

Genome Survey Sequencing of the Wine Spoilage Yeast *Dekkera (Brettanomyces) bruxellensis*^{∇†}

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The hemiascomycete yeast *Dekkera bruxellensis*, also known as *Brettanomyces bruxellensis*, is a major cause of wine spoilage worldwide. Wines infected with *D. bruxellensis* develop distinctive, unpleasant aromas due to volatile phenols produced by this species, which is highly ethanol tolerant and facultatively anaerobic. Despite its importance, however, *D. bruxellensis* has been poorly genetically characterized until now. We performed genome survey sequencing of a wine strain of *D. bruxellensis* to obtain 0.4× coverage of the genome. We identified approximately 3,000 genes, whose products averaged 49% amino acid identity to their *Saccharomyces cerevisiae* orthologs, with similar intron contents. Maximum likelihood phylogenetic analyses suggest that the relationship between *D. bruxellensis*, *S. cerevisiae*, and *Candida albicans* is close to a trichotomy. The estimated rate of chromosomal rearrangement in *D. bruxellensis* is slower than that calculated for *C. albicans*, while its rate of amino acid evolution is somewhat higher. The proteome of *D. bruxellensis* is enriched for transporters and genes involved in nitrogen and lipid metabolism, among other functions, which may reflect adaptations to its low-nutrient, high-ethanol niche. We also identified an adenylyl deaminase gene that has high similarity to a gene in bacteria of the *Burkholderia cepacia* species complex and appears to be the result of horizontal gene transfer. These data provide a resource for further analyses of the population genetics and evolution of *D. bruxellensis* and of the genetic bases of its physiological capabilities.

The flavors and aromas of wine are the products of complex interactions between many microorganisms. *Saccharomyces cerevisiae* is the primary yeast used in wine-making, but other fungi and bacteria, welcome or unwelcome, also contribute to most alcoholic fermentations. Many of these species occur naturally on the skin of grapes and flourish briefly in the initial stages of fermentation before being killed by the rising ethanol concentration (49). Other species appear to make the winery itself a primary habitat, surviving on the walls of the winery, on the interior surfaces of presses and fermentation tanks, or in the wood of the barrels (22), in ideal positions to colonize the fermenting grape must or the maturing wine. *Dekkera bruxellensis*, a hemiascomycete yeast also known as *Brettanomyces bruxellensis*, is one such species.

D. bruxellensis is probably the major microbial cause of wine spoilage worldwide and causes substantial economic losses within the wine industry (22). Wines infected by *D. bruxellensis* are said to have “Brett” character: they may smell mousy or medicinal or of wet wool, burnt plastic, or horse sweat (38). The mousy taint is the result of pyridines synthesized by *D. bruxellensis* from lysine and ethanol (29), while medicinal or barnyard odors are caused by the volatile phenols 4-ethylguaiacol and 4-ethylphenol, secondary metabolites produced by *D. bruxellensis* from phenolic acids naturally present in the grape must (14, 30). *D. bruxellensis* may have been present in wineries for centuries, and a very slight Brett character is traditionally

considered desirable in certain styles of wine (22). The frequency and severity of *D. bruxellensis* contamination have increased in recent decades, however, as winemaking trends have changed to favor the production of wines that contain more residual sugars and that may be unsulfited, unfiltered, or aged on lees (dead yeast cells), all factors that are favorable to *D. bruxellensis* growth.

D. bruxellensis possesses a number of adaptations that allow it to survive in the physiologically challenging environments of fermenting must and maturing wine. Like *S. cerevisiae*, it is ethanol tolerant, facultatively anaerobic, and petite positive (it can survive without mitochondrial DNA). Both species are also Crabtree positive and thus ferment preferentially in the presence of high glucose under aerobic conditions (36, 48). This suite of characteristics has evolved independently in each of the two lineages, possibly in response to similar selective pressures. The species differ in a number of other regards, however. For example, *D. bruxellensis* utilizes glucose less efficiently and grows much more slowly than *S. cerevisiae* (57) but can assimilate a wider variety of alternative carbon sources (16). Together, these characteristics explain the typical ecological succession observed during the course of *D. bruxellensis*-contaminated alcoholic fermentations, with *S. cerevisiae* dominating throughout the primary fermentation phase and then being replaced by *D. bruxellensis* in the maturation phase, when ethanol concentrations are high and only minute amounts of residual sugars remain (51).

Despite its economic importance and physiological interest, *D. bruxellensis* has remained largely unstudied at the genomic level. Previous work has focused on sequencing rRNA regions for phylogenetic analysis (67) or to aid in molecular detection of *D. bruxellensis* contamination (45), and only one nuclear

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protein-coding gene has been sequenced from this species to date (33). Consequently, the genetic bases of the physiological capabilities of *D. bruxellensis* remain largely unknown. To investigate these, and to provide a resource for further research on this species, we undertook genome survey sequencing of strain CBS 2499, isolated from wine. We report here a preliminary analysis of the genome organization and gene content of this strain.

MATERIALS AND METHODS

Sequence data. DNA from Y1031, a petite mutant derived from strain CBS 2499, was used to construct a genomic library of random Sau3AI partial digestion fragments, of average length 5 kb, in the low-copy-number *Escherichia coli* vector pMCL210. Library construction and sequence data generation were done by Agowa (Berlin, Germany). Sequences were obtained from both ends of the insert for 7,381 clones and from one end only for a further 98 clones. Read sequences were base called by Phred and vector masked using Cross_Match (20). Sequence data were assembled into contigs using Phrap (<http://www.phrap.org>), and low-quality sequence at the ends of contigs was trimmed using a purpose-written Perl script.

Gene identification. To identify protein-coding genes in *D. bruxellensis*, we first compared the contig sequences to a database of *S. cerevisiae* protein sequences (5,770 protein-coding genes, excluding dubious open reading frames and transposable element genes, downloaded from the *Saccharomyces* Genome Database in July 2005). Regions of the contigs greater than 100 nucleotides in length were annotated as orthologs to *S. cerevisiae* genes to which they were reciprocal best BLAST hits (using BLASTX and TBLASTN bit scores) (1).

To identify *D. bruxellensis* genes that do not have *S. cerevisiae* orthologs, we repeated this analysis using the nonredundant (NR) protein database (downloaded from the National Center for Biotechnology Information in July 2005). Regions of the *D. bruxellensis* contigs were considered to be orthologs of characterized genes in the NR database to which they were reciprocal best hits if (i) the contig region had no reciprocal best *S. cerevisiae* hit or (ii) the BLASTX bit score of the NR reciprocal best hit to the region was at least 100 bits higher than that of the *S. cerevisiae* hit.

Finally, we used *S. cerevisiae* rRNA and tRNA gene sequences as BLASTN queries against the contigs, with an upper *E*-value threshold of $1e-10$, to identify *D. bruxellensis* homologs of these genes.

Phylogenetic position. We constructed phylogenetic trees using amino acid sequence data from *D. bruxellensis*, *S. cerevisiae*, and eight other fungal species for which the whole genome sequence was available (*Candida glabrata*, *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Yarrowia lipolytica* [19], *Candida albicans* [34], *Ashbya gossypii* [18], *Aspergillus nidulans* [23], and *Schizosaccharomyces pombe* [66]). We accepted as sets of orthologous genes those genes that were best mutual BLASTP hits, with *E* values of $<1e-40$, in all possible pairwise comparisons among all 10 taxa. This yielded 396 sets of orthologous genes.

In those cases where only a partial gene sequence was present in the *D. bruxellensis* data, we extracted the corresponding segment of the orthologous gene in each of the other species based on BLASTP HSP coordinates; in all other cases, complete gene sequences were used. The amino acid sequences of each set of orthologs were aligned using T-Coffee (46), and regions of uncertain alignment were removed using Gblocks (13). Each of the 396 alignments was separately used to estimate the species phylogeny, using PHYML (26) with the JTT model of amino acid substitution and a gamma distribution of substitution rates with four categories.

