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Chloroplast DNA Microsatellites Reveal Contrasting Phylogeographic Structure in Mahogany (*Swietenia macrophylla* King, Meliaceae) from Amazonia and Central America

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Abstract Big-leaf mahogany (*Swietenia macrophylla* King) is one of the most valuable and overharvested timber trees of tropical America. In order to better characterize geographic patterns of genetic variation, we performed a phylogeographic analysis of *S. macrophylla* based on six polymorphic chloroplast genome simple sequence repeat loci (cpSSRs) analyzed in 16 populations (N=245 individuals) distributed across Central America and the Brazilian Amazon. Of the 31 total cpDNA haplotypes identified, 16 occurred in Central America and 15 in Amazonia with no single haplotype shared between the two regions. Populations from Central America showed moderate differentiation (F_{ST}=0.36) while within population genetic diversity was generally high (mean Nei's H_E=0.639). In contrast, the Amazonian populations were strongly differentiated (F_{ST}= 0.91) and contained relatively low genetic diversity (mean H_E=0.176), except for one highly diverse population (H_E= 0.925) from eastern Amazonia. Spatial analysis of molecular variance (SAMOVA) identified a single Central American phylogroup and four Amazonian phylogroups, indicating stronger phylogeographic structure within Amazonia. The results demonstrate distinctive regional patterns of *S. macrophylla* differentiation, and the first evidence of a strong phylogeographic break between Central American and South American mahogany populations. We suggest that the frequent occurrence of hurricanes in Central America, the differences in the glacial histories and in the duration and intensity of anthropogenic disturbance during

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the late Holocene may have played important roles in the geographic structuring of cpDNA lineages in the two regions. The high private haplotype diversity in Brazilian populations suggests that cpSSRs can be used as DNA barcodes for regional timber certification.

Keywords Amazon basin · Mesoamerica · Tropical trees · Big-leaf mahogany · cpSSRs · Phylogeography · DNA barcodes · SAMOVA

Abbreviations

cpDNA	chloroplast genome
cpSSR	simple sequence repeats of the chloroplast genome
AMOVA	Analysis of molecular variance
SAMOVA	Spatial analysis of molecular variance
CITES	Convention on International Trade in Endangered Species

Introduction

Mahogany, *Swietenia macrophylla* King (Meliaceae), is the most valuable hardwood species in Neotropics and is seriously threatened owing to over-exploitation and habitat destruction. S*wietenia macrophylla* has a wide geographic range from Mexico through Central America and across the southern arc of the Amazon basin in Bolivia and Brazil (Lamb 1966; Pennington 1981). The species has wide ecological tolerance and occurs in a variety of habitats from wet to seasonally dry, evergreen to deciduous, tropical to subtropical forests, with typically 800–2,500 mm of annual rainfall and at altitudes ranging from sea level to 1,400 m (Lamb 1966; Whitmore 1983). However, the species reaches its optimum natural development in tropical dry forests (Lamb 1966; Holdrige et al. 1971). Mahogany tends to occur in widely scattered patches and its density within patches is typically less than one commercial-size tree per hectare (Whitmore 1983; Verissimo et al. 1995). This patchy distribution is probably related to its mode of regeneration that requires major disturbances such as river course changes, hurricanes, blowdowns, and fire (Snook 1996). In these situations, stands may be comprised of one or a few cohorts (Grogan et al. 2003).

Swietenia macrophylla has been exploited throughout its natural range since the beginning of the 20th century (Lamb 1966; Rodan et al. 1992). In recent decades, with the depletion of natural stands in Central America, most of the extraction has come from populations in South America, especially in the Brazilian Amazon. Mahogany extraction is based on selective logging, which usually removes only the tallest trees of good form and with a dbh (diameter at breast height) >80 cm (Verissimo et al. 1995; Gullison et al. 1996). In addition to removing the most fecund trees, selective logging may have a significant impact on genetic structure and population size, and compromise the evolutionary viability of natural mahogany populations (Cornelius et al. 2005). The inclusion of *S. macrophylla* in CITES Appendix II in 2002 aimed to control international trade by ensuring that logging will not be detrimental to the survival of the species (Grogan and Barreto 2005).

Studies of the organization of genetic diversity of *S. macrophylla* have been carried out in the Brazilian Amazon (Lemes et al. 2003) and in Central America (Novick et al. 2003) using nuclear microsatellite DNA markers. The studies sampled populations across a similar spatial scale (ca. 1600 km in Central America; 2103 km in Amazonia) using seven loci in common. Both studies showed significant isolation by distance patterns, and moderate levels of population differentiation (R_{ST}). Furthermore, the Central American populations exhibited significantly lower mean genetic diversity than the Amazonian populations, which Novick et al. (2003) suggested may have resulted from the smaller, more dissected nature of suitable habitat in Central America, combined with more severe vegetation changes during the glacial phases of the Pleistocene.

A phylogeographic approach based on chloroplast genome (cpDNA) variation can provide additional insight into the historical patterns of genetic divergence across the range of *S. macrophylla*. Chloroplast DNA is a haploid genome and is maternally inherited in the majority of the angiosperms (Birky 1995; McCauley 1995; Ennos et al. 1999). Because of its four-fold smaller effective population size, chloroplast markers can often detect geographic structure that is not apparent in nuclear DNA markers (Cavers et al. 2003; Petit et al. 2005). Unfortunately the relatively low rates of nucleotide substitution in the chloroplast genome (Wolfe et al. 1987) have often impeded its use in phylogeographic studies (Schaal et al. 1998). However, highly variable mononucleotide repeat loci in the chloroplast genome (cpDNA microsatellites or simple sequence repeats [cpSSRs]) have provided a rich source of variation for studies of phylogeography and gene flow (Provan et al. 2001).

