Botanical Journal of the Linnean Society, 2006, **152**, 533–544. With 3 figures

Genetic diversity and structure of the cycad *Zamia loddigesii* Miq. (Zamiaceae): implications for evolution and conservation

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Received November 2005; accepted for publication June 2006

The genetic diversity and structure of four populations of the cycad *Zamia loddigesii* were studied throughout its range in Mexico. Allozyme electrophoresis of 15 loci was conducted. The mean number of alleles per locus was 1.80 ± 0.09, the percentage of polymorphic loci was 66.6 ± 5.4, and the expected heterozygosity was 0.266 ± 0.02. The results indicated that the genetic diversity was relatively higher, with respect to tropical tree species and other cycads. The genetic variation explained by differences among populations was 18%. On average, gene flow between paired populations was similar ($Nm = 1.6$) to other tropical forest trees and cycad species. Our results indicated that the geographical isolation among populations of *Z. loddigesii* generated allele loss, as well as a clinal variation in the frequencies of two loci (*MDH* and *MNR2*), in relation to the latitudinal distribution of populations. The populations have become fragmented due to increasingly higher pressure of habitat conversion and disturbance. The importance of the establishment of sanctuaries and protected areas and a reduction in deforestation is highlighted in this research as a way of preserving the high genetic diversity of this and other endemic species. *Botanical Journal of the Linnean Society*, 2006, **152**, 533–544.

ADDITIONAL KEYWORDS: allozymic electrophoresis – conservation genetics – fragmentation – Mexico – population genetics – threatened species.

INTRODUCTION

The distribution of genetic variation within and among populations is of increasing interest to ecologists, especially as researchers combine the fundamentals of evolutionary biology with ecology (Hedrick, 2001; Lowe, Harris & Ashton, 2004) and estimate the future success of natural populations (Wright, 1965, 1978; Epperson, 1993). Levels of genetic diversity reflect the genetic resources necessary for short-term ecological adaptation and for long-term evolutionary change (Frankel & Soulé, 1981). The preservation of population differentiation depends on the balance between genetic drift, natural selection, and gene flow, which acts to homogenize genetic variation across the landscape (Slatkin, 1987). In small populations, genetic drift can be a major evolutionary force reducing genetic diversity. The isolation of those populations, which increases genetic drift and reduces gene flow, can thereby cause a significant reduction in genetic variation within populations and promote the evolution of genetic differentiation (Wright, 1931, 1978). The understanding of how these processes affect the species' probabilities of persistence under increasingly disturbed conditions (Lande, 1988, 1999; Frankham, 1995), such as habitat fragmentation (Ballal, Foré & Guttman, 1994; Young, Boyle & Brown, 1996; González-Astorga & Núñez-Farfán, 2001; González-Astorga & Castillo-Campos, 2004; González-Astorga *et al*., 2004) is clearly necessary. The environmental changes resulting from habitat fragmentation

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affect the sizes of local populations and extinction rates, as well as dispersal patterns among them (Sader & Joyce, 1988; Dodson & Gentry, 1991). Such changes can promote genetic drift in local populations if their size is reduced (González-Astorga & Castillo-Campos, 2004; González-Astorga *et al*., 2004; but see Barrett & Kohn, 1991).

Zamia loddigesii Miq. (Zamiaceae) is a dioeceous, 80–100 cm tall, palm-like cycad. The plants produce cones the whole year, which mature from October to February. This species is distributed in tropical dry forests, rainforests, and secondary succession forests along the north-east and south-east coastal plains (*Planicie Costera Nororiental* and *Planicie Costera Suroriental*) from Tamaulipas to Tabasco, Mexico (Vovides, Rees & Vázquez-Torres, 1983; Stevenson & Sabato, 1986). The conservation status of populations of this species is Near Threatened according to the IUCN (2003) red list, and it is covered along with other Mexican cycads by the Official Mexican Norm. (Anonymous, 1994, 2000; INE-SEMARNAP, 2000; Vovides *et al*., 2002).

