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

Geraldine Butler[1], Matthew D. Rasmussen[2], Michael F. Lin[2,3], Manuel A. S. Santos[4], Sharadha Sakthikumar[3], Carol A. Munro[5], Esther Rheinbay[2,6], Manfred Grabherr[3], Anja Forche[7], Jennifer L. Reedy[8], Ino Agrafioti[9], Martha B. Arnaud[10], Steven Bates[11], Alistair J. P. Brown[5], Sascha Brunke[12], Maria C. Costanzo[10], David A. Fitzpatrick[1], Piet W. J. de Groot[13], David Harris[14], Lois L. Hoyer[15], Bernhard Hube[12], Frans M. Klis[13], Chinnappa Kodira[3]†, Nicola Lennard[14], Mary E. Logue[1], Ronny Martin[12], Aaron M. Neiman[16], Elissavet Nikolaou[5], Michael A. Quail[14], Janet Quinn[17], Maria C. Santos[4], Florian F. Schmitzberger[10], Gavin Sherlock[10], Prachi Shah[10], Kevin A. T. Silverstein[18], Marek S. Skrzypek[10], David Soll[19], Rodney Staggs[18], Ian Stansfield[5], Michael P. H. Stumpf[9], Peter E. Sudbery[20], Thyagarajan Srikantha[19], Qiandong Zeng[3], Judith Berman[7], Matthew Berriman[14], Joseph Heitman[8], Neil A. R. Gow[5], Michael C. Lorenz[21], Bruce W. Birren[3], Manolis Kellis[2,3]* & Christina A. Cuomo[3]*

***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 a1/α2 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* infections[1]. 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 leucine[2]. Within this, haploid and diploid species occupy two separate subclades (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 infection[5]; 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

[1]UCD School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland. [2]Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, Massachusetts 02139, USA. [3]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA. [4]Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal. [5]School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK. [6]Bioinformatics Program, Boston University, Boston, Massachusetts 02215, USA. [7]Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota 55455, USA. [8]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA. [9]Centre for Bioinformatics, Imperial College London, Wolfson Building, South Kensington, London SW7 2AY, UK. [10]Department of Genetics, Stanford University Medical School Stanford, California 94305-5120, USA. [11]School of Biosciences, University of Exeter, Exeter EX4 4QD, UK. [12]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, D-07745 Jena, Germany. [13]Swammerdam Institute for Life Sciences, University of Amsterdam, 1090 GB Amsterdam, The Netherlands. [14]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK. [15]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61802, USA. [16]Department of Biochemistry and Cell Biology, SUNY Stony Brook, Stony Brook, New York 11794, USA. [17]Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK. [18]Biostatistics and Bioinformatics Group, Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota 55455, USA. [19]Department of Biology, The University of Iowa, Iowa City, Iowa 52242, USA. [20]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK. [21]Department of Microbiology and Molecular Genetics, The University of Texas Health Science Center at Houston, Houston, Texas 77030, USA. †Present address: 454 Life Sciences, 20 Commercial Street, Branford, Connecticut 06405, USA.
*These authors contributed equally to this work.

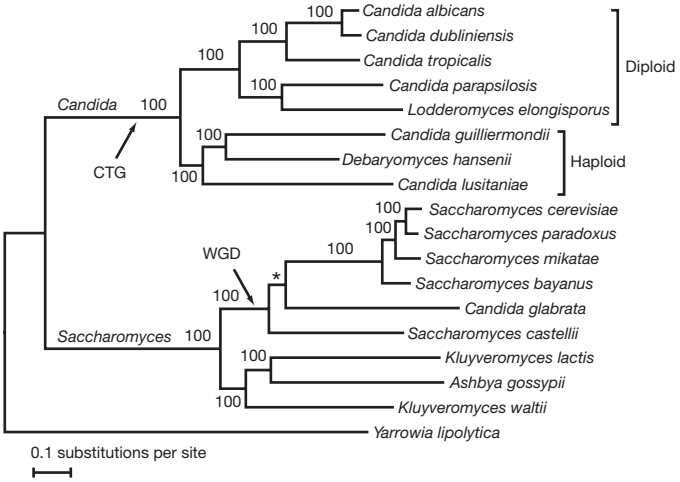


Figure 1 | Phylogeny of sequenced *Candida* and *Saccharomyces* clade species. Tree topology and branch lengths were inferred with MRBAYES (Supplementary Information, section S5). Posterior probabilities are indicated for each branch. The asterisk marks a branch that was constrained on the basis of syntenic conservation[40].

