Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12473–12477, October 1996
Medical Sciences

Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination

(genetic variation/linkage disequilibrium)

YVONNE GRÄSER*, MIGUEL VOLOVSEK†, JENNIFER ARRINGTON†, GABRIELE SCHÖNIAN*, WOLFGANG PRESBER*, THOMAS G. MITCHELL‡, AND RYTAS VILGALYS†

*Department of Microbiology and Hygiene (Charite), Humboldt University, Dorotheenstrasse 96, 10098 Berlin, Germany; †Department of Botany, Duke University, Durham, NC 27708; and ‡Department of Microbiology, Duke University Medical Center, Box 3803, Durham, NC 27710

Communicated by Bruce Wallace, Virginia Polytechnic Institute and State University, Blacksburg, VA, July 30, 1996 (received for review November 1994)

ABSTRACT **The life history of *Candida albicans* presents an enigma: this species is thought to be exclusively asexual, yet strains show extensive phenotypic variation. To address the population genetics of *C. albicans*, we developed a genetic typing method for codominant single-locus markers by screening randomly amplified DNA for single-strand conformation polymorphisms. DNA fragments amplified by arbitrary primers were initially screened for single-strand conformation polymorphisms and later sequenced using locus-specific primers. A total of 12 single base mutations and insertions were detected from six out of eight PCR fragments. Patterns of sequence-level polymorphism observed for individual strains detected considerable heterozygosity at the DNA sequence level, supporting the view that most *C. albicans* strains are diploid. Population genetic analyses of 52 natural isolates from Duke University Medical Center provide evidence for both clonality and recombination in *C. albicans*. Evidence for clonality is supported by the presence of several overrepresented genotypes, as well as by deviation of genotypic frequencies from random (Hardy–Weinberg) expectations. However, tests for nonrandom association of alleles across loci reveal less evidence for linkage disequilibrium than expected for strictly clonal populations. Although *C. albicans* populations are primarily clonal, evidence for recombination suggests that sexual reproduction or some other form of genetic exchange occurs in this species.**

The pathogenic yeast *Candida albicans* causes mild, superficial infections in hosts with normal immune defense, but it can cause persistent and mucocutaneous, as well as life-threatening, systemic diseases in immunocompromised patients. With the increased use of immunosuppressive therapy, opportunistic infections by *C. albicans* have become more frequent. Several characteristics of *C. albicans* have always presented problems for researchers working on its genetics and epidemiology. Although a sexual stage has never been discovered, controversies still exist concerning the role of recombination in the *C. albicans* life cycle (1, 2). The clonal nature of *C. albicans* is even more surprising when one considers the limited genetic investigations that show this species to be diploid (3).

Fungi are famous for exhibiting complex life histories. Since most fungi reproduce both sexually and asexually, evidence for both recombination and clonality may exist even within a single population. Recent studies of fungi known to possess combined clonal and recombining population structure include *Mycosphaerella graminicola* (4), *Cryphonectria parasitica* (5), and *Sclerotinia sclerotiorum* (6). Although sexual stages are known for these groups, the absence of known sexuality in many fungal species including *C. albicans* suggests that recombination may not occur in some groups. However, recent study of genetic variation in another medically important species lacking a known sexual stage, *Coccidioides immitis*, suggests that some mechanism for genetic recombination is present in natural populations (7).

In recent years, molecular methods have demonstrated considerable variation at the DNA level in *C. albicans* (8–12). Methods such as DNA fingerprinting and random amplified polymorphic DNA (RAPD) show much promise for discriminating species and strains in *Candida* (8, 13–16). For epidemiological and population studies, genetic markers also need to address the diploid genetics of *C. albicans*. For this purpose, codominant markers are necessary to allow unambiguous assignment of composite diploid genotypes to individual strains. In this paper we describe a strategy for developing codominant markers at the DNA sequence level based on single-strand conformation polymorphism (*sscp*; ref. 17). We also describe how these markers may be used to assess levels of genetic variation, heterozygosity. and recombination in *C. albicans*.

