In *Candida albicans*, White-Opaque Switchers Are Homozygous for Mating Type

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ABSTRACT

The relationship between the configuration of the mating type locus (*MTL*) and white-opaque switching in *Candida albicans* has been examined. Seven genetically unrelated clinical isolates selected for their capacity to undergo the white-opaque transition all proved to be homozygous at the *MTL* locus, either *MTL***a** or *MTL*α. In an analysis of the allelism of 220 clinical isolates representing the five major clades of *C. albicans*, 3.2% were homozygous and 96.8% were heterozygous at the *MTL* locus. Of the seven identified *MTL* homozygotes, five underwent the white-opaque transition. Of 20 randomly selected *MTL* heterozygotes, 18 did not undergo the white-opaque transition. The two that did were found to become *MTL* homozygous at very high frequency before undergoing white-opaque switching. Our results demonstrate that only *MTL* homozygotes undergo the white-opaque transition, that *MTL* heterozygotes that become homozygous at high frequency exist, and that the generation of *MTL* homozygotes and the white-opaque transition occur in isolates in different genetic clades of *C. albicans*. Our results demonstrate that mating-competent strains of *C. albicans* exist naturally in patient populations and suggest that mating may play a role in the genesis of diversity in this pernicious fungal pathogen.

CANDIDA albicans is carried in the microflora of a majority of healthy individuals as a benign commensal (Odds 1988; Soll *et al.* 1991). When the defense mechanisms of an individual are compromised, this opportunistic pathogen can increase in number and penetrate tissue in one or more body locations, causing a variety of yeast-related diseases (Odds 1988). In severely immunosuppressed individuals, systemic Candida infections are life threatening and difficult to treat (Odds 1988; Zhao and Calderone 2002). The success of this pathogen derives in part from its capacity to switch reversibly and at high frequency between two or more general phenotypes (Slutsky *et al.* 1985, 1987; Soll *et al.* 1991; Soll 1992). Switching has been demonstrated to alter in a coordinated fashion a variety of pathogenic traits and a variety of genes (Soll 1992, 2002b). To understand the molecular basis of switching in *C. albicans*, the "white-opaque transition," first described in strain WO-1 (Slutsky *et al.* 1987), has been employed as an experimental model, since it involves a simple phase transition between two alternative states. In this transition, cells switch from a round budding yeast form with a smooth surface to an elongate, large asymmetric budding yeast form with a pimpled surface (Anderson and Soll 1987; Slutsky *et al.* 1987). White-opaque switching occurs at frequencies of $\sim 10^{-3}$, and the white and opaque phenotypes are typically passed on to progeny cells. The white-opaque transition is spontaneous and reversible and is accompanied by the differential expression of white phase-specific and opaque-phase-specific genes (Morrow *et al.* 1992, 1993; Srikantha and Soll 1993; Balan *et al.* 1997; Sanglard *et al.* 1999). This transition is readily identified on agar containing phloxine B, which differentially stains opaque phase cells and colonies red (Anderson and Soll 1987). Although the white-opaque transition has provided a tractable system for investigating switching, it appeared to represent a minor switching system, expressed in <10% of *C. albicans* isolates (D. R. Soll, unpublished observations).

Hull and Johnson (1999) demonstrated that *C. albicans*, which is diploid, contained genes that corresponded to the mating type (*MAT*) genes *MAT***a**1, *MAT*α1, and *MAT*α2 of *Saccharomyces cerevisiae*. In the strain they analyzed (CAI4, a common patient-derived laboratory strain), the *MTL* locus was heterozygous, containing *MTL***a**1 on one chromosome and *MTL*α1 and *MTL*α2 on the homolog. Subsequently, Hull *et al.* (2000) demonstrated that engineered homozygous *MTL***a** and *MTL*α strains (a/− and α/−, respectively) mated *in vivo* at very low estimated frequencies, and Magee and Magee (2000) demonstrated that laboratory-derived *MTL* hemizygotes mated *in vitro* also at very low estimated frequencies. Recently Miller and Johnson (2002) found that although the original *MTL* heterozygous strain of *C. albicans* employed in their stud-

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ies did not undergo the white-opaque transition, homozygous *MTL* derivatives did. These results indicate that the *MTL* locus controls white-opaque switching and suggest that the majority of *C. albicans* strains, which are heterozygous for mating type and do not undergo the white-opaque transition, are capable of switching if they become homozygous at the mating type locus. In this study, we have tested this proposal by analyzing clinical isolates of *C. albicans* for a correlation between white-opaque switching and *MTL* configuration.

We first identified six new genetically unrelated white-opaque switchers in an epidemiological collection of >70 independent *C. albicans* isolates collected worldwide (Lockhart *et al.* 1996; Blignaut *et al.* 2002; Pujol *et al.* 2002) and tested strain WO-1 and each of the newly identified switchers for allelism at the mating type locus. We next randomly selected 220 isolates representing the five major genetic clades of *C. albicans* (Blignaut *et al.* 2002; Pujol *et al.* 2002) and tested them for allelism at the mating type locus and switching. Our results demonstrate that all identified white-opaque switchers are homozygous at the mating type locus, that the great majority of natural homozygotes undergo the white-opaque transition, and that no natural *MTL* heterozygotes undergo the transition. Therefore, clinical isolates of *C. albicans* show a strong correlation between the ability to carry out white-opaque switching and the configuration of the *MTL* locus.

