Clinical Isolates of *Histoplasma capsulatum* from Indianapolis, Indiana, Have a Recombining Population Structure

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A sample of 30 clinical isolates of *Histoplasma capsulatum* was analyzed to determine (i) whether genetic exchange is important in the life cycle of this fungus and (ii) whether distinct subpopulations which correlate with disease severity or host immune status exist. Eleven biallelic molecular markers were developed, with the frequency of the least common allele at each molecular locus ranging from 10 to 50%. Every isolate had a different, unique multilocus genotype. Data analysis indicated that frequent recombination occurs within the Indianapolis, Ind., population. There were no associations between isolates from the immunocompromised population or from those with different clinical manifestations of histoplasmosis.

The complete adaptation by fungi to parasitize humans, as shown by *Candida albicans* and some of the anthropophilic dermatophyte species, appears to frequently result in a loss of the ability to reproduce sexually (19, 23). Of the mycopathogenic fungi that have retained the ability to live saprophytically, the majority have instead preserved the capacity for sexual reproduction. These fungi are also able to produce large numbers of asexual spores, however, and the relative importance of sexual recombination in the fungal life cycle is not known. In addition to being of interest in population biology and evolutionary studies of pathogenic fungi and other microbes (21, 25), the occurrence of sexual recombination is an important consideration in clinical mycology. Methods developed to investigate and control disease caused by asexual strains or clones of pathogenic organisms are different from those required for pathogen populations that can freely exchange genetic information. In clonal populations virulent strains arise, and diagnostic tests aim to identify these; in sexual populations the search must be for the virulence genes.

*Histoplasma capsulatum* is an example of a true mycopathogen able to reproduce both sexually via ascospores and asexually via macro- and microconidia (13). The fungus has a worldwide distribution but is highly endemic to the Ohio Valley-Mississippi Valley region of the United States, where the majority of inhabitants tested have a skin test positive for the histoplasma antigen (20). Although most infections are asymptomatic or benign, histoplasmosis can cause acute respiratory disease and can progress to a severe, disseminated disease (8). It has become particularly important in the immunocompromised population, in which it is invariably fatal unless treated (28).

Molecular studies aimed at differentiating *H. capsulatum* isolates used restriction fragment length polymorphisms (RFLPs) in mitochondrial and nuclear DNA sequences to group isolates into six broad classes, which appeared to reflect their geographic origins (11). A further study using arbitrarily primed PCR (AP-PCR) (also known as randomly amplified polymorphic DNA [RAPD] PCR) on a collection of 29 North American (class 2) isolates indicated that considerably greater diversity than was revealed by the RFLP analysis may exist. In that study, each of the 29 isolates could be distinguished with a small number of PCR primers (12). Such diversity may be taken as evidence of sexual recombination, although it could also be due to the persistence of a large number of different clonal lineages. In another study which examined RFLPs in *H. capsulatum* isolates taken from immunocompromised patients in the St. Louis, Mo., region, it was found that these isolates did not share the expected class 2 polymorphisms. Instead these isolates were identical to the low-virulence Downs strain, which was previously the only member of the class 1 subdivision (22). This indicated that the immunocompromised population in the St. Louis region may be infected by a distinct subpopulation of low-virulence *H. capsulatum* isolates, suggesting that a large clonal reservoir of *H. capsulatum* which is not normally seen among clinical isolates may exist.

