

Patterns of genetic diversity and biogeographical history of the tropical wetland tree, *Pterocarpus officinalis* (Jacq.), in the Caribbean basin

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Abstract

Studies examining intraspecific variation in plant species with widespread distributions and disjunct populations have mainly concentrated on temperate species. Here, we determined the genetic structure of a broadly distributed wetland tropical tree, *Pterocarpus officinalis* (Jacq.), from eight Neotropical populations using amplified length fragment polymorphisms (AFLP). AFLPs proved highly variable with almost half (48%) of the genetic variation at these loci occurring among individuals within populations. Nonetheless, there was a strong geographical pattern in the distribution of AFLP variation within *P. officinalis*. Caribbean and continental populations fell into two well-defined genetic clusters supported by the presence of a number of unique AFLP bands. Within these two regions, there were also strong genetic differences among populations, caused mainly by frequency differences in AFLP bands, making it difficult to determine the evolutionary relationships among populations. In addition, our analysis of *P. officinalis* revealed striking differences in the levels of AFLP variation among the eight populations sampled. In general, Caribbean populations had lower genetic diversity than continental populations. Moreover, there was a clear loss in AFLP diversity with distance from the continent among Caribbean populations. The overall genetic pattern within *P. officinalis* suggests that past colonization history, coupled with genetic drift within local populations, rather than contemporary gene flow are the major forces shaping variation within this species.

Keywords: AFLP, founder events, genetic structure, phylogeography, stepping-stone colonization

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Introduction

The spatial distribution of genetic diversity in plant populations is determined by life history traits that influence mating patterns and gene dispersal (Hamrick 1989; Hamrick & Loveless 1989; Ouborg *et al.* 1999) and by population history (Schaal *et al.* 1998; Taberlet *et al.* 1998). The relative importance of historical and ecological factors depends on the temporal and spatial scale of the studies. Most genetic studies of tropical plant populations have been restricted to small spatial scales, with a handful of studies over 100 km (Lavin *et al.* 1991; Huff *et al.* 1993; Boshier *et al.* 1995; Schierenbeck *et al.* 1997; Franceschinelli & Kesseli 1999). The relative small spatial scale of the

investigations contrasts with the widespread distribution of many tropical plants, which can include several biogeographical zones (Pittman *et al.* 1999). Thus, the spatial scale of genetic studies needs to be increased to better understand the historical, physical and biological factors that have shaped intraspecific variation, including: (i) migration and colonization routes; (ii) the location of source populations or centres of genetic diversity; and (iii) importance of genetic drift and gene flow in local dynamics.

In this study, we determined the genetic structure of a tropical tree species, *Pterocarpus officinalis* (Fabaceae), from eight populations broadly sampled throughout Neotropics (Fig. 1). *P. officinalis* is one of the dominant tree species in fresh and brackish water wetlands throughout the Caribbean, coastal areas of Central and northern South America. It is characterized by a narrow buttress, compound and

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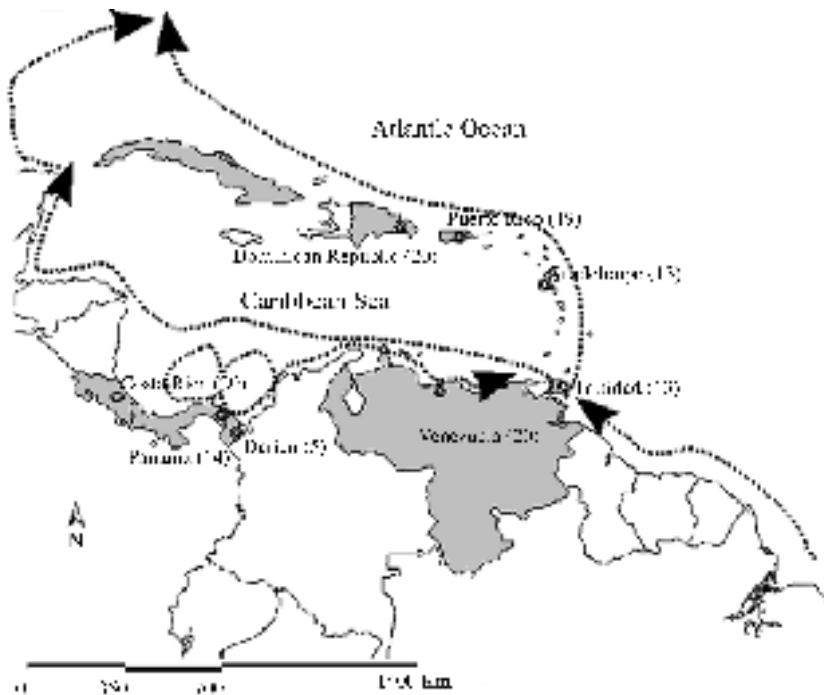


