Two issues that have captured the attention of tropical plant evolutionary biologists in recent years are the relative role of long distance dispersal (LDD) over vicariance in determining plant distributions and debate about the extent that Quaternary climatic changes affected tropical species. Propagules of some mangrove species are assumed to be capable of LDD due to their ability to float and survive for long periods of time in salt water. Mangrove species responded to glaciations with a contraction of their range. Thus, widespread mangrove species are an ideal system to study LDD and recolonization in the tropics. We present phylogenetic and phylogeographic analyses based on internal transcribed spacers region (ITS) sequences, chloroplast DNA (cpDNA), and amplified fragment length polymorphisms (AFLPs) of genomic DNA that demonstrate recent LDD across the Atlantic, rejecting the hypothesis of vicariance for the widespread distribution of the black mangrove (Avicennia germinans). Northern latitude populations likely became extinct during the late Quaternary due to frosts and aridification; these locations were recolonized afterward from southern populations. In some low latitude regions populations went extinct or were drastically reduced during the Quaternary because of lack of suitable habitat as sea levels changed. Our analyses show that low latitude Pacific populations of A. germinans harbor more diversity and reveal deeper divergence than Atlantic populations. Implications for our understanding of phylogeography of tropical species are discussed.

**KEY WORDS:** AFLP, Atlantic–Caribbean–east–Pacific, chloroplast DNA, ITS, mangrove, transoceanic dispersal, tropical phylogeography.
and suggest that the lineage assembly in some modern biotas is more recent than previously thought (de Queiroz 2005).

A debate still exists about the role of Pleistocene climate change in isolating tropical plant populations in refugia during glacial maxima and therefore promoting allopatric speciation (Bermingham and Dick 2001; Richardson et al. 2001; Pennington et al. 2004). Late Quaternary glacial–interglacial cycles entailed cooling of the planet and the spread of ice sheets followed by short warming periods and deglaciation (Imbrie et al. 1993). These climatic oscillations caused population extinctions and range shifts as species were restricted to regions where climate and environment were suitable for their survival (Hewitt 2004a). The most recent cool phase reached its climax in the last glacial maximum (LGM), 22–18 thousand years ago (Yokoyama et al. 2001). Since then many species have expanded their ranges as global climate became warmer, some of them leaving a trace of recolonization that can be followed by fossil and genetic evidence. These recolonization events are widely recorded for temperate species in North America and Europe (Petit et al. 1997; Brunsfeld et al. 2001; Petit et al. 2003; Hewitt 2004a). However, little is known about the effect glacial–interglacial cycles on patterns of recolonization and the subsequent genetic architecture of species in tropical regions (Dutech et al. 2003; Hewitt 2004b).

Mangroves are an ecologically related group of plant species distributed along tropical and subtropical coasts (Tomlinson 1986). Vicariance has been regarded as the main cause for their evolution and diversification (Tomlinson 1986; Duke 1995). As a tropical group whose latitudinal limits are correlated with temperature and aridity, mangroves responded to cooling during the Quaternary with a contraction of their ranges (Duke et al. 1998a; Saenger 1998; Dodd et al. 2002). Because mangroves depend on low-energy, protected coastal environments for their successful establishment, sea level change adds an important dimension to the effects of glacial cycles on mangrove ecosystems (Woodroffe and Grindrod 1991). Rapid marine transgression rates and topography in areas where the continental shelf shelf break remains steeply may have effectively diminished the capacity of mangroves to flourish in West Africa and on oceanic islands (Woodroffe and Grindrod 1991; Versteegh et al. 2004; Scourse et al. 2005). Furthermore, during the LGM, sea levels were 130 m below its present level (Lambeck et al. 2002), and regions where the continental shelf break is less than 130 m deep might have been unsuitable for mangrove establishment.

Although propagules of most mangrove species are able to float and survive in salt water for relatively long periods (Rabinowitz 1978; Ellison 1996; Delgado et al. 2001; Steinke and Ward 2003), little is known about effective seed dispersal in these species. Abrupt discontinuities in allele frequencies among populations of *Avicennia marina* (Forssk.) Vierh. around the Australian coast suggest that, at least for this species, propagule dispersal is limited (Duke et al. 1998b). Here, we use genetic evidence to test for LDD and colonization events in the black mangrove (*A. germinans* [L.] L., Avicenniaceae). *Avicennia germinans* is distributed extensively along tropical and subtropical coasts of the Americas and West Africa, a distribution frequently explained by ancient vicariance events (Tomlinson 1986; Duke 1995). Rabinowitz (1978) showed that seedlings of this species can survive up to 110 days floating in sea water, implying great dispersal potential. There is no evidence of vegetative spread in this mangrove species. Earlier work on *A. germinans* revealed closer similarities between populations of Atlantic South America and those of the east Atlantic (West Africa) than with those of Atlantic North America (Dodd et al. 2000). This geographic pattern was explained under a vicariance scenario, but could also be explained by recent trans-Atlantic dispersal. Here, we hypothesize that if the black mangrove’s distribution is fully explained by vicariance as a result of Gondwanaland separation (∼80 million years ago) or because of the formation of the North Atlantic and the contraction of tropical climates (Duke 1995; ∼50 million years ago), the highest degree of genetic differentiation in neutral molecular markers would be between West African and American populations. Conversely, low divergence between West African and American populations could be the result of repeated genetic exchange between the two regions or recent colonization by trans-Atlantic LDD.

