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Geographic patterns of genetic diversity in *Poulsenia armata* (Moraceae): implications for the theory of Pleistocene refugia and the importance of riparian forest

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Abstract. Tropical forests are species-diverse communities, but we know very little about the geographical distribution of genetic diversity within a species. During the late Pleistocene, lower temperatures and rainfall reduced the distribution of tropical wet forests, and in Central America lowland species may have been limited to riparian habitats. Approximately 12,000 years BP, temperature and rainfall increased in Central America, the distribution of wet forest species expanded, and today the distribution of some species extends into southern Mexico. The distribution of genetic diversity, based on RAPD markers, among ten populations of *Poulsenia armata* (Miq.) Standl. (Moraceae) suggests that these populations did not originate from a single refugium or region in the late Pleistocene. The Central American populations had high genetic diversity and unique bands giving support to the hypothesis that populations of *P. armata* occurred in Central American during the late Pleistocene. The majority of genetic diversity was partitioned among populations and there was no geographical relationship among populations, suggesting that these populations were isolated for a long period and there has been little gene flow. Populations of *P. armata* may have persisted in riparian zones along the Caribbean coast during the late Pleistocene. Given that riparian forest can support high levels of biodiversity in ecological time, and they have played an important role during periods of climate change over geological time, their conservation is of utmost importance particularly with the threat of a rapid shift in climatic patterns.

Key words. Conservation, fragmentation, population genetics, RAPD, tropics.

Resumen. Los bosques tropicales son comunidades de alta diversidad, a pesar de esto, conocemos muy poco sobre la distribución geográfica de la diversidad genética a nivel de especies. Durante el Pleistoceno tardío, una disminución en temperatura y precipitación restringieron la distribución del bosque húmedo tropical y en Centro América, las especies de tierras bajas pudieron estar limitadas a habitats riparios. Hace aproximadamente 12,000 años se produjo un incremento en temperatura y precipitación en Centro América; la distribución de las especies de bosque húmedo tropical se expandió y en el presente la distribución de algunas especies se extiende hasta el sur de México. La distribución de la diversidad genética a través de diez poblaciones de *Poulsenia armata* (Moraceae) basada en 'RAPD', sugiere que estas poblaciones no se originaron de un solo refugio o región en el Pleistoceno tardío. Las poblaciones de Centro América presentaron alta diversidad genética y un alto número de bandas únicas, sosteniendo la hipótesis de que existieron poblaciones de *P. armata* en Centro América durante el Pleistoceno tardío. La mayor parte de la diversidad genética ocurrió entre poblaciones y no se encontró relación geográfica entre las diferentes poblaciones, sugeriendo que estas fueron aisladas por largos periodos de tiempo y que ocurrió poco flujo genético entre ellas. Es posible que poblaciones de *P. armata* se mantuvieron en zonas riparias a lo largo de la costa del Caribe durante el Pleistoceno tardío. Los bosques riparios pueden sostener altos niveles de biodiversidad en un tiempo ecológico y juegan un papel importante durante periodos de cambios climaticos en tiempos geológicos. La conservación de estos habitats riparios es particularmente importante dado a la amenaza de cambios en los patrones climáticos.

Palabras claves. Conservación, fragmentación, genética de poblaciones, RAPD, trópicos.

INTRODUCTION

Palynological studies have shown that the distribution of tropical forest was affected by climate change in the late Pleistocene (25,000–12,000 BP) (van der Hammen, 1974; Kershaw, 1978; Bush *et al.*, 1992). In Colombia, the distribution of montane forest species (e.g. *Quercus* spp.) decreased in elevation and dominated areas that are

presently covered by lowland forest (van der Hammen, 1974). Although Bush *et al.* (1992) identified the presence of montane species around a lowland lake in Panama, lakes in the tropics are uncommon and it has been difficult to determine the distribution of lowland species during the late Pleistocene (Leyden, 1984). Many authors have suggested that tropical lowland forest was greatly reduced due to lower temperatures and precipitation (Prance, 1982). Evidence for lower temperatures is based on palynological cores (van der Hammen, 1974; Colinvaux, 1987; Bush *et al.*, 1992), marine sediments (Hughen *et al.*, 1996), ground water (Stute *et al.*, 1995), and ice cores (Thompson *et al.*, 1995). In the late Pleistocene, estimates of temperatures in tropical regions vary from 4 to 12 °C lower than at present (Stute *et al.*, 1995; Thompson *et al.*, 1995). Evidence from ice cores of the Peruvian Andes suggest that temperatures may have been 12 °C lower than today (Thompson *et al.*, 1995), but pollen (Bush *et al.*, 1992) and ground water (Stute *et al.*, 1995) data from the Amazon basin suggest a decrease of 5–9 °C. Although there is little direct evidence of lower precipitation in the late Pleistocene (Colinvaux, 1987; Salo, 1987), pollen and phytolith records (Leyden, 1984; Piperno *et al.*, 1990), present day distribution of tropical dry seasonal forests (Prado & Gibbs, 1993), and climate models (Clapperton, 1993) suggest that tropical lowlands were drier.