Molecular studies have thus been able to differentiate isolates and have indicated that distinct subpopulations may exist. Thus, as with many other microbial pathogens, strains of *H. capsulatum* have been “typed” by characterizing a few alleles for many individuals, but the contribution of the underlying biological processes responsible for the natural genotypes, meiotic and mitotic recombination, remains unknown. Thus, it is not certain that there are “strains” that persist in the environment; there may be only individuals. To ascertain the relative contributions of sexual and asexual recombination to the population genetics of *H. capsulatum*, i.e., to examine whether the genomes of isolates from a population of *H. capsulatum* were derived clonally or from recombination, we developed an approach for finding DNA sequence level polymorphisms by directly analyzing specific bands from AP-PCRs (7) such that alleles from every individual were recovered and identified. This was used to define multilocus genotypes for 29 clinical isolates collected at the Indiana University Medical School. Twenty-nine of these were collected between 1988 and 1993, and one was taken in 1982. Sampling strategy can influence the
result in studies such as ours; e.g., 30 samples taken from the same patient would certainly appear clonal, regardless of the prevalent reproductive mode of the fungus, as would 30 samples taken from each of 30 genetically isolated populations. We took samples from different patients and from a relatively small area so that individual organisms could have the opportunity to mate (even if they did not do so). The temporal component is not as important as the spatial one; individuals will show the relationships expected of recombining or clonal organisms whether they are collected on the same day or over several years. The isolates were from both immunocompromised and immunocompetent patients and were associated with a range of clinical manifestations.

To distinguish between clonality and recombination, the distribution of molecular markers within this population was analyzed by two different approaches. First, we used a population genetic approach based on that developed for barley by Brown et al. (3) and applied to microorganisms by Maynard Smith et al. (17). This test is based on the pairwise comparison of loci within a multilocus genotype. In a clonal population, two individuals that are identical at one locus have a greater probability of being identical at the second locus than in a recombining population, in which all loci reassort independently. Second, we adapted a phylogenetic approach developed by Archie (1) to make it suitable for population genetic analysis (5). A phylogenetic approach can be used since individuals within a clonal lineage are related in a way similar to that of separate species which have ceased to recombine and are diverging from each other over evolutionary time. This makes it possible to fit the multilocus genotypes of a clonal population to a phylogenetic tree, with forks in the tree arising as new characters arise and become fixed in the descendants. Recombining populations do not fit well to phylogenetic trees, however, as the new characters do not become fixed but are exchanged between members of the population. This forces “branch swapping,” with the result that many more steps are required to accommodate individuals in recombining populations. We report here the results of this study, which indicate that this H. capsulatum population is largely panmictic, with no evidence for correlation between a particular clonal genotype and disease severity or host immune status. This outcome is very similar to what we found for a population of another fungal pathogen, Coccidioides immitis, in Tucson, Ariz. (5).

MATERIALS AND METHODS

H. capsulatum isolates. All isolates included in the Indianapolis, Ind., population were from P. Connolly and L. J. Wheat of the Indiana University Medical School. As Indianapolis is a center of endemicity for H. capsulatum, it is assumed that the majority of isolates collected by the Indiana Medical School originated from the Indianapolis region. Isolates were chosen from patients with different immune statuses and disease severities. The designation, date of isolation, associated disease manifestations, and any underlying host conditions are shown for each isolate in Table 1. The Downes isolate, isolate G-217B (ATCC 26032), and the Indianapolis test isolates (H1 to H6) were supplied by Elizabeth Keath of St. Louis University.

Fungus culture and DNA isolation. Living isolates were manipulated and cultivated under BL2 containment. Isolates were grown for 14 to 21 days in 100 ml of potato dextrose broth with 8 mg of cysteine per ml at 25°C. Flasks were heated to 100°C for 15 min, and 1 ml of heated culture was plated onto potato dextrose agar; the flasks were then frozen. If no growth ensued following 2 weeks of incubation, cultures were considered dead and DNA was extracted under BL2 containment.

DNA extraction was done on approximately 1 g of wet fungal material according to a standard sodium dodecyl sulfate-protease K-phenol-chloroform method (4). Following sonopropanol precipitation, DNA was resuspended in 100 µl of H2O and was diluted 1:10 for PCR.

Sequencing. Sequences were aligned with those in the EMBL, GenBank, and SwissProt databases by using Blastn, Blastx, and Motif.

