

Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics

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Abstract

Many tropical tree species occupy continental expanses of rainforest and flank dispersal barriers such as oceans and mountains. The role of long-distance dispersal in establishing the range of such species is poorly understood. In this study, we test vicariance hypotheses for range disjunctions in the rainforest tree *Ceiba pentandra*, which is naturally widespread across equatorial Africa and the Neotropics. Approximate molecular clocks were applied to nuclear ribosomal [ITS (internal transcribed spacer)] and chloroplast (*psbB-psbF*) spacer DNA sampled from 12 Neotropical and five West African populations. The ITS ($N = 5$) and *psbB-psbF* ($N = 2$) haplotypes exhibited few nucleotide differences, and ITS and *psbB-psbF* haplotypes were shared by populations on both continents. The low levels of nucleotide divergence falsify vicariance explanations for transatlantic and cross-Andean range disjunctions. The study shows how extreme long-distance dispersal, via wind or marine currents, creates taxonomic similarities in the plant communities of Africa and the Neotropics.

Keywords: community assembly, long-distance dispersal, molecular clocks, phylogeography, tropical trees, vicariance

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Introduction

The species richness of tropical rainforest plant communities has been well documented (Wallace 1878; Leigh *et al.* 2004). Until recently, however, little was known about the geographical ranges of most rainforest plant species. Comparisons of regional floras (Ruokolainen *et al.* 2005) and tree inventory plots from the Amazon basin (Pitman *et al.* 1999; Pitman *et al.* 2001) have shown that many rainforest plant species are widespread and flank biogeographical barriers such as oceans and mountains. In lowland Ecuador, for example, approximately 30% of the vascular plant species have populations on both slopes of the Andean cordilleras ($N = 1431$ disjunct species) (Jørgensen & León-Yáñez 1999), and in Panama up to 60% of the rainforest tree

species cross the Andes and are widespread in the Amazon basin (Dick *et al.* 2005).

There are also, remarkably, many taxonomic similarities between Neotropical and African rainforests (Gentry 1993) despite the passage of nearly 100 million years since the fragmentation of western Gondwana (Pitman *et al.* 1993). At least 110 angiosperm genera (from 53 families) and 108 species are restricted to Africa and tropical America (Thorne 1973), of which only 20–25 are weedy or are cultivated by humans. At the scale of two 50-ha forest inventory plots, more than 30 genera and several tree species are shared between rainforests of Ecuador and Cameroon (Pennington & Dick 2004).

The discontinuous ranges of so many tropical rainforest tree species raise fundamental biogeographical questions. Are widespread species the 'weedy' components of rainforest floras most likely to disperse over geographical barriers, as suggested by Gentry (1982)? Or are widespread

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Fig. 1 *Ceiba pentandra* clockwise from left (1) emergent Amazon tree with a person beside the characteristic buttress trunk (photo credit R. Gribel); (2) dehiscent fruit with kapok (photo credit A. Gentry); (3) seed enveloped in kapok (photo credit C. Dick).

Rainforest form	Cultivated form	Savannah form
Trunk spiny (prickles)	Trunk usually spineless	Trunk spineless
Buttress large	Buttress small	Buttress none
Branches horizontal	Branches ascending	Branches strongly ascending
Trunk straight	Trunk straight	Trunk often forked
Reproduction superannual	Reproduction annual	Reproduction annual
Leaves narrow	Leaves broader	Leaves broadest
Fruit capsule dehiscent	Fruit capsule indehiscent	Fruit capsule dehiscent
Chromosomes $2n = 80, 88$	$2n = 72-80$ (variable)	$2n = 72$

Table 1 List of morphological and cytological character differences between three forms of *Ceiba pentandra*. Only the rainforest form (column 1) is found in Africa and Neotropics. The cultivated form, which shows intermediate features, is widely planted in Southeast Asia (modified from Table 1 in Baker 1965)

species old members of rainforest communities, as suggested by vicariance models? Such questions have broad implications for ecology and evolution. For example, if > 30% of Ecuador's lowland flora evolved prior to the rise of the northern Andes, according to vicariance models (Jørgensen *et al.* 1999; Raven 1999), then conspecific populations of more than 1400 species have diverged in genetic isolation and without apparent morphological change for several million years, in a biome renowned for intense adaptive pressures (Dobzhansky 1950; Schemske 2002; Leigh *et al.* 2004).

In this study, we examined the biogeographical history of the widespread rainforest tree *Ceiba pentandra* L. (Fig. 1). *Ceiba pentandra*, also known as the kapok or silk-cotton tree, is widespread in the Neotropics (east and west of the Andes), and in equatorial Africa. We performed a phylogeographical analysis of *C. pentandra* using individuals sampled from throughout the natural geographical range. We then examined levels of cpDNA and nDNA divergence to test vicariance hypotheses for the amphi-Atlantic and cross-Andean disjunctions.