MATERIALS AND METHODS

Maintenance of stock cultures: All yeast cultures were clonally derived from primary clinical isolates. They were stored in sterile water at 25°, in 20% glycerol at −80°, or on agar slants containing supplemented Lee's medium (Bedell and Soll 1979). To assess the white-opaque transition (Slutsky *et al.* 1987), isolates were streaked on fresh agar containing supplemented Lee's medium plus 5 μg/ml of phloxine B, which differentially stains opaque cells (and, hence, opaque colonies and sectors) red, while leaving white cells (and, hence, colonies and sectors) white (Anderson and Soll 1987). To induce opaque phase sectoring in white phase colonies, cells were plated on phloxine B-containing agar at low density (~50 colonies per 85-mm plate) and the plates were wrapped with parafilm and incubated at 25° for 14 days. In some experiments cells were plated on YPD agar (2% dextrose, 2% Bacto-peptone, 1% yeast extract, and 2% agar) and incubated at 25°.

DNA fingerprinting: Select isolates were DNA fingerprinted by Southern blot hybridization with the complex DNA fingerprinting probe Ca3 (Sadhu *et al.* 1991; Anderson *et al.* 1993; Lockhart *et al.* 1995; Pujol *et al.* 1999) according to methods previously described in detail (Schmid *et al.* 1990; Soll 2000; Lockhart *et al.* 2001). In brief, DNA was isolated, digested with the restriction enzyme *Eco*RI and electrophoresed through a 0.8% agarose gel. DNA was transferred to Hybond N^+ membrane (Amersham, Piscataway, NJ) by capillary blotting. Blots were hybridized overnight with randomly primed ^{32}P-labeled Ca3 probe, washed at 45°, and autoradiographed. Autoradiograms were digitized using an Astra 1220U flatbed scanner (UMAX Technologies, Fremont, CA) and analyzed using DENDRON software (Soll 2000), which automatically detects lanes, identifies and links bands, and creates a band data file. All band data were manually edited before analysis. A similarity coefficient (S_{AB}) was computed from comparisons of the banding patterns of every pair of isolates A and B using the formula $S_{AB} = 2E/(2E + a + b)$, where E is the number of bands common between patterns A and B, a is the number of bands in pattern A not in pattern B, and b is the number of bands in pattern B not in pattern A. An S_{AB} of 0.0 indicates patterns with no common bands, while an S_{AB} of 1.00 indicates identical patterns. Values ranging from 0.01 to 0.99 reflect increasing levels of similarity. Dendrograms were generated on the basis of S_{AB} values using the unweighted pair-group method using arithmetic average (Rohlf 1963). Mixed dendrograms were generated by computing S_{AB}'s among newly analyzed isolates and reference isolates that had been previously analyzed, and their band data were stored in the DENDRON database.

PCR analysis of the *MTL* loci: Yeast genomic DNA (~1 ng) prepared by the method of Scherer and Stevens (1987) was used for each 50-μl reaction using Taq DNA polymerase as recommended by the manufacturer (Invitrogen, Carlsbad, CA). The oligonucleotide primers used are described in Table 1. Initial denaturation was for 10 min at 95°, followed by 40 cycles at 94° for 1 min, at 42° for 2 min, and at 68° for 3 min. The final elongation step was performed for 10 min at 68°. All PCR reactions were carried out using a Techne PHC-3 thermocycler (Princeton, NJ). Initially, an isolate was tested for *MTL***a** using the primers MTL**a**longF and MTL**a**longR and for *MTL*α using the primers MTLαlongF and MTLαlongR (Table 1). These primers generated whole open reading frames. This was confirmed for *MTL***a** using the primers OBPaF and OBPaR and for *MTL*α using the primers OBPαF and OBPαR (Table 1). These primers amplified the gene *OBP*, for which heterozygotic alleles are located in the *MTL***a** and *MTL*α loci. For select MTL homozygotes, the primers MTL**a**1F and MTL**a**1R and the primers MTLα2F and MTLα2R were used to amplify shorter regions of the *MTL***a**1 and *MTL*α2 genes, respectively (Table 1). These latter amplifications were performed to test whether there were point mutations or small deletions in the preceding amplified genes, which may have prevented amplification.

Scanning electron microscopy: Cells were grown at 25° in supplemented Lee's medium. Cells were harvested in late log phase, washed twice in double-distilled water and fixed in 2.5% (wt/vol) glutaraldehyde in 0.1 M cacodylate buffer for 1 hr. Cells were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 50 min. After postfixation, cells were washed three times in 0.1 M cacodylate buffer and treated with 6% thiocarbohydrazide at room temperature. A second round of fixation in 1% osmium tetroxide was performed to enhance cell surface architecture. Cells were again rinsed in double-distilled water, dehydrated through increasing concentrations of ethanol solution, chemically dried in hexamethyldisilazane (Polysciences, Warrington, PA), mounted on aluminum stubs, and sputter-coated with gold palladium. Cells were imaged with a Hitachi S-4000 scanning electron microscope (Hitachi, San Diego).

