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Abundance and diversity of *Schizophyllum commune* spore clouds in the Caribbean detected by selective sampling

TIMOTHY Y. JAMES and RYTAS VILGALYS
Department of Biology, Duke University, Durham, NC 27708-0338, USA

Abstract

Selective spore trapping and molecular genotyping methods were employed to examine potential long-distance gene flow among Caribbean populations of the common mushroom *Schizophyllum commune*. Spore-trap samples from five locations were analysed using restriction fragment polymorphisms of five enzymatically amplified gene regions. Successful trappings suggested *S. commune* spores to be abundant in the air, with an estimated sedimentation rate of ≈ 18 spores/m²/h. High levels of genetic diversity characterized the spore-trap samples, with as many as 12 alleles observed at a single locus (chitin synthase) over all samples. In addition, spore-trap samples showed significant among sample heterogeneity including geographical population substructure. The ribosomal DNA (rDNA) intergenic spacer displayed the greatest allele frequency differences among samples, clearly separating the samples into those possessing only a South American-type allele and those segregating for both North and South American-type alleles. The molecular variation provided no clear evidence for dispersal over large, aquatic barriers within the Caribbean region, and instead suggested that spore-trapping experiments are primarily reflective of the local, established population.

Keywords: gene flow, intergenic spacer, mushroom, PCR–RFLP, spore trapping

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Introduction

The long-distance dispersal of fungal spores is both supported and refuted by empirical evidence as well as theory. The widespread dispersal of spores is superficially suggested by the observation that fungi seem to pervade every habitat on the planet via unseen microscopic propagules – taking the form of contaminants, decomposers and parasites. Further suggestive of long-distance dispersal are the wide and often cosmopolitan distributions of fungal species, perhaps explained by aerobiology studies that have uncovered the ubiquity of fungal spores in the air, including the heights of the atmosphere (Hirst & Hurst 1967; Ingold 1971). Contrary to the notion of frequent long-distance dispersal are studies which suggest that a great majority of spores will not disperse far (< 100 m) from their origin (Wolfenbarger 1946, 1959; Gregory 1973; Lacey 1996). Additional support for a limited spore dispersal hypothesis derives from studies that have invoked geographical barriers to explain genetically divergent allopatric species (e.g. Vilgalys & Johnson 1987; Wells & Wong 1989; Vilgalys & Sun 1994a). While the documentation of dispersal patterns from a point source strongly supports the limited dispersal distance hypothesis, more direct observations of spore dispersal, as well as population genetic data, are necessary to understand the influence of long-distance spore dispersal in shaping fungal species distributions and population structure. Although most spores will not be dispersed very far at all (typically < 20 m; Wolfenbarger 1946), most fungal fruiting bodies produce copious spores — a small fraction of which may escape into the upper wind currents and disperse many miles from their source (Wolfenbarger 1946). It is possible that this fraction may be very important for the survival of the species because such spores disperse away from the effects of competition from their own kin as well as from a possibly spent resource.

Our own studies relate to the dispersal biology of a common, cosmopolitan mushroom species, *Schizophyllum commune* Fr., a model genetic organism in the basidiomycete fungi. *S. commune* is a single morphological and biological species with collections from around the world completely interfertile (Raper *et al*. 1958). Besides the lack of apparent

Correspondence: T. Y. James. Fax: +1 919 660 7293; E-mail: tyj2@duke.edu

reproductive barriers among *S. commune* populations, the alleles at the mating incompatibility loci are distributed randomly throughout species' range (Raper *et al*. 1958). These initial observations suggested that long-distance spore dispersal was frequent among intercontinental populations of *S. commune*. The first evidence against panmixia in *S. commune* was the demonstration that crosses between strains from the UK and the US displayed non-allelic interactions in growth rate, leading Simchen (1967) to conclude that this quantitative trait had evolved independently in the two areas (i.e. without trans-Atlantic gene flow). Recently, neutral genetic markers have corroborated Simchen's (1967) assertion by demonstrating strong geographical structuring of populations, with very little gene flow across the Atlantic and Pacific oceans (James *et al*. 1999, 2001). Despite the lack of trans-oceanic gene flow in *S. commune*, population substructure has not been detected within a continent, leading to the conclusion that breeding populations are geographically broad. Within the western hemisphere, two major phylogeographic groups exist. Ribosomal DNA (rDNA) phylogenies group most North American plus Central American samples into a well-supported lineage (James *et al.*, 2001). The other lineage represents South American and Caribbean samples, including several strains from the Caribbean rim of the US, e.g. Florida and North Carolina. However, strains from Florida were found to belong to both the South American and North American rDNA clades. Therefore, we suspected the Caribbean Sea to be an area in which the distributions of two divergent phylogenetic lineages overlap.

