

Population genetics of *Dioon edule* Lindl. (Zamiaceae, Cycadales): biogeographical and evolutionary implications

JORGE GONZÁLEZ-ASTORGA, ANDREW P. VOVIDES*, MIRIAM M. FERRER and CARLOS IGLESIAS

Laboratorio de Genética de Poblaciones, Jardín Botánico Clavijero., Instituto de Ecología, A. C., km 2.5 Antigua Carretera a Coatepec no. 351, Xalapa 91070, Veracruz, Mexico

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Dioon edule Lindl. (Zamiaceae) is a cycad endemic to Mexico, that occurs as one species *D. edule* and the geographical variety *D. edule* var. *angustifolium* (Miq.) Miq. *Dioon edule* has a north to south distribution in eastern Mexico. In this study, we analysed 14 allozymic loci in eight populations of *D. edule* from its total distribution range by sampling all known populations. Patterns of diversity and genetic variability, within and among populations, were obtained. The mean number of alleles per locus (A) was 1.44 and the percentage of polymorphic loci was relatively high ($P = 54.78$). The mean observed (H_o) and expected heterozygosity (H_E) were 0.27 and 0.24, respectively. F -statistics revealed an excess of heterozygous genotypes, locally and globally ($F = -0.17$ and $f = -0.27$, respectively). The genetic variation explained by differences among populations was only 7.5%. We also detected a negative relationship between genetic diversity and latitude. On average, the gene flow between population pairs was relatively high ($Nm = 2.98$); furthermore, gene flow between population pairs was significantly correlated with geographical distances ($r = -0.38$, $P = 0.025$). Therefore, patterns of genetic diversity in *D. edule* appear to be associated with the post-Pleistocene spread of the species, from its southerly (origin) to its northerly range (derived populations, including its central distribution). The biogeographical and evolutionary aspects of the results of this study are discussed. We recognize *Dioon angustifolium* Miq. for the northernmost disjunct populations. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, 80, 457–467

ADDITIONAL KEYWORDS: allozymic electrophoresis – cycads – diversity and genetic structure – floristic refugia – gene flow – Pleistocene.

INTRODUCTION

The joint study of the genetic structure and phylogeography of extant populations provides information to infer different aspects of evolution. In particular, different biogeographical scenarios can be tested on patterns of genetic differentiation among populations in space and time (Hewitt, 1996; Avise, 2000; Irwin, 2002).

It is known that the floristic richness of Mexico is largely due to a variety of physiographic and climatic conditions, which has resulted in a mixture of Neotropical and Holarctic elements. These have been greatly influenced by important climatic changes during the

Quaternary, especially during the Pleistocene glaciations (Rzedowski, 1978). Graham (1976), using palynological analysis, maintained that the modern tropical rain forest of Mexico is recent. The north–south orientation of the principal mountain chains of Mexico, the Sierra Madre Occidental (OCC) and Sierra Madre Oriental (ORI) did not present significant barriers to the southward migration of biota during the glaciations (Fig. 1). The ORI (*D. edule* habitats) is particularly interesting from this point of view, especially the eastern part of the south–north lying mountains of southern Nuevo León, western Tamaulipas and northern San Luis Potosí, which are rich in endemic species (Marshall & Liebherr, 2000). It is believed that the ORI was submerged longer than the OCC and the region eventually lifted above sea level during the Cretaceous. The only important

*Corresponding author. E-mail: vovidesa@ecologia.edu.mx

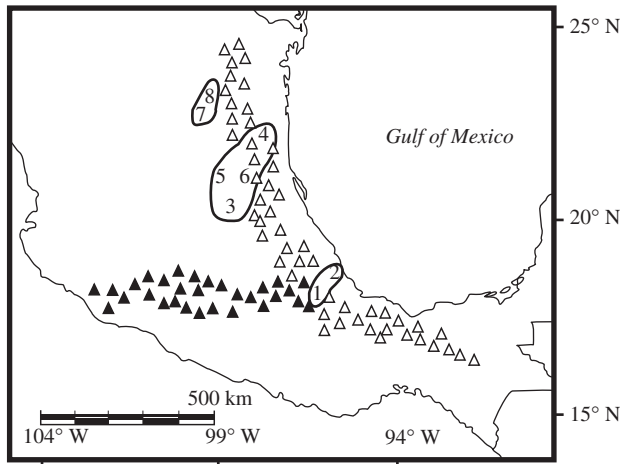


Figure 1. Geographical distribution of *Dioon edule* in Mexico. The open triangles indicate the Sierra Madre Oriental (ORI), and black triangles the Trans Mexican Neovolcanic Mountain Range. The numbers show the geographical position of each population evaluated. (1) Monte Oscuro, Veracruz (2) Farallón, Veracruz (3) Mazacintla, Querétaro (4) Vado del Morro, Tamaulipas (5) El Rascón, San Luis Potosí (6) Tamuin, San Luis Potosí (7) Alamos, Nuevo León, and (8) Iturbide-Linares, Nuevo León.