The distribution of *A. germinans* in the Atlantic basin and along the Pacific coast of America also offers two independent systems for studying the effects of Quaternary climate changes on this species. Dodd et al. (2002) found significant divergence between Atlantic and Pacific populations. These two groups of populations have been isolated since, or before the uplift of the Central American Isthmus (CAI), around three million years ago. (Coates and Obando 1996). Here, our estimates of population divergence and differentiation based on the internal transcribed spacer region (ITS) of nuclear ribosomal DNA, chloroplast DNA (cpDNA), and amplified fragment length polymorphism (AFLP) are consistent with a high level of differentiation between Pacific and Atlantic populations of *A. germinans* and show that low latitude Pacific populations harbor more diversity and reveal deeper divergence than Atlantic populations. Furthermore, we report unexpected low diversity and very low differentiation in some regions of the Atlantic, which are consistent with recent colonization by trans-Atlantic LDD. Divergence time estimations based on a Bayesian framework and coalescent simulations support Quaternary trans-Atlantic colonization. Northernmost latitudes populations also show reduced levels of cpDNA genetic diversity, which might be the result of Holocene recolonization from southern populations. We discuss these results and their
importance for the overall understanding of phylogeographic patterns in tropical regions.

**Materials and Methods**

**SAMPLING STRATEGY**

The black mangrove (*A. germinans*) is distributed along the tropical and subtropical coasts of the American continent, the Caribbean islands, and West Africa (see Fig. 1). Three geographical units can be defined, including east Pacific (American Pacific), west Atlantic (American Atlantic and Caribbean), and east Atlantic (West Africa), comprising the Atlantic–Caribbean–east Pacific (ACEP) biogeographical region. Mature leaf samples were obtained from 33 populations across the natural range of the species. A total of 382 trees were sampled, ranging from four to 20 individuals per population (see Supplementary Table S1 online). One partially sympatric species (*A. schaueriana* Stapf & Leechman ex Moldenke, from Macau, Brazil) and one allopatric species from the Indo–west–Pacific (*A. marina* Forssk. Vierh., from Madagascar) were also included for rooting phylogenetic trees. Leaves were collected from trees at least 50 m apart to maximize the capture of diversity within a population. Samples were fresh or were dried at ambient temperature in a plant press, silica gel, or in an oven at 40 °C. Total genomic DNA was extracted using a simplified CTAB (cetyltrimethyl ammonium bromide) method (Cullings 1992).

**DNA ANALYSIS**

The ITS of nuclear ribosomal DNA (i.e., ITS-1, 5.8S gene, and ITS-2) was amplified in 32 individuals from throughout the range of *A. germinans* and two individuals from the outgroup species. Primers LEU1 and ITS4 (White et al. 1990; Vargas et al. 1998) were used in a standard polymerase chain reaction (PCR) containing bovine serum albumine as adjuvant. The Big Dye 3.1 terminator kit (Applied Biosystems, Foster City, CA) was used for cycle sequencing and the PCR primers plus the internal primers ITS2 and ITS3 (White et al. 1990) were used in separate reactions. Reactions were precipitated and resuspended in 15 μL of Hi-Di formamide before loading them in an automated sequencer. Resulting sequences were deposited in Genbank, accession numbers DQ469837, DQ469839, DQ469840, DQ469842, DQ469844, DQ469845, DQ469847, DQ469849–DQ469855, DQ469857–DQ469863, EF136920–EF136928.

Initial screening for polymorphic polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) fragments was carried out in a subset of 10 populations from throughout the geographic range, using the cpDNA universal primer pairs trnL–trnF (LF) (Taberlet et al. 1991), psaA–trnS (AS), trnH–trnK (HK), trnK1–trnK2 (KK) (Demesure et al. 1995), trnC–trnD (CD), trnS–trnR (SR), and trnF–trnV (FV) (Dumolin-Lapegue et al. 1997) with the restriction enzymes *Alu* I, *α* *Tag* I, and *Hinf* I. The PCR-amplified product was restricted in a 15-μL cocktail containing 1 U of the restriction enzyme (New England Biolabs, Beverly, MA), 1 X buffer provided by the company, and 5 μL of PCR product. Restrictions were incubated for 4 h at 37 °C for *Alu* I and *Hinf* I reactions and 65 °C for *α* *Tag* I, and finalized with a deactivation step of 20 min at 80 °C. All the reactions were performed on a Techne (Cambridge, U.K.) thermocycler. Fragments were separated on a long 8% polyacrylamide gel at a constant 300 V for 3–5 h. Five primer/enzyme combinations (LF/*Alu* I, SR/*α* *Tag* I, HK/*α* *Tag* I, KK/*α* *Tag* I, FV/*Hinf* I) yielded repeatable and consistent polymorphic bands. The PCR products of these primer pairs were of the expected size as assessed in a 1% agarose electrophoresis: LF, ~900 bp (base pair); SR, ~1800 bp; HK, ~1800 bp; KK, ~2500 bp; and FV, ~3500 bp. Primer/enzyme combinations were then applied to 263 samples of *A. germinans*, to eight individuals of *A. schaueriana*, and two of *A. marina*.