Although the knowledge of dispersal in saprophytic fungi falls far behind that for other organisms, several studies have suggested that long-distance dispersal effectively eliminates population structure on a subcontinental scale (Boisselier-Dubayle *et al*. 1996; Vainio *et al*. 1998; Vasiliauskas *et al*. 1998), while others have discovered that on a global scale, saprophytic fungi are rather similar to other phyla in their magnitude and distribution of genetic variation (Spieth 1975; James *et al*. 1999). In contrast, many pathogenic fungi show evidence of strong adaptation to their hosts, resulting in population structure on a finer scale (McDonald & McDermott 1993; Chen *et al*. 1995) and the potential for sympatric speciation through gradual niche divergence onto hosts of different genotype (Brasier 1987).

One of the key difficulties in using molecular population structure as a surrogate for dispersal studies in saprophytic fungi is the difficulty in obtaining large samples, due to the cryptic nature of the fungal mycelium and the ephemeral nature of fungal fruiting bodies. For mycologists studying mushroom-forming basidiomycetes, there exists a novel method for obtaining large population samples from spores settling from the air (Adams *et al*. 1984; Vilgalys & Sun 1994b). This method employs a homokaryotic (genetically haploid) strain of a given species as bait for selectively extracting spores of the same species from the air. These haploid bait strains are capable of picking up spores from the environment and initiating the mating process, the latter of which allows the identification and isolation of dikaryotic or diploid individuals that contain both the bait haploid genetic component and the trapped haploid component as separate nuclei. In this study, we employ this sampling method on spore clouds of *S. commune* in the Caribbean to investigate the genetic diversity of these samples using molecular markers. We attempt to determine the extent of population subdivision in the Caribbean and ask whether long-distance spore dispersal can be detected. Lastly, we compare the samples from the spore cloud with those from naturally occurring fruit bodies in order to assess the utility of the spore-trap technique for understanding gene flow in mushroom-forming fungi and suggest that the technique may be used to contrast indirect and direct measures of gene flow.

Materials and methods

Trapping techniques and culturing

Our method of obtaining *Schizophyllum commune* basidiospores from the air follows the protocol of Adams *et al*. (1984). Briefly, a single homokaryotic or haploid 'bait strain' is inoculated onto the surface of a sterile 6- or 10-cm Petri dish containing a nutrient medium. The trapping medium employed in this study was 1.5% malt extract, 0.3% yeast extract, 0.5% glucose and 1.5% agar (MYG). The bait strain mycelium was allowed to cover the entire surface of the agar media. Inoculated Petri dishes were taken to the sampling sites and their lids were removed so as to expose the bait strain to the environmental air spora for ≈ 12 h. At each site, a single Petri dish was left unexposed as a control. The dishes were then resealed and allowed to incubate for at least 4 days (occasionally longer due to practical circumstances) before being returned to the laboratory for subculturing to determine if dikaryotization had occurred.

After returning to the laboratory, three subcultures were taken from different areas of each previously exposed dish and plated onto a 1.5% malt extract and 1.5% agar medium supplemented with 3 mg/L benomyl and 250 mg/L of both penicillin G and streptomycin sulfate. Like many mushroom-forming fungi, *S. commune* produces a distinct, dikaryotic mycelial morphology that can be discriminated microscopically from the homokaryotic state. After mycelia had grown visibly from the point of inoculation, they were checked to verify that they were not contaminants and were screened for the presence of clamp connections, the hook-like cells that are characteristic of the dikaryotic state.

Dikaryotic subcultures were further subcultured at least twice before genetic analysis. If more than one of the three subcultures from the same Petri dish were dikaryotic,

somatic compatibility pairings were used to determine the number of genetically distinct dikaryons trapped on a single Petri dish. In mushroom-forming fungi, dikaryons of different genetic composition show a distinct rejection reaction when paired on nutrient agar (Todd & Rayner 1978). These compatibility tests were conducted on potato dextrose agar (Difco, Detroit, MI, USA) supplemented with 0.8% glucose and were scored for the presence of a zone of incompatibility indicative of a somatic rejection response (Adams & Roth 1967). All subcultures from the same exposed Petri dish were paired in all combinations to determine the number of genetically distinct individuals. Dikaryons collected from separate Petri dishes were assumed to derive from separate spore-trapping events.

Samples

The bait strain used in this study was a monokaryotic isolate (BEL#1) derived from a fruiting body collected in 1997 in Belize by Wilma Lingle.

Seven samples were collected during the years 1998 and 1999:

1 Field Station in Sabana, Puerto Rico. Twenty-four, 6-cm diameter Petri dishes were exposed for 11 h overnight on 7–8 June 1998. Weather was calm and overcast with a brief period of rain prior to plate closure. Of the 24 plates, six yielded only contaminants, 17 dishes yielded 25 genetically distinct dikaryons and a single dish yielded only the original monokaryotic bait strain.

