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Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast *Candida glabrata*

Simon Wong*, Mario A Fares*, Wolfgang Zimmermann†, Geraldine Butler‡ and Kenneth H Wolfe*

Addresses: *Department of Genetics, Smurfit Institute, University of Dublin, Trinity College, Dublin 2, Ireland. †AGOWA, Glienicker Weg 185, D-12489 Berlin, Germany. ‡Department of Biochemistry and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland.

Correspondence: Kenneth H Wolfe. E-mail: khwolfe@tcd.ie

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Abstract

Background: *Candida glabrata* is a pathogenic yeast of increasing medical concern. It has been regarded as asexual since it was first described in 1917, yet phylogenetic analyses have revealed that it is more closely related to sexual yeasts than other *Candida* species. We show here that the *C. glabrata* genome contains many genes apparently involved in sexual reproduction.

Results: By genome survey sequencing, we find that genes involved in mating and meiosis are as numerous in *C. glabrata* as in the sexual species *Kluyveromyces delphensis*, which is its closest known relative. *C. glabrata* has a putative mating-type (*MAT*) locus and a pheromone gene (*MFALPHA2*), as well as orthologs of at least 31 other *Saccharomyces cerevisiae* genes that have no known roles apart from mating or meiosis, including *FUS3*, *IME1* and *SMK1*.

Conclusions: We infer that *C. glabrata* is likely to have an undiscovered sexual stage in its life cycle, similar to that recently proposed for *C. albicans*. The two *Candida* species represent two distantly related yeast lineages that have independently become both pathogenic and 'asexual'. Parallel evolution in the two lineages as they adopted mammalian hosts resulted in separate but analogous switches from overtly sexual to cryptically sexual life cycles, possibly in response to defense by the host immune system.

Background

The genus *Candida* consists of ascomycete yeast species that lack an apparent sexual (teleomorph) stage in their life cycle and seem to reproduce only mitotically. However, data from the *C. albicans* genome project has recently led researchers to question the asexuality of that species. *C. albicans* was found to have a mating type-like (*MTL*) locus similar to the *Saccharomyces cerevisiae MAT* locus [1]. Natural isolates of *C. albicans* are diploid *MTL***a**/*MTL*α heterozygotes, similar to diploid *S. cerevisiae* but unable to sporulate. By genetic engineering to create *MTL***a** and *MTL*α hemizygotes, *C. albicans* was induced to mate in the laboratory and in infected mice, forming tetraploids [2,3]. In addition, Miller and Johnson [4] showed that *C. albicans MTL* hemizygotes undergo phenotypic switching between the common 'white' form and an 'opaque' form that is a million-fold more active in mating. Further analysis of the almost-complete genome sequence of *C. albicans* revealed that it contains homologs of

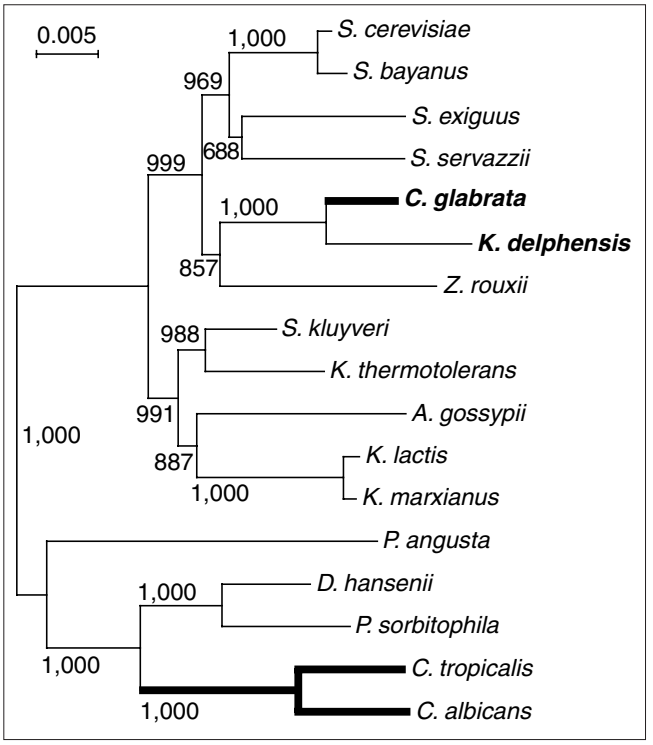

Figure 1
Phylogenetic relationships among ascomycete yeasts, based on the aligned coding regions of the 5S, 18S, 5.8S and 26S rRNAs. Thick lines show 'asexual' lineages. The tree was constructed by the neighbor-joining method and bootstrap values (1,000 replicates) are shown. *A. gossypii*, *Ashbya gossypii*; *D. hansenii*, *Debaryomyces hansenii*; *P. angusta*, *Pichia angusta*; *P. sorbitophila*, *Pichia sorbitophila*; *Z. rouxii*, *Zygosaccharomyces rouxii*.

