

Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes

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Candida species are the most common cause of opportunistic fungal infection worldwide. Here we report the genome sequences of six *Candida* species and compare these and related pathogens and non-pathogens. There are significant expansions of cell wall, secreted and transporter gene families in pathogenic species, suggesting adaptations associated with virulence. Large genomic tracts are homozygous in three diploid species, possibly resulting from recent recombination events. Surprisingly, key components of the mating and meiosis pathways are missing from several species. These include major differences at the mating-type loci (*MTL*); *Lodderomyces elongisporus* lacks *MTL*, and components of the $\alpha 1/\alpha 2$ cell identity determinant were lost in other species, raising questions about how mating and cell types are controlled. Analysis of the CUG leucine-to-serine genetic-code change reveals that 99% of ancestral CUG codons were erased and new ones arose elsewhere. Lastly, we revise the *Candida albicans* gene catalogue, identifying many new genes.

Four *Candida* species, *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, together account for ~95% of identifiable *Candida* infections¹. Although *C. albicans* is still the most common causative agent, its incidence is declining and the frequency of other species is increasing. Of these, *C. parapsilosis* is a particular problem in neonates, transplant recipients and patients receiving parenteral nutrition; *C. tropicalis* is more commonly associated with neutropenia and malignancy. Other *Candida* species, including *C. krusei*, *C. lusitaniae* and *C. guilliermondii*, account for <5% of invasive candidiasis. Almost all *Candida* species, with the exceptions of *C. glabrata* and *C. krusei*, belong in a single *Candida* clade (Fig. 1) characterized by the unique translation of CUG codons as serine rather than leucine². Within this, haploid and diploid species occupy two separate sub-clades (Fig. 1).

To determine the genetic features underlying their diversity of biology and pathogenesis, we sequenced six genomes from the *Candida* clade (Fig. 1). These include a second sequenced isolate of

C. albicans (WO-1) characterized for white–opaque switching, a phenotypic change that correlates with host specificity and mating^{3,4}. We also sequenced the major pathogens *C. tropicalis* and *C. parapsilosis*; *L. elongisporus*, a close relative of *C. parapsilosis* recently identified as a cause of bloodstream infection⁵; and two haploid emerging pathogens, *C. guilliermondii* and *C. lusitaniae*. We compared these with the previously sequenced *C. albicans* strain (SC5314)^{6–8}, *Debaryomyces hansenii*⁹, a marine yeast rarely associated with disease, and nine species from the related *Saccharomyces* clade (Fig. 1). These species span a wide evolutionary range and show large phenotypic differences in pathogenicity and mating, allowing us to study the genomic basis for these traits.

Genome sequence and comparative annotation

We found enormous variation in genome size and composition between the *Candida* genomes sequenced (Table 1). Each genome assembly displayed high continuity, ranging from nine to 27 scaffolds

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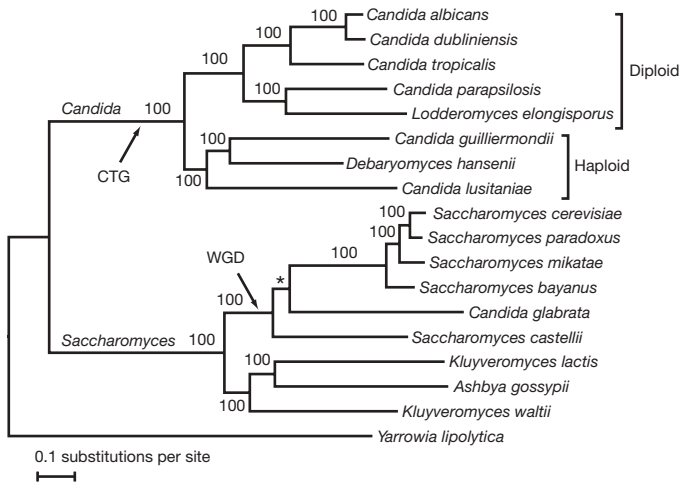


Figure 1 | Phylogeny of sequenced *Candida* and *Saccharomyces* clade species. Tree topology and branch lengths were inferred with MRBAYES (Supplementary Information, section S5). Posterior probabilities are indicated for each branch. The asterisk marks a branch that was constrained on the basis of syntenic conservation⁴⁰.