We visualized the degree of topological conflict among these trees by constructing a consensus network (31) using SplitsTree4 (32). To test whether one of the three best-supported topologies suggested by this consensus network was a significantly better fit to the total sequence data than the others, we performed the Shimodaira-Hasegawa test (56) as implemented in Tree-Puzzle (54). We concatenated the 396 gene alignments to produce an alignment of 115,036 residues and calculated the log likelihood of each of the three topologies given these sequence data and a JTT model of substitution. To ensure that model misspecification did not affect our results, we repeated the Shimodaira-Hasegawa test using four other substitution models (WAG, VT, BLOSUM 62, and Dayhoff).

Genome architecture. For each pair of genes adjacent in *D. bruxellensis*, we determined whether their orthologs were also adjacent (or separated by fewer than five genes) in the *S. cerevisiae* genome. We also used the same method to calculate the degree of gene order conservation between *D. bruxellensis* and *C.*

albicans. For this purpose, we considered as orthologous any pair of *D. bruxellensis* and *C. albicans* genes that shared a mutual *S. cerevisiae* ortholog. The list of *C. albicans*/*S. cerevisiae* orthologs we used here was obtained from <http://www.candidagenome.org>.

We used two methods to identify introns in *D. bruxellensis*. Because many introns occur at conserved genomic locations across the hemiascomycetes (8), we first examined those *D. bruxellensis* genes orthologous to intron-containing *S. cerevisiae* genes. We aligned each pair of orthologous genes and determined whether the *D. bruxellensis* gene contained known hemiascomycete intron splice or branch sites (obtained from reference 27) and/or noncoding, presumably intronic, sequence in the region of the gene corresponding to the *S. cerevisiae* intron. Secondly, to detect *D. bruxellensis*-specific introns, we also identified genes that appeared to contain intron length segments of extra sequence when compared to their *S. cerevisiae* orthologs. To do this, we searched for genes that returned two significant BLASTX HSPs to a single *S. cerevisiae* protein, where the interval between those HSPs was 50 nucleotides (approximately the minimum known hemiascomycete intron length [8]) or greater in *D. bruxellensis* but 10 nucleotides or smaller in *S. cerevisiae*. These genes were then screened for known intronic splice and branch sites, as described above.

We used Tandem Repeats Finder (6) to identify microsatellites in the assembly. To identify transposable elements in this genome, we used the *D. bruxellensis* contigs as BLASTX queries against a database of protein sequences of *TYA* and *TYB* genes from *Ty* elements of multiple hemiascomycete species (43) and protein-coding regions of non-long terminal repeat (LTR) retrotransposons and DNA transposons from *C. albicans* and *Y. lipolytica* (12, 25, 44). Regions of the contigs that had hits with *E* values of $<1e-10$ to any of these proteins were counted as transposable elements. If a region had significant hits to more than one type of element, it was annotated as belonging to the element type of the best hit. Because many of the elements identified were fragmentary and/or substantially diverged from the query sequences used, we identified the longest *D. bruxellensis* elements from each of the three classes found and repeated the analysis, using these sequences as a TBLASTX database, to identify more diverged elements in our data. To ensure that our estimate of the number of elements was not inflated by double counting 5' and 3' ends of a single element on different contigs, we counted only hits that shared a common region of homology to the query as unique elements.

Gene content analyses. To further examine the differences in the sets of proteins encoded by *D. bruxellensis* and other yeasts, we searched for three types of loci: individual genes that have undergone recent lineage-specific duplication in *D. bruxellensis*, gene families that have expanded in *D. bruxellensis* relative to other species, and genes that have been horizontally transferred. To identify genes in the first two categories, we created a database of "putative genes" in *D. bruxellensis*. Each such gene was a region of a contig that was over 100 nucleotides long, had a BLASTX hit to an *S. cerevisiae* protein with *E* $< 1e-10$ (but was not necessarily the reciprocal best BLAST hit to that protein), and did not contain a stop codon.

A pair of genes arising from a lineage-specific gene duplication are likely to be one another's best BLAST hits, to the exclusion of orthologs in other lineages. We used the translated putative *D. bruxellensis* genes as BLASTP queries against a database containing the same putative *D. bruxellensis* proteins plus the proteomes of *S. cerevisiae*, *C. glabrata*, *K. lactis*, *A. gossypii*, *C. albicans*, *D. hansenii*, and *Y. lipolytica*. Any *D. bruxellensis* gene whose top two hits were itself and another *D. bruxellensis* protein was considered a probable lineage-specific duplication.

To assess whether genes with particular functions are more likely to be duplicated in *D. bruxellensis*, we tested whether the *S. cerevisiae* orthologs of the duplicated genes represent a functionally biased subset of the *S. cerevisiae* proteome. We determined the distribution of the orthologs of the duplicates among gene ontology categories, for each of the three ontologies, using GOToolBox (42). For each ontology, we then compared this distribution to that of the 5,770 *S. cerevisiae* proteins used in our analyses, using Fisher's exact test. We corrected for multiple tests (5).

We also searched for gene families that have expanded to a greater size in *D. bruxellensis* than in two closely related species, *C. albicans* and *D. hansenii*. The proteomes of these two species were combined with the translated putative gene set of *D. bruxellensis* into a single database. We queried this database with the protein sequences of *S. cerevisiae* genes belonging to previously defined gene families with two or more members (a subset, defined by phyletic pattern, of the "consensus families" from <http://cbi.labri.fr/Genolevures/fam/index.html>). We then calculated the total number of unique hits with an *E* value of $<1e-10$ to *D. bruxellensis*, *C. albicans*, and *D. hansenii* proteins across all genes in each family. Families that had more hits to *D. bruxellensis* than to either *C. albicans* or *D. hansenii* were considered to have undergone gene family expansion in *D. bruxellensis*. This is a conservative

TABLE 1. Genome analysis summary

Parameter	Value
Assembly	
No. of contigs	5,407
Mean contig length (bp)	1,386
Assembly size (Mb)	7.6
Estimated genome size (Mb)	19.4
% of genome sequenced	~40
Gene content	
Genes with <i>S. cerevisiae</i> orthologs	2,606
Genes without <i>S. cerevisiae</i> orthologs	278
Lineage-specific gene duplicates	89
tRNAs	24
Retrotransposons, Ty1-like	3
Retrotransposons, Ty3-like	16
Retrotransposons, Ty5-like	13
Estimated total no. of protein-coding genes	~7,430
Genome architecture	
Mean intergenic length (bp)	945
Introns, % genes in which present	2
Introns, mean length (bp)	195
% GC content (<i>S. cerevisiae</i> value)	
Introns, genome	40.2 (38.3)
Introns, coding	42.9 (39.7)
Introns, GC3	44.2 (37.0)
Introns, intergenic	39.1 (33.2)
Introns, intronic	34.7 (33.8)
Di/trinucleotide microsatellites	48

threshold, as the *D. bruxellensis* genome data are far less complete than those of either *C. albicans* or *D. hansenii*.

Nucleotide sequence accession numbers. Read sequences have been deposited in GenBank with accession numbers EI011584 to EI026443, and contig sequences are available on request from the corresponding author. In addition to the genome survey data, we completely sequenced a number of clones containing adenyl deaminase genes and nitrate/nitrite utilization genes (GenBank accession numbers EF364424 to EF364429).

RESULTS AND DISCUSSION

Sequence data and assembly. Previous analyses using pulsed-field gel electrophoresis have shown that the karyotypes of *D. bruxellensis* strains vary quite extensively, with estimated total genome sizes ranging from under 20 Mb to over 30 Mb (58). Partial ribosomal DNA (rDNA) sequencing of 30 strains suggests that, nonetheless, most isolates fall into a single major clade (3). We selected strain CBS 2499 for genome analysis because (i) it is a representative of the major *D. bruxellensis* clade, (ii) its estimated genome size, 19.4 Mb, is towards the lower end of the range observed in this species, (iii) it can be easily manipulated in the laboratory, and (iv) we were able to make a petite derivative from this strain, allowing us to construct a genomic DNA library without mitochondrial contamination. We assembled the sequences of 14,860 random reads from this library (see Materials and Methods) into 5,407 contigs, totalling approximately 7.6 Mb of sequence data. Given the estimated genome size of this strain, and assuming that it is haploid, our data contain approximately 40% of the genome. The principal features of our assembly are listed in Table 1.