orographic barrier is the east–west Trans-Mexican volcanic mountain range of more recent Pliocene-Quaternary age (Ferrusquía-Villafranca, 1993). Important floristic refuges are found to the south and south-east of the Trans-Mexican volcanic mountain range (Prance, 1982; Toledo, 1982).

The facts that Mexico lies on geographical tropic extremes, and has a varied topography suggest that climatic stress can be responsible for the development of interesting genotypes and ecotypes within species encouraged by these conditions (Vovides & Gómez-Pompa, 1977).

Genetic diversity maintained within a species is a result of both historical events and recent evolutionary processes. In this context, the genetic structure in natural populations reflects the interactions of different processes such as; natural selection, genetic drift, inbreeding, mating system, mutation, and gene flow (Wright, 1969; Lewontin, 1974; Slatkin, 1987). Those factors can lead to complex genetic structuring at the population level, which is often difficult to resolve. Nevertheless, the use of genetic markers, such as allozyme electrophoresis, provides tools for the investigation of genetic variation between and within populations, and can facilitate our understanding of such complexities (Lewontin & Hubby, 1966; Mitton, 1994). Distribution range is one of the major factors correlated with the genetic variability of plant populations (Hamrick & Godt, 1996a, 1996b; Savolainen & Kuitien, 2000).

Theory predicts that, in small populations, genetic drift and inbreeding can lead to loss of genetic variation (Wright, 1931, 1978). Nevertheless, high gene diversity has been reported for the following rare or endangered plant species: the Hawaiian fern *Adenophorus periens* (Ranker, 1994); the endangered *Caesalpinia echinata* (Cardoso *et al.*, 1998); the narrow endemic fern *Polystichum otomasui* (Maki & Asada, 1998); two endangered pinyon pine species (Delgado *et al.*, 1999) and (Ledig *et al.*, 1999); the endemic *Agave victoriae-reginae* (Martínez-Palacios, Eguiarte & Furnier, 1999); three endemics from Florida (*Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohllingeriae*; Dolan *et al.*, 1999); the endemic annual of Florida, *Warea carteri* (Evans *et al.*, 2000); the endemics *Iris cristata* and *I. lacustris* (Hannan & Orick, 2000); and the monoecious endemic shrub *Brongniartia vazquezii* of the tropical dry forests of Central Mexico (González-Astorga & Núñez-Farfán, 2001).

In other species within the Zamiaceae, reports indicate that diversity and genetic variability are relatively low (e.g. *Macrozamia communis*, Ellstrand, Ornduff & Clegg, 1990; *Zamia pumila*, Walters & Decker-Walters, 1991; *Macrozamia pauli-guilielmi*, Sharma *et al.*, 1998; *M. heteromera*, Sharma *et al.*, 1999). The results for these species correspond to plants with restricted geographical range and relatively low population sizes (Hamrick & Godt, 1996a). Our conclusion appears to be similar to that of Yang & Meerow (1996), for the Asian *Cycas pectinata* complex.

Dioon edule is a long-lived arborescent cycad with trunks up to five metres tall. The plants produce cones all year, which mature from August to December, and are found in ecotone zones between tropical deciduous forest and groves of evergreen oaks, either overhanging or growing on steep slopes, cliffs, in gullies or on less-steep hillsides (Vovides, Rees, Vázquez-Torres, 1983). This species is distributed along the Sierra Madre Oriental, from Nuevo León with disjunct populations in Tamaulipas represented by *D. edule* var. *angustifolium* (Miq.) Miq. and from southern Tamaulipas through San Luis Potosí, Hidalgo Querétaro to Veracruz with *D. edule* (De Luca, Sabato & Vázquez Torres, 1982; González-Astorga, Vovides & Iglesias, 2003) (Fig. 1). The conservation status of populations of this species is rare (R) according to the IUCN (1998) category. It is also enlisted as a protected species and is covered, along with other Mexican cycads by the Official Mexican Norm NOM-059-ECOL-1994–2000, which covers wild species of threatened, rare and endangered flora and fauna that require special protection. Cycads are also considered a priority by the Mexican conservation authorities for conservation and study (INE-SEMARNAP, 2000).