In addition to its utility for phylogeographic studies, conservation and management purposes, cpSSRs may be useful as regionally distinct cpDNA barcodes that could permit forensic verification of timber origins (Deguilloux et al. 2002). Here we report on the phylogeographic structure of *S. macrophylla* populations sampled in Central America (Novick et al. 2003) and Amazonia (Lemes et al. 2003) based on cpDNA microsatellites. The main aims of the study were: (1) to evaluate the utility of chloroplast microsatellite loci for assessing intraspecific variation in

S. macrophylla; (2) to quantify and compare the organization of genetic diversity of *S. macrophylla* populations in Central America and Amazonia ; and (3) to determine if population-specific cpDNA haplotypes are credible as regional DNA barcodes for monitoring timber harvests.

Results

Genetic Variation

Six out of 10 cpSSR loci initially assayed using universal primers (Weising and Gardner 1999) successfully amplified and were found to be polymorphic for *S. macrophylla*. All individuals (n=245) from eight Central American and eight Amazonian populations were analyzed for these six polymorphic cpSSR loci (ccmp 2, ccmp 3, ccmp 4, ccmp 5, ccmp 7, ccmp 10).

A total of 31 different haplotypes and 30 cpSSR alleles were found. The composition of the haplotypes and their occurrence in the populations are given in Table 1. The number of size variants (alleles) per locus varied from three to six. Gene diversity indices (H_E) showed a high range of variation across populations (0.000 to 0.925) (Table 2).

Structuring and Geographical Distribution of Haplotypes

The hierarchical analysis of genetic variation within and among populations performed for each geographical region (Amazonia and Central America) showed contrasting patterns. Most of the variation found in Amazonia was partitioned among populations (91%), while in Central America most variation was partitioned within populations (64%, Table 3).

The pattern of cpSSR haplotype organization provides evidence of a strong phylogeographic break between *S. macrophylla* populations in Central America and Amazonia. Of the 31 haplotypes detected, 15 occurred exclusively in Amazonian populations and the remaining 16 in Central America (Tables 1 and 2), with no single haplotype shared between the two geographical regions. A maximum parsimony median-joining network (Fig. 1), based on the 31 cpDNA haplotypes, exhibited a sole median vector and a total of 69 mutations, 38 of which occurred along the long branch separating the Central American and Amazonian haplotype clusters. The median vector connected the single Boca do Acre haplotype 17 with the other Amazonian haplotypes. Haplotype 17 was separated by 19 mutations from the closest Central American haplotype from Panama (haplotype 7). The long branch between the Central American and Amazonian clusters is further evidence of a deep phylogeographic break.

The organization of haplotype diversity within the two geographical regions also differed. The populations from Central America exhibited a relatively low level of differentiation (F_{ST}=0.36) compared to Amazonian populations (F_{ST}= 0.91) but genetic diversity of populations was generally high (H_E ranging from 0.233 to 0.857). Some common haplotypes were shared among distantly separated Central American populations. For example, haplotypes 4 and 5 were sampled in all eight Central American populations. Haplotype 3, exhibited by 21 individuals and closely related to haplotype 4, was also widespread, occurring in four populations. These three widely distributed haplotypes represented 60% of the individuals sampled in Central America. Despite the generally weak genetic structure observed in Central American populations, haplotypes 9–16 formed a cluster comprised of individuals from the Pacific region of Panama, Costa Rica, and Guatemala, and a few individuals from a north-central Costa Rican population (El Parque).

Consistent with its higher level of population differentiation (F_{ST}=0.91), there were few widespread haplotypes in Amazonia. The most common and widely distributed haplotype 28 was sampled in three adjacent populations (Pimenta Bueno, Cahoeira Parecis E, Resex Chico Mendes). Four Amazonian populations were fixed for one haplotype and three other populations exhibited only two haplotypes. Haplotype diversity within populations was relatively low (mean H_E=0.176), with the exception of Marajoara, which contained nine haplotypes among the sixteen individuals sampled (H_E=0.925). One of haplotypes found in Marajoara was shared with neighboring Agua Azul, located 107 Km to the north. Populations from the western Amazon tended to cluster genetically, except for Boca do Acre, which was relatively isolated in the network and clustered with eastern rather than western populations. Haplotypes from southernmost Amazonian population (Pontes e Lacerda) tended to occupy network tips.

Spatial analysis of molecular variance (SAMOVA) indicated the most likely presence of five genetic groups (F_{CT}= 0.24, P<0.05). Under all values of K, Central American populations grouped together. With K=5, the populations grouped as follows: 1 – Central American populations, 2 - Boca do Acre, 3 - Marajoara, Agua Azul, 4 - Pontes e Lacerda, 5 - Cachoeira Parecis A, Cachoeira Parecis E, Resex Chico Mendes, Pimenta Bueno (Figs. 1 and 2). These haplotype-defined genetic groups tended to cluster the most geographically proximate populations with the exception of Resex Chico Mendes, which grouped with the populations at Cachoeira Parecis and Pimenta Bueno rather than the closer Boca do Acre.