Ceiba pentandra L. Gaertner (Malvaceae)

Neotropical *C. pentandra* L. is a buttressed, canopy-emergent tree that can grow to 70 m in height (Fig. 1). There are two distinctive natural forms of *C. pentandra* in Africa, and one hybrid. The rainforest form is a massive tree with a spiny trunk, as shown in Fig. 1. This is the only form found in the Neotropics and it is the focus of this study. The savanna form is restricted to West African savannas. It is a smaller tree, with a smooth trunk, and its reproductive timing does not coincide with that of the rainforest form. The cultivated form is a cross between the rainforest and savanna forms. The cultivated form has low branches and indehiscent fruits, which facilitate the harvest of kapok fibers used to stuff mattresses, pillows and life preservers. The cultivated form is widely planted outside of the parental species range (e.g. 'java kapok'), but has apparently not established natural populations (Baker 1965; Baker 1983). Under a more refined taxonomy, the savanna and rainforest forms might be classified as separate species based on numerous morphological and cytological differences (Table 1).

Table 2 The observed (K_{obs}) and expected (K_{exp}) levels of nucleotide divergence between disjunct *Ceiba pentandra* populations is presented for ITS and cpDNA under as a test of vicariance hypotheses. The expectations are conservatively based on the slowest published rates of nucleotide substitution for ITS (*Hamamelis*), and the published rate for *Adansonia*, which is close to the mean of published ITS rates. The cpDNA expectations are based on slowest estimates for synonymous substitutions in the chloroplast genome. All values represent percentages of pairwise nucleotide divergence

Vicariance event	Time T	ITS K_{exp} <i>Hamamelis</i>	ITS K_{exp} <i>Adansonia</i>	ITS K_{obs}	cpDNA K_{exp}	cpDNA K_{obs}
Gondwana vicariance	> 96 ma	> 7.30	> 54.91	0–0.4	> 19.2	0
Boreotropical dispersal	> 35 ma	> 2.66	> 20.02	0–0.4	> 7.0	0
Andean uplift	> 3 ma	> 0.23	> 1.72	0	> 0.6	0–0.1

Ceiba pentandra (sensu lato) is one of nine species in the genus *Ceiba*. *Ceiba* is a member of the Malvaceae subfamily Bombacoideae, which contains two Palaeotropical genera, *Adansonia* (Baobab trees) and *Bombax*, and seven Neotropical genera. Most authors have assumed a Neotropical origin of *C. pentandra* (Baker 1965; Gentry 1993; Baum *et al.* 2004) because the Neotropics harbours all *Ceiba* except the African populations of *C. pentandra*. Thorne (1973) suggested that 'often-cultivated plants (in Africa) like *C. pentandra* (L.) Gaertner may represent very early and intentional introductions by man'. In addition to morphological divergence within African *Ceiba*, however, two further lines of evidence indicate that *Ceiba* was not introduced into Africa by European traders: (i) fossil pollen of *Ceiba* occurs in 13 000 years old deposits in Lake Bosumtwi in Ghana (Maley & Livingstone 1983); and (ii) Arab trade of a cultivated form of *C. pentandra* from West Africa dates to the 10th century (Baker 1965).

The rainforest form of *C. pentandra* colonizes riverbanks and grows in both *terra firme* and floodplain habitats. It grows up to 2 m per year in open habitat and can set seed in as little as 2–6 years (Baker 1965). The dehiscent fruits (up to 30-cm long and 5-cm wide) contain large quantities of cottony kapok fibers, which entangle the small seeds as they are transported through wind and water (Fig. 1). A corky floating structure in the oily seed facilitates marine dispersal. During its flowering period, *C. pentandra* attracts bat and moth pollinators with nectar production in excess of 10 L per tree per night, or an estimated 200 L per flowering season (Gribel *et al.* 1999). Phyllostomid bats (Microchiroptera) pollinate Neotropical *C. pentandra* while pteropid bats (Megachiroptera) pollinate *C. pentandra* in Africa (Baker & Harris 1959). *Ceiba pentandra (sensu lato)* is fully capable of self-fertilization (Baker 1965; Murawski & Hamrick 1992; Lobo *et al.* 2005). Reproduction is limited to temperatures above 20 °C during the flowering period (Baker 1965). Breeding populations are therefore restricted to tropical latitudes and to elevations ≤ 1500 m (Baker 1965).

Vicariance models

We evaluated two vicariance hypotheses for the amphiatlantic disjunction: (i) Gondwana vicariance (ii) Boreotropical

dispersal + vicariance (Table 2). Gondwana vicariance posits that the ancestor of an amphiatlantic taxon was widely distributed across the western Gondwana landmass prior to its separation into South America and Africa approximately 96 million years ago (Ma) (Pitman *et al.* 1993). The Boreotropical hypothesis references a warm phase in the Earth's climatic history (Eocene period; 54–35 Ma) during which time frost-intolerant (megathermal) vegetation occupied high latitudes (≤ 50 degrees N), and crossed a land bridge between Europe and North America that disappeared in the late Oligocene (Tiffney 1985; Morley 2000; Davis *et al.* 2002). Both the Boreotropical and Gondwana vicariance hypotheses have been invoked to explain the amphiatlantic distribution of *C. pentandra* (Baker 1965). Low levels of nucleotide divergence (see molecular-clock methods) that correspond to $\ll 35$ Ma of genetic isolation would falsify both of these hypotheses.