The sequences appear to be largely single copy. After removing transposable element sequences (see below), we found 270 regions of the assembly longer than 500 bp that were

matched by a second region with nucleotide identity of 95% or greater. The duplicate copies of these regions add up to a total of 0.23 Mb, or approximately 3% of our sequence data. This will be an underestimate of the total proportion of the genome that is present in duplicate copy, as our data are likely to have sampled only single copies of many regions that are actually present in duplicate in the genome. As a rough correction, we may assume that the probability of observing a region as duplicated, $P(D_0)$, is equal to the probability of the region actually being duplicated, $P(D_A)$, times the probability of observing that second region in our sample (in this case, 0.4). As we observe that $P(D_0)$ is 0.03, this implies that $P(D_A)$ is 0.07, or that approximately 7% of the whole genome is made up of duplicated regions.

Genes and genetic code. We identified complete or partial sequences of 2,606 *D. bruxellensis* protein-coding genes with orthologs in *S. cerevisiae*, listed in Table S1 of the supplemental material. Additionally, 277 genes without *S. cerevisiae* orthologs, but with orthologs from other *Saccharomycetales* species, were identified (most of these genes were annotated as hypothetical; the 50 with functional annotations are listed in Table 2), as was a single example of a gene horizontally transferred from a bacterial species (see below). No other genes without fungal homologs were identified. An almost complete rDNA repeat, including 18S, 5.8S, 25S, and 5S rDNAs, is present in the data, as are at least 24 tRNA genes. If we assume that the 40% of the genome represented in our data are a random sample of the whole genome with respect to gene density, the total protein-coding gene complement of *D. bruxellensis* is likely to be on the order of 7,430 genes, which is comparable to that of other hemiascomycetes.

As a number of hemiascomycete species are known to deviate from the universal genetic code (60), we tested whether there was any evidence of codon reassignment in *D. bruxellensis*. Each *D. bruxellensis* gene with an *S. cerevisiae* ortholog was translated using the universal genetic code, and its product was aligned to the protein sequence encoded by its ortholog. We then returned the *D. bruxellensis* portions of the alignments to codon format and calculated, across all the gene alignments, how frequently each of the 61 sense codons in the *D. bruxellensis* data corresponded to each of the 20 amino acids in the *S. cerevisiae* data. For every codon, the amino acid most frequently observed was that expected under the universal genetic code, suggesting that codon reassignment has not occurred in *D. bruxellensis*. This method correctly identifies the known CUG reassignment in *D. hansenii* (60) (data not shown). The universal genetic code was used in all further analyses of *D. bruxellensis* data.

Phylogenetic position. To determine the phylogenetic position of *D. bruxellensis* among the hemiascomycetes, we constructed 396 trees, based on protein sequences of orthologous genes from *D. bruxellensis* and nine other fungal species (see Materials and Methods). We then calculated a consensus network to quantify the degree of incongruence between these trees (31). This involves generating a list of splits (bipartitions of the taxa corresponding to branches in the trees) present in the complete set of trees and weighting them by the frequency with which they occur; all splits that occur at a frequency above a certain threshold can then be displayed in a network. Boxes in the network indicate the presence of incompatible splits.

TABLE 2. Ascomycete proteins that have orthologs in *D. bruxellensis* but not *S. cerevisiae*

Functional class and NCBI accession no.	Species	Gene name	Function
Nitrate metabolism			
CAA11229	<i>Hansenula polymorpha</i>	<i>YNT1</i>	Nitrate transporter
CAA11232	<i>Hansenula polymorpha</i>	<i>YNR1</i>	Nitrate reductase
CAA11230	<i>Hansenula polymorpha</i>	<i>YNI1</i>	Nitrite reductase
CAA11231	<i>Hansenula polymorpha</i>	<i>YNA1</i>	Nitrate assimilation pathway-specific Zn(II) ₂ Cys ₆ transcription factor
CAC16081	<i>Hansenula polymorpha</i>	<i>YNA2</i>	Nitrate assimilation pathway-specific Zn(II) ₂ Cys ₆ transcription factor
Metabolism of alternative carbon sources			
AAX31178	<i>Candida albicans</i>	<i>NAG1</i>	Glucosamine-6-phosphate deaminase
CAH02587	<i>Kluyveromyces lactis</i>	<i>LAC4</i>	β-Galactosidase
CAE47547	<i>Ambrosiozyma monospora</i>	<i>ALX1</i>	L-Xylulose reductase
CAG88556 ^a	<i>Debaryomyces hansenii</i>		Inositol oxygenase
CAG90181 ^a	<i>Debaryomyces hansenii</i>		Phosphatidylinositol-specific phospholipase C
BAD32688	<i>Hansenula polymorpha</i>		Glycerol dehydrogenase
BAD32689	<i>Pichia ofunaensis</i>		Dihydroxyacetone reductase
Transporters			
EAK93787	<i>Candida albicans</i>	<i>HGT1</i>	High-affinity glucose transporter
AAB17122	<i>Debaryomyces occidentalis</i>	<i>HAK1</i>	High-affinity potassium transporter
EAL02606	<i>Candida albicans</i>	<i>NUP</i>	Purine nucleoside permease
EAK96574	<i>Candida albicans</i>	<i>UAP2</i>	Potential purine permease
Lipid metabolism			
BAD11952	<i>Saccharomyces kluyveri</i>	<i>FAD3</i>	ω-3 fatty acid desaturase
AAU10084	<i>Pichia pastoris</i>		Δ8-(E)-sphingolipid desaturase
AAU10085	<i>Pichia pastoris</i>		Δ4-(E)-sphingolipid desaturase
AAZ08581	<i>Ashbya gossypii</i>		Sphingolipid C9-methyltransferase
AAK73020	<i>Pichia pastoris</i>		Ceramide glucosyltransferase
Respiratory chain complexes			
CAG86996 ^a	<i>Debaryomyces hansenii</i>		Complex I 14-kDa subunit
CAG88782 ^a	<i>Debaryomyces hansenii</i>		Complex I 20-kDa subunit
CAG84577 ^a	<i>Debaryomyces hansenii</i>		Complex I 24-kDa subunit
CAG89146 ^a	<i>Debaryomyces hansenii</i>		Complex I 49-kDa subunit
CAG89064 ^a	<i>Debaryomyces hansenii</i>		Complex I 51-kDa subunit
CAG90271 ^a	<i>Debaryomyces hansenii</i>		Complex I 75-kDa subunit
CAG85893 ^a	<i>Debaryomyces hansenii</i>	<i>AOX1</i>	SHAM-sensitive alternative terminal oxidase 1
Peroxisome			
CAA82928	<i>Hansenula polymorpha</i>	<i>PER1</i>	Peroxisomal matrix protein
AAD52811	<i>Hansenula polymorpha</i>	<i>PEX1</i>	Peroxin
P78723	<i>Hansenula polymorpha</i>	<i>PEX14</i>	Peroxin
CAA65646	<i>Pichia pastoris</i>	<i>PEX2</i>	Peroxin
AAF19606	<i>Pichia pastoris</i>	<i>PEX17</i>	Peroxin
AAD43507	<i>Pichia pastoris</i>	<i>PEX19</i>	Peroxin
Other			
CAG60540 ^a	<i>Candida glabrata</i>	<i>PRN1</i>	Putative pirin
CAG85416 ^a	<i>Debaryomyces hansenii</i>	<i>URA9</i>	Dihydroorotate dehydrogenase 2
CAG86646 ^a	<i>Debaryomyces hansenii</i>		Beta-alanine synthase
CAH00925 ^a	<i>Kluyveromyces lactis</i>		GTP cyclohydrolase II
CAG80587 ^a	<i>Yarrowia lipolytica</i>		FAD-dependent oxidoreductase
BAB12222	<i>Candida boidinii</i>	<i>DAO1</i>	D-Amino acid oxidase
CAH02585	<i>Kluyveromyces lactis</i>		Acid phosphatase
AAS51137	<i>Ashbya gossypii</i>		Calcineurin-like phosphoesterase
EAL00803	<i>Candida albicans</i>		Potential secreted Cu/Zn superoxide dismutase
EAK95140	<i>Candida albicans</i>		Possible thiamine biosynthesis enzyme
EAL02572 ^a	<i>Candida albicans</i>		Ras GTPase-activating protein
CAG85348 ^a	<i>Debaryomyces hansenii</i>		Potential histone binding protein
CAG78240 ^a	<i>Yarrowia lipolytica</i>		Translation initiation factor 3 subunit 7
EAL04857	<i>Candida albicans</i>		Negative regulator of iron uptake genes
EAK96905	<i>Candida albicans</i>		Potential fungal zinc cluster transcription factor
AAQ75382	<i>Hansenula polymorpha</i>	<i>SWI1</i>	Global transcription activator