Table 1. Allelic frequencies of 14 allozyme loci of eight populations of *Dioon edule* in Mexico

Locus	Allele	Populations							
		1 Monte Oscuro (Ver.)	2 Farallón (Ver.)	3 Mazacintla (Qro.)	4 Vado del Morro (Tam.)	5 El Rascón (SLP)	6 Tamuin (SLP)	7 Alamos (NL)	8 Iturbide- Linares (NL)
<i>Mdh 1</i>	A	0.8852	0.9000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	B	0.1148	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Mdh 2</i>	A	0.2181	0.1875	0.0761	0.0000	0.2581	0.0000	0.3472	0.0000
	B	0.7819	0.8125	0.9239	1.0000	0.7419	1.0000	0.6528	1.0000
<i>Pgi 1</i>	A	0.3475	0.3667	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	B	0.6525	0.6333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Pgi 2</i>	A	0.2976	0.3056	0.0000	0.0256	0.0000	0.0000	0.0000	0.0000
	B	0.7024	0.6944	1.0000	0.9744	1.0000	1.0000	1.0000	1.0000
<i>6Pgd</i>	A	0.5548	0.3667	0.4714	0.6034	0.0000	0.4483	0.4730	0.6970
	B	0.4452	0.6333	0.5286	0.3966	0.0000	0.5517	0.5270	0.3030
<i>Dia 1</i>	A	0.3795	0.4375	0.0641	0.5000	0.2745	0.1316	0.2805	0.3718
	B	0.2591	0.1563	0.4615	0.1667	0.2255	0.4342	0.3171	0.2949
	C	0.3614	0.4062	0.4744	0.3333	0.5000	0.4342	0.4024	0.3333
<i>Dia 2</i>	A	0.5000	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	B	0.5000	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Idh</i>	A	0.1200	0.0556	0.1471	0.3888	0.1521	0.0898	0.1277	0.1000
	B	0.8500	0.9444	0.8137	0.5556	0.7609	0.8333	0.6595	0.8714
	C	0.0300	0.0000	0.0392	0.5556	0.0870	0.0769	0.2128	0.0284
<i>Est</i>	A	0.5991	0.5000	0.3953	0.6389	0.5303	0.7262	0.1463	0.2400
	B	0.4009	0.5000	0.6047	0.3611	0.4697	0.2738	0.8537	0.7600
<i>Lap</i>	A	0.2396	0.5769	0.1600	0.2258	0.5326	0.6571	0.6000	0.6618
	B	0.7604	0.4231	0.8400	0.7742	0.4674	0.3429	0.4000	0.3382
<i>Apx 1</i>	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Apx 2</i>	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Apx 3</i>	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Apx 4</i>	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
No. of alleles per locus (mean ± S.D)		1.86 ± 0.66	1.79 ± 0.58	1.36 ± 1.01	1.36 ± 1.01	1.21 ± 1.05	1.29 ± 0.99	1.36 ± 1.01	1.29 ± 0.99

We used allozyme loci to examine the population genetics of *D. edule*. Our objectives were to: (1) determine the amount and distribution of genetic diversity within and among populations; (2) identify the patterns of variation between geographical regions occupied by the species (from south to north), especially in the context of post-glacial migration (post Pleistocene) and establishment patterns in the present; and (3) compare the results and conclusions for *D. edule* with the population genetics of other cycad species within the Zamiaceae.

MATERIAL AND METHODS

SAMPLE COLLECTION

Between 20 and 100 individual plants per population were randomly sampled (Table 2). Tissue sampling was carried out in eight populations along the distribution range of *D. edule* in eastern Mexico. Populations are separated by distances that range from *c.* 50 to 950 km (Table 1 and Fig. 1). The collected leaves were transported in containers with ice, to avoid denaturation of the proteins, then stored in a freezer at -70°C until the moment of extraction.