MATERIALS AND METHODS

Strains. Fifty-two *C. albicans* strains representing a "natural" population sample were isolated, one per person, from nonsymptomatic patients at Duke University Medical Center. Isolates were grown and maintained on yeast peptone dextrose agar. Genomic DNA was isolated from each strain using methods described previously for *C. albicans* (15) and stored at −20°C until use.

Development of Molecular Markers. To provide a source of genetic markers that could be easily sequenced, RAPD markers (18, 19) were screened using two arbitrary primers in each reaction (instead of a single primer) as described by Burt *et al.* (20). Ten-base oligonucleotide primers (primer kits C and F) were obtained from Operon Technologies (Alameda, CA). Amplified fragments of 200–400 bp that were amplified from all strains were excised from low melting agarose (1% SeaPlaque agarose; FMC) and purified using Magic PCR Prep DNA Purification kits (Promega) before sequencing. DNA fragments were sequenced using the same arbitrary primers separately by cycle-sequencing kits (GIBCO) with [γ-^{33}P]ATP (NEN or Amersham) as label. New locus-specific PCR primers of 19–21 nucleotides were designed and synthesized based on these sequences.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: *sscp*, single-strand conformation polymorphisms; RAPD, randomly amplified polymorphic DNA.

Each pair of locus-specific primers was tested for its ability to amplify the primers' corresponding target loci. Conventional PCRs were performed in a total volume of 25 μl, containing 10 mM Tris·HCl (pH 8.3), 2 mM $MgCl_2$, 0.001% gelatin, 100 μM each of dATP, dCTP, dGTP, and dTTP, 7.5 nM of each primer (see Table 1), 10 ng of genomic DNA template, and 0.1 units of Amplitag DNA polymerase (Perkin–Elmer Cetus). PCR was carried out for 35 cycles (1 min at 93°C, 30 sec at 51°C, and 1 min at 72°C), with an initial denaturation step (4 min at 93°C) and a final extension step (5 min at 72°C).

***sscp* Analysis.** A mixture containing 7–10 μl of PCR product (≈40–50 ng of DNA), 0.8–1.5 μl of 1% SDS, 10 mM EDTA (pH 8), and 1–2 μl of stop solution (formamide with bromophenol blue) was heated to 98°C for 10 min and then placed on ice. The samples were run on standard sequencing gels (0.5× TBE buffer and 6% MDE Hydrolink acrylamide; J.T. Baker) under nondenaturating conditions at 230 V for 60–70 hr (at room temperature, 23°C). Gels were stained in ethidium bromide (0.5 mg/ml) for 20–30 min, destained, and photographed under UV light. DNA fragments showing *sscp* were further characterized by sequencing each strain as described above.

RESULTS

Detection of *sscp* and Sequence-Level Variation. From 14 locus-specific PCR primer pairs that were tested, eight primer pairs were successfully able to amplify a single DNA fragment from each *C. albicans* strain. *sscp* were detected in six of these eight fragments (Fig. 1 and Table 1). Sequence analysis of the PCR fragments from every strain confirmed that *sscp* detected all mutations, which consisted of from one to several single nucleotide substitutions or small insertion/deletions (Table 1). Overall sequence diversity in these eight fragments was very low: from a total of 2266 bases, eleven point mutations and one insertion/deletion were detected, for a total rate of variation that was <0.5%.

Diploidy and Heterozygosity in *C. albicans*. Genotypes could be assigned to each strain based on sequence analysis, as described in Fig. 1. Each nucleotide locus was characterized by simple point mutations or by the presence or absence of an insertion/deletion. Strains possessing a single allelic state were assumed to be homozygous, while strains possessing both allelic states were considered to be heterozygous (Table 2). Heterozygosity was evident for 9 of the 12 loci and is consistent with the assumption that most strains of *C. albicans* are diploid.