RESULTS

White-opaque switchers are homozygous at the mating type locus: To test the allelism of white-opaque switchers at the *MTL* locus, >70 isolates from several epidemiological studies (Lockhart *et al.* 1996; Blignaut *et al.* 2002; Pujol *et al.* 2002) were visually screened for white-opaque switchers. In most cases, a few hundred colonies were screened on phloxine B plates after extended incu-

TABLE 1

Oligonucleotides used in this study

MTL**a**1F	5′ CAT ACC CAA ACT CTT ATT TGG G 3′
MTL**a**1R	5′ CAC CTT CAA CCT CCT CGT TTT TTC C 3′
MTL**a**longF	5′ ATC CCC CGG GAA TGA ACT CAG AAA TAG A 3′
MTL**a**longR	5′ TCC CCC CGG CTA CCT TGA ATT TGA ACT 3′
MTLα2F	5′ CAC ATC TGG CAC TCT TTG 3′
MTLα2R	5′ GGT CTT TTT GCA CAT ACG CA 3′
MTLα2longF	5′ AAT CTT CAT GGC TAT TCA GCA CCT 3′
MTLα2longR	5′ AAT ATA CTA GGT ATA AGG ATA CTT CAA 3′
OBPaF	5′ ACA TGT GGT CGC CCA ACT CC 3′
OBPaR	5′ GTG GTC AAT GGA GCT CAT AC 3′
OBPαF	5′ GAA GAT GAC TCA GGT CAT GC 3′
OBPαR	5′ CCT TCA ATT GCA TCG TAA GTA CC 3′

bation at 25° to allow sector formation. Six switchers (L26, 12C, 19F, P37005, P37035, and P78048) in addition to the original white-opaque switching strain WO-1 (Slutsky *et al.* 1987) were identified (Table 3). In Figure 1, A and B, examples are presented of a white and an opaque phase colony, respectively, of strain WO-1, and in Figure 1, C and D, examples are presented of the formation of opaque phase sectors in white colonies of strain WO-1. Each of the six newly selected strains formed white and red colonies on phloxine B-containing agar (Figure 1, E–H). When white colonies were incubated for >10 days, they formed opaque sectors at their peripheries. To test for the unique change in cellular phenotype associated with the switch from white to opaque in strain WO-1 (Anderson and Soll 1987; Slutsky *et al.* 1987), cells from white and opaque phase colonies of the six new strains were examined by scanning electron microscopy. In all six cases, cells from white phase colonies were round to ellipsoidal, with no signs of opaque-phase-specific pimples (Figure 2, A and C), as are cells from white phase colonies of strain WO-1 (Anderson and Soll 1987; Slutsky *et al.* 1987). In all six cases, cells from opaque phase colonies were elongate and larger than white phase cells and exhibited pimples covering their surfaces (Figure 2, B and D–H), as are cells from the opaque phase colonies of strain WO-1 (Anderson and Soll 1987; Slutsky *et al.* 1987). Finally, the frequencies of the transition from the white to opaque phase and opaque to white phase for five of the six newly selected white-opaque switchers (L26, 12C, 19F, P37005, and P78048) were similar to those of strain WO-1 at 25°. In each of these five strains, clonal white phase populations accumulated opaque phase cells at a frequency of $\sim 10^{-3}$, and clonal opaque phase populations accumulated white phase cells at a frequency of 10^{-3}. The one exception was strain P37035. Clonal populations of this strain emanating from the opaque phase contained predominantly white phase cells after 5 days of colony development at 25°, suggesting that in relation to the other strains, the frequency of the transition in the opaque to white direction in this strain was very high. Opaque phase cells of all six newly selected white-opaque phase switchers underwent mass conversion to the white phase when shifted from 25° to 42° (data not shown) in a manner similar to that of strain WO-1 (Morrow *et al.* 1993; Srikantha and Soll 1993).

To test whether WO-1 and the six new white-opaque switching strains were homozygous at the mating type locus, the polymerase chain reaction was used to amplify the *MTL*α2 and *MTL***a**1 genes. DNA amplification of control strain 3153A, which does not undergo the white-opaque transition (Slutsky *et al.* 1985), revealed both genes (Figure 3). However, amplification of DNA from WO-1 and the six newly selected white-opaque switchers produced either *MTL***a**1 or *MTL*α2, but not both (Figure 3). In strains WO-1, 19F, P37035, and P78048, only *MTL*α was detected, while in strains L26, 12C, and P37005, only *MTL***a**1 was detected (Figure 3). All isolates were also analyzed for the presence of the gene *OBP*, which is present in both the *MTL***a** and the *MTL*α locus, but differs enough between *MTL***a** and *MTL*α (Hull and Johnson 1999) to allow distinctions to be made by

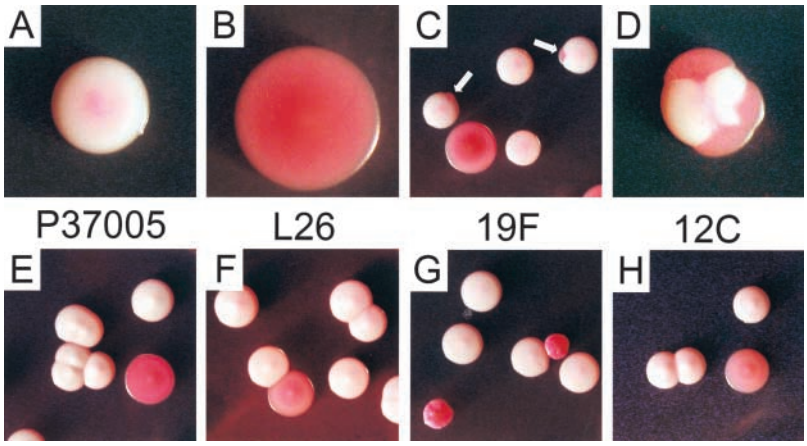

Figure 1.—*MTL* homozygous strains of *C. albicans* undergo the white-opaque transition. The spontaneous transition can be assessed on agar containing phloxine B, which stains white phase colonies white and opaque phase colonies red. (A) White phase colony of strain WO-1; (B) opaque phase colony of strain WO-1; (C and D) opaque phase sectors in white phase colonies of strain WO-1 that have arisen from spontaneous switching; (E–H) white and opaque phase colonies in strains P37005, L26, 19F, and 12C, respectively.