Discussion

The cpSSR haplotype data revealed a strong phylogeographic break between *S. macrophylla* in Central America

Table 1 Chloroplast microsatellite haplotypes observed in *S. macrophylla* populations from Central America and Amazonia. Haplotype, cpSSR loci, population (numbers represent population identification as in Table 2), and *N* = number of individuals. The numbers under the loci represent allele length in nucleotides

Haplotype #	ccmp2	ccmp3	ccmp4	ccmp5	ccmp7	ccmp7	Population	N
1	211	104	123	95	129	111	5, 6	2
2	210	104	123	94	129	111	3, 7	3
3	211	104	123	94	129	111	3, 4, 5,	21
4	211	104	123	94	130	111	1, 2, 3, 4, 5, 6, 7, 8	18
5	211	104	123	95	130	111	1, 2, 3, 4, 5, 6, 7, 8	24
6	211	102	122	92	130	111	2	1
7	211	104	123	94	131	111	8	2
8	211	104	123	95	131	111	8	1
9	211	104	125	94	130	111	8	2
10	211	104	125	94	129	111	4, 6, 7	5
11	210	104	125	94	129	111	6	1
12	211	105	125	94	129	111	4, 6, 7	14
13	210	105	125	94	129	111	7	1
14	211	105	125	94	130	111	8	8
15	211	104	123	94	130	111	4	1
16	211	104	130	94	130	111	4	1
17	211	104	123	94	150	111	16	28
18	211	103	124	95	149	111	9	1
19	210	103	124	95	149	111	9	1
20	210	103	124	95	149	113	9	1
21	210	103	124	95	150	114	9	2
22	210	103	124	98	150	114	9	1
23	211	103	124	98	148	111	9	1
24	211	103	124	95	148	111	9, 10	21
25	210	103	124	95	148	111	9	2
26	210	103	126	95	148	111	9	2
27	211	103	124	94	148	110	12	16
28	211	103	123	93	148	110	11, 13, 14	46
29	212	103	123	93	148	110	11, 14	2
30	211	102	123	93	148	109	15	13
31	211	102	122	93	148	109	15	3

and Amazonia. Similarly large phylogeographic breaks between cis- and trans-Andean populations have been reported for other rain forest tree species (e.g. Dick et al. 2003; Dick and Heuertz 2008; Hardesty et al. 2010). There were also notable differences in the distribution of cpDNA variation within Central America and Amazonia. Central American populations harboured widespread haplotypes that occurred from Mexico to Panama. The Amazonian haplotypes, on the other hand, were more localized and most cpDNA variation was partitioned among populations. This pattern is not likely to be explained by sampling effects, since the sample sizes in Brazil were consistently high (>16 individuals per population) where differentiation was also the highest.

Our results showed some inconsistency with the nuclear SSR (nSSR) analyses of Lemes et al. (2003) and Novick et al. (2003). The nSSR data from Central America showed phylogeographic structure in the form of high levels of differentiation (R_{ST}) across geographic barriers (Novick et al. 2003). Central American populations also had relatively low allelic richness per locus (mean 13 alleles/locus) compared to the Amazonian populations (mean of 18 alleles/locus). In contrast, there was no discernible phylogeographic structure in the Central American cpSSR data and the haplotype diversity (16 haplotypes) was similar to levels found in the Brazilian Amazonia (15 haplotypes). Some of the discrepancy between these results may be explained by differences between the nuclear and chloroplast genomes. First, genetic drift is expected to act more strongly on the chloroplast because of its fourfold lower effective population size. Furthermore, the cpDNA results reflect the sorting of a single genetic locus, whereas the nSSR results were summed over seven nSSR loci and thus provide several independent estimates of population genetic structure.

Geographic structuring of the cpSSR haplotypes does not appear to correspond with contemporary climatic or

Table 2 Sampling locations and genetic variation estimates of the 16 *S. macrophylla* studied populations based on six cpSSR loci. Population number (#); location; country; geographic coordinates (Lat. and Long.); *N* = number of individuals; A = number of alleles observed; N_H = number of haplotypes; and gene diversity (H_E) (±SE)

Pop #	Location	Country	Lat.	Long.	N	A	N_H	Gene diversity (± SE)
01	Nuevo Becal	Mexico	18°48′ N	89°19′W	4	7	2	0.500 (0.265)
02	Las Cuevas	Belize	16°42′ N	88°58′W	6	10	3	0.600 (0.215)
03	Bethel	Guatemala	16°55′ N	90°55′W	16	9	4	0.233 (0.126)
04	Tikal	Guatemala	17°13′ N	89°37′W	16	11	6	0.683 (0.120)
05	Mukuwas	Nicaragua	14°02′ N	84°29′W	16	8	4	0.642 (0.103)
06	Santa Rosa	Costa Rica	10°58′ N	84°46′W	17	11	7	0.853 (0.053)
07	El Parque	Costa Rica	10°53′ N	85°35′W	14	11	6	0.857 (0.056)
08	Tonosi	Panama	7°20′ N	80°29′W	16	10	6	0.742 (0.104)
09	Marajoara, Redenção, PA	Brazil	7°50′S	50°16′W	16	13	9	0.925 (0.047)
10	Água Azul do Norte, PA	Brazil	6°54′S	50°16′W	16	6	1	0.000 (0.000)
11	Pimenta Bueno, RO	Brazil	12°22′S	61°26′ W	16	7	2	0.125 (0.106)
12	Cachoeira Parecis A, RO	Brazil	12°30′S	61°30′W	16	6	1	0.000 (0.000)
13	Cachoeira Parecis E, RO	Brazil	12°34′S	61°30′W	16	6	1	0.000 (0.000)
14	RESEX Chico Mendes, AC	Brazil	10°25′S	69°18′W	16	7	2	0.125 (0.106)
15	Pontes e Lacerda, MT	Brazil	15°05′S	59°09′W	16	7	2	0.233 (0.126)
16	Boca do Acre, AM	Brazil	8°43′S	68°43′W	28	6	1	0.000 (0.000)

altitudinal barriers in Central America. The occurrence of widespread cpDNA haplotypes across Central America strongly implies a role of long distance dispersal and suggests that mountains have not been effective barriers to mahogany seed dispersal here. Hurricanes, which are frequent in Central America, can carry the winged seeds of mahogany over long distances and the accompanying wind throws are thought to play an important role in mahogany dispersal and establishment in this region (Snook 1996), which would lead to the present-day haplotype distribution. In the Amazon basin, on the other hand, hurricanes are absent or very rare.