(Supplementary Table 1). Scaffold number and size largely match pulsed-field gel electrophoresis estimates for all genomes, and telomeric repeat arrays are linked to the ends of nearly all large scaffolds (Supplementary Information, section S2). Genome size ranges from 10.6 to 15.5 megabases (Mb), a difference of nearly 50%, with haploid species having smaller genomes. GC content ranges from 33% to 45% (Table 1). Transposable elements and other repetitive sequences vary in number and type between assemblies (Supplementary Information, section S6). Regions similar to the major repeat sequence (MRS) of *C. albicans* were found only in *C. tropicalis*, suggesting that MRS-associated recombination could contribute to the observed karyotypic variation among *C. tropicalis* strains¹⁰.

Despite the genome size and phenotypic variation among the species, the predicted numbers of protein-coding genes are very similar, ranging from 5,733 to 6,318 (Table 1). Even the small differences in gene number are not correlated with genome size; the smallest genome, *C. guilliermondii*, has more genes than the largest genome, *L. elongisporus*. Instead, genome size differences are explained by an approximately threefold variation in intergenic spacing (Table 1). Large syntenic blocks of conserved gene order were detected among the four diploid species and between two of the haploid species, *C. guilliermondii* and *D. hansenii* (Supplementary Fig. 5). Although syntenic blocks have been shuffled by local inversions and rearrangements, these have been primarily intrachromosomal as chromosome boundaries have been largely preserved across the diploid genomes (Supplementary Fig. 5).

Given the high conservation of protein-coding genes across the *Candida* clade, we used multiple alignments of the related genomes to revise the annotation of *C. albicans*. We identified 91 new or updated genes, of which 80% are specific to the *Candida* clade

(Supplementary Information, section S4). We also corrected existing annotations in *C. albicans*, revealing 222 dubious genes, and also identified 190 probable frame shifts and 36 nonsense sequencing errors in otherwise well-conserved genes (Supplementary Information, section S4). In each case, manual curation confirmed ~80% of these predictions.

Polymorphism in diploid genomes

To gain insights into the recent history of *C. albicans*, we compared the two diploid strains, SC5314 and WO-1, which belong to different population subgroups¹¹. Variation in the karyotype of these strains is primarily due to translocations at MRS sequences (ref. 12 and Supplementary Fig. 1). The two assemblies are largely co-linear with 12 inversions of 5–94 kilobases between them, except that in WO-1 some non-homologous chromosomes have recombined at the MRS (Supplementary Information, section S8). We found similar rates of single nucleotide polymorphisms (SNPs) within each strain (one SNP per 330–390 bases), and twice this rate between them, suggesting relatively recent divergence. Polymorphism rates in the other diploids range from one SNP per 222 bases in *L. elongisporus* and one SNP per 576 bases in *C. tropicalis* to a particularly low one SNP per 15,553 bases in *C. parapsilosis*, more than 70-fold lower than in the closely related *L. elongisporus*.

Notable regions of extended homozygosity are found in three of the four diploid genomes, which may reflect break-induced replication or recent passage through a parasexual or sexual cycle. *Candida albicans*, *C. tropicalis* and *L. elongisporus* each shows large chromosomal regions devoid of SNPs, extending up to ~1.2 Mb (Fig. 2 and Supplementary Figs 6–8). In contrast, the few SNPs in *C. parapsilosis* are randomly distributed across the genome (Supplementary Fig. 9a). A total of 4.3 Mb (30%) of the WO-1 assembly is homozygous for SNPs, approximately twice that found in SC5314 (Supplementary Information, section 7, and refs 7, 13, 14). There is at least one homozygous region per chromosome, none of which spans the predicted centromeres and only one of which starts at a MRS (Fig. 2). Whereas nearly all homogeneous regions are present at diploid levels and are therefore homozygous, WO-1 has lost one copy of a >300-kilobase region on chromosome 3 comprising nearly 200 genes (Supplementary Fig. 10). The pressure to maintain this region as homozygous in both strains is apparently high, as it is diploid but homozygous in SC5314.

