Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica

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Abstract

Spanish Cedar (*Cedrela odorata* L.) is a globally important timber species which has been severely exploited in Mesoamerica for over 200 years. Using polymerase chain reaction–restriction fragment length polymorphisms, its chloroplast (cp) DNA phylogeography was studied in Mesoamerica with samples from 29 populations in six countries. Five haplotypes were characterized, phylogenetically grouped into three lineages (Northern, Central and Southern). Spatial analysis of ordered genetic distance confirmed deviation from a pattern of isolation by distance. The geographically proximate Northern and Central cpDNA lineages were genetically the most differentiated, with the Southern lineage appearing between them on a minimum spanning tree. However, populations possessing Southern lineage haplotypes occupy distinct moist habitats, in contrast to populations possessing Northern and Central lineage haplotypes which occupy drier and more seasonal habitats. Given the known colonization of the proto-Mesoamerican peninsula by South American flora and fauna prior to the formation of the Isthmus of Panama, it seems most likely that the observed population structure in *C. odorata* results from repeated colonization of Mesoamerica from South American source populations. Such a model would imply an ancient, pre-Isthmian colonization of a dry-adapted type (possessing the Northern lineage or a prototype thereof), with a secondary colonization via the land bridge. Following this, a more recent (possibly post-Pleistocene) expansion of moist-adapted types possessing the Southern lineage from the south fits the known vegetation history of the region.

Keywords: *Cedrela odorata*, differentiation, dispersal, Meliaceae, Spanish Cedar, universal cpDNA markers

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Introduction

Phylogeography examines the correspondence between genetic relationships and geographical distribution (Avise *et al.* 1987). Population genetic structure is as much a product of history as of present-day migration patterns and isolation of populations, hence a synthesis of gene-alogical data with independent information, including geology, palynology and archaeology (Avise *et al.* 1987; Bermingham & Moritz 1998) may disentangle the historical component of population structure from that which is the result of contemporary gene flow processes.

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In plants, the recent development of universal primer sets targeting noncoding regions of the chloroplast (cp) genome (Taberlet *et al.* 1991; Demesure *et al.* 1995; Dumolin-Lapegue *et al.* 1997b; Hamilton 1999) has revealed substantial amounts of intraspecific variation (Newton *et al.* 1999a), and cpDNA data have now been successfully used for several phylogeographic studies of plants (Petit *et al.* 1993; Petit *et al.* 1997; Caron *et al.* 2000; Dutech *et al.* 2000; Raspe *et al.* 2000). As a result of its usual maternal inheritance in angiosperms, cpDNA is transmitted only through seeds, and therefore has less potential for gene flow than nuclear genes, which can also move by pollen dispersal. Consequently, genetic variation in the chloroplast genome is often more highly geographically structured than that in the nuclear genome. Furthermore, as the rate of cpDNA

sequence evolution is slow (Wolfe *et al.* 1987), observed patterns reflect the outcome of processes over long time-scales (Ennos *et al.* 1999) so cpDNA is ideal for studying historical patterns of gene flow, in particular migration and colonization.

Several recent studies have taken advantage of these characteristics to investigate vegetation changes, in particular those related to glacial cycles. In Europe (*Alnus glutinosa*, King & Ferris 1998; *Quercus* sp., Dumolin-Lapegue *et al.* 1997a), North America (*Dryas integrifolia*, Tremblay & Schoen 1999; *Liriodendron tulipifera*, Sewell *et al.* 1996) and the tropics (*Vouacapoua americana*, Dutech *et al.* 2000; *Dicorynia guianensis*, Caron *et al.* 2000), cpDNA has been successfully used to detect spatiotemporal patterns of fragmentation and dispersal resulting from climatic variations during the Pleistocene epoch.

The region of interest in this study is tropical Mesoamerica between southern Mexico and northern Colombia, encompassing Guatemala, Honduras, Nicaragua, Costa Rica and Panama. Present day Mesoamerica is a region rich in diversity: there are still more than a quarter of a million square kilometres of primary vegetation, around 24 000 plant species (of which some 5000 are endemic) and nearly 3000 vertebrate species (of which over 1000 are endemic, Myers *et al.* 2000).