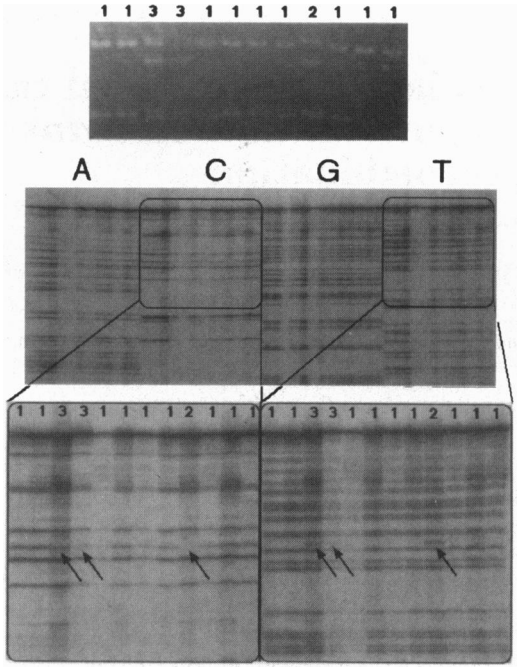

FIG. 1. Detection of *sscp* and their confirmation by DNA sequence analysis. (*Upper*) Three different *sscp* (lanes labeled 1, 2, and 3) observed for 12 strains within a 287-bp fragment (locus C12F10 287–1). Sequence analysis (*Lower*) was performed by grouping the same 12 strains by their four sequencing reactions (A, C, G, and T) to reveal positions at which mutations were present. Arrows in the expanded panel below show that differences between patterns 1 and 2 are due to a single base pair change (C versus T), while pattern 3 represents an individual heterozygous for both alleles (C/T).

Population Structure. From 46 strains that were assigned genotypes at all 12 loci, a total of 27 unique multilocus genotypes were detected. Four genotypes were each represented by more than one individual, with the most common genotype present in 14 individuals, the second most common

Table 1. Polymorphic sequence markers for genetic analysis of *C. albicans*

Locus	Polymorphisms	Length, bp	Primers	EMBL no.
C12F10 287-1, C12F10 287-2	246 (C, T, C/T) 218 (G, T, G/T)	287	5′-ACGTAATAAGGGTATTGTTG, 5′-GCAATTTGTCACTCATCCAG	Y07664
C2F17 290-1, C2F17 290-2, C2F17 290-3	205 (G, A, G/A) 214 (G, A) 87 (C, T)	290	5′-ACTAATCTATCGAGAGAACG, 5′-GTCAGATGGTACGGACAAG	Y07665
C2F10 284-1	151 (G, A, G/A)	284	5′-TTGCTACTACAAATAGTCG, 5′-GCTTAACATTTACCTGCTTC	Y07666
C13F10 340-1	50-55 (indel) (A-ATT-C, AGATTTTC)	340	5′-TGCTATCTTCGTACCGTATC, 5′-ATCTCGTCCTCTACATCATC	Y07667
C15F2 332-1, C15F2 332-2, C15F2 332-3	168 (C, C/G) 174 (G, A, G/A) 207 (G, A, G/A)	332	5′-TAGTTAGTTTGCCTTGTTCC, 5′-GAGAGCTACGTGAGCTCGTG	Y07668
C2F7 282-1, C2F7 282-2	95 (T, C, T/C) 119 (A, G, A/G)	282	5′-GTTTGATCTGGAACGATCTC, 5′-AGAAACCAACCAGCGTCTTC	Y07669
C15F2 250-1	Not detected	250	5′-ATGCTAAGGAAGTTCTTTGAC, 5′-GATGACGCAACGATGATGTC	Y07670
C2F20 213-1	Not detected	213	5′-CACTACCAAACCAAGGTGG, 5′-CATAGACCAAAGCATTGTAC	Y07671

Locus names consist of both primer designations (e.g., C12 and F10) followed by the length (in base pairs) of the resulting PCR fragment (e.g., 287) and an arbitrary number (1, 2 for fragments containing more than one polymorphic site). The location of each polymorphism is indicated (in bp) from the 5′ end of the forward primer, followed by the actual mutations that were observed. Total length of each fragment and sequences for both forward and reverse primers are given.

genotype in five individuals, and two genotypes each represented by two individuals.

