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ORIGINAL ARTICLE

Genetic population structure and distribution of a fungal polypore, *Datronia caperata* (Polyporaceae), in mangrove forests of Central America

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ABSTRACT

Aim We examine the genetic structure of a fungal polypore, *Datronia caperata* (Berk.) Ryvarden (Polyporaceae), colonizing white mangrove, *Laguncularia racemosa* (L.) Gaertn. f. (Combretaceae), of Central America.

Location Mangrove forests of Costa Rica and Panama.

Methods Sequences of elongation factor alpha (EFA), beta tubulin (BTUB) and nuclear ribosomal internal transcribed spacer (ITS) regions were obtained from 54 collections of *D. caperata* collected from Caribbean and Pacific *L. racemosa* forests in Central America. Measures of haplotype and nucleotide diversity, nested clade analyses and coalescent analyses were used to estimate the direction and extent of migration of the fungus, and the factors promoting population divergence. We also conducted phylogenetic analyses using Bayesian estimation to test whether putative *D. caperata* collected from *L. racemosa* was conspecific with *D. caperata* colonizing other hosts from diverse Neotropical forests.

Results Our results demonstrate that there is genetic isolation between *D. caperata* populations from Caribbean mangroves and those from Pacific mangroves. Our data suggest that the best explanation for the observed haplotype distribution is a recent range expansion from the Caribbean to the Pacific coasts, with subsequent isolation. This is supported by the infrequent overlap of haplotypes, unidirectional migration estimates from the Caribbean to the Pacific and the older estimated age of mutations in the Caribbean low-copy BTUB and EFA loci. In addition, our data suggest that *D. caperata* from mangroves are not conspecific with collections from other hosts found in diverse Neotropical forests.

Main conclusions The low frequency of shared haplotypes between coasts, coupled with the incomplete lineage sorting after cessation of gene flow, is consistent with isolation during the last Pleistocene glaciation. We hypothesize that the greater haplotype and nucleotide diversity in the Pacific occurs either because larger effective population sizes of *D. caperata* are maintained in Pacific mangroves or because *D. caperata* populations underwent a significant bottleneck as a result of local extinction followed by recolonization. In addition, we found that *D. caperata* found on *L. racemosa* was not conspecific with *D. caperata* from non-mangrove hosts and suggest that *D. caperata* found on *L. racemosa* may be a host specialist.

Keywords
Coalescent methods, Costa Rica, nuclear DNA, Panama, phylogeography, polypore fungus, white mangrove.

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INTRODUCTION

Wood-decay polypore fungi in Neotropical forests form diverse communities, characterized by the presence of common generalist species colonizing multiple hosts (Lindblad, 2000; Gilbert *et al.*, 2002; Gilbert & Sousa, 2002; Ferrer & Gilbert, 2003). Strong host specialization is expected to be rare in high-diversity forests, where the low density of individual host species reduces the probability of efficient horizontal transmission (May, 1991). An exception to this tropical trend has been observed in mangrove communities of Central America, which comprise only a few dominant tree species (typically three to four; Tomlinson, 1986). In these forests, the polypore composition is strongly dominated by a few host-specialized species (Gilbert & Sousa, 2002). The low diversity of mangrove forests may provide a selective advantage to those fungi that are specialized on locally abundant hosts (Gilbert & Sousa, 2002; Gilbert, 2005), although it is also true that species that locally appear to be host specialists may be global generalists (Fox & Morrow, 1981). One such wood-decay polypore species, *Datronia caperata* (Berk.) Ryvarden, is a reported generalist in diverse Neotropical forests (Lindblad, 2000), but in mangroves it is known to colonize a single plant species [*Laguncularia racemosa* (L.) C.F. Gaertn.; Combretaceae] (Gilbert & Sousa, 2002; Parrent *et al.*, 2004). Although this pattern of host specialization observed in mangrove forests may be atypical when compared with that in diverse Neotropical forests, the dependence on one suitable host in mangrove forests suggests that the demography (including dispersal and horizontal gene transmission) of *D. caperata* is linked to its mangrove host, *L. racemosa*.

The name *D. caperata* has not been widely accepted in the literature, and by the convention established by Index Fungorum it is synonymous with *Coriolopsis caperata* (Berk.) Murrill (1908) (CABI Bioscience, CBS and Landcare Research at http://www.indexfungorum.org). However, we chose to preserve the name *D. caperata* based on morphological characters of the dark context of the trama and the black line separating it from the tomentum (Ryvarden, 1985; L. Ryvarden, personal communications). For most of its life cycle, *D. caperata* is characterized by a dikaryotic mycelium with ploidy = N + N, but karyogamy and meiosis occur in the shelf-like sexual fruit-bodies produced on dying or dead trees. As a result, large numbers of airborne haploid meiospores known as basidiospores are released from the pores underneath the fruit-body, resulting in the infection of new trees and in the spread of the organism. Although our understanding of the infection process and host colonization for this particular organism is limited, most wood-decay pathogens share many attributes. For instance, many wood-decay agents are known to infect trees and to undergo an endophytic phase characterized by limited reproduction. As trees start to decline, the growth of these decay agents becomes more pronounced and leads to the colonization of significant portions of their hosts and to the production of basidiomes.

An unusually high level of genetic isolation for wood-decay fungi has been reported for *D. caperata* populations from coastal mangrove stands in Panama (Parrent *et al.*, 2004). The study of Parrent *et al.* (2004) showed that gene flow was reduced in the coastal populations of *D. caperata* when areas occupied by *L. racemosa* were discontinuous along the coastline, suggesting that populations of mangrove associates may effectively be genetically isolated. *Laguncularia racemosa*, like all mangrove plants, grows only in intertidal areas under strict ecological conditions (Duke *et al.*, 1998; Ellison, 2004). By contrast, other reported hosts for *D. caperata* are common in the widespread lowland tropical forests of Panama (Lindblad, 2000; Gilbert *et al.*, 2002; Gilbert & Sousa, 2002; Ferrer & Gilbert, 2003), and gene flow between mangrove-inhabiting and generalist populations of this fungus would be in conflict with the highly structured populations reported by Parrent *et al.* (2004).

Although similarities in patterns of population genetic structure between hosts and their parasites have been studied (for a synthesis of host–parasite co-evolutionary studies, see Greischar & Koskella, 2007), little is known about the common biogeographical patterns of plant species and their fungal associates. The disjunct distribution of the white mangrove *L. racemosa*, found on both the Caribbean and the Pacific coasts of Central America, offers an opportunity to examine the associated genetic isolation of host-specialized mangrove fungi, such as *D. caperata*. Spores are small compared with pollen, and thus the scale of dispersal and gene flow may differ between fungus and host.