^a For this gene, the best BLAST hit of the *D. bruxellensis* sequence in the NR database is an unannotated or hypothetical gene, and the functional annotation given here is taken from the highest annotated hit, where the bit score of the annotated hit was at least 90% that of the best hit and sequence comparison indicates that the genes are homologs.

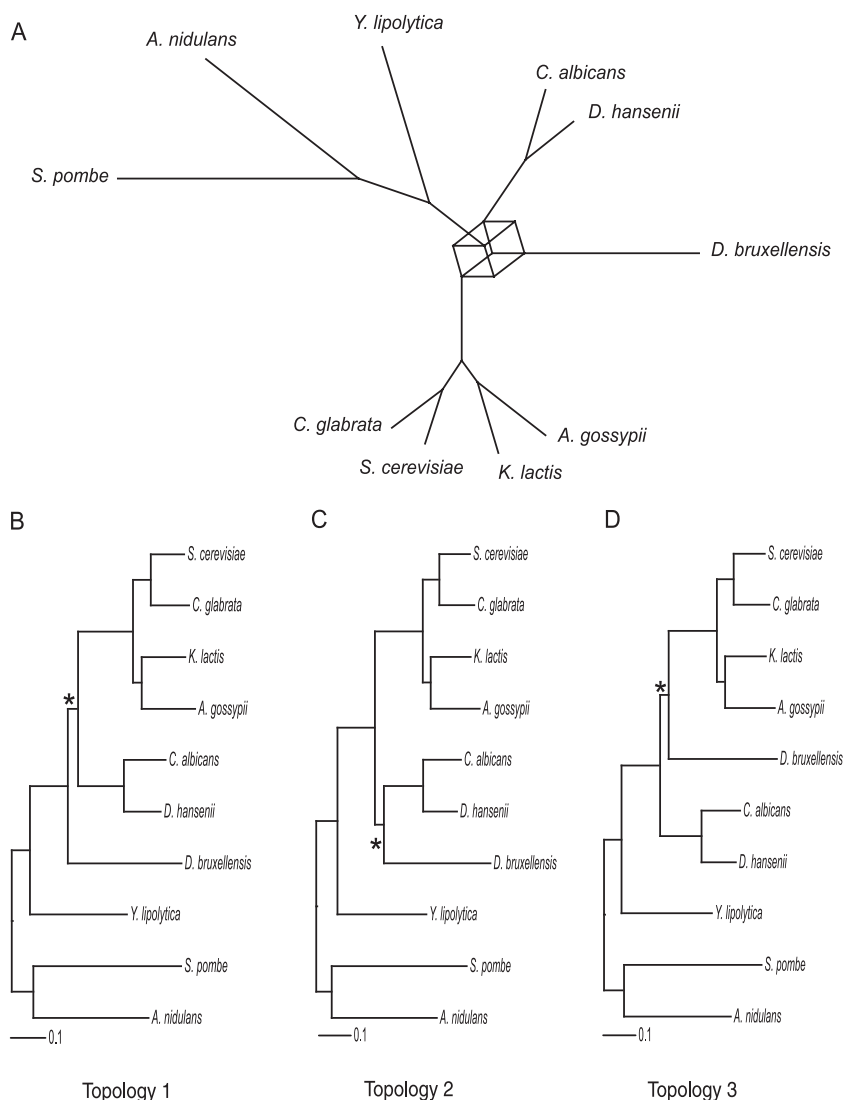


FIG. 1. (A) Consensus network based on 396 protein trees; splits are shown if they are present in 25% or more of the trees. Three incompatible splits involving the placement of *D. bruxellensis* generate a cube in the network. (B to D) Three likely topologies inferred from the consensus network. Each topology contains one of the incompatible splits shown in panel A; in each case the branch corresponding to the split is marked with an asterisk. Branch lengths for these three trees are estimated by maximum likelihood using concatenated amino acid sequence data.

Although the major groupings in our network are well supported, there is conflict between the gene trees regarding the position of *D. bruxellensis*: three incompatible splits are each present in over 25% of trees (Fig. 1A).

From this network, we inferred three probable topologies that could explain the sequence data, each of which includes only one of the three incompatible splits identified above (Fig. 1B to D). The marked splits shown in topologies 1, 2, and 3 occur in 34%, 27%, and 26% of gene trees, respectively. A Shimodaira-Hasegawa test showed that topology 2 is the best fit to the concatenated sequence data (log likelihood, $-1,344,394$), although it is not significantly better than topology 1 ($P = 0.27$; log likelihood, $-1,344,454$). Topology 3, however, can be significantly rejected ($P = 0.01$; log likelihood, $-1,344,558$). The use of different substitution models made no

difference to the outcome of the Shimodaira-Hasegawa test, suggesting that model misspecification is not affecting our results.

Previous estimates of the phylogenetic position of *D. bruxellensis* reflect the uncertainty we observe in our data: separate analyses based on 18S sequence data have supported both topology 1 (11) and topology 3 (47). Our analysis of almost 400 protein-coding genes significantly rejects topology 3, but we remain unable to determine whether *D. bruxellensis* diverged from the hemiascomycete lineage shortly before *C. albicans* and *D. hansenii* (topology 1) or if these three species briefly formed a shared divergent lineage before the speciation of *D. bruxellensis* (topology 2). It is likely that very few substitutions were fixed in the short time periods separating these divergence events, making it difficult to accurately reconstruct the true topology. The proteins used in our analysis were well

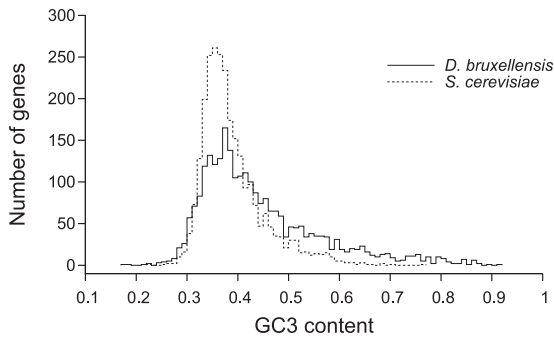


FIG. 2. Distribution of GC content at third-position codon sites in 2,606 orthologous genes in *D. bruxellensis* and *S. cerevisiae*.

conserved across taxa and thus relatively slowly evolving, and it is possible that more rapidly evolving proteins would provide more substitutions on the branches of interest. Such proteins, however, are more difficult to assign to orthology groups and to reliably align and are more likely to be saturated, possibly leading to decreased phylogenetic accuracy.

