

Population genetic structure of the polypore *Datronia caperata* in fragmented mangrove forests

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Datronia caperata, a basidiomycete fungus, is one of the dominant polypore species found in neotropical mangrove forest fragments, where it is locally specialized on *Laguncularia racemosa*. We examined the genetic structure of *D. caperata* populations from four Panamanian mangrove forests using AFLP markers. Using five primer pair combinations, 145 loci were detected, 98.6 % of which were polymorphic. Each of the populations showed a high degree of genetic diversity (Nei's *h* ranging from 0.146 to 0.223). Results from minimum spanning trees and Mantel tests showed little evidence for small-scale spatial structure within sites. A significant amount of total genetic variation was partitioned among populations ($\Phi_{ST}=0.21$) separated by 10s to 100s of km, a considerably greater amount than has been detected in other mushroom and wood-decaying fungi sampled at equal or greater geographic distances. These results suggest that despite production of copious basidiospores capable of long distance dispersal, some homobasidiomycete fungi may be susceptible to genetic isolation due to habitat fragmentation.

INTRODUCTION

Species with tolerance for a narrow range of environmental conditions (e.g. serpentine soil specialists, alpine species, and vernal pool inhabitants) are often distributed among naturally fragmented habitats. Increasingly, anthropogenic land transformation fragments formerly contiguous habitats creating isolated populations. Species in fragmented habitats may experience reduced gene flow among populations with small effective population sizes and high probability of genetic drift (Barrett & Kohn 1991). Decreased gene flow will lead to greater genetic divergence among isolated populations and potentially to a loss of genetic diversity, which in turn may affect population viability and adaptability. The degree to which fragmentation will limit genetic exchange among populations depends upon geographic distance between populations, population size within remaining fragments, and dispersal capability of the organism in question.

Several studies have examined the genetic structure of basidiomycete fungal populations (Stenlid, Karlsson & Höglberg 1994, Höglberg, Stenlid & Karlsson 1995,

Boisselier-Dubayle, Perreau-Bertrand & Lambourdiere 1996, Saville, Yoell & Anderson 1996, Höglberg & Stenlid 1999, James *et al.* 1999), but only a few have addressed the potential role that habitat fragmentation may have on fungal population structure (Höglberg & Stenlid 1999). Results from studies of mushroom fungi indicated only modest genetic divergence among populations separated by 10s or 100s of km (Stenlid *et al.* 1994, Höglberg *et al.* 1995, Boisselier-Dubayle *et al.* 1996, Saville *et al.* 1996, James *et al.* 1999), suggesting that dispersal capabilities of these species may be sufficient to mitigate the isolating effects of habitat fragmentation.

Mangrove forests, coastal flooded forests occurring throughout the subtropical and tropical regions of the world (Thomlinson 1986), have a naturally fragmented distribution due to environmental requirements. Additional anthropogenic factors such as: rapid deforestation, development, and conversion to shrimp farms have left remaining mangrove forests increasingly fragmented (Ong 1995). In contrast to species rich lowland tropical forests, only three or four species comprise the entire tree community in mangrove forests, and the assemblage of polypore fungi in mangroves is overwhelmingly dominated by local host

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specialists (Gilbert & Sousa 2002, Gilbert 2004). Fungal species that are host or habitat specific may be more sensitive to the isolating effects of fragmentation than their generalist counterparts, since they would suffer a disproportionate reduction in the availability of suitable hosts or environmental conditions. The added selective constraints from host specialization that may affect fungi with preferences for mangrove hosts may reduce the effective population size of remaining population fragments of host specialists relative to host-generalist species.

In this study, we have investigated the genetic structure of populations of the polypore *Datronia caperata* in Panama's mangrove forests. A previous study of a Caribbean mangrove forest near Colón, Panama, found *D. caperata* to be locally specialized on *Laguncularia racemosa* (Combretaceae), one of the dominant tree species in these neotropical mangrove forests (Gilbert & Sousa 2002).