The cross-Andean distribution of *C. pentandra* is also of biogeographical interest. The northern Andes of Ecuador, Colombia and Venezuela average more than 2000 m in elevation, while reproductive populations of *C. pentandra* are largely confined to elevations below 1500 m. There are currently no contiguous populations of *C. pentandra* that span the Andes. Large error bars surround estimates of northern Andean palaeo-elevations (Gregory-Wodzicki 2000) but, in broad terms, the uplift of the northern cordilleras began in the early Miocene (~ 24 Ma) and reached modern elevations ≥ 3 Ma (Lundberg *et al.* 1998). To evaluate the hypothesis of cross-Andean vicariance, we anticipated genetic divergence corresponding to ≥ 3 Ma of genetic isolation.

Materials and methods

Collections

Leaf material for DNA extraction was obtained from fresh leaves or herbarium specimens of rainforest *Ceiba pentandra* in West Africa (Cameroon $N = 3$; Gabon $N = 6$; Ghana $N = 1$), Mesoamerica (Panama $N = 4$; Costa Rica $N = 4$; Mexico $N = 1$) South America (Brazil, $N = 18$; Ecuador, $N = 12$; French Guiana, $N = 2$) and the Caribbean (Puerto

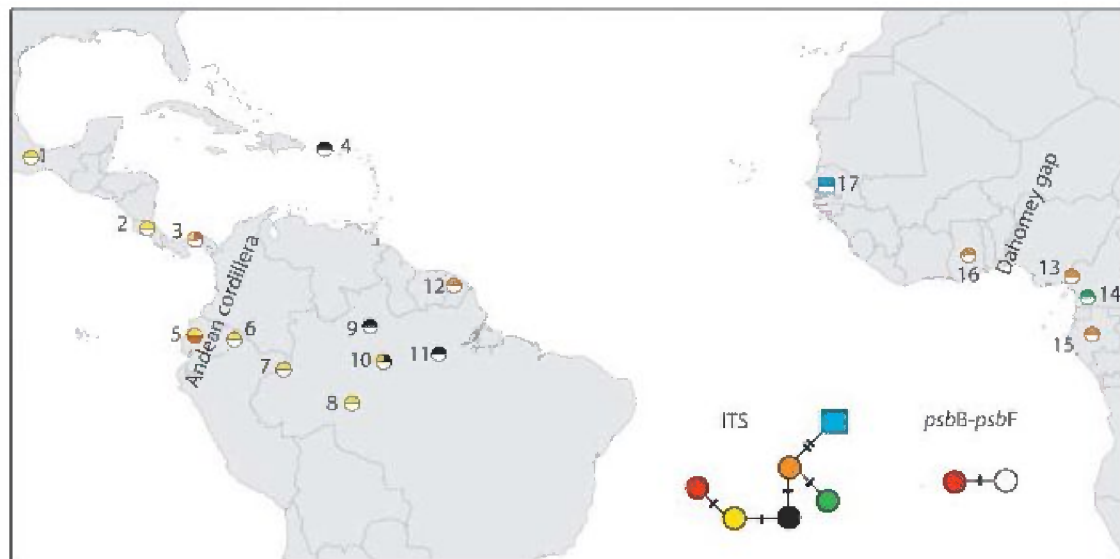


Fig. 2 Geographical distribution of ITS (upper semicircle) and *psbB-psbF* (lower semicircle) haplotypes sampled from Neotropical and Western African *Ceiba pentandra*. The square haplotype (site 17, Senegal) represents the savanna form. Numbered collection sites correspond to column one in Table 3. Hatches correspond to numbers of nucleotide substitutions in the haplotype networks. Identical *psbB-psbF* and ITS haplotypes across the Andes and between Africa and the Neotropics are evidence of long-distance dispersal.

Rico, $N = 3$) (Fig. 2 and Table 3). We obtained a single collection of the savanna form from Senegal. Leaf sampling focused on adult trees separated by at least 100 m, to avoid sampling of closely related individuals. Leaves were collected from massive individuals using slingshots. Most sampling within the sites designated in Fig. 2 spanned large distances. For example, sampling of trees in Panama (site 3) spanned approximately 200 km; west Ecuador samples (site 5) spanned 80–100 km. Sampling within Gabon (site 15) spanned 300 km. The overall Neotropical sampling spanned 5088 km (between Mexico and Rio Madeira, Brazil). The African sampling spanned 1655 km between sites in Senegal and Gabon and sampled Guinean and Congolese rainforest regions, which are separated by the arid Dahomey gap (Fig. 2). The DNA aliquot from each leaf sample was assigned a unique identification number (Laboratory ID), which is linked to information on geographical location, herbarium specimen number, or tag numbers in the case of trees sampled in permanent inventory plots.