Zamia loddigesii is endemic to Mexico, where it is found on the Atlantic side of the Sierra Madre Oriental in Veracruz, Oaxaca, and Tabasco. *Z. loddigesii* occupies various habitats, including disturbed ones within its range. It is apparently the widest distributed *Zamia* in Mexico, with great morphological variation, and for this reason there is controversy on the identity of the species. *Z. loddigesii* covers a wide range in comparison with other *Zamia* spp., but its populations are highly reduced and fragmented (Vovides *et al*., 1983) and in most cases relict populations of very few individuals (less than 12). Cycad taxonomists with a broad species concept have placed many taxa 'as variants' in synonymy under *Z. loddigesii*, as demonstrated by the long list of synonymy (Vovides *et al*., 1983; Whitelock, 2002; Hill & Stevenson, 2004). *Z. lawsoniana* Dyer has also been placed in synonymy under *Z. loddigesii* and this includes one of our sampled populations in the extreme south of the distribution. A variant considered to be *Z. loddigesii* on the Yucatan peninsula and Belize has been described as *Z. polymorpha* D. Stevenson, A. Moretti & Vázq.Torres, 'that appears to be related to but cytologically, morphologically, geographically, and climatically separated from *Z. loddigesii*' (Stevenson, Moretti & Gaudio, 1995–96; Whitelock, 2002). With this in mind and the lack of a recent revision of the species, we designed our sampling to include the extremes and centre of what we consider to be the distribution of the species based upon the neotypification of *Z. loddigesii* by Stevenson & Sabato (1986).

In this study, we examined the diversity and genetic structure of *Z. loddigesii* in its known distribution range. Our objectives were to: (1) evaluate the genetic diversity in four populations across its distribution range, (2) determine the amount and distribution of genetic variation within and among populations; (3) contrast the results of diversity and genetic structure of populations of *Z. loddigesii* with other tropical plant species (trees and cycads), and (4) evaluate the conservation status of the studied populations of this species.

MATERIAL AND METHODS

Study species

The study was carried out in 2003 in four populations of *Z. loddigesii* along the north-east and south-east coastal plains of Mexico, from Tabasco (south) to Tamaulipas (north) (Fig. 1). The cycad is found mostly in tropical dry forest (*sensu* Rzedowski, 1978) and its secondary succession stages, where fragmentation has occurred as a result of recent human activities (Vovides *et al*., 2002). The largest possible populations throughout its geographical range were sampled. Leaflets for electrophoresis were taken from 20 to 25 randomly selected individuals per population when the population exceeded 50 individuals; in smaller populations, all individuals were sampled. Populations of less than 20 individuals were not considered.

Enzyme extraction and electrophoresis

Approximately 250 mg of fresh leaflet tissue was ground with liquid nitrogen to homogenize the tissue. Approximately 250 µl of extraction buffer (0.1 M Tris-HCl pH 7.5, 4% PVP-40, 0.001 M EDTA, 0.01 M $CaCl_2$, 0.01 M $MgCl_2$, and 0.1% β-mercaptoethanol; Chao-Luan *et al*., 1999) was added to dilute and stabilize the enzyme extracts, which were stored on filter paper wicks at –70 °C until used for analyses. Multilocus genotypes of *c*. 25 individuals from each population were obtained through horizontal starch gel electrophoresis (10% w/v) (Müller-Strack, 1998). For each individual plant, allozymic variation was scored in 11 polymorphic loci: malate dehydrogenase (E.C. 1.1.1.37, *MDH*), phosphoglucoisomerase (E.C. 5.3.1.9, *PGI1* and *PGI2*), isocitrate dehydrogenase (E.C. 1.1.1.41, *IDH*), 6–phosphogluconate dehydrogenase (E.C. 1.1.1.44, *6PGD*), menadione reductase (E.C. 1.6.99.–, *MNR1* and *MNR2*), peroxidase anodic (E.C. 1.11.1.7, *APX1* and *APX2*), glutamate oxaloacetate transaminase (E.C. 2.6.1.1, *GOT*), and leucine aminopeptidase (E.C. 3.4.11.1, *LAP*) (Wendel & Weeden, 1989); and four monomorphic loci: diaphorase (E.C. 1.6.99.–, *DIA*), esterase (E.C. 3.1.1, *EST*), aminopeptidase (E.C. 3.4.11.1, *AMP*), and alcohol dehydrogenase (E.C. 1.1.1.1, *ADH*). The R gel/electrode buffer system was used for all 15 loci (Chao-Luan *et al*., 1999). Electrophoresis was carried out at 4 °C for 8 h (constant current of 50 mA, voltage of 80 V).