The Pleistocene Refuge Theory suggests that lowland forest species were restricted to a few refugia near the equator where changes in rainfall and temperatures were not as extreme (Haffer, 1969; Prance, 1982). Studies have attempted to identify the location of these refugia based on levels of diversity and endemism (Haffer, 1969; Prance, 1982). The theory has been attacked on many grounds, and efforts to identify the actual location of refugia or even their existence has led to much controversy (Endler, 1982; Connor, 1986; Colinvaux, 1987; Bush, 1994; Kellman & Tackaberry, 1997). One criticism is that the proposed refugia sites (i.e. areas of high endemism) are correlated with the number of collection trips (Endler, 1982; Beven *et al.*, 1984). In addition, others have questioned the evidence for widespread restriction of lowland forest (Kellman & Tackaberry, 1997).

Although the theory have been refuted on many grounds, there is evidence that lowland forest was replaced by savanna/shrub vegetation in several sites during the last glacial maximum (Kershaw, 1978; Leyden, 1984; Leyden, 1985; Giresse *et al.*, 1994). The possible restriction of forest in the late Pleistocene, would not have caused many speciation events, but it would have had profound effects on species distribution and genetic structure (Whitmore & Prance, 1987). In the neotropics, identifying the locations of the proposed Pleistocene refugia has been difficult, but it may be possible to distinguish regions (i.e. Amazon *v.* Choco) that were important source pools. Gentry (1982) suggested that the Pacific slope of the Andes, an area known as the Choco region in Colombia and Ecuador, was an important source pool for Central American vegetation. High rainfall (up to 12 m $year^{-1}$) and high species diversity in the Choco region are two important reasons why Gentry (1982) has proposed this area as a potential source pool. The late Pleistocene was probably drier, but this area should have had sufficient precipitation to support tropical forest. In addition, there are no major geographical barriers between Choco and Central America. However, Gentry (1986) has shown that the Central American flora has greater affinities with Amazonia than Choco, hence, refugia in Amazonia may have been the major source pools.

If we accept the hypothesis that lower temperature and precipitation restricted neotropical lowland vegetation to either the Choco or Amazon region during the late Pleistocene, then the distribution of genetic diversity in Central American populations should follow the stepping stone model (Kimura & Weiss, 1964). In this model, we assume that during the glacial maximum the total genetic diversity was restricted to the source pool region. Once temperature and rainfall increased, vegetation would expand into Central America, and populations would be founded with a subset of the original genetic diversity. As populations continued to migrate to the north, each successive population would contain less genetic diversity. This would result in low levels of among population variation and a negative relationship between genetic diversity and distance from the source pool. In temperate regions, this pattern has been observed in *Polygonella* spp. (Lewis & Crawford, 1995) and *Pinus contorta* Dougl. ex Loud. (Cwynar & MacDonald, 1987). These studies found the highest genetic diversity in the refugia and the lowest genetic diversity in the most distant populations.

If wet forest species were limited to either the Choco or Amazon region during the late Pleistocene, then rates of seed dispersal must have been high for these species to reach Mexico in ≈10,000–12,000 years. If we use the maximum dispersal rate of late successional species in temperate zones (Davis, 1987; 200 m/yr), it is impossible for tropical forest to have expanded its range from either the Amazon or Choco to Mexico in only 12,000 years.

An alternative hypothesis suggests that lowland wet forest species occurred in riparian habitats along the Caribbean coast of Central America (Meave *et al.*, 1991). Today much of the Caribbean coast receives >4000 mm of precipitation and even if rainfall decreased during the late Pleistocene it is likely that rivers still occurred in the major watersheds. If lowland species occurred within Central America, it is not necessary to invoke extremely high rates of migration to explain the present distribution of this forest type. If migration occurred from populations along the Caribbean coast, then we expect high levels of genetic diversity due to gene flow between populations. To test this hypothesis, we used RAPD markers to compared the genetic diversity within and among ten populations of *Poulsenia armata* sampled from Peru to Mexico.

METHODS

Study species and collection sites

Poulsenia armata (Moraceae) is a monoecious canopy tree species that is shade tolerant and long-lived. It occurs in moist and wet tropical forest from Mexico to Bolivia and is frequently found on fertile soils (Gentry, 1993) and along

FIG. 1. Collection sites and number of individuals used in the analysis of ten populations of *Poulsenia armata*.

streams. This species can be distinguished from other neotropical Moraceae by the presence of prickles on stipules, twigs, and leaves. In Panama, *P. armata* produces flowers and fruits throughout the year (Croat, 1978) and seeds are dispersed by bats.