Random association of alleles within loci was examined by testing genotypic frequencies for goodness of fit with Hardy–Weinberg expectations (21). Significant deviation from random expectation was observed at 8 of 12 loci, with heterozygotes being notably rare in each case (Table 2). However, genotypic frequencies at the four remaining loci (C12F10 287-1; C15F2 332-1; C15F2 332-2; and C15F2 332-3) were not observed to differ from Hardy–Weinberg expectation, suggesting recombination within these loci.

In random-mating populations, associations between marker loci are expected to exist in gametic equilibrium. Because *C. albicans* is diploid, estimation of gametic disequilibrium is complicated, since gametic frequencies cannot be directly observed. Associations between loci were assessed using two approaches. Composite digenic disequilibrium values were estimated and their significance was tested using χ^2 statistics as described by Weir (21). An exact test for association between alleles across loci based on permutation (22) was also employed. Applying both the composite disequilibrium test and the exact test to all 66 pairwise comparisons, only 13 and 18 significant associations were respectively detected (Table 3). This number of significant associations is greater than what would be expected for a random mating population (for 66 multiple comparisons, only 3–4 significant associations would be expected due to chance) and suggests that the overall population structure is clonal. However, because the majority of pairwise comparisons (73–80%) are not significant, many more loci appear to be recombining than would be expected for a strictly clonal population.

Knowledge of linkage relationships can also provide valuable information for understanding associations between alleles. Linkage grouping of the six polymorphic PCR fragments used in the study was determined using hybridization against blots of electrophoretically separated chromosomes (unpublished data). Two PCR fragments (C12F10 287 and C2F10 284, indicated by a box in Table 3) hybridize to a single large chromosome (either chromosome 1 or R according to ref. 23); all of the other PCR fragments hybridize to unlinked chromosomes. Eight of the 12 loci occur on four of the unlinked PCR fragments (indicated in boldface in Table 3).

Patterns of association between loci known to be physically linked (either on the same PCR fragment or chromosome) provide further evidence for recombination in this population. For example, although significant association was detected between loci C15F2 332-2 and C15F2 332-3, neither of these loci was significantly associated with locus C15F2 332-1, even though all three loci are located on the same PCR fragment. Similarly, significant association was detected between loci C2F17 290-1 and C2F17 290-3, while the physically proximal locus C2F17 290-2 was not significantly associated with either.

Direct examination of genotypic frequencies between several loci also indicates that interlocus recombination occurs. Tables 4 and 5 show counts for all 9 possible 2-locus genotypes encountered in the population for two pairs of loci (C15F2 332-3 versus C2F7 282-2, and C2F10 284-1 versus C15F2 332-2). Although a single genotypic class is prevalent in each comparison (representing 27 of 46 and 28 of 51 individuals, respectively), all possible recombinant classes are represented, confirming that the population is recombining to some degree.

Table 2. Genotypic frequencies and test for random segregation in a population sample from *C. albicans*

Locus	Genotype frequencies	*n*	*P*
C12F10 287-1	CC (44); CT (6); TT (1)	51	0.25
C12F10 287-2	GG (46); GT (2); TT (3)	51	0.00*
C2F17 290-1	GG (21); GA (1); AA (30)	52	0.00*
C2F17 290-2	GG (51); GA (0); AA (1)	52	0.01*
C2F17 290-3	CC (11); CT (0); TT (41)	52	0.00*
C2F10 284-1	GG (33); GA (6); AA (13)	52	0.00*
C13F10 340-1	AA (51); AB (0); BB (1)	52	0.01*
C15F2 332-1	CC (46); CG (5); GG (0)	51	1.00
C15F2 332-2	GG (9); GA (32); AA (10)	51	0.10
C15F2 332-3	GG (15); GA (30); AA (6)	51	0.10
C2F7 282-1	TT (39); TC (1); CC (6)	46	0.00*
C2F7 282-2	AA (37); AG (6); GG (3)	46	0.01*

Nomenclature for loci and polymorphisms is given in Table 1. Genotypic counts (in parentheses) show specific polymorphic bases at each locus (A, C, G, T), except for locus C13F10 340-1, in which presence/absence of an indel is shown (A, B). Sample sizes (*n*) and significance values (*P*) are those used to test for deviation from Hardy–Weinberg expectations.
*$P < 0.05$.