most of the *S. cerevisiae* genes involved in the key sexual processes of meiosis and sporulation as well as mating [5]. These findings have led to the hypothesis that the life cycle of *C. albicans* includes a cryptic sexual phase, which perhaps is utilized only occasionally or under particular environmental conditions [6] - infrequently enough not to have been detected during more than a century of research into *C. albicans*, but frequently enough to cause evolutionary conservation of the genes involved in the sexual process. Interestingly, sexual forms of some other *Candida* species were identified long ago by mycologists but their significance has often gone unrecognized by molecular biologists because the anamorphs and teleomorphs are assigned different names - for example, the sexual form of *Candida krusei* is called *Issatchenkia orientalis* [7,8].

Medically, *C. albicans* is still the major fungal agent of human disease, but *C. glabrata* is a species of growing concern. The incidence of *C. glabrata* infections, particularly in the bloodstream, has risen alarmingly over the past decade [9,10]. It has also gained much attention since the discovery of its inherently low susceptibility to the drug fluconazole [11]. The genome of *C. glabrata* appears to be haploid whereas *C. albicans* is diploid [12,13]. But similarly to *C. albicans*, *C. glabrata* also undergoes phenotypic switching [14,15], raising the interesting question of whether it too may have an undiscovered teleomorph form.

In phylogenetic trees drawn from rDNA sequences, most *Candida* species including *C. albicans* fall into one monophyletic group, whereas *C. glabrata* is much more closely related to *S. cerevisiae* than to the *C. albicans* group (Figure 1 and [16-18]). Therefore, the 'asexual' life cycles of *C. glabrata* and *C. albicans* arose independently from sexual ancestors [16,19]. Phylogenetic analysis also showed unexpectedly that *C. glabrata*'s closest relative is *Kluyveromyces delphensis*, a sexual species [17,18]. *C. glabrata* is a commensal resident of the human intestinal tract and an opportunistic pathogen [20,21], whereas *K. delphensis* was first isolated from dried figs [22] and is often found associated with *Drosophila willistoni* [23]. The type strain of *K. delphensis* is homothallic and therefore probably diploid. It has been studied very little at the molecular level.

The aim of the present study was to use genome survey sequencing (GSS) to characterize the genomic differences between the closely related asexual *C. glabrata* and the sexual *K. delphensis*. However, we find that there are no significant differences between the gene repertoires of these species. *C. glabrata* has many genes involved in mating, meiosis and sporulation, including a pheromone gene and a putative mating-type locus. This leads us to propose that, like *C. albicans*, it must have an undiscovered sexual phase in its life cycle.

Results

Genome survey sequencing of *C. glabrata* and *K. delphensis*

We constructed plasmid libraries with random genomic inserts of 7-15 kilobases (kb) from *C. glabrata* and *K. delphensis* and sequenced both ends of about 3,000 plasmids (> 3 megabases (Mb) of primary sequence data, or approximately 0.2x genome coverage) from each species. Phylogenetic analysis of the combined complete 5S, 18S, 5.8S and 26S rDNA sequences from yeasts (Figure 1) confirms that *C. glabrata* and *K. delphensis* are each other's closest known relatives [17,18]. The phylogenetic tree also confirms that *C. glabrata* is more closely related to *S. cerevisiae* than to *C. albicans*, indicating that the two *Candida* species originated independently from sexual ancestors.

Assembled sequence contigs from *C. glabrata* and *K. delphensis* were searched against the complete set of *S. cerevisiae* proteins using FASTY reciprocal best hits to identify putative orthologs (see Materials and methods). The results of the genome surveys of the two species should be roughly comparable to one another, because the same methods and similar numbers of clones were used in both cases. The genomes can

be assumed to be similar in size [24,25], and the two species are equally distantly related to *S. cerevisiae*. Indeed, they yielded similar numbers of sequence contigs (4,481 from *C. glabrata*, 4,202 from *K. delphensis*) and similar numbers of putative genes with unambiguous *S. cerevisiae* orthologs (1,941 and 2,057, respectively).

