

Genomic Structure of *Candida stellatoidea*: Extra Chromosomes and Gene Duplication

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Candida albicans and *Candida stellatoidea* are two closely related imperfect yeasts. Some isolates characterized as *C. stellatoidea* are in fact *C. albicans*, while others differ with respect to virulence and to karyotype, containing extra small chromosomes. Experiments in this study allowed us to infer that a typical *C. stellatoidea* isolate, Y2360, has 12 chromosomes rather than the 7 previously shown for *C. albicans*. The majority of cloned sequences tested hybridized to analogous chromosomes in *C. albicans* and in *C. stellatoidea*, although there were exceptions, and a repeated element isolated as specific for *C. albicans* hybridized to most of the chromosomes of *C. stellatoidea*. Several genes tested hybridized to one of the smaller, *C. stellatoidea*-specific chromosomes as well as to a larger one. The arrangement of restriction enzyme sites around the gene was the same in both the large and small chromosomes. For *ADE2* and *LYS2*, the arrangements were identical to those of a typical *C. albicans* strain, FC18, suggesting a high degree of sequence conservation between the two species. Spheroplast fusion and segregation experiments showed that the *ADE2* genes on both the large and small chromosomes of *C. stellatoidea* are active, implying that the organism is functionally at least triploid for this gene and probably for any others duplicated on the smaller chromosomes.

The taxonomic relationship between *Candida albicans* and *Candida stellatoidea* has been examined in some detail in the past year, largely through the efforts of Kwon-Chung and her collaborators (5, 6). They have shown that the classical distinction between these two imperfect yeasts, the ability to use sucrose as a carbon source (characteristic of *C. albicans* but not *C. stellatoidea*), is insufficient as a criterion for distinguishing the two species, since there are two classes of *C. stellatoidea*. Some *C. stellatoidea* isolates are identical to *C. albicans* in almost every property, except that they are Suc⁻, presumably through mutation. It thus seems most likely that they are misclassified. Kwon-Chung and her co-workers call these latter strains *C. stellatoidea* type II (6). They have found that the other class, called *C. stellatoidea* type I, differs from *C. albicans* in a variety of important properties, including production of the extracellular proteinase, virulence, and karyotype. It also has a different pattern of restriction sites in the rDNA (10). Type II, in keeping with the view that it is misclassified, has a pulsed-field electrophoretic pattern well within the general range found for *C. albicans* (seven large chromosomes), while type I has seven large chromosomes plus from two to four extra chromosomes, all of which are smaller than the smallest type II band (6, 12).

Making the assumption that type II is a Suc⁻ *C. albicans*, it becomes important to make more detailed comparisons of the two similar species *C. albicans* and *C. stellatoidea* (which in this report is used to refer to type I only) to determine how closely related they are. The extra chromosomes found in *C. stellatoidea* amount to more than 1,000 kilobases (kb) of DNA; if the rest of the karyotype is similar to *C. albicans*, the question arises as to what information is carried in this differently arranged DNA. One could imagine that the extra chromosomes contain transposed fragments

removed from one or more of the larger chromosomes. Such fragmentation might not be observable on the electrophoretic karyotype, since the larger chromosomes vary in size from strain to strain and are big enough so that a change of as much as 500 kb of DNA might not alter the mobility too drastically. Alternatively, the five smallest chromosomes of *C. stellatoidea* could contain extra copies of information which is present on the larger chromosomes. A third possibility is that there is information in *C. stellatoidea* which is not present in *C. albicans* and that this information is carried on the extra chromosomes. One way to begin to distinguish among these possibilities is to examine the genomic arrangements of various sequences which are homologous in *C. albicans* and *C. stellatoidea*. This involves determining the location on the *C. stellatoidea* electrophoretic karyotype of several sequences whose localization in *C. albicans* is known. A more detailed analysis can be made by comparing restriction patterns of various sequences between the two species. This report describes such experiments. We found that a remarkable degree of homology is conserved between the two species, down to the level of restriction sites adjacent to functional genes. Many of these sequences hybridized to pulsed-field electrophoretic bands of the same relative position in *C. stellatoidea* as in *C. albicans*, indicating that the general genomic arrangement is similar. We found in addition that some sequences hybridize in *C. stellatoidea* to the extra chromosomes as well as to the large *C. albicans*-like chromosomes. We asked whether the *ADE2* gene, which is one of these sequences, is active in both locations.