DISCUSSION

PCR-Based Markers in *C. albicans*. *Candida albicans* is known to harbor considerable natural genetic variation (9). This variation has been observed at a variety of levels ranging from chromosomal length polymorphism (24, 25), isoenzymes (2, 26, 27), restriction fragment length polymorphisms (RFLPs; refs. 11 and 28), DNA fingerprinting (10, 29), PCR fingerprinting (30), and RAPD (8). Although all of these approaches are useful for genetic analysis in *Candida,* the results are not always directly comparable for different methods and even among laboratories using similar techniques. DNA polymorphisms generated by methods such as DNA fingerprinting or RAPD are usually dominant, and null alleles are not detectable in heterozygous individuals (18), which make them less desirable for population studies (31, 32). To avoid problems of dominance, our strategy was to develop codominant locus-specific DNA markers, which could be readily amplified from diverse sources, and which could provide information about frequencies of alleles and genotypes in populations at the nucleotide sequence level. These data make it possible to study the life history and epidemiology of *C. albicans* using the powerful framework of population genetics.

Two criteria guided our initial selection of single-copy nuclear DNA markers. First, markers should represent a random sample of neutral genetic variation present in natural populations. Anonymous DNA sequences generated using RAPD seem to meet this criterion. In addition, hybridization against electrophoretically separated chromosomes also provided information about linkage groups among marker loci. Knowledge of linkage relationships for different markers can strengthen conclusions about the extent of recombination. Secondly, the markers should be easily amplified using conventional PCR techniques. The use of RAPD employing pairs of arbitrary primers facilitates screening, since amplified fragments can be sequenced directly without the need for cloning (20). Of 14 primer pairs initially tested, eight produced PCR fragments of the intended size, and six of these were found to be polymorphic. This success rate (6/14) is comparable with the rates reported for PCR-based markers from other organisms by Karl and Avise (33). In that study, 7 of 15 primer pairs tested were successfully able to amplify polymorphic DNA sequences from green turtles, and 6 of 11 pairs were able to amplify in American oyster populations.

Sequence analysis confirmed the molecular basis for all polymorphisms (Table 1 and Fig. 1). A total of 12 sequence-level polymorphisms were identified in the population sample. Interpretation of sequencing gels was relatively straightforward, enabling each strain to be assigned a multilocus diploid genotype at all 12 loci. Although our results do not preclude the possibility that some strains might be aneuploid or even polyploid, interpretation of the sequence data from this study is most consistent with strains being diploid. These results agree with other studies (2, 27) that demonstrate typical

Table 3. Tests for gametic disequilibrium among pairs of individual nucleotide loci in a population of *C. albicans*