Gene functions in *C. glabrata* and *K. delphensis* were inferred from the known functions of their *S. cerevisiae* orthologs, using the 'cellular role' categories of the Yeast Proteome Database (Table 1). Orthologs were found for approximately 40% of the *S. cerevisiae* genes involved in most cellular roles, reflecting the level of GSS coverage. The numbers of genes found in *C. glabrata* and *K. delphensis* for each cellular role are highly similar (Table 1), and the two genomes are not significantly different in any of the 42 categories ($p > 0.05$ by χ^2 test). Importantly, *C. glabrata* does not have significantly fewer genes than *K. delphensis* in the categories of mating response and meiosis, which relate to sexual functions.

Mating pathway genes

We identified *C. glabrata* orthologs of many genes in the *S. cerevisiae* mating response pathway including the Ste11, Ste7 and Fus3 kinases and the Ste12 transcription factor (Figure 2). Because some components of the mating pathway also participate in other pathways (such as filamentous growth) that might legitimately be expected to be present in an asexual organism, we focus here on genes that have no other known functions apart from mating. The GSS data identified *C. glabrata* orthologs of 13 *S. cerevisiae* genes that may be involved exclusively in mating (Table 2). These include an α-factor pheromone gene (*MFALPHA2* [26]), *STE13*, whose sole function appears to be maturation of prepro-α-factor [27], and *STE6*, whose only known role is in **a**-factor export [28]. The complete *C. glabrata MFALPHA2* gene was sequenced and codes for a signal peptide and three repeats of a candidate mature pheromone sequence WHWV(R/K)(L/I)RKGQGLF (single-letter amino-acid notation) flanked by processing sites for Kex2 [29], Kex1 and Ste13 proteases. The ortholog in *K. delphensis* was also sequenced and has four copies of the sequence WHWLSVRPGQPIY. The two precursor proteins share 49% sequence identity.

C. glabrata appears capable of responding to pheromones as well as synthesizing them, because it has genes for the polarity-establishment proteins Far1 and Cdc24 [30], for Sgv1 (a kinase acting in the pheromone adaptation pathway [31]), and Akr1 (a protein with an inhibitory effect on the pheromone signal transduction pathway [32]). At the end of the signal transduction cascade it has orthologs of Fus3 (the final MAP kinase in the mating response pathway, which activates Ste12 and Far1 [33]), as well as the nuclear fusion protein Kar5 [34].

We identified putative mating-type (*MAT*) loci in both *C. glabrata* and *K. delphensis*, containing orthologs of the *S. cerevisiae* genes for the α1 transcription activator and the α2 repressor (*MATALPHA1* and *MATALPHA2*, respectively), oriented divergently (Figure 3). As expected from the species phylogeny, the level of amino-acid sequence identity between *S. cerevisiae* and *C. glabrata* (38% in α1, and 40% in α2) is greater than that between *S. cerevisiae* and *K. lactis* or *C. albicans* in the same proteins [1,35]. Between *C. glabrata* and *K. delphensis*, there is 59% amino-acid sequence identity in α1 and 76% in α2.

In *K. delphensis* the α2 and α1 genes are flanked on one side by a series of five genes whose orthologs are beside the *MAT* locus on *S. cerevisiae* chromosome III (Figure 3), in the same arrangement except that *K. delphensis* lacks *PHO87*. These genes include *BUD5*, which is almost twice as large in *K. delphensis* as in *S. cerevisiae* (1,241 amino acids versus 642). The predicted KdBud5 protein includes an extra SH3 domain near its amino terminus, giving it an overall structure more similar to Cdc25 [36]. On the other side of the α2 and α1 genes in *K. delphensis* there is a series of five genes whose orthologs are on *S. cerevisiae* chromosome XII, beginning with *YLR186W* (*EMG1* [37]). The same breakpoint between chromosome III and chromosome XII orthologs is also seen in *C. glabrata* (Figure 3). It therefore seems likely that a chromosomal rearrangement occurred on the right-hand side of the *MAT* locus either in an ancestor of *K. delphensis* and *C. glabrata*, or in an ancestor of *S. cerevisiae*. Interestingly, the coding regions of the α1 gene and *EMG1* overlap by 28 nucleotides at their 3′ ends in both *K. delphensis* and *C. glabrata*.