Three pairs of universal primers for the amplification of chloroplast microsatellites developed by Weising and Gardner (1999) were tested on the same 263 samples (ccmp4, ccmp7, and ccmp10) following the PCR protocol reported by these authors. The PCR product (0.75 μL) was mixed with a solution of 8 μL of formamide and 0.5 μL of 350 ROX size standard.

![Figure 1. Geographic range of Avicennia germinans. Numbers correspond to sampled populations. See Supplementary Table S1 for sampling site information.](image-url)
Fluorescent AFLP was studied in 377 samples using the method of Vos et al. (1995) with modifications, as presented elsewhere (Douhovnikoff and Dodd 2003). Three sets of selective primers that had proven useful for diversity screening on A. germinans (Dodd et al. 2002) were used (Mse I-CAA/EcoR I-GTA, Mse I-CGTG/EcoR I-AC, Mse I-CGTA/ EcoR I-GTG). Products from the selective amplification were electrophoresed on an ABI 3100 automated sequencer. Peak categories were selected manually and several control runs were performed to select those that were consistent in presence, size, and shape.

**DATA ANALYSIS**

**Phylogenetic analysis**

Sequence data were aligned manually with the aid of GENEDOC version 2.6 (Nicholas and Nicholas 1997). Phylogenetic relationships among ITS sequences were reconstructed with the maximum parsimony criterion using a heuristic search in PAUP* version 4.04b (Swofford 1998) with 100 random-addition sequences and using the tree bisection reconnection (TBR) branch swapping; node support was obtained by nonparametric bootstrapping (Felsenstein 1985; 1000 replicates and 100 random addition sequences per replicate) and by decay index (DI) values calculated using PRAP version 1.21 (Müller 2004) and PAUP*. Trees were rooted using A. schaueriana and A. marina as the outgroup. A 7 bp indel mutation was treated as a fifth base in the tree search. The best model of evolution of the ITS region was assessed using the Akaike information criterion (AIC) on different substitution models with the aid of MODELTEST (Posada and Crandall 1998). The hypothesis of a molecular-clock on a most parsimonious tree was examined by a likelihood ratio test (Felsenstein 1988) using PHYLIP version 2.6 (Felsenstein 1985; 1000 replicates and 100 random taxon additions per replicate) and decay indices. Global and regional average within-population genetic diversity (h_S), total genetic diversity (h_T), and overall genetic differentiation (G_ST) based on haplotypes were calculated for A. germinans using the software Permut (Petit 2004).

Each AFLP fragment was assumed to represent one locus within the 75 bp and 350 bp size range. Presence (or absence) of peaks of the same length and comparable shape and intensity were considered to be homologous. Genetic diversity was estimated as percent of polymorphic loci (P) among all scored AFLPs using ARLEQUIN version 2.0 (Schneider et al. 2000). Heterozygosities and G_ST were obtained using the Bayesian approach of Holsinger et al. (2002), which does not assume Hardy–Weinberg equilibrium and does not require previous knowledge of the intrapopulation fixation index (F_IS). The “relaxation” of these assumptions in the estimation seems pertinent in analyzing dominant genetic data from A. germinans populations because a mixture of outcrossing and inbreeding has been detected in populations of another species in the genus, A. marina (Maguire et al. 2000), and some recently isolated microsatellites of A. germinans showed deviations from Hardy–Weinberg equilibrium in one population in the east Pacific (Nettel et al. 2005). The HICKORY version 1.0 (Holsinger and Lewis 2003) software was used for this estimation. Default running parameters under the “F-free model” were used, and several runs were performed. An unrooted neighbor-joining tree was constructed using Reynolds et al. (1983) genetic distance between pairs of populations with the aid of the PHYLIP package (Felsenstein 2004).

Partition of genetic diversity at global and at regional levels was investigated by hierarchical analysis of molecular variance (Excoffier et al. 1992) using ARLEQUIN version 2.0 (Schneider et al. 2000). Different regional groupings were compared to test for significant differentiation and to detect how much of the genetic diversity in both genomic and cytoplasmic markers they explained.