To assess the scale of genetic structure of *D. caperata* on *L. racemosa* among fragmented stands of mangroves, we sampled *D. caperata* from four mangrove forest sites located on the Pacific and Caribbean coasts of Panama. Based on the presence of fruit bodies along transects that included all mangrove tree species occurring at each site, we assessed the abundance and host preference of *D. caperata*. All *D. caperata* basidiomata encountered were collected, and a subset of samples were grown in culture and analysed using amplified fragment length polymorphism (AFLP) to examine how genetic diversity was partitioned within and among *D. caperata* populations. We also sequenced the internal transcribed spacers (ITS) and 5.8S rRNA gene for each strain to determine the genetic relatedness among strains and to confirm that these samples represent a single phylogenetic species.

MATERIALS AND METHODS

Study sites and fungal isolates

In July–Aug. 1998, four mangrove forest sites in Panama were surveyed. Pon Sok, Manantí, and Coco Solo are located along the Caribbean coast, and David is in the Southwest corner of the country along the Pacific Ocean (Fig. 1). These forests are almost entirely comprised of three host species; *Rhizophora mangle* (red mangrove, Rhizophoraceae), *Avicennia germinans* (black mangrove, Avicenniaceae), and *Laguncularia racemosa* (white mangrove, Combretaceae). Trunk morphology is distinct enough to reliably identify even dead, decomposing trunks to species. *D. caperata* basidiomata were collected along 20 m wide, 200 m long transects in each mangrove forest site. Two transects were completed at the Pon Sok site, one at Manantí, four at Coco Solo, and four at David, with the number of transects varying according to mangrove extent and time available at a site. Data from all transects were used to evaluate *D. caperata* host

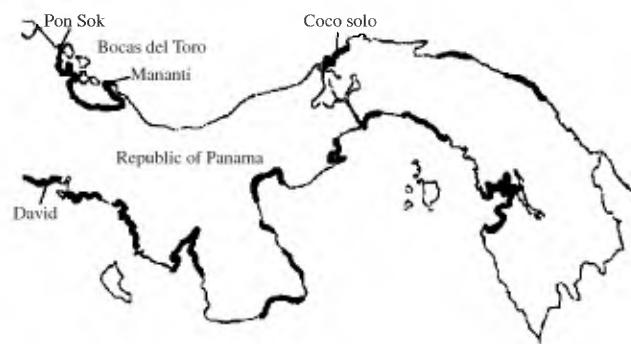


Fig. 1. Map of the Republic of Panama. Mangrove forests located along the coastlines are highlighted in black (distribution data from Wolf & Jackson 1996).

preference, but isolates used for AFLP analysis and ITS sequencing were only available from a subset of the transects: Pon Sok transects 1, 2, Manantí 1, Coco Solo 1, 2, 4, and David 1, 2. Only one collection was made per tree trunk. All mangrove trees and *D. caperata* fruit body collections made were mapped using GPS (Trimble XR-Pro, Trimble, Boulder, CO) and a laser rangefinder (Criterion 300; Laser Technology, Englewood, CO). Basidiospores were collected and germinated on 1.5% malt extract agar plates amended with 200 ppm Benlate and 200 ppm ampicillin antibiotics. Germinated spores were isolated and maintained on 1.5% malt extract agar slants. To confirm that all cultures were derived from haploid single-basidiospore isolates, all samples were examined for the presence or absence of clamp connections. Heterokaryotic strains of *D. caperata* readily produce clamp connections, so determination of monokaryons by this method is quite reliable. 42 monokaryotic isolates were ultimately used in the analysis; ten from Coco Solo, seven from Manantí, 11 from Ponsok, and 14 from David. Voucher specimens and cultures are deposited in the University of California Berkeley herbarium.

DNA extraction

Samples were grown in 1.5% malt extract + 0.5% dextrose broth for 8 wk in stationary culture. Mycelia were harvested by vacuum filtration, lyophilized, and DNA was extracted using the protocol of Garbelotto *et al.* (1993), except that 50–100 µl of 0.25× TE in 0.3 M ammonium acetate was used for the first pellet resuspension.