Development of molecular markers. (i) AP-PCR/RAPD and SSPC. AP-PCR/RAPD amplification, band isolation, and reamplification were performed as described in reference 7. Briefly, DNA from one H. capsulatum isolate was first amplified with a number of RAPD primers (from the University of British Columbia collection, Nucleic Acid-Protein Service Unit), both alone and in combination with the M13-40 sequencing primer. Amplified DNA was electrophoresed in a 2% NuSieve agarose gel, stained with ethidium bromide, and visualized under UV transillumination. Primer combinations producing bands of approximately 200 to 500 bp that were not produced by the RAPD primer alone (and were therefore assumed to have been amplified by the two primers because the M13-40 primer alone does not amplify H. capsulatum DNA) were chosen to amplify the six test isolates. If the desired band was present in all six isolates, it was excised from the amplification profile of each isolate for analysis by single-stranded conformational-polymorphism (SSCP) analysis (18). DNA in the excised gel fragment was first reamplified with the same RAPD primer plus the M13-40 primer alone (and were therefore assumed to have been amplified by the two primers because the M13-40 primer alone does not amplify H. capsulatum DNA) were chosen to amplify the six test isolates. If the desired band was present in all six isolates, it was excised from the amplification profile of each isolate for analysis by single-stranded conformational-polymorphism (SSCP) analysis (18). DNA in the excised gel fragment was first reamplified with the same RAPD primer plus the M13-40 primer alone (and were therefore assumed to have been amplified by the two primers because the M13-40 primer alone does not amplify H. capsulatum DNA) were chosen to amplify the six test isolates. If the desired band was present in all six isolates, it was excised from the amplification profile of each isolate for analysis by single-stranded conformational-polymorphism (SSCP) analysis (18). DNA in the excised gel fragment was first reamplified with the same RAPD primer plus

- **TABLE 1.** Designations, host statuses, and clinical manifestations associated with isolates present in the Indianapolis population

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Yr isolated</th>
<th>Status or underlying condition</th>
<th>Disease*</th>
</tr>
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<tbody>
<tr>
<td>H14</td>
<td>1988</td>
<td>AIDS</td>
<td>D</td>
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<tr>
<td>H19</td>
<td>1989</td>
<td>P</td>
<td>P</td>
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<td>H20</td>
<td>1993</td>
<td>Pediatric</td>
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<tr>
<td>H21</td>
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<td>P</td>
<td>P</td>
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<tr>
<td>H23</td>
<td>1993</td>
<td>AIDS</td>
<td>D</td>
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<tr>
<td>H24</td>
<td>1989</td>
<td>AIDS</td>
<td>D</td>
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<tr>
<td>H28</td>
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<td></td>
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<tr>
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<tr>
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<td>P</td>
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<tr>
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</tr>
<tr>
<td>H58</td>
<td>1988</td>
<td>AIDS</td>
<td>D</td>
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* D, disseminated; P, pulmonary.

(ii) Sequencing. Manual sequencing reactions used biotinylated M13-40 as the primer for PCR. A single-stranded template was prepared by adsorption of the PCR product to streptavidin-coated magnetic beads (Promega) followed by release of the nonbiotinylated strand with alkali (2). The released strand was sequenced using ABI model 373 automated sequencer. Sequencing primer was done in the ABI model 373 automated DNA sequencer, and results were analyzed with Genescan software. The reamplified, fluorescently labelled DNA was diluted to approximately 20 ng/ml and mixed 1:1 with 95% formamide–5 mM EDTA. Samples were heated to 95°C for 5 min and snap-cooled on ice, and 1 ml was applied to the gel along with 0.5 ml of undenatured ABI BOX-labelled Genescan size standards. Gels were run at 150 V for 20 h at room temperature. The RAPD primers that were used in this study are listed in Table 2.

(iii) Design of specific primers. Specific primer pairs were designed to amplify the sequence polymorphism directly from additional H. capsulatum isolates, and these are listed in Table 2. In some cases, it was necessary to design only a single specific primer, which, when paired with M13-40, amplified only the polymorphic band. Oligo 5.0 software was used to optimize the primer design.