(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* strains[10].

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 synteny 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 subgroups[11]. 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 homogenous in SC5314.

Usage and evolution of CUG codons

All *Candida* clade species translate CUG codons as serine instead of leucine[15]. 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†
C. albicans WO-1	14.4	33.5	6,159	1,444	921	diploid	++
C. albicans SC5314	14.3	33.5	6,107	1,468	858	diploid	++
C. tropicalis	14.5	33.1	6,258	1,454	902	diploid	++
C. parapsilosis	13.1	38.7	5,733	1,533	752	diploid	++
L. elongisporus	15.4	37.0	5,802	1,530	1,174	diploid	−
C. guilliermondii	10.6	43.8	5,920	1,402	426	haploid	+
C. lusitaniae	12.1	44.5	5,941	1,382	770	haploid	+
D. hansenii	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 GenBank[9]. 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.

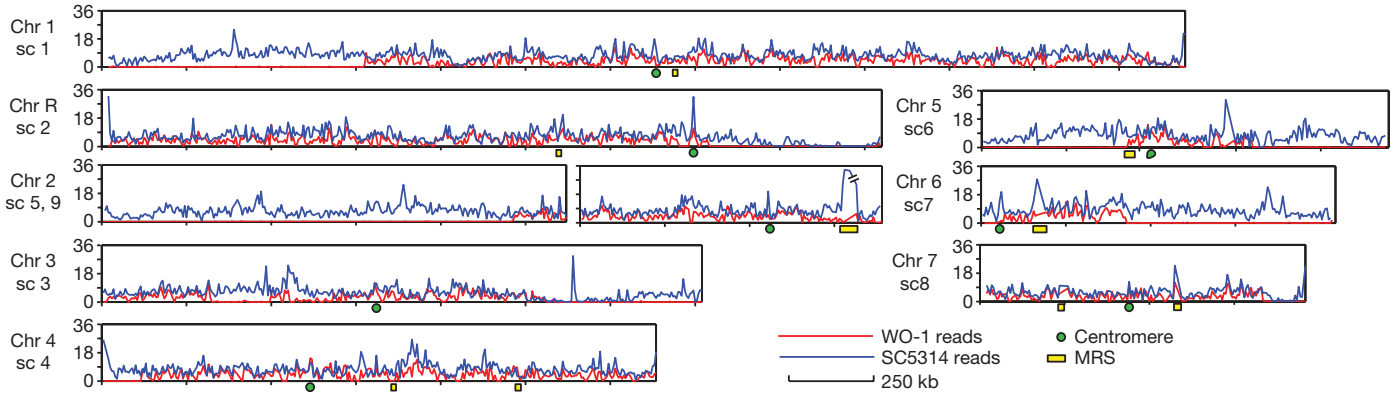


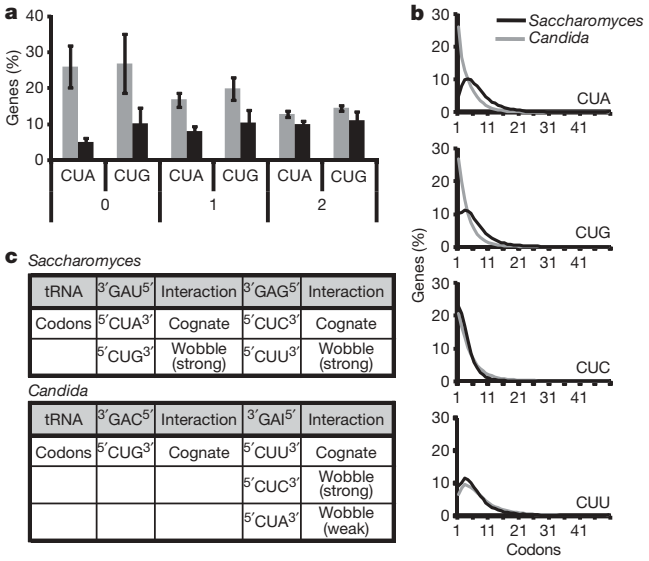
Figure 2 | *C. albicans* WO-1 is highly homozygous. Red lines show SNPs per kilobase, normalized by coverage, within WO-1, and blue lines show SNPs per kilobase between WO-1 and SC5314. Although both copies of chromosome 5 have rearranged at the MRS (yellow box) in WO-1, we show this as a single chromosome 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 pathogenicity[8], as well as poorly characterized families not previously associated with pathogenesis.



