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The genetic structure of the tropical understory herb *Dieffenbachia seguine* L. before and after forest fragmentation

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ABSTRACT

Hypothesis: Theoretically, forest fragmentation is expected to reduce genetic diversity and increase population differentiation through isolation and reduction of habitat.

Assumption: Assessing the effects of fragmentation on the genetic structure makes it necessary to know the former structure in the undisturbed, continuous habitat.

Organism: *Dieffenbachia seguine* (Araceae), an understory herb of the primary tropical rain forests.

Field site: Los Tuxtlas Biosphere Reserve, Veracruz, Mexico.

Methods: Four populations of the continuous forest (in an area >700 ha) and six populations in remnant fragments of a different area were sampled. The variability of nine allozyme loci was surveyed for 35 plants in each population.

Conclusions: *F*-statistics indicated that populations of *D. seguine* are highly structured as a result of random processes ($F_{st} = 0.3052$) and local inbreeding ($F_{is} = 0.2905$) both in fragments and in the continuous forest. However, genotypic richness was significantly lower in populations of fragments than in the continuous forest, and positively related to the fragment area. The population in the smallest fragment showed the highest genetic distance from all other populations. This suggests an exacerbation of the random differentiation process in fragments.

Keywords: Araceae, clonality, *F*-statistics, genetic drift, genotypic richness, isozymes, Los Tuxtlas.

INTRODUCTION

Habitat fragmentation may lead to changes in genetic diversity and in population structure of a species by altering the magnitude and/or direction of gene flow, the potential for colonization, and by reducing effective population size (Young *et al.*, 1996; Lande, 1999; Hanski and Ovaskainen, 2000).

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Following subdivision by fragmentation, local populations could change their level of genetic differentiation depending on the rate of gene flow (Young *et al.*, 1996). If gene flow increases among fragmented populations, it may reduce or eliminate population differentiation, maintaining genetic diversity (Foré *et al.*, 1992; White *et al.*, 1999) and acting against drift or diversifying selection (Richards, 2000). In contrast, if gene flow is reduced by fragmentation, differentiation among populations may increase. A reduction in gene flow can occur through changes in the abundance and activity pattern of pollinator species (Murcia, 1996; Knutsen *et al.*, 2000). Reduced gene flow and small effective sizes of populations in fragments may lead to inbreeding and drift, and a subsequent erosion of genetic variation (Ellstrand and Elam, 1993; Young *et al.*, 1996; Rosquist and Prentice, 2000).

Loss of genetic diversity can compromise the persistence of local populations (Young *et al.*, 1996; Young and Clarke, 2000) by limiting their ability to respond adaptively to the varying local conditions (Rosquist and Prentice, 2000) and increasing the risk of extinction due to inbreeding depression (Ellstrand and Elam, 1993). Thus, knowledge about the impact of habitat fragmentation on genetic diversity and structure of populations has direct implications for the conservation and management of species, by offering insights into the evolutionary and ecological dynamics (Neel and Ellstrand, 2001).

Empirical studies of the genetic consequences of habitat fragmentation in plants have revealed that genetic variability tends to decrease in small populations, although fragmentation does not necessarily lead to genetic losses (Young *et al.*, 1996). These different responses to fragmentation are due to the variety and complexity of reproductive strategies (sexual and asexual) and mating system (outcrossing, selfing or mixed), combined with differences in population sizes, seed dispersal, and pollination systems (Young and Clarke, 2000). Moreover, the population genetic structure of a species before fragmentation is often not known (due to the lack of large areas of forest); in cases where genetic structure as produced by fragmentation can be identified, the former structure of populations preceding habitat change may be evident.

The effects of fragmentation on genetic variation and structure are well known for temperate herbs (e.g. van Treuren *et al.*, 1991; Raijman *et al.*, 1994; Young *et al.*, 1999; Rosquist and Prentice, 2000) and tropical tree species (Prober and Brown, 1994; Boshier *et al.*, 1995; Hall *et al.*, 1996; Aldricht *et al.*, 1998; Shapcott, 1998; White *et al.*, 1999). However, the genetic effects of fragmentation have not been evaluated in tropical herbs (Shapcott, 1998; Young *et al.*, 1999), despite understory species being the most diverse structural component in the tropical rain forests (Ibarra-Manríquez *et al.*, 1997). Moreover, the shorter life span of herb plant species makes it possible to evaluate the effect of fragmentation on the genetic structure of the derived generations compared with long-lived trees or shrub species.

The aim of the present study was to assess the impact of habitat fragmentation on the genetic diversity and structure of a tropical understory herb, in the Los Tuxtlas tropical rain forest. Given the effect of fragmentation on gene flow, we anticipated: (1) a reduction in genetic diversity, an increment in the genetic differentiation among populations, and increased inbreeding in populations of fragments compared with populations in the continuous forest; and (2) an inverse relationship between the size of the fragment and the magnitude of the genetic effect.