Usage and evolution of CUG codons

All *Candida* clade species translate CUG codons as serine instead of leucine¹⁵. This genetic-code change altered the decoding rules of CUN codons in the *Candida* clade: whereas *Saccharomyces cerevisiae* uses two transfer RNAs, each of which translates two codons, *Candida* species use a dedicated tRNA_{CAG}^{Ser} for CUG codons and a single tRNA_{IAG}^{Leu} for CUA, CUC, and CUU codons, as inosine can base-pair with A, C and U (Fig. 3). This alteration in decoding rules forced the reduced usage of CUG—and also CUA, probably as a result of the weaker wobble—in *Candida* genes (Fig. 3a). CUU and CUC codons do not display the same bias for infrequent usage

Table 1 | *Candida* genome features

Species*	Genome size (Mb)	GC content (%)	No. of genes	Ave. gene size (bp)	Intergenic ave. (bp)	Ploidy	Pathogen†
<i>C. albicans</i> WO-1	14.4	33.5	6,159	1,444	921	diploid	++
<i>C. albicans</i> SC5314	14.3	33.5	6,107	1,468	858	diploid	++
<i>C. tropicalis</i>	14.5	33.1	6,258	1,454	902	diploid	++
<i>C. parapsilosis</i>	13.1	38.7	5,733	1,533	752	diploid	++
<i>L. elongisporus</i>	15.4	37.0	5,802	1,530	1,174	diploid	–
<i>C. guilliermondii</i>	10.6	43.8	5,920	1,402	426	haploid	+
<i>C. lusitaniae</i>	12.1	44.5	5,941	1,382	770	haploid	+
<i>D. hansenii</i>	12.2	36.3	6,318	1,382	550	haploid	–

bp, base pair.

**C. albicans* SC5314 assembly 21 and gene set dated 28 January 2008 downloaded from the *Candida* Genome Database; *D. hansenii* assembly from GenBank⁹. The remaining assemblies are reported as part of this work, and are available in GenBank and at the Broad Institute *Candida* Database website.

† Relative level of pathogen strength: ++, strong pathogen; +, moderate pathogen; –, rare pathogen.