The distribution and composition of the Mesoamerican flora and fauna have been strongly influenced by geological and climatic events (Burnham & Graham 1999). Prior to the formation of the Isthmus of Panama, there was considerable interchange of flora between the separate land masses of North and South America (Raven & Axelrod 1974), possibly via an island chain. At this time, populations would have been isolated in the proto-Mesoamerican peninsula, by the sea to the south and by the more temperate climate to the north (Savage 1982). Following the formation of the Panamanian land link [between 5 and 3 million years ago (Ma), Coney 1982] the Great American Interchange resulted in numerous invasions of Mesoamerica by South American angiosperm flora (Burnham & Graham 1999). Later, the climatic fluctuations of the Pleistocene (1.6–0.01 Ma) had a substantial influence on the Mesoamerican flora (Prance 1982a,b; Toledo 1982). Major fragmentation of the extensive tropical forest took place (Toledo 1982; Leyden 1984; Islebe & Hooghiemstra 1997; Williams *et al.* 1998; Hewitt 2000), with many species restricted to refuge populations in the region of present day Guatemala and northwest Colombia during glacial maxima. In general, for many plant and animal species of Mesoamerica, distinct biogeographic patterns remain, reflecting the significant influence of dispersal and isolation, extinction and colonization on the populations of this dynamic and diverse region (Savage 1982; Bermingham & Martin 1998; Burnham & Graham 1999).

Spanish Cedar (*Cedrela odorata*) is a neotropical member of the hardwood family Meliaceae (Swietenioideae), well known for its high-quality, high-value timber. The species (and family) has a long history of human exploitation and is still a valuable commodity, used in furniture-making and construction (Lamb 1968; Rodan *et al.* 1992; Valera 1997). *Cedrela odorata* is naturally distributed from the Mexican Pacific coast at 26° N and the Mexican Atlantic coast at 24° N, throughout the Caribbean islands, the Yucatan and lowland Central and South America to northern Argentina at 28° S. The tree is deciduous and grows in both dry and moist lowland areas where soils are not flooded, up to around 1200 m altitude. It is a fast-growing, light-demanding species (Lamb 1968; Chaplin 1980; Valera 1997) reaching 40 m in height and 120 cm in diameter. As with other Meliaceae, the species is monoecious (flowers are unisexual, Pennington *et al.* 1981; pollinated by small bees, wasps and moths, Navarro *et al.* 2002; Bawa *et al.* 1985). Flowering occurs annually in 10–15-year-old trees, and a good seed crop is produced every 1–2 years (ICRAF Agroforestree database http://www.icraf.cgiar.org/). Seed is wind dispersed.

The value of *C. odorata* has resulted in over-exploitation of the species in its natural habitat for two centuries. A number of studies have been carried out on the species within Costa Rica, focusing on its known intraspecific variation, manifest primarily as tolerance for both dry and moist habitats. Common garden experiments have indicated that this environmental 'tolerance' has a genetic basis and that ecotypic differentiation has occurred within the species. For example, apical dominance experiments (Newton *et al.* 1995), *Hypsipylla grandella* resistance trials (Newton *et al.* 1999b) and morphological studies (Navarro *et al.* 2002) all identify two distinct groups within Costa Rica that are correlated with habitat. Random amplified polymorphic DNA (RAPD) analysis of Costa Rican populations (Gillies *et al.* 1997) showed differentiation between these ecotypes for neutral loci. To date, there has been no large-scale molecular study of *C. odorata* and the extent of intraspecific variation in the Mesoamerican population is unknown. Given the threat posed to the species by unsustainable logging practices and habitat loss, and evidence indicating significant population structuring, there is a clear need to assess the current levels and distribution of diversity in the Mesoamerican population.