tRNA	3′GAU5′	Interaction	3′GAC5′	Interaction
Codons	5′CUA3′	Cognate	5′CUC3′	Cognate
	5′CUG3′	Wobble (strong)	5′CUU3′	Wobble (strong)

tRNA	3′GAC5′	Interaction	3′GAI5′	Interaction
Codons	5′CUG3′	Cognate	5′CUU3′	Cognate
			5′CUC3′	Wobble (strong)
			5′CUA3′	Wobble (weak)

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*.

Three cell wall families are enriched in the pathogens: those encoding Hyr/Iff proteins (ref. 16), Als adhesins[17] 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 surfaces[18], invasion of host cells[19] and iron acquisition[20]. 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.

Als[17] 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.

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

No.	Annotation	Pathogen genes	Non-pathogen genes	*P* val.	Dup.	Loss	Gene rate	*C. alb.*	*C. tro.*	*C. par.*	*L. elo.*	*C. gui.*	*C. lus.*	*D. han.*	*C. gla.*	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. lusitaniae* 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 resistance[23].

Structure of the *MTL* locus

Pathogenic fungi may have limited their sexual cycles to maximize their virulence[24], and the sequenced *Candida* species show tremendous diversity in their apparent abilities to mate. Among the four diploids, *C. albicans* has a parasexual cycle (mating of diploid cells followed by mitosis and chromosome loss instead of meiosis[25]), *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. lusitaniae* are heterothallic (cross-mating only) and have a complete sexual cycle, whereas *D. hansenii* is haploid and homothallic[27] (Supplementary Information, section 14c).

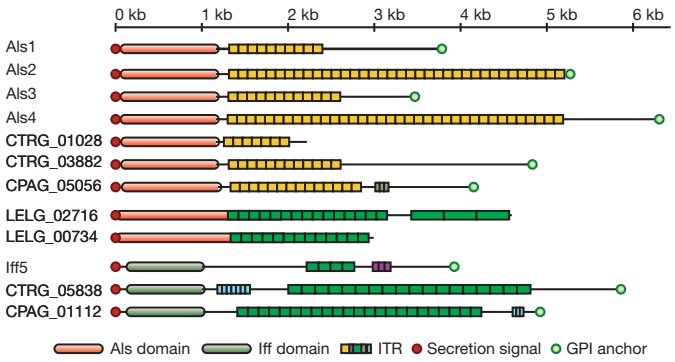


Figure 4 | Conserved domains of Als and Hyr/Iff cell wall families. The amino-terminal Als domain (red) and Hyr/Iff domain (green) are shown as ovals. Intragenic tandem repeats (ITRs; see Supplementary Information, section S11) are shown as rectangles, coloured to represent similar amino-acid sequences.

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 **a**1, α1 and α2, respectively. *Candida albicans MTL**a*** also encodes **a**2, 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 *MTL**a*** 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 α1 activates α-specific mating genes, the high-mobility group (HMG) factor **a**2 activates **a**-specific mating genes, and the **a**1/α2 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). *MTL**a**1* has become a pseudogene in *C. parapsilosis*[31], and is probably a recent loss because target genes retain predicted **a**1/α2 binding sites (Supplementary Information, section S14). *MTL*α*2* is missing in both *C. guilliermondii* and *C. lusitaniae* (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 *MTL**a*** in other species, but this contains only 508 base pairs of apparently non-coding DNA,