Ten to twenty individuals of ten populations of *P. armata,* were collected in Peru, Colombia, Panama, and Mexico (Fig. 1). The collection sites included: two sites in the Amazonia region of Colombia and Peru (Amacayacu National Park and Cuzco Amazonico, respectively), two sites in the Choco region of Colombia (Rio Naranjo, Utria National Park), one site along the Caribbean coast of Colombia (Sierra Nevada de Santa Marta), four sites in Panama (Nusagandi, Barro Colorado Island, Rio Calovebora, Rio Changuinola), and one site in Mexico (Los Tuxlas). In the Amacayacu and Calovebora populations, only eight and ten individuals, respectively, were located after an exhaustive search. Tropical trees often occur at low population densities and although the sample size is small, we believe that most of the individuals in each population were collected. To standardize the number of individuals per population in the analyses, we randomly selected eight to ten individuals per population even if more individuals were sampled.

Field collection of leaf material

When possible, leaf samples were collected from plants that were at least 20 m apart to avoid sampling closely related individuals. Young leaves without any obvious phytophylls were selected, the surface was cleaned, and cut into $\approx 2 \times 2$ cm pieces. The leaf fragments of each individual were stored in air tight plastic bags with DriRite desiccant. Even in high humidity conditions, the leaves dried quickly preventing DNA degradation.

DNA isolation, purification, and quantification

Total DNA was extracted from all leaf samples following the extraction protocol of Edwards *et al.* (1991). A small piece of dry leaf (0.005 g) was macerated, resuspended in 400 μL of extraction buffer (200 mM Tris-HCl (pH 7.5), 25 mM EDTA, 0.5% SDS, 250 mM NaCl), and was left for 1 h at room temperature. Samples were centrifuged for 5 min at 13,000 r.p.m. and 300 μL of the supernatant was transferred to a new tube. Isopropanol (300 μL) was added to precipitate the DNA and left for 2 min. The isopropanol/DNA mix was centrifuged for 5 min at 13,000 r.p.m. The isopropanol was removed and the DNA pellet was dried

TABLE 1. Primers (Operon Technologies-Kit B) used to generate bands with ten populations of *P. armata* using a Coy and a Rapid Cycler (Idaho Technologies) thermocyclers.

Primer	Nucleotide sequence 5′ to 3′	Number of polymorphic bands produced with Coy Thermocycler	Number of polymorphic bands produced with Rapid Cycler
B-01	GTTTCGCTCC	22	8
B-02	TGATCCCTGG	11	6
B-04	GGACTGGAGT	19	10
B-05	TGCGCCCTTC	16	9
B-06	TGCTCTGCCC	20	14
B-07	GGTGACGCAG	15	8
B-08	GTCCACACGG	8	6
B-10	CTGCTGGGAC	21	8
B-17	AGGGAACGAG	19	10
B-18	CCACAGCAGT	12	6
Total		163	85

for 3 h. The pellet was then resuspended in 20 μL of ddH_2O and stored at 4 C°.

DNA extracts were purified using 1% Sea Plaque GTG low melting agarose. The extract was electrophoresed during 1 h at 60 V. A sample of *P. armata* was placed in the first and last well of the gel. These lanes were stained with ethidium bromide to determine the position of the large molecular weight DNA band (≈23,000 bp). The band of each sample was excised using pipette tips cut to match the width of the wells. After removing the bands the gel was stained to verify that all bands had been correctly removed. The excised bands were diluted in 100 μL of ddH20.

The DNA concentrations were quantified by comparing the intensity of sample DNA with a known concentration of DNA (Gibco – DNA Mass Ladder) on a ethidium bromide stained gel. Band intensities were compared by using a video image of the gel and Band Leader software (Aharoni, 1994). All samples were diluted to a final concentration of 5 ng/μL.

RAPD\PCR procedure

Using a Coy thermocycler, a series of trails were conducted in which template, primer, $MgCl_2$, polymerase, and dNTPs concentrations were varied to determine the optimal amplification conditions. The optimal PCR reaction mixtures contained a final volume of 25 μL with final concentrations of 0.2 mM each dATP, dGTP, dCTP, dTTP, 0.2 μM of primer, 1.25 U of Promega polymerase, and 20 ng of DNA [5 ng/μL]. Ten 10 bp primers were used for amplifications (Table 1). The amplification program was: 3 min of denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min for annealing at 36 °C, and 2 min for elongation at 72 °C. Amplification products were electrophoresed in 3% agarose gels (3:1-Sigma 9539 and Sigma 2790) at 80 V for 2 h in 1X TAE buffer. A 100 bp DNA ladder (Gibco BRL) was used as a molecular size standard. Gels were stained with ethidium bromide for 15 min and destained in H_2O for at least 20 min