This study investigates the phylogeographic structure of the Mesoamerican population of *C. odorata*. Universal chloroplast markers are employed to identify patterns of population structure that reflect the seed dispersal history of the species, assuming maternal inheritance of the chloroplast. The results are interpreted in the light of the known geological and climatic history of the Mesoamerican Isthmus to infer colonization dynamics of *C. odorata*.

Fig. 1 Map of the populations and distribution of haplotypes of *Cedrela odorata* sampled in Mesoamerica. Inset shows the location of Mesoamerica on a global map. Population markers are proportional to the number of samples analysed: large circles, 20 individuals; small circles, five individuals.

Materials and methods

Samples were collected from a total of 580 *Cedrela odorata* individuals in 29 populations throughout Mesoamerica (Fig. 1 and Table 1). Populations were defined as groups of trees at least 100 m apart but within a coherent geographical area, such that they were in potential reproductive contact. Twenty trees were sampled per population. Four populations were sampled from each of Mexico, Guatemala, Honduras and Nicaragua. Three populations were sampled from Panama and 10 from Costa Rica. Individuals were sampled by collecting either leaf tissue (dried on silica gel) or cambium tissue [immersed in 70 : 30, ethanol : cetyltrimethylammonium bromide (CTAB) buffer containing 100 mM Tris–HCl pH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 1% polyvinylpyrrolidone-40T (PVP–40T), 2% CTAB]. Genomic DNA was extracted using a modified CTAB protocol (Gillies *et al.* 1997).

Screening for variation in the cpDNA used the universal primers described in Demesure *et al.* (1995), Dumolin-Lapegue *et al.* (1997b) and Hamilton (1999). The polymerase chain reaction (PCR) protocol was as described in Demesure *et al.* (1995) and fragment patterns were visualized on 8% nondenaturing polyacrylamide gel in a Hoefer

SE600 electrophoresis unit (300 V) using Tris–borate EDTA buffer (1×). As a result of amplification problems, not all populations could be screened for all 20 individuals collected and those from Guatemala and Panama were assessed using only five individuals. Pons & Petit (1995, 1996) emphasize the importance of increased numbers of populations as opposed to increased within-population sample sizes for studies of population subdivision, so the reduced data sets from Guatemala and Panama were included in the analysis.

All individuals were characterized for cpDNA haplotype, and the data set was analysed for within-population (H_S) and total (H_T) diversity and for the level of population subdivision (G_{ST}). A minimum spanning tree (Excoffier & Smouse 1994) was constructed using MINSPNET (available at http://lgb.unige.ch/software), scoring fragments as multistate characters then preparing a pairwise distance matrix based on the number of mutational (indel or restriction site) differences between haplotypes. By incorporating the relationships between haplotypes from the minimum spanning tree, an estimate of population subdivision for phylogenetically ordered alleles (N_{ST}) was obtained and a test statistic, *U*, comparing the values of N_{ST} and G_{ST} was calculated (Pons & Petit 1996). All statistics were calculated using the program HAPLONST, which is available at http://www.pierroton.inra.fr/genetics/labo/Software/).

The geographical component of population structure was investigated through spatial analysis of genetic variation. Pairwise values of F_{ST} (Wright 1969), unordered genetic distance, D_G (Gregorius 1978; where

$$D_G(i,j) = \frac{1}{2} \sum_{k=1}^{n} \left| p_{ik} - p_{jk} \right|$$

and *i*, *j* are two populations, *n* is the number of haplotypes and p_{ik} is the frequency of the *i*th haplotype in the *k*th population) and ordered genetic distance (taking the distance between haplotypes as the number of mutational steps between them along the minimum spanning network; an average distance between populations was then determined based on this distance and the frequency of each haplotype in the populations) were plotted against geographical distances. The plots of F_{ST} and unordered genetic distance were prepared using the program SGS (Degen *et al.* 2001; available at http://kourou.cirad.fr/genetique/software.html).

Finally, environmental data for each of the population sites (mean annual precipitation, mean number of dry months and mean annual temperature, Table 1) were investigated with sites grouped according to their population cpDNA haplotype. A one-way analysis of variance (ANOVA; using MINITAB) was carried out to look for associations between the environmental data and cpDNA haplotype group.