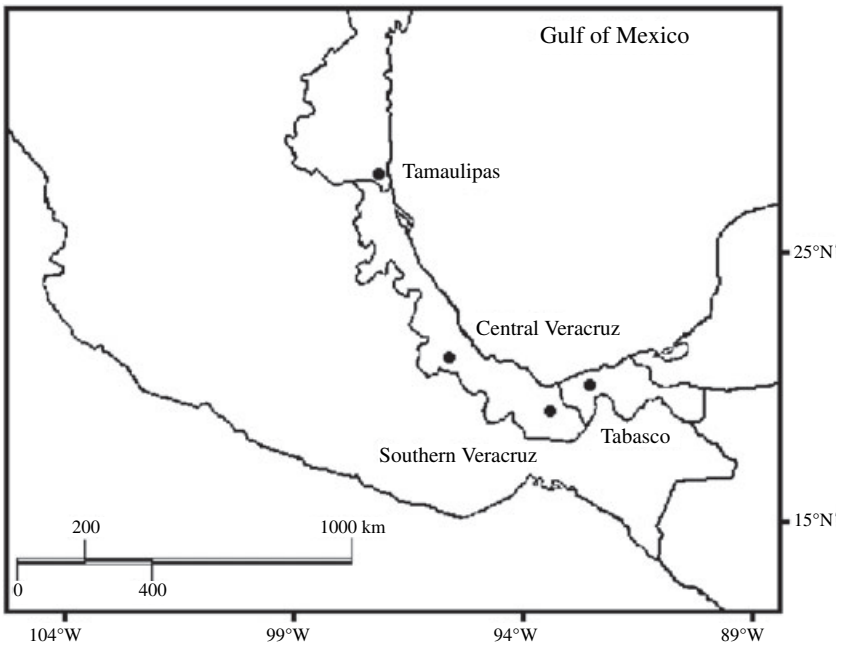

Figure 1. Geographical distribution of *Zamia loddigesii* in north-east and south-east Mexico. The dots show where the populations were sampled.

DATA ANALYSIS

The bands from each allozyme system were assigned to alleles and genotypes based on theoretical expectations and observed banding patterns. The TFPGA 1.3 software (Miller, 1997) was used to obtain the genetic estimators. The genetic diversity of *Z. loddigesii* was described by the percentage polymorphism (P), the expected and observed heterozygosity (H_E and H_O), and the mean number of alleles per locus (A) (Hartl & Clark, 1997). A locus was considered polymorphic if the largest allele frequency was less than 0.95 (Hedrick, 2000). The genotypic frequencies obtained were used to estimate the observed mean heterozygosity (H_O) and allelic frequencies. Also, the mean expected heterozygosity (H_E), based on Hardy–Weinberg expectations (Hartl & Clark, 1997; Hedrick, 2000), was estimated. The partitioning of genetic variability was performed using F-statistics (Wright, 1978). To determine whether F_{is} and F_{it} 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 F_{st} statistic per locus, the chi-square statistic was used: $\chi^2 = (2N)F_{st}(k-1)$, with $(k-1)(s-1)$ degrees of freedom, where s is the number of populations (Workman & Niswander, 1970). The 95% confidence intervals of the F-statistics were obtained by bootstrapping over loci for the multilocus estimate, and jackknifing over populations for the single-locus estimates (Weir & Cockerham, 1984; Weir, 1990). The average gene flow among populations (Nm) was estimated from F_{st} values, as $F_{st} = 1/(4Nm\alpha + 1)$, where $\alpha = (s/s-1)^2$ and s is the number of populations (Crow & Aoki, 1984). Nm is interpreted as the number of migrants per generation among two given populations (Slatkin, 1993).

We tested for genetic differentiation among populations using the exact test of Raymond & Rousset (1995), which is analogous to Fisher's exact test (Fisher, 1935), but uses a Markov chain to explore all potential states of an $s \times t$ contingency table based on s populations and t genotypes. The test was conducted using the TFPGA 1.3 software (Miller, 1997) and 10 000 Markov steps for all pairwise combinations. Finally, phenetic clustering of the populations was performed using Nei's (1972) genetic distances and UPGMA (Sneath & Sokal, 1973).

