Low Genetic Variation and No Detectable Population Structure in *Aspergillus fumigatus* Compared to Closely Related *Neosartorya* Species†

Carla Rydholm,[1]* George Szakacs,[2] and François Lutzoni[1]

Department of Biology, Duke University, Durham, North Carolina 27708,[1] and Technical University of Budapest, Budapest, Hungary[2]

Aspergillus fumigatus is an anamorphic euascomycete mold with a ubiquitous presence worldwide. Despite intensive work to understand its success as a pathogen infecting immunosuppressed patients, the population dynamics and recent evolutionary history of *A. fumigatus* remain understudied. We examined patterns of genetic variation at three intergenic loci for 70 natural isolates from Europe, North America, South America, Asia, Africa, and Australia. The same loci were used to analyze within-population genetic variation for 33 isolates obtained from five geographic locations. Neither data set detected evidence of population differentiation or found any association between the genetic and geographic distances among these isolates. No evidence for genetic differentiation within the two *A. fumigatus* mating types was detected. The genetic diversity of *A. fumigatus*, contrasted with that of its close teleomorphic relatives, *Neosartorya fischeri* and *Neosartorya spinosa*, is remarkably low.

Population genetic studies of fungal species have held applied and theoretical interest since the first mycological application of molecular markers by Spieth in 1975 (51) and are now established as a vital component to accurate and complete understanding of a species' biology. With such data, global population structure, intraspecific genetic differentiation, and genetic diversity may be estimated. We applied a population genetic approach to study patterns of genetic variation in the cosmopolitan haploid mold *Aspergillus fumigatus*. The primary focus was to gauge patterns of genotypic diversity and differentiation at global and local scales.

A. fumigatus is a euascomycete (*Pezizomycotina*) species of the order *Eurotiales* for which no sexual reproduction structures have yet been observed. It is abundant in soil rich with organic materials and upon decaying vegetation undergoing aerobic decomposition (54). It is a cosmopolitan species but is more commonly reported from the air column in the central latitudes of the Northern Hemisphere (23). Most research on *A. fumigatus* has focused on the molecular basis for its success as a common airborne fungus isolated clinically (2). Genotyping efforts (for a review, see reference 56) characterizing pathogenic strains have found clinical isolates to be generally indistinguishable from strains obtained from the hospital and external environment. The majority of these data sets were isozyme and arbitrary PCR product profiles. When evaluated for evidence of an exclusively clonal reproductive mode, the majority of analyses detected patterns consistent with historical recombination (37, 41, 56).

For our population genetic survey of *A. fumigatus*, we sampled at two spatial scales. A global sampling of strains was made from across the breadth of the species' geographic range. A second set of *A. fumigatus* strains was isolated from soil at three North American and two European sites. The latter sampling was done to address the intraspecific distribution of genetic variation for environmental isolates of *A. fumigatus*. The genetic markers used for both data sets were three nuclear intergenic regions. The intergenic regions were used instead of coding regions to maximize polymorphic sites within a locus; in coding regions, variation is usually limited to third-base positions.

Our evaluations of the population genetic parameter values estimated for *A. fumigatus* are made with consideration given to an interspecific comparison with its close relatives *Neosartorya fischeri* and *Neosartorya spinosa*. These species have a different generic name owing to the mycological tradition of confining species for which a sexual state is known (teleomorphs) to separate taxonomic groups from the species without a known sexual state (anamorphs; also referred to as mitosporic fungi). Recent phylogenetic work on the form genus *Aspergillus* that included most anamorphic *Aspergillus* species and the associated taxa with known sexual states infers that *A. fumigatus*, *N. fischeri*, and *N. spinosa* are part of the *Aspergillus* subgenus *Fumigati* subgroup *Fumigati* (38). The subgroup has a total of 24 species (58). Phylogenetic studies find the two species *A. fumigatus* and *N. fischeri* to be sister taxa with good branch support (15, 58). These two taxa are a potential example of the species pair concept predicted for fungi (5, 6, 14, 24). The species pair hypothesis suggests that strictly or predominantly asexual species emerge from sexual lineages that have adapted to selective forces in a given environment (27, 31). Knowledge of the evolutionary history associated with both lineages may inform our understanding of the emergence of *A. fumigatus* as a species.

The *A. fumigatus* multilocus sequence data obtained for this study were also analyzed within the context of mating compatibility groups. Regulatory regions, termed mating-type loci, control sexual compatibility between individuals and their initiation into the sexual cycle. Characterization of mating-type loci has proved crucial to understanding mating, pathogenicity,

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* Corresponding author. Mailing address: Biology Department, Duke University, Box 90338, Durham, NC 27708. Phone: (919) 660-7382. Fax: (919) 660-7285. E-mail: clr@duke.edu.

and global population dynamics of common fungi (1, 7, 26, 29, 33, 43). Two versions of the mating-type locus exist (referred to in *Aspergillus* as MAT1-1 and MAT1-2) in most ascomycete fungi (55, 61). They have little or no sequence similarity (16), and both are required for a successful mating. Fungi can be either homothallic, where both mating types are present within an individual, or heterothallic, where only one mating type is present in an individual. These loci have recently been characterized for *A. fumigatus* (37, 39, 57). Each strain carries only a single mating-type locus, either MAT1-1 or MAT1-2, and thus appears to have a heterothallic mating system architecture. A broad survey of environmental and clinically isolated strains finds no significant difference in abundance of the two mating types and provides evidence of their co-occurrence within individual patients and environmental samples (37). The evolutionary history of strains of the two mating types could differ if sexual recombination is infrequent and/or if the fitness of the two types is different.