AFLP protocol

AFLP detection was conducted using the AFLP Analysis System I kit (Gibco BRL, Grand Island, NY). Restriction digest, adapter ligation, and preamplification reactions were performed according to manufacturer's specifications. PCR conditions used for preamplification were 20 cycles of: 30 s denaturing at 94 °C, 60 s annealing at 56 °, and 60 s extension at 72 °. For selective amplification primer labelling was not

performed; instead five pairs of unlabelled *Mse*I and *Eco*RI primers were used: M-CAC/E-3B, M-CAT/E-AT, M-3B/E-3B, M-2/E-3B, M-2/E-AG. Primers M-CAC, M-CAT, E-AT, and E-AG were from the AFLP core reagent kit (Gibco BRL), while the remaining primers used, M-3B, M-2, and E-3B, were synthesized separately (Operon Technology, Alameda, CA). Selective amplification was performed using the following PCR conditions: one cycle of denaturing at 94 ° for 30 s, 65 ° annealing for 60 s, and 72 ° extension for 60 s, after which 23 additional cycles were performed at 94 ° denaturing for 30 s, 56 ° annealing for 30 s with a touchdown of 0.7 ° per cycle for the first 12 cycles, and 72 ° extension for 60 s. Fragments were separated on a 2.5% Metaphor agarose gel (BioWhittaker Molecular Applications, Rockland, ME) at 120 mV for 5 h at 4 °. AFLP products were stained with Gelstar (BioWhittaker Molecular Applications), visualized with an ultraviolet transilluminator, and digitized with Kodak ID digital software (Eastman Kodak, Rochester, NY). Although an explicit test of AFLP reproducibility was not conducted for this study, AFLPs for a subsample of the strains were reproduced at least once for all five primer pair combinations.

ITS sequencing

The ITS and 5.8S rRNA gene region was amplified in 50 µl reactions containing a final concentration of: 2 mM dntp (Promega, Madison, WI), 25 µM MgCl₂ (Promega), 5 µl of 10× reaction buffer, 50 µM each of primers ITS 1 and ITS 4 (White *et al.* 1990), 0.5 µl taq polymerase (Promega), and 12.5 µl template DNA. PCR amplification was carried out according to the following conditions: one cycle of denaturing at 94 ° for 60 s, 58 ° annealing for 60 s, and 72 ° extension for 60 s, after which 34 additional cycles were performed at 93 ° denaturing for 30 s, 58 ° annealing for 30 s, and 72 ° extension for 60 s. PCR products were cleaned using the Qiaquick PCR cleanup kit according to manufacturer's specifications (Qiagen, Valencia, CA). Cycle sequencing was performed using Big dye chemistry version 3.1 and sequences were visualized on an ABI3100 (Applied Biosystems, Foster City, CA).

Data analysis

Polymorphisms were scored visually as present (1) or absent (0), assisted by profile data provided by the Kodak ID imaging system. The binary data were transformed into a matrix of interindividual distances using AMOVAPREP v. 1.01 (M. N. Miller, Arizona University, Flagstaff, AZ). Analysis of molecular variance was performed with WINAMOVA software (Excoffier 1993). Genetic variation was partitioned into within and among populations, and significance values were assigned to variance components based on 1000 random permutations assuming no genetic structure. Similarity matrices were calculated using the Jaccard

coefficient and an unrooted neighbour-joining dendrogram was generated using PAUP (PAUP 4.0b3a; Sinauer Associates, Sunderland, MA). ITS sequences were manually aligned and parsimony analysis was conducted using PAUP (PAUP 4.0b3a; Sinauer Associates) to generate an unrooted network. For parsimony analyses and the calculation of genetic distances, gaps were treated as a fifth character state. Genetic diversity (Nei 1973) was computed for each population using POPGENE version 1.31 (Yeh & Boyle 1997). NTSYS-pc (Rohlf 1993) was used to generate minimum spanning trees and perform Mantel tests to compare geographic and genetic distances for each population. Due to geographic proximity and small sample size for the Manantí and Pon Sok sites, isolates from these locations were consolidated into a single population prior to AMOVA analysis (hereafter referred to as Bocas).

RESULTS

Distribution and host preference

In all, 1923 mangrove trees were included in this survey: 1057 *Rhizophora mangle*, 445 *Avicennia germinans*, and 421 *Laguncularia racemosa* individuals. Out of the 113 *D. caperata* fruit bodies encountered in this study, all 113 were found fruiting only on *L. racemosa* host trees, confirming the highly host specialized nature of *D. caperata* within mangrove forests previously observed at one site (Coco Solo) by Gilbert & Sousa (2002).