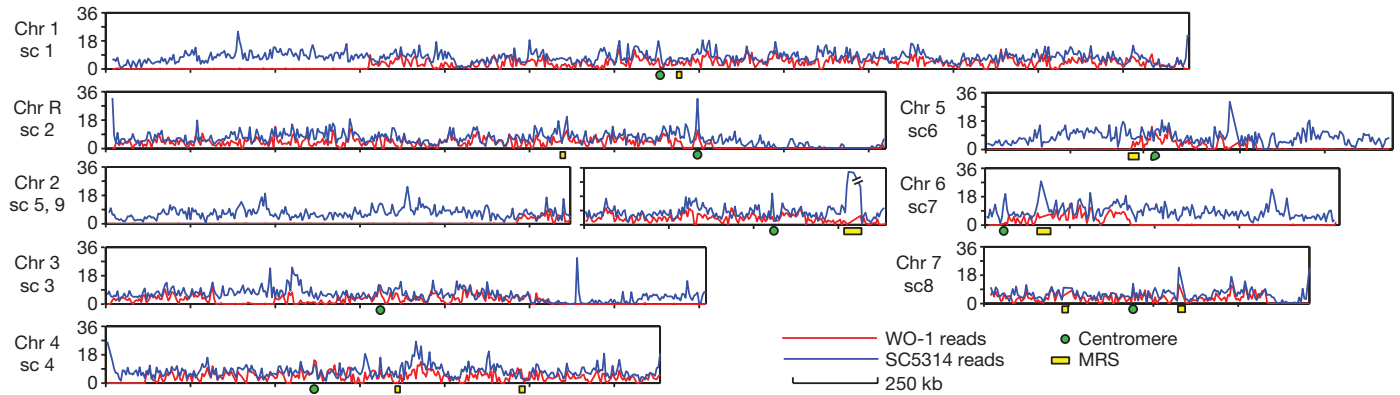


Figure 2 | *C. albicans* WO-1 is highly homozygous. Red lines show SNPs per kilobase, normalized by coverage, within WO-1, and blue lines show SNPs per kilobase between WO-1 and SC5314. Although both copies of chromosome 5 have rearranged at the MRS (yellow box) in WO-1, we show

this as a single chromosome to allow a haploid reference for polymorphism. Relative to SC5314, chromosomes 1, 4 and 6 have the opposite orientation (Supplementary Fig. 6). Chr, chromosome; sc, supercontig; kb, kilobase.

(Fig. 3b). An additional pressure influencing codon usage may be the GC content, as usage of leucine codons in *Candida* species is correlated with the percentage GC composition (Supplementary Table 11).

We also examined the evolutionary fate of ancestral CUG codons and the origin of new CUG codons (Supplementary Table 12). CUG codons in *C. albicans* almost never (1%) align opposite CUG codons in *S. cerevisiae*. Instead, CUG serine codons in *C. albicans* align primarily to *Saccharomyces* codons for serine (20%) and other hydrophilic residues (49%). CUG leucine codons in *S. cerevisiae* align primarily to leucine codons in *Candida* (50%) and to other hydrophobic-residue codons (30%). This suggests a complete functional replacement of CUG codons in *Candida*.

Gene family evolution

To identify gene families likely to be associated with *Candida* pathogenicity and virulence, we used a phylogenomic approach across

seven *Candida* and nine *Saccharomyces* genomes (Supplementary Information, section S10). Among 9,209 gene families, we identified 21 that are significantly enriched in the more common pathogens (Table 2). These include families encoding lipases, oligopeptide transporters and adhesins, which are all known to be associated with pathogenicity⁸, as well as poorly characterized families not previously associated with pathogenesis.

Three cell wall families are enriched in the pathogens: those encoding Hyr/Iff proteins (ref. 16), Als adhesins¹⁷ and Pga30-like proteins (Table 2 and Supplementary text S11). The Als family (family 17 in Supplementary Tables 22 and 23) in *C. albicans* is associated with virulence, and in particular with adhesion to host surfaces¹⁸, invasion of host cells¹⁹ and iron acquisition²⁰. All these families are absent from the *Saccharomyces* clade species and are particularly enriched in the more pathogenic species (Supplementary Table 18). All three families are highly enriched for gene duplications (Supplementary Table 19), including tandem clusters of two to six genes, and show high mutation rates (fastest 5% of families) (Supplementary Information, section S10d). This variable repertoire of cell wall proteins is likely to be of profound importance to the niche adaptations and relative virulence of these organisms.

Als¹⁷ and Hyr/Iff genes frequently contain intragenic tandem repeats, which modulate adhesion and biofilm formation in *S. cerevisiae*²¹ (Fig. 4 and Supplementary Figs 19 and 20). The sequence of intragenic tandem repeats is conserved at the protein level across species (Supplementary Figs 19 and 20). Two proteins contain both an Als domain and repeats characteristic of the Hyr/Iff family.

Candida clade pathogens show expansions of extracellular enzyme and transmembrane transporter families (Table 2 and Supplementary Table 22). These families are either not found in *Saccharomyces* (including amino-acid permeases, lipases and superoxide dismutases) or are present in *S. cerevisiae* but significantly expanded in pathogens (including phospholipase B, ferric reductases, sphingomyelin phosphodiesterases and GPI-anchored yapsin proteases, which have been linked to virulence in *C. glabrata*²²). Several groups of cell-surface transporters are also enriched (including oligopeptide transporters, amino-acid permeases and the major facilitator superfamily). Overall, these family expansions illustrate the importance of extracellular activities in virulence and pathogenicity. Genes involved in stress response are also variable between species (Supplementary Information, section S12).