(iv) Restriction analysis. Polymorphic sequences were analyzed to determine whether the sequence change responsible for the SSCP mobility shift fell within
a restriction endonuclease recognition site and could be used to generate an RFLP. If it could, the specific primer pairs were used to amplify polymorphic regions from the remaining H. capsulatum isolates, and the alleles were scored by digestion with the appropriate enzyme. With the exception of AluI, all restriction enzymes gave complete digestion of DNA in the PCR buffer, making it unnecessary to purify the DNA prior to digestion. AluI digests were done by first ethanol precipitating 20 μl of PCR product and then resuspending the pellet in 9.5 μl of 1× AluI buffer plus 0.5 μl of enzyme and incubating it at 37°C for 3 h. Polymorphism in all RFLPs was scored as the presence (numerical score of 1) or absence (numerical score of 0) of the restriction enzyme site.

The 610.1 band, which contained a 7-bp insertion/deletion polymorphism, was analyzed by electrophoretic mobility through 3% NuSieve–0.5% SeaKem, with the polymorphism recorded as an insertion (1) or deletion (0). The 3-bp deletion associated with the 648.2 polymorphism was analyzed by allele-specific amplification with primer 648.2U, which contains the three inserted bases at the 3′ end. Polymorphism was scored as the presence (numerical score of 1) or absence (numerical score of 0) of an amplification product.

### Data analysis
The multilocus genotype data were analyzed by two different methods which distinguish between clonal and recombining populations. The first method uses the traditional test for association between loci by calculating the variance of the similarities determined for all possible pairs of multilocus genotypes. Because their genomes are not subject to mating and recombination, clonal populations should show much more association between loci than should recombining populations. This method was developed by Brown et al. (3) and applied to microbes by Maynard Smith et al. (17) as an index of association (IA). The second method uses the computer program PAUP (24) to build phylogenetic trees by using parsimony from the multilocus genotypes. In this method the individual fungi are the taxa and each locus is treated as a phylogenetic character with two character states, representing the two alleles. Parsimony analysis aims to find the tree relating the taxa for which the fewest character state changes, or steps, are necessary. Because clonal populations evolve in a tree-like fashion, they are expected to support trees with fewer steps and better resolution than are recombining populations, whose evolutionary pattern is more like a net. This approach is based on a method used to estimate the phylogenetic signal present in a collection of nucleotide sequences from different organisms (1). Both methods compared the observed data set to data sets that would be expected if H. capsulatum were recombining in nature. These artificially recombined data sets were constructed by starting with the observed data set and randomizing alleles for each locus between members of the population while keeping the proportion of alleles constant. Inability of the analyses to distinguish between the observed data set and a distribution of 1,000 to 10,000 artificially recombined data sets would not allow us to reject the null hypothesis of recombination in H. capsulatum; a significant difference between the data sets would allow us to reject it and would thus support the alternative explanation of clonality.

### Nucleotide sequence accession numbers
The GenBank accession numbers for the polymorphic sequences are as follows, with polymorphisms in parentheses:
- L604 (A), U40220; L604 (T), U40067; L610.1 (with a 7-bp deletion), U39624; L610.1 (without a 7-bp deletion), U39625; L620.1 (G), U39626; L620.1 (A), U39627; L626 (G), U39628; L626 (C), U39629; L642 (C), U39630; L642 (T), U39631; L649.3 (A), U39632; L649.3 (G), U39633; L649.3 (T), U39830; L652 (C), U39831; L655.3 (C), U39832; L655.3 (T), U39833; L667.1 (C), U40068; L687.1 (G), U40089.

### RESULTS
Amplification and selection of polymorphic bands. PCR amplification with the 10-base RAPD primers in combination with primer M13-40 was performed in order to generate bands that were suitable for SSCP analysis and subsequent sequencing. Such bands were present in each of the six tester strains, were of 200 to 500 bp in length, and were clearly produced by the two primers in combination (that is, they were not seen when only the RAPD primer or the M13-40 primer was used in the amplification reaction). Initially, the six tester strains, chosen at random from our collection, were all from the St. Louis region; later, as the study focused on Indianapolis, six new tester strains were chosen from the collection of Indianapolis isolates. Loci that were polymorphic in the St. Louis isolates were fortunately also polymorphic in the Indianapolis isolates, but the St. Louis isolates were not subjected to further analysis. SSCP analysis uncovered sequence polymorphism in approxi-
imately one of every five bands isolated. Usually, mobility polymorphisms were seen as two distinct mobility types for a given band. Up to three isolates of each mobility type were then sequenced, and in all cases a single sequence polymorphism which correlated with the mobility polymorphism was found. No additional polymorphisms were seen in any of the sequenced bands, and only two alleles were seen at all of the loci used in this analysis, indicating that the loci are not hot spots for mutation and are unlikely to have undergone frequent allelic changes. This is supported by recent work on South American isolates of *H. capsulatum*, in which all loci but L626 are monomorphic (6a).