Amplifications were repeated using a Rapid Cycler thermocycler (Idaho Technologies). The Rapid Cycler uses much shorter annealing time than the Coy and, thus, we expected greater specificity. The optimal PCR reaction mixtures for the Rapid Cycler contained a final volume of 10 μL with final concentrations of 0.1 mM each dATP, dGTP, dCTP, dTTP, 0.4 mM of primer, 0.6 U of Promega polymerase, 1.0 μL 10X BSA buffer, 1.0 mM $MgCl_2$, 1.0 μL of 10X buffer/BSA-20 mM $MgCl_2$ (Ficoll/Dye), and 1.6 μL of DNA. The reaction mixture was loaded into a capillary tube of 10 μL volume. The same ten 10 bp primers were used in the amplifications (Table 1). Amplifications were done in a Rapid Cycler with the following program: 40 cycles, 30 s of denaturation at 94 °C, 30 s for annealing at 36 °C, and 70 s for elongation at 72 °C followed by 4 min at 72 °C after the last cycle. The electrophoresis procedure was the same as described above.

Band scoring

The gel images were saved as PC compatible files using the UVP Imagestore 500 system. The images were analysed using the Band Leader software (Aharoni, 1994). This program permits the comparison of different gels simultaneously. The molecular weight of all bands was determined and bands were scored for presence or absence using their molecular size. In the analyses, we assumed that bands of the same molecular weight represent the same DNA sequence, an assumption that appears to be appropriate at the intraspecific level (Thormann *et al.*, 1994). RAPD amplification can produce many bands, but our analyses are limited to bands between 550 and 2000 bp. Bands less than 550 were usually too faint to confidently score and it was difficult to determine the molecular weight of bands greater than 2000 bp using the 100 bp DNA reference ladder.

Analyses

Analyses were carried out with two data sets. The first set included 163 bands produced with the Coy thermocycler. The second set was the eighty-five bands that were produced by both the Rapid Cycler and Coy thermocyclers. Band frequencies were used to calculate the expected

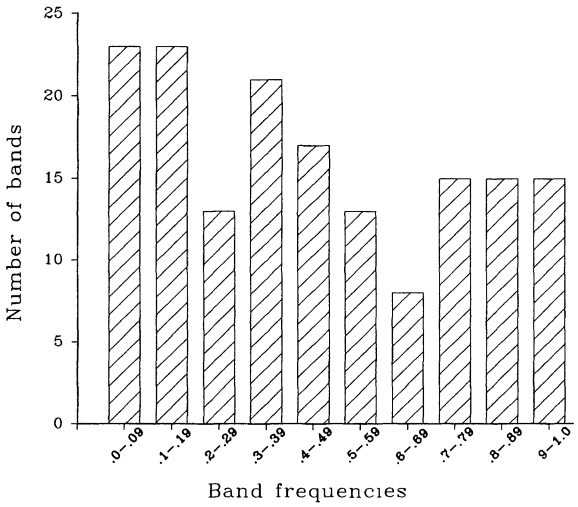

FIG. 2. Distribution of band frequency for ten populations of *Poulsenia armata*. A total of 163 bands were produced with ten primers.

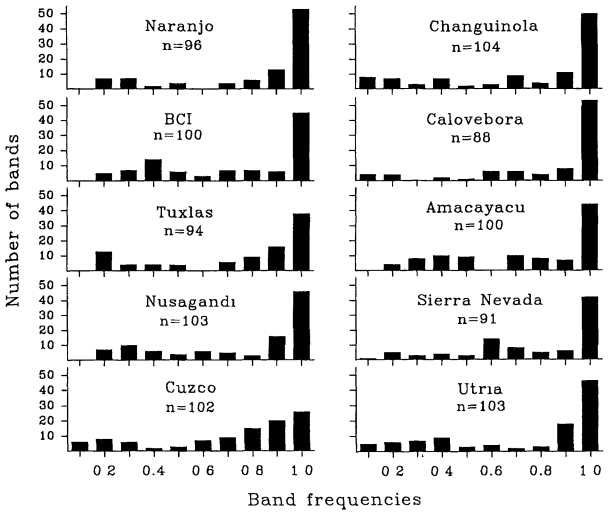

FIG. 3. Distribution of band frequency for each of the ten populations of *Poulsenia armata*. *N* equals the number of bands present in each population.

heterozygosity (Ht) under Hardy–Weinberg equilibrium following Lynch & Milligan (1994) for comparison with published allozyme studies. Band frequencies were also used to calculate Shannon's diversity index and to partition genetic diversity within and among populations (Chalmers *et al.*, 1992). Genetic distance matrices, using the Nei & Li (Nei & Li, 1979) and Euclidean (Excoffier *et al.*, 1992) formulas, were constructed and used as input files for an analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). In addition, Phylip 3.5 (Felsenstein, 1993) was used to determine the relationship among populations. Presence/absence data were analysed using Mixed parsimony (Wagner parsimony method). Frequency data were used to calculated Nei's genetic distance and trees were created with Neighbour-Joining (UPGMA method version 3.55c). In both analyses, the bootstrapping routine was used to create 100 trees. These trees were used by the consensus tree program to construct the majority rule consensus tree for the two data sets.

