REVIEW

Genomic Insights into the *Saccharomyces sensu stricto* Complex

Anthony R. Borneman*,[1] **and Isak S. Pretorius**†
*The Australian Wine Research Institute, Adelaide, South Australia 5064, Australia, and †Macquarie University, Sydney, New South Wales 2109, Australia

ABSTRACT The *Saccharomyces sensu stricto* group encompasses species ranging from the industrially ubiquitous yeast *Saccharomyces cerevisiae* to those that are confined to geographically limited environmental niches. The wealth of genomic data that are now available for the *Saccharomyces* genus is providing unprecedented insights into the genomic processes that can drive speciation and evolution, both in the natural environment and in response to human-driven selective forces during the historical "domestication" of these yeasts for baking, brewing, and winemaking.

KEYWORDS yeast; genomics; *Saccharomyces*; industrial fermentation

THE *Saccharomyces sensu stricto* complex is currently composed of at least seven distinct species with origins ~10–20 MYA (Kellis *et al.* 2003). *Saccharomyces uvarum* and *S. eubayanus* are the most basal members of the *Saccharomyces sensu stricto* clade, while the division between *S. paradoxus* (encompassing *S. cariocanus*) and *S. cerevisiae* is the most recent (Figure 1A).

Members of the *Saccharomyces sensu stricto* group range from the important industrial and laboratory species *S. cerevisiae* to those that, to date, are found in specific, geographically limited environmental ranges. However, all members share the common attributes of ease of laboratory propagation, short generation times, and small genome sizes that make them appealing for evolutionary and functional genomics studies. This has resulted in a wealth of information of the genomes of these yeasts and an unrivaled framework for comparative investigation.

S. cerevisiae Model for Fundamental Biology and Industrial Workhorse

S. cerevisiae is the most prominent of the *Saccharomyces sensu stricto* clade due to its historically intimate association with human activities such as brewing, baking, and winemaking. In addition to its industrial role, by way of its eukaryotic biology, ease of propagation, and well-defined genetics, *S. cerevisiae* represents one of the most intensively studied biological model systems and the first eukaryote for which a fully characterized genome sequence was available (Goffeau *et al.* 1996).

Following the introduction of next-generation sequencing, the economic importance of industrial strains of *S. cerevisiae* has driven the large-scale sequencing of many industrial isolates. Genome sequence information is now available for >80 strains of *S. cerevisiae* in some form (complete, draft, or raw data).

Examination of subsets of these genomic data sets has shown that the population structure of *S. cerevisiae* is complex, composed of both clearly defined "pure" lineages based around either strictly geographic (Africa, North America, or Southeast Asia) or industrial limits (wine or sake) and mosaic strains that appear to be the result of outcrossing between multiple pure lineages (Liti *et al.* 2009; Schacherer *et al.* 2009). The presence of a large proportion of mosaic strains is very different from the situation observed in *S. paradoxus*, in which outcrossing appears to be very rare (Koufopanou *et al.* 2006; Liti *et al.* 2009), and may be due to the close association of *S. cerevisiae* with human activity. This association with humans is almost certainly also the basis of the discovery of links between industry and geography such as that found between strains from Europe and wine and vineyard isolates from around the world (Figure 2A).

Due to their far greater demands, *de novo* assembled and curated genomes still represent the minority of genomic data available for *S. cerevisiae*. However, annotation of small numbers of industrial strains from the wine, brewing, and biofuel industries has begun to uncover the genetic variation that has accumulated in the *S. cerevisiae* genome. Relative to the *ad hoc*

Copyright © 2015 by the Genetics Society of America
doi: 10.1534/genetics.114.173633
Manuscript received September 11, 2014; accepted for publication December 11, 2014.
[1]Corresponding author: The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia. E-mail: anthony.borneman@awri.com.au