In this study we report an analysis of intraspecific DNA sequence variation in a global sample of 70 strains of *A. fumigatus* using three intergenic regions. These data are compared to values obtained from the same loci for six strains of *N. fischeri* and five strains of *N. spinosa*, homothallic species also belonging to the genus *Aspergillus*, subgenus *Fumigati*, section *Fumigati*. In culture collections, isolations for both species have been recorded from soil, spoiled food, and skin of human patients from the Northern and Southern Hemispheres, as well as both sides of the Pacific and Atlantic Oceans. Of the two, only *N. fischeri* is reported as a species causing invasive infection, with a long history of such records (54). Our purpose here was to relate the genetic diversity present in *A. fumigatus* to its close and sexually reproducing relatives as a context to interpret the estimates obtained. Loci were also analyzed for deviations from neutrality. The relative divergence times of the species *A. fumigatus* and *N. fischeri* were estimated. Further data for the three intergenic loci were collected for 33 *A. fumigatus* strains isolated from five different geographic sites in North America and Europe. From this site-specific sampling, isolation by distance and distribution of genetic variation within the species were evaluated. The global and local surveys of *A. fumigatus* were pooled by mating types. Their genetic diversity and their mutation frequency spectra and deviation from neutrality were separately analyzed to evaluate genetic differentiation within the species correlated with mating compatibility group. With these data and comparisons we sought to better define the population genetic patterns and evolutionary history of *A. fumigatus*.

MATERIALS AND METHODS

Strains. Seventy strains of *A. fumigatus* isolated from the environment were utilized in the global survey of this study (see Table S1 in the supplemental material). These strains were received from the mycological collections of the Technical University of Budapest, Martha Christensen at the University of Wyoming, or Maren Klich at the USDA Agriculture Research Service or were isolated for this study by C. Rydholm or G. Szakacs from soil samples. C. Rydholm also isolated strains used in the site-specific survey of multiple isolates from each of five locations from soil samples (see Table S1 in the supplemental material). Soil was collected from Finland, Germany, the territory of Nunavut in the Canadian Arctic, Northwestern Ontario, and North Carolina. Nine soil samples were collected from 10-by-10-m plots, with samples being taken 5 m apart in a grid formation. From each soil sample one single spore isolation was attempted. A total of 33 isolates were obtained for the site-specific sampling. Soil

was initially spread in serial dilution on MEA medium with antibiotics (12 g/liter malt extract, 20 g/liter agar, 90 μg/ml tetracycline) and incubated for 2 to 4 days at 37°C. Isolates were then streaked on MEA medium with Triton X-100 (12 g/liter malt extract, 20 g/liter agar, 1 ml/liter Triton X-100), incubated for 2 days, and transferred to another Triton X-100 plate; this was repeated four times. *Neosartorya fischeri* and *N. spinosa* strains were obtained from the Centraalbureau voor Schimmelcultures (CBS) and Agricultural Research Service Culture Collection (NRRL) (see Table S1 in the supplemental material). One isolate from each of four *Aspergillus* subgenus *Fumigati* species (*N. aureola*, *N. pseudofischeri*, *N. spathulata*, and *N. stramenia*) were used as outgroup taxa.

DNA isolation and sequencing. Genomic DNA was prepared from mycelium grown in liquid malt medium (12 g/liter). Lyophilized fragments were transferred with forceps to 1.5-ml tubes containing 500 μl of 1% sodium dodecyl sulfate extraction buffer. Mycelium was ground with a polypropylene micropestle and incubated at room temperature. DNA was purified with phenol-chloroform (1:1, vol/vol) as described by Raeder and Broda (42). Next, 300 μl of chloroform-isoamyl alcohol (24:1, vol/vol) was added, and the solution was emulsified and centrifuged for 15 min. The DNA was precipitated from the aqueous phase by the addition of an equal volume of cold isopropanol. The pellet was washed with 70% ethanol, and the DNA was resuspended in 50 μl water.

To infer the phylogenetic affinities of strains, the internal transcribed spacer region (ITS) and a portion of the β-tubulin gene were amplified via PCR from genomic DNA. Oligonucleotide primers used to amplify ITS were ITS1 and ITS4 (60). Conditions used for PCR of ITS were according to Vilgalys and Hester (59). The primers used to amplify the β-tubulin region were benA1 and benA2 (15). The PCR protocol used to amplify the β-tubulin region was that of Geiser et al. (15).