The majority of polymorphisms were single base substitutions (7 transitions and 3 transversions), and 9 of 10 of these altered a restriction endonuclease recognition site, generating a useful RFLP. Two additional insertion/deletion polymorphisms were found. An example of the type of mobility polymorphisms seen on an ABI SSCP gel is shown in Fig. 1A, and the distribution of the associated RFLPs in the Indianapolis population is shown in Fig. 1B. Additional polymorphisms in which more than two mobility polymorphisms were seen on the SSCP gel were also found. Sequencing indicated that these involved repetitive DNA. These were not included in the analysis because the mode of their evolution is not clear, and analyses of these DNA types suggest that they may be subject to frequent mutations, including reversions.

**Homology searches.** The Blastx search indicated significant homology between the translated sequences of bands L604 and L667.1 with protein sequences in the Swiss Prot database. The band L604 translation product was homologous with the endoplasmic reticulum lumen receptor 2 protein from a variety of different organisms (*P < 10^-15*). This was confirmed with Motif; the endoplasmic reticulum lumen receptor 2 motif pattern LE(SA)VAILPOL was present in the 604 sequence as LESVA ILPOL. The A-to-T base substitution occurred in the third position of an alanine codon upstream from the conserved motif block and thus did not alter the coding sequence of this gene.

The translation product from band L667.1 was homologous (*P < 10^-15*) with isoleucine-valine tRNA synthetase from numerous organisms, including nucleus-encoded mitochondrial isoleucine tRNA synthetase from *Saccharomyces cerevisiae*. The G/C polymorphism resulted in substitution of a glutamine for a glutamic acid residue within the sequence encoded by the conserved portion of the synthetase gene. Glutamine occurred more frequently at this position than glutamic acid in the homologous protein from other organisms, although this residue was frequently replaced by other amino acids, including serine in *S. cerevisiae*. The replacement of the glutamic acid residue was widespread in the Indianapolis population, with 30% of isolates containing this substitution. There was no correlation with any detectable phenotype.

**Assignment of multilocus genotypes.** Use of specific primer pairs resulted in the amplification of single polymorphic bands, which were then analyzed for the presence or absence of the appropriate RFLP or insertion/deletion. The alleles at each locus for each isolate in the Indianapolis population are shown in Table 3. There was no evidence of more than one allele at each locus in any of the isolates examined, which is consistent with haploidy in *H. capsulatum*. In most cases the two alleles at each locus were present in the population in equal or nearly equal frequencies, the only notable exception being the SpeI polymorphism at locus L652, for which only 10% of the isolates were positive for the restriction enzyme site.

In only two cases was it not possible to determine an allele: the L603 locus could not be amplified from isolate H53 (even when the original 603 RAPD primer--M13-40 primer pair was used), and isolate H24 produced a band of a size which was different from that expected when amplified with the 648U--M13-40 primer pair. These missing data were scored as “unknown” in the analysis.
Some of the RFLPs were also analyzed in the Downs isolate to determine whether it was identical to any of the isolates obtained from immunocompromised hosts. Several of the primer pairs did not amplify the appropriate band from this strain, confirming that it is considerably different from the majority of North American isolates. The multilocus genotype that was obtained with seven primer pairs that were able to amplify the correct band was not the same as any of the genotypes present in the Indianapolis population.