ELECTROPHORESIS

Approximately 250 mg of fresh leaflet tissue was ground with liquid nitrogen. About 250 µL of extraction buffer (0.1 M Tris-HCl pH 7.5, 4% PVP-40, 0.001 M EDTA, 0.01 M CaCl₂, 0.01 M MgCl₂, 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 individuals from each population were obtained through horizontal starch gel electrophoresis (10% w/v). For each individual plant allozymic variation was scored for 14 loci: malate-dehydrogenase (E.C. 1.1.1.37, *Mdh1* and *Mdh2*), phosphoglucose isomerase (E.C. 5.3.1.9, *Pgi1* and *Pgi2*), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44, *6Pgd*), diaphorase (E.C. 1.6.99.-, *Dia1* and *Dia2*), isocitrate dehydrogenase (E.C. 1.1.1.41, *Idh*), Esterase (E.C. 3.1.1, *Est*), leucine aminopeptidase (E.C. 3.4.11.1, *Lap*) and peroxidase anodic (E.C. 1.11.1.7, *Apx1*, *Apx2*, *Apx3* and *Apx4*) (Wendel & Weeden, 1989). Two gel/electrode buffers systems were used: (1) the system R (Chao-Luan *et al.*, 1999), for *Mdh*, *Pgi*, *6Pgd*, *Dia* and *Idh*; and (2) the system 9 (Soltis *et al.*, 1983) for *Est* and *Apx*. Electrophoresis was carried on at 4°C for 8 h (constant current of 50 mA, and voltage of 80 V).

STATISTICAL METHODS

The bands or alleles were inferred from the banding patterns and genotypes observed. The *TFFPGA* 1.3 package (Miller, 1997) was used to estimate the genetic variation. Allele frequencies were obtained 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 percentage of polymorphic loci (P), and the expected mean heterozygosity with respect to Hardy-Weinberg (H_E ; Hartl & Clark, 1997; Hedrick, 2000). We used χ^2 to test for deviations from genotypic frequencies expected under Hardy-Weinberg equilibrium (Snedecor & Cochran, 1967) and for heterogeneity of allelic frequencies among populations (Workman & Niswander, 1970).

Partitioning of genetic variation was done with F statistics (Wright, 1965, 1978) that were estimated according to the equations of Weir & Cockerham (1984). These measure genetic structure by partitioning variation in the same way as a regular analysis of variance. The θ statistic measures the divergence in allele frequencies among populations, whereas f and F measure heterozygote excess (<0) and deficit (>0) relative to the Hardy-Weinberg expectation 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 θ statistic per locus, the chi-square statistic was used: $\chi^2 = (2N)\theta(k-1)$, with $(k-1)(s-1)$ degrees of freedom, where s is the number of populations (Workman & Niswander, 1970). The confidence intervals (at 95%) of the F -statistics were obtained by bootstrapping over loci for the multilocus estimate, and jack-knifing over populations for the single-locus estimates (Weir & 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 = the number of analysed populations (Crow & Aoki, 1984). Nm is interpreted as the number of migrants per generation between two given populations (Slatkin, 1993, 1994). The significance of this relationship cannot be evaluated using the typical statistical methods because values of Nm from different pairs of populations are not independent (Smouse, Long & Sokal, 1986), so we used Mantel's test (Mantel, 1967) to overcome this limitation. Finally, phenetic clustering of the populations was performed using Nei's (1972) genetic distances and UPGMA (Sneath & Sokal, 1973) algorithms as implemented in *TFFPGA* 1.3 package (Miller, 1997).

We performed regression models (Sokal & Rohlf, 1995) for the latitude and genetic diversity estimates (A , P and H_o) to explore the relationship between these variables.

RESULTS

GENETIC DIVERSITY

Allelic frequencies for the 14 loci (*6Pgd*, *Idh*, *Est* and *Lap*, one locus each, *Mdh*, *Pgi* and *Dia*, two loci, and *Apx*, four loci) were scored for each population. Ten of the 14 putative loci were polymorphic in at least one population. *Apx* loci (i.e. *Apx1*, *Apx2*, *Apx3* and *Apx4*) were monomorphic in all populations. Three loci (*Mdh1*, *Pgi1* and *Dia2*) were detected only in southern populations (1 and 2, both in Veracruz State; see Fig. 1), and not in northern and central populations. The *Pgi2* locus has the B allele fixed in all northern populations, except population 4, and the *Mdh2* locus has the B allele fixed in three out of the six northern populations (Table 1). The average number of alleles per locus was 1.44 ± 0.19 . The percentage of polymorphic loci per population varied from 45.45% (populations 4, 6 and 8) to 71.43% (populations 1 and 2), with an average of $54.78\% \pm 10.94$ (Table 2). Observed mean heterozygosity was 0.273 ± 0.097 (range 0.202–0.450) and expected mean heterozygosity was 0.239 ± 0.039 (range 0.193–0.298) (Table 2).