Genetic diversity

Monokaryotic isolates of *D. caperata* (*n*=42) were genotyped using five primer pairs, yielding 145 loci, 98.6% (143/145) of which were polymorphic (Fig. 2). Results for David and Coco Solo populations were similar in both total number of loci detected and proportion of polymorphic loci, but were slightly higher for David (104 polymorphic loci/108 loci detected=96.3% polymorphic loci) than for Coco Solo (96/102=94.1% polymorphic loci). Although Bocas had the greatest sample size in this study, only 81.4% of the 92 loci were found to be polymorphic, the lowest value of the three populations (Manantí, 45/88=51.1% polymorphic loci; Pon Sok, 77/101=76% polymorphic loci). Results for population genetic diversity are similar to the polymorphism data, with Coco Solo and David populations possessing similar values for mean genetic diversity, *h* (Nei 1973). In this instance, Coco Solo had the highest mean genetic diversity (*h*=0.223) (Table 1); mean genetic diversity for David was slightly lower, *h*=0.215. Bocas had the lowest genetic diversity, *h*=0.146. The Coco Solo population also possessed the greatest proportion of unique polymorphisms (14/102=13.7%) compared to the David and Bocas populations (13/108=12.0% and

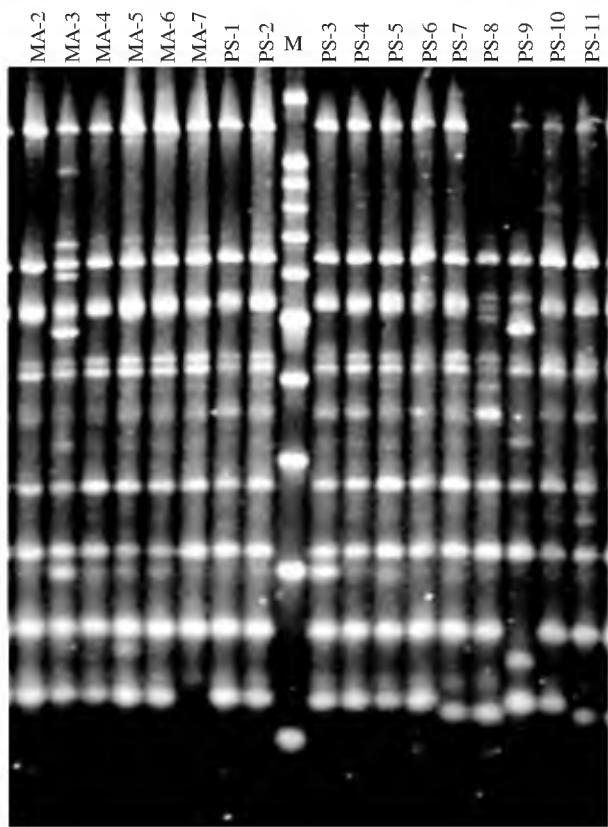


Fig. 2. Representative gel of AFLP fragments *Datronia caperata* isolates from the Bocas population (Manantí and Pon Sok) amplified with the *MseI* and *EcoRI* primer pair M-CAC/E-3B. Fragments are separated on a 2.5% Metaphor agarose gel and stained with Gelstar. Strains are labelled above figure. M, marker; MA, Manantí; and PS, Pon Sok. Marker fragment sizes from bottom to top of figure range from 100 to 1000 bp in length increasing at 100 bp increments, and the largest fragment is 1500 bp.

13/113 = 11.5%, respectively). Although there were a number of bands that were monomorphic within a population, none of them were exclusive to the population for which the locus was fixed.

Population subdivision

The AMOVA analysis of AFLP patterns revealed that 79% of the total genetic variance was partitioned among individuals within a population (Table 2). The remaining 21% was partitioned among populations ($\Phi_{ST}=0.21$): this amount of variance is highly significant ($P<0.001$) and reveals substantial population genetic structure.