Laboratory methods

DNA was extracted using the DNeasy kit (QIAGEN Corporation) following manufacturer protocols. The internal transcribed spacers ITS1 and ITS2 and the 5S ribosomal gene were amplified using the primers ITS4, ITS5 (White *et al.* 1990) and ITSi (Urbatsch *et al.* 2000), which anneal to the flanking 18S and 26S ribosomal genes. Chloroplast intergenic spacers were amplified using the *psbB* and *psbF*

primers of Hamilton (1999a). Polymerase chain reactions (PCR) for ITS and *psbB-psbF* were performed on an MJ Research thermal cycler with the following conditions: 94 °C for 4 min, followed by 30 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 3 min. Amplification products were extracted from low melting point agarose, cleaned up with Gelase (Epicentre Technologies), and sequenced using Big Dye chemistry [ABI (Applied Biosystems Incorporated)] on an ABI automated DNA sequencer. Chromatograms were aligned and edited with SEQUENCHER 4.1 (Gene Codes Corporation). All polymorphisms were confirmed by sequencing with forward and reverse primers. Absolute and maximum-likelihood (ML) genetic distances were calculated in PAUP* 4.10b. The near complete lack of nucleotide divergence among haplotypes obviated the need for additional DNA sequence analyses.

Substitution rates

As there are no estimates of nucleotide substitution rates for *Ceiba*, we used an approximate molecular clock based on a broad range of published ITS and noncoding chloroplast DNA (cpDNA) nucleotide substitution rates. Estimated substitution rates for the ITS region (ITS1, 5.8S, and ITS2) range from 0.38 to 8.34×10^{-9} substitutions per site per year (subs/site/year), based on surveys of 29 time-calibrated phylogenies of herbaceous and woody plant lineages drawn from 22 angiosperm families (reviewed in Richardson *et al.* 2001; Dick *et al.* 2003; Kay *et al.* 2006). The ITS rate estimate for *Adansonia* (Bombacoideae) of

Table 3 Voucher information for sequenced individuals of *Ceiba pentandra*. The laboratory ID is a voucher number for the leaf tissue and DNA maintained at the University of Michigan. The GenBank Accession nos for *psbB-psbF* and the ITS region are presented in the last two columns

Map	Collection site	Coordinates	Laboratory ID	<i>psbB-psbF</i>	ITS accession
4	Camuy, Puerto Rico	18°22'N, 66°45'W	690	DQ284781	DQ284818
4	Camuy, Puerto Rico	18°22'N, 66°45'W	662	n/a	AY635499
4	Quebradillas, Puerto Rico	18°22'N, 67°00'W	663	AY642683	n/a
4	Quebradillas, Puerto Rico	18°22'N, 67°00'W	691	DQ284782	DQ284819
1	Morelos, Mexico	18°51'N, 99°05'W	1110	DQ284783	DQ284820
2	La Suerte, Costa Rica	10°26'N, 83°46'W	893	n/a	DQ284821
2	La Suerte, Costa Rica	10°26'N, 83°46'W	894	DQ284784	DQ284822
2	La Suerte, Costa Rica	10°26'N, 83°46'W	895	DQ284785	DQ284823
2	La Suerte, Costa Rica	10°26'N, 83°46'W	896	n/a	DQ284824
2	Jobero, Panama	07°36'N, 80°33'W	518	AY642675	AY635497
2	Jobero, Panama	07°36'N, 80°33'W	519	AY642682	AY635498
3	Jobero, Panama	07°36'N, 80°33'W	520	DQ284786	DQ284825
3	Naos Causeway, Panama	08°55'N, 79°32'W	879	DQ284787	DQ284826
5	Endesa reserve, Ecuador	00°08'N, 79°03'W	63	n/a	AY635495
5	Endesa reserve, Ecuador	00°08'N, 79°03'W	298	AY642681	AY635494
5	Borbon, Ecuador (km 29)	01°05'N, 78°06'W	867	DQ284788	DQ284827
5	Borbon, Ecuador (km 31)	01°05'N, 78°06'W	871	DQ284789	DQ284828
5	Borbon, Ecuador (km 10)	01°05'N, 78°06'W	875	DQ284790	DQ284829
5	Borbon, Ecuador (km 31)	01°05'N, 78°06'W	883	DQ284791	DQ284830
6	Yasuni park, Ecuador	00°38'S, 76°30'W	64	DQ284792	n/a
6	Yasuni park, Ecuador	00°38'S, 76°30'W	65	DQ284793	AY635500
6	Yasuni park, Ecuador	00°38'S, 76°30'W	66	DQ284794	AY635501
6	Yasuni park, Ecuador	00°38'S, 76°30'W	67	DQ284795	DQ284831
6	Yasuni park, Ecuador	00°38'S, 76°30'W	68	DQ284796	DQ284832
6	Yasuni park, Ecuador	00°38'S, 76°30'W	1021	DQ284797	DQ284833
7	Tabatinga, Brazil	00°03'S, 69°40'W	914	AY642680	DQ284834
7	Tabatinga, Brazil	00°03'S, 69°40'W	915	DQ284798	AY635493
10	Manaus, Brazil	02°50'S, 59°49'W	910	AY642676	DQ284835
10	Manaus, Brazil	03°04'S, 60°00'W	911	AY642677	DQ284836
10	Manaus, Brazil	03°04'S, 59°50'W	1088	DQ284799	AY635504
10	Manaus, Brazil	03°04'S, 59°58'W	1089	DQ284800	DQ284837
10	Manaus, Brazil	03°12'S, 59°57'W	1095	DQ284801	DQ284838
9	Caracaraí, Brazil (Rio Branco)	01°36'N, 61°13'W	916	DQ284802	DQ284839
9	Caracaraí, Brazil (Rio Branco)	01°36'N, 61°13'W	917	DQ284803	DQ284840
9	Caracaraí, Brazil (Rio Branco)	01°36'N, 61°13'W	1098	n/a	DQ284841
11	Castanhal Veado, Brazil (Rio Trombetas)	01°18'S, 56°30'W	918	DQ284804	DQ284842
11	Castanhal Veado, Brazil (Rio Trombetas)	01°18'S, 56°30'W	919	DQ284805	DQ284843
11	Castanhal Veado, Brazil (Rio Trombetas)	01°18'S, 56°30'W	1092	DQ284806	DQ284844
11	Castanhal Veado, Brazil (Rio Trombetas)	01°18'S, 56°30'W	1093	DQ284807	DQ284845
8	Remanso, Brazil (Rio Madeira)	05°06'S, 59°27'W	912	DQ284808	n/a
8	Remanso, Brazil (Rio Madeira)	05°06'S, 59°27'W	1091	DQ284809	DQ284846
8	Remanso, Brazil (Rio Madeira)	05°06'S, 59°27'W	913	AY642679	DQ284847
12	Kourou, French Guiana	05°09'N, 52°39'W	468	DQ284810	DQ284848
12	Kourou, French Guiana	05°09'N, 52°39'W	469	DQ284811	AY635496
13	Korup Park, Cameroon	05°04'N, 08°51'E	61	AY642673	AY635502
13	Korup Park, Cameroon	05°04'N, 08°51'E	62	AY642674	AY635503
14	Bimbia, Cameroon	03°06'N, 09°16'E	1101	DQ284812	DQ284849
15	Gabon (OH 91)	02°34'S, 10°44'E	1118	DQ284813	DQ284850
15	Gabon (OH 92)	01°20'S, 13°07'E	1119	DQ284814	DQ284851
15	Gabon (OH 93)	00°99'S, 12°44'E	1120	DQ284815	DQ284852
15	Gabon (OH 94)	00°32'N, 11°27'E	1121	DQ284816	DQ284853
15	Gabon (OH 95)	00°50'N, 12°80'E	1122	DQ284817	DQ284854
15	Gabon (OH 97)	00°11'S, 11°59'E	1124	n/a	DQ284855
16	Elmina, Ghana	06°02'N, 01°04'W	1199	n/a	EF432375
17	Oussouye, Senegal	12°26'N, 16°45'W	1200	EF432373	EF432374