Gene sequence statistics. We calculated the amino acid identity between each *D. bruxellensis* protein and its *S. cerevisiae* ortholog by aligning their amino acid sequences and removing gapped sites. *D. bruxellensis* and *S. cerevisiae* orthologous proteins have a mean amino acid identity of 48.8% ($N = 2,615$, standard deviation, 14.6). This is significantly lower than the amino acid identity between *C. albicans* and *S. cerevisiae* orthologs for the subset of proteins that are present in all three species (mean *C. albicans*-*S. cerevisiae* identity, 52.9%; mean *D. bruxellensis*-*S. cerevisiae* identity, 49.9%; $N = 2,115$; $P < 1 \times 10^{-16}$, Wilcoxon signed-rank test). If we accept that the divergence of *D. bruxellensis*, *S. cerevisiae*, and *C. albicans* is effectively a trichotomy (or that the best-supported tree, topology 2, reflects the real phylogeny), this suggests that the rate of amino acid sequence evolution in *D. bruxellensis* is higher than that of *C. albicans*.

D. bruxellensis genes tend to have higher GC content than their *S. cerevisiae* orthologs at all classes of sites (Table 1). The difference in GC content across orthologous sites is statistically significant ($P < 1 \times 10^{-16}$ for all genic sites and for codon third position sites; Wilcoxon signed-rank test). GC content also varies among *D. bruxellensis* genes substantially more than among the orthologous *S. cerevisiae* genes, a trend that is particularly obvious at third position sites (Fig. 2).

Genome architecture. We examined four aspects of genome organization in *D. bruxellensis*: the conservation of gene order in *D. bruxellensis* relative to that in *S. cerevisiae* and in *C. albicans*; the lengths of intergenic regions; the positions, lengths, and motif sequences of introns; and the repeat content of the genome.

We compared the order of genes along *D. bruxellensis* contigs with that of their orthologs on the *S. cerevisiae* genome to determine the degree of synteny conservation between these two species. Most of our *D. bruxellensis* contigs contain only a single gene, but 286 contain two or more genes, allowing us to analyze 318 pairs of adjacent genes. Of these gene pairs, 19% are also immediate neighbors in *S. cerevisiae* and a further 4% are close neighbors separated by one to five genes. Because we expect gene order relationships between *D. bruxellensis* and *S.*

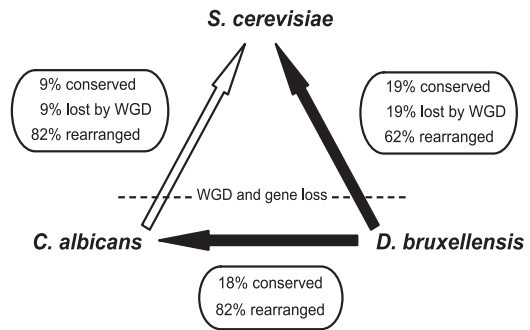


FIG. 3. Estimated rates of chromosomal rearrangements between *D. bruxellensis*, *C. albicans*, and *S. cerevisiae*. Black arrows indicate analyses performed in this study, while the white arrow refers to a previous study (55). The percentage of adjacent gene pairs conserved between species was calculated from the data, and the percentage of gene pair adjacencies disrupted by gene loss following whole genome duplication (WGD) in *S. cerevisiae* is estimated to be approximately equal to the percentage conserved. The remaining gene pairs have been disrupted by chromosomal rearrangements.

cerevisiae to have been affected by the whole genome duplication that occurred on the *S. cerevisiae* lineage (65), we also assessed the degree of gene order conservation between *D. bruxellensis* and *C. albicans*, a species that has not undergone whole genome duplication. In this case, of 233 pairs of adjacent *D. bruxellensis* genes with *C. albicans* orthologs, 18% have adjacent *C. albicans* orthologs and an additional 8% are separated by one to five genes. Only 17 pairs of adjacent genes in *D. bruxellensis* are also adjacent in both *C. albicans* and *S. cerevisiae* (see Table S2 in the supplemental material); these genes show no obvious functional bias.

These results suggest that *D. bruxellensis* has a somewhat lower rate of small-scale rearrangements than *C. albicans* (Fig. 3). An earlier analysis using the same method determined that only 9% of gene pairs adjacent in *C. albicans* were also adjacent in *S. cerevisiae* (55). As we expect approximately half of the links between gene pairs in pre-whole-genome duplication species to be broken due to random gene loss in the *S. cerevisiae* lineage subsequent to duplication (65), we can estimate that approximately 62% of *D. bruxellensis* and 82% of *C. albicans* neighboring gene pairs have been disrupted in *S. cerevisiae* due to chromosomal rearrangement. The lower value for *D. bruxellensis* is not unusual: it has been shown that the rate of small chromosomal inversions differs by over an order of magnitude among six completely sequenced yeast genomes (21), and our estimate of the percentage of adjacent gene pairs in *D. bruxellensis* conserved in *S. cerevisiae* (19%) is consistent with those for *D. hansenii* (16%) and *C. tropicalis* (18%) (39).

The mean intergenic distance for the set of 318 adjacent *D. bruxellensis* genes is 700 nucleotides. This may be an unrealistically low estimate of the average genomic intergenic distance, however, as the relatively short lengths of the contigs mean that long intergenic regions (complete with flanking genes) are unlikely to be detected. Approximately 1,300 contigs have no significant BLASTX hit to any protein in the NR database, nor do they contain rRNAs or tRNAs. If we assume that these contigs consist of intergenic sequence and include their lengths in our calculation of mean intergenic distance, this value becomes 945 nucleotides. This may represent a substantial un-