RESULTS

Genetic diversity

Amplifications with ten primers produced a total of 163 polymorphic bands (between 550 and 2000 bp) using a Coy thermocycler. Eighty-five bands were produced by both the Coy and Rapid Cycler thermocyclers (Table 1). The mean number of bands produced by each primer was 16.3 and ranged from eight (B-08) to twenty-two (B-01) with the Coy Thermocycler (Table 1). Approximately 28% of the bands had frequencies less than 0.2 and 18% of the bands had frequencies greater than 0.8 (Fig. 2). Eighty-seven bands (53%) had frequencies between 0.2 and 0.8 (Fig. 2). The distributions of band frequencies based on eighty-five or 163 bands were not significantly different ($\chi^2 = 6.67$, d.f. = 9, $P = 0.67$). Band frequencies for the individual populations (Fig. 3) showed a different pattern in comparison with the species band frequency distribution (Fig. 2). On average, the individual populations had ninety-eight of the 163 bands (Fig. 3). Within each population, the majority of bands occurred at high frequencies and few bands had intermediate frequencies (Fig. 3). Only eighty-eight bands were scored in the Calovebora population and most of these bands occurred at high frequencies (Fig. 3).

The mean expected heterozygosity (Ht) for both data sets (85 and 163 bands) was 0.15 (Table 2). Ht was 0.16 if only bands with frequencies less than 0.7 were considered (Lynch & Milligan, 1994; Table 2). Based on the three calculations of Ht, Cuzco Amazonico, Amacayacu, and Barro Colorado were the populations with the highest diversity (Table 2). Genetic diversity based on Shannon's H index showed a similar pattern with the same three populations having the highest diversity (Table 3). The mean diversity for the ten populations was 19.5 and 10.5 for 163 and eighty-five bands, respectively (Table 3). The Amacayacu population had one of the highest levels of diversity and was located in a potential Pleistocene refugium, but Naranjo, which was located in the proposed Choco refugium, had one of the lowest levels of diversity. The genetic results reject two of the predictions of the 'stepping stone model'. There was no relationship between distance from the either one of these potential refugium and genetic diversity (Amacayacu: $r = -0.08$, $P = 0.81$; Naranjo: $r = 0.38$, $P = 0.28$) and the most northerly population, Los Tuxlas, Mexico, did not have the lowest levels of genetic diversity. In addition, Barro Colorado, a Central American population, had a high level of total diversity (Tables 2, 3). The majority of unique bands occurred in the Central American populations, suggesting that these populations are not subsets of South American populations, refuting another prediction of the stepping stone model (Table 3).

The majority of the genetic diversity is distributed among populations (Table 3). Depending on the data set (163 or

TABLE 2. Estimates of expected heterozygosity (Ht) for *P. armata* calculated following Lynch & Milligan (1994) and estimates of Ht from allozyme studies.

Populations of *Poulsenia armata*	Number of bands		
	85[a]	163[b]	53[c]
Amazon			
Cuzco Amazonico	0.17	0.17	0.15
Amacayacu	0.17	0.16	0.19
Sierra Nevada	0.13	0.14	0.14
Choco			
Naranjo	0.14	0.12	0.15
Utria	0.12	0.14	0.14
Central America			
Nusagandi	0.12	0.14	0.12
Barro Colorado	0.18	0.15	0.19
Calovebora	0.12	0.16	0.19
Changuinola	0.15	0.13	0.16
Los Tuxlas	0.16	0.14	0.15
Mean	0.15	0.15	0.16

	Ht
Allozyme studies	
Poulsenia armata[d]	0.25
Tropical species[d]	0.21
Uncommon tropical speices[e]	0.14

[a] Estimate based on eight-five bands.
[b] Estimate based on 163 bands.
[c] Estimate based on fifty-three of 163 band with overall frequencies less than 0.70.
[d] Hamrick & Loveless (1989).
[e] Hamrick & Murawski (1991).

eighty-five bands), 33–36% of the variation occurred within populations and 64–66% occurred among populations (Table 3). The Calovebora population deviated from this pattern, with only 20–28% of the genetic diversity within the population. Barro Colorado (38%) and Amacayacu (39%) were the two populations with the highest within population diversity. The analysis of molecular variance (AMOVA) also showed that the majority of genetic variation was among populations (Table 4). The estimates of within and among population variation were virtually identical using the Nei & Li (1979) and Euclidean distance matrices (Table 4). Depending on the number of regional groups, the among populations variations ranged from 60.2 to 62.1% and the within population variations ranged from 37.9 to 38.4% (Table 4).

Relationship among populations

The analyses of molecular variation based on genetic distances did not show any significant regional effect when populations were divided into two (South America, Central America) or three (Amazon, Choco, Central America) regional groups (Table 4). Consensus trees, based on presence/absence data and Nei's genetic distance, did not show any obvious geographical relationship among populations (Fig. 4). Most of the branches occurred less than 60% and in neither tree do clades represent populations from the same geographical region.