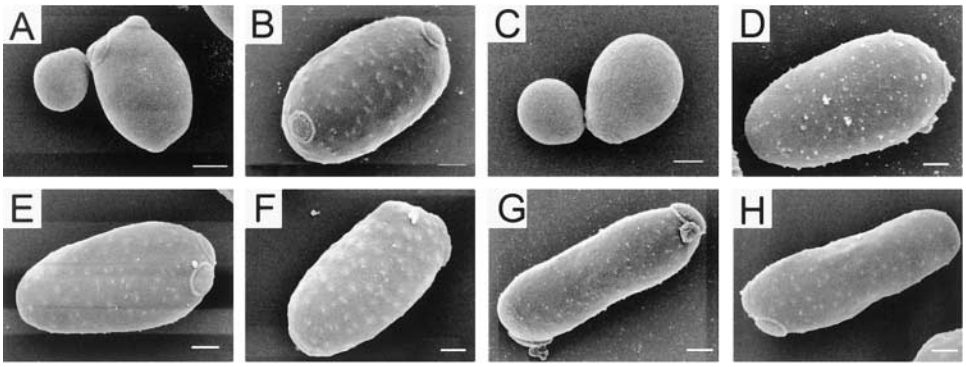

FIGURE 2.—Opaque phase cells of *MTL* homozygous strains of *C. albicans* possess distinguishing opaque-phase-specific pimples on their cell surfaces. Pimples were visualized by scanning electron microscopy. (A) White phase cell of strain WO-1; (B) opaque phase cell of strain WO-1; (C) white phase cell of strain L26; (D) opaque phase cell of strain L26; (E–H) opaque phase cells of strains P78048, 12C, 19F, and P37005, respectively. Note that white phase cells are devoid of pimples.

PCR. In every case, the *OBP***a** allele segregated with the *MTL***a**1 gene (L26, 12C, and P37005) and the *OBP*α allele segregated with the *MTL*α2 gene (strains WO-1, 19F, P37035, and P78048; data not shown), confirming that strains undergoing the white-opaque transition contained either *MTL***a** or *MTL*α, but not both. We could not, however, determine from these PCR assays whether WO-1 and the six additional strains possessed one copy of the mating type locus *MTL***a** or *MTL*α or contained two copies of one or the other. A deletion analysis of strain WO-1 indicated the presence of two *MTL*α loci (data not shown), which favors a mechanism in which *MTL* becomes homozygous at the two alleles rather than a mechanism in which one allele is lost. On the basis of this result, we will refer to **a** or α strains as "*MTL* homozygous" for simplicity, keeping in mind that we have not distinguished between homozygosity and hemizygosity, except in the case of strain WO-1.

Switching, homozygosity, and genetic relatedness: In analyses of population structure using DNA fingerprinting with the complex probe Ca3, it has been demonstrated that *C. albicans* isolates cluster into five major genetically unrelated groups: I, II, III, SA, and E (PUJOL *et al.* 1997, 2002; BLIGNAUT *et al.* 2002). In North America, the prevalent clades are groups I, II, and III, with very little group SA or E representation (2 and 3%, respectively). In Europe, groups I, II, III, SA, and E are represented, and group E is most common (22%). In South Africa, the prevalent clades are groups I, II, and SA, with little group III or E representation, and group SA is most common (35–55%). To examine the distribution of switchers and *MTL* homozygotes among the five groups, the seven white-opaque switchers were DNA fingerprinted with the complex probe Ca3 (Figure 4), and the data were used to generate a mixed dendrogram with DNA fingerprinting data from previously analyzed isolates representing the five clades. Mixed dendrograms facilitate the identification of clade affiliation of new isolates (SOLL 2000; BLIGNAUT *et al.* 2002; PUJOL *et al.* 2002). The seven white-opaque switchers were divided between clades I and II. In addition, both homozygous *MTL***a** strains and *MTL*α strains were present in clade I (Figure 5). These results demonstrate that (1) white-opaque switching occurs in isolates from different clades, (2) *MTL* homozygotes occur in different clades,

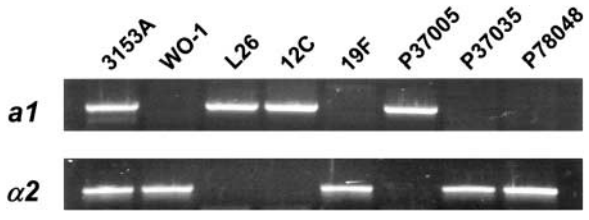

FIGURE 3.—PCR analysis of *MTL* allelism revealed that the seven strains of *C. albicans* selected for their capacity to undergo the white-opaque transition were all homozygous for the mating type locus. Selected strains were analyzed by the polymerase chain reaction for both *MTL***a** and *MTL*α. Laboratory strain 3153A is *MTL* heterozygous and exhibits both *MTL***a** and *MTL*α products. In contrast, the selected white-opaque switching strains (WO-1, L26, 12C, 19F, P37005, P37035, and P78048) exhibited either the *MTL***a** or the *MTL*α product.