TABLE 3. Introns in *D. bruxellensis* genes

<i>S. cerevisiae</i> ortholog		5' Motif ^a	Branch Motif	3' Motif	Intron length ^b (bp)	
Systematic name	Standard name				<i>D. bruxellensis</i>	<i>S. cerevisiae</i>
YBL001C	<i>ECM15</i>	GTAAGT	TACTAAC	CAG	65	NA
YBL050W	<i>SEC17</i>	—	TATTAAC	TAG		
YBR159W	YBR159W	GTATGT	TACTAAC	CAG	79	NA
YBR189W	<i>RPS9B</i>	GTAAGT	TACTAAC	TAG	344	413
YDL082W	<i>RPL13A</i>	—	TACTAAC	TAG		
YDL108W	<i>KIN28</i>	—	TAATAAT	AAG		
YDL125C	<i>HNT1</i>	GTAAGT	TACTAAC	AAG	65	111
YDR025W	<i>RPS11A</i>	GTATGC	TACTAAC	TAG	403	339
YDR064W	<i>RPS13</i>	—	TACTAAC	TAG		
YDR092W	<i>UBC13</i>	GTATGT	TACTAAC	AAG	61	268
YDR367W	YDR367W	—	GACTAAC	TAG		
YDR424C	<i>DYN2</i>	—	TACTAAC	TAG		
YDR424C	<i>DYN2</i>	GTAAGT	TACTAAC	CAG	125	80
YDR471W	<i>RPL27B</i>	—	TACTAAC	TAG		
YER009W	<i>NTF2</i>	GTAAGT	TACTAAC	TAG	260	NA
YGL076C	<i>RPL7A</i>	—	TACTAAC	TAG		
YGL076C	<i>RPL7A</i>	GTAAGT	TACTAAC	CAG	276	468
YGL103W	<i>RPL28</i>	GTAAGT	TACTAAC	TAG	419	511
YGR029W	<i>ERV1</i>	GTAAGT	TACTAAC	TAG	143	83
YGR243W	YGR243W	GTAAGT	TACTAAC	AAG	118	NA
YHL001W	<i>RPL14B</i>	—	TACTAAC	TAG		
YHR001W-A	<i>QCR10</i>	GTAAGT	TACTAAC	CAG	67	63
YHR016C	<i>YSC84</i>	GTATGT	TACTAAC	CAG	113	168
YHR021C	<i>RPS27B</i>	—	AACTAAT	TAG		
YIL018W	<i>RPL2B</i>	—	TACTAAC	CAG		
YIL069C	<i>RPS24B</i>	—	TACTAAC	TAG		
YIL133C	<i>RPL16A</i>	—	TACTAAC	CAG		
YJL191W	<i>RPS14B</i>	—	TACTAAC	CAG		
YKR057W	<i>RPS21A</i>	—	TACTAAC	TAG		
YLR185W	<i>RPL37A</i>	—	TACTAAC	CAG		
YLR306W	<i>UBC12</i>	GTAAGT	TACTAAC	CAG	65	134
YLR344W	<i>RPL26A</i>	GTATGT	TACTAAC	TAG	357	447
YLR448W	<i>RPL6B</i>	—	TACTAAC	TAG		
YML067C	<i>ERV41</i>	GTAAGT	TACTAAC	CAG	92	93
YML094W	<i>GIM5</i>	GTAAGT	TACTAAC	CAG	75	83
YMR116C	<i>ASC1</i>	—	TACTAAC	TAG		
YMR143W	<i>RPS16A</i>	GTATGT	TACTAAC	TAG	359	544
YMR194W	<i>RPL36A</i>	GTATGT	AACTAAC	CAG	472	463
YNL310C	YNL310C	GTAAGT	TACTAAC	TAG	77	NA
YNR053C	<i>NOG2</i>	—	TACTAAC	AAG		
YOL120C	<i>RPL18A</i>	GTAAGT	TACTAAC	TAG	328	447
YOL127W	<i>RPL25</i>	—	TACTAAC	TAG		
YOL132W	<i>GAS4</i>	GTAGGT	TACTAAC	CAG	62	NA
YOL146W	<i>PSF3</i>	GTAAGT	TACTAAC	TAG	65	NA
YOR122C	<i>PFY1</i>	GTAAGT	TACTAAC	CAG	161	209
YOR182C	<i>RPS30B</i>	—	TACTAAC	CAG		
YPL090C	<i>RPS6A</i>	GTATGT	TACTAAC	CAG	357	394
YPL143W	<i>RPL33A</i>	GTAAGT	TACTAAC	TAG	359	525
YPR028W	<i>YOP1</i>	—	TACTAAC	TAG		
YPR062W	<i>FCY1</i>	GTAAGT	TACTAAC	CAG	100	NA

^a —, the start of the intron is not present in our sequence data; for these cases, the intron length cannot be given.

^b Where the intron length is known in *D. bruxellensis*, the length of the corresponding intron in *S. cerevisiae* is given for comparative purposes. NA, the orthologous gene in *S. cerevisiae* does not contain an intron.

derestimate (if many intergenic regions are much longer than individual contigs) or a slight overestimate (if fragments of genes at the ends of contigs are not detected by BLAST analysis) of the true average intergenic length in this species. Although higher than the mean intergenic distance in *S. cerevisiae*, this value is well within the range observed in other hemiascomycete species (10).

We identified introns in 40 *D. bruxellensis* genes whose *S. cerevisiae* orthologs also contain introns (Table 3). Of these, two (*DYN2* and *RPL7A* and their *D. bruxellensis* orthologs)

each contain two introns. We found an additional eight introns that appear to be specific to *D. bruxellensis* (Table 3). This may be an underestimate of the true number of novel introns, as our detection method requires that exons both upstream and downstream of the intron be identified as HSPs by BLAST. Hemiascomycete introns (including those we observe in our data) have a strong bias towards the 5' end of genes, and in many cases the first exon encodes five or fewer amino acids; such first exons may not be detected as an HSP.

In total, approximately 2% of the *D. bruxellensis* genes we

TABLE 4. Gene families that are expanded in *D. bruxellensis* relative to both *C. albicans* and *D. hansenii*

Gene family ^a	<i>S. cerevisiae</i> genes in family	No. of unique BLASTP hits ^b		
		<i>D. bruxellensis</i>	<i>C. albicans</i>	<i>D. hansenii</i>
GLC.1379	BNI1 BNR1	4	2	2
GLC.1390	ITC1 YPL216W	2	1	1
GLC.1609	DIN7 EXO1 RAD2 RAD27 YEN1	5	4	4
GLC.1623	SEC1 SLY1 VPS33 VPS45	5	4	4
GLC.1700	CDC46 CDC47 CDC54 MCM3 MCM6	9	8	6
GLC.1702	CDC16 CDC27	4	3	3
GLC.1711	RDS2 YBR239C YJL103C	4	3	3
GLC.1749	CCC2 DNF1 DNF2 DNF3 DRS2 ENA1 ENA2 ENA5 NEO1 PCA1 PMA1 PMA2 PMC1 PMR1 SPF1 YOR291W	19	18	18
GLC.1765	NCL1 NOP2 YNL022C	4	3	3
GLC.1826	SHC1 SKT5	3	1	1
GLC.1900	ECM22 UPC2	2	1	1
GLC.1967	HSP26 HSP42	2	0	0
GLC.2074	GDE1 PHO81	4	2	2
GLC.2091	APL1 APL2 APL3 APL4 APL5 APL6 SEC26	8	7	7
GLC.2291	HMG1 HMG2 NCR1	5	2	2
GLC.2339	GEA1 GEA2 SEC7	7	2	2
GLC.2379	DHR2 ECM16 PRP16 PRP2 PRP22 PRP43 YLR419W	11	9	8
GLC.2424	ARX1 MAP1 MAP2	5	3	3
GLC.2529	SCS3 YDR319C	2	1	0
GLC.2545	ACC1 HFA1	6	4	3

^a Gene families are from reference 19.

^b Total number of unique BLASTP hits (with *E* values of <1e-10) of *S. cerevisiae* proteins in that family to each of the three species, respectively. Note that as the *D. bruxellensis* genome is not completely sequenced, the numbers of hits given for this species will be a substantial underestimate of the true figure.

identified contain introns, and the mean length of the introns is 195 nucleotides. These values are consistent with those found in other hemiascomycetes: the proportion of genes that contain introns varies from 1% in *D. hansenii* to 4% in *S. cerevisiae*, with a concomitant increase in average intron length from around 100 to over 250 nucleotides (8). The *D. bruxellensis* introns have 5', 3', and branch site motifs that are very similar to the consensus in *S. cerevisiae*.

We examined two kinds of repeated sequences in the *D. bruxellensis* genome: microsatellites and transposable elements. The assembly contains 48 microsatellites with between 8 and 34 dinucleotide or trinucleotide repeat units. On the basis of results from other hemiascomycete species (37), it is likely that at least some of these are polymorphic and may thus be of use in future analyses of population diversity.

The great majority of transposable elements so far described in hemiascomycete yeasts are LTR retrotransposons, although non-LTR retrotransposons and DNA transposons have been found in *C. albicans* and *Y. lipolytica* (12, 25, 44). The LTR retrotransposons fall into two broad categories: Ty1/*cop* (including Ty1, Ty2, T4, and Ty5 elements) and Ty3/*gypsy* (43). The *D. bruxellensis* data contain at least 42 transposable elements, all of which are LTR retrotransposons: three Ty1-like, 16 Ty3-like, and 13 Ty5-like. This number includes only those elements that retain at least fragments of the *TYA* or *TYB* protein-coding genes and is thus a minimum estimate of the number of elements present; many solo LTR sequences are likely to be present but too degenerate to identify by comparison with LTR sequences from other species.

It has previously been suggested that Ty1 elements first appeared in the genomes of hemiascomycetes at the base of the *S. cerevisiae*/*K. lactis* clade (43), after the divergence of *D. bruxellensis* from the main hemiascomycete lineage. The pres-

ence of three putative Ty1 elements in our data may indicate that elements of this class arose at an earlier stage in hemiascomycete evolution and have subsequently been lost from other species in the *C. albicans*/*D. hansenii* clade (as they have been from *Saccharomyces bayanus*, for example). The Ty1-like elements present in *D. bruxellensis* are substantially diverged from one another and from their closest homologs in other species (the most similar has 26% amino acid identity to a Tkm1 element from *Kluyveromyces marxianus*), indicating that they are unlikely to be the result of a recent horizontal gene transfer event.