Three nuclear intergenic loci were developed with the help of Fred Dietrich and Jason Stajich (Molecular Genetics and Microbiology Department, Duke University). A BLASTX search was done with the assembled contigs from the *Aspergillus fumigatus* genome project and the protein database of *Saccharomyces cerevisiae*. From this search, genes that were closer than 1,000 bp apart in *A. fumigatus* but not found together in *S. cerevisiae* were targeted for primer design. The loci used in this study are the intergenic regions between the following gene pairs (as annotated in *S. cerevisiae*): *inter1* (found between *sec61* and *ecm40* on chromosome 5), *inter2* (found between *yll034C* and *erb1* on chromosome 1), and *inter3* (found between *glc3* and *atp2* on chromosome 5). The three loci are unlinked. Primers used for *inter1* were secFa 5′ GCGGTATGGTATCCTC CTTG 3′ and ecmRb 5′ CGATTTCAGCCACGAGTACG 3′. Primers used for *inter2* were yllF 5′ CTAATCCCTTGGAGCGTGTG 3′ and erbRa 5′ CGCTT GAGCCACATAACCTG 3′. Primers used for *inter3* were glcFa 5′ GCTTCGG TCGTAATCTCAAGG 3′ and atpRa 5′ GCAGTTTCAAGGCCATCATC 3′. Amplification reactions were prepared for a 25-μl final volume containing 1.25 μl of 10 mM primer, 2.5 mg/ml bovine serum albumin (B9001S; New England Biolabs), 2.5 μl PCR buffer 10× with MgCl₂, 2.5 μl deoxynucleoside triphosphates, 0.125 μl *Taq* polymerase, 13.875 μl sterile distilled water, and 1 μl of template genomic DNA. PCR was performed on Peltier Thermal Cycler PTC-200 (MJ Research) under the following conditions: one cycle of 1 min at 95°C linked to 25 cycles of 45 s at 95°C, 40 s at 52°C, and 2 min at 72°C, followed by 15 cycles of 45 s at 95°C, 40 s at 52°C, and 2 min at 72°C, with additional extension time of 5 s per cycle. Samples were held for a final 10 min at 72°C to complete primer extensions, after which the samples were kept at 4°C until electrophoresis was performed on a 1% agarose gel prepared with Tris-acetate-EDTA and stained with SyBr Green. Following PCR, the QIAGEN clean-up kit was used.

Sequencing reactions were performed in a 10-μl final reaction volume as follows: 2 μl of Big Dye (Big Dye Terminator cycle sequencing kit, ABI PRISM; Perkin-Elmer, Applied Biosystems), 2 μl of Big Dye buffer, 1 μl of 10 mM primer, 3 μl of distilled water, and 2 μl of purified PCR product. Sequencing primers were the same as those used for PCR, and sequencing was performed on Peltier Thermal Cycler PTC-200 (MJ Research).

Inference of phylogenetic affinities and divergence. Two data sets were used to infer the evolutionary relationships between strains of the species *A. fumigatus*, *N. fischeri*, and *N. spinosa*. Sequence fragments of ITS and β-tubulin were assembled in Sequencher 3.0 (Gene Code Corporation, 1995), and sequences were aligned in MacClade 4.01 (25). Bayesian Metropolis coupled Markov chain Monte Carlo (B-MCMCMC) analysis was done with MrBayes 3.0b4 (18). Models of evolution were selected with Modeltest 3.06 (40). No topological conflicts were detected for these data when a reciprocal 95% posterior probability criterion was used (22), and therefore a Bayesian analysis of the combined data set of ITS and β-tubulin was performed. Posterior probabilities of 95% or higher were considered significant. The analysis used a random tree, four separate chains that each ran for 5 million generations, and trees sampled every 100th generation.

Likelihood scores and model parameters were evaluated to determine that the run had reached stationarity. The length of the burnin interval was 1 million generations.

The program r8s 1.7 (46) was used to estimate relative divergence times from the most recent common ancestor of the two species *A. fumigatus* and *N. fischeri* and from the most recent common ancestor of each species. This approach uses a penalized likelihood method and the truncated Newton algorithm to enable independence from the molecular clock while estimating divergence times, meaning that different branches may have different rates of evolution (45). A maximum-likelihood tree was constructed with PAUP* (52), based on *inter1* sequences for a clone-corrected 528-bp data set of 21 unique haplotypes of *A. fumigatus*, *N. fischeri*, *N. spinosa*, and *A. clavatus*. The locus *inter1* was chosen over the combined data set due to its higher genetic variation within species. The best evolutionary model for these data was estimated by MODELTEST (40) to be HKY (17). The single tree obtained was used to estimate relative divergence points. The outgroup *A. clavatus* was pruned prior to relative time point estimation. The optimal smoothing parameter was set to 1, permitting rate variation. Five random starting values were given. The variance of values obtained was evaluated with a bootstrap script written by David Hearn (personal communication), as recommended in the program manual. A total of 993 bootstrap replicates were used to estimate standard deviation values. Two standard deviations are used to indicate variance for the time points estimated.