The Los Tuxtlas region has been heavily deforested over the last 30 years, producing a highly patchy landscape. However, the region constitutes an excellent system to test our hypothesis because there are still some large areas of continuous undisturbed forest (> 640 ha) and fragment remnants of different sizes (range 0.3–134 ha). The Los Tuxtlas is

an important area for preservation because it is the boreal limit of the tropical forests in America, and because it has a high species diversity of tropical and temperate origins and some endemics (Gonzáles *et al.*, 1997).

We used isozyme variation to analyse four populations within the continuous forest to determine the former genetic variation and structure of the populations in an undisturbed forest, and six populations in fragments of different sizes to assess the effect of fragmentation on genetic structure. The comparison of the genetic dynamics of populations in continuous forest in relation to fragments allows us to assess, without overestimating the results, changes in the genetic variation and structure in populations of this species due to habitat reduction and isolation.

METHODS

Species description

Dieffenbachia seguine is an understory clonal herb of neotropical rain forests. It ranges from southern Mexico to Guyana, Brazil, and Ecuador (Mayo *et al.*, 1997). This species forms large aggregations. New ramets arise from broken rhizomes. Each ramet produces one to four inflorescences in a flowering season, depending on the plant's size (Cuartas, 2002). The opening of a given inflorescence on a ramet does not coincide with another inflorescence of the same ramet. Female and male flowers are located at the base and the tip of the spadix, respectively. Female flowers are receptive before pollen is released – that is, they are protogynous. To our knowledge, no studies have been published regarding the reproductive biology and mating system of *D. seguine*. The genus *Dieffenbachia* is pollinated by scarab beetles of the genera *Cyclocephala* and *Erioscelis*. The reproductive biology of *Dieffenbachia* is detailed in Young (1988, 1990). Fruits are red when mature and dispersed by understory birds of the genera *Turdus* and *Habia* (R. Estrada-Coates, personal communication).

Study site and field collection

The study was conducted at the Los Tuxtlas Biosphere Reserve located in the state of Veracruz in the Gulf of Mexico (Fig. 1). In the Los Tuxtlas region, continuous tropical rain forest has been reduced to some isolated remnants due to the conversion of land into cattle grazing and sugar cane plantations (Gonzáles *et al.*, 1997). However, three large areas of undisturbed forest still remain (San Martín Volcano, San Martín Pajapan Volcano, and Sierra Santa Martha).

To asses the effect of geographic isolation on the genetic structure, ten populations of *D. seguine* from a wide geographic area were sampled within the Biosphere Reserve (Table 1, Fig. 1): four populations in the continuous forest within the Los Tuxtlas Tropical Biology Station (separated by a mean distance of 6.08 km from one another) and six populations from the few remaining remnant fragments with minimal disturbance. Four fragments are located in the vicinity of the Station (5.98 km in average), while two are located some distance from the Station (24.7 km on average) in the Sierra Santa Martha. Sampling was carried out in the centre of each fragment.

For genetic analyses, leaves of 35 plants on average were sampled in each population. Haphazardly, a plant was collected every 10 m to prevent the collection of two ramets of the same genet. Clonality was not specifically examined; however, some conclusions could be made from our results.