RESULTS

GENETIC DIVERSITY

Of 15 loci, 11 were polymorphic: *MDH*, *PGI1*, *PGI2*, *IDH*, *6PDG*, *MNR1*, *MNR2*, *APX1*, *APX2*, *GOT*, and *LAP*. The remaining four loci were monomorphic (*DIA*, *EST*, *AMP*, and *ADH*) (Table 1). For eight polymorphic loci (*MDH*, *PGI1*, *PGI2*, *IDH*, *MNR1*, *MNR2*, *GOT*, and *LAP*), the test for allelic heterogeneity frequency among populations showed significant differences (χ^2 test with 1 d.f., $\alpha = 0.01$); for the remaining three

Table 1. Allelic frequencies of 15 allozyme loci for four populations of *Zamia loddigesii* in north-east and south-east Mexico. Asterisks indicate the statistical significance for genetic differentiation among populations using the conventional Monte Carlo method (ten batches, 1000 permutations per batch, and 10 000 total permutations)

Population	Allele	*MDH*	*PGI1*	*PGI2*	*IDH*	*6PGD*	*MNR1*	*MNR2*	*APX1*	*APX2*	*GOT*	*LAP*	*DIA*	*EST*	*AMP*	*ADH*
Tamaulipas	1	0.4286	0.5714	0.1667	0.3095	0.6429	0.3095	0.9737	0.5714	0.9286	0.9000	0.7857	1.0000	1.0000	1.0000	1.0000
	2	0.5714	0.3571	0.3095	0.3810	0.3571	0.6905	0.0263	0.4286	0.0714	0.1000	0.2143	0.0000	0.0000	0.0000	0.0000
	3		0.0715	0.5238	0.3095							0.0000				
Tabasco	1	1.0000	0.5263	0.6500	0.5333	0.9000	0.5500	0.6750	0.8421	0.6579	0.9000	0.5625	1.0000	1.0000	1.0000	1.0000
	2	0.0000	0.4737	0.3500	0.4667	0.1000	0.4500	0.3250	0.1579	0.3421	0.1000	0.3125	0.0000	0.0000	0.0000	0.0000
	3		0.0000	0.0000	0.0000							0.1250				
Southern	1	0.9545	0.3913	0.4545	0.7778	0.8158	0.8235	0.7500	0.5750	0.5435	0.9565	0.3810	1.0000	1.0000	1.0000	1.0000
Veracruz	2	0.0455	0.6087	0.5455	0.2222	0.1842	0.1765	0.2500	0.4250	0.4565	0.0435	0.2381	0.0000	0.0000	0.0000	0.0000
	3		0.0000	0.0000	0.0000							0.3810				
Central	1	0.6053	0.8421	0.8750	0.8947	0.9000	0.7000	0.8250	0.8158	0.8889	0.4250	0.6053	1.0000	1.0000	1.0000	1.0000
Veracruz	2	0.3947	0.1579	0.1250	0.1053	0.1000	0.3000	0.1750	0.1842	0.1111	0.5750	0.3947	0.0000	0.0000	0.0000	0.0000
	3		0.0000	0.0000	0.0000							0.0000				
			*	*	*	ns	*	*	ns	ns	*	*	–	–	–	–

*$P < 0.01$; ns, not significant.

Figure 2. Allele frequencies at two loci in four populations of *Zamia loddigesii* in north-east and south-east Mexico. A, locus *MDH* allele 1 (shaded area). B, locus *MNR2* allele 1 (shaded area). Note that *MDH* allele 1 decreased with latitude, whereas *MNR2* allele 1 increased.

polymorphic loci (*6PGD*, *APX1*, and *APX2*), the differences were not significant (Table 1).

The population of Tamaulipas presented three exclusive alleles (*PGI1*-3, *PGI2*-3, and *IDH*-3); whereas Tabasco and southern Veracruz populations had one exclusive allele (*LAP*-3) (Table 1). The frequency of allele 1 at locus *MDH* proportionally decreased from northern to southern populations. The pattern was inversed for allele 1 of the *MNR2* locus (Table 1, Fig. 2). This suggests isolation by distance; this process probably generated genetic differences among populations.

The mean number of alleles per locus was 1.80 ± 0.09 (Table 2). The percentage of polymorphic loci was 66.6 ± 5.4. The observed mean heterozygosity was 0.263 ± 0.04 and the expected mean heterozygosity was 0.266 ± 0.02 (Table 2).