Both geological and climatic changes may have had profound effects on the biogeographical history of Central American mangrove species. The gradual closing of the Isthmus of Panamá lasted from 13 to 1.9 Ma (million years ago) (Collins *et al.*, 1996). The isolation between Caribbean and Pacific coastal populations was precipitated by the final closure of the Isthmus of Panamá (Jackson & Budd, 1996), leading to divergence among trans-isthmus populations (Marko, 2002). Although the minimum width of the Isthmus of Panamá is only 35 km, much of the interior is dominated by a mountainous divide, a formidable barrier to plant dispersal (Dodd *et al.*, 2002; Dodd & Afzal-Rafii, 2002; Takayama *et al.*, 2006; Nettel & Dodd, 2007). As recently as 19,000 years ago, the glacial periods that dominated the Pleistocene lowered global sea levels to as much as 120 m below present levels (Siddall *et al.*, 2003). The lack of intertidal environments in areas where the continental shelf drops off sharply along the Caribbean coast and on Caribbean islands may have limited the capacity for mangroves to thrive (Woodroffe & Grindrod, 1991; Versteegh *et al.*, 2004; Scourse *et al.*, 2005; Nettel & Dodd, 2007). Thus, the current Caribbean mangrove populations in Central America may be the result of isolation as a consequence of isthmus closure, coupled with local recolonization after extinction in shallow coastal regions (Nettel & Dodd, 2007). Here, we explore whether the genetic structure of an important fungal mangrove associate, *D. caperta*, is reflective of a genetic history similar to that observed in its host.

Nested clade analyses (NCAs) and coalescent methods are used in the present study. NCAs provide a framework with which to test alternative hypotheses about historically driven mechanisms affecting patterns of gene diversity (Templeton *et al.*, 1992; Donnelly & Tavaré, 1995; Templeton, 1998; Edwards & Beerli, 2000). NCA involves an overlay of geography and phylogeny to differentiate the contribution of historical events from the effects of ongoing recurrent processes in shaping observed genetic patterns (Avise, 2000). Coalescent analysis more precisely reconstructs the processes underlying observed genetic patterns, including the determination of ancestral populations (Kingman, 1982a,b) and the direction of gene flow (Beerli, 1998), while factoring in population subdivision and migration.

MATERIALS AND METHODS

Collections and DNA extraction

Single-spore haploid isolates were obtained from four mangrove populations sampled from Caribbean (Coco Solo, $n = 7$; Mananti, $n = 6$; PonSok, $n = 10$) and Pacific (David, $n = 10$) coastal populations as previously described by Parrent *et al.* (2004) (Fig. 1, Appendix S1 in Supporting Information). We sampled an additional population from dikaryotic (N + N) basidiomes from two coastal mangrove forests within two kilometres of one another on the Pacific coast of Costa Rica, which were collectively designated as Guacalillo ($n = 21$) (Fig. 1, Appendix S1). Additional dikaryotic *D. caperata* cultures from non-mangrove hosts collected from Venezuela were provided by the Center for Forest Mycology Research, Forest Products Lab, USFS (Madison, WI, USA). We also sequenced three dikaryotic basidiomes from Barro Colorado Island, located in the Panama Canal, that were sampled from two lowland tropical plant species [*Tachigali versicolor* (Fabaceae) and *Trichilia tuberculata* (Meliaceae)] (Gilbert *et al.*, 2002). Tissue was excised and lyophilized for 48 h and then ground in a Retsch Mixer Mill MM301 (Retsch, Hann, Germany) at 30 rps for 90 s. Genomic DNA was extracted using the CTAB (cetyl trimethyl ammonium bromide)-modified phenol/chloroform method, followed by purification on GeneClean Turbo columns (MPBiomedicals, Solon, OH, USA) as previously described in Hayden *et al.* (2004). Genomic DNA was eluted in 0.1× TE. Extracts were stored at −20°C.

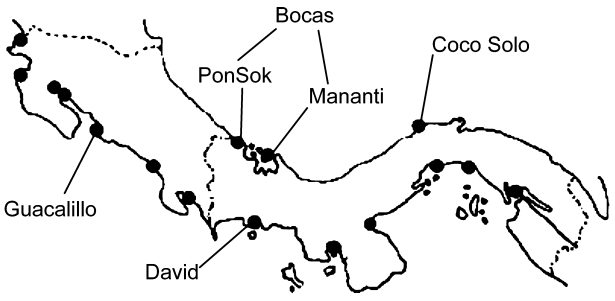

Figure 1 Distribution of large populations of mangroves in Costa Rica and Panama (adapted from Ellison, 2004) and the five populations of *Datronia caperata* sampled in this study: Coco Solo, David, Guacalillo, Mananti and PonSok (the last two collectively referred to as Bocas).

Polymerase chain reaction amplification and sequencing

Three loci were amplified: elongation factor 1-alpha (EFA), beta-tubulin (BTUB) and the nuclear internal transcribed spacer region (ITS) including the intervening 5.8S ribosomal DNA. Polymerase chain reaction (PCR) amplification of the BTUB loci was carried out in 50-μl reactions containing 1× reaction buffer (0.5 M KCl, 0.1 M Tris pH 8.3, 1 mg mL^{-1} gelatin), 0.2 mM dNTPs (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 0.5 μM each primers F-ßtub3 and F-ßtub4r (Einax & Voigt, 2003), 2 U *Taq* polymerase and 6.25 μL of template DNA. Thermocycling conditions required one cycle at 95°C for five min, followed by 35 cycles of 95°C for 30 s, 50°C for 60 s and 72°C for 60 s, with a final extension at 72°C for 10 min. ITS amplifications using primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) were carried out with similar PCR conditions after adjustment of the *Taq* concentration to 2.5 U per reaction. Thermocycling conditions for ITS were one cycle of denaturing at 94°C for five min, followed by 35 cycles of 93°C for 35 s, 58°C for 55 s and 72°C for 50 s, with a final extension at 72°C for 10 min. PCR amplifications of the EFA locus were similar to those for the BTUB locus but required adjustment of the $MgCl_2$ concentration to 1.5 mM, and 1 μM each primer ef-gr (designed by S. Rehner and available from http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf) and ef595f (Kauserud & Schumacher, 2003) and 2.5 U *Taq* polymerase. Thermocycling was performed by initial denaturing at 95°C for 90 s, followed by 35 cycles of 95°C for 30 s and 60°C for 90 s, with a final extension at 72°C for 10 min. A subset of four dikaryotic samples with heterozygous nuclear alleles (BTUB, EFA) were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) and PCR amplified with plasmid primers T7 and M13R using the PCR amplification and sequencing protocols previously described (Bergemann *et al.*, 2006).

Alignments and nucleotide polymorphisms

All variable sites were confirmed by visual inspection of the chromatograms, and sequences were aligned manually using Se-Al (Rambaut, 1996). A subset of BTUB heterozygotes sampled from N + N Guacalillo basidiomes ($n = 4$) were resolved by cloning and sequencing using the protocols previously described. In addition, we sequenced both EFA alleles from a subset ($n = 3$) of heterozygous loci in the Guacallilo population (Table 1). In all cases, both alleles were found using the cloning methods and were included in the analyses. The remaining haplotypes that were heterozygous

Table 1 Estimates of haplotype (h) and nucleotide diversity (π) estimates ± standard deviation (Schneider *et al.*, 2000), Tajima's D (Tajima, 1989), and Fu and Li's D^* and F^* (Fu & Li, 1993) and departure from neutrality for all mutations in *Datronia caperata* from the Caribbean and Pacific mangrove populations.