2.48×10^{-9} subs/site/year (Baum *et al.* 1998) is close the mean rate of 2.86×10^{-9} subs/site/year estimated by Kay *et al.* (2006). We compared the observed levels of divergence in disjunct *C. pentandra* with the *Adansonia* rate (2.48×10^{-9} subs/site/year) and the slowest published ITS rate (0.38×10^{-9} subs/site/year) in the Kay *et al.* (2006) survey, which is derived from a fossil-calibrated study of *Hamamelis* (Wen *et al.* 1999). By using the slowest published rate, our analysis is likely to overestimate divergence times, thereby favouring acceptance of vicariance hypotheses. The per-site substitution rates in *Hamamelis* and *Adansonia* correspond to 0.076 and 0.572% divergence/Ma, respectively. Expected levels of nucleotide divergence are derived from the relationship $K = RT/2$, where K is the nucleotide divergence between two sequences, R is the rate of nucleotide substitution and T is the divergence time.

There are no similar reviews for substitution rates in the *psbB-psbF* cpDNA spacer. Therefore, we considered a broad range of synonymous substitution rates (R_S) published for angiosperm cpDNA: Wolfe *et al.* (1987) estimated a range of $1.0\text{--}3.0 \times 10^{-9}$ substitutions per silent site per year (subs/silent-site/year) based on a comparison of eight chloroplast genes from monocots and dicots (divergence time $T = 140$ Ma) and from wheat and maize ($T = 20\text{--}40$ Ma). Zurawski & Clegg (1987) calculated an R_S of 1.3×10^{-9} subs/silent-site/year based on cpDNA divergence between spinach and tobacco. Xiang *et al.* (2000) estimated R_S of 1.02 to 1.42×10^{-9} subs/silent-site/year for the *rbcL* gene, based on fossil calibration of the *Cornus* phylogeny, and obtained similar estimates of R_S from 10 sister taxa (from 10 families) that display the same Asia/North America disjunction. We applied the slowest rates of synonymous substitution (1.0×10^{-9} subs/silent-site/year) to *psbB-psbF* as a con-

servative test of the vicariance hypotheses. This corresponds to a divergence rate of 0.2%/Ma.