Table 2. Genetic variation for 15 allozyme loci of four populations of *Zamia loddigesii* in north-east and south-east Mexico

Population	N	P	A	H_O	H_E
Tamaulipas	20.8	66.6	1.93	0.251	0.291
Tabasco	19.2	66.6	1.73	0.270	0.268
Southern Veracruz	21.5	60.0	1.80	0.310	0.273
Central Veracruz	19.5	73.3	1.73	0.220	0.233
Mean ± standard deviation	20.25 ± 1.1	66.6 ± 5.4	1.80 ± 0.09	0.263 ± 0.04	0.266 ± 0.02

N, mean number of plants evaluated; P, percentage of polymorphic loci; A, number of alleles per locus; H_O, H_E, observed and expected mean heterozygosity, respectively.

Table 3. Wright's F-statistics for 11 polymorphic loci in four populations of *Zamia loddigesii* in north-east and south-east Mexico. All values are different from zero ($P < 0.05$)

Loci	F_{it}	F_{st}	F_{is}
MDH	0.182	0.164	0.022
PGI1	0.220	0.186	0.041
PGI2	0.215	0.164	0.060
IDH	0.205	0.176	0.035
6PGD	0.230	0.186	0.054
MNR1	0.201	0.181	0.025
MNR2	0.206	0.187	0.024
APX1	0.233	0.188	0.054
APX2	0.230	0.181	0.059
GOT	0.199	0.168	0.037
LAP	0.228	0.189	0.047
Mean (± standard deviation)	0.213 (0.048)	0.179 (0.029)	0.041 (0.014)
95% confidence interval	0.124–0.300	0.128–0.230	0.001–0.082

Table 4. Exact χ^2 test for significance of genetic differentiation (Raymond & Rousset, 1995) among four populations of *Zamia loddigesii*. The test was performed for 10 000 Markov steps

Contrast among populations	χ^2 test	Probability (P)
Tamaulipas vs. Tabasco	116.1	0.0001
Tamaulipas vs. southern Veracruz	141.1	0.0001
Tamaulipas vs. central Veracruz	111.8	0.0001
Tabasco vs. southern Veracruz	44.9	0.01
Tabasco vs. central Veracruz	85.7	0.001
Southern Veracruz vs. central Veracruz	132.2	0.0001

GENETIC STRUCTURE

Considering the polymorphic loci, Wright's F-statistics (F_{it}, F_{is}, and F_{st}) were positive and significantly different from zero. The mean (± standard deviation) values of F_{it} and F_{is} were 0.213 ± 0.048 and 0.041 ± 0.014, respectively, and showed a significant deficit of heterozygosity. The mean F_{st} value was 0.179 ± 0.029; this indicated that 18% of the genetic variation in *Z. loddigesii* was due to differences among populations (Table 3). Also, the exact test of allelic differentiation among populations was significant (Table 4).

GENE FLOW AND GENETIC DISTANCES

The mean Nm value or migrants per generation was 1.60 ± 1.45 between population pairs. The lowest Nm value was between the Tamaulipas and central Veracruz populations ($Nm = 0.71$) separated by 593 km,

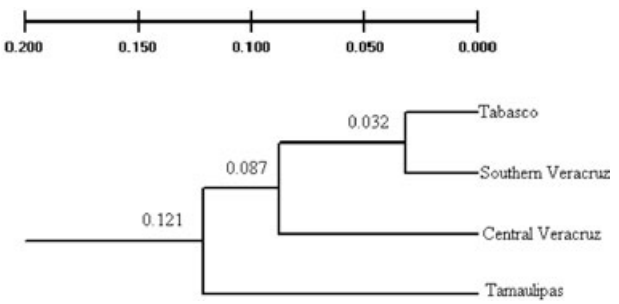

Figure 3. UPGMA phenogram based on Nei's genetic distances between four populations of *Zamia loddigesii* in north-east and south-east Mexico.

and the highest was between Tabasco and southern Veracruz ($Nm = 4.52$) separated by 167 km. The mean genetic distance among populations of *Z. loddigesii* was 0.095 ± 0.04. The UPGMA cluster analysis, based on genetic distances, showed north–south grouping of populations according to their geographical distribution (Figs 1, 3).