Haplotype networks and summary statistics: global *A. fumigatus*, *N. fischeri*, and *N. spinosa* data sets. Sequences were aligned by hand with MacClade 4.01, and haplotype networks were constructed based on the detected polymorphic sites. The haplotype networks were constructed by hand based on the polymorphic sites shared and confirmed using maximum parsimony as implemented in PAUP* (52). The lengths of the three intergenic regions were 514 bp for *inter1*, 615 bp for *inter2*, and 950 bp for *inter3*, from 70 strains of *A. fumigatus* isolated from locations across the globe. The same loci were sequenced from six strains of *N. fischeri* and five strains of *N. spinosa* isolated from diverse locations (see Table S1 in the supplemental material). Bootstrap support values were estimated for the haplotype networks of the species *N. fischeri* and *N. spinosa* by using 1,000 replicates with two random-addition sequences per replicate.

DnaSP, version 3.53, software (44) was used to estimate the population genetic parameters of nucleotide diversity and effective population size (32) and to perform neutrality tests based on the frequency distributions of segregating sites (12, 13, 53). Neutrality tests based on haplotype number (K) and haplotype diversity (H) were conducted by comparing observed values of K and H to their 95% confidence intervals under strict neutrality (8). For each locus and each species, nucleotide diversity was estimated for each intergenic locus sequenced (πt). Approximate 95% confidence intervals were obtained for πt using Monte Carlo simulations based on the coalescent process, as implemented in DnaSP, version 3.53. These simulations assumed a neutral, infinite-sites model, with a large and constant population size and no recombination. All simulations were conducted by fixing the number of segregating sites to that observed in the sample. Simulating the evolution of 10,000 independent replicate populations generated the empirical distribution of the statistic. This distribution was used to determine approximate confidence intervals.

To conduct a nonparametric test for the hypothesis that the anamorphic species (*A. fumigatus*) harbors less genetic variation overall than either of the teleomorphic species (*N. fischeri* and *N. spinosa*), the Mann-Whitney U test was performed with Statview, version 5, software (SAS Institute) on the basis of nucleotide diversity estimates for each species and locus.

Analyses of population structure. The software package Arlequin V2.00 (47) was used to estimate population parameters for each of the data sets of multiple strains isolated from five sites. Population substructure was examined with F_{ST}, which does not explicitly take into account a mutational mechanism, as well as R_{ST} (50), which does. Correlation between genetic and geographic distances was examined by using a Mantel test with 10,000 permutations, also with Arlequin V2.00.

Nucleotide sequence accession numbers. All sequences determined in this study have been deposited in the GenBank database (accession numbers DQ020660 to DQ020968 and DQ378068 to DQ378094).

RESULTS

Isolates compared in a phylogenetic framework. When analyzed together, ITS and β-tubulin sequences from isolates of *A. fumigatus*, *N. fischeri*, and *N. spinosa* clustered into three monophyletic groups (Fig. 1, top) that correspond to each of
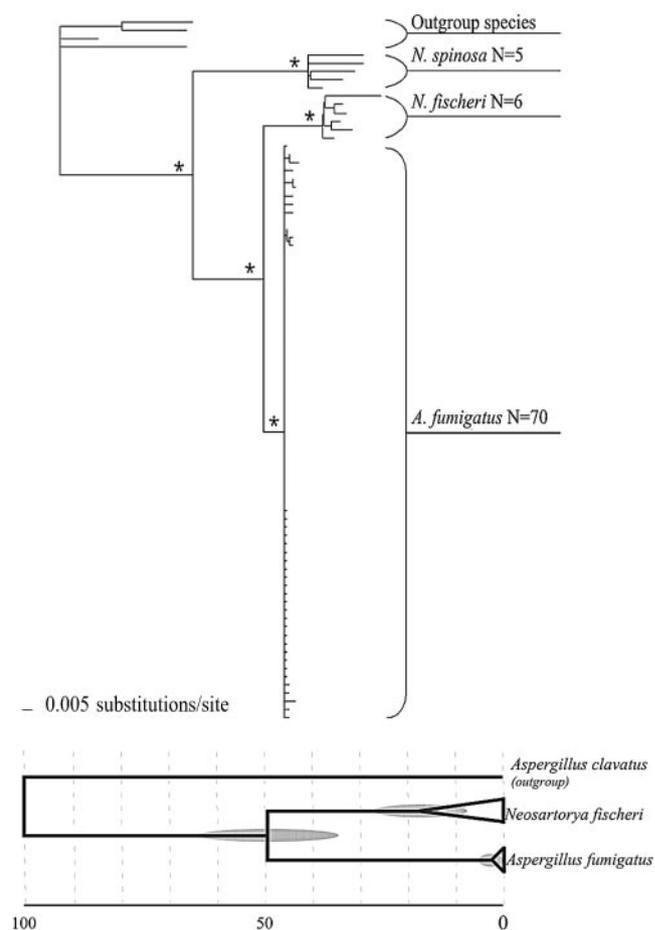

FIG. 1. Phylogenetic reconstructions for *A. fumigatus* and *Neosartorya* species. (Top) Molecular phylogeny of the species *A. fumigatus*, *N. fischeri*, and *N. spinosa*. This phylogenetic reconstruction is based on a B-MCMCMC analysis of ITS and β-tubulin with outgroup taxa from the genus *Aspergillus* subgenus *Fumigati* (*N. aureola*, *N. pseudofischeri*, *N. spathulata*, and *N. stramenia*). Branches with asterisks represent nodes supported by 95% or greater posterior probability. (Bottom) Representation of relative divergence times from the most recent common ancestor of *N. fischeri* and *A. fumigatus* and from the most recent common ancestor of isolates sampled for each of the species *N. fischeri* and *A. fumigatus*. Two standard deviations for each of the estimated divergence times are indicated by shaded ovals. The relative divergence times are plotted on a 100-unit scale with, 0 being the present day and 100 being the most recent common ancestor for *A. fumigatus*, *N. fischeri*, and the outgroup taxon *Aspergillus clavatus*.

the three species. These groups were each significantly supported, but no subdivision within any species clade was supported. Branch lengths indicate low genetic variation in the *A. fumigatus* lineage compared to *N. fischeri* and *N. spinosa*.