Individuals collected from the same geographic location generally clustered together in the unrooted neighbour-joining dendrogram generated from the Jaccard-transformed similarity matrix (Fig. 3). Individuals from the Manantí and Pon Sok sites within Bocas have greater genotypic similarity than the other populations denoted by the shorter branch lengths of Bocas isolates compared to isolates from Coco Solo and David. This result is reinforced by comparing

Table 1. Summary of sample size and mean genetic diversity (Nei 1973) for each *Datronia caperata* population included in this study. Number of loci and percentage of polymorphic loci are summed over all five primer pairs. PS, Pon Sok; Ma, Manantí.

Population	Number of isolates	Number of loci	Percentage of polymorphic loci	Mean genetic diversity (h)
Bocas (PS+Ma)	18	113	81.4	0.146
Pon Sok	11	101	76.2	
Manantí	7	88	51.1	
Coco Solo	10	102	94.1	0.223
David	14	108	96.3	0.215

average pairwise genetic distances within each population: Bocas, average pairwise distance = 0.30 ± 0.11 ; Coco Solo = 0.52 ± 0.09 and David = 0.50 ± 0.18 . Although most isolates from the same geographic location cluster together, three individuals from the David site (DA-3, DA-12, DA-13) grouped together with those from Coco Solo, and CS-8 grouped with the Bocas strains (Fig. 3).

The ITS sequence alignment was 632 nucleotides in length, with a mean sequence divergence of 0.4% (range 0–1.1% sequence divergence). Parsimony analysis yielded one most parsimonious reconstruction 10 steps in length. In the unrooted ITS network, 20 isolates from the Coco Solo and Bocas populations share an identical sequence type, with the remaining Coco Solo and Bocas samples possessing 1–3 substitutions differentiating them from this large group (Fig. 4). For the David isolates, there are four fixed differences that separate the entire population from all of the Coco Solo and Bocas isolates.

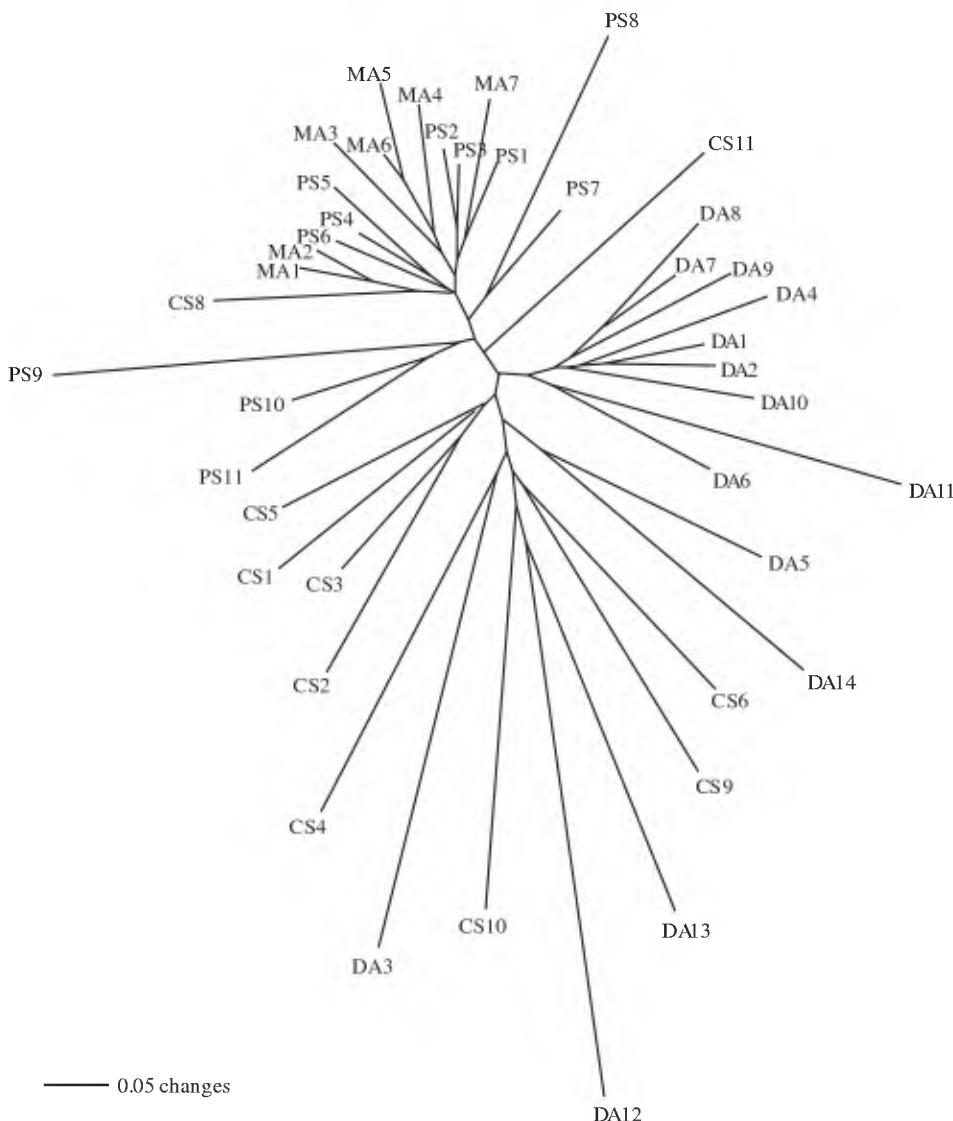
Along each transect, we recorded the spatial position of every *D. caperata* fruit body. Minimum spanning trees and Mantel tests comparing genetic and geographic distances between isolates within sampling locations revealed little evidence of population structure at a small spatial scale (100s of m), except for one of the Pon Sok transects. At this location a group of three *D. caperata* isolates collected from three closely spaced trees (<10 m apart) were more closely related to each other than to the other strains collected further away along this transect (Fig. 5). The association of genetic distance with geographic distance was statistically significant for this transect: Mantel test $P=0.01$; all others had P values greater than 0.45.