2 Beach, LaParguera, Puerto Rico. Twenty-six, 6-cm diameter Petri dishes were exposed for 12 h overnight on 8–9 June 1998. A constant breeze blew from the southwest over the sea. Two dishes yielded only contaminants, 15 dishes produced 28 unique dikaryons and nine dishes yielded only the original bait strain.

3 Georgetown, Guyana. Twenty-four, 6-cm diameter Petri dishes were exposed for 11 h overnight on 20–21 June 1998. Seven dishes yielded only contaminants, 12 dishes produced 26 unique dikaryons and five yielded only the original bait strain.

4 Chubb Cay, Bahamas. Twenty-five, 10-cm diameter Petri dishes were exposed for 13 h overnight on the deck of the vessel *Edwin Link*, anchored 1 mile off shore. A light breeze blew from the southwest. Eight dishes yielded only contaminants, 15 dishes yielded 27 dikaryons and two dishes yielded only the original bait strain.

5 The Kampong, Coral Gables, Miami, Florida, USA. Thirty-six, 10-cm diameter Petri dishes were exposed for 10 h overnight on 18–19 March 1999. Weather was clear skies with a constant breeze coming southeasterly over the sea. Two dishes yielded only contaminants, two dishes returned only the original bait strain and 32 dishes yielded 48 dikaryons.

6 John Pennekamp State Park, Key Largo, Florida, USA. Twenty-three, 10-cm diameter Petri dishes were exposed for 12 h overnight on 30 June–1 July 1999. Weather was warm and humid with mild winds. All dishes yielded only the original bait strain.

7 San Salvador Island, Bahamas. Twenty-four, 10-cm diameter Petri dishes were exposed for 10.5 h during the day on 4 August 1999 on the *Edwin Link*, < 1 mile offshore. A light breeze blew from the southeast. All 24 dishes were contaminated.

Fruiting body samples from several locations in Puerto Rico were collected during 1997–1998. Ten fruiting bodies were used to derive 10 dikaryotic cultures. These dikaryotic strains were used to estimate the allele frequencies in the established population. Likewise, eight fruiting bodies from southeastern Florida were collected in 1999 to derive eight monokaryotic cultures to compare with the spore cloud sampled from Miami, Florida, USA.

DNA techniques

Each genetically unique spore-trap dikaryon was subject to genetic analysis of selected loci targeted by polymerase chain reaction (PCR) amplification. DNA extracts were obtained from strains that were grown on MYG agar. Approximately 0.1–0.5 g wet weight of mycelia was scraped from the surface of the agar and mycelia were dehydrated in a Speed-vac concentrator (Savant Instr. Inc., Farmingdale, USA). Mycelia were ground using a sterile pipette tip and microcentrifuge pestle and DNA extracted following Zolan & Pukkila (1986). Five loci were analysed for each individual using the PCR amplification protocol of Vilgalys & Hester (1990). Amplification was accomplished using primers: LR12R and 5SRNASC (James *et al.*, 2001) for the intergenic rDNA spacer region (IGS), the primers LCS1F (5′-CCATTCACTGGCATGGATTCTTCC) and LCS1R (5′-GGTTGGCGCGGATCCAGTAGTTG) for laccase (LACC), and the primers B36F and B12R (Thon & Royse 1999) for beta-tubulin (BTUB). Amplification of super oxide dismutase (SOD) used the primers SOX1R (5′-GAGCAGTACGCCAAGCTGCAGAG) and SOX1L (5′-ATGATGGGGACGTGGGCTG) at a modified annealing temperature of 60 °C. Amplification of chitin synthase (CHS) was with primers CHS1F (5′-GCGCACTTGTGCAAGCGCGAT) and CHS1R (5′-AGAGCGCACCGAGGGGATTCAGGA), at an annealing temperature of 58 °C. Amplification of the mitochondrion encoded NADH-ubiquinone oxidoreductase chain 5 (NAD5) was with primers NAD53F (5′-GGWCCTACWCCYGTATCWG) and NAD57R (5′-CCTAWTRWAATATATAAAGCATAAG).

Genetic analysis of amplification products (amplicons) was accomplished via their digestion using restriction enzymes following the manufacturer's instructions (Promega, Madison, USA and New England Biolabs, Beverly, USA).