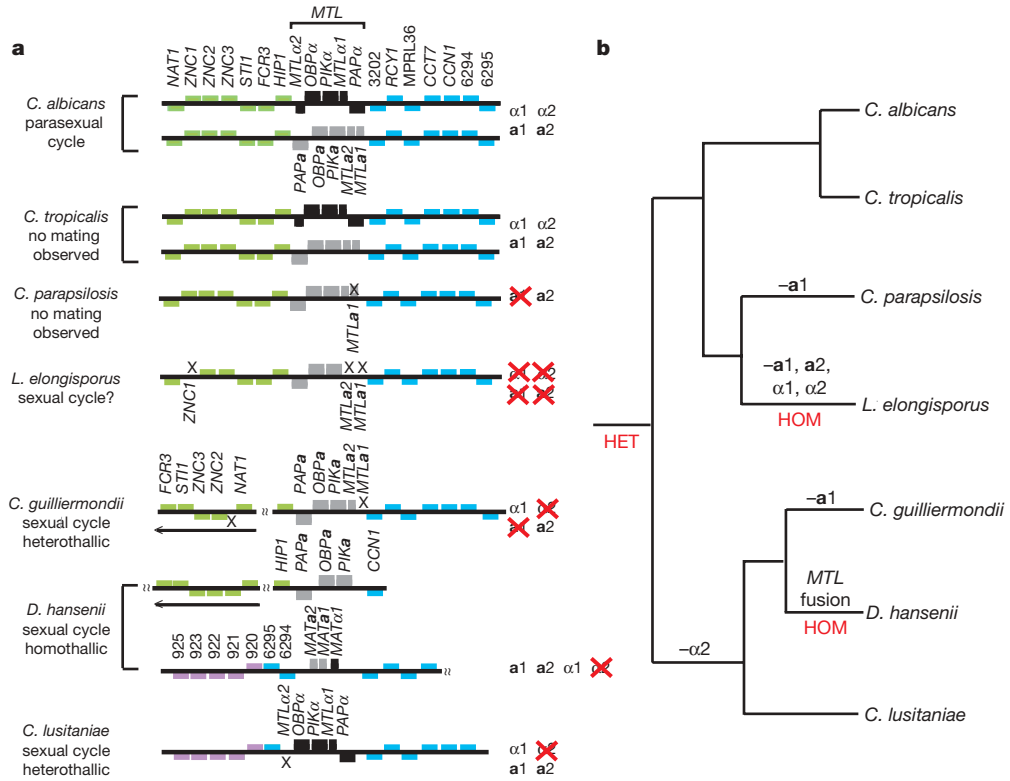


Figure 5 | Organization of *MTL* loci in the *Candida* clade. a, *MTLa*-specific genes are shown in grey, *MTLα*-specific genes are shown in black and other orthologues are shown in colour. Two idiomorphs from *C. albicans* and *C. tropicalis* are shown. Arrows indicate inversions relative to *C. albicans*. Crosses show gene losses and the *C. parapsilosis MTLa1* pseudogene. There is no *MTL* locus in *L. elongisporus*; gene order around the *PAPa*, *OBPa* and *PIKa* genes is shown. For *C. guilliermondii* and *C. lusitaniae*, the genome project sequenced one idiomorph; the second was obtained by J. Reedy. In *D. hansenii*, *PAP*, *OBP* and *PIK* are separate from the fused *MTL* locus[32]. **b**, Placement of gene losses on the phylogenetic tree. HOM, homothallic; HET, heterothallic.

a length insufficient to encode **a**1 or **a**2 even if they were extensively divergent in sequence. We confirmed this finding in seven other *L. elongisporus* isolates (not shown). The sexual state of *L. elongisporus* has been assumed to be homothallic because asci are generated from identical cells[5,26], but the absence of an **a**-factor pheromone, receptor and transporter (Supplementary Table 34) as well as *MTL*, suggests that it may not have a sexual cycle. Alternatively, mating may require only one pheromone and receptor (α), and *L. elongisporus* may be the first identified ascomycete that can mate independently of *MTL* or *MAT*.