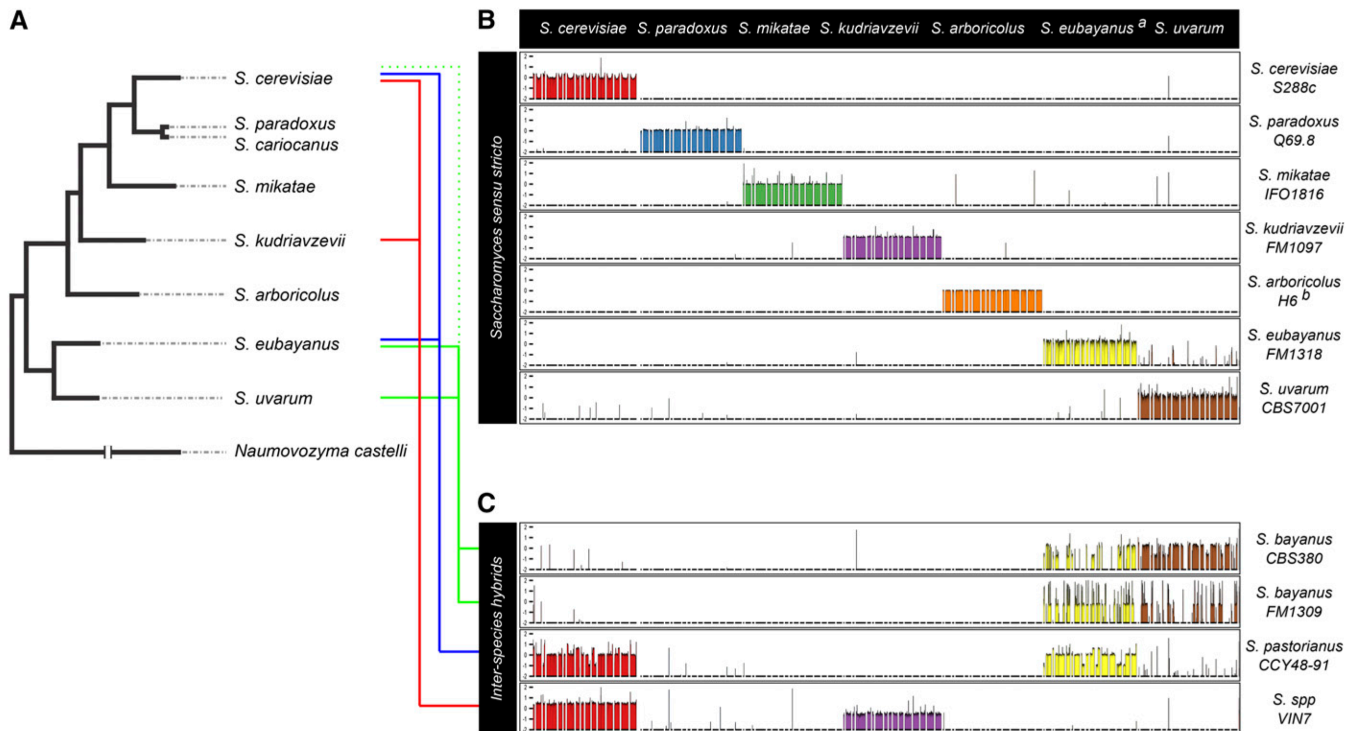

Figure 1 The *Saccharomyces sensu stricto* clade. (A) A schematic representation of the phylogenic structure of the *Saccharomyces sensu stricto* members, with *Naumovozyma castelli* as an outgroup. (B) Genomic representation of the *Saccharomyces sensu stricto* clade. Short read sequencing data from individual strains (as indicated to the left of the plot) were aligned to a common reference sequence composed of ordered, chromosomal-based scaffolds for each of the seven *Saccharomyces sensu stricto* species (listed at the top of the plot). The $\log_2$ ratio of sequence coverage compared to the genome-wide average across this reference is shown for 10-kb sliding sequence windows. For each "pure" species, sequence reads primarily map to the expected single reference genome, with an even level of coverage indicating equal relative chromosomal copy number. Small, isolated regions of coverage may be indicative of small-scale introgression events between species in individual strains. (C) Genomic representation of *Saccharomyces* interspecific hybrids, including the hybrid "species" *S. bayanus* and *S. pastorianus*. Sequencing data from individual hybrid strains were analyzed as in B. Each hybrid strain displays sequence reads that map to large portions of chromosomes from multiple distinct pure species. In addition, uneven sequence coverage indicates genomic copy number variation due to aneuploidy or chromosomal rearrangement. The *S. eubayanus* reference genome was estimated from the *S. eubayanus* portion of the *S. pastorianus* genome and therefore lacks several genomic loci that have been lost in this strain.

reference strain, S288c, there are at least 200 kb of additional DNA spread across numerous distinct genomic loci (encoding single genes to multigene clusters) present in other strains of *S. cerevisiae* (Table 1). Interestingly, the common theme across all of these comparisons to S288c is that this strain appears to represent an almost minimal core of common genes, displaying no ORFs that are absent in the majority of other strains (except for an extremely high number of transposon integration events), and is likely to reflect gene loss during its transition to laboratory cultivation under ideal growth conditions.