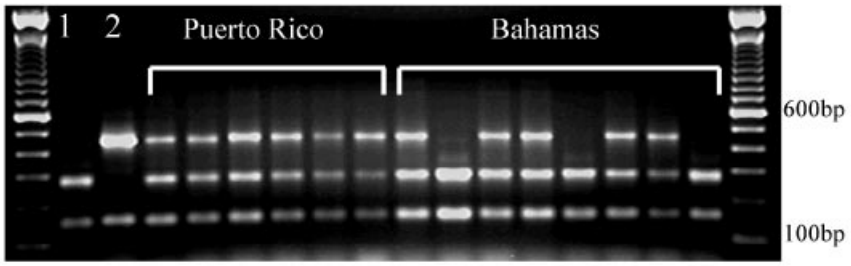

Fig. 1 Genotypes of the co-dominant *IGS1* locus digested with *Hin*fI. In the first and last lanes are shown a molecular mass marker. Lane 1, NAM restriction pattern of the bait strain, with a larger ≈ 290 bp band and two co-migrating fragments of ≈ 140 bp. Lane 2, the homozygous South American type (SAM) pattern of a fruiting body collected in Puerto Rico with fragments of ≈ 450 and 140 bp. Lanes marked 'Puerto Rico' are spore-trap dikaryons from Puerto Rico that display a heterozygous pattern with a North American type (NAM) allele component from the bait strain and a SAM component from the trapped nucleus. Lanes marked (Bahamas) show variation among the Bahamas spore-trap dikaryons at the *IGS1* locus, where both NAM and SAM nuclei were trapped.

Enzymes producing the most polymorphic restriction patterns amongst a subset of diverse strains were selected for further analyses. The IGS region was digested solely with *Hin*fI; SOD amplicons were digested with *Rsa*I and *Cfo*I, CHS with *Hae*III and *Mbo*I, LACC with *Hae*III and *Eco*RI, and BTUB with *Hin*fI, *Mse*I and *Hae*III. Digestion products were separated by electrophoresis on 1–2% agarose gels. The scoring of digestion profiles was aided by the co-dominant nature of the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique. Each enzyme/locus combination was scored for unique restriction patterns following the discounting of the bait strain haplotype. Between two and six patterns were found for each enzyme/locus combination. Alleles for a given locus were assigned based upon unique multienzyme combinations. No attempt was made to map or assign particular patterns to the presence or absence of specific restriction sites.

Data analysis

Estimates of genetic differentiation (θ_{ST}), exact tests of linkage disequilibrium, and genetic distance were calculated using the Genetic Data Analysis software package (version 1.0d15; Lewis & Zaykin 2000). A population matrix of Nei's unbiased genetic distance estimator (Nei 1978) was used to generate a phenogram with the neighbour-joining algorithm included in the PHYLIP software package (Felsenstein 1993).

Results

Occurrence and sedimentation of Schizophyllum commune *spores*

The results of our spore-trapping experiments show spores of *Schizophyllum commune* to be extremely abundant in the air. Five of the seven attempted samplings trapped dozens of spores, while two samplings resulted in no trapped spores. We attribute the latter to the possibility of either technical error or inappropriate weather conditions. The failed attempt to trap spores near San Salvador Island, Bahamas was conducted during the day, whereas spores of most mushrooms are believed to be released in highest abundance at night (Haard & Kramer 1970; Burge 1986). Regardless, from all seven spore-trap samplings, we sampled for a total of 79.5 h and trapped 154 dikaryons, providing an average estimate that 18.24 spores of *S. commune* land on each m^2 of land per hour. Because most of these spore-trap samples were conducted away from forested areas, these estimates probably apply generally to any and all outdoor areas.

Genetic diversity of spore-trapped samples

Molecular techniques revealed that in every instance (except one, discussed below), the haplotype of the bait strain was observed following PCR amplification and restriction enyzme digestion of amplicons from spore-trap dikaryons (Fig. 1). These results suggest that our results are unlikely to be sensitive to preferential amplification of only one of the two alleles within a dikaryon. The ability to identify the bait strain allele, and its common RFLP difference from trapped spores, allowed the genotyping of the 154 trapped dikaryons and the assignment of multilocus haplotypes to each of the trapped spores. For the 10 dikaryotic strains derived from fruiting bodies from Puerto Rico, single-locus haplotypes could not be assigned in instances in which the strain appeared heterozygous for more than one restriction enzyme at a particular locus (for a discussion of this problem see Karl *et al*. 1992). These loci with unassignable haplotypes were scored as missing data for that particular strain. For the other loci of the fruit body samples for which haplotypes were unambiguously assignable, alleles were randomly chosen from the pair to facilitate analysis at the haploid level.

Population	Number of alleles	Number of genotypes	Allelic diversity (average expected heterozygosity)
Sabana, Puerto Rico (n = 25)	11	13	0.430
LaParguera, Puerto Rico (n = 28)	11	11	0.363
Puerto Rico, fruiting bodies (n = 10)	10	6	0.381
Guyana (n = 26)	14	17	0.467
Bahamas (n = 27)	26	22	0.609
Miami (n = 48)	20	20	0.509
Florida, fruiting bodies (n = 8)	18	8	0.616

Table 1 Summary of the genetic diversity of both spore-trap and fruiting body samples. The number of alleles per population is summed over the five PCR–RFLP loci. The number of genotypes refers to the number of unique five locus genotypes

Using PCR–RFLP of five selected loci on the spore-trap dikaryons, 67 unique multilocus genotypes were detected among the 154 spore-trap dikaryons. Most of the spore-trap dikaryons that derived from the same exposed Petri dish possessed different multilocus genotypes, suggesting that the somatic compatibility tests were able to distinguish genetically unique individuals.