On the other hand, topography may provide physical and climatic barriers for pollinator movements, as suggested by the significant divergence among Central American populations across geographical barriers found by Novick et al. (2003) using nSSR markers. Similarly, the divergences among three close populations from different valleys of the Parecis mountains in west-central Brazil, studied by Lemes et al. (2003) using nSSRs, were also highly significant, although these populations belong to the same cpSSR haplotype genetic group (group 5) in the present study.

A non-exclusive alternative explanation for to the observed phylogeographic structure in Central America is the severity of the impact of Pleistocene glaciations in the regional climate (Whitmore and Prance 1987) coupled with the relatively small areas of suitable habitat for mahogany establishment during that period. The reconstructed vegetation of lowland Central America between 20,000 and 10,500 B.P. (Piperno and Pearsall 1998) showed restricted areas with moist and dry forests and widespread thorn woodlands, low scrub, and wooded savanna vegetation in the region. These factors are expected to have caused local extinctions and much more dramatic reduction in effective population size for Central American than for Amazonian mahogany populations. Under this scenario, any ancient

Table 3 Analysis of molecular variance (AMOVA) based on six cpSSR loci for eight populations of *Swietenia macrophylla* from Central America and eight populations from Amazonia. The percentage of variation was estimated by two methods: (*) the number of different alleles (F_{ST}), and (#) the sum of squared size difference (R_{ST})

Source of variation	Percentage of Variation*	Percentage of Variation#	Genetic differentiation
Central America			F_{ST}=0.360; R_{ST}=0.359
Among populations	36.02	35.96	
Within populations	63.98	64.04	
Amazonia			F_{ST}=0.906; R_{ST}=0.842
Among populations	90.59	84.24	
Within populations	9.41	15.76	

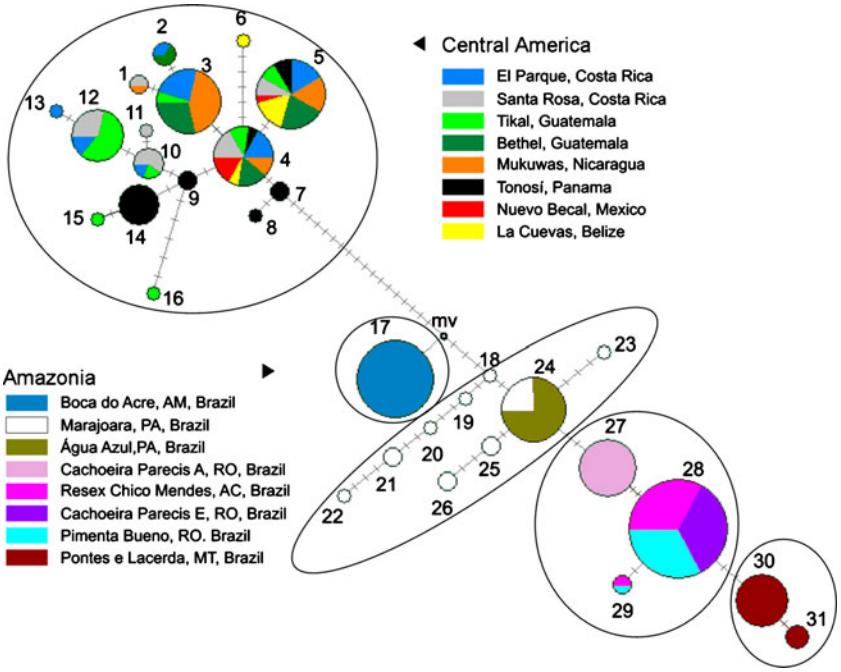

Fig. 1 Maximum parsimony median-joining network based on 31 haplotypes of *Swietenia macrophylla* from Central America and Amazonia. The sizes of the circles are roughly proportional to the haplotype frequencies. Numbers correspond to haplotypes. Colors indicate populations. Big circles represent five SAMOVA groupings