Meiotic genes

The GSS data from *C. glabrata* also identified orthologs of many *S. cerevisiae* genes involved in meiosis, a central step in the sexual cycle that leads ultimately to the production of gametes (sporulation). We found *C. glabrata* orthologs of 19 *S. cerevisiae* genes whose only known functions are in meiosis or sporulation (Table 3), including the master regulatory switch gene *IME1* [38]. *S. cerevisiae IME1* expression is induced by the **a**1/α2 heterodimer representing the genetic signal from a diploid cell, in combination with nutritional signals. We found *C. glabrata* orthologs of *MCK1* and *RIM9*, which are inducers of *IME1* expression, and *UME6* which negatively regulates meiosis-specific genes during vegetative growth but is converted into an activator of early meiosis genes when Ime1 is present [39,40]. *C. glabrata* also has orthologs of *IME2*, which can promote sporulation in the absence of *IME1* [41], and *IDS2* and *RIM4*, whose products promote Ime2-dependent activation of many downstream targets [42,43].

C. glabrata has orthologs of *RIM4* and *MUM2*, both of which are needed for premeiotic DNA replication [44,45], and *HOP2*, which acts to prevent synapsis between non-homologous chromosomes [46]. We also found *MSH4*, which is implicated in synaptonemal complex formation and

Table 1

Numbers of *C. glabrata* and *K. delphensis* orthologs found in different YPD 'cellular role' categories

YPD 'cellular role' categories	*C. glabrata*	*K. delphensis*	CG/SC*	KD/SC†	*S. cerevisiae*‡
Aging	16	15	0.41	0.38	39
Amino acid metabolism	92	96	0.42	0.44	217
Carbohydrate metabolism	70	84	0.28	0.33	254
Cell adhesion	1	3	0.25	0.75	4
Cell cycle control	96	91	0.45	0.43	213
Cell polarity	91	97	0.42	0.45	216
Cell stress	98	114	0.30	0.34	331
Cell structure	51	51	0.43	0.43	120
Cell wall maintenance	68	75	0.37	0.41	184
Chromatin/chromosome structure	111	119	0.41	0.43	274
Cytokinesis	21	26	0.53	0.65	40
Differentiation	36	25	0.35	0.24	104
DNA repair	59	76	0.38	0.49	154
DNA synthesis	53	55	0.51	0.53	104
Energy generation	107	103	0.37	0.36	290
Lipid and sterol metabolism	76	99	0.37	0.48	206
Mating response§	45	54	0.34	0.41	132
Meiosis§	56	56	0.38	0.38	147
Membrane fusion	15	16	0.38	0.40	40
Mitochondrial transcription	3	4	0.60	0.80	5
Mitosis	72	73	0.49	0.50	147
Nuclear-cytoplasmic transport	49	48	0.53	0.52	92
Nucleotide metabolism	26	35	0.27	0.37	95
Other	27	26	0.47	0.46	57
Other metabolism	62	71	0.32	0.37	192
Phosphate metabolism	8	9	0.31	0.35	26
Pol I transcription	14	14	0.47	0.47	30
Pol II transcription	148	158	0.39	0.41	384
Pol III transcription	15	19	0.38	0.49	39
Protein complex assembly	22	20	0.47	0.43	47
Protein degradation	86	90	0.47	0.49	183
Protein folding	33	42	0.36	0.46	91
Protein modification	99	106	0.40	0.43	246
Protein synthesis	99	106	0.27	0.28	373
Protein translocation	37	31	0.43	0.36	87
Recombination	29	39	0.33	0.44	88
RNA processing/modification	126	144	0.45	0.51	280
RNA splicing	44	50	0.38	0.43	116
RNA turnover	19	28	0.37	0.54	52
Signal transduction	54	55	0.40	0.41	135
Small molecule transport	138	144	0.34	0.35	407
Vesicular transport	136	146	0.45	0.48	305
		Average	0.40	0.44	

*Proportion of *S. cerevisiae* genes in the category with orthologs in *C. glabrata*. †Proportion of *S. cerevisiae* genes in the category with orthologs in *K. delphensis*. ‡Number of *S. cerevisiae* genes assigned to the category in the YPD database. §Lists of the mating response and meiosis genes identified in *C. glabrata* and *K. delphensis* are given in the additional data file.