The number of alleles was highest at *CHS* (12), followed by *SOD*, *LACC* and *BTUB* each with six. Only two alleles were found at *IGS1*, however, only one restriction enzyme was used to genotype this locus. Each locus was polymorphic within spore-trap samples with the exception of *IGS1*, which was monomorphic within both Puerto Rico samples and the Guyana sample. The Bahamas sample had the highest allelic diversity with 26 alleles found over all loci including eight alleles unique to the sample. Table 1 presents the measures of allelic diversity among the five spore-trap samples plus the two samples derived from fruiting bodies. Estimates of genetic diversity for the two samples of fruiting bodies were similar to those of spore-trap samples. Puerto Rican fruiting bodies provided an estimate of expected heterozygosity similar to those from spore-trap samples from the same island; in contrast, the Florida fruiting bodies displayed the highest diversity (Table 1).

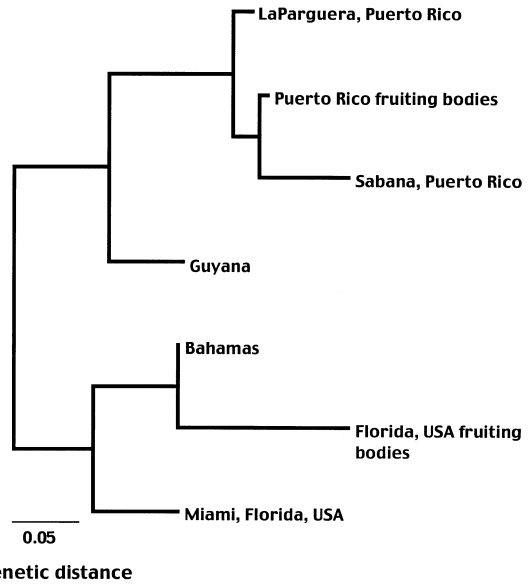

Fig. 2 Neighbour-joining phylogram of the spore trap and fruiting body samples.

Sample differentiation

The *IGS1* locus has been used to extensively characterize the global geographical structure of *S. commune* (James *et al.*, 2001). Caribbean and South American samples were found to have larger (≈ 30 bp) IGS1 spacers and to lack a *Hin*fI site that was nearly fixed in the group of samples from North America. The bait strain used in this study possesses a North American (NAM) type *IGS1* allele. The use of co-dominant markers allowed the easy identification of spore-trap dikaryons possessing the South American (SAM) type *IGS1* haplotype (Fig. 1). For both samples from Puerto Rico and the sample from Guyana, all spore-trap dikaryons revealed two *IGS1* alleles following *Hin*fI digestion, the NAM allele from the bait-strain and the SAM allele from the trapped spore nucleus. Therefore, we infer that all strains from these regions inherited the SAM allele from a trapped spore (Fig. 1). In contrast, the Bahamas and Miami samples revealed polymorphism of the *IGS1* haplotype, with several spore-trap strains producing only the NAM homozygous phenotype following digestion (Fig. 1). Differentiation among the five spore-trap samples at *IGS1* as measured by θ_{ST} (0.268) was higher than other loci, with *LACC* (0.229) and *BTUB* (0.223) higher than *CHS* (0.151) and *SOD* (0.092). The multilocus estimate of θ_{ST} was 0.181. This value was significantly different from zero with a confidence interval of 0.242–0.125.

Genetic differentiation among samples was further characterized by phenetic analysis of genetic distances using Nei's (1978) unbiased distance estimator and the neighbour-joining algorithm. This method also allowed the comparison of fruit bodies collected from nature to samples generated from spore trapping. The phenetic tree shown in Fig. 2 suggested that those populations displaying polymorphism at *IGS1* (i.e. the Bahamas, Miami and southern Florida samples) were distinct from those for which the South American allele was fixed (i.e. Guyana

and both Puerto Rico samples). These data also suggested that the spore-trap cultures were at least partially reflective of the naturally occurring fruiting bodies from each region, because the fruit body samples cluster near the most geographically proximal spore-trap samples. However, Puerto Rican fruiting bodies were more similar to the spore-trap dikaryons from Puerto Rico than fruiting bodies from southern Florida were to the Miami spore-trap sample.

Linkage disequilibrium

We used the PCR–RFLP data to test the null-hypothesis that spores trapped from the atmosphere represent random alleles drawn from the distributions of local allele frequencies vs. the hypothesis that certain allelic associations among loci occur more frequently than expected. Such linkage disequilibrium could result from: (i) a minimal number of fruiting bodies from the same population that provided the spore sample; (ii) differentiation among populations of fruiting bodies that contributed to the spore sample; or (iii) physical genetic linkage.