Differences in gene content. The variations in physiological capabilities between *D. bruxellensis* and other yeasts are caused by differences in gene complement and regulation in these species. To investigate the extent of gene content differences, we identified genes in *D. bruxellensis* that are absent from *S. cerevisiae* (Table 2), and we also sought evidence for increases in gene number that could underlie various traits. We identified 89 pairs of lineage-specific duplicated genes (see Table S3 in the supplemental material). Enzymes and transporters were notably overrepresented in this set of genes, among other significantly enriched GO categories (see Tables S4a to c in the supplemental material). Notably, we found duplicated copies of three genes in the allantoin catabolism pathway (*DAL1*, *DCG1*, and *MLS1*), all with 97% DNA sequence identity between copies. We also identified 18 gene families that are expanded in *D. bruxellensis* relative to both *C. albicans* and *D. hansenii*, including several families functioning in sterol, phosphocholine, and phospholipid synthesis (Table 4).

A number of the genes present in our data that do not have *S. cerevisiae* orthologs (Table 2) probably underpin known species-specific differences in metabolic capabilities between *S. cerevisiae* and *D. bruxellensis* (36). For example, a minority of

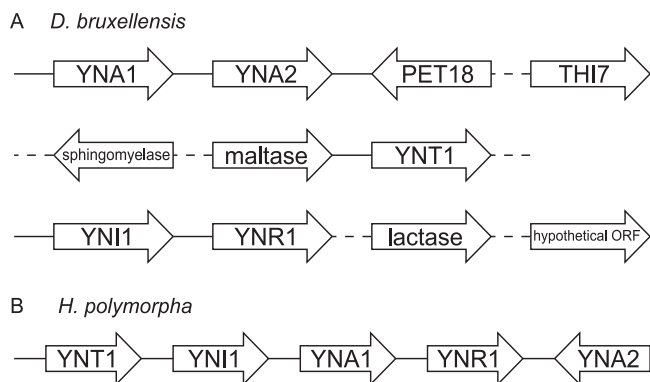


FIG. 4. (A) Gene order relationships (not drawn to scale) of five nitrate assimilation genes and their neighboring genes in the *D. bruxellensis* data. See Table 2 for full gene names. The arrowheads indicate the direction of transcription. Solid lines between genes indicate sequenced intergenic regions; dotted lines indicate that the gene order is inferred from clone end pair information. (B) Order and orientation of the cluster of orthologous genes in *Hansenula polymorpha* (based on information provided in reference 4).

D. bruxellensis strains have recently been shown to grow on lactose or arabinose (16), and the presence in our data of genes coding for β -glucosidase (lactase) and L-xylulose reductase (required for L-arabinose catabolism [64]) suggests that, unlike *S. cerevisiae*, strain CBS 2499 may be able to utilize both these carbon sources. *S. cerevisiae* cannot utilize nitrate as a nitrogen source, while *D. bruxellensis* can; five genes required for nitrate assimilation are present in the *D. bruxellensis* sequence data. These five genes (*YNT1*, *YNR1*, *YNI1*, *YNA1*, and *YNA2*) are clustered in the genome of the yeast *Hansenula polymorpha* (4), as are subsets of these genes in a number of other more distantly related fungi (2, 17). Due to the fragmented nature of the *D. bruxellensis* sequence data, we cannot currently determine the exact degree of clustering of these genes in this species. It is clear, however, that all five genes do not occur in a single uninterrupted cluster and that the relative positions and orientations of the genes differ between *D. bruxellensis* and *H. polymorpha* (Fig. 4).

Genes involved in lipid metabolism appear to be substantially enriched in the *D. bruxellensis* genome: it possesses genes in these pathways that are not found in *S. cerevisiae* (Table 2), and known families of lipid metabolism genes that are present in *S. cerevisiae* have undergone expansion in *D. bruxellensis* (Table 4). Some of these genes may contribute to the high ethanol tolerance of this species (35). Numerous others have orthologs in *C. albicans* whose precise role in the cell is currently unknown (e.g., the sphingolipid desaturase and methyltransferase genes) (9).

D. bruxellensis also possesses genes coding for several subunits of the respiratory chain complex I and for a SHAM-sensitive alternative oxidase (*AOX1*) (Table 2), suggesting that this species is capable of alternative respiration. This pathway bypasses complexes III and IV of the respiratory chain; instead, electrons from the ubiquinone pool are used by the alternative oxidase to reduce oxygen to water. Alternative respiration and aerobic fermentation have previously been hypothesized to represent two alternative responses to glycolytic overflow (63). Under this theory, when high levels of glucose

are available to the yeast cell, rapid glycolysis may lead to saturation of the respiratory pathway and subsequent accumulation of pyruvate and NADH. To prevent glycolysis from stalling, this NADH may then be reoxidized to NAD^+ either via alternative respiration (in Crabtree-negative yeasts) or by the reduction of pyruvate to ethanol (in Crabtree-positive yeasts). The presence of complex I and *AOX1* in *D. bruxellensis*, a Crabtree-positive yeast capable of aerobic fermentation, is therefore somewhat unexpected (62), although alternative respiration has previously been demonstrated in some strains of *Dekkera* and *Brettanomyces* yeasts (7). It has been suggested that this alternative respiratory pathway may be implicated in the Custers effect (anaerobic inhibition of fermentation) shown by these yeasts (7) or NADH reoxidation through glycerol production, but little is known of the mechanisms of these processes.

Finally, seven *D. bruxellensis* genes coding for peroxisome structural and regulatory proteins were also identified as having ascomycete but no *S. cerevisiae* orthologs. Peroxisomes are found in almost all eukaryotes, and some peroxins (including that encoded by *PEX19*) are conserved across this taxonomic spectrum (53), suggesting that *S. cerevisiae* orthologs to at least some of these genes are likely to exist. Many of the ascomycete peroxisome genes in Table 2 have in fact been previously annotated as homologous to *S. cerevisiae* genes (59), although their products often have low amino acid identity. Phylogenetic analyses suggest that *S. cerevisiae* peroxin genes are indeed orthologous to the *D. bruxellensis* genes but are too divergent to be detected using our method (data not shown).

Horizontal gene transfer. While comparing our contig sequences to the entire NCBI database, we identified a gene fragment in *D. bruxellensis* that was the reciprocal best BLAST hit to a *Burkholderia cenocepacia* (α -Proteobacteria) gene annotated as encoding an adenosine deaminase (ADA). We sequenced three additional plasmid clones to obtain the full sequence of this gene, the surrounding intergenic regions, and portions of the neighboring genes. The gene is 1,053 nucleotides long and is located downstream of a *URA4* homolog and upstream of a transporter of the *DAL5* family. Both of the neighboring genes show clear evidence of fungal origin, which rules out the possibility that the ADA gene-like gene arose from bacterial contamination of our *D. bruxellensis* library. The complete gene sequence identifies the gene as a member of the adenylyl deaminase gene subfamily, which includes both adenosine deaminase and adenine deaminase (ADE) genes (41, 52).

We constructed a phylogenetic tree using sequences of the *D. bruxellensis* gene, its close BLAST hits, and a number of known ADA and ADE genes (Fig. 5). The *D. bruxellensis* gene clusters with neither the ADA nor the ADE gene clade but forms a separate clade with sequences from four species of the *Burkholderia cepacia* complex (40) and one sequence from *Kineococcus radiotolerans*. The clear separation of this clade from the fungal ADE genes, together with the existence of a canonical ADE gene in *D. bruxellensis* (*DbADE1*) (Fig. 5), suggests that horizontal gene transfer from a bacterial species is the most likely origin for the *Burkholderia*-like gene, which we named *DbHAD1* for *D. bruxellensis* horizontally transferred adenylyl deaminase gene.