For the *Hamamelis*- and *Adansonia*-derived ITS rates, expected percentage of nucleotide divergence between disjunct ITS haplotypes are > 7.30 and > 54.91 under Gondwana vicariance, > 2.66 and > 20.02 under the Boreotropical model, and > 0.23 and > 1.72 for Andean vicariance. The expectations for *psbB-psbF* are > 19.2 under the Gondwana model, > 7.0 under the Boreotropical model, and > 0.6 for Andean vicariance (Table 2).

Results

ITS sequences averaging 750 bp were obtained for 51 individuals (Table 3). The ITS sequences were G-C biased (nucleotide frequencies: A = 0.17, C = 0.32, G = 0.33 and T = 0.18). Five polymorphic sites were found among all ITS sequences, including a single nucleotide indel (site 141, Fig. 3). Several individuals from the Amazon basin were heterozygous at two ITS nucleotide sites (a G/T polymorphism at site 224; and a T/C polymorphism at site 299) as indicated by dual chromatogram peaks on both forward and reverse strands. Two of the five ITS haplotypes (haplotypes 1 and 4 in Fig. 3) were represented by single individuals from Panama (GenBank DQ284825) and Cameroon (DQ284849). DNA sequences from the *psbB-psbF* spacer were obtained from 49 individuals (Table 3) and averaged 742 bp in length. The *psbB-psbF* sequences displayed a T-A bias in base composition (A = 0.30, C = 0.17, G = 0.19, T = 0.34). A single polymorphic site (T/A substitution at site 219) distinguished the western Ecuador cpDNA haplotype ($N = 5$) individuals from the widespread cpDNA haplotype (Fig. 2).

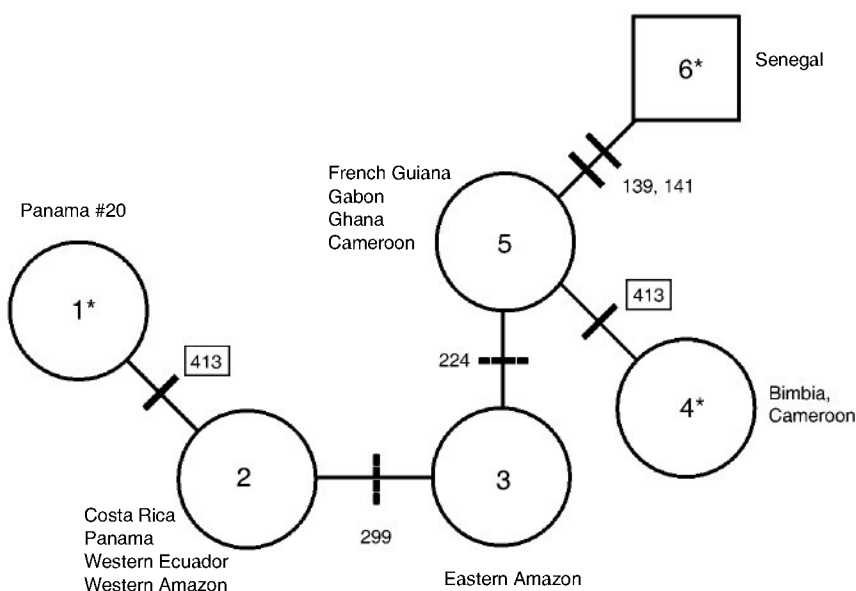


Fig. 3 Shallow phylogeographical structure of *Ceiba pentandra* is indicated by the ITS haplotype network (see also Fig. 2). Hatch marks indicate nucleotide changes at numbered positions in the consensus sequence. Sites 299 and 224, represented by dashed lines, were heterozygous in some individuals in Manaus. The substitution at site 413 is homoplasic in haplotypes 1 and 4 in this network. The squared haplotype is from the savanna form of *C. pentandra*. *represented by a single individual.

Three individuals were excluded from the analysis because of anomalous ITS and *psbB-psbF* sequences. Ecuador sample no. 66, collected from a juvenile tree, was divergent at both ITS (AY635501) and *psbB-psbF* (DQ284794) and was most likely collected from *Ceiba saumauma*, which has similar leaf morphology and is sympatric with *Ceiba pentandra* in lowland Ecuador. The ITS2 sequence of Puerto Rican sample no. 662 (AY635499) differed from the common haplotype by 14-bp differences, and it matched unpublished ITS sequence of *Ceiba aesculus* (K. Walsh, R. Oldham, and D. Baum, unpublished data). Gabon sample 1122 was highly divergent at ITS (DQ284854; > 83-bp differences or ~13% nucleotide divergence), and its *psbB-psbF* sequence (DQ284817) differed from the widespread haplotype by three nucleotide changes. The Gabon ITS sequence matched the published ITS sequences of *Bombax malabaricum* (DQ826447 and AF460192).