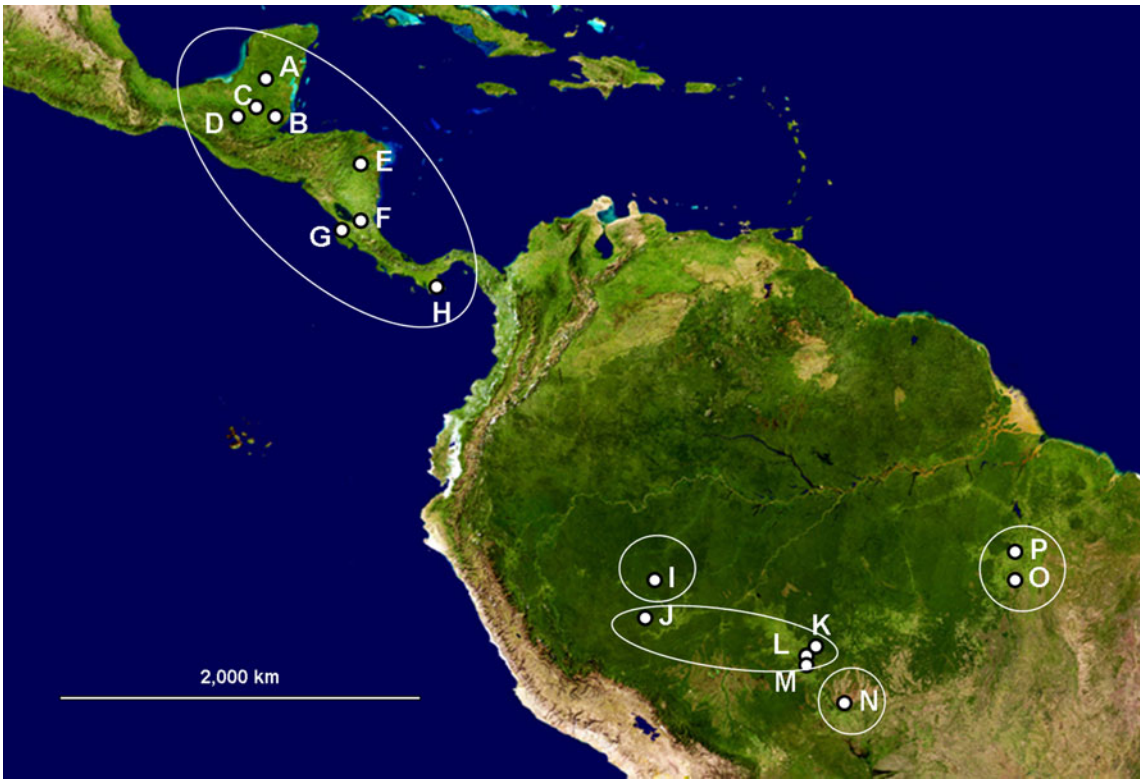

Fig. 2 Locations of the sixteen populations of S. *macrophylla* sampled in Central America and Amazonia . A - Nuevo Becal, B – Las Cuevas, C – Tikal, D – Bethel, E – Mukuwas, F- El Parque, G - Santa Rosa, H – Tonosi, I – Boca do Acre, J – Resex Chico Mendes, K – Pimenta Bueno, L - Cachoeira Parecis A, M - Cachoeira Parecis E, N - Pontes e Lacerda, O – Marajoara, and P – Agua Azul do Norte. Circles represent groups of populations based on haplotype distribution indicated by SAMOVA

signal of structuring and diversification would have been modified by Pleistocene vegetation changes. Thus, the current pattern of genetic variation may reflect only the most recent geographic expansion of a few founder haplotypes from a limited refugial source and the subsequent formation of newly derived haplotypes. It is worth noting the occurrence of a few rare and highly differentiated lineages (e.g. haplotypes 6 and 16) in Central America that may be a relict of the ancestral polymorphism.

The significantly lower number of nuclear microsatellite alleles and lower heterozygosity in Central American (Novick et al. 2003) than Amazonian populations (Lemes et al. 2003) suggests either a more recent geographic expansion, or lower effective population sizes in the more topographically dissected Central American region. Mating system analysis has shown that mahogany is somewhat tolerant of selfing (Lemes et al. 2007) and the lower nuclear microsatellite diversity in Central America may be influenced by ecological pressures favoring inbreeding in individuals colonizing new areas in this region.

In addition to the impacts of the Pleistocene glaciations, Holocene events may have contributed to the current phylogeographic pattern observed for mahogany in Central America. For at least 1500 years before European conquest, tens of millions of Pre-Columbian agriculturalists practiced shifting agriculture, cultivating maize and other light-demanding crops in this region (Denevan 1992). Notably, four of our sample sites (sites A-D) are in what was the most highly populated core Mayan zone of influence where several loosely associated city-states coexisted and rapid forest clearance began about 2800 B.P. (Hodell et al. 2000; Islebe et al. 1996). As a result of the intensive land use by these dense sedentary agrarian communities, Central America was probably covered, at the time of the European first arrival, by a mosaic of crop lands and abandoned fields with secondary vegetation at different successional stages. Despite the death of adult trees caused by forest clearances, this type of anthropogenic landscape will likely have enhanced dispersal of mahogany, a long-lived pioneer tree (Grogan et al. 2003), over the region. With the population decline accompanying the collapse of the Classic Mayan society between A.D. 800 and 900 (Hodell et al. 1995) and the demographic collapse experienced by Amerindian populations after European contact (Denevan 1992), human pressures were strongly curtailed and most of the fragmented landscape in Central America was abandoned. The subsequent large-scale forest regeneration (Nevle and Bird 2008) would also have accelerated the expansion of the remnant mahogany lineages in this region.

CpSSR variation in the peripheral Amazon basin exhibited a comparatively stronger phylogeographic structure than in Central America. Most of the Amazonian populations were fixed for one haplotype, or exhibited only a few related haplotypes. Spatial analysis indicates that there is significant within-region structuring in South America, primarily reflecting geographic proximity. The exception to this general pattern is the clustering of 'Resex Chico Mendes' with Rondonian populations rather than the more proximate 'Boca do Acre'. These two populations, 200 km apart and having the Juruá River as the sole geographical barrier between them, were probably derived from separate lineages. Resex Chico Mendes, located in the transitional zone between the Brazilian Shield and the Tertiary deposits of the Amazon basin was likely formed by lineages coming from the relict populations in the Serra dos Parecis mountains. Boca do Acre, located at the northwestern limit of the species distribution in Brazil, seems to be more genetically related to populations from eastern Amazonia.

It seems most likely that cycles of demographic expansion followed by population bottlenecks and isolation have shaped the phylogeographic pattern in the region. Clearly, there has been a distinctly different demographic history in Amazonia compared with Central America, with an older colonization suggested by longer branch lengths and a higher level of geographic isolation for the Amazonian populations as indicated by higher F_{ST}.