Fig. 1 Collection sites and sample size of the eight *Pterocarpus officinalis* populations used in this study. The dotted line shows the average general direction of the sea currents in the Caribbean.

alternate leaves, yellow bee-pollinated flowers and blood-red sap (Little & Wadsworth 1964). The seed is flat and irregularly rounded with small wings and a woody cover that allows it to float. Seeds are tolerant to salt water and have germinated after 6 weeks in salt water providing a potent mechanism for long-distance dispersal (E. Rivera, personal observation).

Based on pollen records, *P. officinalis* arrived in the Neotropics during the Quaternary (10 000 to 1.8 Ma), being a relatively recent colonizer in comparison with other wetland species (Graham 1995). Given its widespread distribution, its capacity for water dispersal and the presence of a closely related subspecies in West Africa, *P. officinalis gillettii*, it is likely that *P. officinalis* colonized the Neotropics via oceanic dispersal from Africa. The Benguela current that originates off the western coast of South Africa first contacts South America in north-eastern Brazil (Garzoli & Gordon 1996). At this point, the sea currents splits, one current continues to the north along the Antilles and the other passes along the Caribbean coast of South and Central America (Fig. 1). Under this scenario, *P. officinalis* probably colonized the north-east coast of South America and then spread through the Caribbean basin. This colonization scenario makes strong predictions about the distribution of genetic variation among extant populations of *P. officinalis*. Populations along the north-east coast of South America should have received the majority of immigrant seeds and should harbour most of the genetic diversity within Neotropical populations of *P. officinalis*. These populations should be the source for subsequent expansion of the species within the Neotropics. Thus, if we assumed that

colonization occurred unidirectionally, following sea currents, then we should expect a decrease in genetic diversity with distance from these source populations.

Other studies used contemporary patterns of genetic diversity to infer historical colonization and migration routes (Glover & Barrett 1987; Barrett & Husband 1990; Lavin *et al.* 1991; Dolan 1994; Giles & Goudet 1997; Gillies *et al.* 1997; Taberlet *et al.* 1998; Caron *et al.* 2000; Dutech *et al.* 2000; Lowe *et al.* 2000). These studies clarified the population genetic consequences of postglacial range expansion and migration in Europe and North America, and generally predict that genetic diversity should decrease with increasing distance from refugial sources (Cwynar & MacDonald 1987).

Several models have been developed to describe the effects of colonization on the genetic structure of populations (Kimura & Weiss 1964; Barrett & Husband 1990; Le Corre & Kremer 1998). The island and stepping-stone models have been used to explain the effect of migration and colonization on genetic structure (Kimura & Weiss 1964; Slatkin 1977; Barrett & Husband 1990). The island model assumes random migration between subpopulations with the same effective population size, and does not consider the distance between populations (Wright 1931). The stepping-stone model assumes that migration occurs between adjacent populations, and that colonized islands contain only a subset of the genetic information of the source population (Kimura & Weiss 1964). Migration and colonization events often result in the loss of genetic diversity due to founder effects (Wright 1969; Nei 1987). The magnitude of genetic decay depends on the number of colonists, population

growth rates, population isolation and dispersal ability (Barrett & Husband 1990; Le Corre & Kremer 1998). Although several studies have determined the effects of colonization and migration in plant species with widespread distributions and disjunct populations (e.g. island-continent), few describe these processes in tropical species (Glover & Barrett 1987; Barrett & Husband 1990; Lavin *et al.* 1991; Aide & Rivera 1998).