MATERIALS AND METHODS

The strains used were Y2360, a *C. stellatoidea* strain provided by C. Kurtzman; FC18, a standard *C. albicans* strain in this laboratory (11); and *C. albicans* hOG300 (*ade2 met pro*), a gift from Russell Poulter (16). Cells were grown as described previously (10).

CHEF and OFAGE were generally done as previously

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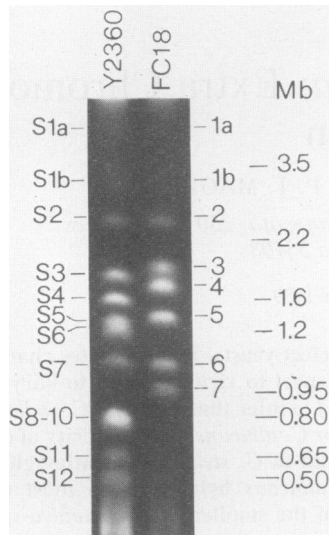


FIG. 1. Comparison of the CHEF separations of the chromosomes of *C. stellatoidea* Y2360 and *C. albicans* FC18. The *C. stellatoidea* chromosomes are numbered according to the convention for *C. albicans* (10), but they are preceded by an S, since they are not identical to the *C. albicans* chromosomes. Band 8, Y2360, is considered to contain three chromosomes based on brightness and on the data presented in this report. Molecular weights of standards from *Schizosaccharomyces pombe* and *S. cerevisiae* are shown (Mb).

described (10, 11). Variations from previously described protocols are noted in the figure legends. DNA transfer, hybridization, and autoradiography were done by standard procedures (1). Hybridization of *C. stellatoidea* DNA with *C. albicans* genes was done at normal stringency. Sequences were labeled by random oligonucleotide priming or nick translation. DNA electrophoresis was done as described previously (10).

Mutants were isolated by UV irradiation to 15% survival on YEPD plates. Auxotrophs were identified and characterized in standard fashion (3). Spheroplast fusion was done by the method of Poulter et al. (15). Heat shock was done as described by Hilton et al. (2), but conditions were optimized for 10% survival (in this case, 51°C for 90 s). UV-induced crossing-over was as described previously (20).

Restriction enzymes were used according to the instructions of the suppliers.

RESULTS

Genomic organization of *C. stellatoidea* compared with that of *C. albicans*. Fourteen sequences, 11 of which contain genes which complement mutations in *Saccharomyces cerevisiae*, have been assigned to the seven linkage groups in *C. albicans* (11). These include the cognate genes *ADE2*, *URA3*, and *TUB2*. We examined the location of each of these on the *C. stellatoidea* electrophoretic karyotype and found that the general arrangement is quite similar. Figure 1 shows a pulsed-field separation of the chromosomes of *C. stellatoidea* Y2360, a type I isolate, and FC18, a typical *C. albicans*. The chromosomes are numbered in accordance with the convention we have adopted for *C. albicans*, with the largest (rDNA-containing) band numbered S1 (to distinguish it from chromosome 1 of *C. albicans*) (10). There are three bands smaller than chromosome 7, the fastest-migrating band in most *C. albicans* strains, and there are some

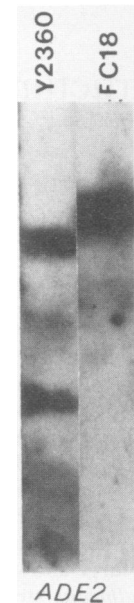


FIG. 2. Hybridization of the *ADE2* sequence from *C. albicans* to CHEF separations of *C. stellatoidea* (Y2360) and *C. albicans* (FC18) chromosomes. The Y2360 strip is from the separation shown in Fig. 3A; the FC18 strip is from a standard CHEF separation.

size differences among the first seven. The first band after chromosome 7 appears to be much brighter than any of the others. Evidence presented below suggests that it contains three unresolved chromosomes. This assumption implies that there are five smaller chromosomes in Y2360.