The common *psbB-psbF* haplotype was widespread in Africa and the Neotropics, and was also present in the savanna tree. An ITS haplotype was shared between populations in French Guiana and West Africa (Ghana, Cameroon and Gabon). The other ITS haplotypes found in Africa and the Neotropics differed by a maximum of four nucleotides (including one indel; $K = 0.53\%$). The ITS haplotype from the savanna tree from Senegal (EF432374) showed 0.3–0.6% divergence from the rainforest haplotypes. For reference, in a limited sample of six *Ceiba* species the level of interspecific ITS divergence varied from 2.8 (*C. pentandra* and *C. crispifolia*) to 6.0% (*C. pentandra* and *C. aesculifolia*) (K. Walsh, R. Oldham, and D. Baum, unpublished data), supporting the expected sister relationship between the rainforest and savannah forms of *C. pentandra*.

Discussion

Ceiba pentandra has the weakest phylogeographical structure yet reported for a widespread rainforest tree species. Apart from the cluster of sites in Western Ecuador having a variant *psbB-psbF* haplotype, there was no cpDNA variation across Mesoamerica, the Amazon basin, and West Africa. In fact, this study found less cpDNA variation across three continents than some rainforest tree species exhibit within putative breeding populations in French Guiana and Brazil (Hamilton 1999b; Dutech *et al.* 2000; Latouche-Halle *et al.* 2003) or among sample sites at regional scales in French Guiana and across Mesoamerica (Caron *et al.* 2000; Cavers *et al.* 2003). Neotropical ITS haplotypes extended from Mexico into the Amazon basin, and the French Guiana ITS haplotype was encountered in multiple West African sites. The levels of divergence between distinct haplotypes across major range disjunctions fell below the vicariance expectations, even using the slowest published rates of cpDNA and ITS substitution (Table 2). These results falsify Gondwana vicariance, Boreotropical dispersal-vicariance,

and Andean vicariance as applied to *C. pentandra*. Extreme long-distance dispersal, via wind or marine currents, appears to be the primary mode of geographical range expansion in *C. pentandra*.

Oceanic dispersal

Fossils and phylogeographical analysis of ITS in the amphi-Atlantic rainforest tree *Symphonia globulifera* (Clusiaceae) suggest that its populations invaded the Neotropics via oceanic dispersal at least once during the mid-Miocene, at which time *Symphonia* fossil pollen abruptly appears in Mesoamerica and South America. Despite a relatively slow estimated rate of nucleotide substitution in *Symphonia* ITS (0.70×10^{-9} subs/site/year), there was > 4% nucleotide divergence between African and Neotropical populations (Dick *et al.* 2003). As with *Symphonia*, most transatlantic plant dispersal is thought to proceed from Africa to the Neotropics (Renner 2004), with possible exceptions found in the genera *Andira* (Pennington 2003), *Drepanocarpus*, *Hernandia*, *Hymenaea*, *Sacoglottis* and *Thalia* (Renner 2004). The Neotropical origin of *C. pentandra* is supported by the Neotropical distribution of all recognized *Ceiba* species. Furthermore, there is a much higher level of divergence among the Neotropical species (e.g. 6.0% between *C. pentandra* and *C. aesculifolia*) than expected if African *C. pentandra* were the progenitor of a Neotropical radiation. Under the latter scenario, there should be > 6% divergence between African and Neotropical *C. pentandra*. The occurrence of identical ITS haplotypes in French Guiana and West Africa suggests recent dispersal to Africa from northern South America. Alternatively, the French Guiana haplotype may represent secondary dispersal from Africa.

Exceptionally strong winds between northeast Brazil and West Africa may have transported kapok-entangled seeds. Wind transport is a plausible explanation for the African range expansion of the Neotropical bromeliad *Pitcairnia feliciana* (Bromeliaceae), the herb *Maschalocephalus dinklagei* (Rapateaceae) (Givnish *et al.* 2004) and woody lineages of Melastomataceae (Renner & Meyer 2001). *Ceiba pentandra* fruits and/or seeds could have floated to Africa as effluent from large Neotropical rivers (Houle 1998) such as the Amazon or Orinoco. *Ceiba pentandra* must have dispersed or transported by people to Puerto Rico, since this is a volcanic island that has never been connected to mainland (Iturralde-Vinent & MacPhee 1999).

Ceiba pentandra is typically found in low population densities, but large individuals can produce hundreds of thousands of seeds. Rapid growth, longevity, the mixed mating system, and extensive seed and pollen dispersal all favour rapid colonization. Once a founding population has been established, wind dispersal of seeds and bat pollination along rivers may rapidly increase the size of breeding

populations. In a microsatellite-based paternity analysis of *C. pentandra* along the Rio Negro in Brazil, Gribel and colleagues (referenced by Wilson *et al.* 1997) demonstrated bat-mediated pollen dispersal of nearly 20 km among spatially isolated trees. Assuming a Neotropical origin, the founding individuals in Africa could have lost recessive deleterious alleles through genetic drift. The genetic purge would result in less inbreeding depression and a lower rate of abortion of self-fertilized ovules (Hufford & Hamrick 2003; Ward *et al.* 2005). In agreement with this model, Baker (1965) reported high levels of successful self-fertilization (up to 100%) in Africa compared to South American *C. pentandra*.