Table 1 Details of locations and environmental data for all populations sampled

Population	Latitude (N)	Longitude (W)	Sampling method	Altitude (m)	No. of dry months	Precipitation (mm/year)	Mean annual temperature (°C)
Panama							
Gualaca	8°35′	82°14′	cambium	150	4	4000	26.9
Las Lajas	8°12′	81°52′	cambium	20	4	3500	26.9
San Francisco	8°14′	80°59′	cambium	100	4	2500	26.7
Costa Rica							
Horizontes	10°44′	85°35′	leaf	200	5	1500	27.0
Puriscal	09°56′	84°17′	leaf	900	5	2400	25.0
Canas	10°12′	84°57′	leaf	100	5	1829	27.0
Palo Verde	10°21′	85°21′	leaf	50	5	1824	27.0
Hojancha	10°05′	85°22′	cambium	250	5	2232	27.0
Jimenez	10°13′	83°37′	leaf	240	1	4465	22.1
Talamanca	09°38′	82°51′	leaf	75	0	2467	25.6
Upala	10°47′	85°02′	cambium	150	4	2558	25.0
Pacifico Sur	08°32′	82°51′	cambium	40	0	4089	26.8
Perez Zeledon	09°20′	83°39′	cambium	700	4	2934	23.3
Nicaragua							
Ometepe	11°29′	85°29′	leaf	40	6	1500	27.3
Masatepe	11°54′	86°08′	leaf	450	6	1880	27.3
Wabule	12°53′	85°41′	leaf	600	6	1750	27.3
La Trinidad	12°59′	86°14′	leaf	600	6	1700	27.3
Honduras							
Meambar	14°50′	88°06′	leaf	600	1	2425	22.0
Taulabe	14°50′	88°06′	leaf	633	1	2425	22.0
Comayagua	14°09′	87°37′	leaf	579	5	1052	24.4
La Paz	14°25′	87°03′	leaf	726	3–4	1976	22.0
Guatemala							
Los Esclavos	14°15′	90°17′	leaf	737	6	1552	23.6
El Idolo	14°26′	91°23′	leaf	300	5	3010	26.0
Tikal	17°14′	89°37′	leaf	250	5	2500	30.0
San Jose	17°11′	89°52′	leaf	235	5	1300	26.0
Mexico							
Calakmul	18°27′	88°19′	leaf	300	5	1450	24.5
Bacalar	18°17′	89°09′	leaf	15–300	5	1450	24.0
Zona Maya	19°22′	88°01′	leaf	50	5	1450	24.0
Escarcega	18°24′	90°54′	leaf	100	5	1198	24.0

Results

Six polymorphic primer/enzyme combinations were identified (Table 2). These contained a total of 11 indels (insertion/deletion mutations) and one restriction site mutation, characterizing five cpDNA haplotypes (Table 2). Half of the mutations differentiated Mexican and Guatemalan populations from all other populations. The five haplotypes could be clearly resolved using just three primer/enzyme combinations and these alone were used to screen the whole collection.

Almost all of the populations were fixed for a single haplotype (Table 3), hence average within-population diversity was very low, $H_S = 0.03$ (Table 4). Only three of the 29 populations screened showed any within-population diversity: San Francisco in Panama, Upala in Costa Rica and Los Esclavos in Guatemala. Of these, the first two are at boundaries between populations fixed for different haplotypes, while Los Esclavos had the only case of a private haplotype detected during the study. The total diversity (H_T) was 0.70 and the level of population subdivision (G_{ST}) was 0.96 (Table 4).