The relative divergence point of the most recent common ancestor for the *A. fumigatus* and *N. fischeri* isolates examined is estimated to be 49.51 ± 13.66. The relative divergence point of the most recent common ancestor of the *N. fischeri* isolates is 17.43 ± 10.12, and for the *A. fumigatus* isolates the estimated relative divergence point is 2.64 ± 3.18. There is no overlap between the estimated divergence point and variance (2 standard deviations) of *A. fumigatus* and that of *N. fischeri*.
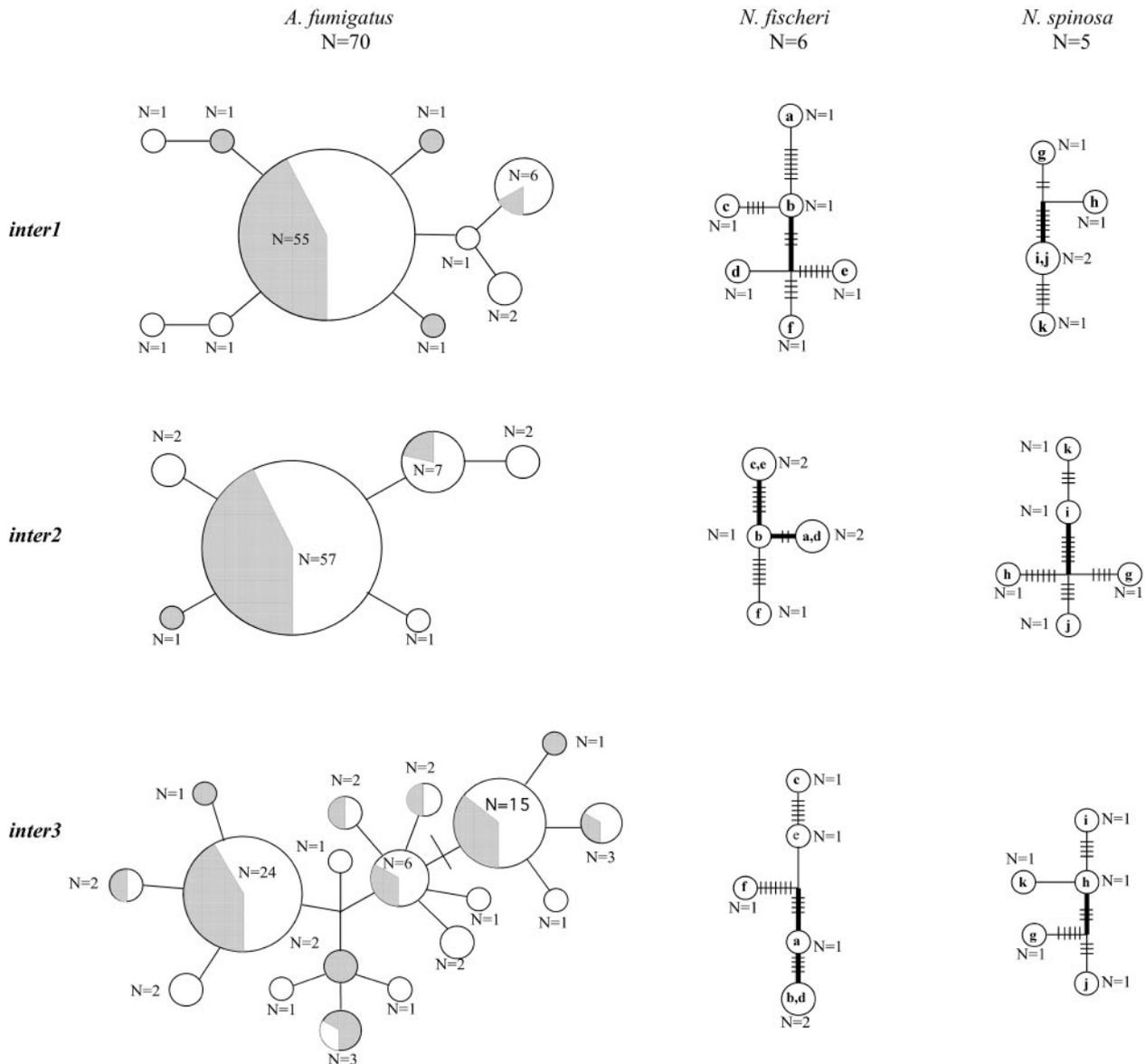
FIG. 2. Haplotype networks for *A. fumigatus*, *N. fischeri*, and *N. spinosa* isolates. Each branch represents one mutation, and each hash mark signifies one additional mutation. Circle sizes and the numbers (N) reported beside or within the circles indicate the total number of times an allele was sampled. Circles of *A. fumigatus* are shaded gray to indicate the relative proportion of individuals that are mating-type MAT1-1 and unshaded to indicate the proportion of individuals that are mating-type MAT1-2. One individual has an unknown mating type and so, for the shading of the haplotype circle it is in, the total used to determine proportions is one less than the total number of individuals. Bootstrap support values of 70% or greater for particular nodes are indicated by bolding of the internode. *N. fischeri* and *N. spinosa* isolates are labeled as follows: a, NRRL 4075; b, CBS316.89; c, CBS317.89; d, CBS525.65; e, CBS681.77; f, CBS832.88; g, CBS161.88; h, CBS448.75; i, CBS580.90; j, CBS483.65; k, CBS 586.90 (see Table S1 in the supplemental material).