	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's D	Fu & Li's D^*	Fu & Li's F^*
BTUB					
Caribbean	0.3953 ± 0.1279	0.0009 ± 0.0007	−1.875*	−2.469*	−2.455*
Pacific	0.9983 ± 0.0074	0.0020 ± 0.0013	−1.161	−1.041	−1.175
Combined	0.8966 ± 0.0387	0.0029 ± 0.0017	−1.889*	−2.985*	−2.918*
EFA					
Caribbean	0.7391 ± 0.1015	0.0012 ± 0.0096	−1.393	−2.015	−1.958
Pacific	0.7576 ± 0.0816	0.0024 ± 0.0015	−1.801*	−2.869*	−2.018*
Combined	0.9041 ± 0.0372	0.0031 ± 0.0019	−1.376	−1.589	−1.697
ITS					
Caribbean	0.5850 ± 0.0122	0.0019 ± 0.0014	−1.792*	−2.267*	−2.276*
Pacific	0.2452 ± 0.1012	0.0004 ± 0.0005	−1.057	−1.619	−1.570
Combined	0.9266 ± 0.0322	0.0052 ± 0.0031	−2.069*	−2.008*	−2.268*

BTUB, beta tubulin; EFA, elongation factor alpha; ITS, nuclear ribosomal internal transcribed spacer; *$P < 0.05$.

(BTUB, $n = 13$; EFA, $n = 17$) were collapsed into existing haplotypes using the criteria proposed by Steeves *et al.* (2005). Haplotypes were collapsed into identical haplotypes (if the haplotype was identical to another haplotype with the exception of one ambiguous nucleotide site) or collapsed into the most common haplotype (if the haplotype was otherwise identical to two or more haplotypes). This procedure allowed conservation of nucleotide polymorphisms without artificial inflation by the addition of new haplotypes. All aligned regions (BTUB, EFA, ITS) were analysed separately without the concatenation into a single alignment.

Phylogenetic relationships between *Datronia caperata* on mangrove and non-mangrove hosts

A phylogenetic analysis of *D. caperata* was undertaken using ITS sequences from samples collected on *L. racemosa* hosts, samples collected from Barro Colorado Island, Venezuela, and sequences from GenBank with unknown host associations (see Appendix S1 for host, voucher and GenBank accession numbers) to test whether *D. caperata* from non-mangrove hosts and *L. racemosa* were conspecific. We rooted the tree with *Trametes*, based both on prior analyses (Ko & Jung, 1999; Hibbett & Binder, 2002) and on tests using a subset of polypore fungi from another study (Guglielmo *et al.*, 2007). Bayesian posterior probabilities were calculated using MrBayes ver. 3.1.2 (Huelsenbeck *et al.*, 2006) using a general time-reversible model with a gamma-distributed rate, as selected in MODELTEST ver. 3.7 (Posada, 2006) under the Akaike information criterion (Posada & Buckley, 2004). Two runs of 1,000,000 generations were performed, saving 10,000 visited trees. Scatterplots were generated to determine 'stationarity' or the lack of improvement in maximum likelihood scores. All samples taken prior to stationarity (specified by burn-in = 2000) were discarded, and the remaining samples ($n = 8001$) were used to determine posterior probability distributions. From these distributions, a 50% majority rule consensus tree was computed.

Nested clade analyses

We carried out NCA (Templeton *et al.*, 1992, 1995; Templeton, 1998) to infer geographical patterns of gene flow in *D. caperata*. The NCA is an objective statistical analysis that tests the null hypothesis of no association between haplotype and geographic range, and that helps to distinguish between the effects of recurrent or restricted gene flow and non-recurrent historical events (e.g. range expansion, fragmentation). Haplotype networks were converted into nested clades following nesting rules (Templeton *et al.*, 1987, 1992). Two-way, contingency permutation analysis using GeoDis ver. 2.5 (Posada *et al.*, 2000) was used to test the null hypothesis of no geographic structure within each nested clade. Statistically significant large or small D_c values (a measure of the geographic range of a clade) and D_n values (the geographic range of a clade relative to its closest sister clades) were compared with the differences between the average interior tip (T) distance $[(1 - T)_{D_c}]$ and the average tip distance $[(1 - T)_{D_n}]$. Significance values were interpreted using an updated inference key provided with GeoDis ver. 2.5.

Haplotype and nucleotide diversity

It has been hypothesized that centres of origin should display a greater diversity of haplotypes (Hewitt, 2000). We computed haplotype (h) and nucleotide (π) diversity from Caribbean, Pacific and combined populations using ARLEQUIN ver. 2.000 (Schneider *et al.*, 2000). To test the assumption that our estimates of time since initial divergence reflect historical isolation and not past selective pressures, Tajima's D (Tajima, 1989) and Fu and Li's D^* and F^* (Fu & Li, 1993) were calculated for all populations, and the statistical significance of test statistics was assessed using DnaSP (Rozas *et al.*, 2003). Tajima's D-test compares nucleotide diversity with the number of segregating sites. If they are equal, it is presumed that mutations are selectively neutral (Tajima, 1989). Fu and Li's D^* and F^* compare differences

between the number of singleton mutations (those that occur once) and the average number of nucleotide differences between pairs of sequences (Fu & Li, 1993). Significant positive values of these three tests may indicate balancing selection or population differentiation, and significant negative values may indicate recent selective sweeps or recent population expansion following a genetic bottleneck (Tajima, 1989; Fu & Li, 1993).

Coalescent analyses and gene flow

We used a suite of software available in SNAP Workbench to estimate population genetic parameters, to test overlying assumptions of the coalescent model, to estimate migration rates and to perform coalescent simulations (Price & Carbone, 2005). Sequences were collapsed into unique haplotypes after removing insertions and deletions (INDELS) (SNAP Map; Aylor *et al.*, 2006). Pairwise tests of geographic subdivision between all populations were conducted using Permtest after excluding INDELS (Hudson *et al.*, 1992). INDELS were present only in the nuclear rDNA ITS sequences but were excluded because of the difficulty of modelling the mutation process (Aylor *et al.*, 2006).

Estimates of the maximum likelihood value for θ [($4 \times$ the net effective population size (N_e) multiplied by μ (mutation rate)], time to the most recent common ancestor (T) and migration (M) [$2 \times$ the net effective population size (N_e) multiplied by m (migration rate)] were performed using the MDIV software program, which uses a maximum likelihood MCMC (Markov chain Monte Carlo) approach based on the standard coalescent model (Nielsen & Wakeley, 2001). Populations with no genetic subdivision based on Hudson's test ($P < 0.05$) were combined (Mananti and PonSok, David and Guacalillo) prior to analyses. The parameters θ, M and T were estimated using a 2,000,000-generation MCMC with an additional 500,000-generation burn-in. These contrasts used a finite-sites model, and *a priori* maxima were set for M and T, with $M_{max} = 10$ and $T_{max} = 5$. These *a priori* maxima were based on the assumption that these populations must have low migration rates (Parrent *et al.*, 2004), with two notable exceptions, where no population structure was observed ($P > 0.05$) (Mananti and PonSok were combined into a single population designated Bocas, and David and Guacalillo were combined into a single Pacific population). In order to ascertain whether the Markov chains had reached stationarity, 10 independent runs were computed with 10 random starting seeds.