DISCUSSION

Understanding the genetic structure of fragmented populations may provide insights into how a species maintains gene flow over significant geographic distances, and how it will respond to increasing habitat fragmentation. Outside of mangrove forests, *Datronia caperata* can be found elsewhere in Panama including

Table 2. Results from the Analysis of Molecular Variance (AMOVA) indicating percentage of total genetic variation partitioned within and among populations.

Source of variation	Degree of freedom	Sums of squared deviations	Mean squared deviations	Variance component	% of total	P-value
Among populations	2	135.0	67.5	3.9	21.0	<0.001
Among individuals within populations	39	569.9	14.6	14.6	79.0	<0.001

**Fig. 3.** Unrooted neighbour-joining dendrogram generated from the Jaccard-transformed similarity matrix. CS, Coco Solo; DA, David; MA, Manantí; and PS, Pon Sok.

upland forests adjacent to the Coco Solo mangroves (Gilbert & Sousa 2002, Ferrer & Gilbert 2004, Gilbert 2003), but is highly abundant and specialized exclusively on *L. racemosa* within mangrove forests. The strong genetic structure detected among fragmented mangrove forests for this species suggests that fungi such as *D. caperata* may be susceptible to the isolating effects of additional habitat fragmentation despite the potential for long-distance dispersal or colonization of other host species in adjacent habitats.

Each of the *D. caperata* populations sampled in this study were found to have a large proportion of polymorphic bands, suggesting that these are diverse, highly outcrossing populations. The Bocas population (Pon Sok and Manantí combined) shows lower genetic diversity ($h=0.146$) than found in Coco Solo ($h=0.223$) and David ($h=0.215$) populations, despite having the largest sample size and geographic area covered in this study. The reduced genetic diversity observed in the Bocas population could be the result of either a founder