Our discovery that *MTLα2* and *MTLa1* are frequently absent challenges our understanding of how the mating locus operates. The model derived for mating regulation in *C. albicans*, including the role of **a**1/α2 in white–opaque switching[3], must differ substantially in the other sexual species. This is particularly interesting because the major regulators of the white–opaque switch in *C. albicans* (*WOR1*, *WOR2*, *CZF1* and *EFG1*[34]) are generally conserved in other species, but Wor1 control over white–opaque genes appears to be a recent innovation in *C. albicans* and *Candida dubliniensis*[35]. Our data also suggest that the loss of α2 occurred early in the haploid sexual lineage.

Mating and meiosis

To gain further insight into their diversity in sexual behaviour, we examined whether 227 genes required for meiosis in *S. cerevisiae* and other fungi have orthologues in the *Candida* species (Supplementary Information, section S14). A previous report[36] that some components of meiosis, such as the major regulator *IME1*, are missing from *C. albicans* led us to propose that their loss could be correlated with lack of meiosis. Surprisingly, however, we find that these genes are missing in all *Candida* species, suggesting that sexual *Candida* species undergo meiosis without them. Conversely, even seemingly non-mating species showed highly conserved pheromone response pathways, suggesting that pheromone signalling plays an alternative role such as regulation of biofilm formation[37]. These findings suggest considerable plasticity and innovation of meiotic pathways in *Candida*.

Moreover, we find that sexual *Candida* species have undergone a recent dramatic change in the pathways involved in meiotic recombination, with loss of the Dmc1-dependent pathway in the heterothallic species *C. lusitaniae* and *C. guilliermondii* (Supplementary Information, section 14c). We also found that mechanisms of chromosome pairing and crossover formation have changed recently in these two species, because they (and to a lesser extent *D. hansenii*) have lost several components of the synaptonemal and synapsis initiation complexes (Supplementary Table 35). They have also lost components of the major crossover-formation pathway in *S. cerevisiae* (*MSH4*, *MSH5*), but have retained a minor pathway (*MUS81*, *MMS4*)[38,39]. Overall, if *Candida* species undergo meiosis it is with reduced machinery, or different machinery, suggesting that unrecognized meiotic cycles may exist in many species, and that the model of meiosis developed in *S. cerevisiae* varies significantly, even among yeasts.

The genome sequences reported here provide a resource that will allow current knowledge of *C. albicans* biology, the product of decades of research, to be applied with maximum effect to the other pathogenic species in the *Candida* clade. They also allow many of the unusual features of *C. albicans*—such as cell wall gene family amplifications, and its apparent ability to undergo mating and a parasexual cycle without meiosis—to be understood in an evolutionary context that shows that the genes involved in virulence and mating have highly dynamic rates of turnover and loss.

METHODS SUMMARY

The methods for this paper are described in Supplementary Information. Here we outline the resources generated by this project.

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).

Received 22 February; accepted 15 April 2009.
Published online 24 May 2009.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the US National Human Genome Research Institute (NHGRI) for support under the Fungal Genome Initiative at the Broad Institute. We thank C. Kurtzman for providing the sequenced strains of *L. elongisporus*, *C. guilliermondii* and *C. lusitaniae*, M. Koehrsen for Broad Institute website support, D. Park for informatics support and K. Wolfe and A. Regev for comments on the manuscript. We acknowledge the contributions of the Broad Institute Sequencing Platform and A. Barron, L. Clark, C. Corton, D. Ormond, D. Saunders, K. Seeger and R. Squares from the Wellcome Trust Sanger Institute for the *C. parapsilosis* sequencing and assembly. N.A.R.G., A.J.P.B., M.B. and co-workers were supported by the Wellcome Trust; M.G., S.S., Q.Z., C.K., B.W.B. and C.A.C. were supported by NHGRI and the National Institute of Allergy and Infectious Disease, US National Institutes of Health (NIH), Department of Health and Human Services; G.B. and co-workers were supported by Science Foundation Ireland; J.B., A.F., J.H., A.M.N. and co-workers were supported by the NIH; and M.K., M.D.R. and M.F.L. by the NIH, the US National Science Foundation and the Sloan Foundation.

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*), AAFT00000000 (*C. lusitaniae*), CABE01000001–CABE01000024 (*C. parapsilosis*). Reprints and permissions information is available at www.nature.com/reprints. 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).