We examined the population genetic architecture of continental and island populations of *P. officinalis* using amplified fragment length polymorphisms (AFLP). The AFLP technique reveals hundreds of dominant marker loci rapidly and relatively inexpensively (Vos *et al.* 1995; Mueller & Wolfenbarger 1999). It is highly replicable and sensitive at detecting fine-scale genetic structure within plant and animal populations (Jones *et al.* 1997; Blears *et al.* 1998). For *P. officinalis*, the AFLP data allowed us to: (i) identify geographical regions of high genetic diversity and possible sources of population expansion within the Neotropics; (ii) test predictions about levels of genetic variation within local populations; (iii) determine the evolutionary relationship among populations; (iv) assess the relative importance of gene flow and genetic drift in population structure in this species; and (v) identify populations with high genetic diversity that can be used for future conservation of the species.

Materials and methods

Population sampling

Leaf samples from eight populations of *Pterocarpus officinalis* were collected across the species distribution in the Neotropics (Fig. 1). The collection sites were selected based on the geographical range of *P. officinalis*, and the position of the populations in relation to the predicted migration route between the New World and Old World populations in West Africa (Fig. 1). Most population samples were collected in wetland areas associated with mangrove forests; however, the Costa Rican samples (CR) and Panamanian samples from the Darien region (PD) were collected from riparian sites. Leaf samples were cut into square pieces and stored in sealed plastic bags with DRIERITE at -20°C .

DNA extraction and purification

P. officinalis leaves have a high concentration of polysaccharides which complicated the DNA extraction and subsequent restriction enzyme digestion. DNA was extracted following a modification of the Edwards *et al.* (1991) protocol. Leaf tissue was ground in lysis buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and extracted once with equal volumes of chloroform/phenol. To reduce the amount of polysaccharide contaminants, we first added 1:2 volume of isopropanol and removed the mucus-like polysaccharide precipitate. We then added another half volume of isopropanol and centrifuged for 20 min at 13 000 r.p.m. on a Beckman GS-15r with a 014491 fixed-angle rotor. The DNA extracts were then run in 1% Sea Plaque GTG (FMC) low-melting agarose to separate DNA from RNA and remaining polysaccharides. The high molecular mass DNA band was excised and digested for 3 h at 37°C with 0.4 units of gelase (Epicentre Technologies). Genomic DNA concentration was determined by comparing the intensity of each band with Lambda DNA bands of known concentrations.

AFLP protocol

Approximately 60 ng of genomic DNA was cut using the restriction enzymes *EcoRI* and *MseI* to yield fragments in the 100–600 bp range. Two polymerase chain reaction (PCR) linker sequences were ligated onto the resulting pool of DNA fragments following the protocol of Vos *et al.* (1995). To reduce the complexity of DNA fingerprints we performed a two-step PCR procedure. Preselective PCR amplification was carried out using 1:10 template dilutions and single base extension primers complementary to the linker and restriction site sequence. Preselective amplifications were performed for 35 cycles, starting with 94°C for 2 min and followed by 35 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min, and a final extension cycle of 72°C for 10 min.

A second amplification was performed using three-base extension primers for *EcoRI* and *MseI* fragments (Table 1) and 1:20 template DNA dilutions of the preselective PCR

AFLP primer combination	Total no. of loci	No. of polymorphic loci	Percent polymorphism
Eco-CTC\Mse-CAT	82	43	52
Eco-CTC\Mse-CAC	55	41	75
Eco-CTC\Mse-CCT	37	22	59
Eco-CTT\Mse-CAC	94	75	80
Eco-CTT\Mse-CCG	72	49	68
Total	340	230	Mean = 68

Table 1 The five AFLP primer combinations used to generate genetic data for the eight populations of *Pterocarpus officinalis*. Polymorphic loci were bands with frequencies between 0.05 and 0.95 in the 120 individuals of eight populations

products. *EcoRI* selective primers were end-labelled with (γ - ^{32}P) ATP using T4 polynucleotide kinase. The kinase reaction conditions are identical to those described in Vos *et al.* (1995). The selective amplification programme was: an initial 2 min denaturation at 94 °C followed by 12 cycles of 94 °C for 30 s, 65 °C for 30 s (with a reduction of 0.7 °C at the annealing temperature in each cycle) and 72 °C for 1 min. This programme was followed by 23 cycles with a constant annealing temperature of 56 °C and a final extension cycle of 72 °C for 10 min. We loaded 1.5 mL of each PCR product onto a 6% denaturing acrylamide gel. Samples were electrophoresed at 100 W, 50 mA, 50 °C for 75 min and the resulting banding pattern was visualized in X-OMAT autoradiography film (Kodak). A 30–330 AFLP molecular mass marker (Life Technology) was used to determine band size. In addition, we included 5–8 individuals from different populations in each gel to more accurately identify homologous loci across different gels.