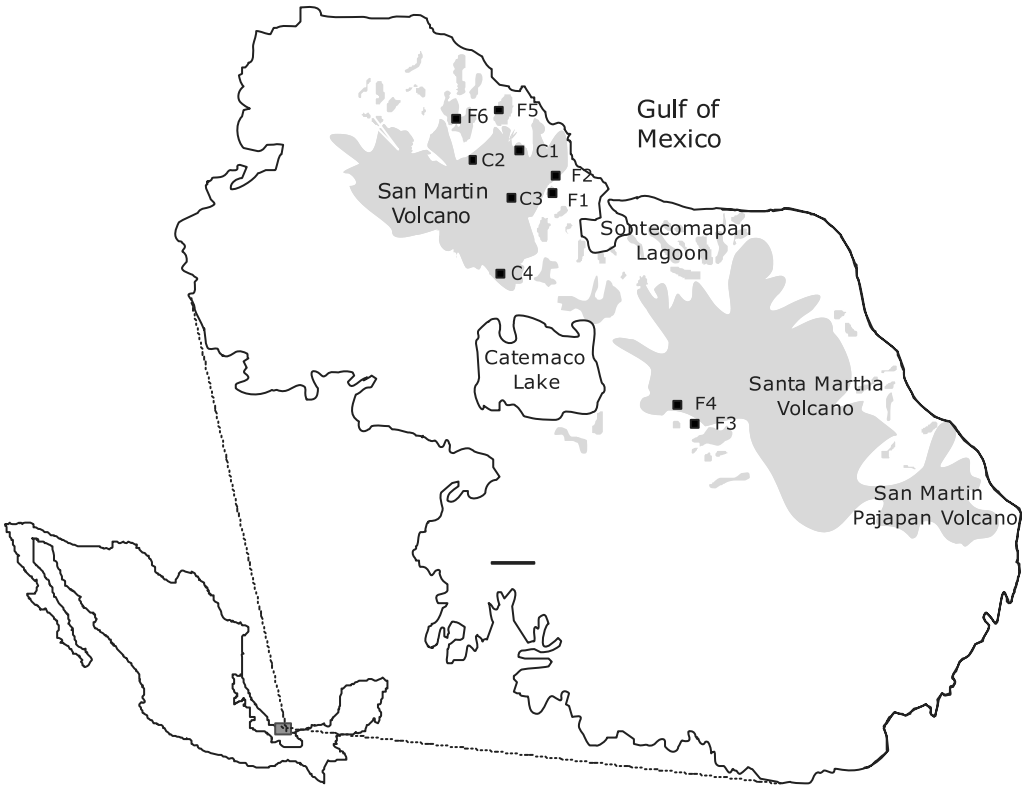

Fig. 1. Map of the Los Tuxtlas Biosphere Reserve in the State of Veracruz, Mexico. The populations of *Dieffenbachia seguine* sampled in the fragments (F) and continuous forest (C) are indicated. The stippled area highlights the area of remaining tropical forest. The horizontal line is equivalent to 4 km.

Table 1. Geographic position and area of *D. seguine* populations sampled in Los Tuxtlas, Mexico

Population	Symbol	*N*	Geographic position	Area (ha)
Estación	C1	40	18°35′240 N, 95°04′620 W	640
Laguna	C2	30	18°35′245 N, 95°05′912 W	640
Pedregal	C3	30	18°34′347 N, 95°07′776 W	640
San Martín	C4	39	18°31′357 N, 95°10′675 W	640
Cascada	F1	30	18°34′692 N, 95°03′973 W	0.3
Playa	F2	30	18°35′050 N, 95°02′600 W	3
Santa Martha 1	F3	40	18°32′676 N, 95°07′328 W	5
Santa Martha 2	F4	40	18°22′025 N, 94°57′781 W	20
Cerro	F5	30	18°37′739 N, 95°05′157 W	37.4
Ruíz Cortínez	F6	30	18°36′730 N, 95°05′821 W	114.6

Note: N is the number of sampled individuals.

Genetic markers

Horizontal starch-gel electrophoresis was used to obtain allozyme data for 35 plants per population following standard methods (Cheliak and Pitel, 1984). The extraction buffer was a 3 : 1 mixture of the YO and VEG II buffers, respectively (Yeh and O'Malley, 1980; Cheliak and Pitel, 1984). Extracts were centrifuged at 7000 rev · min^{-1} for 7 min and the supernatant was absorbed by paper wicks. Samples were run on starch gels (9% w/v) at 4°C. Nine polymorphic loci of seven enzymes displayed reliable banding patterns. The buffer system Histidine pH 6.5 (run for 7 h) (Stuber *et al.*, 1988) was used for the enzymes glutamate dehydrogenase (GDH) E.C. 1.4.1.2 (one locus), malate dehydrogenase (MDH) E.C. 1.1.1.37 (two loci), and malic enzyme (ME) E.C. 1.1.1.40 (one locus). The buffer system Poulik pH 8.6 (run for 4 h) (Hakim-Elahi, 1976) was used for anodal and cathodal peroxidase (APX and CPX) E.C. 1.11.1.7 (one and two loci, respectively), glutamate oxalacetate dehydrogenase (GOT) E.C. 2.6.1.1 (one locus), and esterase (EST) E.C. 3.1.1 (one locus).

Statistical analysis

We obtained the multi-locus genotype for each individual of *D. seguine*, based on the observed banding patterns for each enzyme locus. Allelic frequencies were estimated from the genotypic frequencies and used to calculate the genetic parameters using the program TPFGA 1.3 (Miller, 1997). Four measures of genetic variation were estimated for each population: polymorphism (P, 95% criterion), mean number of alleles per locus (A), observed mean heterozygosity (H_o), and the expected mean heterozygosity (H_e) at the Hardy-Weinberg equilibrium. The deviation of the observed genotypic frequencies from random mating expectations was tested using a χ^2 goodness of fit (Zar, 1999), and then calculating the fixation index $F = 1 - (H_o/H_e)$ (Wright, 1951).