The minimum spanning network of haplotype relationships (Fig. 2) shows three haplotype lineages. The first is in Mexico and Guatemala (including two haplotypes; henceforth called 'Northern' lineage), the second in Honduras, Nicaragua and northwestern Costa Rica (a single haplotype; 'Central') and the third in eastern and southwestern

Table 2 Description of the five cpDNA haplotypes identified

Haplotype	Polymorphic fragments							
	PF1 1–510 2–240 3–230 indel	PF2 1–760 2–740 indel	PF3 1–380 2–360 indel	PF4 1–370 2–340 indel	PF5 1–410 2–400 3–390 indel	PF6 1–390 2–330 3–320 indel	PF7 1–250 2–absent site	PF8 1–415 2–410 indel
1	2	1	1	1	1	2	1	1
2	2	1	1	1	3	1	1	1
3	1	2	2	2	1	3	2	2
4	3	2	1	2	2	3	2	2
5	3	2	1	2	2	2	2	2

Numbers 1, 2, 3 indicate character state of fragment, in decreasing order of size (bp).
Full details of primers/enzymes available from authors.

Costa Rica and Panama (including two haplotypes; 'Southern'). The network does not reflect geographical distribution, as the Northern lineage was most strongly differentiated from the geographically proximate Central lineage, rather than from the geographically distant Southern lineage.

The N_{ST} estimate ($N_{ST} = 0.98$) was greater than the G_{ST} estimate ($G_{ST} = 0.96$, Table 4), although the difference was not significant. Nevertheless, the phylogenetic component of the geographical distribution was clearly evident in the contrast between spatial analysis of ordered and unordered data (Fig. 3). Pairwise F_{ST} remained fairly constant around the overall G_{ST} of 0.96 (Fig. 3, top panel). However, the unordered genetic distance increased steadily with pairwise geographical distance, reaching 1 for distance classes above 880 km (Fig. 3, top panel). Unordered genetic distance reflected only the number of haplotypes shared between populations and did not take into account phylogenetic relationships. Hence it increased with distance, as comparisons between populations from different geographical regions are unlikely to share any haplotypes. In contrast, the ordered genetic distance increased with geographical distance between populations, up to around 700 km, but then fell above distances of 1000 km (Fig. 3, bottom panel). This is because of the large genetic distance between the geographically proximate Northern and Central lineages in comparison to the lesser divergence between the Northern and Southern lineages (when haplotype relationships are taken into account, Fig. 2).

Investigation of environmental data showed significant differences between sites with populations possessing different cpDNA lineages (Fig. 4, population Upala was classed as being of the Southern lineage) for both mean annual rainfall (ANOVA: $F = 17.60$, $P < 0.000$, d.f. = 2) and number of dry months (ANOVA: $F = 5.92$, $P < 0.008$, d.f. = 2). The analysis of variance for mean annual temperature was not significant. Therefore, populations possessing Northern or Central lineage haplotypes were found to experience generally low annual rainfall (means of 1738 and 1884 mm/year, respectively) and pronounced seasonality (greater annual number of dry months, mean 4.04). Populations possessing Southern lineage types occupied wetter sites, with shorter dry seasons (mean rainfall 3314 mm/year, mean duration of dry season 2.6 months).

Discussion

The level of population differentiation within *Cedrela odorata* ($G_{ST} = 0.96$) is one of the highest yet obtained (cf. *Quercus* sp. $G_{ST} = 0.828$, Dumolin-Lapegue *et al*. 1997a; *Vouacapoua americana* $G_{ST} = 0.89$, Dutech *et al*. 2000; *Argania spinosa* $G_{ST} = 0.60$, El Mousadik & Petit 1996). Considering the geographical distances (maximum interpopulation distance approx. 1500 km) and environmental gradients involved, relative to seed and pollen dispersal distances in the species (maximum distance approx. hundreds of metres), the collection cannot be panmictic, and some genetic structuring was to be expected because of genetic drift. However, several aspects of the haplotypic distribution indicate divergence from a neutral pattern of isolation by distance.