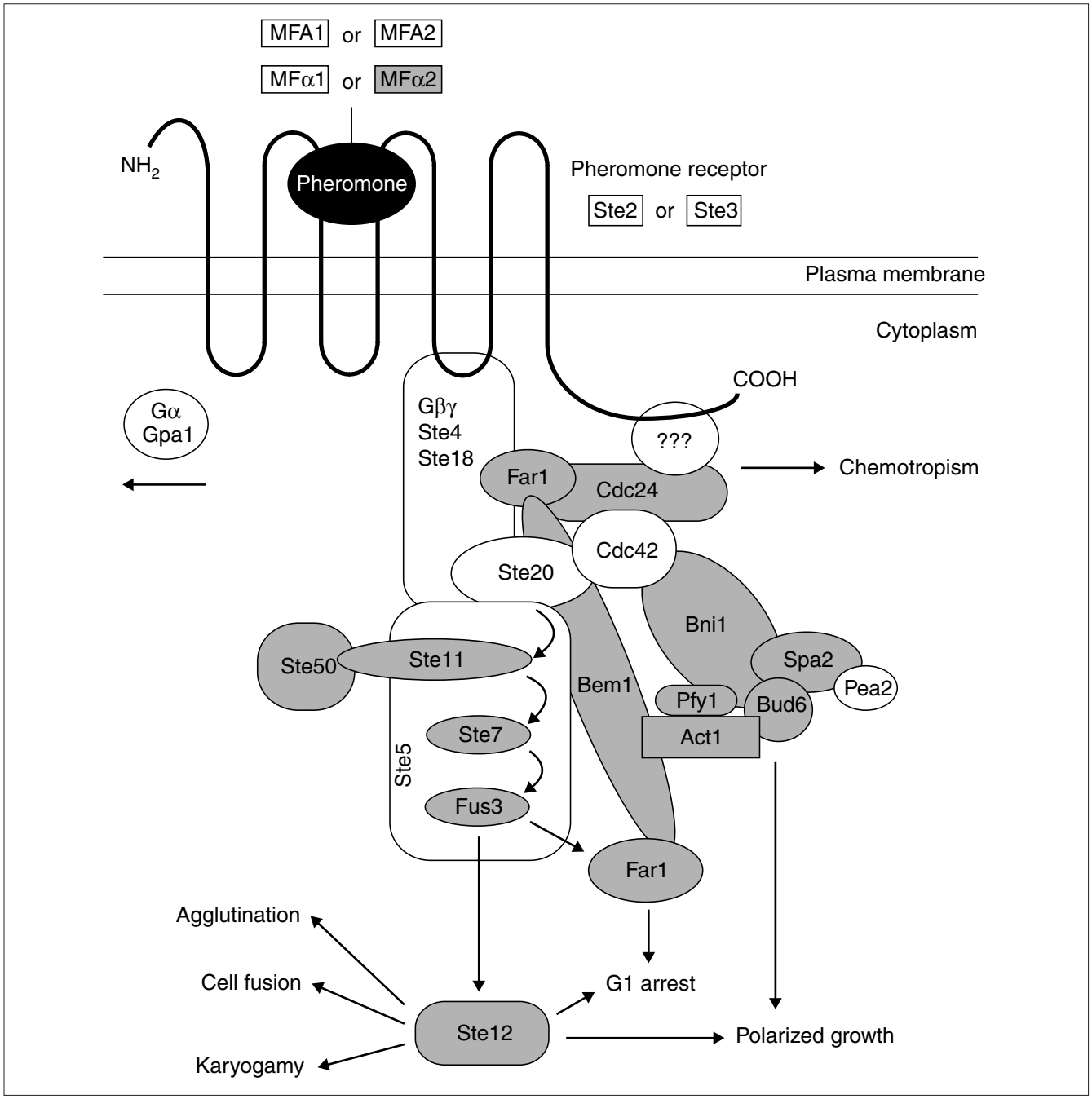

Figure 2
Model of the *S. cerevisiae* pheromone response pathway (adapted from [30]). Genes whose orthologs were identified in *C. glabrata* are indicated in gray.

meiotic recombination [47]. The presence of these genes suggests that critical events required for the unique process of reductional division during meiosis I, such as recombination and chromosome synapsis, occur in *C. glabrata*.