The four *B. cepacia* complex species that have HAD genes also have canonical ADE genes present elsewhere in their

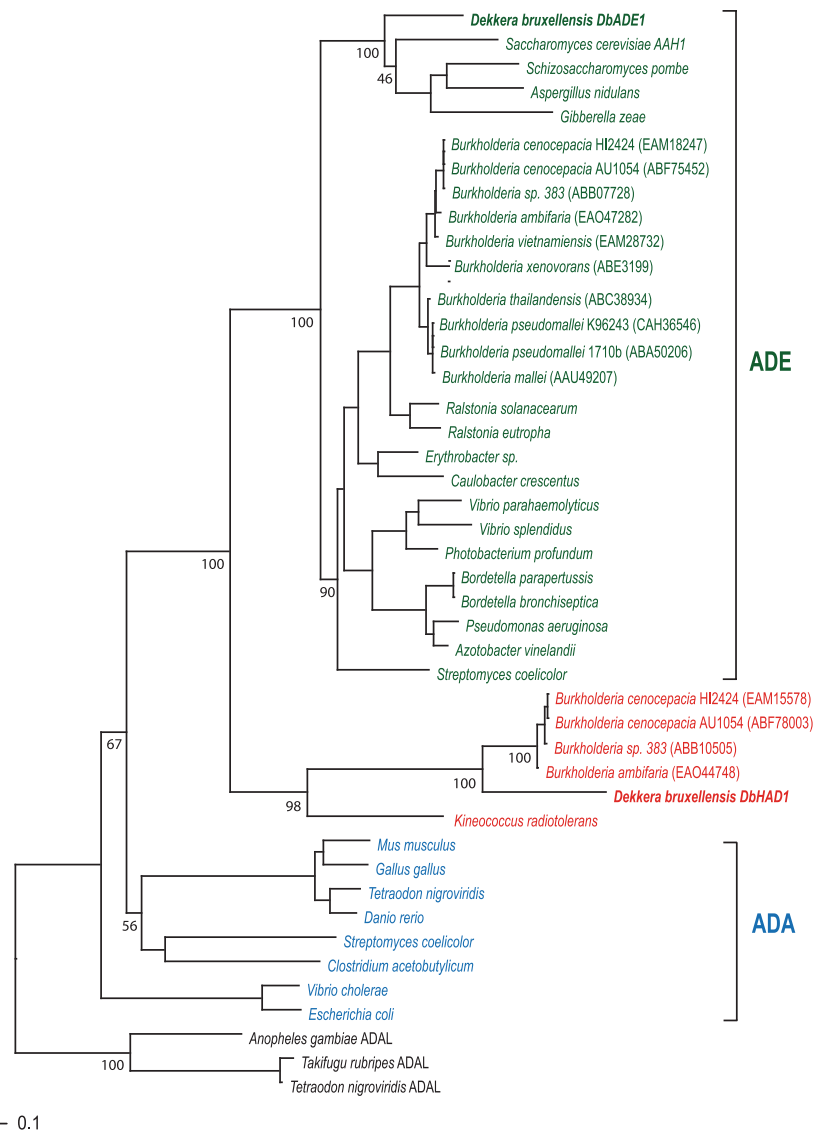


FIG. 5. Maximum likelihood phylogenetic tree, based on amino acid sequences, showing the relationships between adenine deaminases (ADEs, green), adenosine deaminases (ADAs, blue), and the cluster of horizontally transferred genes (red). Bootstrap values (1,000 replicates) are given for branches of interest. GenBank accession numbers are given in parentheses for *Burkholderia* genes.

genomes (Fig. 5). We could not identify orthologs of the HAD gene in any of the other *Burkholderia* species for which whole genome sequences are available, although canonical ADE genes are present in those species. This distribution of the HAD gene among *Burkholderia* species suggests that it was acquired by a relatively recent common ancestor of these four species. Inspection of the genomic region surrounding the HAD gene in the four *B. cepacia* complex species suggests that it is part of an operon specific to these species (e.g., genes Bcep18194_B0386 to Bcep18194_B0389 in the *Burkholderia* sp. strain 383 genome; GenBank accession number NC_007511.1) (data not shown), but none of the other genes in the operon can be detected in the *D. bruxellensis* sequence data or among publicly available *K. radiotolerans* sequences.

It seems likely that both *D. bruxellensis* and the four *B. cepacia* complex species received this gene as a horizontal

transfer, either independently or sequentially, from some other species not yet represented in the public sequence databases. The four *B. cepacia* complex species, *K. radiotolerans*, and *D. anomala* (the sister species of *D. bruxellensis*) have all been isolated from soil (15, 50, 61), a common habitat that provides the opportunity for genetic transfers, and *B. cepacia* complex species are known to have genomic characteristics that facilitate the receipt and transfer of nonendogenous DNA (15). Comparative analysis of conserved amino acid residues gave no indication of the substrate specificity of DbHAD1, although the residues required for catalytic function are conserved (see Table S5 in the supplemental material). It therefore seems likely that this gene remains functional in *D. bruxellensis*; it is difficult to speculate on precisely what role it plays, however.

While the genomes of some other hemiascomycete species have been shown to contain a number of horizontally transferred

genes (19, 28), *DbADE1* was the only example found in our data. We found no evidence of an ortholog of *URA1*, the horizontally transferred cytosolic dihydroorotate dehydrogenase (DHODase) gene present in *S. cerevisiae* and closely related species (24). Most basal hemiascomycetes, like other eukaryotes, have a mitochondrial DHODase, encoded by *URA9*; the activity of this enzyme is coupled to the mitochondrial respiratory chain because a quinone is the terminal electron acceptor. As *URA1* uses fumarate as an electron acceptor, the presence of this gene effectively decouples uracil biosynthesis from respiration, and it has been proposed that the transfer of *URA1* from an anaerobic bacterium to an ancestor of *S. cerevisiae* and *K. lactis* was an essential step in the evolution of facultative anaerobiosis in this group of yeasts (24, 28, 48).

Some species in the *URA1*-possessing clade, such as *K. lactis*, retain both *URA1* and *URA9*, while in others, like *Saccharomyces cerevisiae*, *URA9* has been lost and only *URA1* has been retained (24, 28). Our sequence data contain a complete *URA9* gene (Table 2), indicating that a parallel gene displacement event has not occurred in *D. bruxellensis*. It is not possible to determine from our data, however, whether *D. bruxellensis* has an undetected *URA1* gene that allows it to live anaerobically or whether it has found an alternative evolutionary solution to this challenge.

Conclusions. The genome survey sequence data analyzed here show that *D. bruxellensis* strain CBS 2499 has genome characteristics typical of other hemiascomycetes in many respects, including estimated gene number, intron size and number, intergenic length, and gene content. Its rate of sequence evolution is faster than that of its relative *C. albicans*, though it has a lower rate of genomic rearrangement; neither rate, however, is extreme. Although aneuploidy may contribute to the variation in karyotypes observed between strains of *D. bruxellensis* (58), the relatively low proportion of duplicated sequence we see in our data, together with the small number of lineage-specific duplicate genes identified, suggests that this strain is haploid; this is consistent with its relatively small estimated genome size when compared with other strains.

These data should additionally provide a resource for future genetic study of this species. For example, although our sequences do not include the gene coding for the cinnamate decarboxylase required for the production of ethylphenols and ethylguaiacols (30), they contain numerous uncharacterized enzymes, some of which may be involved in the generation of the other volatile compounds that contribute to “Brett” character in wine (29, 38). These data may also facilitate analyses of population structure and variation in *D. bruxellensis*. Such analyses are essential if we are to develop optimal strategies to control contamination and to determine whether strains of *D. bruxellensis* that are neutral or even beneficial to wine character exist and may be harnessed by the viticultural industry (22).

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