First, seed flow between populations possessing the Northern and Central lineages appears to be restricted. There is no clear ecological or physical reason why gene flow via seed should not occur, yet the boundary is distinct. In European oaks the recolonization of northern Europe has occurred in parallel from three genetically differentiated refugial populations since the Pleistocene, establishing a distinct pattern in cpDNA variation (Dumolin-Lapegue *et al*. 1997a; Petit *et al*. 2002a,b). At and beyond contact zones between migrating fronts, significant mixing of haplotypes has occurred, as all populations are colonizing new territory. However, following the establishment of a population, immigration is rare (most recruitment is from local sources) and an enduring patch structure results. For

Table 3 Distribution of haplotypes within populations of *Cedrela odorata* in Mesoamerica

Population	Haplotypes					
	1	2	3	4	5	Total
Panama						
Gualaca					5	5
Las Lajas				5		5
San Francisco				1	4	5
Costa Rica						
Horizontes				20		20
Puriscal				20		20
Canas				20		20
Palo Verde				20		20
Hojancha				20		20
Jimenez		20				20
Talamanca		20				20
Upala		19		1		20
Pacifico Sur		20				20
Perez Zeledon		20				20
Nicaragua						
Ometepe		20				20
Masatepe		20				20
Wabule		20				20
La Trinidad		20				20
Honduras						
Meambar		20				20
Taulabe		20				20
Comayagua		20				20
La Paz		20				20
Guatemala						
Los Esclavos	4	1				5
El Idolo	5					5
Tikal	5					5
San Jose	5					5
Mexico						
Calakmul	20					20
Bacalar	20					20
Zona Maya	20					20
Escarcega	20					20
Total	99	1	259	106	9	475

Details of the haplotypes are given in Table 2.

Table 4 Mean levels of population and total diversity and level of population subdivision, for 29 populations of *Cedrela odorata* throughout Mesoamerica

Number of populations:	29
Arithmetic mean population size:	16.4
Harmonic mean population size:	11.60
H_S	0.03 (0.02)
H_T	0.70 (0.05)
G_{ST}	0.96 (0.03)
v_S	0.01 (0.01)
v_T	0.70 (0.04)
N_{ST}	0.98 (0.01)
U	1.41

Standard errors are given in brackets. All estimates were calculated using the software HAPLONST. N_{ST} is based on the minimum spanning tree in Fig. 2.
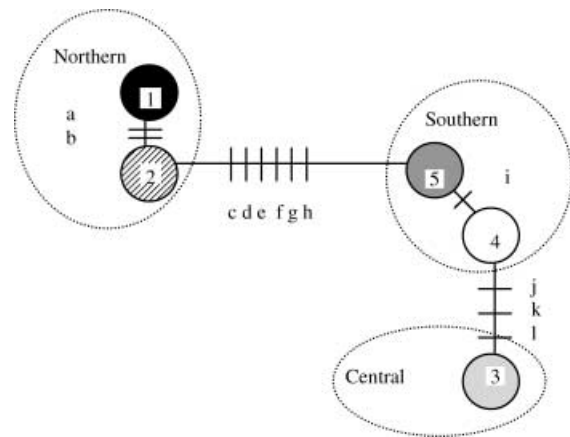

Fig. 2 Minimum spanning tree for the five haplotypes identified in *Cedrela odorata*. The geographical distribution of the haplotypes is shown in Fig. 1. Bars on connecting spans indicate minimum numbers of individual mutations (see Table 2 for details): a, PF4 (state 1–state 3); b, PF6 (1–2); c, PF3 (1–2); d, PF5 (1–2); e, PF4 (1–2); f, PF2 (1–2); g, PF7 (1–2); h, PF8 (1–2); i, PF6 (2–3); j, PF1 (1–2); k, PF1 (1–3); l, PF4 (1–2).

C. odorata, the lack of cpDNA mixing at the contact zone suggests that, rather than parallel colonization, secondary contact has occurred between an established population and a colonizing front.