In general, the strain-specific loci reside in the subtelomeric regions of the *S. cerevisiae* genome, a location that appears to be the epicenter of genetic diversity in this species. This is presumably due to the presence of large numbers of subtelomeric repeats that act as the seeds for the integration, duplication, and/or loss of genomic segments between strains. The fact that these uncommon, gain-of-genome events are commonly observed in *S. cerevisiae* relative to the other members of the *Saccharomyces sensu stricto* group likely reflects the disruptive influence of human activity, whereby the selective forces imposed by the development of various industrial fermentations inadvertently selected for rare mutations with large phenotypic impact. This is highlighted by high-throughput phenotypic analysis that has shown *S. cerevisiae* to display a greater phenotypic plasticity than other *Saccharomyces sensu stricto* strains, such that industrial and wild strains of *S. cerevisiae* are often more distinct from each other than they are from other *Saccharomyces* species (which form tight, species-specific phenotypic clades) (Warringer *et al.* 2011).

There are three main loci that seem to define specific industrial classes of yeast, the *RTM1* cluster found predominantly in ale and distilling strains and the wine strain-specific circular cluster (see below) (Novo *et al.* 2009; Borneman *et al.* 2011). The *RTM1* gene was originally isolated as a subtelomeric gene, associated with the sucrose utilization (*SUC*) locus that was absent in laboratory strains of *S. cerevisiae*, which provided specific distilling strains with the ability to resist the effects of inhibitory compounds present in molasses (Ness and Aigle 1995). Subsequent genomic sequencing identified *RTM1* as a member of a three-gene cluster that is present in the opportunistic human pathogen YJM789 (Wei *et al.* 2007), ale yeast strains (FostersB and FostersO) (Borneman *et al.*