The separation shown in Fig. 3A was transferred to nitrocellulose and cut into strips to allow the assignment of cloned genes to specific chromosomes. Figure 2 compares the hybridization of the *C. albicans* *ADE2* gene to the chromosomes of Y2360 and of FC18. As previously shown, *ADE2* hybridized to chromosome 3 of FC18. In Y2360, however, the sequence showed homology not only to chromosome S3 (upper band) but also to the area of the bright band after chromosome S7. To be sure that the *ADE2* sequences on chromosome S8, S9, or S10 included the coding region, rather than some repeated sequence fortuitously on the plasmid, we used the fragment internal to the *ADE2* coding region as a probe and obtained the same results as with the whole sequence (data not shown). Thus, the smaller chromosomes of *C. stellatoidea* contain at least some sequences which duplicate information found on the larger *C. albicans*-like chromosomes.

One explanation for this arrangement would be that the smaller chromosomes are persisting duplicated fragments of the larger ones. Evidence presented below supports this view. One question is whether the smaller bands are all derived from one chromosome or whether they contain duplicated information from several. Figure 3 suggests that information from at least two chromosomes is presented in the small bands. Two strips from the separation shown in Fig. 3A were probed with *URA3* and *LYS2*. *URA3* hybridized solely to chromosome S3, as it does in *C. albicans*. Thus, the smaller chromosome does not contain all the information on chromosome S3 (nor could it, given the sizes of the two bands), nor is all the information on chromosome S3 found in the region of the smaller chromosomes. *LYS2*, on the other hand, hybridized to chromosome S5, analogous

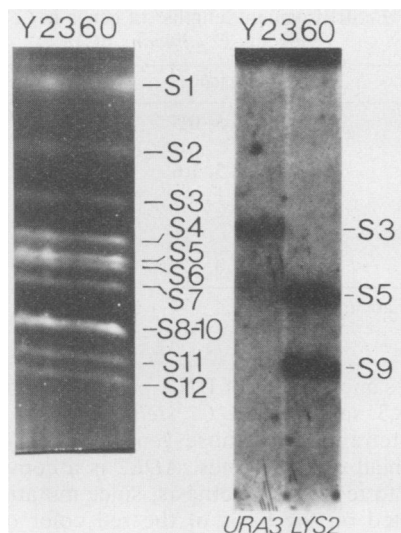


FIG. 3. (A) CHEF separation of the chromosome of Y2360. (B) Hybridization of the *C. albicans* *URA3* and *LYS2* sequences to this separation.

to its *C. albicans* location, and also to chromosome S8, S9, or S10. Thus, it is possible that one of these chromosomes contains information from at least two of the larger chromosomes. Experiments described below, however, suggest that the two genes hybridize to separate chromosomes.

We examined the chromosomal locations in *C. stellatoidea* of several other cloned *C. albicans* sequences. The general rule that we found is that the arrangement of genes on the larger chromosomes is similar but not identical in the two species, while some genes appear to be duplicated on the small chromosomes of *C. stellatoidea*. Table 1 shows the assignment of several genes in *C. stellatoidea* and compares their location with that in *C. albicans*. (The data for *C. albicans* are largely taken from reference 11, but the assignments of several additional sequences are given.) Only the genes shown were tested for *C. stellatoidea*. It is evident

TABLE 1. Chromosome location of cloned *C. albicans* genes in *C. stellatoidea* and *C. albicans*^{a,b}