Significant ($P < 0.05$) among-locus disequilibria were observed within three spore-trap populations. No disequilibrium was found among alleles in either Sabana or Guyana populations. At LaParguera, allelic combinations at *SOD* and *LACC*, and *LACC* and *BTUB* were in disequilibrium and the three-locus disequilibrium was also significant.

Large amounts of disequilibria were uncovered in both Bahamas and Miami samples. In the Bahamas sample, the rare alleles at *SOD* were associated with rare alleles at *BTUB*. Likewise at *CHS*, a rare allele was associated with the rare allele at *BTUB*. In addition, a significant five-locus disequilibrium was observed in the Bahamas sample.

In the Miami sample, very strong disequilibrium was observed between *IGS1* and *LACC*. The (NAM) allele at *IGS1* was never associated with the (4) allele at *LACC*, although both alleles were in relatively high frequency. *SOD* and *CHS*, *SOD* and *BTUB*, and *CHS* and *LACC* also showed disequilibria. Higher-order, including five-locus, disequilibria were also significant.

The differences in disequilibrium relationships among loci from different populations suggested that physical linkage was an unlikely source of the linkage disequilibrium among alleles. In order to understand whether the disequilibria uncovered within populations were the result of sampling a small number fruit bodies from a larger, more diverse population, we attempted to determine the minimal number of fruiting bodies that could have produced each spore-trap sample. An exact solution was used to determine the minimum number of parents that could have generated each of the spore-trap samples by maximizing the number of genotypes produced by hypothetical parents, while simultaneously minimizing the number of different contributing parents. Results for all of the samples suggested that each sample could have possibly been derived from a small number of fruiting bodies. The values ranged from two parents for both Sabana and LaParguera samples to six parents for the Bahamas sample. Thus, while linkage disequilibrium within three of the spore-trap samples suggests the trapping of spores from multiple, genetically differentiated populations, it is impossible to exclude the explanation that only a small number of genetically different strains actually formed the basis for such disequilibrium.

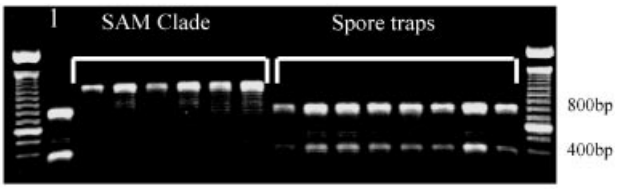

Fig. 3 Inheritance of the mitochondrion in *Schizophyllum commune* spore-trap dikaryons as revealed by *Eco*RI digestion of *NAD5* amplicons. In the first and last lanes are shown a molecular weight marker. Lane 1, the restriction pattern of the bait strain with two fragments of ≈ 800 and 400 bp. Lanes marked 'SAM clade' are wild collected strains from the Caribbean and South America that do not digest with *Eco*RI. Lanes marked 'spore traps' are the digestions of spore-trap dikaryons that shown a similar pattern to the bait strain.

An anomalous strain suggests nuclear sorting

The spore-trap dikaryon Guy.21.2 produced an unusual multilocus genotype that suggested that it was the product of the mating of two nuclei, neither of which was the original bait strain. This dikaryon was found to possess only the SAM *IGS1* haplotype, and further genetic dissection revealed that the culture possessed two alleles at *BTUB* and *CSYN*, neither of which was present in the original bait strain. We investigated the cytoplasmic inheritance of the spore-trap strains, including Guy.21.2, by PCR–RFLP analysis of the mitochondrial-encoded *NAD5* gene. The original bait-strain possessed an *Eco*RI site within the ≈ 1200 bp NAD5 amplicon which was absent from the *NAD5* locus of 12 members of the SAM clade (from throughout the Caribbean and South America) that were genotyped. Each of 22 genotyped spore-trap dikaryons, including Guy.21.2, also possessed the *Eco*RI site in NAD5 amplicons, suggesting the mitochondria of all these dikaryons were inherited from the bait strain (Fig. 3). We infer from the NAD5 data that Guy.21.2 derives from the mating of two genetically different trapped nuclei that displaced the resident nucleus but adopted the cytoplasm of the bait strain.