The current distribution of mahogany in the Amazonia is characterized by aggregations of trees in deciduous and semi-deciduous forests along an arc following the southern boundary of the basin (Grogan 2001; Grogan et al. 2002). These seasonally dry forests are areas of "ecological tension" between the Amazonian and Cerrado biomes and are bounded by the evergreen rain forests to the north and by savannas to the south. Seasonally dry forests, which provide optimum habitat for mahogany, probably expanded during the cool and dry glacial intervals (Pennington et al. 2000; Bush and Silman 2004; Bush and Oliveira 2006; Colinvaux et al. 1996, 2001) and mahogany population sizes would have been correspondingly larger and possibly more continuous in some areas. During the wetter and warmer Holocene, rainforests expanded south and eastwards replacing the deciduous forests and savannas in the Amazonian lowlands and in the foothills of the Brazilian Shield. Thus, it is possible that the retraction and isolation of mahogany populations since the end of the Last Glacial Maximum and the lack of long distance seed dispersal among the remaining mahogany aggregations have influenced phylogeographic structure in the Brazilian Amazon.

The high level of private haplotype diversity found in the Marajoara population, in contrast to other Amazonian populations, suggests additional intricacy to the pattern. One possible explanation concerns the stability of a dry and seasonal climate in this region, even during interglacial periods. Marajoara is located on the SW border of the dry transverse corridor that crosses central Amazonia in a NW-

SE direction, separating humid upper and lower Amazonia (Haffer 2008). The private haplotype diversity of the Marajoara population may be explained by the greater size and stability of the dry forests in this area, which could have led to the accumulation and maintenance of cpSSR diversity.

The Pre-Columbian cultivation practices in Amazonia also changed the environment in different ways especially along of the headwater basins of the main rivers coming from the northern flanks of the Brazilian Shield (Heckenberger et al. 2007). This region is dominated by semi-deciduous forests and represents the natural area of distribution of *S. macrophylla* in the Brazilian and Bolivian Amazon. Several small to medium-sized complex societies flourished in this broad region (Heckenberger et al. 2003; Erickson 2006) with earthworks, agricultural and parkland landscapes occurring around villages (Parssinen et al. 2009). However, the lower Amerindian density in this vast region, the large distances between the main settlements, the prevalence of a hunter-gatherer subsistence strategy in many groups, and the management of the landscape using agricultural systems that did not require intensive forest clearance (Denevan 2001; Balée 2006) suggest that the impact of anthropogenic disturbance in the southern Amazon during the Late Holocene was likely smaller than in Central America. One would expect that there were proportionally fewer anthropogenically-altered habitats available for mahogany colonisation in the Amazon basin than in Central America during Pre-Columbian times. Thus the present-day haplotype distribution in Amazonian mahogany appears to have been most likely shaped by Pleistocene events.

In addition to providing genetic evidence of regional demographic history, our study should also be useful for genetic conservation and management. In mahogany, important traits such as resistance to shoot borers, growth rate and degree of branching show heritable variation (Newton et al. 1999). The deep phylogeographic break between Central and South American mahogany populations suggests that there may be major genomic differences between these sources of mahogany. While the major provenance trials for mahogany (CATIE, Turrialba, Costa Rica) contain only Central American samples, our study strongly suggests that Central and South American provenances should be jointly studied for silviculture programs.

The findings also have relevance for the conservation of natural mahogany populations. Recent advances in DNA extraction technology permit genotyping of DNA from dried timber samples (Deguilloux et al. 2002). Using a DNA barcoding approach, it is possible to determine the species origin of tropical timbers. However, standard plant DNA barcodes often display little variation among closely related species, let alone between populations within a species (Dick and Kress 2009), making it difficult to determine the provenance of timber, which is essential in order to monitor illegal logging activities. Our study demonstrates that combinations of cpSSR loci can provide distinct regional cpDNA haplotypes for mahogany. With the six cpSSR loci in this population, it was possible to definitively assign samples to either Central American or South American provenances. With more loci, it should be possible to provide distinct genotypes at finer geographic scales. These DNA barcode approaches should be especially useful in the Amazon basin, which displays the highest level of cpDNA phylogeographic structure, and which contains the largest commercial and protected tracts of mahogany.

In summary, our data have highlighted a strong phylogeographic break and an intriguing contrast between Amazonian and Central American mahogany populations in terms of phylogeographic structure. In order to clarify the occurrence of points of historical dispersal between the two geographical regions, more extensive sampling in South America is needed, particularly from the Peruvian, Ecuadorian, Colombian and Venezuelan Amazon. Based on the data available so far we suggest that differences in glacial history for Central America and Amazonia may have been a key factor in determining these very divergent patterns. Differences in terms of duration and intensity of anthropogenic disturbance between the two regions during the late Holocene may have also affected the vegetation history and played an important role in structuring cpDNA lineages. In addition, these findings indicate that any in situ conservation program or germplasm collection initiatives for this valuable and endangered tree should take into consideration the distinct genetic structures shaped by the contrasting historical biogeography of the species in these regions.

Methods

Study Sites and Collection

Leaves of 245 plants were collected from eight populations from six countries in Central America and from eight populations spread across 2,100 km in the southern arc of the Amazon drainage basin in Brazil (Fig. 2). The samples were used in previous nuclear microsatellite analyses of Lemes et al. (2003) and Novick et al. (2003). In addition, new leaf material from adult trees was sampled from Boca do Acre in Brazil (N=28). The leaf material came from adult trees from natural populations in Amazonia except for Cach E and Agua Azul or from progeny arrays for the Central American populations and Cach E and Agua Azul, in which a single progeny was used as a proxy for an adult tree. Living material from the Central American popula-

tions is maintained by CATIE in Turrialba, Costa Rica. The leaves were dried in silica gel and stored at −20°C until DNA extraction.