Chromosome ^c	<i>C. stellatoidea</i>	<i>C. albicans</i> ^d
1	rDNA, <i>HIS3</i> , ACT	rDNA, <i>TUB2</i> , <i>GAL1</i> , <i>MGL1</i> , <i>GAL1</i> , <i>SOR9</i> , ACT
2	TS	<i>HIS3</i> , TS, PRA, (<i>SOR9</i>), (<i>MGL1</i>)
3	<i>URA3</i> , <i>ADE2</i> , <i>SOR2</i>	<i>URA3</i> , <i>ADE2</i> , <i>SOR2</i>
4	pCHR4, <i>SOR2</i>	pCHR4
5	<i>LYS2</i>	<i>LYS2</i>
6	pCHR6	Ben ^r , Mtx ^r
7	pCHR7, DHFR	pCHR7, DHFR
8	<i>ADE2</i>	
9	<i>LYS2</i>	
10		
11	<i>SOR9</i>	
12	DHFR, pCHR6	

^a Those genes not assigned to *C. stellatoidea* chromosomes were not tested on this organism.

^b ACT, Actin gene (13; S. Scherer, unpublished data); TS, thymidylate synthase (18); PRA, aspartyl proteinase (9); and DHFR, dihydrofolate reductase.

^c The *C. stellatoidea* chromosomes are S1 to S12.

^d Parentheses indicate variation by strain.

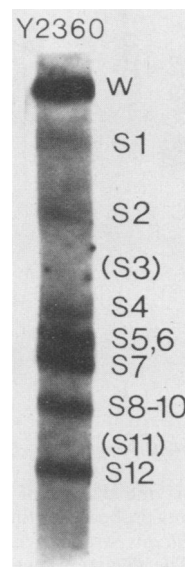


FIG. 4. Hybridization of a *C. albicans* mid-repeat sequence to the chromosomes of *C. stellatoidea* Y2360. The CHEF separation shown in Fig. 3A was transferred to nitrocellulose and probed with the sequence described by Scherer and Stevens (17). The numbers at the right indicate the positions of the chromosomes on the original separation. Chromosomes S3 and S11 are in parentheses to indicate that they do not contain sequences homologous to the probe.

that the overall arrangement of the genes on the larger chromosomes is similar, although there are some differences in location (*HIS3*, *SOR9*) and one duplication on the large chromosomes (*SOR2*). Chromosome S12 appears to contain information which is contained in chromosomes 6 and 7 in *C. albicans*. Two other *C. stellatoidea* strains were tested for some of these genes, and the arrangement was similar.

Scherer and Stevens (17) have identified a moderately repetitive sequence from *C. albicans* which hybridizes to all but one of the chromosomes and appears to be species specific. An indication of the similarity of *C. stellatoidea* and *C. albicans* is that this sequence is also found in the *C. stellatoidea* genome. Figure 4 shows that it hybridizes to all but 2 or 3 of the 11 chromosomes. (We cannot say for sure that it hybridizes to both S8 to S10 or only one of the chromosomes in that band. Interestingly, the one chromosome in *C. albicans* which lacks the sequence is chromosome 3 (unpublished data).

Restriction fragment analysis of *ADE1* and *LYS2* genes. The smaller, unique chromosomes of *C. stellatoidea* are thus composed, at least in part, of information which is a duplication of that on the larger chromosomes. This material could be arranged as small fragments of the larger chromosomes, consisting of pieces approximately the size of intact and functional genes, or as sequences containing large stretches of information, including several genes. In the first case, one would expect that the sequences on the small chromosomes might vary significantly from those on the larger chromosomes in their flanking restriction sites. Figure 5 shows that for *ADE2* and *LYS2*, both duplicated genes, the restriction patterns for *C. stellatoidea* genomic DNA are indicative of a single pattern of sites. This result suggests that the homologous sequences on the small chromosome consist of fragments bigger than single genes. Furthermore, both the repeated genes have restriction fragment lengths