Discussion

This study uncovered an abundance of spores of the common mushroom species *Schizophyllum commune* in the Caribbean air spora and characterized the population

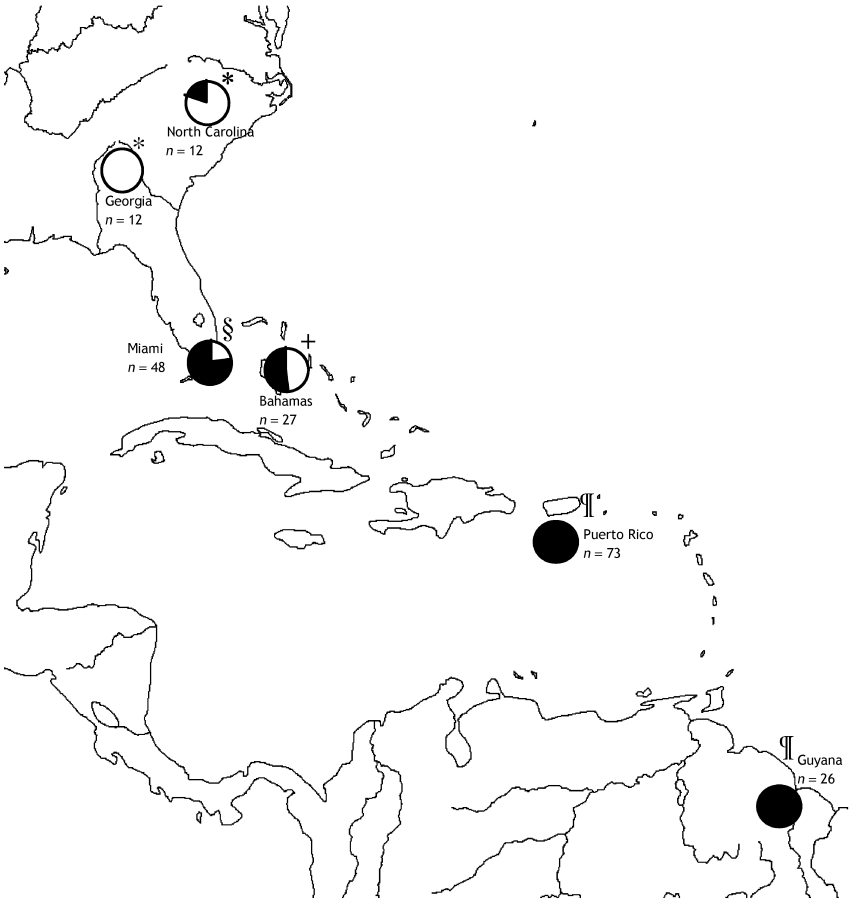

Fig. 4 Distribution of *Schizophyllum commune IGS1* haplotypes in the Caribbean. *n*, sample sizes used to estimate frequencies. Black fraction represents the South American type *IGS1* haplotype (SAM allele) and the white fraction the North American haplotype (NAM allele). The symbols (*, §, + , ¶) are used to indicate populations whose allele frequencies are not significantly different from each other. All comparisons were significant at $P < 0.05$ using Fisher's exact test (Raymond & Rousset 1995), except North Carolina vs. the Bahamas which was marginally significant ($P < 0.10$). Data from Georgia and North Carolina derived from a global ribosomal (r)DNA survey (James *et al*., 2001) and James and Vilgalys (unpublished).

genetics of these spores. Other studies have used spore-trap techniques to suggest that basidiomycete spores are abundant in the air column. A higher success rate in trapping dikaryons of *S. commune* was found relative to spore trapping of *Pleurotu*s spp. (Vilgalys & Sun 1994b), although technical differences prevent the two rates from being compared directly. The sedimentation rate of spores recorded in this study (18.24 spores/m^2/h) is lower than that recovered for another common mushroom species, *Coriolus versicolor*, in which 636 dikaryons were obtained over 660 h of sampling, providing an estimated sedimentation rate of (112.92 spores/m^2/h; Williams *et al*. 1984). Although these results are not the normal volumetric estimates desired for aerobiological studies, they do suggest that most available substrates receive a high spore load very soon after they become available for decay.

The *IGS1* locus has previously demonstrated a strong population genetic subdivision between North America and South America, with Caribbean samples grouping with those from South America (James *et al*., 2001). South American type *IGS1* alleles are also found as far north as North Carolina, USA. The frequency of the South American type *IGS1* allele increases at lower latitudes, suggestive of an allele frequency cline at this locus (Fig. 4). The South American type allele at *IGS1* increases in frequency to 0.75 among southern Florida fruiting bodies and is similarly 0.77 among the spore-trap sample from Miami. Interestingly, the South American allele is found at a lower frequency in the Bahamas sample (0.52) and the differences in frequencies are significant ($P < 0.05$). This sampling site was approximately the same latitude and 2° east of the Miami sample. Southward to Puerto Rico and lower latitudes, the South American type *IGS1* allele appears fixed.