To assess the amount of clonal diversity within populations, we estimated the proportion of distinguishable genotypes G/N (i.e. probability that a newly sampled individual would have a new genotype), where G is the number of different multi-locus genotypes and N is the number of individuals sampled in each population. G/N ranges from nearly zero in populations where only a single genotype is encountered, to 1 in populations where each individual is unique (Pleasants and Wendel, 1989). Additionally, we calculated the average diversity of genotypes for fragments and continuous forest using the Shannon-Weiner index; this approximation is appropriate when sample sizes are different (Kikkama, 1986).

The hierarchical population genetic structure is described by means of the unbiased statistics F, θ, and f, corrected for population size, number of alleles per locus, and number of populations (Weir and Cockerham, 1984), and are equivalent to Wright's F-statistics F_{it}, F_{st}, and F_{is}, respectively (Wright, 1951, 1965). We calculated 95% confidence intervals for these estimates by means of a jackknife procedure across loci (Weir, 1990). To determine whether the F_{it} and F_{is} values were significantly different from zero, a χ^2 test was performed for each locus:

$$\chi^2 = F[2N]^*[k-1] \qquad \text{with } k(k-1)/2 \text{ degrees of freedom}$$

where N is the sample size and k is the number of alleles (Weir, 1990). The departure of the F_{st} statistic from zero was determined using the χ^2 method of Workman and Niswander (1970):

$$\chi^2 = (2N)\, F_{st}(k-1) \qquad \text{with } (k-1)(s-1) \text{ degrees of freedom}$$

where s is the number of subpopulations.

The average gene flow *Nm* (number of individuals exchanged between populations per generation) was estimated from F_{st} values, using the equation

$$F_{st} \cong 1/(4Nm\alpha + 1)$$

where $\alpha = [n/(n-1)]$ and n is the number of populations (Wright, 1951, 1965; Crow and Aoki, 1984). In addition, we estimated the rate of outcrossing (t), assuming that self-fertilization is the only source of inbreeding as

$$t = (1-f)/(1+f)$$

where f is the inbreeding coefficient (Haldane, 1924). Finally, Nei's (1972) genetic distance (D) was estimated among all pairs of populations. A population dendrogram was constructed using an UPGMA (unweighted pair-group method with arithmetic averaging) procedure, based on the genetic distances among populations (Hedrick, 2000). The correlation between the matrix of genetic distance and the matrix of geographical distance among populations was tested using a Mantel's test (Manly, 1994).

Estimates of genetic diversity and population structure were obtained across individual populations (local scale), type of habitat (continuous forest and fragments), and all populations (global scale). Differences in the estimates of genetic variation between fragmented and continuous forest populations were tested with a t-test, given that all variables, except A and H_o, are normally distributed and with equal variances. For A and H_o, we used a Wilcoxon test. Spearman's rank correlation was estimated to assess the magnitude of the association between the amount of genetic diversity and the size of the fragment (Zar, 1999).

RESULTS

Genetic diversity in *D. seguine*

Nine loci analysed for *D. seguine* were polymorphic, possessing two or three alleles. Some alleles had frequencies less than 5% or were absent in some populations, either in the fragmented or in the continuous forest (Fig. 2). For most loci, allele frequencies showed marked differences between populations. There was no pattern in the allelic frequencies (e.g. fixation) in relation to the type of habitat (continuous forest or fragments) or the size of the fragment (Fig. 2).

An average of 2.01 alleles per locus across all populations was observed (Table 2). Allelic richness did not differ between fragments and continuous forest ($z = 0.113$, $p = 0.9095$). Polymorphism at the global level was $P = 84.44\%$ and ranged from $P = 66.66\%$ in fragments of intermediate size (F2 and F5) to $P = 100\%$ in some populations of both habitats (Table 2). The average polymorphism between continuous forest and fragments was not significantly different ($t = -0.313$, $p = 0.7626$). Observed heterozygosity (H_o) ranged from 0.19 to 0.38, with a global mean of 0.24. Expected heterozygosity (H_e) varied from 0.27 to 0.43, with a global mean of 0.33. There were no significant differences in heterozygosity between populations in the continuous forest or fragments (H_o: $z = -1.385$, $p = 0.1650$; H_e: $t = 0.920$, $p = 0.3839$). The heterozygosity values did not show any dependence on the size of the fragment (Table 2).