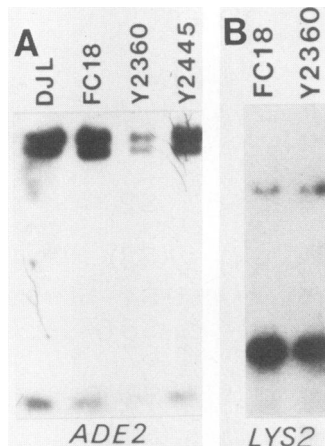


FIG. 5. Restriction enzyme digests of genomic DNA from *C. stellatoidea* and *C. albicans* probed with the *ADE2* and *LYS2* genes. DJL and FC18 are *C. albicans* strains, while Y2441 and Y2360 are *C. stellatoidea* strains. The digestions were done as described in Materials and Methods. (A) *EcoRI* digest probed with *ADE2*. Band sizes are 3.8, 3.5, and 0.5 kb. (B) *EcoRV* digest probed with *LYS2*. Bands are 6.1 and 2.3 kb.

similar or identical to those of *C. albicans*, underscoring the similarity between *C. albicans* and type I *C. stellatoidea*. In looking for *EcoRI* restriction fragment length polymorphisms between two species, we found that of seven genes examined, four have polymorphisms, while *SOR2*, *LYS2*, and *ADE2* are identical (Table 2). However, there are bands in common in all but *URA3*, suggesting that strains have similar or identical gene copies in one chromosomal location for these genes. (FC18 shows one of several *C. albicans* patterns in its restriction fragment lengths for *URA3*; the bands in Y2360 are similar [possibly identical] to two of those reported by Kelley et al. [4] for other *C. albicans* strains, 4.8 and 3.3 kb.)

C. stellatoidea is functionally triploid or tetraploid for *ADE2*. Since *C. albicans* is functionally diploid for at least a large part of its genome and *C. stellatoidea* contains approx-

TABLE 2. *EcoRI* fragment lengths^a of genes in *C. stellatoidea* Y2360 and *C. albicans* FC18

Gene	Y2360	FC18
<i>ADE2</i>	3.8, 3.5, 0.5	3.8, 3.5, 0.5
<i>HIS3</i>	8.5	9.8, 8.5, 2.6
<i>LYS2</i>	3.7, 2.5, 1.6	3.7, 2.5, 1.5
Ben ^r	8.5, 5.1	5.1
<i>URA3</i>	5.1, 3.5	10, 2.2
<i>SOR2</i>	3.7	3.7
rDNA	6.0, 4.0, 2.5	6.4, 3.7, 2.5

^a All lengths are in kilobases.

imately the same amount of DNA per cell (7), the results in Fig. 2 and 3 suggest that *C. stellatoidea* is functionally triploid or tetraploid for those genes located on both the large and small chromosomes. *ADE2* is a convenient gene with which to test this hypothesis, since mutations in it are easily isolated on the basis of the red color of the auxotrophic colonies. We therefore set out to isolate *ade2* mutants of *C. stellatoidea* Y2360. We were not successful, although we screened 54,000 survivors after UV treatment. While this result is compatible with a ploidy higher than 2, it is not conclusive, since several explanations, such as a closely linked recessive lethal gene which renders homozygosity difficult, are also possible. We therefore decided to use parasexual genetics to determine whether both sets of *ADE2* genes were active. We fused Y2360R1, an Arg⁻ derivative of Y2360, with hOG300, a strain which is *ade2 met pro*. Prototrophic fusions are selected on minimal plates with sucrose as the sole carbon source. We then used heat shock to induce chromosome loss. We expected that if both genes were active, red segregants would have to have lost both chromosome 2 and chromosome 8 of *C. stellatoidea*. Several red auxotrophs were isolated. Figure 6A shows a CHEF separation of the chromosomes from Y2360R1 and five *ade2* segregants from the fusant. While several of the *ade2* products retained a band in the region of chromosomes S8 to S10, one (segregant 13) did not.