**Divergence time estimation**

To investigate the hypothesis of vicariance versus recent LDD between Atlantic populations in A. germinans, we estimated the time to the most recent common ancestor (TMRCA) of the smallest ITS clade that contained populations from both sides of the Atlantic. The most recent common ancestor was estimated under a Bayesian framework using Markov chain Monte Carlo (MCMC) integration as implemented in BEAST version 1.3 (Drummond and Rambaut 2005a). An uncorrelated exponential relaxed clock (Ho et al. 2005) analysis was performed using a general time reversible model of substitution with variable sites (GTR + I) as specified by the AIC. We assumed a constant population size.
and a fixed mean of the prior for the substitution rate. We did separate runs for each of the five different mutation rates that cover the range of reported rates for the ITS region: (1) 6.4 × 10⁻¹⁰ substitutions per site per year (s/s/y); (2) 2.2 × 10⁻⁹ s/s/y; (3) 4.0 × 10⁻⁹ s/s/y; (4) 5.9 × 10⁻⁹ s/s/y; (5) 7.8 × 10⁻⁹ s/s/y (Wendel et al. 1995; Baldwin and Sanderson 1998; Richardson et al. 2001; Dick et al. 2003). Priors for the population size and variance parameter of the lognormal distribution were drawn from uniform distributions over [0, 10,000] and [0, 2.0], respectively. We used a most parsimonious tree as starting tree and defined the outgroup by forcing monophyly for *A. germinans*. We further estimated the ITS mutation rate for *Avicennia* by using the age of the oldest known fossil (Early Eocene of France, ∼50 million years ago; Gruas-Cavagnetto et al. 1988), as prior information for the base of the tree (uniform distribution over [45, 55]) and allowed the mutation rate to vary. Posterior distributions of TMRCA and ITS mutation rate were approximated by sampling values at every 1000 updates over 10 million MCMC updates, after discarding a burn-in of 100,000 updates. The software TRACER version 1.3 (Drummond and Rambaut 2005b) was used to analyze the results. Results are presented as the mean and the 95% highest posterior density (HPD) limits.

**Results**

**ITS ANALYSIS**

Sequencing of the ITS region yielded a dataset of 658 bp. None of the obtained sequences had pseudogene characteristics based on the GC content, 5.8S region substitutions, and free-energy values (results not shown). Within *A. germinans*, 34 (5.22%) sites were variable and 31 (4.76%) were parsimony informative. A 7 bp insertion, not present in the outgroup species, was found in individuals from Galeta and Portobelo from Atlantic Central America.

Maximum parsimony analysis resulted in one most parsimonious tree with 87 steps (see Fig. 2), which separates the species into three main clades. Clade 1, includes black mangroves from

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**Figure 2.** Phylogram of the most parsimonious tree of *Avicennia germinans* based on ITS region sequences. Bold numbers correspond to bootstrap node support, numbers below the line correspond to decay index (DI) values. Arrow indicates the node selected for trans-Atlantic divergence time estimation. Numbers in parentheses correspond to the population number. Consistency index excluding uninformative characters (CI) = 0.97, retention index (RI) = 0.99.

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and Excoffier 2004). Infiles and simulated files are available from the authors.
Pacific Costa Rica and Mexico, clade 2 contains samples from Pacific Panama and Pacific Costa Rica, and clade 3 is formed entirely of individuals collected within the Atlantic basin. Bootstrap support and DI values were substantially higher for clades 1 (100%, DI = 9) and 3 (98%, DI = 5) than for clade 2 (68%, DI = 2). Support for monophyly of *A. germinans* in relation to the outgroup species was weak (71%, DI = 1). Within the Atlantic lineage (clade 3), east Atlantic and Atlantic South American samples formed a monophyletic group sister to a lineage of Caribbean and Atlantic Central American samples.

The best model of evolution selected for the ITS data was the general time reversible model of substitution with invariable sites (GTR + I). The hypothesis of a molecular clock was rejected ($X^2 = 191, df = 28, P < 0.001$).

### cpDNA DIVERSITY AND DIFFERENTIATION

Five of 21 primer/enzyme combinations tested (LF/Alu I, SR/αTaq I, HK/αTaq I, KK/αTaq I, FV/Hinf I) yielded eight polymorphic fragments. Only indel mutations were detected in six of these polymorphic fragments; KK/αTaq I and FV/Hinf I had restriction site mutations, showing presence or absence of a band (see Table 1). Two microsatellite loci, ccmp4 and ccmp10, were polymorphic with five (136 bp, 142 bp, 143 bp, 144 bp, 145 bp) and three alleles (96 bp, 97 bp, 98 bp), respectively. Primer set ccmp7 was monomorphic for the species. The combination of restriction fragments and microsatellites resulted in 19 unique character state combinations or haplotypes (A–S) in all the assessed *A. germinans* samples.

Parsimony analysis of chloroplast haplotypes resulted in a single most parsimonious tree, but bootstrap support and decay values were low (see Fig. 3). Only *A. schaueriana* was used as the outgroup because comparison of haplotypes from *A. marina* was not possible due to the lack of putative homologous fragments for the regions tested.