Observed heterozygosities were significantly lower than expected heterozygosities in all *D. seguine* populations (global: $\chi^2 = 8.56$, d.f. = 1, $p < 0.005$; continuous forest: $\chi^2 = 4.93$,

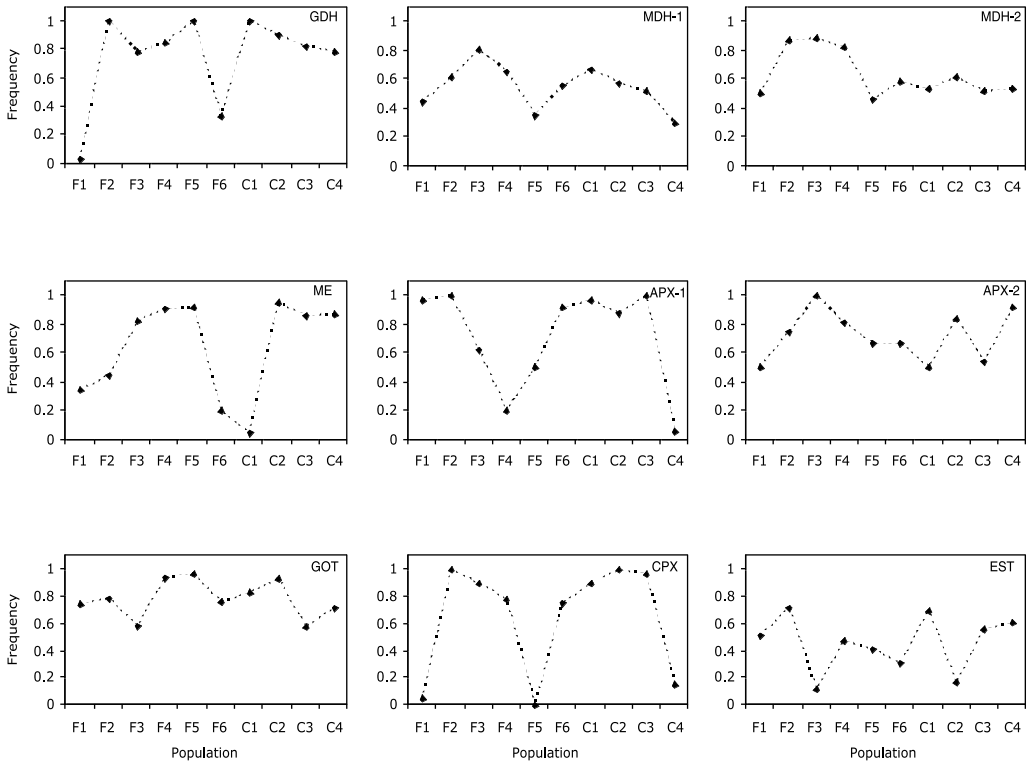

Fig. 2. Frequency of the most common allele at nine loci in ten populations of *Dieffenbachia seguine*. Populations are arranged from the smallest to the largest in area (see Table 1). F, fragments; C, continuous forest.

d.f. = 1, $p < 0.025$; fragments: $\chi^2 = 3.95$, d.f. = 1, $p < 0.05$). Deviation of genotypic frequencies from Hardy-Weinberg expectations showed no tendency to be grouped in any particular population or habitat type.

The fixation index was positive in all populations studied, indicating inbreeding and the average values did not differ significantly between populations in the continuous forest and fragments ($t = -1.362$, $p = 0.2103$) (Table 2).

Genetic diversity of *D. seguine* expressed as the number of different multi-locus genotypes was very high. A total of 296 multi-locus genotypes was observed over all populations. The average genotypic diversity was $G/N = 0.853$ (Table 2), indicating a probability of 85% that any individual chosen at random had a new multi-locus genotype (Pleasants and Wendel, 1989). At the population and type of habitat level, G/N showed high values (> 0.73). The G/N ratio was statistically lower in populations of fragments than in continuous forest ($t = -2.373$, $p = 0.0451$), indicating a negative effect of fragmentation on genotypic diversity. The Shannon-Weiner index showed the same trend, indicating lower diversity indices for populations in fragments than those in continuous forest ($t = 3.511$, $p = 0.0082$) (Fig. 3a). This implies higher genotypic diversity and/or more even frequencies in the continuous forest. Furthermore, G/N was significantly and linearly related to the area of the fragment ($r = 0.65$, $p = 0.0243$) (Fig. 3b).

Table 2. Genetic diversity estimates for *D. seguine* populations in Los Tuxtlas, Mexico

Population	*A*	*P*	H_o	H_e	*F*	*G*/*N*
Continuous forest						
C1	2.00	77.77	0.1961	0.2883	0.320	0.875
C2	2.00	88.88	0.2069	0.285	0.274	0.900
C3	2.00	77.77	0.2357	0.3548	0.336	0.923
C4	2.11	100.00	0.2150	0.3343	0.357	0.949
Mean	2.03	86.11	0.2134	0.3156	0.322	0.912
Standard error	0.06	6.88	0.026	0.023	0.044	0.032
Fragments						
F1	2.11	77.77	0.2594	0.3630	0.285	0.931
F2	1.78	66.66	0.2175	0.2718	0.200	0.833
F3	2.00	88.88	0.2812	0.3334	0.157	0.775
F4	2.11	100.00	0.1863	0.3267	0.430	0.750
F5	1.88	66.66	0.2460	0.3329	0.261	0.733
F6	2.11	100.00	0.3759	0.4326	0.131	0.867
Mean	2.00	83.33	0.2610	0.3434	0.244	0.815
Standard error	0.05	5.61	0.022	0.019	0.036	0.026
Global						
Mean	2.01	84.44	0.2420	0.3323	0.275	0.853
Standard error	0.03	4.13	0.018	0.015	0.029	0.025

Note: A is the mean number of alleles per locus, *P* is the percentage of polymorphic loci (95%), H_o is the observed heterozygosity, H_e is the expected heterozygosity, *F* is Wright's fixation index, *G*/*N* is genotypic richness.