DISCUSSION

Population genetics and refugia hypothesis

If lowland tropical tree species of Central America were restricted to refugia in South America during the Late

TABLE 3. Distribution of genetic diversity and number of unique bands in ten populations of *P. armata* based on 163 and eighty-five bands.

Population	Shanon's Diversity Index (H pop)		% Within population variation		% Among population variation		Number of bands unique to a single population	
	163	85	163	85	163	85	163	85
Amazon								
Cuzco Amazonico	24.3	12.0	41	41	59	59	0	0
Amacayacu	23.2	12.9	39	44	61	56	0	0
Sierra Nevada	19.0	9.0	32	31	68	69	1	0
Choco								
Naranjo	14.9	9.6	25	32	75	67	1	0
Utria	20.1	8.4	34	29	66	71	1	1
Central America								
Nusagandi	20.6	8.3	35	28	65	72	1	0
Barro Colorado	22.7	13.4	38	46	62	54	2	0
Calovebora	11.7	8.2	20	28	80	72	3	0
Changuinola	19.3	11.7	33	40	67	60	2	0
Los Tuxlas	19.0	11.4	32	39	56	61	2	1
Mean	19.5	10.5	33	36	67	64		
Total	59.1							

TABLE 4. Analysis of Molecular Variance (AMOVA) for ninety-two individuals, from ten populations of *P. armata*, using eighty-five RAPD markers. In the first analysis no regional groups were defined. In the second analysis the populations were divided into two groups: Central America ($n = 5$) and South America ($n = 5$). In the third analysis the populations were divided into three groups: Central America ($n = 5$), Choco ($n = 2$), and Amazon ($n = 3$).

Number of groups	Source of variation	*df*	SSD	MSD component	Variance	% Total	*P*-value
1	Euclidean distance						
	Population	9	930	103.4	10.5	61.8	$P < 0.009$
	Individuals	82	535	6.5	6.5	38.2	
	Nei and Li distance						
	Population	9	865	96.2	9.8	61.6	$P < 0.009$
	Individuals	82	501	6.1	6.1	38.4	
2	Euclidean distance						
	Group	1	117	117.4	0.3	1.8	$P = 0.21$
	Populations	8	813	101.6	10.4	60.2	$P < 0.009$
	Individuals	82	535	6.5	6.5	37.9	$P < 0.009$
	Nei and Li distance						
	Group	1	108	108.7	0.3	1.8	$P = 0.21$
	Populations	8	757	94.6	9.6	60.1	$P < 0.009$
	Individuals	82	501	6.1	6.1	38.1	$P < 0.009$
3	Euclidean distance						
	Group	2	207	103.5	0.0	−0.2	$P = 0.42$
	Populations	7	724	103.4	10.6	61.9	$P < 0.009$
	Individuals	82	535	6.5	6.5	38.3	$P < 0.009$
	Nei and Li distance						
	Groups	2	189	94.7	−0.1	−0.6	$P = 0.6$
	Populations	7	676	96.6	9.9	62.1	$P < 0.009$
	Individuals	82	501	6.1	6.1	38.5	$P < 0.009$

Pleistocene we expected that the stepping stone model would explain the distribution of genetic diversity in present day populations. This would result in low among population variation and a decrease in total genetic diversity with distance from the source pool. The distribution of genetic diversity of *P. armata* does not support this hypothesis. Among population variation was high and there was no relationship between distance from two proposed refugia and genetic diversity. The high among population variation suggests that these populations have been isolated and that there is little gene flow. In addition, some of the Central American populations had high levels of diversity and unique bands, which also contradict the stepping stone model.

Although our results are based on DNA and the majority of previous studies have used allozymes, these genetic markers usually detect similar patterns (Puterka *et al.*, 1993; Heun *et al.*, 1994; Peakall *et al.*, 1995). The estimates of genetic diversity for *P. armata*, using RAPDs, are within the range of other tropical trees, although Hamrick & Loveless (1989) reported slightly higher estimates for *P. armata* possibly due to a larger sample size (Table 2). Allozyme studies of tropical trees have shown that the majority of genetic diversity is found within populations (Hamrick & Loveless, 1989). These studies have suggested that long distance pollen movement (Hamrick & Murawski, 1990) and animal seed dispersal (Hamrick & Loveless, 1989; Rocha & Lobo, 1996) contribute to high levels of gene flow which reduces the among population variation (Hamrick & Loveless, 1989). Tropical trees tend to have high levels of outcrossing (O'Malley & Bawa, 1987; Loveless, 1992; Murawski & Bawa, 1994; Boshier *et al.*, 1995) which are associated with high gene flow and low among population variation (Loveless & Hamrick, 1984; Loveless, 1992). Contrary to these previous studies we detected high levels of among population variation.