Four main lineages were recognized; one of them included all Atlantic samples, and the other three included the east Pacific samples. Within the Atlantic clade, haplotype A (Guiana) and haplotype B (Brazil and east Atlantic) formed a group sister to an Atlantic Central America–North America–Caribbean (ACNC) clade. In the east Pacific, all the haplotypes from Costa Rica and Mexico formed a monophyletic group; Panamanian haplotypes potentially belonged to two different lineages. The geographic location of haplotypes is shown in Figure 4.

Chloroplast diversification estimates for the species and for regional groups of populations were high ($G_{ST}$ global 0.78, $G_{ST}$ regional 0.69–0.80), except for the east Atlantic, in which only a single haplotype was detected (see Table 2). Intrapopulation diversity was relatively low ($h_S$ total 0.19, $h_S$ regional < 0.24).

### Table 1. Chloroplast DNA (cpDNA) haplotypes identified using eight polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) fragments and two microsatellites in Avicennia germinans (A–S) and *A. schaueriana*. Length variation of fragments is represented by numbers (1–3) or the size in base pairs (bp) for microsatellites. A0 denotes absence of the fragment. Each polymorphic fragment for a primer enzyme combination is labeled in decreasing order of molecular weight in roman letters.

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with only one or two haplotypes detected in most populations. AMOVA’s partition of genetic diversity at the global level showed the same pattern; high population divergence (Global $\Phi_{ST}$ 0.95) and low intrapopulation variation. Partitions of haplotype variance into a hierarchy of regional groupings were all significant except for the Atlantic subgroup partition between South America and east Atlantic ($P = 0.37$, see Supplementary Table S2 online).

**GENOMIC DNA DIVERSITY AND DIFFERENTIATION**

From three selective primer combinations, we scored 263 polymorphic fragments in 33 populations. Each individual had a unique banding pattern. Mean percent of polymorphic loci was 36 with a minimum of 14 from Bermuda, in the Atlantic, and a maximum of 69 from the Chiriqui population on the Pacific Coast of Panama (polymorphic criterion of 0.95). Mean heterozygosity for the species was 0.12. Heterozygosites were highest for populations along the Pacific Coast of Panama and Atlantic Coast of Costa Rica (see Supplementary Table S1 online), whereas Angola and Bermuda were the least diverse. The estimate for overall genetic differentiation was $G_{ST} = 0.33$. The highest estimate of regional differentiation was for the west Atlantic ($G_{ST} = 0.27$), followed by east Atlantic ($G_{ST} = 0.26$), whereas the lowest estimate of differentiation was for the east Pacific ($G_{ST} = 0.22$).

An unrooted neighbor-joining tree revealed a major division between Atlantic and Pacific populations (see Fig. 5). Atlantic populations formed three main groups corresponding to the following delineated geographic areas: Caribbean Islands; Atlantic Panama, Yucatan Peninsula, Florida, and Bermuda; and northern West Africa–Atlantic South America. Angola from southern West Africa and Atlantic Costa Rican populations did not branch within the major Atlantic groups.

Global $\Phi_{ST}$ was 0.38, which is higher than the estimated $G_{ST}$. Contrary to the cpDNA AMOVA results, most of the genetic variance for AFLP markers was attributed to within populations at the global and regional levels. The partition of AFLP molecular variance into a hierarchy of regional groups resulted in the South American and West African groups failing to show significant differentiation ($P = 0.063$, see Supplementary Table S2 online).
Table 2. Chloroplast diversity and differentiation. Chloroplast haplotype global and regional diversity and differentiation statistics according to Pons and Petit (1996) from 33 A. germinans populations. $h_S$, intrapopulation diversity; $h_T$, total population diversity. SE, standard error.

<table>
<thead>
<tr>
<th>Avicennia</th>
<th>East</th>
<th>West</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>$h_S$</td>
<td>0.19</td>
<td>0.05</td>
<td>0.24</td>
<td>0.1</td>
</tr>
<tr>
<td>$h_T$</td>
<td>0.87</td>
<td>0.03</td>
<td>0.77</td>
<td>0.13</td>
</tr>
<tr>
<td>$G_{ST}$</td>
<td>0.78</td>
<td>0.05</td>
<td>0.69</td>
<td>0.09</td>
</tr>
</tbody>
</table>

DIVERGENCE TIME ESTIMATION

The smallest amphi-Atlantic clade included samples from South America and the east Atlantic (see Fig. 2). Mean estimates of the TMRCA for this clade using previously reported ITS mutation rates ranged from $2.3 \times 10^{-10}$ s/s/y to 170 thousand years (95% HPD: 380–40, mean substitution rate of $7.8 \times 10^{-9}$ s/s/y). A summary of the results is presented in Table 3. The mean estimated ITS substitution rate and TMRCA for the smallest amphi-Atlantic clade using the oldest known fossil as prior information for the root of the tree were $7.8 \times 10^{-9}$ s/s/y (95% HPD: 1.2–3.9 $\times 10^{-10}$) and 1.89 million years (95% HPD: 3.67–0.29), respectively.