DISCUSSION

Genetic diversity

The results presented in this paper show that *Z. loddigesii* populations have relatively high levels of genetic diversity ($A = 1.80$, $P = 66.6$, $H_E = 0.26$), when compared with the mean genetic diversity of 16 tropical trees [cf. Appendix 1; $A = 1.60 \pm 0.42$ (range 1.06–2.40), $P = 42.1 \pm 19.2$ (range 100–20.5), and $H_E = 0.156 \pm 0.09$ (range 0.044–0.369)]. Also, the estimates of genetic diversity of *Z. loddigesii* were relatively higher than the overall mean values for 13 cycad species (cf. Appendix 2; $A = 1.47$, $P = 40.2$, and $H_E = 0.100$). This implies that *Z. loddigesii* has higher genetic diversity when compared with the previously mentioned groups.

Only the cycad *Macrozamia riedlei* (Byrne & James, 1991) showed higher levels of genetic diversity than *Z. loddigesii*. This may be due to a greater number of populations and plants analysed (15 vs. four) and low or no fragmentation of the *Macrozamia riedlei* populations. Contrarily, populations of *Z. loddigesii* were highly fragmented and eliminated in the tropical dry forest, owing to extensive agricultural expansion and cattle pasture, as well as frequent fires in the region (Castillo-Campos, 1995; Masera, Ordóñez & Dirzo, 1997).

On the other hand, genetic diversity in *Z. loddigesii* is similar to those reported by González-Astorga *et al*. (2003) for *Dioon edule* ($A = 1.44$, $P = 54.8$, and $H_E = 0.240$) and Yang & Meerow (1996) for *Cycas siamensis* ($A = 1.48$, $P = 58.5$, and $H_E = 0.134$). These results are notable, owing to the fact that in *Z. loddigesii* only four populations were analysed, whereas for *Dioon edule* and *Cycas siamensis*, 14 and 17 populations were analysed, respectively.

The geographical isolation among populations of *Z. loddigesii* and the reduction in individual numbers may have generated the differential loss of alleles among populations (*PGI1*-3, *PGI2*-3, and *IDH*-3 in Tabasco, central and southern Veracruz; *LAP*-3 in central Veracruz and Tamaulipas). In addition, the latitudinal patterns found for loci frequencies of *MDH* (Fig. 2A) and *MNR2* (Fig. 2B), which increase and decrease, respectively, with latitude, reflect a clinal variation in those loci. Our results of genetic distances among populations confirm this north–south pattern, as populations were consistently grouped according to their geographical distribution (see Figure 3). This supports the assumption of a differential action of natural selection, gene flow, and genetic drift at the population level (Slatkin, 1973; Endler, 1977). Similar latitudinal clinal patterns were reported for *Elymus caninus* in Scandinavia, Russia, and Asia (Díaz, Björn & Von Bothmer, 1999) and *Glycine soja* in Japan (Ohara & Shimamoto, 2002). Latitudinal clinal variation has also been reported for other species in the Gulf of Mexico (*Rhizophora mangle*, Núñez-Farfán *et al*., 2002; the cycad *Dioon edule*, González-Astorga *et al*., 2003). This is also in keeping with the biogeographical distribution patterns of the eastern Mexican flora (Graham, 1993; Marshall & Liebherr, 2000).

Genetic structure and gene flow

Zamia loddigesii has significant levels of genetic differentiation among populations, with 18% of the total variation due to differences among populations. The mean F_{st} value for *Z. loddigesii* is similar to those reported for 16 plant species trees in tropical zones (19.9%; Appendix 1) and 13 cycad species (21.3%; Appendix 2). However, the populations of *Z. loddigesii* have relatively high levels of genetic differentiation compared with other cycads, such as *Macrozamia riedlei* (9.2%; Byrne & James, 1991), *Macrozamia parcifolia* (9%; Sharma *et al*., 1998), *Macrozamia pauliguilielmi* (3%; Sharma *et al*., 1998), *Cycas guizhouensis* (8%; Yang & Meerow, 1999), and *Cycas taitungensis* (3.4%; Lin *et al*., 2000).

These results and comparisons illustrate the effect of habitat fragmentation and, therefore, isolation among the populations of *Z. loddigesii*, a process typical of tropical zone plant species (Alvarez-Buylla *et al*., 1996). In this context, White, Boshier & Powell (1999) proposed that this phenomenon can be reduced, through an increase in pollen flow among remnant populations. However, in the case of *Z. loddigesii*, this is relatively complicated due to the cycads having highly specific insect pollinators that are poor flyers

(Norstog, 1987). A more efficient seed dispersal mechanism might mitigate the effects of habitat fragmentation to a lesser extent and *Zamia* seeds are known to be dispersed by mocking birds (Eckenwalder, 1980).