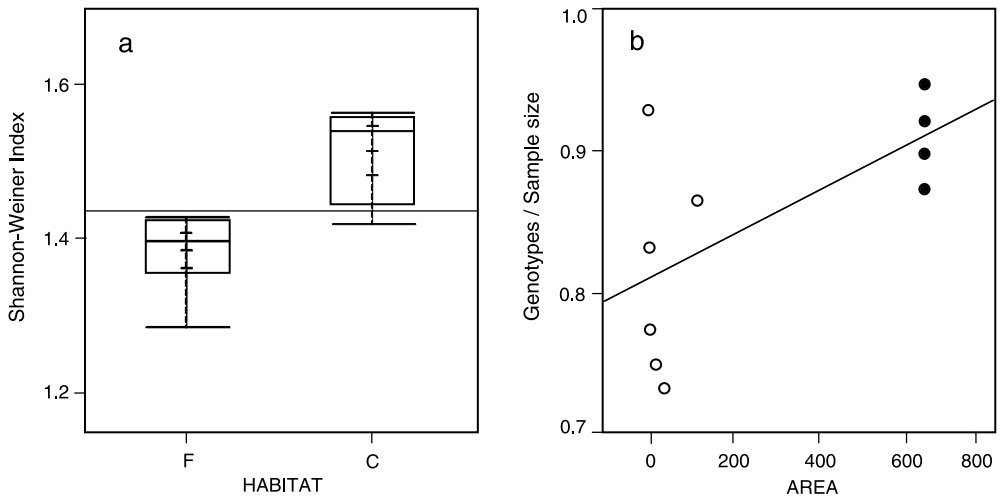

Fig. 3. (a) Box-and-whiskers plot of the genotypic diversity (Shannon-Weiner Index) of fragments (F) and continuous forest populations (C). The figure shows median genotypic diversity (lines), 25% to 75% quartiles (boxes), and ranges (whiskers). Contained within the boxes are the mean genotypic diversity and the mean standard error. (b) Relationship between fragment size and genotype richness ($G/N = 0.81 + 0.00016\ area$). ○, populations in fragments; ●, populations in continuous forest.

Genetic structure of *D. seguine* populations

Populations of *D. seguine* are highly structured (Table 3). The global and type of habitat F_{it}, F_{st}, and F_{is} estimates were significantly different from zero for all loci ($p < 0.05$). Overall population estimates of *F*-statistics indicate an equivalent contribution of local inbreeding (F_{is}) and population differentiation (F_{st}) to the global inbreeding (F_{it}) (Table 3). However, the extent of population differentiation was similar in magnitude for continuous forest and fragments (Table 3). Similarly, there were no significant differences in local inbreeding between continuous forest and fragment populations. The outcrossing rate estimated at the global level was 0.568, suggesting a mixed mating system in *D. seguine* populations. The indirect estimates of average gene flow at the global level ($Nm = 0.784$), among populations in the continuous forest ($Nm = 0.874$), and fragments ($Nm = 0.724$) suggest low migration rates and possible effects of genetic drift (Wright, 1951).

Genetic distances among populations were high in most cases (global average $D = 0.225$), ranging from 0.043 to 0.491. The average genetic distance (D) among populations was 0.250 and 0.241 for the continuous forest and fragments, respectively. High values of D were obtained even between populations in the continuous forest (e.g. C1 vs. C4, $D = 0.4866$). The minimum D value was observed between the C4 and F5 populations, despite the large geographic distance between them (13.75 km). The dendrogram based on genetic distances among populations (Fig. 4) shows that fragment 1 (F1) is separated from the rest of the populations by an average distance of 0.36 km. Similarly, it shows that populations do not group together by proximity (with the exception of F3 and F4, 0.5 km apart, both in the Sierra Santa Martha) or type of habitat (F or C), as illustrated by C4 and F5. Thus, contrary to expectations, there was no correlation between genetic and geographic distances among populations (Mantel test: $r = -0.1515$, $p = 0.8030$).