Table 3. Internal transcribed spacers region (ITS) divergence time estimation. Summary of the results of the Bayesian estimation of the time to the most recent common ancestor (TMRCA) for the smallest monophyletic group in the most parsimonious ITS tree, which contains samples from both sides of the Atlantic. TMRCA for each of the different mutation rates used is presented as mean values (bold) and the lower and upper 95% highest posterior density (HPD) limits. s/s/y, substitutions per site per year.

<table>
<thead>
<tr>
<th>ITS substitution rate (s/s/y)</th>
<th>Mean million years ago</th>
<th>95% HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$6.4 \times 10^{-10}$</td>
<td>2.3</td>
<td>(0.46–4.59)</td>
</tr>
<tr>
<td>$2.2 \times 10^{-9}$</td>
<td>0.65</td>
<td>(0.14–1.32)</td>
</tr>
<tr>
<td>$4.0 \times 10^{-9}$</td>
<td>0.32</td>
<td>(0.78–0.72)</td>
</tr>
<tr>
<td>$5.9 \times 10^{-9}$</td>
<td>0.25</td>
<td>(0.05–0.49)</td>
</tr>
<tr>
<td>$7.8 \times 10^{-9}$</td>
<td>0.19</td>
<td>(0.04–0.38)</td>
</tr>
</tbody>
</table>

All the populations from the east Atlantic and the two populations from Brazil shared the same, fixed cpDNA haplotype (haplotype B, see Fig. 4). A second closely related haplotype (A), differing by only 1 bp in the microsatellite ccmp10 from haplotype B, was detected in the population from French Guiana. We compared the pattern of Brazilian and east Atlantic populations with that of two simulated populations at different divergence times, effective population sizes ($N_e$), and mutation rates ($\mu$). Results were considered significantly different from the observed data if identical, fixed character states were shared in the two simulated populations in less than 5% of the 100 simulated datasets ($P$-value of 0.05). All the simulations for a divergence time earlier than 0.5 million years ago are significantly different from the observed data, regardless of the selected $N_e$ or $\mu$. For $N_e = 1000$ and $N_e = 10,000$, all the simulations with a divergence time earlier than 60,000 years and 250,000 years, respectively, were significantly different. Figure 6 shows results for $N_e = 100$, the setting at which simulated data most closely fitted the observed data.

Discussion

The relatively low divergence between South American and east Atlantic populations is consistent with Quaternary trans-Atlantic propagule dispersal and establishment of A. germinans. This result is unexpected considering the prevailing hypothesis that the widespread distribution of A. germinans in the Atlantic is due to vicariance (Tomlinson 1986; Duke 1995). For our ITS divergence time estimation, only the lowest mutation rate ($6.4 \times 10^{-10}$ s/s/y) places the divergence of South American and east Atlantic populations earlier than the Pleistocene. This mean substitution rate was calculated by Dick et al. (2003) using trees with a generation time longer than 10 years. Whereas the reported peak rate of propagule production for A. marina is at 7.5 years (Clarke 1993).
number following slash corresponds to the mutation rate of two
ulated restriction fragment length polymorphism (RFLPs) and the
constant effective population size (Ne) of 100 are shown. Number
would have to be invoked to explain the low divergence and low
have been separated. Extremely low rates of molecular evolution
the more than 80
noncoding regions of the cpDNA to accumulate mutations over
Atlantic when the Atlantic was formed, we would expect
erative scenario, therefore, these hypothesis was rejected. Even
ative time estimation are in contradiction to the expectations of the vi-
sence of Atlantic South America and east Atlantic and the relatively reduced extension
of mangrove swamps south of Gabon, limiting the possibility of
nflow. Furthermore, the Southern Equatorial Current and
in south Atlantic and east Atlantic populations are apically
lith and the ITS and chloroplast genealogies likely reflect
the inherent hypervariability of AFLP markers (Douhovnikoff and
and, therefore, the lack of enough characters to fully resolve lineage relationships.

The fossil record for Avicennia in the ACEP supports dis-
persal over vicariance also in Tertiary times. Most authors agree
that the origin of extant mangrove taxa was along the shores of the
Tethys Sea in the late Cretaceous between 100 and 80 million years
ago (Duke 1995; Ellison et al. 1999; Saenger 2002). Nevertheless, the first fossil record of Avicennia appears much later in the Early
Eocene of France (~50 million years ago; Gruss-Cavagnetto et al.
1988). The Tertiary mangrove fossil record has been reviewed by
different authors (Duke 1995; Rull 1998; Saenger 1998; Ellison
et al. 1999; Plaziat et al. 2001; Dodd and Afzal-Rafii 2002; Saenger
2002), and if we regard the trends shown by fossils as trustwor-
thy, Avicennia would not have been present in the Americas or
the east Atlantic earlier than 15 million years ago, when the con-
tinents were almost in their present position (Plaziat et al. 2001).
The earliest records of Avicennia fossils in the ACEP are from the
late Miocene (10–12 million years ago; van Steenis 1969; Muller
et al. 1987). Assuming a Tethyan origin of the genus, LDD would
necessarily be the means by which black mangroves arrived and
established along coastlines of the American continent. Never-
theless, we acknowledge that the mangrove fossil record is far
from complete, particularly in the case of Avicennia (Plaziat et al.
2001), and new fossil discoveries could change our understanding
of mangrove history.