Genetic data analyses

After an initial screen of 36 different primer combinations, five primer combinations that yielded a moderate number of bands (<100 bands) in the 100–500 bp range were chosen for genetic analysis (Table 1). For each of these five combinations, we scored all loci showing a clear and easily readable banding pattern. For all individuals, we scored the presence or absence of a band at these loci. After scoring all loci for all 120 individuals, loci with frequencies <0.05 and >0.95 in the total population were excluded from further analysis.

Levels of genetic diversity within populations, represented by average heterozygosity (H), per cent of polymorphic loci (P) and genetic differences among populations (Nei 1972), were determined using the program TFPGA (Miller 1997a). Because AFLPs generate dominant markers and heterozygotes cannot be distinguished directly, we used Lynch and Milligan's Taylor expansion to estimate allele frequencies and determined indirect levels of heterozygosity (Lynch & Milligan 1994; Miller 1997a). This approach assumes that populations are in Hardy–Weinberg equilibrium and that AFLPs produce two alleles per locus (Lynch & Milligan 1994). Band presence represents the dominant homozygous, whereas band absence represents the recessive homozygotes and the heterozygotes are estimated indirectly.

UPGMA cluster analysis was used to generate a dendrogram of the relationships among the eight sampled populations using Nei's genetic distance (Nei 1972). Support for branching pattern was determined using 1000 bootstrap permutations of the data set using the program TFPGA (Miller 1997a). A Mantel test (Mantel 1967) was used to determine if there was an association between Nei's genetic distance and geographical distance for the eight

populations. A correlation coefficient (r) and one tailed P -value were determined using 1000 random permutations in NTSYS-PC version 1.8 (Rohlf 1993).

In addition, the distribution of genetic variation within *P. officinalis* was analysed using an analysis of variance (AMOVA) (Excoffier *et al.* 1992). For this analysis, we used the computer program WINAMOVA, Version 1.55 (Excoffier 1995). The pairwise Euclidean distance matrix (number of mismatches/total number of bands) and other input files used in the AMOVA analysis were prepared using AMOVA-PREP (Miller 1997b).

Results

The five AFLP primer combinations produced a total of 340 bands in 120 individuals (mean \pm SE, 68 ± 10 per primer combination), 230 (68%) of which were polymorphic (Table 1). An average of 46 polymorphic bands was generated for each primer combination (mean \pm SE, 46 ± 8.5). The percentage of polymorphism across the five primers ranged from 52 to 80% (Table 1).

Although the sample size varied among populations (5–20 individuals/population) there was no relationship between sample size and average heterozygosity (Pearson's correlation test; $n = 8$, $r = -0.12$, $P = 0.7$). The levels of genetic diversity represented by the average heterozygosity ranged from 0.15 to 0.27 among the eight populations of *P. officinalis* (Table 2). The Trinidad and Venezuela populations had the highest levels of diversity ($H = 0.27$ and 0.25 , respectively). The Dominican Republic and Puerto Rico populations had the lowest levels of diversity ($H = 0.15$ and 0.17 , respectively). Overall, Caribbean populations (excluding Trinidad) had lower diversity than continental populations (mean \pm SE, $H = 0.17 \pm 0.01$ and 0.23 ± 0.01 , respectively). The levels of genetic diversity within the regions decreased toward the extremes of the geographical distribution (Table 2, Fig. 2). In the Caribbean region, genetic diversity decreased from the Trinidad population to the Dominican Republic population ($H_T > H_C > H_{PR} > H_{DR}$). There was no sign of loss of diversity among continental populations (Fig. 2). Continental populations also had a higher percentage of polymorphic loci than the Caribbean populations (mean \pm SE, 69 ± 6.4 and 57 ± 6.1 , respectively) (Table 2).