Microsatellite Analysis

Total genomic DNA was extracted using a Fast Prep (Bio101 Corporation) following standard CTAB procedure (Doyle and Doyle 1987) or alternatively using Plant DNeasy kits (Qiagen Corporation, Valencia, CA). DNA quantification was performed by comparison with known concentrations of a DNA standard (Lambda DNA) in ethidium bromide-stained 1% agarose gels.

PCR was initially performed using 10 universal angiosperm primers developed by Weising and Gardner (1999) for cpSSR analysis in tobacco. Reactions were carried out in a total volume of 10 µl containing 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM dNTPs, BSA (2.5 mg ml^{-1}), 1.25 µM of each forward and reverse primers, 1 U Taq DNA polymerase and 5.0 ng of genomic DNA using a MJ Research Incorporated PTC 200 thermal cycler under the following parameters: (1) initial denaturation at 94°C for 4 min; (2) 30 cycles of denaturation at 94°C for 1 min, annealing at primer-specific T_m for 1 min and extension at 72°C for 1 min; (3) final extension at 72°C for 10 min. PCR products were eletrophoresed on 5% polyacrylamide gels in an Applied Biosystem Incorporated (ABI) Prism 377 sequencer and analysed with Genescan and Genotyper softwares (ABI). The cpSSR allele sizes were binned and normalized using *AlleloBin* software (Idury and Cardon 1997).

Data Analysis

Unique multi-locus combinations of cpSSR alleles (size variants) were considered as distinct haplotypes. Genetic diversity was estimated for each population based on the number of alleles (A), the number of haplotypes (N_H), and gene diversity index (H_E, Nei 1987). Partitioning of genetic variation within and among populations was tested for each geographical region (Amazonia and Central America) separately by analysis of molecular variation (AMOVA; Excoffier et al. 1992) using *Arlequin 2.001* (Schneider et al. 2001). The significance of the fixation index was tested with 1000 permutations. Relationships among the haplotypes were inferred using median-joining network analysis (Bandelt et al. 1999) implemented by *Network* software (Forster et al. 2000). Spatial structuring of variation at chloroplast loci was examined using SAMOVA (Dupanloup et al. 2002), considering values of K (phylogroup number) between 2 and 10, using 100 initial conditions for each run and the sum of squared size differences as a measure of molecular distance.

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References

Balée W (2006) The research program of historical ecology. Annu Rev Anthropology 35:75–98

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Birky CW (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci USA 92:11331–11338

Bush MB, Oliveira PE (2006) The rise and fall of the Refugial Hypothesis of Amazonian Speciation: a paleo-ecological perspective. Biota Neotropica 6(1):bn00106012006

Bush MB, Silman MR (2004) Observations on Late Pleistocene cooling and precipitation in the lowland Neotropics. J Quaternary Sci 19:677–684

Cavers S, Navarro C, Lowe AJ (2003) Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica. Mol Ecol 12:1451–1460

Colinvaux PA, De Oliveira PE, Moreno JE, Miller MC, Bush MB (1996) A long pollen record from lowland Amazonia: forest and cooling in glacial times. Science 274:85–88

Colinvaux PA, Irion G, Räsänen ME, Bush MB, Nunes De Mello JAS (2001) A paradigm to be discarded: geological and paleoecological data falsify the Haffer and Prance refuge hypothesis of Amazonian speciation. Amazoniana 16:609–646

Cornelius J, Navarro C, Whightman K, Ward S (2005) Is mahogany dysgenically selected? Environ Conserv 32:129–139

Deguilloux MF, Pemonge MH, Petit RJ (2002) Novel perspectives in wood certification and forensics: dry wood as a source of DNA. Proc R Soc Lond B Biol Sci 269:1039–1046

Denevan WM (1992) The pristine myth: the landscape of the Americas in 1492. Ann Assoc Am Geogr 82:369–385

Denevan WM (2001) Cultivated landscapes of Native Amazonia and the Andes. Oxford University Press, New York

Dick CW, Heuertz M (2008) The complex biogeographic history of a widespread tropical tree species. Evolution 62:2760–2774

Dick CW, Kress WJ (2009) Dissecting tropical plant diversity with forest plots and a molecular toolkit. Bioscience 59:745–755

Dick CW, Abdul-Salim K, Bermingham E (2003) Molecular systematics reveals cryptic Tertiary diversification of a widespread tropical rainforest tree. Am Nat 162:691–703

Doyle JJ, Doyle JL (1987) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dupanloup L, Schneider S, Excoffier L (2002) A simulated annealing approach to define de genetic structure of populations. Mol Ecol 11:2571–2581

Ennos RA, Sinclair WT, Hu X-S, Langdo A (1999) Using organelle markers to elucidate the history, ecology and evolution of plant populations. In: Hollingsworth PM, Bateman RM, Gornall RJ (eds) Molecular Systematics and Plant Evolution. Taylor & Francis Ltd, London