However, the intensity of the chromosome S8 band in some of the *ade2* segregants appears significantly lower than

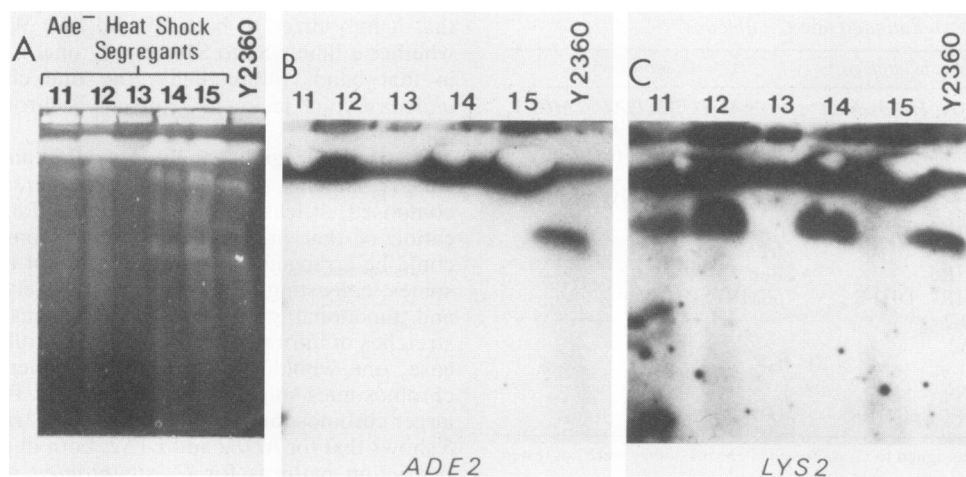


FIG. 6. CHEF separation of the chromosomes of *C. stellatoidea*-*C. albicans* fusions. The fusants were constructed and *ade2* segregants were obtained as described in the text. The parents and segregants were subjected to CHEF (A), blotted, and probed with *ADE2* (B) and *LYS2* (C). (The CHEF separation was at a pulse time of 60 s for 15 h, then 90 s for 8 h. This regimen separates the small chromosomes but not the large ones which constitute the bright bands at the top of the gel.)

in lane 6, which contains the parent strain Y2360R1. This could be due to the loss of the chromosome containing the *ADE2* gene during heat shock. We therefore probed a blot of the CHEF gel shown in Fig. 6A with the *ADE2* gene. Figure 6B shows that all the red *ade2* segregants have lost the sequence homologous to *ADE2*, suggesting that the sequence on chromosome S8 is functional and that the chromosome must be lost for the recessive *ade2* phenotype to be expressed. To explore the nature of the band migrating in the area of chromosomes S8 to S10, we probed the CHEF separation with *LYS2*. Figure 6C shows that in three of the segregants (11, 12, and 14), the *LYS2* sequence was retained, while in two (13 and 15) it was lost. Very interestingly, although segregant 15 lacks both the *ADE2* and the *LYS2* sequences, a band in the region of chromosome S8 to S10 remains. We therefore conclude that the extra copies of *LYS2* and *ADE2* are on different chromosomes in Y2360 and that all the heat shock segregants have lost the *ADE2*-containing one (arbitrarily named S8), while several have retained the *LYS2*-bearing one (chromosome S9). We also conclude that this band contains a third chromosome, S10, which contains neither *ADE2* nor *LYS2*.

DISCUSSION

The similarity of *C. albicans* and *C. stellatoidea* from the point of view of morphology, ability to form germ tubes, overall physiology, and DNA homology is striking and has led to *C. stellatoidea* being classified as a subspecies of *C. albicans*. The discovery that some *C. stellatoidea* strains contain large chromosomes similar to those of *C. albicans* as well as two to five chromosomes smaller than any of the *C. albicans* chromosomes posed the question whether the arrangement of genes varied between the two organisms (6). It seems clear from the results reported here that the general genomic arrangement is the same, with the small chromosomes containing at least some material that duplicates that on the larger ones. We numbered the *C. stellatoidea* chromosomes S1 to S12, to distinguish them from the *C. albicans* ones. (We continued to consider that *C. albicans* normally contains only seven chromosomes, despite a report to the contrary [8], because the extra large chromosome reported there seems to be absent in most strains [B. Magee, unpublished data]). More evidence for the nearness of the relationship is that the mid-repeat sequence of Stevens and Scherer (17), which was isolated as being specific for *C. albicans*, is highly repeated in the *C. stellatoidea* genome.