Table 3. Wright's *F*-statistics for *D. seguine* populations in Los Tuxtlas, Mexico

Locus	F_{it}	F_{st}	F_{is}
Gdh	0.7447***	0.4612***	0.5261***
Mdh-1	0.9387***	0.0595***	0.9348***
Mdh-2	0.7585***	0.0873***	0.7354***
ME	0.7578***	0.4779***	0.5360***
Cpx-1	0.7870***	0.7013***	0.2871***
Cpx-2	0.2875***	0.0580***	0.2437***
Got	−0.1097***	0.0992***	−0.232***
Apx	0.7515***	0.7100***	0.1431***
Est	−0.2297***	0.1067***	−0.3765***
Mean	0.5113***	0.3058***	0.2960***
Jackknife ± standard error			
Global	0.5082 ± 0.1600*	0.3052 ± 0.0964*	0.2905 ± 0.2125*
Continuous forest	0.5314 ± 0.1644*	0.3154 ± 0.1326*	0.3157 ± 0.2088*
Fragments	0.4825 ± 0.1779*	0.3121 ± 0.0922*	0.2420 ± 0.2335*

* $P < 0.05$, *** $P < 0.0001$.

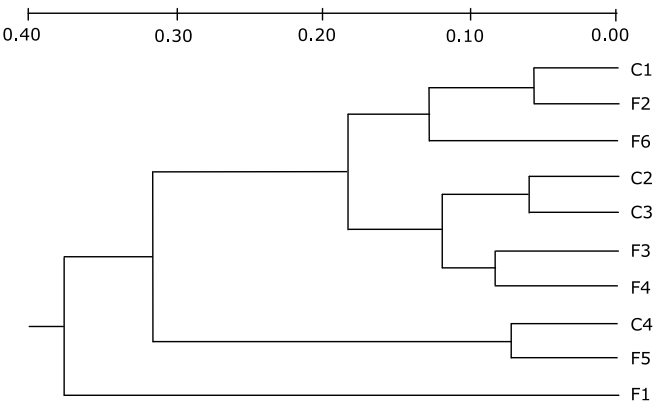

Fig. 4. UPGMA cluster analysis based on Nei's genetic distance among populations of *Dieffenbachia seguine* populations in Los Tuxtlas, Mexico.

DISCUSSION

The most striking result of this study is that populations of *D. seguine* are highly structured even in the continuous forest and were probably structured before fragmentation. Thus, fragmentation has yet to exacerbate genetic differentiation among populations beyond that prior to population subdivision, contrasting with theoretical expectations (Ellstrand and Elam, 1993; Young *et al.*, 1996). Also, we detected that habitat fragmentation has produced a reduction in the diversity of genotypes of *D. seguine*, but all other estimates of genetic diversity (allelic richness, polymorphism, and heterozygosity) showed no reduction in relation to isolation or any reduction in population size.

Genetic diversity

Populations of *D. seguine* from Los Tuxtlas are highly genetically diverse compared with other perennials or monocot species (Hamrick and Godt, 1996). There were no significant differences in genetic diversity among *D. seguine* populations in continuous forest and fragments. In addition, we did not detect any relationship between genetic variation estimates and the size of the fragments. A similar response to fragmentation has been reported in the tropical tree *Symphonia globulifera* in Costa Rica (Aldricht *et al.*, 1998) and the palm *Carpentaria acuminata* in Australia (Shapcott, 1998). However, a positive relationship between genetic variation and reproductive population size has been observed in many species [*Salvia pratensis* and *Scabiosa columbaria* (van Treuren *et al.*, 1991), *Eucalyptus albens* (Prober and Brown, 1994), *Gentiana pneumonanthe* (Raijman *et al.*, 1994), *Phitecellobium elegans* (Hall *et al.*, 1996), *Swetenia humilis* (White *et al.*, 1999), and *Rutidosis leptorrhynchoides* (Young *et al.*, 1999)], and this trend has been suggested to result from the elimination of rare alleles in fragmented populations. In contrast, in *D. seguine*, most loci had alleles at intermediate frequencies, and low-frequency alleles were not associated with fragmentation.

Genotypic diversity in populations of *D. seguine* is high compared with other clonal plants (Pleasants and Wendel, 1989; Pappert *et al.*, 2000). Despite the expectation that a neighbourhood in a clonal plant would contain many identical genotypes (McLellan *et al.*, 1997), the low proportion of redundant genotypes found within and among populations of *D. seguine* suggests a low

level of clonality. This high genotypic diversity can be the result of (1) a high number of genets established prior to fragmentation, (2) different sources of propagules (migration), and (3) outcrossing (McLellan *et al.*, 1997). *Dieffenbachia seguine* possess reproductive mechanisms that promote outcrossing: protogyny, sequential flowering in a ramet, a long flowering period, and a small number of open inflorescences every day (Cuartas, 2002), obligating pollinators to move between distant inflorescences (Handel, 1985; Young, 1988; Goulson, 2000).