Locus	C2F10 284-1	C2F17 290-1	C2F17 290-2	C2F17 290-3	C12F10 287-1	C12F10 287-2	C13F10 340-1	C15F2 332-1	C15F2 332-3	C15F2 332-2	C2F7 282-1	C2F7 282-2
C2F7 282-2	0.106*	0.009	0.006	0.036	0.009	0.040*	0.006	0.021	0.018	0.014	**0.159**†	
C2F7 282-1	0.144‡	0.021	0.006	0.012	0.021	0.050*	0.006	0.009	0.061	0.068		**0.00***
C15F2 332-2	0.043	0.008	0.020	0.044	0.001	0.001	0.020	**0.001**	**0.126**†		0.01*	0.01*
C15F2 332-3	0.092*	0.007	0.023	0.001	0.006	0.025	0.023	**0.001**		**0.00***	0.02*	0.02*
C15F2 332-1	0.028	0.059‡	0.002	0.038*	0.012	0.008	0.002		**0.21**	**0.41**	0.43	0.14
C13F10 340-1	0.027	0.016	0.001	0.008	0.003	0.0031		0.99	0.13	0.18	0.99	0.99
C12F10 287-2	0.049	0.004	0.003	0.005	**0.012**		0.99	0.99	0.01*	0.00*	0.05*	0.06
C12F10 287-1	0.029	0.074‡	0.003	0.064‡		**0.99**	0.99	0.20	0.99	0.99	0.99	0.72
C2F17 290-3	0.199*	**0.210**†	0.008		0.00*	0.73	0.99	0.06	0.99	0.49	0.99	0.21
C2F17 290-2	0.012	**0.016**		**0.99**	0.14	0.10	0.99	0.99	0.12	0.19	0.99	0.99
C2F17 290-1	0.073*		**0.99**	**0.00***	0.05*	0.99	0.99	0.01*	0.49	0.78	0.99	0.86
C2F10 284-1		0.66	0.99	0.99	0.05*	0.07	0.36	0.19	0.00*	0.00*	0.01*	0.00*

Numbers above the diagonal are measures of composite digenic disequilibrium (D[AB]) together with significance level. Numbers below diagonal are *P* values for the exact test of association between alleles conditional on allelic counts. Values in boldface indicate nucleotide loci that are located on the same PCR fragment. Boxes enclose groups of loci belonging to the same linkage group (based on hybridization to electrophoretically separated chromosomes).
*$P < 0.05$; †$P < 0.001$; and ‡$P < 0.01$.

diploid heterozygous patterns for enzyme markers in *C. albicans*.

Clonal Versus Recombining Population Structure in *C. albicans*. Much controversy exists about the extent to which recombination can occur in predominantly asexual fungi and other microorganisms (1, 7, 34). Because a sexual stage is not known in *C.albicans*, its population structure is expected to be primarily clonal. Tibayrenc *et al.* (1) outlined several criteria for assessing clonal population structure in microorganisms such as *C. albicans*. These criteria include: (*i*) presence of overrepresented genotypes due to clonal propagation; (*ii*) lack of evidence for segregation at individual loci (e.g., deviation of genotypic frequencies from Hardy–Weinberg expectations); and (*iii*) absence of recombination between loci (linkage disequilibrium).

Using the above criteria, Pujol *et al.* (2) used isozyme evidence from 13 polymorphic loci to analyze the population structure of 55 *C. albicans* strains from AIDS patients in Montpellier, France. From the 41 genotypic classes that were detected in their study, one class was represented by 13 strains and another by 3 strains. Analysis of genotypic frequencies for individual loci found significant deviations from Hardy–Weinberg expectations for 6 of the 13 loci. Finally, significant patterns of linkage disequilibrium were detected among genotypes. Because all three of the above criteria were met, they concluded that the Montpellier population structure is primarily clonal.

In our study, population genetic analyses of PCR-based genetic markers also support a predominantly clonal population structure for *C. albicans*. Twenty-seven unique genotypic classes were detected from our sample. Of these, one class was represented by 14 strains, another by 5 strains, and two classes by 2 strains each. Thus, some genotypic classes appear to be overrepresented in our sample, meeting the first criterion above. Strains belonging to a common genotypic class might be expected to be clonally derived and thus genetically similar to each other. Additional support for this conclusion is available using other genotyping methods, such as RAPD or PCR fingerprinting (13, 14, 30). Data from PCR fingerprints using the M13 primer to randomly amplify polymorphic fragments indicates that at least some of the strains belonging to common genotypic classes from this study also share similar DNA fingerprints (unpublished data).