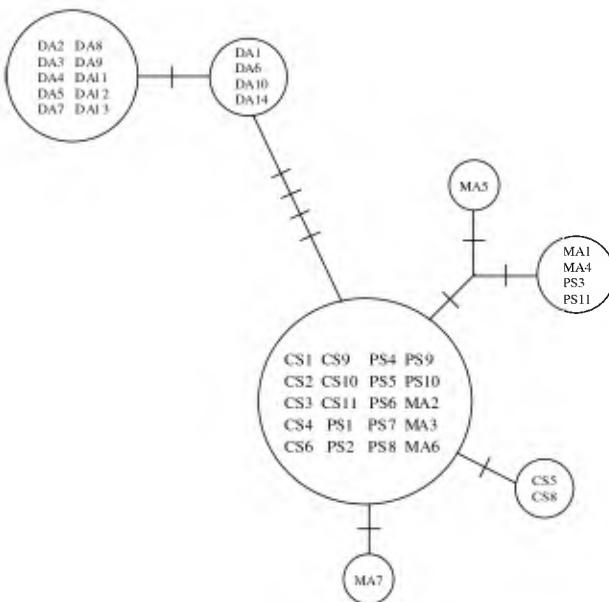


Fig. 4. Unrooted ITS network inferred by parsimony analysis. Each hatch mark along a branch represents a single mutational step. CS, Coco Solo; DA, David; MA, Manantí; and PS, Pon Sok.

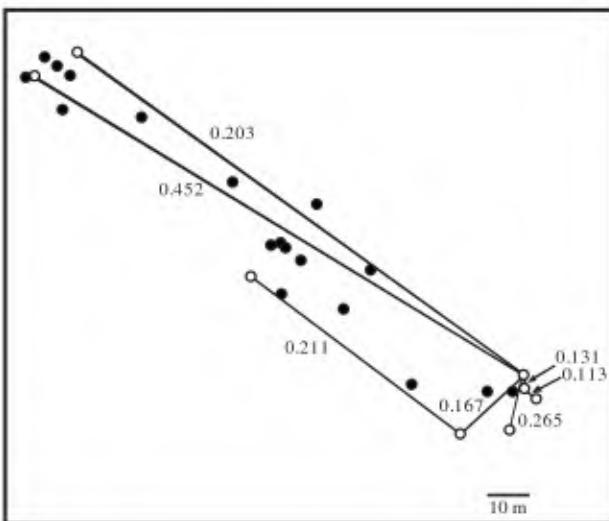


Fig. 5. Minimum spanning tree and genetic distances for Pon Sok transect 2 (Bocas population) overlaid upon the physical map of the site. Filled circles are *Laguncularia racemosa* trees without *Datronia caperata* fruit bodies. Open circles represent *L. racemosa* trees from which *D. caperata* fruit bodies were collected. Numerical values represent the genetic distance between *D. caperata* individuals. Bar = 10 m.

event or a recent genetic bottleneck. Alternatively, Bocas may simply be more isolated and have a smaller effective population size than that of David or Coco Solo, resulting in lower overall genetic diversity. The smaller number of well developed mangrove forest fragments along the Caribbean coast of Panama (Wolf & Jackson 1996) and strong human pressures for wood and charcoal from the Bocas mangroves (pers. obs.)

Table 3. Genetic and physical (geographic) distances between pairs of populations. For some analyses, the Manantí and Pon Sok sites are combined into one Bocas site. Geographic distances (km) are given above the diagonal. Below the diagonal are pairwise Φ_{ST} values. The probability random genetic distance > observed distance (1000 iterations) for each population pair is <0.0001.

	Coco Solo	Manantí	Pon Sok	David	Bocas
Coco Solo	0	237	274	307	255
Manantí	0.2311	0	57	89	NA
Pon Sok	0.1739	0.1192	0	111	NA
David	0.1348	0.3599	0.24	0	101
Bocas combined	0.218	NA	NA	0.253	0

could be responsible for a decreased *D. caperata* population size.

Results for this study revealed the genetic variation partitioned among *D. caperata* populations (Φ_{ST}) to be 0.21. In comparison, average genetic differentiation among populations relative to total genetic variation (G_{ST}) was found to be only 0.07 for populations of the mushroom species *Mycena rosea* sampled across France (Boisselier-Dubayle *et al.* 1996). For populations of the polypore *Fomitopsis pinicola* from Finland and Sweden $\Phi_{ST} = 0.0768$ (Högberg *et al.* 1995). James *et al.* (1999) determined F_{ST} values from a global sampling of the mushroom *Schizophyllum commune* to be 0.214. Thus *D. caperata* populations separated by 200–300 km are experiencing a much lower amount of gene flow between them than has been reported for other basidiomycete fungi sampled at greater distances.