Similarly, we found orthologs of genes involved in the middle and late stages of meiosis. The middle-stage genes include *SPO1*, a phospholipase B homolog that promotes spindle-body duplication exclusively during meiosis [48], and *SPO22*, *CSM1* and *CSM3* which are less well characterized but show meiosis-specific expression with deletion mutants exhibiting varying degrees of chromosome mis-segregation [49]. *C. glabrata* also has a homolog of *SMK1*, which in *S. cerevisiae* encodes a MAP kinase involved in a sporulation-specific signal transduction cascade, necessary for proper spore morphogenesis and full expression of late

Table 2

Mating-specific *S. cerevisiae* genes with orthologs in *C. glabrata*

Gene	Function
MFALPHA2	Mating pheromone α-factor
STE13	Peptidase, α-factor processing
STE6	Exporter of **a**-factor
FIG4	Lipid phosphatase, pheromone induced
FAR1	Inhibitor of Cdc28-Cln1/2 kinase complexes
CDC24	GTP-GDP exchange factor for Cdc42
SGV1	Kinase, pheromone adaptation
AKR1	Negative regulator in the pheromone response pathway
FUS3	Kinase, activates Ste12 and Far1
KAR5	Required for nuclear membrane fusion during karyogamy
LSG1	Required for normal morphology, mating and sporulation
OPY2	Overproduction causes insensitivity to α-factor arrest
SAG1	α-Agglutinin

meiotic genes [50,51]. Another surprising finding is an ortholog of *DIT1*, which is required for dityrosine biosynthesis [52]. In yeasts, the dimerized amino acid dityrosine has only been found on the outer surface layer of the ascospore wall but not in vegetative cell walls [53]. The maintenance of these genes in *C. glabrata* is highly indicative of an ability to sporulate.

Discussion

The results from survey sequencing of the *C. glabrata* and *K. delphensis* genomes show that they have very similar repertoires of genes in all categories of cellular roles (Table 1), including mating and meiosis. More detailed analysis showed that *C. glabrata* has orthologs of at least 31 genes that in *S. cerevisiae* have no known functions apart from mating or meiosis (Tables 2 and 3), and that it has intact genes for α-factor and a putative mating-type locus. Together, these results suggest that *C. glabrata* has an undiscovered sexual cycle. Although it is possible that future studies in *S. cerevisiae* will discover new roles for some of these genes other than in mating or meiosis, it seems more reasonable to propose that *C. glabrata* has a sexual cycle than to propose that it is asexual and that all 31 genes have been preserved in its genome because they have undiscovered roles in nonsexual processes. The compact nature of yeast genomes makes it unlikely that all the sexual genes we identified by GSS are pseudogenes, and the MFALPHA2, MATALPHA1 and MATALPHA2 genes certainly are not pseudogenes.

Even though we did not find orthologs of some other genes that are central to mating (for example, *STE2*/*STE3* and *MFA1* [54]) or meiosis (for example, *NDT80*), it should be noted that the genome was only surveyed to 0.2x sequence coverage, so that only 1,941 genes (roughly one-third of the expected number of genes in the genome) were detected in this study. It is interesting that *C. glabrata* has orthologs of *MFALPHA2* and *IME1*, which were not found in *C. albicans* [5]. However, this is possibly just due to extensive sequence divergence, rather than gene loss, in *C. albicans*. The complete sequence of *C. glabrata* Ime1 has only 27% amino-acid sequence identity to *S. cerevisiae* Ime1. Very recently, a candidate *C. albicans* pheromone gene was described [55,56].

Despite the evolutionary distance between them (Figure 1) and gross differences such as the fact that *C. glabrata* is haploid whereas *C. albicans* is diploid, there are remarkable parallels between the evolution of *C. glabrata* and *C. albicans*. The two species have evolved independently from sexually reproducing yeast ancestors that are unlikely to have been pathogenic, because the majority of lineages in this phylogenetic group are not pathogenic (Figure 1). Thus, in becoming human pathogens, both *C. glabrata* and *C. albicans* have adopted a lifestyle where the sexual phase is hidden. Miller and Johnson [4] proposed that, in *C. albicans*, this is because the white (asexual) form survives better in the mammalian host. By analogy, it is tempting to speculate that one of the forms produced by phenotypic switching in *C. glabrata* [14,15] might be mating-competent. It is interesting to note that in other species of *Candida* for which sexual forms (teleomorphs) have been identified, such as *Candida krusei*, the form isolated in clinical settings is invariably the asexual one [7,8]. We hypothesize that having a sexual cycle may be essential for the long-term evolutionary viability of all yeast species because of the evolutionary advantages conferred by recombination [57,58], but that mating confers a disadvantage on those individuals that mate because they are somehow more vulnerable to the host's immune response. The result of these opposing forces seems to have been the evolution of cryptically sexual pathogens in which the frequency of mating in the population has been reduced to a low but optimal level.