There was a clear geographical pattern of genetic variation within *P. officinalis*, with the Caribbean and continental populations falling into two distinct and well-supported clusters (Fig. 3). The separation of the two regions was due to differences at $\approx 10\%$ of the polymorphic loci and moderate frequency differences at many more loci. The Caribbean cluster, which included Trinidad, was supported by 25 loci, 13 of which were unique to the region. The continental cluster was supported by 30 loci, 11 of which were unique to the region. Within each cluster,

Table 2 Genetic diversity estimates for the eight populations of *Pterocarpus officinalis*. Estimates were based on 230 AFLP polymorphic loci using the program TRGPA (Miller 1997a). *H* = average heterozygosity, *N* = sample size, % Polymorphism = loci with frequencies < 0.95 in each population. Hardy–Weinberg equilibrium was assumed for the estimation of *H* and % polymorphic loci parameters

Region	Population	Popn ID	<i>N</i>	<i>H</i>	% Polymorphism	Distance from Trinidad (km)
Caribbean	Trinidad	T	13	0.27	74	—
	Guadeloupe	G	12	0.18	55	625
	Puerto Rico	PR	19	0.17	50	970
	Dominican Republic	DR	20	0.15	47	1175
	Subtotal		64			
Continental	Venezuela	V	18	0.25	74	13
	Darien	PD	5	0.20	50	1770
	Panama	P	13	0.25	72	1970
	Costa Rica	CR	20	0.24	79	2470
	Subtotal		56			
Total		120				

Table 3 Nested AMOVA results for 120 individuals of *Pterocarpus officinalis* from eight populations. Results are based on 230 polymorphic loci. For this analysis, populations were separated in two regions: Caribbean (DR, PR, G and T) and continent (V, P, PD and CR). Statistics included sum of squared deviations (SSD), mean squared deviations (MSD) and estimation of variance components. The probability of obtaining a more extreme estimate by chance was determined by 1000 permutations of the original distance matrix (see Excoffier 1995)

Source of variation	d.f.	SSD	MSD	Variance component	% Total	<i>P</i> -value
Between regions	1	940.5	950.5	12.0	26	<0.001
Among population within regions	6	1191.2	198.5	12.2	26	<0.001
Among individuals within population	112	2506.4	22.4	22.4	48	<0.001
Total	119	4638.1	1171.4	46.6		

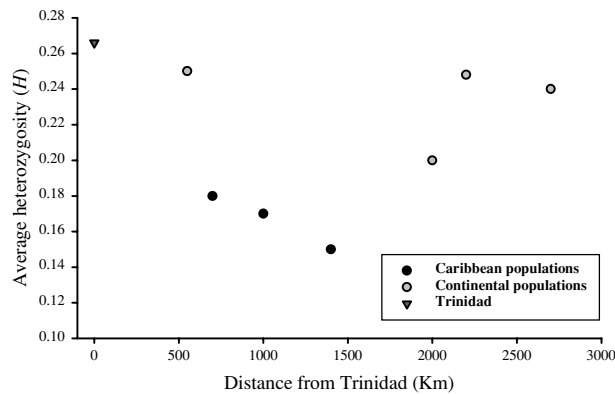


Fig. 2 Relationship of average heterozygosity (*H*) and distance from Trinidad for Caribbean and continental populations of *Pterocarpus officinalis*.

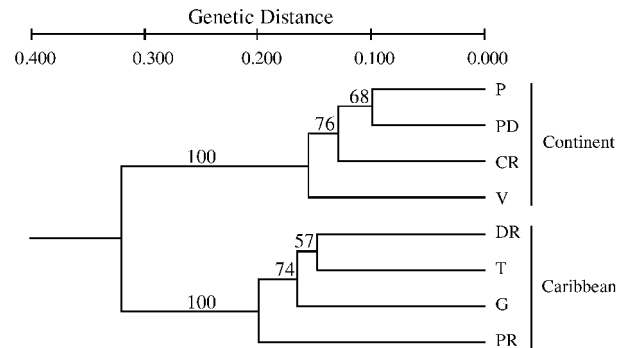


Fig. 3 UPGMA dendrogram based on 230 AFLP polymorphic loci from 120 individuals of *Pterocarpus officinalis*. Genetic distance was calculated using Nei’s genetic distance (Nei 1972). Support for branching pattern was determined using 1000 bootstrap permutations. Population ID are listed in Table 2.

internodal branches were short relative to terminal branches and there was less bootstrap support for the branching network, resulting in poor phenetic relationship between populations (Fig. 3). In the continental cluster only the two Panama populations consistently grouped together under a variety of genetic distance models (Fig. 3).