We used the MIGRATE program ver. 2.0.6 (Beerli & Felsenstein, 1999) to estimate the direction and extent of gene flow between populations. MIGRATE calculates maximum likelihood estimates of effective population size θ (defined as $4N_e\mu$, where μ represents mutation rate) and of migration M (defined as m/μ, where m represents migration rate) based on the coalescent theory and allows detection of asymmetries in migration rates over the history of the population (Beerli, 1998; Beerli & Felsenstein, 1999). We ran 10 short chains, each with a total of 50,000 genealogies and with a sampling increment of 20 genealogies, after which we ran five long chains, each with a total of 100,000 genealogies and a sampling increment of 20 genealogies. The first 10,000 genealogies in each chain were discarded (Beerli & Felsenstein, 2001). For all other settings, we used the default parameters in MIGRATE.

The ancestral history of each gene genealogy was constructed using GENETREE ver. 9.01 (Griffiths, 2002). This method assumes an infinite-site mutation model and is thus sensitive to both recombination and the rate of mutation. We tested for the underlying assumption of recombination for each locus by plotting site compatibility matrices using SNAP Clade and Matrix. For the GENETREE analyses, we estimated the population mutation parameter μ under a subdivided population model with migration nearly zero ($M = 0.01$), assuming equal population sizes and an estimate of the number of segregating sites calculated by computing the mean value of Watterson's θ between Pacific and Caribbean populations ($\theta_{BTUB} = 2.4$; $\theta_{EFA} = 2.4$; $\theta_{ITS} = 0.7$) (Watterson, 1975). The tree with the highest root probability was estimated by performing 1,000,000 simulations of the coalescent with 10 different random number seeds.

RESULTS

Haplotype and nucleotide diversity

Thirteen, seven and seven haplotypes were distinguishable by 17, eight and seven variable sites in the BTUB, EFA and ITS, respectively. Sequences for each collection (either single-spore haploid isolates, alleles cloned from heterozygous loci, or haplotypes with ambiguities collapsed into common haplotypes) were deposited in GenBank (BTUB, EU030066–EU030123; EFA, EU030009–EU030065; ITS, EU030124–EU030183) (see Appendix S1 for accession numbers and details of isolates collected). We found greater haplotype and nucleotide diversity in Pacific populations for BTUB and EFA, but greater ITS haplotype diversity in Caribbean populations. The significance of neutrality tests varied depending on locus and region. Within the Pacific region, Tajima's D, and Fu and Li's D^* and F^* were negative and significant ($P < 0.05$) for the EFA locus but not for the BTUB and ITS loci (Table 1). Within the Caribbean, these measures were significantly negative using the BTUB and ITS loci, but not using the EFA locus (Table 1). All tested values within both regions had negative values, which suggests that the distribution of haplotypes is the result of recent range expansion after a significant genetic bottleneck (Tajima, 1989; Fu & Li, 1993).

Phylogenetic relationships between *Datronia caperata* on mangrove and non-mangrove hosts

Internal transcribed spacer sequence similarity between *D. caperata* colonizing *L. racemosa* and putative *D. caperata* collections obtained from other studies was low, ranging from