C. albicans also showed species-specific expansion of some families, including two associated with filamentous growth, a leucine-rich repeat family and the Fgr6-1 family (Table 2). *Candida albicans* forms hyphae whereas *C. tropicalis* and *C. parapsilosis* produce only pseudohyphae, so these families may contribute to differences in hyphal growth.

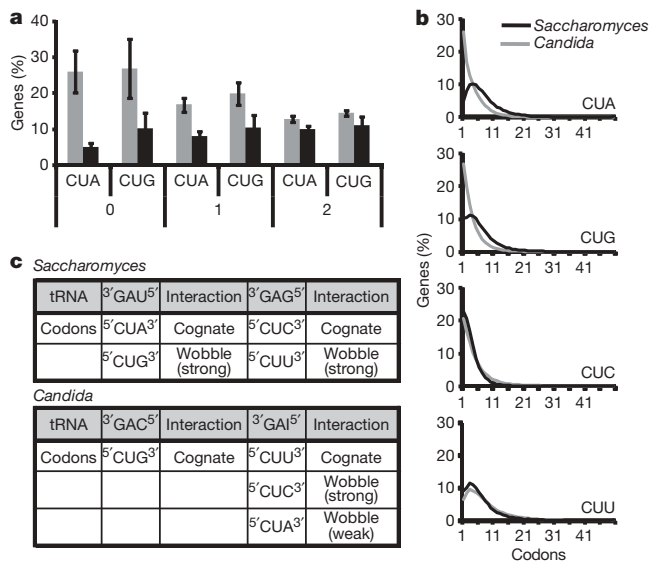


Figure 3 | Evolutionary effects of CUG coding. **a**, Average percentage of genes with zero, one or two CUG and CUA codons in *Candida* (grey bars) and *Saccharomyces* (black bars). Error bars, s.d. All differences are significant with $P \leq 0.0004$ (t -test, $N = 8$ *Candida* spp. and 6 *Saccharomyces* spp.) except for those in genes with two CUA codons ($P = 0.02$). ‘*Candida*’ and ‘*Saccharomyces*’ here refer to the CTG and WGD clades in Fig. 1, but including *Pichia stipitis* and excluding *C. dubliniensis*. **b**, CUN codon usage for all codon counts. **c**, Decoding rules for CUN codons in *Saccharomyces* and *Candida*.

Table 2 | Gene families enriched in pathogenic *Candida* spp.