Table 2. Geographic location and genetic variation for eight populations of *Dioon edule* in Mexico. N_i : average sample size; P : percentage of polymorphic loci; H_O and H_E : observed and expected mean heterozygosity, respectively. Deviations from Hardy–Weinberg equilibrium test ($P < 0.05$), deficiency (–) and excess of heterozygotes (+)

Population	Geographical position	N_i	P	H_O	H_E	H–W deviations		
						Test	–	+
(1) Monte Oscuro (Ver.)	19°19'27" N 96°44'46" W 485 m a.s.l.	84.5	71.43	0.4073	0.2982	10	–	5
(2) Farallón (Ver.)	19°36'49" N 96°22'40" W 6 m a.s.l.	15.0	71.43	0.4502	0.2893	10	–	3
(3) Mazacintla (Qro.)	21°10'21" N 99°19'34" W 1100 m a.s.l.	45.5	54.54	0.2079	0.2053	6	4	–
(4) Vado del Morro (Tam.)	23°34'32" N 98°35'38" W 282 m a.s.l.	29.8	45.45	0.2283	0.2262	4	1	–
(5) El Rascón (SLP)	21°55'27" N 99°14'07" W 300 m a.s.l.	42.2	50.00	0.2404	0.2393	5	2	–
(6) Tamuin (SLP)	21°58'53" N 98°55'02" W 216 m a.s.l.	39.2	45.45	0.2321	0.2036	5	2	1
(7) Alamos (NL)	24°41'09" N 99°41'32" W 560 m a.s.l.	40.4	54.54	0.2021	0.2585	6	4	–
(8) Iturbide-Linares (NL)	24°44'55" N 99°48'29" W 815 m a.s.l.	35.6	45.45	0.2191	0.1935	6	4	–
Mean ± SD		41.52 ± 19.80	54.78 ± 10.94	0.2734 ± 0.0973	0.2392 ± 0.0397			

TEST FOR HARDY–WEINBERG EQUILIBRIUM

Of the 52 valid tests for deviation from genotypic frequencies expected under the Hardy–Weinberg equilibrium, 17 indicated a significant deficiency of heterozygotes and nine indicated significant excess. The excess corresponds mostly to southern populations, and deficiency occurs in the populations from the central and northern areas of the *D. edule* geographical distribution (Table 2, Fig. 1).

GENETIC DIVERSITY AND LATITUDE

Regression models for the latitude and genetic diversity estimates (allele number, polymorphism and observed heterozygosity) were significant and explained up to 50% of the variance in all cases. The negative β coefficients indicate that genetic diversity decreases as latitude increases (Table 3).

Table 3. Coefficient of lineal regression ($\beta \pm E.E.$) and determination coefficient (R^2), between diversity genetic parameters on latitude in populations of *Dioon edule* in Mexico

Indexes of genetic diversity	$\beta \pm E.E.$	R^2	P
Alleles per locus	-0.086 ± 0.033	0.523	0.042
Percentage of polymorphic loci	-4.081 ± 1.401	0.585	0.027
Observed heterozygosity	-0.037 ± 0.011	0.637	0.017

GENETIC STRUCTURE

Wright's F -statistics, F and f , were negative and significantly different from zero for ten loci ($P < 0.05$ and < 0.01), except for the four loci of *Apx*, indicating an