Biogeographical studies of the flora (Raven & Axelrod 1974) and fauna (Savage 1982) and fossil pollen data (Graham 1999) indicate that there was considerable dispersal from South America to Mesoamerica prior to the formation of the Panama land bridge (5–3 Ma, Coney 1982). Given the large differentiation between the Northern and Central

lineages (suggesting a timescale in the order of millions of years, King & Ferris 1998; Ennos *et al*. 1999; Dutech *et al.* 2000) it seems feasible to suggest that the Northern lineage colonized the proto-Mesoamerican Isthmus prior to formation of the land bridge, via long-distance dispersal or migration across an early island arc, and that the Central lineage followed later, probably across the land bridge (as occurred for most lowland Mesoamerican flora, Gentry 1982a; Burnham & Graham 1999). Several other studies have noted the distinctiveness of Mexican/Yucatan populations from southern Mesoamerican/Pacific coast populations (e.g. *Cordia alliodora*, Boshier 1984; Chase *et al.* 1995; *Gliricidia sepium*, Lavin *et al.* 1991; *Calliandra calothyrsus*, Chamberlain 1998; *Swietenia macrophylla*, Gillies *et al.* 1999)
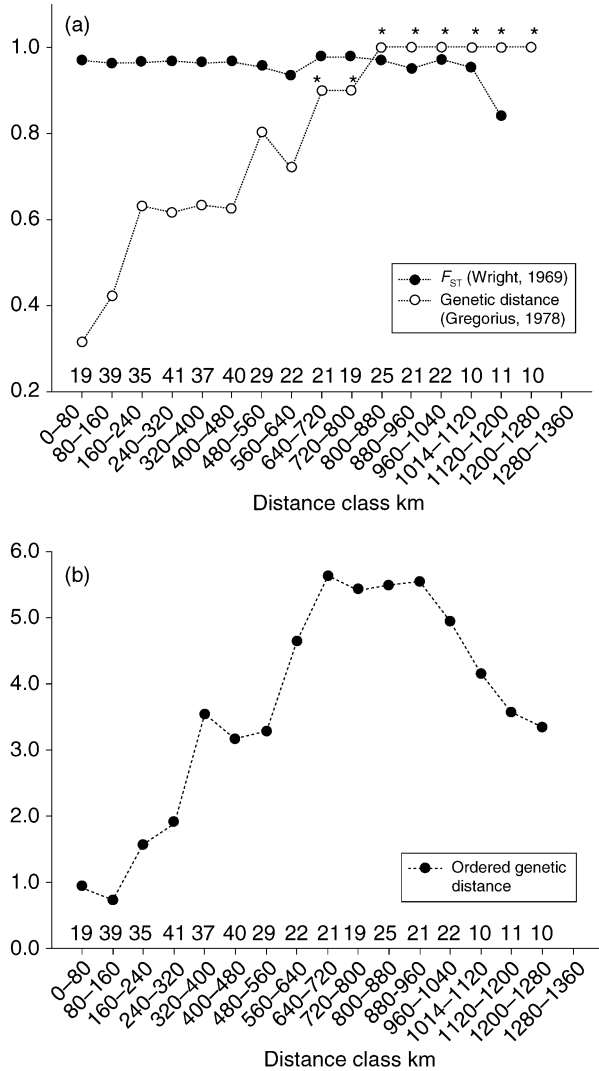
Fig. 3 Spatial analysis of genetic variation in *Cedrela odorata* cpDNA. Top panel, average pairwise comparisons of genetic distance and F_{ST} by distance class (calculated using sgs software, Degen *et al*. 2001). Asterisk indicates significant positive correlation (at 95% confidence level). Bottom panel, average pairwise ordered genetic distance by distance class based on the minimum spanning tree (Fig. 2). All 95% confidence intervals less than 0.03. In both plots, the number of pairwise population comparisons in each distance class is shown above the *x*-axis.

Fig. 4 Top panel, group means and 95% confidence intervals for annual rainfall for all populations in the three principal haplotype regions. Bottom panel, group means and 95% confidence intervals for number of dry months for all populations in the three principal haplotype regions. Results from the corresponding ANOVA are given in the text.

possibly indicating a general pattern, which should be further investigated.