The report presented by Renner (2004), which summarizes
numerous LDD events across the Atlantic for plants (most of
which are not specialized to disperse by water), combined with the force and directionality of the equatorial Atlantic current system and prevailing winds, provides a plausible and supportive framework for our findings. Our results and analyses show that LDD occurred relatively recently between Africa and South America in A. germinans, probably during the Quaternary. This raises possibilities of Quaternary trans-Atlantic migration for populations of other water-dispersed tropical woody plant species present in the Atlantic. This would be particularly true for mangrove and mangrove-associated species such as Laguncularia racemosa C.F. Gaertn. (Combretaceae), Conocarpus erectus L. (Combretaceae), Rhizophora mangle L., R. racemosa G. Meyer & Leechm., and R. harrisonii Leechm. (Rhizophoraceae), whose ranges extend through the CAI and the Atlantic Ocean (Tomlinson 1986). Furthermore, Takayama et al. (2006) reported a similar pattern for the water-dispersed mangrove-associate Hibiscus L. with strong cpDNA differentiation between east Pacific and Atlantic populations and the sharing of a chloroplast haplotype across the Atlantic Ocean. Their interpretation of the data is also consistent with recent (< 3.5 million years ago) trans-Atlantic LDD.

Our data show signals of northward leading-edge recolonization for populations from the northern latitudinal extremes of the distributional range of A. germinans (Baja California, Mexico in the east Pacific; Bermuda in the west Atlantic). Each of these populations had low cpDNA diversity and formed a monophyletic ITS clade with southern, undifferentiated populations (Chautengo, Mexico, and the Dominican Republic, respectively). Mangrove populations may have gone extinct at high latitudes in the Americas during cold phases in the Quaternary because of the prevalence and intensity of frost events (Sherrod and McMillan 1985; Ellison 1996; Dodd et al. 2002). Frosts in Bermuda were more common during glacial maxima due to the contraction of tropical sea currents toward the equator (Ellison 1996; Trend-Staïd and Prell 2002). Even though seedlings of some populations of A. germinans are able to tolerate up to five days at 2–3°C (McMillan and Sherrod 1986), a few days of subfreezing temperatures are highly detrimental (80–85% population decline, Sherrod and McMillan 1985).

East Atlantic populations of A. germinans presented only one fixed cpDNA haplotype, which is also consistent with population extinction and range expansion from a refugium after glaciations. The latitudinal distribution of east Atlantic mangrove ecosystems is limited by aridity (Saenger 1998). Sea surface temperature decrease during glacial maxima may have prompted large-scale aridification in West Africa (Scheufub et al. 2003) with corresponding adverse conditions for mangrove populations.

Unfortunately, the fossil pollen of Avicennia, as for most animal-pollinated plants, is not well represented in Quaternary fossil records. In contrast, pollen and a biochemical marker from the anemophilous mangrove genus, Rhizophora, are readily found in offshore deposits in the east Atlantic (Dupont et al. 2000; Versteegh et al. 2004; Scourse et al. 2005). Important cyclical fluctuations in Rhizophora fossil pollen (from 0% to 16%) have been observed in cores that date back to the middle Pleistocene. However, recently, these changes have been correlated to the rate of marine transgression and not to mangrove swamp extension (Scourse et al. 2005) and, therefore, they may not be helpful in determining the extent of mangrove population changes.

Even though some populations with the lowest AFLP diversity (i.e., Bermuda, Soyo, and La Paz1) are at the latitudinal extremes of the range of the species and the populations with highest diversity were from Central America, reduced diversity at higher latitudes was not a strict pattern as it was for chloroplast haplotypes (see Supplementary Table S1, see Fig. 4). Also, in contrast to cpDNA results, AFLP diversity was high in some east Atlantic populations. We interpret this discrepancy as mainly the result of the different characteristics between markers; AFLPs are inherently hypervariable and are suitable for studies involving individual assignment tests (Campbell et al. 2003), parentage analysis (Gerber et al. 2000), and clone identification (Douhouvnikoff and Dodd 2003) and, therefore, are expected to be much more variable at the within-population level than cpDNA.