Analysis of segregation indicates that most of the loci we examined deviate significantly from random expectations (Table 2). Much of the deviation is attributable to underrepresentation of the heterozygous class at each locus. This result is consistent with the second criterion for clonality described above, suggesting limited segregation in clonal populations. However, other explanations are also available to explain the apparent low levels of heterozygosity in our sample. Even in outcrossing populations, inbreeding can produce lower levels of heterozygosity through selfing or population subdivision. In *C. albicans* and other yeasts, mitotic gene conversion could also be partly responsible for elimination of heterozygosity in otherwise randomly segregating populations. Although mitotic recombination is well documented for laboratory strains of *C. albicans* (3), its prevalence and importance for genetics of natural populations is not known.

Absence of recombination between loci constitutes the strongest evidence for clonality in populations. Under a strictly clonal mode of reproduction, recombination should not occur, or at least it will be greatly reduced. Our data show little evidence for linkage disequilibrium, as most pairwise associations among loci are not significant (Table 3). Detection of all possible recombinant genotypes between several loci (Tables 4 and 5) also supports the view that recombination occurs in natural populations. The most surprising outcome of this study and the previous one (2) is not that *C. albicans* is clonal, which was certainly expected, but that any recombination at all might be detectable in a species for which no known meiotic mechanism is known to exist. This is similar to the situation in some bacteria such as *Escherichia coli*, which is known to have a primarily clonal population structure yet still shows evidence

Table 4. Combined genotypic counts for paired loci C15F2 332-3 and C2F7 282-2 showing evidence for recombination

C2F7 282-2 genotype	C15F2 332-3 genotype		
	GG	GA	AA
GG	4	27	6
GA	1	1	4
AA	1	1	1

Nomenclature of loci is the same as in Table 1. Numbers show counts for all possible recombinant genotypic classes.

Table 5. Combined genotypic counts for paired loci C2F10 284-1 and C15F2 332-2 showing evidence for recombination

C15F2 332-2 genotype	C2F10 284-1 genotype		
	GG	GA	AA
GG	3	2	4
GA	28	1	3
AA	1	3	6

Nomenclature of loci is the same as in Table 1. Numbers show counts for all possible recombinant genotypic classes.

for limited recombination (35). Different levels of clonality versus recombination may occur within some species due to variation in geographic sampling (36) or due to seasonal changes in the mode of reproduction for species which have both asexual and sexual spores (37). In their study, Pujol *et al.* (2) indicated the possibility of limited interlocus recombination in *C. albicans*. Based on their results and those presented here, further searches for a possible meiotic stage or other recombinatory apparatus might be warranted in this important human pathogen.

We would like to thank John Perfect for collecting and providing the strains used in this study; Bruce Weir, Dimitri Layvin, and Paul Lewis for assistance with statistical analysis; and Anja Forche for assistance with electrophoretic separation of chromosomes. This work was supported by Grant AI 28836 from the National Institutes of Health, DEB 91-07812 from the National Science Foundation, and Scho 448/3-1 from the Deutsche Forschungsgemeinschaft.

1. Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Breniere, S. F., Darde, M. L. & Ayala, F. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5129–5133.
2. Pujol, C., Reynes, J., Renaud, F., Raymond, M., Tibayrenc, M., Ayala, F. J., Janbon, F., Mallié, M. & Bastide, J.-M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9456–9459.
3. Barton, R. C. & Gull, K. (1992) *Mol. Microbiol.* **6,** 171–177.
4. Chen, R.-S. & McDonald, B. A. (1996) *Genetics* **142,** 1119–1127.
5. Milgroom, M. G., Lipari, S. E., Ennos, R. A. & Liu, Y. C. (1993) *Heredity* **70,** 385–392.
6. Kohn, L. M. (1995) *Can. J. Bot.* **73** Suppl. 1, S1231–S1240.
7. Burt, A., Carter, D. A., Koenig, G. L., White, T. J. & Taylor, J. W. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 770–773.
8. Lehmann, P. F., Lin, D. & Lasker, B. A. (1992) *J. Clin. Microbiol.* **30,** 3249–3254.
9. Magee, P. T., Bowdin, L. & Staudinger, J. (1992) *J. Clin. Microbiol.* **30,** 2674–2679.
10. Soll, D. R., Galask, R., Schmid, J., Hanna, C., Mac, K. & Morrow, B. (1991) *J. Clin. Microbiol.* **29,** 1702–1710.
11. Wickes, B. L., Hicks, J. B., Merz, W. G. & Kwon-Chung, K.-J. (1992) *J. Gen. Microbiol.* **138,** 901–907.
12. Wilkinson, B. M., Morris, L., Adams, D. J., Evans, E. G. V., Lacey, C. J. N. & Walmsley, R. M. (1992) *J. Med. Vet. Mycol.* **30,** 123–131.
13. Schönian, G., Meusel, O., Tietz, H.-J., Meyer, W., Gräser, Y., Tausch, I., Presber, W. & Mitchell, T. G. (1993) *Mycoses* **36,** 171–179.
14. Thanos, M., Schönian, G., Meyer, W., Schweynoch, C., Gräser, Y., Mitchell, T. G., Presber, W. & Tietz, H.-J. (1996) *J. Clin. Microbiol.* **34,** 615–621.
15. Scherer, S. & Stevens, D. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1452–1456.
16. Schmid, J., Rotman, M., Reed, B., Pierson, C. L. & Soll, D. R. (1993) *J. Clin. Microbiol.* **31,** 39–46.
17. Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. (1989) *Genomics* **5,** 874–879.
18. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. (1990) *Nucleic Acids Res.* **18,** 6531–6535.
19. Welsh, J. & McClelland, M. (1990) *Nucleic Acids Res.* **18,** 7213–7218.
20. Burt, A., Carter, D. A., White, T. J. & Taylor, J. W. (1994) *Mol. Ecol.* **3,** 523–525.
21. Weir, B. S. (1990) *Genetic Data Analysis* (Sinauer Associates, Sunderland, MA).
22. Zaykin, D., Zhivotovsky, L. & Weir, B. S. (1995) *Genetica (The Hague)* **96,** 169–178.
23. Wickes, B., Staudinger, J., Magee, B. B., Kwon-Chung, K. J., Magee, P. T. & Scherer, S. (1991) *Infect. Immun.* **59,** 2480–2484.
24. Doi, M., Homma, M., Chindamporn, A. & Tanaka, K. (1992) *J. Gen. Microbiol.* **138,** 2243–2251.
25. Iwaguchi, S.-I., Homma, M. & Tanaka, K. (1990) *J. Gen. Microbiol.* **136,** 2433–2442.
26. Lehman, P. F., Kember, B. J., Hsiao, C.-H. & Dev, S. (1989) *J. Clin. Microbiol.* **27,** 2514–2521.
27. Caugant, D. A. & Sandven, P. (1993) *J. Clin. Microbiol.* **31,** 215–220.
28. Magee, B. B., D'Souza, T. M. & Magee, P. T. (1987) *J. Bacteriol.* **169,** 1639–1643.
29. Lasker, B. A., Page, L. S., Lott, T. J., Kobayashi, G. S. & Medoff, G. (1991) *Gene* **102,** 45–50.
30. Meyer, W., Mitchell, T. G., Freedman, E. & Vilgalys, R. (1993) *J. Clin. Microbiol.* **31,** 2274–2280.
31. Clark, A. G. & Lanigan, C. M. S. (1993) *Mol. Biol. Evol.* **10,** 1096–1111.
32. Lewis, P. O. & Snow, A. A. (1992) *Mol. Ecol.* **1,** 155–160.
33. Karl, S. A. & Avise, J. C. (1993) *Mol. Biol. Evol.* **10,** 342–361.
34. Burdon, J. J. & Roelfs, A. P. (1985) *Phytopathology* **75,** 1068–1073.
35. Selander, R. K., Musser, J. M., Caugant, D. A., Gilmour, M. N. & Whittam, T. S. (1987) *Microb. Pathog.* **3,** 1–7.
36. Maynard-Smith, J., Smith, N. H., O'Rourke, M. & Spratt, B. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4384–4388.
37. McDonald, B. A. & McDermott, J. M. (1993) *Bioscience* **43,** 311–319.