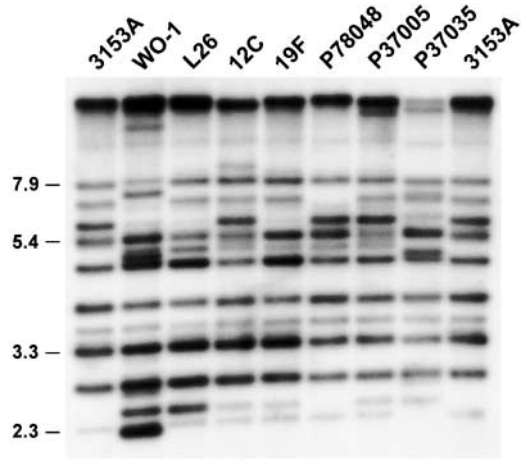

FIGURE 4.—Southern blot analysis of total genomic DNA probed with the complex DNA fingerprinting probe Ca3 revealed that the seven *MTL* homozygous strains selected originally for their capacity to undergo the white-opaque transition were genetically unrelated.

and (3) homozygous *MTL***a** and *MTL*α strains can occur in the same clade.

Selected *MTL* homozygotes undergo the white-opaque transition: Demonstration that WO-1 and the six additional strains selected for the white-opaque transition were homozygous at the mating type locus suggests that all or most switchers will prove to be *MTL* homozygous. However, this does not prove the converse, namely that all *MTL* homozygotes are white-opaque switchers. To test the latter, we used PCR amplification to assess allelism at the mating type loci of 220 clinical isolates and then tested all identified *MTL* homozygotes and randomly chosen *MTL* heterozygotes for the white-opaque transition. Fifty of the tested isolates were from group I, 50 from group II, 50 from group III, 50 from group SA, and 20 from group E. Of the 220 tested isolates, 7 were *MTL* homozygotes (3.2%), 3 were *MTL***a** strains, and 4 were *MTL*α strains (Table 2); 213 isolates (96.8%) were *MTL* heterozygous (Table 2). Homozygous *MTL***a** strains were identified in groups I, II, and SA, and homozygous *MTL*α strains were identified in groups II and SA (Table 3). No *MTL* homozygous strains were identified in groups III and E. Of the 7 identified *MTL* homozygotes, 5 (GC75, OKP90, P60, P57072, and P78048) underwent the white-opaque transition (Table 2). One was an *MTL*α from group I, 2 were *MTL***a**'s from group II, 1 was an *MTL*α from group II, and 1 was an *MTL*α from group SA (Table 2). One *MTL***a** homozygote from group SA and 1 *MTL*α homozygote from group SA formed colonies that stained pink on phloxine B plates, a color midway between white and opaque (Table 2). The colony morphologies of these two strains were irregular or wrinkled, and the cell population contained pseudohyphae and budding cells, but no opaque cells. No white-opaque switching was evident in these two strains.

To test whether *MTL* heterozygotes (*MTL***a**/*MTL*α) switched, ~250 cells of each of 20 randomly selected *MTL* heterozygotes were grown on agar medium containing phloxine B for 14 days at 25° to allow formation of opaque sectors, the result of switching. Of the 20

TABLE 2

Genotypes at the mating type locus

		MTL genotype		
Clade	*N*	**a** (%)	α (%)	**a**/α (%)
I	50	0 (0)	1 (2)	49 (98)
II	50	1 (2)	2 (4)	47 (94)
III	50	0 (0)	0 (0)	50 (100)
SA[a]	50	2 (4)	1 (2)	47 (94)
E[b]	20	0 (0)	0 (0)	20 (100)
Total		3 (1.4)	4 (1.8)	213 (96.8)

[a] Clade found primarily in South Africa.
[b] Clade found primarily in Europe.