Table 4. Wright's F -statistics for eight populations of *Dioon edule* in Mexico

Loci	F	θ	f
<i>Mdh 1</i>	-0.1742*	0.0804*	-0.2768*
<i>Mdh 2</i>	-0.1895**	0.0728**	-0.2829**
<i>Pgi 1</i>	-0.1258*	0.0862*	-0.2320*
<i>Pgi 2</i>	-0.1701**	0.0692**	-0.2571**
<i>6Pgd</i>	-0.1854**	0.0833*	-0.2932**
<i>Dia 1</i>	-0.0998**	0.0797*	-0.1951**
<i>Dia 2</i>	-0.0501*	0.0860*	-0.1489*
<i>Idh</i>	-0.2346**	0.0685**	-0.3253**
<i>Est</i>	-0.2076**	0.0660*	-0.2929**
<i>Lap</i>	-0.2143**	0.0637**	-0.2970**
<i>Apx 1</i>	-0.1658	0.0755	-0.2611
<i>Apx 2</i>	-0.1658	0.0755	-0.2611
<i>Apx 3</i>	-0.1658	0.0755	-0.2611
<i>Apx 4</i>	-0.1658	0.0755	-0.2611
Mean \pm S.D.	-0.1724* \pm 0.1647	0.0749* \pm 0.0246	-0.2700* \pm 0.1553
95% CI	-0.4624–0.1345	0.0292–0.1223	-0.5311–0.0292

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

excess of heterozygotes (Tables 1, 4). All loci showed θ estimates that were positive and significantly different from zero ($P < 0.05$ and < 0.01), except for the loci *Apx1* to *Apx 4*. For all loci, the average θ was 0.0749 ± 0.025 (95% CI: 0.03–0.122). Approximately 7.5% of the genetic diversity within *D. edule* was distributed among populations (Table 4).

GENETIC DISTANCE

The mean genetic distance (Nei, 1972) among populations of *D. edule* was 0.040 ± 0.015 . The UPGMA cluster analysis based on the genetic distances showed the populations grouping geographically from south to north latitude (Fig. 2).

GENE FLOW

Average Nm or migrants per generation was 2.98 ± 1.50 between pairs of populations (Table 5). The lowest estimate was obtained between populations 6 and 7 ($Nm = 1.01$) separated by *c.* 418 km, and the highest estimate was found between populations 1 and 2 ($Nm = 8.0$) separated by *c.* 58 km. Correlation between Nm matrixes and the geographical distance between pairs of populations indicated that genetic differentiation had occurred as expected under the isolation by distance model, as demonstrated by Mantel's test ($r = -0.38$; $P = 0.025$; Table 5). Also, the relationship between gene flow and geographical distances between pairs of populations was negative and statis-

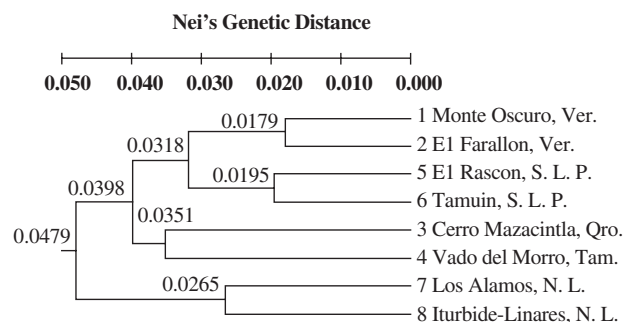


Figure 2. UPGMA phenogram based on Nei's genetic distances between eight populations of *Dioon edule* estimated from 14 loci (see Fig. 1).

tically significant ($\beta = -0.232$; $P = 0.05$; $R^2 = 37.2\%$) (Fig. 3).

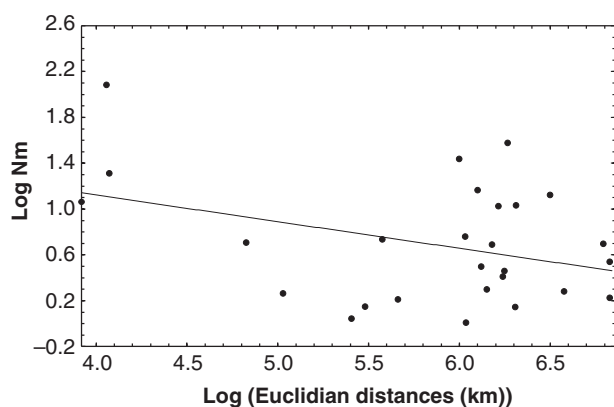
DISCUSSION

As a long lived, dioecious plant, *D. edule* should have relatively high levels of diversity and genetic variation, compared with other cycads within the Zamiaceae: such as *Macrozamia communis* (Ellstrand *et al.*, 1990); *Zamia pumila* (Walters & Decker-Walters, 1991); *M. parcifolia*, *M. pauli-guilielmi*, *M. crassifolia* (Sharma *et al.*, 1998) and *M. heteromera* (Sharma *et al.*, 1999).