The variation in the degree of population differentiation could be related to the spatial scale of the different studies. In the present study, populations of *P. armata* were sampled from Peru to Mexico and the minimum distance between populations was 150 km. Most population genetic studies of tropical trees have focused on a single population or multiple populations separated by < 10 km (O'Malley & Bawa, 1987; Hamrick & Loveless, 1989; Murawski & Bawa, 1994; Boshier *et al.*, 1995; Loiselle *et al.*, 1995). Studies that have compared populations over a larger spatial scale in Costa Rica have measured low population differentiation in *Pithecellobium elegans* (Hall *et al.*, 1996), *Enterolobium cyclocarpus* (Rocha & Lobo, 1996), and *Carapa guianensis* (Hall *et al.*, 1994*b*), but high population differentiation was detected in *Pentaclethra macroloba* (Hall *et al.*, 1994*a*). A comparison of populations of *Cordia alliodora* from Guatemala to Costa Rica found low levels of among population variation, but there were significant differences between Atlantic and Pacific populations (Chase *et al.*, 1995). Differentiation was even greater when populations of *Acacia mangium* were compared across the distribution in South-East Asia and Australia (Moran *et al.*, 1989*a*). Although studies at larger spatial scales are expected to detect greater population differentiation, more studies over larger geographical areas are need to determine the generality of these results.

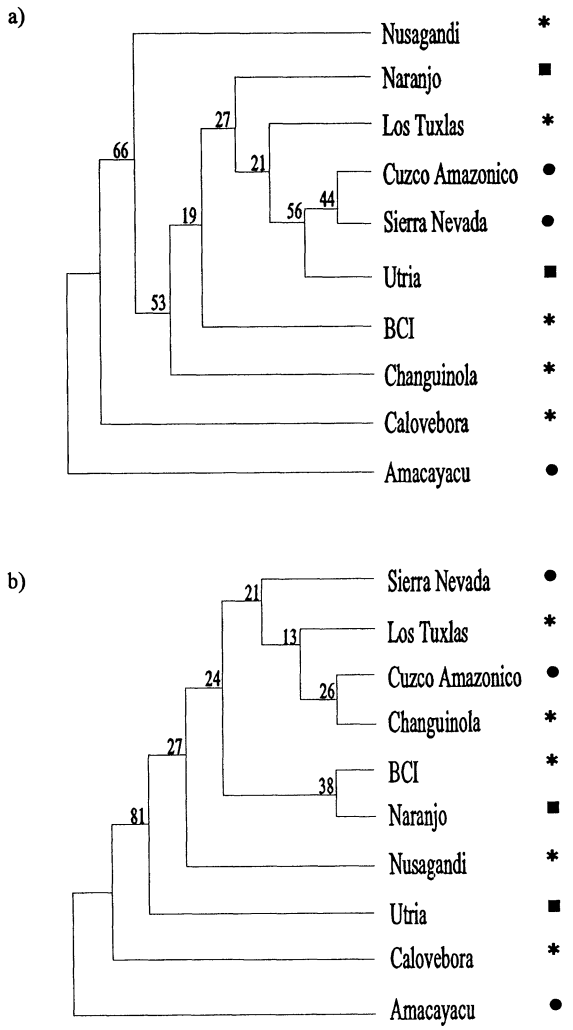

FIG. 4. Consensus trees base on: (a) 100 bootstraped maximum parsimonly trees, and (b) 100 bootstrapped Nei genetic distance–UPGMA Neighbour joining trees. The numbers on the branches indicate the number of times out of 100 that the clade occurred. '★' represents the Central American populations, '■' the Choco populations, and '●' the Amazon populations.

Gene movement has been detected up to 2–3 km (Hamrick & Loveless, 1989), but it is not clear to what extent it will occur over greater distances. Species that have a large geographical range do not necessarily have a continuous distribution and if pollen and seed movement are restricted this could lead to population differentiation. For example, Hamrick & Loveless (1989) demonstrated high gene movement in *P. armata* in three populations on Barro Colorado Island and high within population variation, but when we compared the Barro Colorado Island population with more distant populations there was high among population variation. High levels of among population variation in *P. armata* may be due to a distribution that is associated with riparian communities as has been suggested for *Acacia auriculiformis* (Moran *et al.*, 1989*b*). Riparian species may have high levels of gene flow within a watershed, but little gene movement between watersheds. The mosaic distribution of soils, variation in climate, geographical barriers, and historical events are other factors that can create disjunct species distributions and effect the genetic structure. Many other tropical trees have large geographical distributions and it is possible that similar patterns of population differentiation may occur.