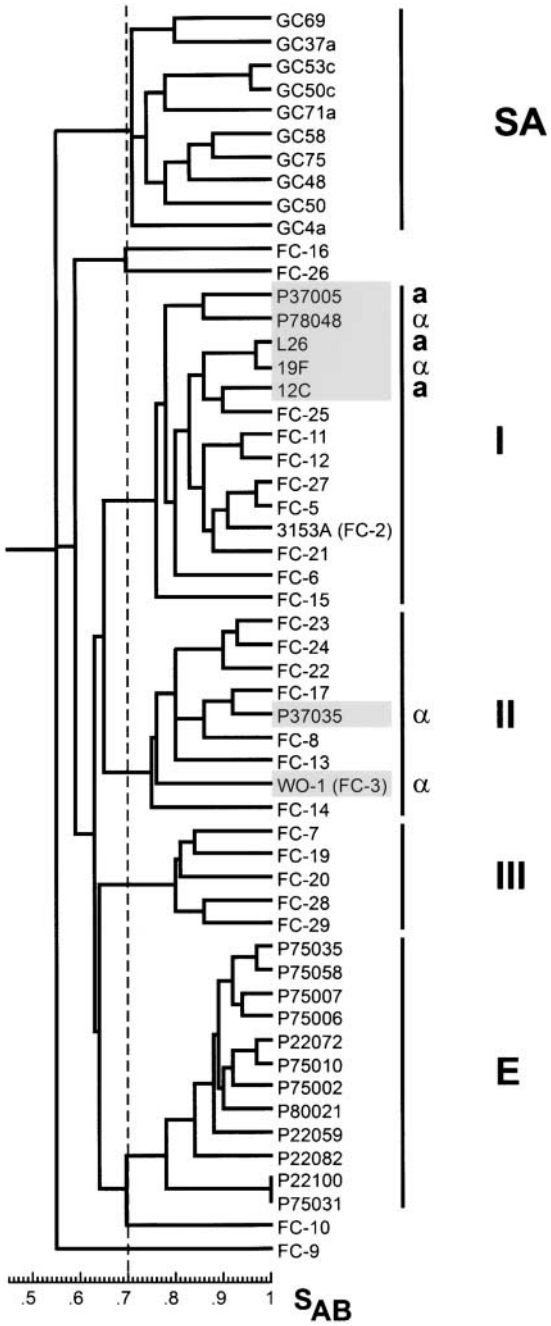

FIGURE 5.—Cluster analysis reveals that the seven identified white-opaque switchers separate into two of the major five clades of *C. albicans* and that both homozygous *MTL***a** and *MTL*α strains can be members of the same clade. A mixed dendrogram was generated from the Ca3 fingerprinting data of the seven selected white-opaque switchers (see Figure 6) and 47 random isolates spanning the five major clades of *C. albicans* (groups I, II, III, SA, and E; PUJOL *et al.* 1997, 2002; BLIGNAUT *et al.* 2002). The dendrogram is based on similarity coefficient (S_{AB}) values computed for each pairwise combination of strains. Solid vertical lines denote clades. An S_{AB} threshold of 0.70 indicated by a dashed vertical line was used to distinguish clades (SOLL 2000).

TABLE 3

Strains characterized for switching and allelism at the *MTL* locus

Group selection	Isolate	Origin[a]	Geo. locale[b]	*MTL* genotype	Clade[c]	Wh/Op transition[d]	Reference
Wh/Op Switch.	WO-1	BSI	IA (USA)	α	II	+	Slutsky *et al.* (1987)
	L-26	VP (v)	IA (USA)	**a**	I	+	This study
	12C	VP (or)	MI (USA)	**a**	I	+	Lockhart *et al.* (1996)
	19F	VP (v)	MI (USA)	α	I	+	Lockhart *et al.* (1996)
	P37005	H (or)	FL (USA)	**a**	I	+	This study
	P37035	H (or)	FL (USA)	α	II	+	This study
	P78048	BSI	Winnipeg (Canada)	α	I	+	Pujol *et al.* (2002)
Homozyg.	G106	HIV+ (or)	South Africa	**a**	SA	(−)	Blignaut *et al.* (2002)
	GC75	H (or)	South Africa	α	SA	+	Blignaut *et al.* (2002)
	OKP90	H (or)	South Africa	**a**	II	+	Blignaut *et al.* (2002)
	P60	HIV+ (or)	South Africa	**a**	II	+	Blignaut *et al.* (2002)
	P87	HIV+ (or)	South Africa	α	SA	(−)	Blignaut *et al.* (2002)
	P57072	BSI	IA (USA)	α	II	+	Pujol *et al.* (2002)
	P78048	BSI	Winnipeg (Canada)	α	I	+	Pujol *et al.* (2002)
Heterozyg.	G5	HIV+ (or)	South Africa	**a**/α	SA	−	Blignaut *et al.* (2002)
	G59	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	GC37	H (or)	South Africa	**a**/α	SA	−	Blignaut *et al.* (2002)
	K18	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	K36	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	K136	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	K277	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	K347	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	K684	HIV+ (or)	South Africa	**a**/α	II	−	Blignaut *et al.* (2002)
	P22092	BSI	Germany	**a**/α	I	−	Pujol *et al.* (2002)
	P48076	BSI	MA (USA)	**a**/α	III	−	Pujol *et al.* (2002)
	P48090	BSI	IA (USA)	**a**/α	I	−	Pujol *et al.* (2002)
	P57039	BSI	Brazil	**a**/α	III	−	Pujol *et al.* (2002)
	P57047	BSI	MA (USA)	**a**/α	II	−	Pujol *et al.* (2002)
	P75037	BSI	Turkey	**a**/α	SA	−	Pujol *et al.* (2002)
	P75063	BSI	France	**a**/α	SA	(+)	Pujol *et al.* (2002)
	P75071	BSI	Italy	**a**/α	SA	−	Pujol *et al.* (2002)
	P76004	BSI	TX (USA)	**a**/α	III	−	Pujol *et al.* (2002)
	P76019	BSI	NY (USA)	**a**/α	I	−	Pujol *et al.* (2002)
	P76023	BSI	NY (USA)	**a**/α	II	−	Pujol *et al.* (2002)
	P76066	BSI	Ottawa (Canada)	**a**/α	II	−	Pujol *et al.* (2002)
	P76068	BSI	Ottawa (Canada)	**a**/α	III	−	Pujol *et al.* (2002)
	P78042	BSI	IN (USA)	**a**/α	III	−	Pujol *et al.* (2002)
	P78056	BSI	VA (USA)	**a**/α	I	−	Pujol *et al.* (2002)
	P80001	BSI	NY (USA)	**a**/α	III	(+)	Pujol *et al.* (2002)
	P80004	BSI	NY (USA)	**a**/α	SA	−	Pujol *et al.* (2002)
	P80042	BSI	TX (USA)	**a**/α	III	−	Pujol *et al.* (2002)

