

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

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• **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 ($Nm = 0.26 \pm 0.09$), 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.

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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 1996a, 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.*, 1996b; Lande, 1999; Hedrick, 2001).

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

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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 anti-septic, 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.

METHODS

Study sites

The study was conducted during 1998–99, in the localities of Jalcomulco and Apazapan ($96^{\circ}41'–99^{\circ}08'W$; $19^{\circ}18'–19^{\circ}27'N$) 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 Köppen (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

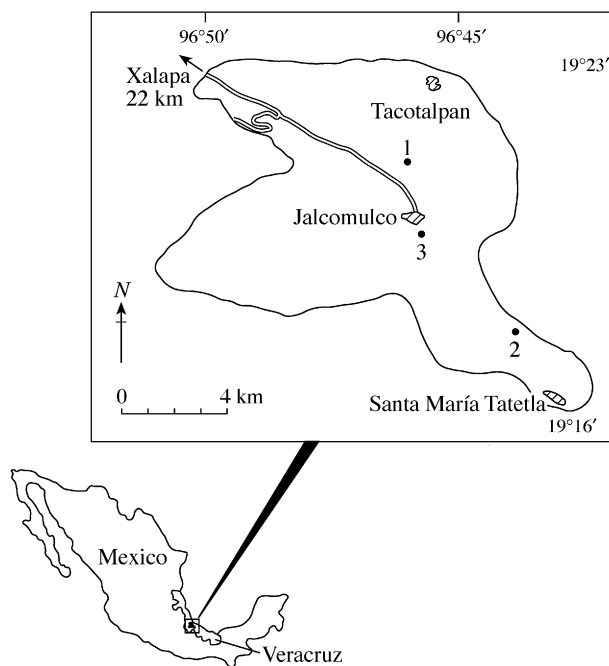


FIG. 1. Geographical distribution of populations examined of *Antirhea aromatica* in Veracruz, Mexico.

in ice-filled containers, and then stored in a freezer at $-70^{\circ}C$ 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*), phosphoglucosomerase (E.C. 5.3.1.9, loci *Pgi1* and *Pgi2*), glutamate oxaloacetate transaminase (E.C. 2.6.1.1, locus *Got*), and phosphoglucosomutase (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 *Dial* 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 $-70^{\circ}C$ 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 $4^{\circ}C$ 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 ($P < 0.05$) 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 ($P < 0.05$). The mean F was higher for the adult than for the seedling population (0.64 ± 0.049 vs. 0.46 ± 0.04 ; $F_{(1,28)} = 1330$, $P < 0.00001$). Similarly, the f -statistic was higher for adult than for seedling populations ($F_{(1,28)} = 101$, $P < 0.00001$) (Table 3).

The average genetic differentiation among adult populations ($\theta = 0.51$) was higher than for seedling populations ($\theta = 0.35$). 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 ($\chi^2 = 178.3$; $df = 18$; $P < 0.00001$), and seedling ($\chi^2 = 161.2$; $df = 18$; $P < 0.00001$) 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 ($\theta \approx 1$), 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.*, 1996b). 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.*, 1996b; 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
<i>Mdh1</i>				
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
<i>Mdh2</i>				
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
<i>Est1</i>				
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
<i>Est2</i>				
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
<i>Est3</i>				
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
<i>Pgi1</i>				
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
<i>Pgi2</i>				
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
<i>Got</i>				
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
<i>Pgm</i>				
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
<i>6Pgd</i>				
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
<i>Dia 1</i>				
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

TABLE 1. Continued

Locus	Allele	Populations		
		1	2	3
<i>Dia 2</i>				
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
<i>Idh</i>				
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
<i>Lap</i>				
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
<i>Apx</i>				
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 (\pm 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, 1996a). 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, 1996a). 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 (\pm 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 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 than 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 ($\theta = 0.51$, adults; and $\theta = 0.35$, 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 *Brosimum 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).

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

Loci	F	q	f
<i>Mdh1</i>			
Adults	0.6565**	0.4867**	0.3308**
Seedlings	0.4563*	0.3261*	0.1932**
<i>Mdh2</i>			
Adults	0.6386**	0.4961**	0.2827*
Seedlings	0.4503*	0.3229*	0.1881*
<i>Est1</i>			
Adults	0.6640**	0.5089**	0.3158**
Seedlings	0.4686**	0.3489	0.1838*
<i>Est2</i>			
Adults	0.6397**	0.5267*	0.2387*
Seedlings	0.4477**	0.3238*	0.1832*
<i>Est3</i>			
Adults	0.6420**	0.5216**	0.2517**
Seedlings	0.4620**	0.3666*	0.1506*
<i>Pgi1</i>			
Adults	0.6213**	0.4735*	0.2807**
Seedlings	0.4531*	0.3331*	0.1800**
<i>Pgi2</i>			
Adults	0.6277**	0.5086**	0.2423**
Seedlings	0.4764*	0.3584*	0.1840**
<i>Got</i>			
Adults	0.6379*	0.5439**	0.2060**
Seedlings	0.4980*	0.3760*	0.1955*
<i>Pgm</i>			
Adults	0.6037**	0.4729*	0.2481**
Seedlings	0.4744*	0.3467*	0.1955*
<i>6Pgd</i>			
Adults	0.6370	0.5050	0.2666
Seedlings	0.4654	0.3450	0.1838
<i>Dia 1</i>			
Adults	0.6370	0.5050	0.2666
Seedlings	0.4654	0.3450	0.1838
<i>Dia 2</i>			
Adults	0.6370	0.5050	0.2666
Seedlings	0.4654	0.3450	0.1838
<i>Idh</i>			
Adults	0.6370	0.5050	0.2666
Seedlings	0.4654	0.3450	0.1838
<i>Lap</i>			
Adults	0.6370	0.5050	0.2666
Seedlings	0.4654	0.3450	0.1838
<i>Apx</i>			
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

* $P < 0.05$; ** $P < 0.01$.

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