowers (Combretaceae): a cornucopia resource for bird pollinators in the Pantanal, western Brazil. *Plant Systematics and Evolution* **227**: 195–208.

Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG. 1994. Evaluation approaches to the conservation of rare and endangered plants. *Ecology* **75**: 584–606.

Slatkin M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**: 264–279.

Slatkin M. 1994. Gene flow and population structure. In: Real L, ed. *Ecological genetics*. New Jersey: Princeton University Press, 3–17.

Sobrevila C, Ramirez N, de Enrech NX. 1983. Reproductive biology of *Palicourea fendleri* and *P. petiolaris* (Rubiaceae), heterostylous of a tropical cloud forest in Venezuela. *Biotropica* **15**: 161–169.

Soltis DE, Haufler CH, Darrow DC, Gastony GJ. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* **73**: 9–27.

Vovides AP, Iglesias CG. 1994. An integrated conservation strategy for the cycad *Dioon edule* Lindl. *Biodiversity and Conservation* **3**: 137–141.

Vovides AP, Iglesias CG, Pérez-Farrera M, Vazquez-Torres M, Schippmann U. 2002. Peasant Nurseries: a concept for an integrated conservation strategy for cycads in Mexico. In: Maunder M, Clubbe C, Hankamer C, Groves M, eds. *Plant conservation in the tropics*. Kew, UK: Royal Botanic Gardens, 421–444.

Waller DM, O'Malley DM, Gawler SC. 1987. Genetic variation in the extreme endemic *Pedicularis furbishiae* (Scrophulariaceae). *Conservation Biology* **1**: 335–340.

Weir BS. 1990. *Genetic data analysis*. Sunderland, MA: Sinauer Associates.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of populations structure. *Evolution* **38**: 1358–1370.

Wendel JF, Weeden NF. 1989. Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS, eds. *Isozymes in plant biology*. Portland, Oregon, USA: Discorides, 5–45.

Workman PL, Niswander JD. 1970. Population studies on southwestern Indian tribes II**.** Local differentiation in the Papago. *American Journal of Human Genetics* **22**: 24–29.

Wright S. 1951. The genetic structure of populations. *Annals of Eugenetics* **16**: 97–159.

Wright S. 1965. The interpretation of population structure by *F*-statistics with spatial regard to system of mating. *Evolution* **19**: 355–420.

Wright S. 1978. *Evolution and the genetics of populations. Vol. 4*. Chicago and London: University of Chicago Press.

Young A, Boyle T, Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**: 413–418.