Patterns of haplotype variation. In *A. fumigatus*, the intergenic regions *inter1* and *inter2* have one dominant haplotype, though multiple similar and, in some cases, shared (shared by two or more isolates) haplotypes were also recovered (Fig. 2). At *inter3*, three haplotypes predominate, though other similar, but often unique, haplotypes were recovered. From the 70 *A. fumigatus* individuals analyzed, we found a total of 33/2,079 (0.016%) sites to be polymorphic (10, 5, and 18 for *inter1*, *inter2*, and *inter3*, respectively), with only two states recovered at each polymorphic site. The number of nucleotide differences between any two sequences ranged from 1 to 6 substitutions

across the loci. The haplotype networks have consistency indices of 1 (no homoplasy) when generated with maximum parsimony as the optimization criterion.

In comparison, the three intergenic loci from *N. fischeri* isolates had proportionally more unique haplotypes. At each locus, at least four of the six strains analyzed had a novel haplotype (Fig. 2). In *N. fischeri*, 72/2,169 (0.034%) sites were polymorphic, with only two states recovered at each polymorphic site. Similarly, the three intergenic loci from *N. spinosa* had proportionally more unique haplotypes than *A. fumigatus*, with at least four of the five strains analyzed having a novel

TABLE 1. Nucleotide variation and diversity of *Aspergillus fumigatus*, *Neosartorya fischeri*, and *Neosartorya spinosa*, based on three intergenic loci

Locus	Species	No. of strains	Length (bp)	No. of haplotypes	No. of polymorphic sites	No. of shared substitutions[a]	Tajima's D[b]	πt (10^{-3})
inter1	*A. fumigatus*	70	514	10	9	5	1.03	1.16
	N. fischeri	6	523	6	30	4	−1.28	67.32
	N. spinosa	5	526	4	17	7	−0.84	74.57
inter2	*A. fumigatus*	70	615	6	5	6	1.02	0.71
	N. fischeri	6	620	4	17	11	−0.40	41.61
	N. spinosa	5	628	5	30	8	−0.75	49.08
inter3	*A. fumigatus*	70	950	18	19	12	0.9	2.37
	N. fischeri	6	977	5	25	17	−0.5	79.25
	N. spinosa	5	989	5	20	4	−0.86	66.49

[a] Total number of substitutions shared by at least two isolates.
[b] No significant departures from the frequency distribution expected under neutrality were observed.

haplotype (Fig. 2). In *N. spinosa*, 67/2,143 (0.031%) sites are polymorphic, with only two states recovered at each polymorphic site. Branches with bootstrap support values of 70 or greater were recovered for some nodes within each genealogy for *N. fischeri* and *N. spinosa*, but these relationships were not consistent across the three loci for either species.

Nucleotide diversity. The data revealed a significant relationship between predominant reproductive mode and nucleotide diversity ($P = 0.017$, Mann-Whitney U test). Estimates of nucleotide diversity were always greater in *N. fischeri* and *N. spinosa*, the two species with known teleomorphic states, than in *A. fumigatus*, the anamorphic species (Table 1). The approximate 95% confidence intervals for πt in *A. fumigatus* did not overlap with those for *N. fischeri* or *N. spinosa* in any of the three loci examined (Fig. 3).

Neutrality tests. None of the neutrality tests indicated a significant deviation from the neutral model, based on frequency distributions of segregating sites (12, 13, 53) or haplotypes (8). Estimates of Tajima's D statistic for each sample are provided in Table 1.

Genetic differentiation. Three analyses to assess genetic differentiation in the species *A. fumigatus* were done. The first used the 70 isolates of *A. fumigatus* from the global population and assigned each to one of the geographic regions: North America, South America, Europe, Africa, Australasia. A second analysis used the 33 isolates of *A. fumigatus* obtained from soil at five locations in Europe and North America. Isolates were grouped by site of origin. In the third analysis, all 103 isolates of *A. fumigatus* used in the first two analyses were grouped by mating type.