Average fruit-set in *D. seguine* was reduced in small fragments compared with continuous forest (Cuartas, 2002). This, together with the reduction in the number of multi-locus genotypes, suggests that fragmentation is affecting pollinators (Murcia, 1996; Knutsen *et al.*, 2000). Changes in pollination may alter the mating system and thus genotypic frequencies (Lloyd and Schoen, 1992) and reproductive success through pollen limitation (Matsumura and Washitani, 2000). Thus, it is likely that fragmentation affects the reproductive biology of natural populations of *D. seguine*, changing genotypic structure in the short term and the genetic structure in the long term (Young *et al.*, 1999).

Genetic structure

Although the populations of *D. seguine* studied are separated by short geographic distances, they are highly structured in continuous forest as well as in fragments. The level of differentiation in populations of *D. seguine* exceeded that observed by Hamrick and Godt (1996) for monocots in a mixed mating system with widely distributed plants. The high significant F_{st} values over loci and the small *Nm* values estimated between pairs of populations suggest random population differentiation (Cabe and Alstad, 1994), although selection cannot be ruled out. The high F_{st} value estimated for the populations in the continuous forest indicate that even in the undisturbed habitat, there is restricted gene flow. This remarkable result can be explained by considering the combined effects of two aspects related to the dynamics of the populations: First, colonizing events of *D. seguine* may depend upon forest gap dynamics, since *D. seguine* invade or flourish in nearby gaps (Núñez-Farfán and Dirzo, 1988). Additionally, recent studies on the demography of this herb suggest that the performance of populations (clonal growth) is enhanced in small forest patches with greater light availability (Cuartas, 2002). Second, genetic contact among populations in the continuous forest or fragments can be broken as a result of disperser or pollinator behaviour. The disperser birds of *D. seguine* have contrasting habitat preferences: *Habia* sp. is forest-restricted, whereas *Turdus* sp. prefers non-forested habitats (Graham and Blake, 2001). Similarly, beetle pollinators of *D. seguine* are unable to move among fragments or over long distances as observed in other Scarabaeidae beetles (Klein, 1989; Young, 1990; Halffter *et al.*, 1992), and their abundance is very low (S. Cuartas, personal observation).

This dependence of *D. seguine* on the creation of gaps implies that different genetic contingents could colonize recently formed gaps, through seed dispersal by *Turdus* sp. (long-distance gene flow), which explains the high genetic variability within populations. However, gene flow accomplished by *Habia* sp. and the pollinators (short-distance gene flow) following the colonization event could produce genetic differentiation, even between close populations, because these species do not fly outside the forest (Klein, 1989; Halffter *et al.*, 1991) and they only rarely reach other fragments following riparian corridors (E. Figueroa, personal communication).

Inbreeding at the global scale is high in *D. seguine* (29%) compared with some perennial herbs (Neel and Ellstrand, 2001). This level of inbreeding can result from: (1) self-pollination as

suggested by the estimated outcrossing rate ($t = 0.54$); (2) mating between relatives due to the scarcity of mates (Goulson, 2000); (3) genetic drift; and (4) bi-parental inbreeding, given that clonal growth may produce a spatial genetic structure (Ellstrand and Elam, 1993). Finally, (5) clonality can produce a similar effect to inbreeding due to the lack of genetic recombination (Abrahamson, 1980). However, the high genotypic diversity in *D. seguine* suggests that the deficit of heterozygotes is not an artefact of clonality.

The causes of inbreeding are not known for *D. seguine* and for other tropical herbs in fragmented landscapes. Thus it is imperative to investigate the reproductive biology and mating system within populations of *D. seguine*.

The absence of any habitat type or geographic pattern among populations revealed by the UPGMA analysis based on genetic distances is consistent with the explanation that *D. seguine* possesses a demographic dynamics coupled with complex colonizing events, but is not dependent upon forest fragmentation. However, population F1 branched apart from the other populations, suggesting that genetic drift could accelerate differentiation in fragmented small populations (Young *et al.*, 1999), and that the loss of genetic variability may be exacerbated through time.

Finally, because individual plants of *D. seguine* can take up to 2 years to reproduce, current populations most probably constitute a mixture of ancient genotypes established before fragmentation and new ones introduced by seed dispersal. Thus, the observed genetic structure reflects, at least in part, the post-fragmentation processes within populations.

Considering the high genetic differentiation and the loss of genotypes in small fragments, conservation strategies at the Los Tuxtlas should focus on maintaining as many large fragments as possible. This strategy will sustain both the genetic diversity distributed within and among populations and plant–pollinator interactions (Tsharntke and Brandl, 2004), which are critical in the long-term persistence of populations. For the particular case of *D. seguine*, it is of utmost importance to maintain the processes that promote the creation of available patches of habitat for colonization (e.g. gap dynamics) and stability of species with a metapopulation structure.

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