If we compare the results of *D. edule* with other plant species, the percentage of polymorphic loci is

Table 5. Number of individual migrants per generation (Nm) (above diagonal) and geographical distances (below diagonal) between pairs of populations of *Dioon edule* in Mexico

	1	2	3	4	5	6	7	8
	Monte Oscuro (Ver.)	Farallón (Ver.)	Maza- cintla (Qro.)	Vado del Morro (Tam.)	El Rascón (SLP)	Tamuin (SLP)	Alamos (NL)	Iturbide- Linares (NL)
1 Monte Oscuro (Ver.)		8.00	2.77	3.07	2.80	1.58	1.25	1.32
2 Farallón (Ver.)	57.7		1.35	1.32	4.84	1.99	2.00	1.71
3 Mazacintla (Qro.)	500.6	469.5		2.13	2.02	1.30	1.51	1.16
4 Vado del Morro (Tam.)	664.3	717.5	416.4		2.10	1.24	1.04	1.16
5 El Rascón (SLP)	550.5	525.7	124.5	263.9		3.72	4.21	3.20
6 Tamuin (SLP)	517.4	482.4	152.6	287.9	58.6		1.01	1.64
7 Alamos (NL)	923.1	891.4	512.2	222.7	403.4	418.1		2.89
8 Iturbide-Linares (NL)	949.3	923.1	548.8	240.0	446.2	454.8	50.4	

**Figure 3.** Linear regression between individual migrants per generation (Nm) and Euclidian distances (d) among pairs of populations (both transformed to natural logarithm), for eight populations of *Dioon edule*. ($\log Nm = 2.05 - 0.232 \log d$, $P = 0.05$; $R^2 = 37.2\%$).

higher (54.8%) than that reported for other long-lived narrow endemic perennials (40–45.1%, Hamrick & Godt, 1996a). The mean expected heterozygosity within populations of *D. edule* is also higher (0.24), than that reported for other regionally distributed tropical long-lived trees, and even higher than in temperate long-lived coniferous trees (0.125 and 0.145, respectively; Hamrick, Schnabel & Wells, 1994). Heterozygosity is also higher than in other long-lived perennial endemics (0.105; Hamrick & Godt, 1996a). This shows that *D. edule* has higher genetic variation, when compared to the aforementioned groups.

Our study represents the second allozyme survey for a cycad species, where genetic variability indicated an excess of heterozygous genotypes, locally and globally ($F = -0.172$, and $f = -0.270$) (cf. Lin *et al.*, 2000;

Cycas taitungensis). Dioecy favours a Hardy–Weinberg equilibrium resulting in low positive or zero fixation indices (Hedrick, 2000). We suggest that in *D. edule* the negative values are probably due to natural selection that favours a higher percentage of heterozygous individuals that can adapt to environmental changes. Negative values of those indices have also been described for other plant species, mainly long-lived perennials (*Pinus ponderosa*: Linhart *et al.*, 1981; *Carduus mutans*: Smyth & Hamrick, 1984; *Eucalyptus rhodantha*: Sampson, Hopper & James, 1989; *Astrocaryum mexicanum*: Eguiarte, Perez-Nasser & Piñero, 1992; *Cecropia obtusifolia*: Alvarez-Buylla *et al.*, 1996).

In *D. edule* only 7.5% of the variation accounts for the differences among populations. This can be explained by the species life history traits which include dioecy, pollination by specific insects *Rhopalotria* spp. and *Pharaxonotha* spp. (Tang, 1987; Norstog & Fawcett, 1989; Vovides, 1991), the long life cycles of *D. edule* (Vovides & Peters, 1987; Norstog, 1987; Vovides, 1990; Vovides, 1991; Fawcett, Norstog & Vovides, 1995; Schneider *et al.*, 2002). These appear to promote homogeneity or hinder divergence among populations (Loveless & Hamrick, 1984; Eguiarte *et al.*, 1992; Hamrick & Godt, 1996b).

We propose that the southern populations of the species of Veracruz are precursory, based on having the most genetic variation. These correspond to the base of the phenogram with respect to the loci *Mdh 1*, *Pgi 1* and *Dia 2*, which are exclusive to the Veracruz populations (Table 1, Fig. 2). The northern populations are believed to be derived because there is less genetic diversity than expected relative to the southern populations. The occurrence of high species diversity in the genus *Dioon* in the northern region of Oaxaca state supports this proposition. Oaxaca bor-

ders the state of Puebla, and south central Veracruz (Fig. 1) (cf. Vovides *et al.*, 1983; Moretti *et al.*, 1993; Gregory *et al.*, 2003; Vovides *et al.*, in press).