The high levels of genetic diversity in the Central American populations of *P. armata* are consistent with the hypothesis that lowland tropical forest were present in the region during the late Pleistocene (Bush & Colinvaux, 1990; Kellman & Tackaberry, 1997). If lowland forest occurred within Central America, it is not necessary to invoke extremely high dispersal rates to explain the present day distribution of lowland forest. The Pleistocene Refugia Theory predicts that extreme aridity would have restricted lowland tropical forest to areas near the equator and savanna/shrub vegetation would have replaced lowland forest. Although some studies have documented this shift in vegetation (Kershaw, 1978; Leyden, 1985; Giresse *et al.*, 1994), these studies occurred near the extremes of the distribution of lowland tropical forest (Kellman & Tackaberry, 1997). The Peten region of Guatemala, was dominated by savanna/shrub vegetation in the Late Pleistocene, but at the beginning of the Holocene there was a quick change to lowland tropical species (Leyden, 1984). This suggests that lowland species migrated from nearby sites and not the proposed refugia thousands of kilometres away. Palaeoecological studies in Central Panama have shown shifts in vegetation composition with montane species occurring at lower elevation, but some lowland species were present throughout the Late Pleistocene (Bush & Colinvaux, 1990; Bush *et al.*, 1992). Although there is evidence for cooler temperature ($\approx 5\,^{\circ}C$) in Central America there is little evidence for extreme aridity (Bush & Colinvaux, 1990; Bush *et al.*, 1992). These observations suggest that the reduction in precipitation was not sufficient to eliminate lowland forest from Central America, but the limited number of adequate palynological site has made it difficult to predict the distribution of this forest type (Leyden, 1984).

Bush & Colinvaux (1990) and Bush *et al.* (1992) argued that lowland forest did not respond as a community to late Pleistocene climate change, but that each species had a unique response. Some lowland species could tolerated 5 °C decrease in temperature and did not migrate to lower elevations (Bush & Colinvaux, 1990; Bush *et al.*, 1992). Other species that were more sensitive to changing temperatures may have migrate to lower elevations. Meave *et al.* (1991) and Kellman *et al.* (1996) have suggested that under these cooler and possibly drier conditions, many species maintained populations in riparian habitats. High humidity, low frequency of fires, and fertile soils are some of the characteristics that may have permitted lowland species to remain in riparian habitats in Central America (Meave *et al.*, 1991).

Although the genetic data are from a single species, *P. armata*, we believe that these results are representative for other wet forest species. Presently, *P. armata* occurs in areas of high rainfall and is usually associated with riparian vegetation (T. M. Aide personal observation; Iremonger *et al.*, 1995) or areas of high soil fertility (Gentry, 1993). In

Panama, *P. armata* occurs mainly in tropical wet forest along the Caribbean coast. The distribution extends into tropical moist forest and a population occurs on Barro Colorado Island (BCI) where it occurs in areas of high soil moisture. During the 1982 El Niño, which caused an extended dry season on BCI, *P. armata* had the second highest rate of mortality in comparison with forty-six common species (Hubbell & Foster, 1990). This species appears to be very sensitive to conditions of water stress. If *P. armata* mainly occurs in the riparian zone today and was able to survive during the drier Late Pleistocene within Central America, then other wet forest species may have survived in the riparian zones as well.

Conservation implications

Studies of tropical trees at a local scale have demonstrated that gene flow is high and much of the genetic diversity is represented in individual populations (O'Malley & Bawa, 1987; Hamrick & Loveless, 1989), but the present study shows that among population variation can be high when populations are compared across larger distances. The high among population diversity in *P. armata* demonstrates that each population contributes to the overall genetic diversity of the species by having a unique combination of bands. The uniqueness of the individual populations suggests that conservation efforts directed only at species preservation can result in the lose of genetic diversity. The possible isolation of species in riparian forest during the Late Pleistocene, may have contributed to the high among population variation in many species within Central America.

Riparian forests are important habitats for many species. As tropical forests become more fragmented due to deforestation these areas play a crucial role in providing habitats and corridors between forest patches. In addition to their importance in conserving biological diversity, riparian forests also provide important ecosystems functions (e.g. carbon storage, erosion control) in ecological time (Naiman *et al.*, 1993) and riparian forests may have helped to maintain and create biodiversity in evolutionary time. Riparian forest are typically narrow, but can have high species diversity, and palaeoecological data suggest that this diversity can be maintained for thousands of years, even during dry periods (Kellman *et al.*, 1996). These riparian forest patches are very different from patches created by deforestation. The edge effects in recently formed patches can change the microclimate (Kapos, 1989) and permit fires to enter the forest (Uhl & Buschbacher, 1985). Patches of riparian forest differ in that they are protected by a river, have high levels of humidity, and often occur within steep ravines (Meave *et al.*, 1991). These factors contribute to making riparian forest an important habitat during periods of climate change.

Changes in the global climate during the next 50–100 years could affect the distribution of tropical vegetation (Peters, 1991). If riparian forests played a critical role in maintaining biodiversity for more than 10,000 years in the past (Kellman *et al.*, 1996), then we expect that these forest may be able to do the same in the future. Unfortunately, few National Parks and Reserves in Central America occur along the Atlantic coast, an area of potential refugia. The establishment of reserves in this region would be an important step in conserving the biodiversity of the Central American flora and fauna.

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