Partitioning of genetic variation within and among the 70 isolates grouped geographically was examined by using an analysis of molecular variance (AMOVA) (10), starting from a matrix of pairwise genetic distances. Using pairwise distances measured as the number of shared alleles, which corresponds to the infinite-alleles model, all pairwise F_{ST} values were smaller than 0.08 and none were significant after the permutation process. The AMOVA, pooling isolates according to geographical regions, showed that 71% of the genetic variance observed was within regions and the variance component was not significant ($P = 0.09$). Therefore, the overall F_{ST} value (0.38) was not larger than those obtained from random permutations of haplotypes between populations, indicating no genetic structure.

For the 33 strains isolated from the five sites in North America and Europe, similarity between strains was calculated as $S =$ (the number of alleles shared over all loci)/(the number of loci). Pairwise distances were then calculated as $1 − S$. Geographic distances between the sites in Finland, Germany, Nunavut, Ontario, and North Carolina were obtained from a web-based program (available at http://www.indo.com/distance/), and a 33-by-33 geographic distance matrix was constructed. As expected from the lack of geographical structure and the presence of large local differentiation, no correlation was detected between the geographic and genetic distance matrices ($r = 0.06$, $P = 0.290$; Mantel test, 10,000 permutations).

Our results found no evidence to support a mating-type-based trend of genetic differentiation for the species *A. fumigatus*. Using pairwise distances measured as the number of shared alleles, which corresponds to the infinite-alleles model, an F_{ST} of 0.21 is not significant at the 0.05 level. About 79% of the total variance is contained within a mating-type locus.
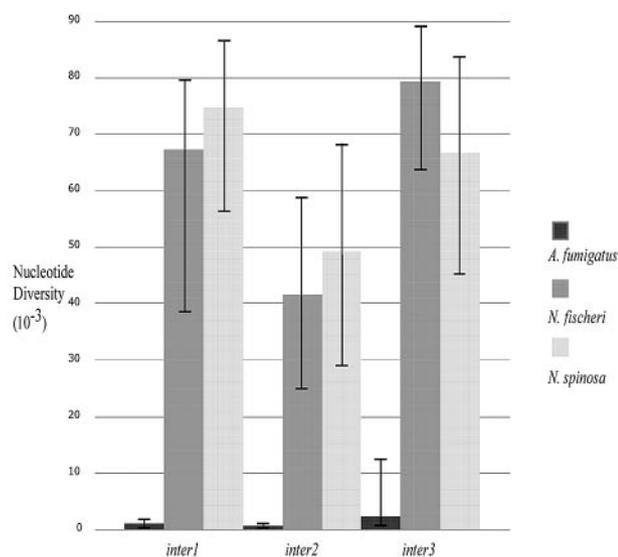

FIG. 3. Nucleotide diversity for three intergenic regions in *A. fumigatus*, *N. fischeri*, and *N. spinosa*. Thick bars represent estimates of nucleotide diversity based on pairwise sequence comparisons. Thin bars indicate ranges of error represented by the approximate 95% confidence intervals for this estimate, determined by Monte Carlo simulation of the coalescent process.

When using the sum of squared allele-sized differences, a distance measure corresponding to the stepwise mutation model, 82% of the variance is among regions, and an R_{ST} of 0.40 is not significant at the 0.05 level.

DISCUSSION

This study assessed genetic variation and genetic differentiation for the common mold *A. fumigatus* with the aim to further our understanding of its population genetics and evolutionary history. We found genetic variation to be low in the anamorphic *A. fumigatus* compared to its close teleomorphic relatives *N. fischeri* and *N. spinosa*. From our data, no geographic pattern for genetic differentiation within *A. fumigatus* was observed when isolates were associated according to broad geographical regions or by geographic distance. When *A. fumigatus* isolates were partitioned by mating type, no genetic differentiation within the mating type was found.

Genetic variation in *A. fumigatus*, *N. fischeri*, and *N. spinosa*. *A. fumigatus* is an anamorphic species, and though population genetic results consistent with historical recombination have been detected in this species, it is assumed that its predominant reproductive mode is by the asexual production of conidial spores (41, 56). Clonality is a characteristic of this species' life history that is expected to reduce variation within populations but may increase variation among populations if migration is low. In the absence of high levels of gene flow, local selective sweeps will remove variation within populations but will not remove variation between populations. We find, however, that levels of variation on a global scale appear to be very low for *A. fumigatus*. A significant difference (Fig. 3) was found between the genetic diversity of unlinked loci in the anamorphic *A. fumigatus* species and each of the teleomorphic species, *N. fischeri* and *N. spinosa*.

The haplotype networks for each of the three loci illustrate the distribution of mutations and the number of haplotypes present within each species (Fig. 2). At each of the three loci, *A. fumigatus* has fewer mutations and proportionally fewer haplotypes present than either *N. fischeri* or *N. spinosa*. Based on the modest samplings of *N. fischeri* and *N. spinosa* isolates, this suggests that these species hold greater genetic variation than *A. fumigatus*.