[a] BSI, bloodstream isolate; VP, vaginitis patient; v, vaginal sample; or, oral sample; HIV+, HIV-positive; H, healthy individual.
[b] Geographical locale in which isolate was collected.
[c] Genetic clade.
[d] +, homozygous isolates that underwent the white-opaque transition; (−), homozygous isolates that did not undergo the white-opaque transition; −, heterozygous isolates that did not undergo the white-opaque transition; (+), isolates originally found heterozygous that underwent the white-opaque transition, but only after homozygosis.

isolates, 18 did not undergo the white-opaque transition. Because we found no opaque colonies out of the 250 colonies plated, the estimated switching frequency would be $<4 \times 10^{-3}$. When it is considered that a switching strain generates two to four sectors per colony after 14 days of incubation and that no sectors were detected in the 18 isolates that did not switch, the estimated switching frequency would be reduced to $<4 \times 10^{-6}$. Two isolates (P80001 and P75063) did, however, form white and opaque colonies and sectors. One isolate was from group III, and one was from group SA (Table 3). When cells from opaque colonies of the two isolates that switched were analyzed for *MTL* allelism, they proved to be *MTL* homozygous, indicating that the original *MTL*

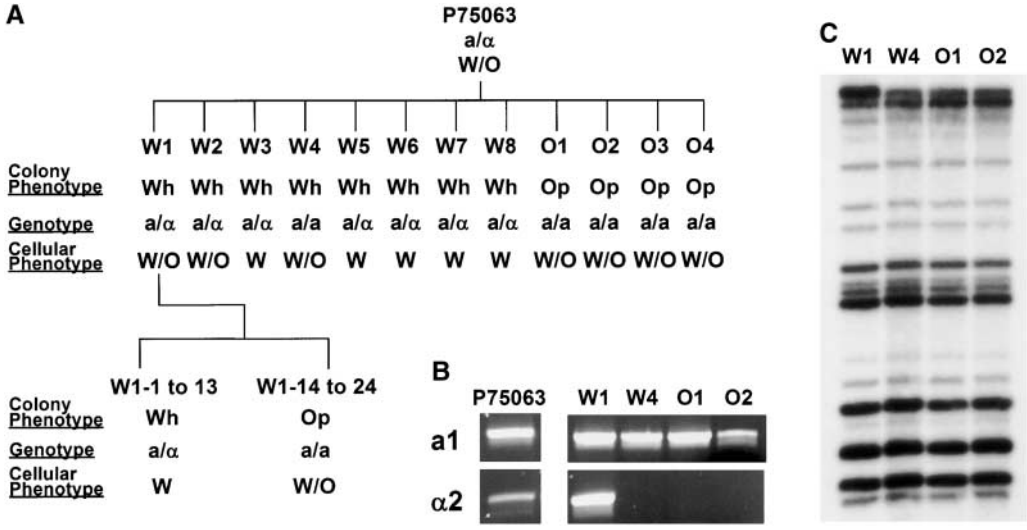

FIGURE 6.—Heterozygous strains that formed opaque phase colonies did so through the spontaneous high-frequency formation of *MTL* homozygotes. (A) The lineage of one of two such strains, P75063, is presented. P75063 was originally *MTL* heterozygous but formed white and opaque colonies. Eight white (W1–W8) and four opaque (O1–O4) colonies were analyzed for genotype and cellular phenotype. One apparently white colony contained white and opaque phase cells. When plated, it formed white and opaque phase colonies. Note that while white colonies in the lineage were either *MTL* heterozygous or *MTL* homozygous, all opaque colonies were *MTL* homozygous. (B) Mating type. PCR analysis demonstrating an *MTL* heterozygous and an *MTL* homozygous white colony and *MTL* homozygous opaque colonies. (C) Ca3 fingerprints. Southern blot analysis with the complex probe Ca3 revealed that heterozygous and homozygous isolates represented the same strain (P75063).

heterozygous clones had become homozygous at the *MTL* locus. A lineage of one of these isolates, P75063, is presented in Figure 6A. The original clinical isolate was cloned prior to storage in water. It was then subcultured as a patch and analyzed for mating type. It was demonstrated to be heterozygous for the *MTL* locus (Figure 6B). Cells plated from this patch formed white and opaque phase colonies on agar containing phloxine B. Eight individual white phase colonies (clones) and four individual opaque phase colonies (clones) were in turn picked and analyzed for mating type allelism and switching. Seven of the eight white colonies proved to be heterozygous (*MTL***a**/*MTL*α) and one homozygous (*MTL***a**) for *MTL*, while all four of the opaque colonies were homozygous (*MTL***a**; Figure 6A). When cells from 13 white colonies (W1-1 to -13) and 11 opaque colonies (W1-14 to -24) of original clone W1 were in turn analyzed for mating type and switching, the former proved to be heterozygous (*MTL***a**/*MTL*α) and to exhibit the white phenotype only, while the latter proved to be homozygous (*MTL***a**) and capable of switching (Figure 6A). To be sure that all tested clones emanated from strain P75063, one white *MTL* heterozygous clone and three *MTL* homozygous clones (one white and two opaque) were DNA fingerprinted with the species-specific probe Ca3. All four exhibited similar DNA fingerprints (Figure 6C), demonstrating that they were all derived from the same progenitor. These results demonstrate that while a majority (90%) of *MTL* heterozygotes do not normally undergo the white-opaque transition, a minority (10%) are capable of doing so. These latter strains appear to become homozygous at high frequency, again supporting the idea that only cells homozygous for the *MTL* locus can undergo the white-opaque transition.