Erickson C (2006) Domesticated landscapes of the Bolivian Amazon. In: Balée W, Erickson C (eds) Time and complexity in historical

ecology: studies in the Neotropical lowlands. Columbia University Press, New York
Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. Genetics 131:479–491
Forster P, Bandelt HJ, Rohl A (2000) NETWORK 3.1.1.0. Software free available via www.fluxus-engineering.com. Fluxus Technology Ltd., Cambridge.
Grogan J (2001) Big-leaf mahogany (*Swietenia macrophylla* King) in southeast Para, Brazil: a life history study with management guidelines for sustained production from natural forests. PhD thesis, Yale University, New Haven, CT, USA
Grogan J, Barreto P (2005) Big-leaf mahogany on CITES Appendix II: big challenge, big opportunity. Conserv Biol 19:973–976
Grogan J, Barreto P, Verıssimo A (2002) Mogno na Amazonia Brasileira: Ecologia e Perspectivas de Manejo. IMAZON, Belém, PA
Grogan J, Ashton MS, Galvão J (2003) Big-leaf mahogany (*Swietenia macrophylla*) seedling survival and growth across a topographic gradient in southeast Pará, Brazil. For Ecol Manag 186:311–326
Gullison RE, Panfil SN, Strouse JJ, Hubbell S (1996) Ecology and management of mahogany (*Swietenia macrophylla* King) in the Chimanes Forest, Beni, Bolivia. Bot J Linn Soc 122:9–34
Hardesty BD, Dick CW, Hamrick JL, Degen B, Hubbell SP, Bermingham E (2010) Geographic influence on genetic structure in the widespread Neotropical tree, *Simarouba amara* (Simaroubaceae). Tropical Plant Biol 3(1):28–39
Haffer J (2008) Hypotheses to explain the origin of species in Amazonia. Braz J Biol 68(4, Suppl):917–947
Heckenberger MJ, Kuikuro A, Kuikuro UT, Russell JC, Schmidt M, Fausto C, Franchetto B (2003) Amazonia 1492: pristine forest or cultural parkland. Science 301:1710–1714
Heckenberger MJ, Russell JC, Toney JR, Schmidt MJ (2007) The legacy of cultural landscapes in the Brazilian Amazon: implications for biodiversity. Philos Trans R Soc Lond, Ser B 362:197–208
Hodell DA, Curtis JH, Brenner M (1995) Possible role of climate in the collapse of classic Maya civilization. Nature 375:391–394
Hodell DA, Brenner M, Curtis JH (2000) Climate change in the northern American tropics and subtropics since the last ice age. In: Lentz DL (ed) Imperfect balance: landscape transformations in the Precolumbian Americas. Columbian University Press, New York
Holdrige LR, Grenke WC, Hatheway WH, Liang T, Tosi JA (1971) Forest environments in tropical life zones: a pilot study. Pergamon Press, Oxford
Idury RM, Cardon LR (1997) A simple method for automated allele binning in microsatellite markers. Genome Res 7:1104–1109
Islebe GA, Hooghiemstra H, Brenner M, Curtis JH, Hodell DA (1996) A Holocene vegetation history from lowland Guatemala. Holocene 6:265–271
Lamb FB (1966) Mahogany of tropical America: its ecology and management. The University of Michigan Press, Ann Arbor
Lemes MR, Gribel R, Proctor J, Grattapaglia D (2003) Population genetic structure of mahogany (*Swietenia macrophylla* King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. Mol Ecol 12:2875–2883
Lemes MR, Grattapaglia D, Grogan J, Proctor J, Gribel R (2007) Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical tree species. Plant Ecol 192(2):169–179
McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol Evol 10(5):198–202
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
Nevle RJ, Bird DK (2008) Effects of syn-pandemic fire reduction and reforestation in the tropical Americas on atmospheric CO2 during European conquest. Palaeogeogr Palaeoclimatol Palaeoecol 264:25–38
Newton AC, Watt AD, Lopez F et al (1999) Genetic variation in host susceptibility to attack by the mahogany shoot borer, *Hypsipyla grandella* (Zeller). Agric For Entomol 1:11–18
Novick RR, Dick CW, Lemes MR, Navarro C, Caccone A, Bermingham E (2003) Genetic structure of Mesoamerican populations of big-leaf mahogany (*Swietenia macrophylla*) inferred from microsatellite analyses. Mol Ecol 12:2885–2893
Parssinen M, Schaan D, Ranzi A (2009) Pre-Columbian geometric earthworks in the upper Purus: a complex society in western Amazonia. Antiquity 83:1084–1095
Pennington TD (1981) A monograph of the Neotropical Meliaceae. In: Pennington TD, Styles BT, Taylor DAH (eds) Flora neotropica monograph 28: Meliaceae. The New York Botanical Gardens, New York
Pennington RT, Prado DE, Pendry CA (2000) Neotropical seasonally dry forests and Quaternary vegetation changes. J Biogeogr 27:261–273
Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Mol Ecol 14:689–701
Piperno DR, Pearsall DM (1998) The origins of agriculture in the lowland Neotropics. Academic, London
Provan J, Powell W, Hollingsworth PM (2001) Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends Ecol Evol 16:142–147
Rodan BD, Newton AC, Veríssimo A (1992) Mahogany conservation: status and policy initiatives. Environ Conserv 19:331–338
Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. Mol Ecol 7:465–474
Schneider S, Kueffer JM, Rosseli D, Excoffier L (2001) Arlequin version 2.001: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland
Snook LK (1996) Catastrophic disturbance, logging and the ecology of mahogany (*Swietenia macrophylla* King): grounds for listing a major tropical timber species in CITES. Bot J Linn Soc 122:35–46
Verissimo AC, Barreto P, Tarifa R, Uhl C (1995) Extraction of a high-value natural resource in Amazonia: the case of mahogany. For Ecol Manag 72:39–60
Weising K, Gardner RC (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. Genome 42:9–19
Whitmore JL (1983) *Swietenia macrophylla and S. humilis* (caoba, mahogany). In: Janzen DH (ed) Costa Rican natural history. University of Chicago Press, Chicago
Whitmore TC, Prance GT (1987) Biogeography and quaternary history in tropical America. Oxford Science Publications, Clarendon Press
Wolfe KH, Li WH, Sharp PM (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast and nuclear DNA. Proc Natl Acad Sci USA 84:9054–9058