Bootstrap support values of 70% or higher were inferred for some branches in the haplotype networks of the species *N. fischeri* and *N. spinosa* but not for *A. fumigatus* (Fig. 2). The supported topological partitions for the two teleomorphic species were, however, discordant among loci and therefore are not considered candidates for cryptic species by a criterion proposed by Dettman et al. (9) wherein well-supported clades that are not contradicted by other loci are considered candidate cryptic species. The supported topological partitions are also not consistently supported in the majority of loci, another criterion for intraspecific genetic differentiation suggestive of a cryptic species (9).

A weakness inherent to our comparison between *A. fumigatus* and the species *N. fischeri* and *N. spinosa* is that the *Neosartorya* isolates used were all from culture collections. Multiple mycologists isolated the *Neosartorya* strains over the past century from diverse geographic locations and ecological habits. For many decades in the early 1900s, maintenance of cultures was done with slant agar media by serial transfer. After many dozens of such transfers, the strain might not be identical, genetically or morphologically, to the original strain deposited, especially if preservation conditions influenced the mutation rate or the pressures of natural selection. The cultures of *Neosartorya* we used for this study range in age over the past century, whereas almost all *A. fumigatus* isolates were obtained within the past five years. In addition, it is possible that these isolates had an unusual morphology or growth habits that led them to be candidates for deposition at a culture collection and that, along with phenotypic diversity, the isolates are biased for greater genotypic diversity. Another drawback of the *Neosartorya* data set is that both species are homothallic (self-compatible), meaning that *A. fumigatus* was being compared to sexual species that could outcross or self; the implications of this mating system would have some impact regarding their population genetics, which were not accounted for.

The estimated relative divergence point for the *A. fumigatus* isolates analyzed here is determined to be more recent, with no overlap when 2 standard deviations are applied, compared to the isolates of *N. fischeri* (Fig. 1, bottom). This result supports the interpretation that *A. fumigatus*, as it persists now, is a species with a more recent common ancestor for all extant strains than that of *N. fischeri*. Having a more recent intraspecific diversification, *A. fumigatus* may have had less time to accrue mutations within the species.

Population structure of *A. fumigatus*. A lack of genetic differentiation was observed for *A. fumigatus* in both the broad sampling of mainly environmental isolates and in the data set of multiple isolates from each of five sites in North America and Europe. AMOVA did not detect any pattern of genetic differentiation between the sites for which multiple isolates were obtained. The lack of population structure could be solely the result of rampant and effective dispersal patterns, or it could reflect (cryptic) genetic exchange of individuals sufficient to prevent population divergence.

The population genetics portrait that begins to emerge from this and previous studies is of a widespread species that exhibits little variation, either within geographic regions or on a global scale. This result agrees with the recent findings of Pringle et al. (41) regarding *A. fumigatus* and the arguments made by Raper et al. (43) that fungal species with a broad geographic distribution have little potential for genetic structure. Eukaryotic microbes such as *C. elegans* (48) and alga (49) are examples of widely distributed and free-living nonfungal species with little population structure and support the generalized hypothesis presented by Finlay et al. (11) regarding global dispersal patterns of microbes. Findings presented here contrast with examples of fungal species where a global population was hypothesized but then disproved following molecular systematic or population genetic treatment (3, 19, 21, 30, 36).

A possible explanation to account for the low levels of variation in our data and those from other studies is that *A. fumigatus* has only recently spread throughout the world. General trends for its isolation from soil find *A. fumigatus* more readily recovered from disturbed soils, correlating with anthropogenic activity, than from undisturbed sites (G. Szakacs, unpublished data). Another interpretation to explain the low

levels of variation worldwide is that *A. fumigatus* occurs in very low numbers in nature, but given its rapid growth rate observed in laboratory cultures, its ease of isolation from soil, and documentation of its ready isolation from the air column, this seems an unlikely explanation. Fertile *A. fumigatus* conidia are commonly recovered from the air column and soil of disturbed areas and may be a candidate for rampant clonal dispersal (41).

The patterns of genetic variation in *A. fumigatus* can also be considered in light of models of natural selection. If recombination is low due to low rates of outcrossing, then both selective sweeps (20, 28) and background selection (selection against deleterious alleles that takes with it neutral polymorphism) (4, 34, 35) can greatly reduce levels of genetic variation. However, two points argue against selective sweeps as the main cause of low polymorphism. First, selective sweeps that are associated with local adaptation will remove variation only over the local geographic area, increase population differentiation, and promote the presence of more widespread polymorphisms. However, *A. fumigatus* reveals low levels of polymorphism globally and no indication of population structure. Second, a recent selective sweep exerts an effect on patterns of variation at linked neutral loci very similar to that of a recent population expansion. In effect, a selective sweep may cause a severe bottleneck for linked loci. However, the frequency of shared mutations provided only scant evidence for recent population growth, as would be expected if much of the genome was linked to regions that had recently experienced selective sweeps on a global scale.

Aspergillus fumigatus occurs in areas with widely different climates and environments. The low genetic variation and lack of population genetic differentiation on a global scale observed in this species could be due to continual gene flow across continents. Its conidia are buoyant in the air column and have some protection from UV light due to their melanin pigmentation. The tolerance of these mitospores to a range of temperatures makes them well suited for survival in the air column and soil. The data presented here are also consistent with small population effects typical of bottleneck events or a recent divergence of the *A. fumigatus* lineage.

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