No.	Annotation	Pathogen genes	Non-pathogen genes	P val.	Dup.	Loss	Gene rate	<i>C. alb.</i>	<i>C. tro.</i>	<i>C. par.</i>	<i>L. elo.</i>	<i>C. gui.</i>	<i>C. lus.</i>	<i>D. han.</i>	<i>C. gla.</i>	Yeast (ave.)
1	GPI family 18 (Hyr/Iff-like)	56	10	1.4×10^{-16}	52	11	16.2	11	18	17	9	3	7	1	0	0.0
2	Leucine-rich repeat (Ifa/Fgr38-like)	34	0	4.2×10^{-16}	32	5	18.3	33	1	0	0	0	0	0	0	0.0
3	Ferric reductase family	45	10	1.9×10^{-12}	30	25	2.5	12	19	7	7	3	4	2	0	0.1
4	Reductase family	43	11	3.2×10^{-11}	31	30	2.3	7	12	9	6	13	2	4	0	0.1
5	GPI family 17 (Als-like adhesins)	31	5	4.4×10^{-10}	29	4	20.5	8	16	5	4	2	0	1	0	0.0
6	GPI family 13 (Pga30-like)	34	7	5.0×10^{-10}	25	5	14.8	12	14	6	6	1	1	1	0	0.0
7	Unclassified	20	0	9.0×10^{-10}	13	3	15.9	9	9	0	0	2	0	0	0	0.0
8	Cell wall mannoprotein biosynthesis	38	18	7.2×10^{-7}	19	34	2.1	8	7	8	8	11	4	9	0	0.1
9	Major facilitator transporters	25	7	9.2×10^{-7}	14	17	2.0	3	3	7	3	10	2	4	0	0.0
10	Oligopeptide transporters	31	13	2.2×10^{-6}	23	11	6.7	6	9	9	4	4	3	1	0	0.9
11	Unclassified	25	9	6.3×10^{-6}	15	6	11.1	7	9	3	5	3	1	4	2	0.2
12	Amino-acid permeases	27	11	7.7×10^{-6}	11	18	1.7	6	6	6	4	6	3	6	0	0.1
13	Sphingomyelin phosphodiesterases	18	5	3.2×10^{-5}	11	9	7.4	4	5	4	2	3	2	1	0	0.2
14	Fgr6 family (filamentous growth)	12	1	3.3×10^{-5}	7	1	14.5	8	1	1	0	1	1	1	0	0.0
15	Secreted lipases	20	7	4.6×10^{-5}	17	8	9.6	10	5	4	4	1	0	3	0	0.0
16	Cytochrome P450 family	34	21	5.5×10^{-5}	23	22	6.0	6	8	10	7	5	4	6	1	1.0
17	Amino-acid permeases	16	4	5.6×10^{-5}	14	10	1.5	2	3	6	3	2	3	1	0	0.0
18	Zinc-finger transcription factors	31	18	6.2×10^{-5}	17	14	12.3	5	8	7	7	7	4	11	0	0.0
19	Unclassified	13	2	6.3×10^{-5}	8	0	8.1	3	1	6	1	2	1	1	0	0.0
20	Predicted transmembrane family	17	5	7.2×10^{-5}	9	2	7.5	4	4	5	3	3	1	2	0	0.0
21	Unclassified secreted family	20	8	1.1×10^{-4}	7	6	9.3	4	4	6	4	4	2	4	0	0.0

Pathogen genes: total genes in family for *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitanae* and *C. glabrata*. Non-pathogen genes: total genes in family for *L. elongisporus*, *D. hansenii* and all *Saccharomyces* clade species (Fig. 1) except *C. glabrata*. P val., P value of the hypergeometric test; all families shown above have a false discovery rate of less than 0.05 (Supplementary Information, section S10c). Dup., duplications; Loss, losses (Supplementary Information, section S10b). Gene rate: average mutation rate for each family (Supplementary Information, section S10d); the average gene rate across all families is 5.8. Yeast (ave.): average count for all *Saccharomyces* species. GPI, glycosyl phosphatidylinositol.

We identified 64 families showing positive selection in the highly pathogenic *Candida* species (Supplementary Table 32). These are highly enriched for cell wall, hyphal, pseudohyphal, filamentous growth and biofilm functions (Supplementary Information, section S13). Six of the families have been previously associated with pathogenesis, including that of *ERG3*, a C-5 sterol desaturase essential for ergosterol biosynthesis, for which mutations can cause drug resistance²³.

Structure of the *MTL* locus

Pathogenic fungi may have limited their sexual cycles to maximize their virulence²⁴, and the sequenced *Candida* species show tremendous diversity in their apparent abilities to mate. Among the four

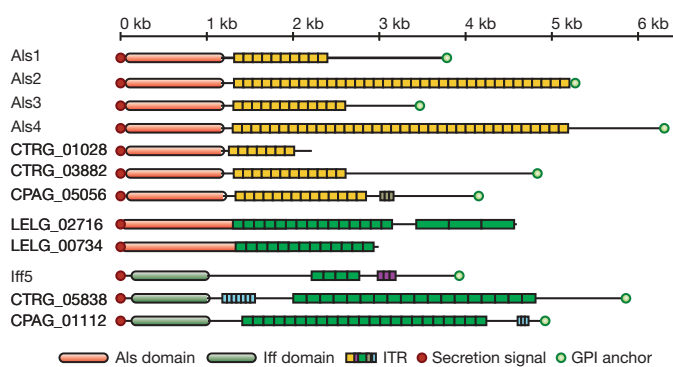


Figure 4 | Conserved domains of Als and Hyr/Iff cell wall families. The amino-terminal Als domain (red) and Hyr/Iff domain (green) are shown as ovals. Intragenic tandem repeats (ITRs; see Supplementary Information, section S11) are shown as rectangles, coloured to represent similar amino-acid sequences.