Materials and methods

The type strains of *C. glabrata* (CBS 138) and *K. delphensis* (CBS 2170) were purchased from the Centraalbureau voor Schimmelcultures (Utrecht, Netherlands). High-molecular-weight DNA was prepared using standard procedures and partially digested with *Sau*3AI. Fragments in the size range 7-15 kb were isolated and used to make random genomic libraries in the low copy number *Escherichia coli* vector pMCL210 (AGOWA, Germany). Sequences were obtained from both ends of the insert for 2,939 *C. glabrata* (CG) and 2,974 *K. delphensis* (KD) clones, with a further 449 CG and 290 KD clones sequenced successfully from one end only. The average lengths of sequence reads used for analysis were 548 base-pairs (bp) (CG) and 515 bp (KD). Representation of

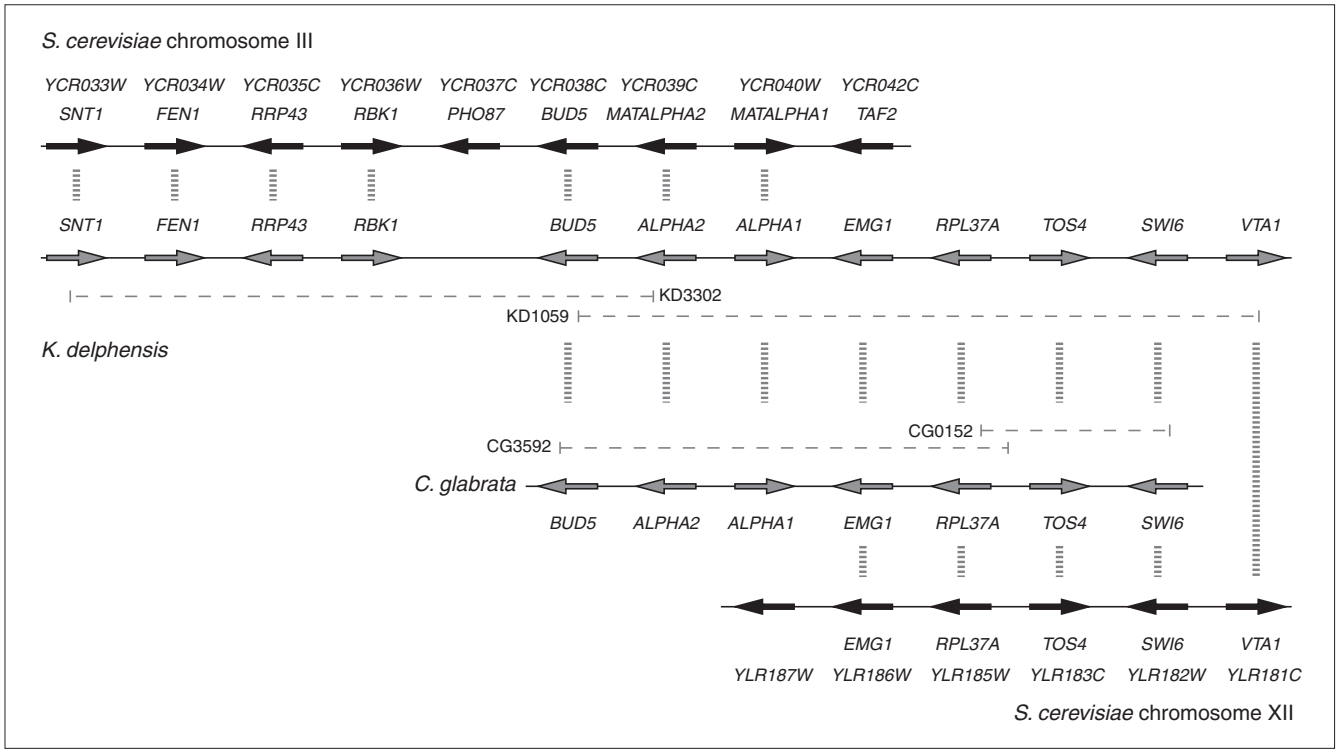

Figure 3
Gene organization (not to scale) around the *MAT* locus of *S. cerevisiae* and the putative *MAT* loci of *K. delphensis* and *C. glabrata*. Dashed horizontal lines indicate the extents of the clones sequenced.