One striking fact about the repeated information on the small chromosomes is that it appears to consist of reasonably large fragments, since for two repeated genes restriction fragment analysis with enzymes cutting relatively frequently showed no evidence of polymorphism, even though there were in principle four copies of the gene present and polymorphisms are often found in *C. albicans*, in which genes are only diploid (4). A second surprising finding was that the similarity between the two species *C. stellatoidea* and *C. albicans* can extend to the level of equivalent restriction fragment length when enzymes which cut frequently are used. Among seven sequences examined, three were identical in their patterns, while two others had one band in common between the two strains. The common band probably indicates that the sequence on one of the two homologs of one species is similar to the other species.

One question which we have not been able to resolve is whether the small chromosomes specific to *C. stellatoidea* are present in diploid numbers. They appear to be roughly

equal in intensity to the larger chromosomes by ethidium bromide staining (Fig. 1) or blotting (Fig. 2 and 3). However, two pieces of evidence suggest that they are haploid, at least for some genes. First, we were able to get *ade2* segregants from the Y2360R1-hOG300 fusion at a reasonable rate after UV treatment. If four mitotic crossovers or gene conversions were needed, one might expect a much lower frequency of appearance. The argument is weakened by the possibility that in the process of fusion, one homolog of chromosome S8 could have been lost. Such chromosome loss has been observed before in fusion experiments with *C. albicans* (3). The second bit of evidence is found in Fig. 6 in which a band corresponding to chromosomes S8 to S10 occurs in segregant 15, but the band does not hybridize to *LYS2* or *ADE2*. It is conceivable that there is an extra chromosome which is not homologous to either chromosome S8 or chromosome S9. We are therefore tentatively assigning three chromosomes to this band.

Thus, a number of puzzles about the *C. stellatoidea* karyotype remain unanswered, but two conclusions can be drawn from this work. First, *C. albicans* and *C. stellatoidea* (type I) share a great deal of genetic identity, including the arrangement of genes on the karyotype and often extending to the level of restriction fragment identity. Second, *C. stellatoidea* differs by having up to five extra, smaller chromosomes which contain at least some information which duplicates that on the larger chromosomes. The *ADE2* results suggest that these smaller chromosomes contain at least some functional genes, not pseudogenes. The taxonomic relationship between the two species is, of course, not resolved by these studies. The close relationship between these two yeasts raises the question of what constitutes a species in the imperfect fungi. *C. albicans* and *C. stellatoidea* differ in a number of physiological properties, such as growth rate at 30°C, proteinase secretion, growth on indicator medium, and virulence (6). They also, as shown previously and in this report, differ in electrophoretic karyotype. On the other hand, at the molecular level they appear to be very similar. We confirmed the observation by Poulter and Hanrahan (14), reported before the distinction between type I and type II *C. stellatoidea* was shown, that the *ADE1-MET* linkage in *C. albicans* is conserved in *C. stellatoidea* (type I) (E. Rikkerink, unpublished data). The two species share high sequence homology in genes, frequent identity in restriction enzyme fragment size, and a similar, albeit not identical arrangement of genes on chromosomes of similar size. We carefully investigated two strains of *C. albicans*, C9 and FC18. There is almost no variation in gene-chromosome assignment between those two. For WO-1, however, a strain which undergoes the phenotypic transition (19), the variation in gene location is greater than in Y2360 (B. B. Magee, unpublished data). An interesting quandary is thus presented. At the molecular level, the variation in randomly chosen genes appears to be as great within the *C. albicans* group as between *C. albicans* and *C. stellatoidea*, while at the organismal level there are clear distinctions between the two groups, although not terribly large ones. It is conceivable that the difference lies in information on the smaller chromosomes, but the fact that these contain a large fraction of duplicated information makes this rather unlikely. Whether these differences are significant enough to justify the species distinction is debatable.

Are the smaller chromosomes dispensable? If they are, heat shock experiments could allow one to examine whether they play a role in the properties of *C. stellatoidea* which

differ from those of *C. albicans*. Such experiments would also reveal more about what information they do carry.

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