diploids, *C. albicans* has a parasexual cycle (mating of diploid cells followed by mitosis and chromosome loss instead of meiosis²⁵), *L. elongisporus* has been described as sexual and homothallic (self-mating)²⁶, whereas *C. tropicalis* and *C. parapsilosis* have never been observed to mate. Among the three haploids, *C. guilliermondii* and *C. lusitanae* are heterothallic (cross-mating only) and have a complete sexual cycle, whereas *D. hansenii* is haploid and homothallic²⁷ (Supplementary Information, section 14c).

To understand the genomic basis for this diversity, we studied the *Candida MTL* locus, which determines mating type, similar to the *MAT* locus in *S. cerevisiae*. In both *C. albicans* and *S. cerevisiae*, the mating locus has two idiomorphs, **a** and α , encoding the regulators **a1**, $\alpha1$ and $\alpha2$, respectively. *Candida albicans MTLa* also encodes **a2**, and both idiomorphs in this species contain alleles of three additional genes without known roles in mating: *PAP*, *OBP* and *PIK*²⁸. The *MTL α* and *MTLa* genes, alone or in combination, specify one of the three possible cell-type programs (**a** haploid, α haploid, **a**/ α diploid). In *C. albicans*, the alpha-domain protein $\alpha1$ activates α -specific mating genes, the high-mobility group (HMG) factor **a2** activates **a**-specific mating genes, and the **a1**/ $\alpha2$ homeodomain heterodimer represses mating genes in **a**/ α cells^{29,30}.

Despite extended conservation of the genomic context flanking the mating-type locus, there is great variability in *MTL* gene content (Fig. 5). *MTLa1* has become a pseudogene in *C. parapsilosis*³¹, and is probably a recent loss because target genes retain predicted **a1**/ $\alpha2$ binding sites (Supplementary Information, section S14). *MTL $\alpha2$* is missing in both *C. guilliermondii* and *C. lusitanae* (J. L. Reedy, A. Floyd and J. Heitman, submitted). A fused mating-type locus containing both **a** and α genes is found in *D. hansenii* and *Pichia stipitis*^{32,33}.

Most surprisingly, all four mating-type genes are missing in *L. elongisporus*. It contains a site syntenic to *MTLa* in other species, but this contains only 508 base pairs of apparently non-coding DNA,

Assemblies, gene sets, and single nucleotide polymorphisms are available in GenBank and at the Broad Institute *Candida* Database website (http://www.broad.mit.edu/annotation/genome/candida_group/MultiHome.html). The Broad Institute website provides search, visualization, BLAST and download of assemblies and gene sets. Gene families can be accessed by searching either for individual genes or with family identifiers (CF#####). The *C. parapsilosis* assembly is also available at the Wellcome Trust Sanger Institute website (<http://www.sanger.ac.uk/sequencing/Candida/parapsilosis/>). The revised annotation of *C. albicans* (SC5314) is available at the *Candida* Genome Database (www.candidagenome.org).

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Author Information Assemblies reported here have been deposited in GenBank under the following project accession numbers: AAFO00000000 (*C. albicans* WO-1), AAFN00000000 (*C. tropicalis*), AAPO00000000 (*L. elongisporus*), AAFM00000000 (*C. guilliermondii*), AAF000000000 (*C. lusitaniae*), CABE01000001–CABE01000024 (*C. parapsilosis*). Reprints and permissions information is available at www.nature.com/reprints. This paper is distributed under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence, and is freely available to all readers at www.nature.com/nature. Correspondence and requests for materials should be addressed to C.A.C. (cuomo@broad.mit.edu), M.K. (manoli@mit.edu) or G.B. (geraldine.butler@ucd.ie).