Analysis of the overall multilocus genotype for each isolate indicated that no two isolates had an identical genotype. Even when isolates that had been collected in a single year (e.g., the nine isolates collected in 1989) were examined, no similarity in genotype was seen. Fisher’s exact test was performed to compare allele frequencies of isolates from normal hosts with those of isolates from hosts suffering from AIDS or other forms of immunosuppression. No significant difference was seen at any of the loci, indicating that the population is not subdivided with respect to host immune status.

**Population structure.** If *H. capsulatum* has a clonal population structure, population genetic theory predicts that there should be strong correlations between alleles found at different loci (i.e., linkage disequilibrium). Maynard Smith has proposed an $I_A$ to describe this correlation (17), with “0” reflecting no linkage disequilibrium and higher values reflecting linkage disequilibrium between loci. As can be seen in Fig. 2, the $I_A$ for the observed data set (0.0428) is well within the distribution of $I_A$s for 1,000 artificially recombined data sets. Because the $I_A$ for our data cannot be distinguished from those calculated for recombined data sets, we infer that the genomes of the *H. capsulatum* population from Indianapolis are recombining in nature.

Again, if *H. capsulatum* has a clonal population structure, the multilocus genotypes determined for individuals should be accommodated on a short, well-resolved phylogenetic tree (where tree length is defined as the number of steps required to accommodate the acquisition or loss of a given allele at each polymorphic locus in this population). As seen in Fig. 3, a short, well-resolved tree could not be fitted to the Indianapolis population. No single most parsimonious tree was defined, and instead Fig. 3 is a consensus of the 2,225 most parsimonious trees. The tree is not well resolved, with only a few internal branches and a large polytomy of 19 isolates. There are 45 steps in the tree of the observed data set, whereas the shortest possible tree, i.e., one without reversals or convergences (homoplasy in the lexicon of cladistics [10]), is 11 steps. The length

![Fig. 2. Position of the $I_A$ value for the observed data set within $I_A$ values for 1,000 artificially recombined data sets. The recombined data sets were produced by randomizing alleles at each locus among members of the Indianapolis population.](image-url)
of the tree determined for the observed data was also compared with the distribution of tree lengths for trees fitted to 1,000 artificially recombined data sets (Fig. 4). Again, the observed length (45 steps) was much longer than the minimum length (11 steps) but was at the low end of the distribution of lengths for artificially recombined data sets ($P = 0.05$). These features are consistent with recombination in *H. capsulatum* but with some deviation from complete panmixia.

Isolates that were taken from patients with AIDS or other underlying immunosuppressive illnesses are indicated on the consensus tree determined from parsimony analysis (Fig. 3). There are no obvious relationships among any of these isolates.

**DISCUSSION**

AP-PCR (29), or RAPD PCR (27), can be used as a means of generating homologous DNA fragments from different isolates of a given organism. SSCP analysis (18) and direct sequencing of these bands allow robust markers to be generated on the basis of sequence polymorphisms (6, 7). These techniques utilize the advantages of AP-PCR/RAPD amplification, whereby only minute quantities of even partially degraded DNA material may be used, but they avoid the problems associated with reproducibility and interpretation of arbitrarily amplified band profiles (9, 26). Also, as all alleles are observed and defined as single, polymorphic nucleotide positions or small insertions or deletions, there is little ambiguity about the nature of the mutations responsible for the different alleles. Furthermore, amplified bands in a given AP-PCR/RAPD profile may not be independent, and a loss or gain of one band may influence the loss or gain of other bands. In this study each locus was the result of amplification by a different primer pair; thus, independence of each marker may be assumed. Independence of markers was further confirmed by the test of association between loci, which failed to detect physical linkage between any of the different loci.

Sequence analysis of bands showing mobility polymorphisms frequently uncovered useful RFLPs which could then be rapidly scored in amplified bands from additional isolates. Homology searches of the sequences with the GenBank, EMBL, and Swiss Prot data banks also uncovered two structural genes among the arbitrarily primed DNA fragments. Thus, in a method analogous to shotgun cloning, AP-PCR/RAPD amplification may be used to obtain DNA in which gene sequences can be found. Similar results have been reported by Lorenzet al. (16), who found two open reading frames among five sequenced randomly amplified bands from *Prochlorococcus marinus*.