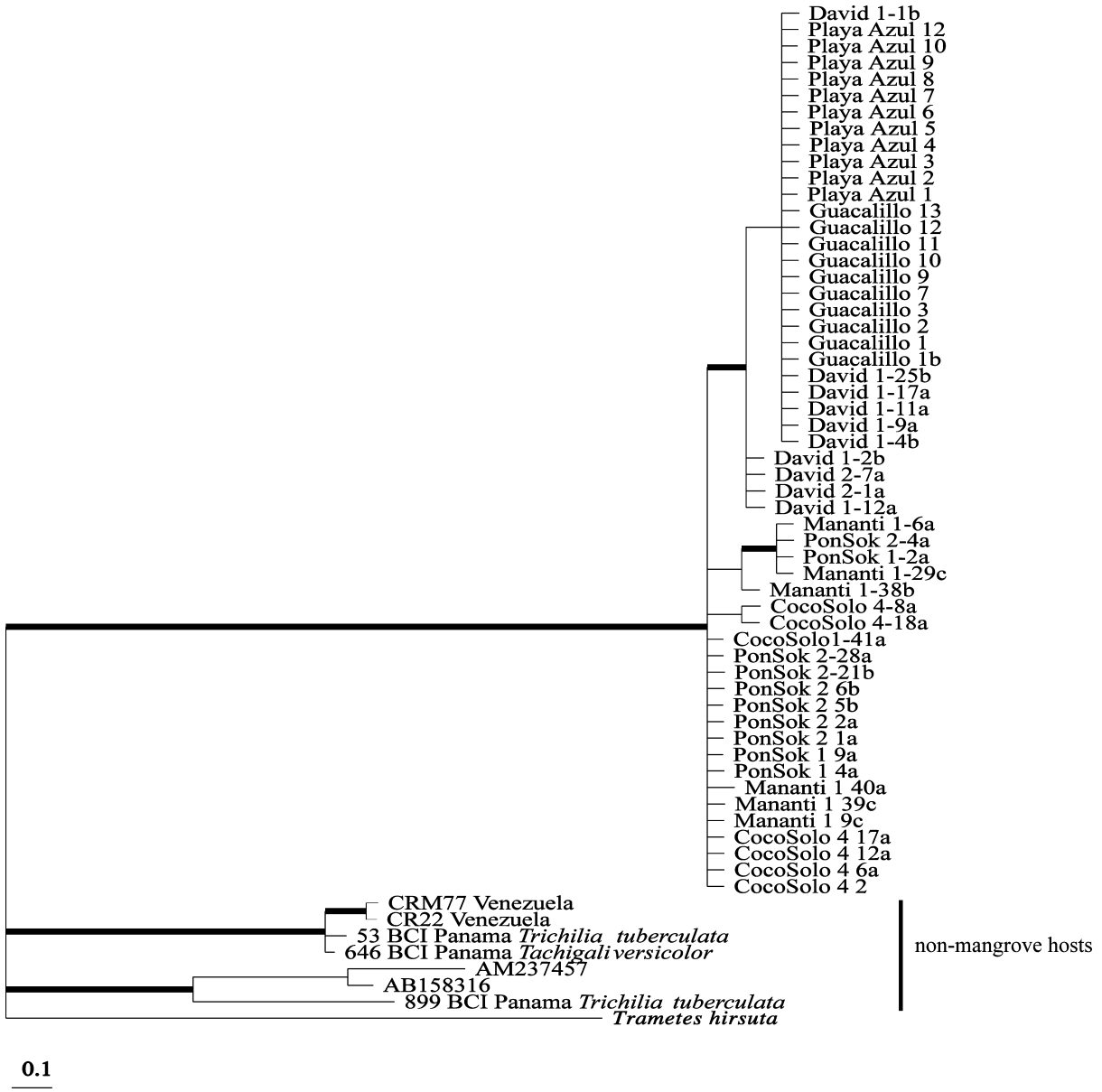

Figure 2 Majority consensus tree of 8001 trees inferred from Bayesian posterior probabilities of the alignment of *Datronia caperata* ITS1, 5.8S, ITS2. Bayesian posterior probabilities greater than or equal to 95% are shown in bold. *Datronia caperata* colonizing *Laguncularia racemosa* is classified according to the population sampled (Coco Solo, David, Guacalillo, Mananti, and PonSok). Non-mangrove collections are shown indicated by country collected, host where known and either GenBank accession number (AB158316, AM237457), voucher number for BCI = Barro Colorado Island (Panama) or culture identifier (CRM77, CR22) for Venezuela isolates. The length of branches corresponds to the number of substitutions per site (scale bar: 0.1 substitutions per site).

67% to 78%. The majority rule consensus tree generated from 8001 trees shows that *D. caperata* colonizing *L. racemosa* forms a monophyletic clade that is distinct from *D. caperata* collections from Barro Colorado Island, Venezuela and other unknown hosts (Fig. 2).

Nested clade analyses

The gene genealogies inferred by the higher-order nesting of the statistical parsimony network are shown in Fig. 3. Many of the haplotypes common to both the Caribbean and Pacific coasts have a central interior position in the one-step clade, and the remaining haplotypes are connected by short branches (typically a single mutational step). In the BTUB and EFA loci, although the two most frequent haplotypes were common to both Caribbean and Pacific populations, their frequency differed greatly between coasts, so that common haplotypes on one coast were always rare in the other coastal population (Fig. 3). For example, the two shared EFA haplotypes (VI, VII) common to the Caribbean and Pacific populations were

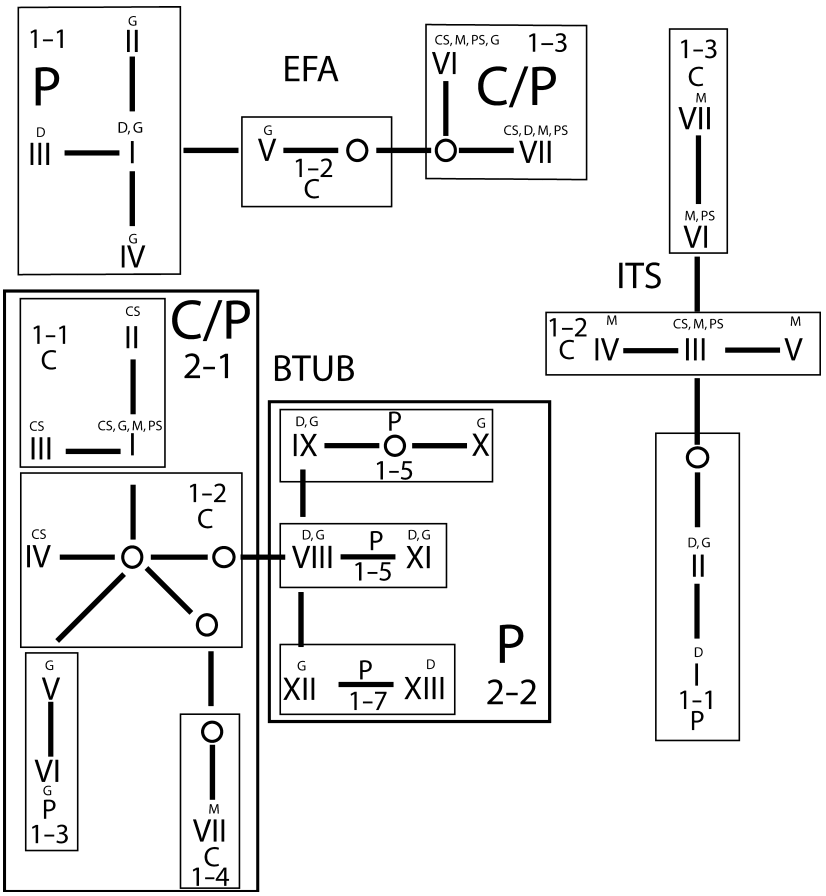

Figure 3 Nested clade analysis of beta-tubulin (BTUB), elongation factor alpha (EFA) and internal transcribed spacer (ITS) regions with nesting design. Haplotypes belonging to the same clade level are boxed up to 2-step clade levels. Clade-level designations are given within each box that contains observed haplotypes and are labelled with the corresponding coastal population (Caribbean, C; Pacific, P). Missing haplotypes are indicated by ○, and each line indicates one mutational step. The coastal population from which a haplotype was sampled (CS, Coco Solo;, David; G, Guacalillo; M, Mananti; PS, PonSok) is shown above each haplotype labelled with roman numerals.

frequent in the Caribbean, but were rare in the Pacific. In the BTUB locus, haplotype I, shared between Caribbean and Pacific populations, occurred at the highest frequency in the Caribbean population but was sampled only once in the Pacific (Guacalillo) (Fig. 3). In the ITS locus, there were no shared haplotypes from the two sampled coasts; that is, all haplotypes were unique to either coast (Fig. 3). There were significant associations between haplotype and geographic distribution in some of the two-step clades (BTUB) and one-step clades (EFA, ITS) (Table 2). In BTUB Clade 2-1 and EFA Clade 1-3 (Fig. 3), we detected a significantly large D_n value in the tip clades, but the outcomes were inconclusive (Table 2). In the ITS total cladogram, significantly large D_n values were found in Clade 1-1 and in the total cladogram (Table 2). Geographic patterns could be explained by long-distance dispersal (LDD), range expansion (RE) or fragmentation (FRG) (Table 2).

Coalescent analyses and gene flow

Although MIGRATE can estimate the direction and extent of migration, it is unable to differentiate between shared ancestral polymorphisms and recurrent gene flow (Brito, 2005). MDIV was used to determine if Caribbean and Pacific haplotypes were the result of recent gene flow or of the retention of ancestral polymorphism in the Pacific populations. The estimated MIGRATE values detected asymmetrical migration (Caribbean to Pacific) in BTUB and EFA loci, and nearly symmetrical migration among the Caribbean coastal populations (Table 3). Asymmetrical migration from the Pacific to Caribbean populations was detected in the ITS locus (Table 3). Our results of the posterior distribution using MDIV suggest the absence or low levels of gene flow among Caribbean and Pacific populations because estimates of M were typically <0.05, with a confidence interval typically encompassing zero values (Fig. 4). Thus, the asymmetrical migration generated by MIGRATE was interpreted as a result of recent range expansion rather than of recurrent gene flow, given that MDIV estimates of migrants are nearly zero. In contrast to results obtained for intercoastal gene flow, nearly symmetrical estimates of migration were found between Caribbean populations (Table 3), and MDIV estimates were greater than 0.05 migrants, indicating the presence of migration within the Caribbean coast (Fig. 4). For T and θ, the interpretation using the maximum likelihood estimate was problematic, because of the probability that T first increased and then stabilized before reaching zero (data not shown). Thus, estimates of T could not be used to approximate the divergence between the Caribbean and Pacific coasts.