Significant linkage disequilibrium within three of the five samples suggested that spores were being trapped from genetically divergent populations. However, it was unclear whether such disequilibrium could have also occurred through trapping spores from a small number of genetically different fruiting bodies from within the same population. Additional evidence as to the number of parents contributing to the spore-trap sample could be gathered by genotyping the mating incompatibility loci. These loci are highly polymorphic in mushroom-fungi with up to hundreds of alleles per locus (Raper 1966; Murphy & Miller 1997). In the study of Williams *et al*. (1984), all of the tested spore-trap dikaryons contained different mating alleles, suggesting that a large number of fruiting bodies actually contributed to the spore-trap sample.

The only evidence for disequilibrium between *IGS1* and other loci was the association of the *IGS1* (SAM allele) with the *LACC-4* allele in the Miami spore-trap sample. The *LACC-4* allele occurred at high frequency in the Miami sample but was practically absent from all other samples. This suggests that the SAM *IGS1* alleles found in the Miami spore-trap sample did not come from spores originating in Guyana, Puerto Rico, or other Caribbean or South American populations with similar allele frequencies. Thus, this study provides no genetic evidence for trans-Caribbean spore dispersal of *S. commune*. In addition, because the spore-trap samples displayed significant among-sample genetic heterogeneity, the populations from which the spores derived from show some measure of subdivision and hence lack of gene flow.

It is important to note that spore-trap samples are reflective of spore dispersal and thus represent potential and not actual gene flow. For gene flow to be realized, spores must successfully establish within the population to which they migrate. Differences in quantitative traits of potential fitness effects between populations of *S. commune* (e.g. Simchen 1967) may suggest that long-distance migrants may be selected against. However, the similarity of spore-trap samples to the fruiting body samples from the same geographical area suggest that the lack of gene flow among populations would be the effect of barriers to dispersal rather than barriers to establishment.

Circumstantial evidence, however, stresses that *S. commune* is able to disperse long distances. Because attempted spore trappings were conducted in areas without obvious fruiting bodies in the vicinity, the success rate and genetic diversity of the samples suggest that spores do travel greater than km distances. Our Bahamas sample was collected on the deck of a research vessel 1 mile from land. While *S. commune* is known to habit off-shore, mangrove islands (D. Porter, personal communication), the high diversity of the sample suggests that not all of the trapped spores derived from the most nearby source. The high diversity in the Bahamas sample might be explained because it was more distant from a large source of potential fruiting bodies. Therefore, the spore cloud may have been more mixed than spore clouds from areas of high woody substrate. In addition, relatively fast winds associated with open seas could also provide greater mixing of the spore cloud. Although it was not studied, it is possible that some of the spores trapped in the Bahamas could actually have come from quite far away. Sequence analysis of *IGS1* from the Bahamas spore-trap sample would allow this possibility to be distinguished.

Few studies have sought to address the dispersal of mushroom spores over large bodies of water. Kallio (1970) was able to trap viable *Heterobasidion annosum* spores on a lighthouse island that was ≈ 17 km from any potential spore source. Although we present no direct data regarding long distance dispersal of *S. commune* spores in the Caribbean, we have found that the distribution of *IGS1* types agrees with the regional wind patterns reported for the region. Namely, the northeastern trade winds that drive air westerly in the North Atlantic would be expected to provide unidirectional spore dispersal from northern South America towards the Caribbean islands and prevent the opposite pattern (Young & Holland 1996). The observation that the North American *IGS1* allele has never been recovered in South America or the Caribbean outside of the US or the Bahamas supports the possibility of unidirectional gene flow of spores in the Caribbean. The hypothesis of unidirectional dispersal due to Caribbean tropical air and sea currents also agrees well with biogeographic patterns observed in less vagile organisms, such as vertebrates (Hedges 1996).

The use of the spore-trap sampling has tremendous potential, but is uniquely only applicable to those studying basidiomycete fungi. Its use hinges on the ability to work at both the haploid and diploid level and the ability to distinguish their nuclear condition using morphological criteria. Spore trapping has great promise for generating large samples for population studies that cannot be achieved through the collection of fruit bodies. Moreover, the use of spores rather than fruiting bodies allows for the direct estimation of gene flow, and such results can be compared with indirect estimates of gene flow using fruiting bodies. The intersection of these two estimates may provide evidence for selection against immigrants. Further experiments are needed to verify the robustness of the procedure for estimation of true population parameters. Remaining questions include: (i) is there temporal heterogeneity among spore clouds that is determined by the fruiting phenology of the established population; (ii) where are the most appropriate places to collect spore-trap samples when attempting to estimate allele frequencies of the established population; and (iii) does sexual selection occur between the bait strain and the spores which it may potentially mate with?

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This work formed part of the PhD research by Tim James on population and mating genetics of hymenomycete fungi. This study is part of a larger effort to understand the potential for long-distance dispersal of mushroom basidiospores. Rytas Vilgalys is an investigator in the Duke University Mycology Research Unit. Interests of the Vilgalys group include molecular phylogeography and systematics, speciation and mating genetics of mushrooms and medically important fungi.