Cycad populations that become highly fragmented or subjected to frequent fires become vulnerable to pollinator population crashes. Ground-level fires that burn surface humus also kill the diapause stages of the pollinator beetle larvae that 'overwinter' in the male cone debris (Norstog & Fawcett, 1989; Norstog *et al*., 1995; Vovides *et al*., 1997). Also, severe fragmentation and great distances between fragments can result in low pollinator activity, owing to the poor flying capability of the insects, as well as the loss of 'brood and shelter' for the beetles that rely on male cones to breed (Norstog & Fawcett, 1989).

Higher F_{st} values can result from reduced population sizes and gene flow (Slatkin, 1993). The mean gene flow per generation ($Nm = 1.60$) estimated in *Z. loddigesii* is similar to that of endemic or narrowly distributed plant species, and is lower than that reported for other species with outcrossed breeding systems by animals, in this case insects (cf. Norstog & Fawcett, 1989) and seed dispersal by gravity (Hamrick, Godt & Sherman-Broyles, 1995). This indicates that there is little genetic exchange among populations, although there may be enough to prevent complete isolation among them.

Therefore, a few reproductive plants in each population may disproportionately affect the level of genetic isolation among the populations. Preliminary results appear to confirm this, where in the central Veracruz population, only 6.5% (i.e. 21 plants) of the total population (321 plants) presented cones (15 females and six males). Also, a low recruitment of seedlings occurs in this population. A Lefkovitch projection matrix detected a minor finite growth rate ($\lambda = 0.78 \pm 0.08$), which suggests that this population of *Z. loddigesii* decreases at a rate of 22% per generation (Aguirre-Fey, 2004). Moreover, if the model proposed by Slatkin & Barton (1989) for computing the effective neighbourhood size ($Nb = 4\pi Nm$) is considered, the mean effective population size for *Z. loddigesii* is $Nb = 20 \pm 15$ (range 9–57). This is indicative of a low number of reproductive individuals in all populations. Those results support the idea that only a few individuals reproduce, yielding a low effective population size. Similar results have been reported for *Z. amblyphyllidia* by Negrón-Ortiz, Gorchov & Breckon (1996) and for *Encephalartos villosus* and *E. cycadifolius* by Raimondo & Donaldson (2003).

Conservation implications

Because of destruction and fragmentation of their habitats, many plant species have been forced into small and isolated populations. These populations generally face appreciable risk from the affects of environmental variation, demographic stochasticity, and reduced genetic diversity (Lande, 1988, 1999; Gray, 1996). This also appears to be the case for *Z. loddigesii*, where the loss and fragmentation of habitat affected both demographic and genetic traits.

Lande (1988) and others (e.g. Dobson *et al*., 1992; Caro & Laurenson, 1994) have argued that inbreeding plays a minor role in extinctions, because demographic and environmental stochasticity, as well as catastrophes, will drive small populations to extinction before genetic factors become important. Our data and results indicate that the sizes of *Z. loddigesii* populations have become reduced giving rise to subsequent isolation, and as a consequence, the loss of alleles. Thus, the main short-term threat this species faces is anthropogenic habitat loss (Lande, 1999).

The fragmentation and its effects on the genetic variation of the populations of *Z. loddigesii* along the Gulf of Mexico are probably quite recent and anthropogenic. The importance of the establishment of sanctuaries and protected areas and a reduction in deforestation is highlighted in this research as a way of preserving genetic variation of this and other endemic species.

ACKNOWLEDGEMENTS

The research was supported by grant CONACyT-SEMARNAT no. 2002-C01-0183. We thank Daniel Piñero, Mikael Hedrén, Isabel Mateu-Andrés, Vania Jiménez-Lobato, and one anonymous reviewer for their critical review of this manuscript. Our sincere gratitude is expressed to Julia Hernández Villa for technical assistance in the laboratory. This study is part of the bachelor dissertation of Daniel Aguirre-Fey and was supported by a thesis grant by the Instituto de Ecología, A. C.