Ecological divergence

Our study includes one Senegalese representative of the savanna form of *C. pentandra*. The ITS sequence in this sample differs by two nucleotides (<0.3%) from the most similar rainforest haplotype (Fig. 2), and it shares the *psbB-psbF* sequence found in African rainforest and most Neotropical samples. The savanna form probably derives from African populations of the rainforest form. It is possible, however, that the savanna form originated from Neotropical populations in a separate founder event. Testing for multiple founder events will require further geographical and genomic sampling.

Ceiba pentandra exhibits considerable morphological and cytological variation (Table 1), despite relatively low levels of nucleotide divergence. Chromosome counts of Neotropical ($2n = 88$) and African rainforest trees ($2n = 80, 88$) are similar and may overlap. The African savanna form has a chromosome count of $2n = 72$, however, and chromosome counts for cultivated *Ceiba* are variable ($2n = 72, 74, 75, 76, 80$) (Baker 1965). Savanna and rainforest forms differ in morphology of their leaves, flowers, trunk, and canopy architecture. The flowering periods are not synchronous, and both African forms (savanna and rainforest) have lost sulphuric compounds used to attract Microchiropteran bats (Pettersson *et al.* 2004). While this level of variation seems unusual in light of its recent origin, it should be noted that many plant taxa have experienced recent and rapid adaptive radiations. The species-rich tree genus *Inga*, for example, is comprised of nearly 300 Neotropical rainforest species that probably share a common ancestor less than 2 million years ago (Lavin 2006). Some closely related *Inga* species share identical ITS haplotypes (Richardson *et al.* 2001).

Mountain crossings

Although both many wind- and animal-dispersed rainforest tree species exhibit strong genetic differentiation around the Central American cordilleras (Cavers *et al.* 2003; Dick

et al. 2003; Novick *et al.* 2003; Cavers *et al.* 2005) no such pattern was detected in *C. pentandra*. In South America, the *C. pentandra* population in coastal Ecuador contained a variant *psbB-psbF* haplotype; but these same individuals shared widespread ITS haplotypes that provide evidence for dispersal over or around the northern Andes. In animals (Brower 1994; Zamudio & Greene 1997; Slade & Moritz 1998) and in the rainforest tree *S. globulifera* (Dick *et al.* 2003) levels of cross-Andean genetic divergence were consistent with Andean vicariance. However, some bats (Ditchfield 2000; Hoffmann & Baker 2003), and euglossine bees (Dick *et al.* 2004) show the dispersal pattern found in *C. pentandra*. Further studies of lowland tree populations sampled from either side of the Andes will determine the extent to which cross-Andean communities have been genetically isolated. The present study nevertheless provides one exception to Raven's (1999) Andean vicariance hypothesis.

Some contemporary cross-Andean dispersal routes are possible. The Las Cruces mountain pass, for example, narrowly connects the Chocó region with the lowland Colombian Amazon via the Magdalena valley. Las Cruces is approximately 20-km wide and 1874 m in elevation. While exceeding *C. pentandra*'s altitudinal threshold for reproduction (≤ 1500 m), lowland tropical trees like *C. pentandra* may have reproduced and dispersed at higher altitudes during the mid-Holocene (6000 years before present), which was warmer (1–4 °C) and drier than present (Bush *et al.* 2004). A temperature increase of only 1 °C may increase the elevation of lowland rainforest species by more than 200 m (M. Bush, personal communication). Upwelling winds may also facilitate seed dispersal through mountain passes. Marine currents provide an alternative route; *C. pentandra* seeds could have reached the Caribbean coast of Mesoamerica via outflow of the Orinoco River, then dispersed over land through the Isthmus of Panama.

Community assembly

Long-distance dispersal has recently become an accepted biogeographical explanation for many amphi-Atlantic plant and animal disjunctions (de Queiroz 2004; Givnish & Renner 2004; Pennington & Dick 2004) and it may explain taxonomic similarities between rainforest tree communities. Pennington & Dick (2004) evaluated the role of long-distance dispersal in structuring the community composition of trees in a 25-ha forest inventory plot in Yasuní, Ecuador (Valencia *et al.* 2004). 'Immigrants' were defined as species belonging to lineages that originated outside of South America long after its separation from Africa. The immigrant group included the Leguminosae (Schrire *et al.* 2004; Lavin *et al.* 2005), Annonaceae (Richardson *et al.* 2004), some lineages of Lauraceae (Chanderbali *et al.* 2001) and Melastomataceae s. str. (Renner *et al.* 2001). Of the 1104 tree species identified in the Yasuní forest, 232 (21%)

belonged to families and genera classified as immigrants. Some of the immigrant lineages may have arrived directly from Africa via marine dispersal, as in the case of *S. globulifera*, while others may have crossed the Panama land bridge. Summed over geological time, intercontinental migration and long-distance dispersal events contributed substantially to the taxonomic composition of a species-rich Amazon tree community. Thus, *C. pentandra*'s dispersal and range expansion in Africa is a snapshot of a process that has created many other taxonomic similarities between rainforests throughout the world.

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