The history of the vegetation of southern Mexico has been studied by Lundell (1945) and Miranda (1957, 1959), and they suggest that the region contains relict floral elements of great age. The presence of very old tropical floristic and faunistic refuges (40 000 years) has been more recently postulated in southern Mexico (Toledo, 1982, 1988; Wendt, 1987). Refuges of this type are apparently absent from north-eastern Mexico (north of the Trans Mexican Neovolcanic Mountain range). Toledo (1982, 1988) identified three Pleistocene refuges: (i) the Los Tuxtlas region of southern Veracruz (ii) the Sierra de Juárez of Oaxaca and (iii) the Córdoba region in central Veracruz at the southern foothills of the Neovolcanic range. The latter is adjacent to populations 1 and 2 of *D. edule* distributed in central Veracruz (Fig. 1). This suggests that southern populations remained unaltered during the Pleistocene and could have maintained relatively high diversity and genetic variation. However, populations in the centre and northern areas of the cycads' distribution were probably influenced by climate stress during glaciation, drastically reducing population density and consequently reducing genetic diversity (Table 2). After the glaciers retreated at the end of the Pleistocene, migration probably occurred from southern populations to the central and northern range of *D. edule* (Tables 3, 5; cf. Hewitt, 1996). Our data on gene flow suggest that large numbers of historical alleles were shared and the actual gene flow occurs between geographically close populations. Similar trends have been reported for the Asian *Cycas pectinata* complex by Yang & Meerow (1996). In the southern populations of *D. edule* there is greater gene flow between adjacent populations than in those of the north, even though the geographical distances are similar (Table 5). One of the factors that can explain these results is a probable reduction in population size of the cycad pollinator in the northern populations of *D. edule* (cf. Vovides *et al.*, 1997). Another factor could be that, considering migration rates are constant throughout the whole range of the cycad, major allelic richness in the southern populations of *D. edule* probably causes a higher gene flow northwards.

The present distribution of genetic diversity within populations of *D. edule* appears to be correlated with factors such as life history and biogeographical history (cf. González-Astorga *et al.*, 2003). We envisage a scenario of southern immigration of warm tropical floras during the Pleistocene glaciations followed by a loss of biological diversity among northern populations of *D. edule* reducing their population sizes (and genetic variability) to a minimum through climate change. Therefore a bottleneck effect could induce genetic drift

and significant reduction in genetic diversity. Alternatively, recent colonization in the northern range and an associated founder effect could also explain this lower genetic variation. This colonization-and-spread scenario has also been reported for *Cycas seemanii* in the Vanuatu archipelago by Keppel, Lee & Hodgskiss (2002), possibly explained by recent stepwise migration from Australasia to the Pacific (Hill, 1996). Additionally, the northward migration of tropical floras, including *D. edule*, during the postglacial amelioration of climates occurred, and only genotypes adapted to these conditions are present today. Similar trends have been found in *Pinus flexilis* (Jørgensen, Hamrick & Wells, 2002), in *Lophocereus schottii* (Nason, Hamrick & Fleming, 2002), and in *Rhizophora mangle* (Núñez-Farán *et al.*, 2002). DNA sequencing data for the genus *Ceratozamia* showed a similar trend where the basal clades suggest a probable ancestral geographical area in southern Mexico with taxa associated to the aforementioned Pleistocene refuges. A single unresolved clade of *Ceratozamia* in north-eastern Mexico north of the Trans Mexican Neovolcanic Mountain range suggests a recent speciation process in the genus (González & Vovides, 2002).

In conclusion, our results indicate that the current pattern of genetic diversity throughout the geographical range of *D. edule* is complex. This can be attributed to the interaction of a multiplicity of factors such as the geographical distribution of Pleistocene and post Pleistocene populations, the influence of corresponding climatic changes, as well as high gene flow among neighbouring populations, in this long lived palaeoendemic plant species. Finally, based on the study of González-Astorga *et al.* (2003) and this study we recognize species status for the disjunct northern populations of *D. edule* var. *angustifolium* by referring to the basionym *Dioon angustifolium* Miq.

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