Nine RFLPs and two insertion/deletion polymorphisms were used to generate multilocus genotype profiles for 30 clinical isolates from Indianapolis. A comparison of the distribution of
alleles in the observed data set with those of 10,000 randomly recombined data sets indicated no significant difference when the population biology approach of association of loci, or the I_{2} (17), was used. By using a phylogenetic approach, the population was shown to be far from completely clonal, but there was a small but statistically significant difference from complete panmixia. This conflict illustrates the importance of analyzing data by as many means as possible. A similar difference between the two approaches was seen in our work on C. immitis and may indicate that the phylogenetic approach is more sensitive to deviations from panmixia. The results of the phylogenetic analysis suggest that the Indianapolis population might contain subpopulations among which genetic exchange has been less frequent. An example of this was noted by Maynard Smith et al., who found epidemic clones within a panmictic population of Neisseria meningitidis (17). Further analysis with a larger number of isolates will be necessary to confirm this hypothesis.

H. capsulatum exists as two compatibility (or mating) types, + and −, which mate to form loose ascocarps containing ascospores (13). The occurrence of sexual recombination in the population is therefore not unexpected and may, in fact, be necessary for the perpetuation of the fungus during adverse conditions, as sexual spores are generally more resistant than asexual spores and hyphae. Clearly, asexual reproduction must also be important, as both compatibility types may also sporulate to release numerous asexual micro- and macroconidia. In our previous analysis of C. immitis it was found that even moderate levels of sexual recombination could result in a panmictic population structure (5).

The mating type of the isolates used in this study is not known; however, a previous study of the frequency of each compatibility type in areas of the United States where H. capsulatum is endemic indicated that among clinical isolates the − type predominated by a factor of 7:1 (15). However, skewed ratios were not observed in environmental isolates, which contained 50% of each mating type. This is further evidence that sexual recombination, which results in equal numbers of each mating type, is important in nature. The unequal distribution of mating types in clinical isolates may indicate that the − type is associated with increased virulence, either directly through the properties of the − mating type locus or through another closely linked gene which segregates with the − mating type. A similar association between mating type and virulence was recently established for another human mycopathogen, Cryptococcus neoformans var. neoformans (14). That the H. capsulatum mating type ratios are not correspondingly skewed in environmental isolates may indicate that the parasitic cycle is of relatively limited importance in maintaining H. capsulatum populations. As the fungus is not shed from the host and cannot be transmitted horizontally from animal to animal, it can return to the environment only when histoplasmosis results in the death of the host. Human infection may thus have little influence on the population dynamics of this organism.

A high level of panmixia within the Indianapolis population suggests that infection is not due to the occurrence of genetically distinct virulent strains or clones but may instead be due to virulence-associated genes which can be exchanged between members of the population. The fact that even isolates collected in a single year were no more closely related to each other than to the rest of the collection indicates that new clones do not arise and spread over short periods and only later recombine with the resident population. This is in contrast to findings for parasitic protozoa (25) and the obligate yeast pathogen C. albicans (19), which have been found to be clonal, but it is similar to our results for C. immitis, another dimorphic fungal pathogen with a saprobic as well as a parasitic phase in its life cycle (5). In addition, the lack of homology between isolates associated with immunosuppressive illnesses does not point to the presence of a large pool of low-virulence strains, as has been postulated to exist in St. Louis (22). Instead, these results suggest that until markers which segregate with certain clinical manifestations can be found, all isolates of H. capsulatum must be considered potentially capable of causing human disease.

Populations of the true human mycopathogens have remained poorly characterized, mostly as a result of the difficulties associated with working with large numbers of hazardous microorganisms. The development of simple, reliable PCR-based techniques like those used in this study, combined with the growing importance of fungal diseases as causes of human morbidity and mortality, provides impetus for further analyses of these organisms at the population level. We intend to extend our analysis to clinical and environmental samples of H. capsulatum from different geographic regions in order to further clarify the population structure and substructure of this important mycopathogen.

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