For GENETREE construction of the BTUB, EFA and ITS gene genealogies, we assumed a low level of migration

Table 2 Chi-squared test of geographical association of clades and inference from the nested clade analysis of haplotypes of *Datronia caperata*.

Locus	Clades	Permutation χ^2-statistic	*P*-value	Inference key steps	Inference
BTUB	1-1	9.26	0.18		
	1-5	0.75	1.00		
	1-6	2.52	0.17		
	1-7	5.00	0.09		
	2-1	22.60	0.00	2-11-17	Inconclusive
	2-2	0.23	1.00		
	Total	47.33	0.00	Interior status cannot be determined	Inconclusive
EFA	1-1	7.16	0.07		
	1-2	N/A			
	1-3	11.44	0.01	Interior status cannot be determined	Inconclusive
	Total	33.21	0.00	2-11-17	Inconclusive
ITS	1-1	9.64	0.01	2-3-4-9-10	FRG/IBD
	1-2	8.63	0.05		
	1-3	1.00	1.00		
	Total	65.22	0.00	2-3-5-6-7-13-14	RE/LDD/FRG

P is the probability of obtaining a χ^2-statistic larger than or equal to the observed statistic by randomly permuting the original contingency table 1000 times. Inferences were obtained following the key provided in GeoDis ver. 2.5 (Posada *et al.*, 2000).
BTUB, beta tubulin; EFA, elongation factor alpha; ITS, nuclear ribosomal internal transcribed spacer; FRG, fragmentation; IBD, isolation by distance; LDD, long-distance dispersal; RE, range expansion.

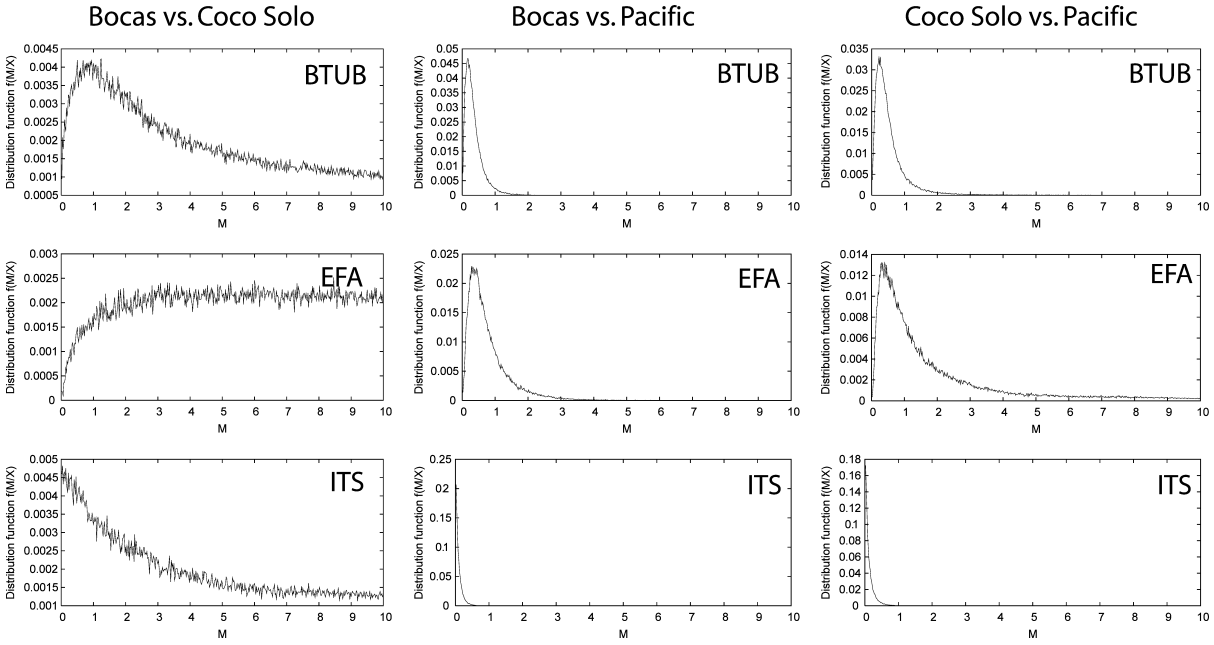

Figure 4 The migration posterior probability distributions generated by MDIV within Caribbean (Bocas vs. Coco Solo) and between Caribbean and Pacific (Bocas vs. Pacific, Coco Solo vs. Pacific) populations of *Datronia caperata* estimated for beta tubulin (BTUB), elongation factor alpha (EFA), and nuclear ribosomal internal transcribed spacer (ITS) loci using MDIV. Likelihood functions for migration f(M/X) are shown on the *y*-axes, and the *x*-axes represent time measured in 2*N* generations.

between Caribbean and Pacific clades ($M = 0.01$). The coalescence-based gene genealogy of the ITS region was informative for inferring the mutational history with respect to population subdivision in Caribbean and Pacific populations (Fig. 5). In the ITS gene genealogy, the mutations in the Caribbean and Pacific populations were of similar age (Fig. 5).