In the Caribbean cluster, the two extreme populations (Dominican Republic and Trinidad) were grouped together, although this cluster showed the lowest bootstrap value (Fig. 3). Even though the lack of geographical correspondence showed by the cluster analysis, the Mantel

test showed a very weak, but significant, relationship between genetic distance and geographical distance ($r = 0.5$, $P = 0.03$).

The nested AMOVA supported the separation of the Caribbean and continental populations, and regional differences accounted for 26% of the AFLP variation (Table 3). Moreover, a significant percentage of the genetic variation (26%) was attributed to differences among populations within the two regions ($P < 0.001$). Nonetheless, approximately half (48%) of the variation within *P. officinalis* can be attributed to differences among individuals within populations (Table 3).

Discussion

Despite the relatively recent colonization of *Pterocarpus officinalis* into the Neotropics suggested by pollen data (Graham 1995), our AFLP analysis detected striking genetic differences among the eight populations sampled. Although nearly half of the variation of *P. officinalis* was caused by differences among individuals within populations, there was a clear pattern of genetic differentiation between continental and Caribbean populations. Populations within the two regions fell into clusters defined by a number of unique AFLP bands and regional differences accounted for 26% of the variation at AFLP markers (Table 3). In addition, there were clear differences among populations within the two regions; however, in this case, differentiation was caused by frequency differences in AFLP bands. Genetic diversity was on average 1.5 times lower in the Caribbean in comparison with continental populations. Within Caribbean populations there was a loss in AFLP diversity with increasing distance from Trinidad, but this pattern was not observed in the continental populations (Fig. 2). The separation of the Caribbean and continental populations together with the high differentiation among populations within each region suggests that past colonization history coupled with genetic drift within local populations are the major forces shaping variation within this species.

Possible scenarios of P. officinalis colonization in the Neotropics

The most likely colonization scenario is that *P. officinalis* seeds were transported from West Africa by the Benguela current, to the north-western South America and subsequent colonization occurred along the coastal areas of South and Central America and throughout the Antilles (Fig. 1). Trinidad is the closest sampled population to the hypothesized African source (Rojo 1977). Thus, under this scenario, Trinidad was likely colonized early and represents the potential source for the other Caribbean and continental populations. Indeed, our genetic diversity

results support this hypothesis. The Trinidad population showed the highest genetic diversity of all populations sampled. Moreover, although Trinidad was grouped strongly with insular Caribbean populations, it shared many loci with populations sampled along the continental margins. Intrapopulation diversity decreases with distance from Trinidad suggesting that a general loss of diversity is associated with colonization. The linear decline in genetic diversity with distance from Trinidad probably reflects the action of genetic drift associated with sequential founder events during the colonization of the Caribbean.

It is also possible that the Neotropical populations are the result of several independent colonization events. This could explain the strong divergence between Caribbean and continental populations. In this case, the AFLP patterns are consistent with two independent colonization events. One event resulted in the colonization of the Caribbean islands and the other in the colonization of the continental margins. Each region was defined by a number of unique AFLP bands that were fixed or nearly fixed (> 0.85) among all the populations within a region. The presence of these unique loci in all populations within a region suggests that they were present at the beginning of the colonization. Under a scenario with multiple colonization events, it is most likely that seeds arrived first at Trinidad and spread up the Antilles. Another dispersal event may have resulted in the colonization of north-eastern South America and subsequent colonization along the Caribbean coast of South and Central America. This model assumes little connection between the continental and Caribbean populations, except in Trinidad, which may be a zone of mixing. Trinidad is ≈ 13 km from South America and directly adjacent to the mouth of the Orinoco river, which has large riparian populations of *P. officinalis*. Clearly, information on genetic variation in populations of *P. officinalis* from the eastern extreme of the distribution in the Neotropics (e.g. northern Brazil and the Guyanas) and from African populations is necessary to further clarify the colonization history of this species.

Colonization models

Regardless of whether the genetic patterns we observed in Neotropical populations of *P. officinalis* were the result of one or two colonization events, there is support for population colonization following the stepping-stone model. Our genetic diversity results coincide with the expected under this colonization model. Le Corre & Kremer (1998) have discussed the cumulative effects of founder events on genetic structure and differentiation expected by different colonization models. They used computer simulations to compare two colonization models: the linear stepping-stone model and the island model (multidirectional migration between populations).

These simulations demonstrate that the linear stepping-stone model results in faster loss of genetic diversity and more population differentiation for small number of colonists in comparison with island model (Le Corre & Kremer 1998; Whitlock & McCauley 1999).