mitochondrial DNA in the libraries appeared to be very low, even though we did not take any specific measures to exclude it. After data analysis, the inserts of selected plasmid clones were sequenced completely on both strands by primer walking. Sequences have been deposited in GenBank with accession numbers BZ293019-BZ299345 (*C. glabrata* GSS), BZ299346-BZ305583 (*K. delphensis* GSS), AY181247-AY181250 (complete sequences of *MAT* and *MFALPHA2* loci), and AJ535506 (*C. glabrata* IME1).

Trace files from the random genomic clones were base-called using PHRED [59,60] and vector clipping was done by CROSS_MATCH. Clipped sequences shorter than 100 bp were discarded from the dataset. Contigs were assembled using PHRAP with the original trace quality files and are available on request. Contigs were filtered to eliminate mitochondrial DNA as well as known repetitive sequences such as rDNA and Ty elements, which may cause misassembly. This was achieved using the contigs as queries in BLASTN and BLASTX searches [61] against the relevant *S. cerevisiae* sequences. Any contig with a significant expect value (E-value) of < 1e-5 was excluded from ortholog assignment.

We used a recent annotation of the *S. cerevisiae* genome [62], containing 5,583 annotated proteins (excluding 'very hypothetical' proteins and pseudogenes), downloaded from [63]. Orthologs of these genes in the *C. glabrata* and *K. delphensis* filtered contigs were identified using FASTY version 3.4t05 [64], after a low complexity masking step using the NSEG and PSEG programs [65]. For any gene-sized region in a contig, we considered the *S. cerevisiae* protein with the strongest FASTY hit to be the ortholog, provided that the E-value for this hit was < 1e-5 and was more than 1e3 times lower than the E-value for the second-best hit to the same region of the contig. For each ortholog identified, its function in *S. cerevisiae* was examined using the 'cellular role' categories of the Yeast Proteome Database (YPD) of the Incyte BioKnowledge Library [66]. It should be noted that in this functional annotation scheme, a single protein can be classified into more than one cellular role. While we adhered strictly to the YPD classification of genes in our initial analyses (Table 1), we also discovered some genes that can be reclassified on the basis of the literature (for example, *SPS2* was classified under 'differentiation' in YPD but was found to be meiosis-specific on further examination) and included these reclassified genes in Tables 2 and 3.

Among the full set of contigs, those that contained rDNA sequences were identified. These were used in conjunction with publicly available rDNA sequences, isolated from the *C. glabrata* and *K. delphensis* type strains, to create the complete rDNA repeating unit for both species. The two

Table 3

Meiosis-specific *S. cerevisiae* genes with orthologs in *C. glabrata*

Gene	Function
IME1	Master regulatory switch of meiosis
MCK1	Kinase required for maximal *IME1* expression
RIM9	Stimulates expression of *IME1*
IME2	Kinase, stimulates meiotic gene expression
UME6	Transcriptional regulator of meiotic genes
RIM4	Activation of sporulation-specific genes
IDS2	Ime2-dependent signaling protein
MUM2	Required for premeiotic DNA synthesis and sporulation
HOP2	Prevents nonhomologous synapsis in meiosis
MSH4	Meiosis-specific MutS homolog, recombination
SPO1	Required for spindle body duplication
SPO22	Required for meiotic chromosome segregation
CSM1	Required for meiotic chromosome segregation
CSM3	Required for meiotic chromosome segregation
SMK1	Sporulation-specific MAP kinase
DIT1	Dityrosine biosynthesis enzyme
SPO75	Required for spore and ascus formation
SMA2	Required for proper prospore membrane formation
SPS2[*]	Putative prospore membrane protein

[*]We are not certain whether plasmid CG4206 contains an ortholog of *SPS2* or the similar gene *YCL048W*, but we have included it in the table because both *SPS2* and *YCL048W* encode prospore membrane proteins induced during sporulation [70].

rDNA sequences were combined with those from 14 other hemiascomycete yeast species used in our previous study [67] and aligned using T_COFFEE [68]. Phylogenetic trees were constructed using the NJ method as implemented in CLUSTALW [69].

Additional data files

Tables of the mating response and meiosis genes identified in *C. glabrata* and *K. delphensis* are available as one additional data file with the online version of this paper.

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