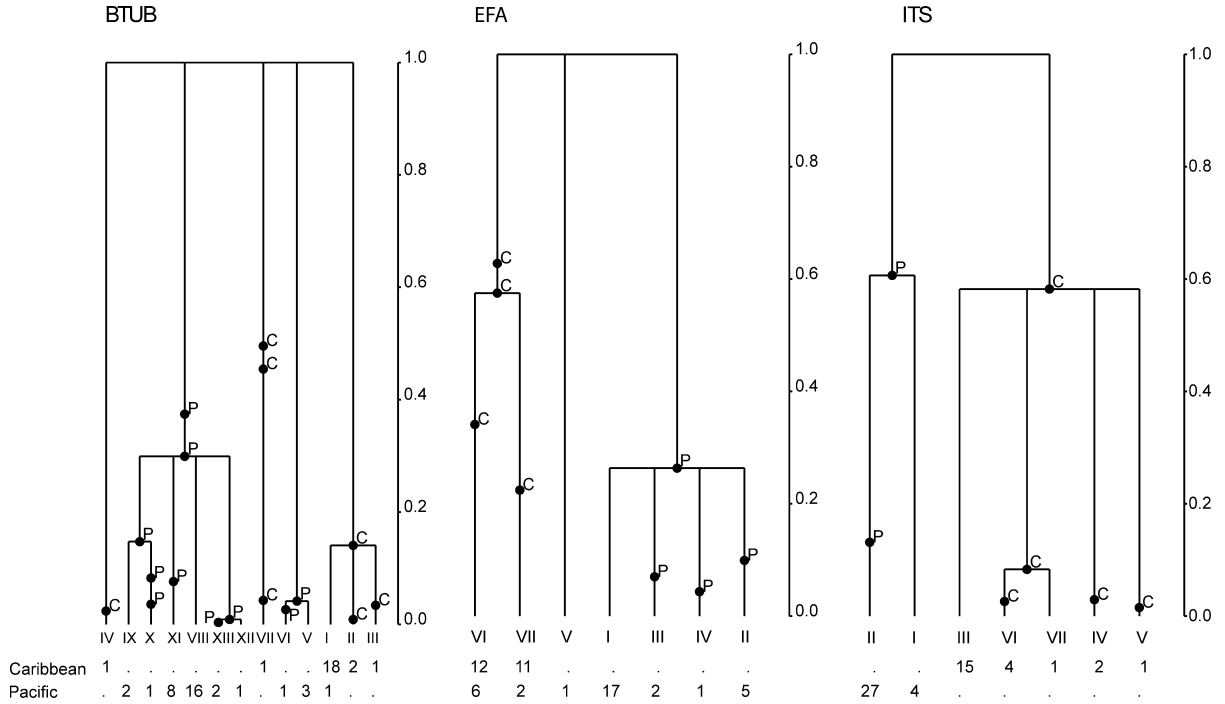

Figure 5 Coalescent-based gene genealogies with the highest root probability [beta tubulin (BTUB) likelihood = 3.9017×10^{-26}, SD = 2.5727×10^{-23}; elongation factor alpha (EFA) likelihood = 4.5065×10^{-21}, SD = 4.1438×10^{-18}; nuclear ribosomal internal transcribed spacer (ITS) likelihood = 1.8167×10^{-11}, SD = 3.95575×10^{-10}] showing the distribution of mutations and the number of samples corresponding to each haplotype in the Caribbean (C) and Pacific (P) for the BTUB, EFA and ITS loci. Mutations indicated by ● are shown with respect to generation time (*y*-axis; the timescale is in coalescent units of $2N$, where N is population size). Haplotypes are indicated by roman numerals and correspond to the haplotypes in the nested cladogram (see Fig. 3). The respective coastal population of each haplotype (C, Caribbean; P, Pacific) is provided.

Table 3 The migration parameter $4N_em$ estimated using MIGRATE for each locus. Asymmetric migration rates for source (1+) and receiving (2+) populations of *Datronia caperata* are given.

	1+		
	Mananti and PonSok (Caribbean)	Coco Solo (Caribbean)	David and Guacalillo (Pacific)
2+			
BTUB			
Manati, PonSok (Caribbean)	–	3.7×10^3	0
Coco Solo (Caribbean)	3.7×10^3	–	0
David, Guacalillo (Pacific)	6.3×10^2	6.8×10^2	–
EFA			
Manati, PonSok (Caribbean)	–	2.6×10^4	0
Coco Solo (Caribbean)	3.0×10^4	–	0
David, Guacalillo (Pacific)	1.8×10^3	1.0×10^3	–
ITS			
Manati, PonSok (Caribbean)	–	1.5×10^4	0
Coco Solo (Caribbean)	1.5×10^2	–	4.8×10^2
David, Guacalillo (Pacific)	0	0	–

All estimates were the result of running analyses with 10 replicates using a different random number seed for each estimate run.
BTUB, beta tubulin; EFA, elongation factor alpha; ITS, nuclear ribosomal internal transcribed spacer.

On the basis of estimates of the mean ages of mutations, the oldest mutations in the nuclear BTUB and EFA loci are found in the Caribbean population (Fig. 5). In the BTUB and EFA coalescence-based gene genealogies, it was not possible to resolve the relative age of divergence, measured in number of generations (N).

DISCUSSION

A previous study based on nuclear amplified fragment length polymorphisms (AFLPs) showed high population differentiation among the three sites studied, one on the Pacific coast (David) and two in the Caribbean (Bocas and Coco Solo) (Parrent *et al.*, 2004). The current study expands the sampling, allowing for a comparison based on three independent loci of population genetic structure both within and between coasts. The nuclear loci (BTUB, EFA) point to the Caribbean as the centre of origin (site of oldest mutations), from which *D. caperata* dispersed over the mountainous divide, colonized the Pacific, after which gene flow ceased. Population differentiation was also observed on the Caribbean coast, whereas no evidence of population structure was detected among Pacific populations.

The discovery of a shared ancestral haplotype that has persisted in nuclear BTUB and EFA loci from Caribbean and Pacific coasts, resulting in a lack of reciprocal monophyly, can be interpreted either as recurrent gene flow among populations or as gene flow between populations prior to isolation and divergence (Hudson, 1990). We argue that recent gene flow is unlikely to have contributed significantly to the observed haplotype distribution across coasts. If ongoing intraspecific gene flow between Caribbean and Pacific populations were frequent, we would expect to find significant overlap of haplotypes common to both coasts. Contrary to this expectation, BTUB and EFA haplotypes shared between Caribbean and Pacific populations were frequent in Caribbean populations, but rare in Pacific populations. Furthermore, the abundance of rare alleles across Caribbean and Pacific populations of *D. caperata* conflicts with the genetic pattern expected in the presence of recurrent gene flow among populations (Tajima, 1989). Thus, the greater similarity between intercoastal populations in terms of overlapping haplotypes, and the tests of neutrality, along with migration estimates using MDIV (nearly zero) suggest that the persistence of ancestral polymorphism in the low-copy nuclear genes (BTUB, EFA) is probably the result of a past demographic expansion followed by a cessation of gene flow.

Coalescent-based gene genealogies and haplotype networks showed limited numbers of mutational steps (1–3) between Caribbean and Pacific populations. The limited divergence among haplotypes is indicative of a recent interruption of gene flow (probably in the last 0.02 Myr; see discussion below) among populations of *D. caperata* on these two coasts. However, when testing for the direction of migration among distinct populations, historical migration was detected from the Caribbean to the Pacific in the nuclear BTUB and EFA (although contrary to the pattern observed in the ITS; see discussion below), but not in the opposite direction. This pattern would be expected if fungal spores had occasionally been carried from coast to coast by strong wind gusts. Strong trade winds blow across Panama consistently from the north (Caribbean to Pacific) during the dry season (January–May); wet-season winds are much weaker and of varying direction (Windsor, 1990). Interestingly, significant migration, although at different rates, was measured in both directions between these two distinct populations studied on the Caribbean coast.