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APPENDIX 1

SUMMARY OF GENETIC DIVERSITY AND STRUCTURE IN 16 TROPICAL TREE SPECIES

Species	*N*	*L*	*A*	*P*	H_E	F_{st}	Reference
Astrocaryum mexicanum	4	11	1.36	31.8	0.153	0.040	Eguiarte, Pérez-Nasser & Piñero (1992)
Pentaclethra macroloba	7	14	1.06	21	0.044	0.038	Hall, Chase & Bawa (1994)
Cordia alliodora	11	8	1.72	44	0.143	0.117	Chase, Boshire & Bawa (1995); Boshier, Chase & Bawa (1995a, b)
Ocotea tenera	6	18	1.54	44.4	0.225	0.128	Gibson & Wheelwright (1995)
Cecropia obtusifolia	8	5	1.30	27.1	0.050	0.034	Alvarez-Bullya & Garay (1994); Garay & Alvarez-Bullya (1997)
Pithecellobium elegans	8	6	1.24	35	0.130	0.101	Hall, Walker & Bawa (1996)
Calliandra calothyrsus	17	23	1.22	20.5	0.075	0.802	Chamberlain (1998)
Tachigali versicolor	6	13	1.33	29.6	0.073	0.069	Loveless, Hamrick & Foster (1998)
Carpentaria acuminata	7	14	1.60	41	0.143	0.379	Shapcott (1998)
Shorea leprosula	9	8	2.60	100	0.369	0.101	Lee *et al.* (2000)
Rhizophora mangle	14	6	1.50	38.1	0.125	0.287	Núñez-Farfán *et al.* (2002)
Intsia palembanica	6	14	2.40	55.9	0.242	0.048	Lee *et al.* (2002)
Antirhea aromatica	3	15	1.76	51.1	0.185	0.512	González-Astorga & Castillo-Campos (2004)
Hymenaea courbaril	9	11	1.39	32.5	0.101	0.079	Dunphy, Hamrick & Schwagerl (2004)
Phoenix canariensis	9	18	1.59	41.8	0.158	0.249	González-Pérez, Cuajápe-Castells & Sosa (2004)
Phoenix dactylifera	4	18	1.95	60.1	0.277	0.205	González-Pérez *et al.* (2004)
Mean (± standard deviation)	8 (3.7)	12.6 (5.2)	1.60 (0.42)	42.1 (19.2)	0.156 (0.09)	0.199 (0.21)	

N, number of populations; *L*, number of loci; *A*, mean alleles per locus; *P*, percentage of polymorphic loci; H_E, expected heterocigosis; F_{st}, genetic differentiation among populations.

APPENDIX 2

SUMMARY OF GENETIC DIVERSITY AND STRUCTURE OF 13 CYCAD SPECIES

Species	*N*	*L*	*A*	*P*	H_E	F_{st}	Reference
Macrozamia communis	5	18	1.61	50.0	0.045	0.270	Ellstrand, Ornduff & Clegg (1990)
Macrozamia riedlei	15	14	2.43	93.0	0.274	0.092	Byrne & James (1991)
Cycas pectinata	11	17	1.82	58.5	0.076	0.387	Yang & Meerow (1996)
Cycas siamensis	13	17	1.48	58.9	0.134	0.291	Yang & Meerow (1996)
Macrozamia parcifolia	2	17	1.20	17.6	0.037	0.090	Sharma *et al.* (1998)
Macrozamia pauli-guilielmi	3	17	1.30	31.3	0.081	0.030	Sharma *et al.* (1998)
Cycas panzhihuaensis	3	5	1.13	14.3	0.061	0.139	Chao-Luan *et al.* (1999)
Macrozamia heteromera	5	16	1.30	26.0	0.077	0.100	Sharma *et al.* (1999)
Cycas guizhouensis	3	17	1.61	58.3	0.100	0.080	Yang & Meerow (1999)
Cycas taitungensis	2	19	1.07	2.5	0.013	0.034	Lin *et al.* (2000)
Cycas seemannii	5	20	1.20	21.3	0.057	0.594	Keppel (2002); Keppel, Lee & Hodgskiss (2002)
Dioon edule	8	14	1.44	54.8	0.240	0.075	González-Astorga *et al.* (2003)
Macrozamia plurinervia	9	17	1.50	36.6	0.111	0.588	Sharma, Jones & Foster (2004)
Mean (± standard deviation)	6.5 (4.4)	16 (3.7)	1.47 (0.36)	40.2 (24.8)	0.100 (0.08)	0.213 (0.20)	

N, number of populations; *L*, number of loci; *A*, mean alleles per locus; *P*, percentage of polymorphic loci; H_E, expected heterocigosis; F_{st}, genetic differentiation among populations.