In contrast, the contact zone between the Central and Southern lineages is quite different. Trees possessing the two lineages occupy significantly different habitats in terms of both annual rainfall and seasonality (Figs 1 and 4, Table 1). Previous studies have demonstrated significant morphological (Navarro *et al*. 2002), physiological (Newton *et al*. 1995, 1999b) and nuclear sequence (Gillies *et al*. 1997) differentiation between populations that possess the Central and Southern cpDNA lineage types, indicating
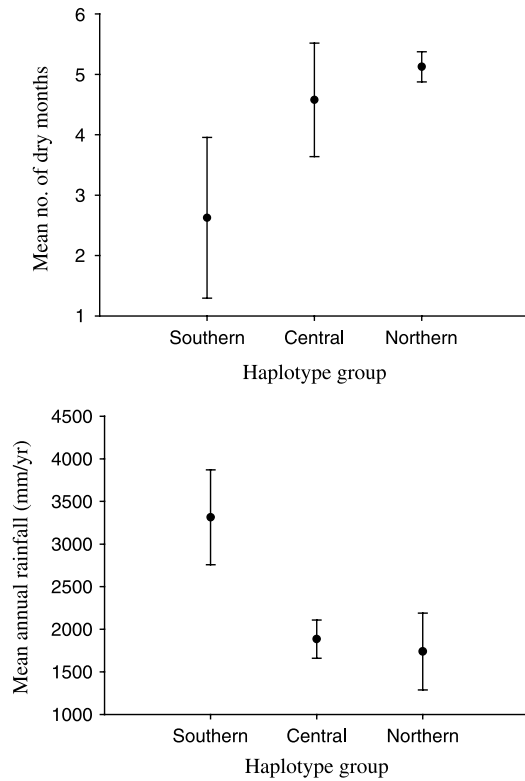
probable reproductive isolation and the existence of ecotypes. On the basis of the current evidence, the most likely scenario for generation of the observed pattern is repeated colonization of the Isthmus during known fluctuations in vegetation assemblages associated with the climatic changes of the Pleistocene epoch (Graham 1991; Williams *et al*. 1998; Hewitt 2000). Under such a model, the Central lineage could have colonized during a period that favoured expansion of dry-adapted vegetation, following the formation of the Isthmus of Panama (see above). The Southern lineage would then represent a much more recent northward colonization of a wet-adapted type from a previously differentiated southern American source population during a period favouring expansion of moist forest assemblages. This could have occurred recently, following the last glacial maximum (13 000 years ago), from a source in the geographically proximate wet Choco region of Colombia. Interestingly, within the Southern lineage, two haplotypes are present in Panama, whereas only one is present in Costa Rica, a pattern which might be expected from a northern expansion and associated reduction in diversity in this region.

As is the case for many other Neotropical species, biogeographical evidence suggests that *C. odorata* has colonized Mesoamerica from South America. The species is distributed widely throughout the Neotropics but its range is centred in South America. Evidence for 'waves' of colonization, some from divergent South American sources, has been noted for other Central American species, for example freshwater fish (Bermingham & Martin 1998). In addition, the tropical moist forest was substantially and repeatedly fragmented into refugial areas throughout Mesoamerica and northern South America (Gentry 1982b; Prance 1982a,b; Toledo 1982). Hence, it seems likely that the haplotype distribution pattern in Mesoamerica is caused by repeated northward colonization from previously differentiated South American source populations, although further data to test this model are needed.

To this end, a thorough assessment of nuclear variation is warranted, covering the same range as for the cpDNA, with particular attention to the northern contact zone. An analysis of variation in the nuclear genome would also indicate whether populations had experienced a bottleneck in the past and thereby help to refute or verify the colonization hypothesis. It would be useful to assess variation using a phylogenetically informative nuclear marker, such as an intron sequence, to permit preparation of a genealogy and an intraspecific phylogeographic hypothesis. Clearly, it will be necessary to extend the study to cover the whole range of the species, including South America and the Caribbean. The significance of the differentiation within Mesoamerican populations for both chloroplast and nuclear markers could then be assessed in a rangewide context. Finally, it would be helpful to extend the phylogeography of Mesoamerica to cover additional tree species and potentially other organisms. This would shed light on any shared patterns of the colonization of Mesoamerica and contribute to the understanding of tropical forest response to changing environment.

Acknowledgements

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