The unrooted neighbour-joining dendrogram (Fig. 3) shows that individuals from the same geographic location generally cluster together, illustrating the pattern of genetic differentiation among populations. This result is also supported by the pairwise Φ_{ST} values, which demonstrates that there is significant genetic differentiation among all three populations (Table 3).

Despite high among population Φ_{ST} values, low ITS sequence divergence (less than 1%) confirms that all strains are conspecific. The four ITS sequence differences found only in David strains affirms their genetic isolation from Coco Solo and Bocas samples (Fig. 4). This isolation of the Pacific coast David population may be a consequence of the mountain range that runs the entire length of Panama, creating a geographic barrier between the Caribbean and Pacific mangrove forests. These ITS sequence results, showing little differentiation between the Coco Solo and Bocas populations, conflict with the AFLP data, which suggest Bocas and Coco Solo are genetically distinct populations. However, the ITS and 5.8S rRNA gene region is a single locus, whereas AFLP analysis surveys a large number of loci from across the genome and is therefore more likely to accurately reflect overall population structure.

The underlying mechanism responsible for the relatively low levels of genetic exchange detected between *D. caperata* populations may be due to either a lack of

basidiospore dispersal between mangrove forest fragments or to divergent selection pressures (i.e. local adaptation). The high degree of genetic diversity found within these populations would argue against strong local adaptation being the sole factor responsible for decreased genetic exchange between populations. Given the large number and small size of basidiospores, basidiomycete fungi have the potential to be very effective at long distance dispersal, as has been shown for a number of species (Kallio 1970, James & Vilgalys 2001). A possible explanation for the observed population differentiation is that *D. caperata* that are highly specialized on *L. racemosa* in mangrove forests have diverged from *D. caperata* in non-mangrove forests. Such host (and thus habitat) specialization, in conjunction with the fragmented distribution of mangrove forests, may be sufficient to depress successful colonization between populations, thereby decreasing gene flow. It is possible that abiotic factors, such as wind patterns, may also be an important determinant in the degree of gene flow among *D. caperata* populations. Strong trade winds in Panama come from the north (Windsor 1990). Caribbean populations would thus be isolated from spore immigration from other mangrove forests by the open sea to the north, whereas there would be greater potential for long distance transport from northerly Caribbean mangroves (such as Coco Solo), to southerly Pacific forests (such as David). Such transport may help explain the appearance in David of three isolates genetically very similar to the Coco Solo population.

The apparently low gene flow among *D. caperata* populations in fragmented mangrove forests may lead to reductions in interfertility among isolates from different populations and hosts. Though ITS sequence divergence suggests that these samples represent a single species, future work should include measuring frequency of dikaryon formation among paired isolates from mangrove forests of different distances, as well as interfertility with isolates from non-mangrove forests.

By comparing geographic and genetic distances within populations we detected little spatial structure at the local scale. This suggests that within *D. caperata* populations, clonal expansion, perhaps through underground root infection, is not likely to be the primary means of local spread, and successful dispersal and infection by basidiospores extends beyond the nearest neighbouring trees.

The degree of genetic structure observed for *D. caperata* populations in this study is higher than that of other polypore or mushroom fungi investigated thus far at similar geographic scales. Both physical distance and intervening topography between fragments should have a large impact on genetic isolation. In our study, we saw no evidence of genetic differentiation among the two sites at Bocas (Manantí and Pon Sok, separated by approximately 57 km across open ocean with some intervening mangrove islands), but significant isolation between the Bocas sites and David, which are separated

not only by the slightly longer 101 km, but also by a mountain range and large agricultural areas. The other sites are significantly farther apart – 255 km (Bocas to Coco Solo) and 307 km (Coco Solo to David). Although the population structure observed in this study is significant, expanding the study to include additional isolates from within existing populations, and additional populations from both mangrove and non-mangrove forests in the region is needed to confirm the observed pattern of population differentiation, and to determine which of the potential mechanisms is responsible for generating this pattern.

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