One of the underlying hypotheses of this study is the interdependence of the phylogeographic histories of a plant pathogen and of its host. The validity of this hypothesis is dependent on the idea that *D. caperata* populations from mangroves may not be conspecific with those reported from other hosts. The ITS rDNA sequences showed that *D. caperata* from *L. racemosa* is probably not conspecific with samples from other host species. Although unresolved, the ITS phylogeny clearly identifies distinct clades, with one clade exclusively containing all of the samples from mangroves, and other clades containing isolates from other hosts. Thus, we conclude that *D. caperata* on *L. racemosa* and morphologically similar fungi found on other hosts are different taxa. Our analyses support a more restricted taxonomic concept of *D. caperata sensu stricto*; however, our sampling is insufficient to establish the phylogenetic relationships among *D. caperata* in diverse Neotropical systems. The taxonomic uncertainty highlighted by this study warrants further investigation.

In the presence of a strict evolutionary interdependence of pathogen and host, significant insights into the evolutionary history of the host may be gained by understanding that of the host. Nettel & Dodd (2007) showed that Pacific and Caribbean black mangrove [*Avicennia germinans* (L.) L.] populations were different and that Pacific populations had a higher genetic diversity than those of the Caribbean basin. Lower levels of genetic diversity in Caribbean populations were attributed to rapid local extinction along steep continental margins during the Quaternary sea rise and retraction (Nettel & Dodd, 2007). Under this scenario, it is assumed that the mangrove populations on the Atlantic coast are of recent origin. Had *D. caperata* associated with *L. racemosa* followed a similar pattern of local extinction, we would expect the following: (1) fungal re-establishment would postdate the arrival of *L. racemosa* and (2) the source population would be of Caribbean origin.

Greater haplotype diversity and nucleotide diversity is expected in an ancestral population (Hewitt, 2000). However, we found greater haplotype and nucleotide diversity in the Pacific population than in the presumed ancestral Caribbean populations. One possible explanation is that larger effective population sizes of *D. caperata* are maintained in the Pacific, perhaps in part owing to the extensive range occupied by its host, *L. racemosa* (Ellison, 2004). Another possible alternative is that Caribbean populations may have undergone a significant bottleneck not experienced by Pacific populations owing to a reduced density of mangroves during the significant changes in sea levels experienced during the last interglacial cycle, as described above (Nettel & Dodd, 2007). Furthermore, the negative values of Fu and Li's D^* and F^* values are characteristic of species that have undergone recent bottlenecks followed by a range expansion.

Although it was impossible to estimate formally the time since divergence between populations on the Pacific and Caribbean coasts, two results suggest that speciation between coasts is in progress and further confirm the lack of current intercoastal migration. The divergence of populations from the ancestral population is often defined by the cessation of gene flow (Nielsen & Slatkin, 2000). Whether this cessation corresponds to particular geological events or occurs some time after such events is usually not known (Knowlton & Weigt, 1998); however, we propose that our data are consistent with a more recent historical isolation between Caribbean and Pacific populations (within the last 2 Myr) rather than with trans-isthmus divergence. There are multiple lines of evidence to corroborate this finding, including (1) the observation of the differences in gene flow estimates, which suggests that cessation of gene flow has precipitated divergence; (2) evidence of incomplete lineage sorting in nuclear (BTUB, EFA) loci, often consistent with speciation in progress (Bowen, 1998); and (3) the fact that organisms occupying mangrove habitats, although amongst the last trans-isthmus habitats to diverge (3–9 Ma) (Knowlton & Weigt, 1998), typically show complete loss of ancestral haplotypes marked by complete reciprocal monophyly (Knowlton & Weigt, 1998; Marko, 2002). Most of the studies of trans-isthmus divergence have based their estimates on mtDNA sequences (Knowlton & Weigt, 1998; Marko, 2002), which have an effective population size one-quarter of that of the nuclear genes used in this study (Crawford, 2003); however, comparative studies using single-copy nuclear loci were similar in that loss of ancestral polymorphisms have led to complete reciprocal monophyly (Williams *et al.*, 2001). In our case, a useful comparison that may help to resolve the timing of divergence could include the addition of mtDNA loci. For fungi, efforts to uncover genetic structuring using mitochondrial sequences have been limited and perhaps somewhat mired by the predominance of mobile introns in cytochrome oxidase I (COI) (Seifert *et al.*, 2007), and many other mtDNA loci are typically characterized by low substitution rates (Clark-Walker, 1991). Consequently, researchers have often relied on the nuclear ribosomal ITS or intergenic spacers (IGS) for phylogeographic inference (e.g. Geml *et al.*, 2006; Carbone & Kohn, 2001). We recognize that the nuclear ribosomal rDNA ITS or IGS may not be ideal for phylogeographic studies. For one, rDNA are repetitive segments found on multiple chromosomes and are known for their rapid homogenization of tandem repeats, leading to fixation of alternative rDNA alleles (concerted evolution) (Liao, 1999). Second, the rate and direction of homogenization cannot be predicted across different lineages (Feliner & Rossello, 2007). Third, studies based on nuclear ribosomal ITS or IGS data may result in incongruence with those based on single-copy nuclear markers owing to the various evolutionary mechanisms that influence ITS but are not inherent in these other markers (Feliner & Rossello, 2007).

Our conclusions regarding the interdependence of the biogeographical histories of the fungal polypore *D. caperata* and its host *L. racemosa* are of interest not only because host and parasite differ in their dispersal abilities, but also because the dispersal of *D. caperata* is not directly linked to or dependent on the dispersal *per se* of the host itself. *Datronia caperata*, in fact, does not colonize plant seeds; instead, it is found colonizing both live and recently dead *L. racemosa* tissues (Gilbert & Sousa, 2002; Parrent *et al.*, 2004). Because fungal spread is attained through infection primarily by airborne basidiospores produced in basidiomes (Parrent *et al.*, 2004), and because this fungus colonizes the wood and not the seeds of its host, we conclude that dispersal does not occur by means of infected mangrove propagules or plant parts as described for other wood-decay fungi such as *Lentinula* spp. (Hibbett, 2001). In contrast to the population structure observed in *D. caperata*, the generalist wood-decay fungus *Schizophyllum commune* has been shown to traverse large distances from South America to the southern continental United States (James & Vilgalys, 2001), and *Fomitopsis pinicola*, a polypore species that has a broad host range, has been reported to have a limited population structure within large continental regions (Högberg *et al.*, 1999). We propose that a limiting factor of successful dispersal for *D. caperata* is the availability of suitable hosts.

The implications of the biogeographical patterns described here for this fungal polypore are significant not only from an evolutionary but also from a conservation perspective. Some immediate implications of the dependence between the dispersal of the fungus and the availability of its host are evident and supported by our findings. Eliminating mangrove populations that constitute bridges between other populations will decrease the frequency of regional gene flow of this host-dependent parasitic fungus and lead to a potential loss of allelic richness caused by genetic drift. Furthermore, because of the unidirectional gene flow detected in our study, Caribbean populations may represent an important source of genetic variation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Isolate identifier, herbarium accession numbers and GenBank accessions for three loci [beta-tubulin (BTUB), elongation factor alpha (EFA) and internal transcribed spacer (ITS) region] for all single-spore isolates (cs, ms, ps, da) and basidiomes (gu, PA) of *Datronia caperata* collected from white mangroves and Neotropical forests.

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BIOSKETCH

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