In general, cpDNA diversity was low in the Atlantic basin in comparison to the Pacific basin. The Yucatan Peninsula was the most diverse area for chloroplast haplotypes in the Atlantic, including private haplotypes that differed by only 1 bp in chloroplast microsatellite fragments (see Fig. 4, Table 1). The Atlantic coast of Central America included only two cpDNA haplotypes, in contrast to the Pacific coast in which 11 haplotypes were detected. Reflecting this pattern of haplotype diversity, the ITS phylogeny revealed that Central American populations in the Pacific came from two divergent lineages, whereas the whole Atlantic basin formed a monophyletic group. If we assume that A. germinans was present around the emerging CAI during its gradual uplift around three million years ago, the lack of shared haplotypes between Central American coasts and the low diversity in Atlantic Central America indicate complete differentiation of Atlantic and Pacific populations and probably a higher rate of extinction in the Atlantic over this time period.

Besides the intensity of frosts and aridity, another plausible cause for extinctions is lack of habitat availability during the Quaternary due to rapid sea level change and steepness of the continental shelf (Scourse et al. 2005). Ellison and Stoddart (1991) proposed that mangroves are unable to keep pace with a sea level rise rate of 12 cm/100 years, nevertheless rates as much as 30 times higher have been recorded during the Holocene (Bard et al. 1990). Mangroves are inherently linked to intertidal, protected environments and, therefore, are likely to respond to sea level fluctuations and topography: steady inundation of gently sloping shelves and protected areas would favor mangrove development, in contrast
to sudden inundation of steep slopes where mangroves would be unable to establish (Scourse et al. 2005). Along with other regions in the species’ range, Atlantic Central American, Atlantic South American, and east Atlantic coasts have a narrow continental shelf followed by a steep slope. Furthermore, in some areas the continental shelf break occurs at a shallow depth (< 80 m below the present sea level) and during the LGM, when sea level reached a low-stand up to 130 m below the present sea level (Lambeck et al. 2002), protected low-energy environments would have been scarce and probably prevented mangroves from establishing.

The higher genetic diversity observed in ITS and cpDNA data for the Pacific populations was also reflected in the AFLP markers; mean diversity indices were higher for the Pacific (P[%] = 44.4, h = 0.14, 12 populations) than the Atlantic (P[%] = 33.6, h = 0.12, 22 populations). Nevertheless, a more detailed assessment is needed to estimate the significance of this difference. On the other hand, a study of Colombian populations of A. germinans reports the highest AFLP genetic diversity in this species (Cerón-Souza et al. 2005). Their estimates of average heterozygosity (He = 0.25) and mean percent of polymorphic loci (P[%] = 74.3) exceed our estimates (see Supplementary Table S1). In our study, populations in Panama presented the highest diversity in chloroplast haplotypes and high estimates of AFLP heterozygosity. Our sampling did not include the southernmost regions of Panama, which might be as diverse as those in neighboring Colombia.

Hybridization with A. bicolor Standl., a locally restricted species, may contribute to the high genetic diversity in Pacific Central American populations of A. germinans. Although A. bicolor is phenotypically and ecologically distinct from A. germinans (Jimenez 1994) and we sampled individuals that were phenotypically determined to be A. germinans, we cannot rule out introgressive hybridization between these two species. We are currently conducting a thorough reexamination of Pacific Central American populations to address the phylogenetic relationship and extent of genetic exchange between these two species.

Our data illustrate the different processes that affected genetic diversification of a tropical species during the Quaternary. We have shown that, except for low latitudes in the American Pacific, black mangrove diversity is relatively low. Our data are consistent with the hypothesis that LDD is responsible for the wide-range distribution of A. germinans. Our data are also consistent with the interpretation that northern populations went extinct during cold phases of the late Quaternary resulting in a contraction of their range. This contraction was followed by northward leading-edge recolonization—a pattern shared with temperate species. Major, recent range expansions in South America since the Pleistocene also have been reported for terrestrial rain forest species. For example, in Symphonia globulifera L.f. (Clusiaceae), a single ITS haplotype is shared in populations that extend throughout Brazil (Dick et al. 2003). Also, major extinctions and recolonization were detected for Vouacapoua americana Aubl. (Leguminosae) in French Guiana, possibly due to periods of drought during the Holocene (Dutech et al. 2003). On the other hand, studies of the rain forest tree genus, Inga Mill. (Leguminosae) (Richardson et al. 2001), and other trees from the dry forest in South America show a high degree of lineage diversity that predates the Quaternary (Bermingham and Dick 2001; Pennington et al. 2004). Wide-range phylogeographic studies of tropical plant species supported by paleoclimatic and fossil evidence are needed to further advance our understanding of the causes for the different patterns of diversification within tropical regions.

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**Supplementary Material**

The following supplementary material is available for this article:

**Table S1.** Characteristics of the 33 Avicennia germinans populations sampled.

**Table S2.** AMOVA results. Results from hierarchical AMOVA of Avicennia germinans populations based on chloroplast haplotypes, and AFLP markers.

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(This link will take you to the article abstract).

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