DISCUSSION

We have found that the original white-opaque switching strain WO-1 and six additional clinical strains that were selected for their capacity to undergo the white-opaque transition were homozygous at the mating type locus, supporting the suggestion that all strains that undergo the white-opaque transition are homozygous at the mating type locus. Since our results were obtained with naturally occurring clinical isolates, they complement experiments carried out using a genetically manipulated laboratory strain demonstrating that the *MTL* locus controls white-opaque switching (MILLER and JOHNSON 2002). Analysis of the genetic relatedness of strain WO-1 and the six naturally occurring white-opaque switchers revealed that they were all genetically distinct. A cluster analysis (PUJOL *et al.* 1997, 2002; BLIGNAUT *et al.* 2002) further revealed that the seven *MTL* homozygous switchers were distributed among two of the five major clades of *C. albicans*, group I and group II, and that *MTL***a** and *MTL*α homozygotes occurred in the same clade. These results demonstrate that strains in different clades can undergo the white-opaque transition and should further dispel past reservations that the white-opaque transition was unique to strain WO-1.

Although our analysis of *MTL* allelism of the seven selected white-opaque switchers supported the conclusion that all white-opaque switchers are homozygous for the mating type locus, it did not prove the converse, namely that all *MTL* homozygotes undergo the white-opaque transition. To examine the latter suggestion, we analyzed the *MTL* allelism of 220 independent *C. albicans* isolates and tested all identified *MTL* homozygotes for the white-opaque transition. Of the tested collection, 96.8% were heterozygous and 3.2% homozygous, the

latter including both *MTL***a** strains and *MTL*α strains. Of the seven identified *MTL* homozygotes, five underwent the white-opaque transition. No *MTL* homozygotes were obtained from group III or group E, but because the general frequency of *MTL* homozygotes among the entire collection of isolates was so low, no conclusion can be made on the absence of *MTL* homozygotes in a particular clade. In fact, one of the *MTL* heterozygous isolates that spontaneously became homozygous at high frequency (P80001) was from group III. What is noteworthy, however, is the apparent absence of white-opaque switching in two SA isolates, one *MTL***a** and one *MTL*α. These two isolates formed irregular wrinkled colonies, which contained high levels of pseudohyphae. It is not clear whether these strains did not undergo the white-opaque transition or whether the white-opaque transition was masked by expression of a variant phenotype in an alternative phenotypic switching system not under the regulation of the *MTL*, in this case the irregular wrinkle phenotype in the 3153A switching system (Slutsky *et al.* 1985). The presence of multiple switching systems within the same strain that can affect one another has been suggested in both *C. albicans* (Soll 2002b; Zhao *et al.* 2002; Zhao and Calderone 2002) and *C. glabrata* (Lachke *et al.* 2002).

Of 20 randomly selected *MTL* heterozygotes, 18 did not undergo the white-opaque transition. Two *MTL* heterozygotes, however, switched. An analysis of white and opaque colonies obtained from these strains revealed that they spontaneously generated *MTL* homozygotes at high frequency, which in turn underwent the white-opaque transition. In each of these strains, only *MTL***a** or *MTL*α colonies were exclusively generated, suggesting the presence of a recessive lethal allele on the homologous chromosome (Whelan and Soll 1982). Alternatively, the bias may reflect a mechanism that fosters mating between unrelated strains.

Our results, therefore, generalize the original finding by Miller and Johnson (2002) that strains heterozygous at the mating type locus do not undergo the white-opaque transition, but can do so when they become *MTL* homozygous. Our results suggest that only *MTL* homozygotes undergo the white-opaque transition, although not all *MTL* homozygotes may be capable. Our study has also identified *MTL* heterozygous strains that become *MTL* homozygous at very high frequency. Finally, our results demonstrate that in nature the majority (96.8%) of isolates in the five major clades are *MTL* heterozygous. Only 3.2% of clinical isolates are *MTL* homozygous. *C. albicans* strains that contain *MTL***a** or *MTL*α, but not both, have the ability to mate (Hull *et al.* 2000; Magee and Magee 2000). White-opaque switching is intimately involved in mating in two respects. First, it is controlled by the *MTL* locus, the same locus that controls mating type. Obviously, *MTL* heterozygosity suppresses the white-opaque transition. Second, opaque phase cells mate much more efficiently than white phase cells, suggesting that cells must undergo the white to opaque transition as a normal part of the mating process (Miller and Johnson 2002). In this article, we have shown that ~3% of clinical isolates of *C. albicans* are homozygous at the *MTL* locus and carry out white-opaque switching. Moreover, we have identified strains of *C. albicans* that are *MTL***a**/*MTL*α heterozygotes, but generate *MTL***a** or *MTL*α homozygous strains at very high frequency. All of these results suggest that mating-competent strains of *C. albicans* exist naturally in patient populations, and preliminary results indicate that a majority are capable of mating (S. R. Lockhart and D. R. Soll, unpublished observations). Studies of population structure suggest that although reproduction is primarily clonal, recombination does occur at low frequency (Pujol *et al.* 1993; Graser *et al.* 1996; Anderson *et al.* 2001). Mating of *C. albicans* would lead to recombination, but would require cocolonization. Although few DNA fingerprinting studies have been performed to assess the degree of cocolonization of hosts by multiple strains of *C. albicans*, there are reasons to believe that cocolonization occurs more frequently than assumed (Soll *et al.* 1988, 1991; Soll 2002a) and may, through mating, be an important mechanism to generate diversity.

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