In general, the diversity and differentiation patterns within the Caribbean correspond to the linear stepping-stone model of colonization. The pattern of AFLP variation suggests that the Trinidad population was an important source for the subsequent colonization of the region, given that this population had the highest genetic diversity. Our data also show that the process of colonization resulted in progressive loss of genetic diversity north through the Antillean chain (Fig. 2). Furthermore, the genetic distance among populations increased with geographical distance, as expected by stepping-stone colonization. Although for *P. officinalis* populations this correlation was very weak, suggesting the effect of other local factors.

Diversity decreased with distance from Trinidad more notably in the Caribbean populations than the continental populations (Fig. 2). Although the Caribbean populations are geographically closer, they are more isolated because of their insular condition. The Caribbean populations probably had fewer initial migrants and greater effects of genetic drift in comparison with the continental populations. Genetic diversity (H) decreased by $\approx 50\%$ between the populations of Trinidad and Dominican Republic, and although there is a clear trend we must be cautious in interpreting the absolute values due to the assumptions and the bias introduced by indirect estimates.

Diversity did not decrease from east to west in the continental populations. Although these populations are currently discontinuous, pollen records suggest that this fragmentation is relatively contemporary, resulting from either historical climatic changes or anthropogenic destruction of wetlands (Bacon 1990; Graham 1995). If continental populations were formerly continuous, then they should experience greater gene flow, which would reduce the effect of founding events. The low genetic divergence among these populations and similar levels of diversity suggest that migration was not entirely unidirectional, contrasting with the Caribbean, which appears to have had more restricted and unidirectional migration.

The lack of a clear geographical and genetic correspondence among populations within each region could reflect the limitations of AFLP or the influence of secondary currents on colonization patterns. AFLP markers evolve very rapidly as suggested by the number of unique bands within the Caribbean populations that were much less diverse than those in the continental populations. This rapid evolution may have reduced the evolutionary signal within the regional populations. In contrast, local currents and stochastic colonization events may be responsible for the weak geographical and genetic correspondence. For

example, the large river plume of the Orinoco River, which extends up to the Greater Antilles, could be responsible for the similarity between Trinidad and Dominican Republic populations (Muller-Karger *et al.* 1989; Blough *et al.* 1993).

Islands vs. continental populations

Very few studies have compared island and continental plant populations; but in most cases, island populations have had less genetic diversity than continental populations (Ledig & Conkle 1983; Glover & Barrett 1987; Barrett & Shore 1989; Affre *et al.* 1997). Barrett & Shore (1989) compared the genetic diversity of continental (South America) and island populations (Dominican Republic and Puerto Rico) of *Turnera ulmifolia* and the island populations had less isozyme variation ($H_S = 0.04$) than the continental populations ($H_S = 0.27$). The loss of variation was attributed to bottlenecks events associated with island colonization (Barrett & Shore 1989). The results of our study also show less genetic diversity in the inland populations than in the continental populations, except for Trinidad, which is close to the continent and could represent a mixing area. The reduction in genetic diversity in island populations may be a general pattern of species with island-mainland distributions. Frankham (1997) compared data of genetic diversity of different organisms (e.g. mammals, birds, reptiles, fishes, amphibians, molluscs, insects and plants) that have mainland-island distribution, and, in most cases, island populations were less diverse than mainland populations. These results suggest that lower genetic diversity in islands is related to the island colonization process and restricted gene flow.

*Caribbean biogeography and the conservation of *P. officinalis**

In the Caribbean, the biogeographical patterns of the fauna have been studied extensively (Briggs 1984; Hedges 1996; Seidel 1996), but biogeography studies of the flora are very limited (Lavin 1993; Santiago-Valentín 1999). Our finding that AFLP variation within *P. officinalis* falls into two distinctive regional clusters with strong diversity differences among populations has important conservation implications. Furthermore, the Caribbean-continental separation and rapid loss of genetic diversity in the islands populations may represent a general pattern for other species with similar dispersal mechanisms (e.g. mangroves). Information on the biogeographical patterns of other Caribbean plant species using genetic markers could be used as an important tool to help manage plant populations threatened by habitat destruction and climate change, across larger spatial scales. For example, within the Caribbean region, the highly diverse Trinidad population

of *P. officinalis* should be a high priority for regional conservation.

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