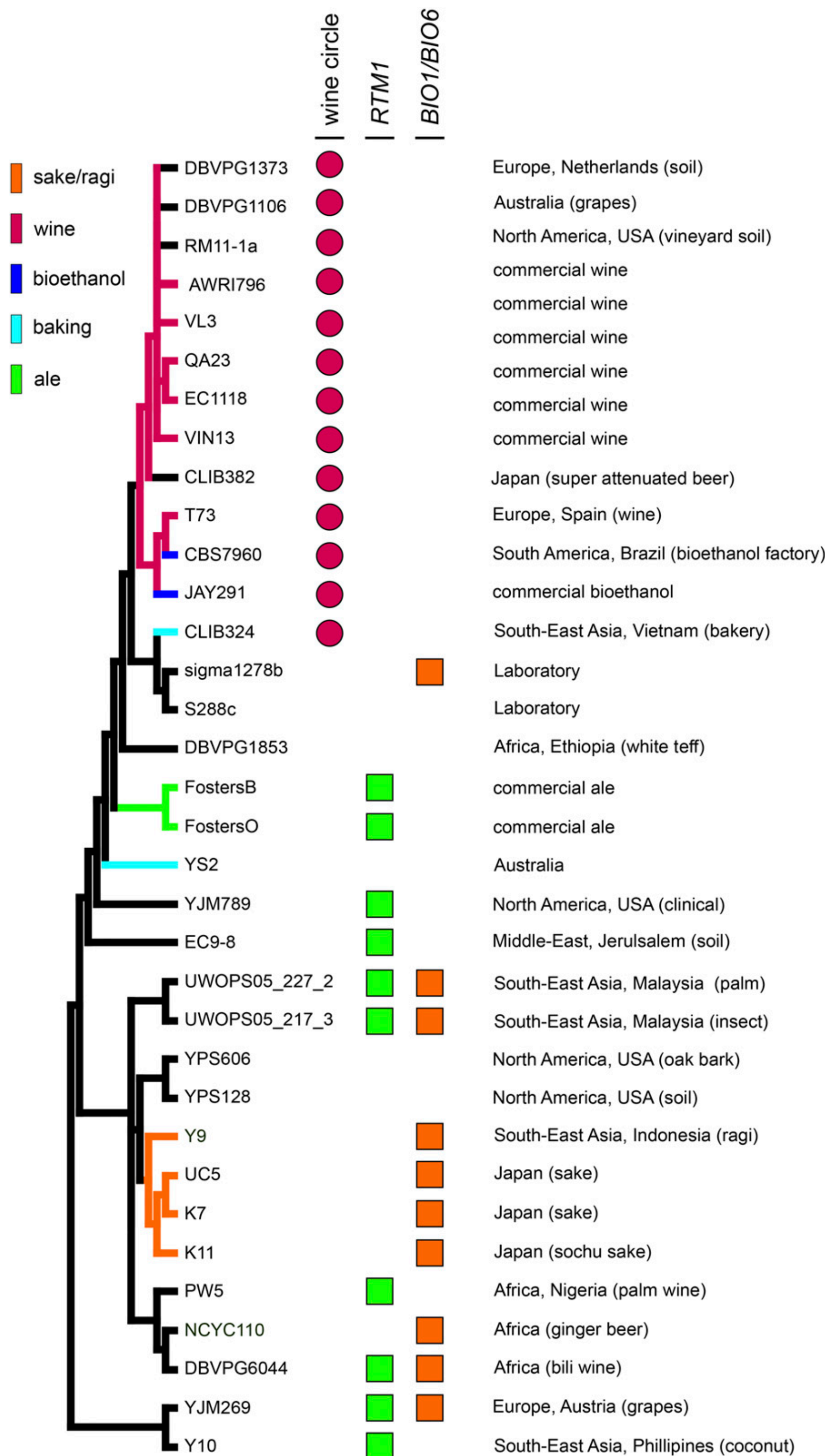

Figure 2 Genomic comparison of various strains of *S. cerevisiae*. A maximum-likelihood phylogeny was constructed for a variety of *S. cerevisiae* strains for which whole-genome sequence data are available. Branches involving industrial strains are shaded according to their documented use (sake/ragi, grape-based wine, bioethanol, baking, or ale production). The presence of three strain-specific genomic loci (wine circle, *RTM1* cluster, and *BIO1/BIO6*) and their source of isolation are also indicated for each strain.

2011), and several environmental isolates (Figure 2), but is absent from the genome of the biofuel strain JAY291, despite the common use of molasses for yeast propagation in brewing and biofuel production (Argueso *et al.* 2009). Interestingly, an ORF directly adjacent to *RTM1* (SCY_1426 in YJM789, FostersB_5069 in FostersB, and FostersO_5019:5020 in FostersO) is predicted to encode a large, ~800-amino-acid protein that has no detectable homology to proteins currently in the GenBank protein database, although it does contain sequence characteristics consistent with a role as a transcription factor. As such, the function of this protein and its relationship to *RTM1*, if any, remain to be determined.

Table 1 Strain-specific genomic loci in *S. cerevisiae*

Locus chromosomal location(s)	Locus size (kb)	No. predicted ORF(s)	Strain(s)	Strain type	Source
VI (L)[a]	38	19	EC1118	Wine	Novo *et al.* (2009)
			Lalvin QA23	Wine	Borneman *et al.* (2011)
			Vin7	Wine hybrid	
XV (R)[b]	60	>20	EC1118	Wine	Novo *et al.* (2009)
			Lalvin QA23	Wine	Borneman *et al.* (2011)
			Vin13 (partial)	Wine	Borneman *et al.* (2011)
			VL3 (partial)	Wine	Borneman *et al.* (2011)
			Vin7	Wine hybrid	Borneman *et al.* (2012)
			CLIB382		
XV	45	18	AWRI796	Wine	Borneman *et al.* (2011)
XI, XII, XIII, XIV, often multicopy	17	5	RM11-1a	Vineyard	
			EC1118	Wine	Novo *et al.* (2009)
			Lalvin QA23	Wine	Borneman *et al.* (2011)
			VL3	Wine	Borneman *et al.* (2011)
			AWRI796	Wine	Borneman *et al.* (2011)
			Vin13	Wine	Borneman *et al.* (2011)
			T73	Wine	
			CLIB382	Ale contaminant	
			JAY291	Biofuel	Argueso *et al.* (2009)
			CBS7960	Biofuel	
			CLIB324	Baking	
Various, often multicopy	4	2 (*RTM1*)	FL100	Laboratory	
			YJM789	Human	Wei *et al.* (2007)
			FostersB	Ale	Borneman *et al.* (2011)
			FostersO	Ale	Borneman *et al.* (2011)
			YJM269	Wine grapes	
			Y10	Coconut	
			PW5	Palm wine	
			EC9-8	Soil	
			UWOPS05_227_2	Palm	Liti *et al.* (2009)
			UWOPS05_217_3	Insect	Liti *et al.* (2009)
			NCYC110	Ginger beer	Liti *et al.* (2009)
			DBVPG6044	Bili wine	Liti *et al.* (2009)
VI	19	>3	JAY291	Biofuel	Argueso *et al.* (2009)
			PW5	Palm Wine	
			T7	Oak	
			Y10	Coconut	
			YJM269	Wine Grapes	
			EC9-8	Soil	
			YPS163	Biofuel	
VI, X, XIV, often multicopy	<1	1 (*MPR1* or *MPR2*)	Σ1278b	Laboratory	Dowell *et al.* (2010)
			RM11-1a	Vineyard	
			EC1118	Wine	Novo *et al.* (2009)
			QA23	Wine	Borneman *et al.* (2011)
			AWRI796	Wine	Borneman *et al.* (2011)
			VL3	Wine	Borneman *et al.* (2011)
			Vin13	Wine	Borneman *et al.* (2011)
			AWRI1631	Wine	Borneman *et al.* (2008)
			Vin7	Wine hybrid	Borneman *et al.* (2012)
			JAY291	Biofuel	Argueso *et al.* (2009)
			CBS7960	Biofuel	
			EC9-8	Soil	
			T7	Oak	
			CLIB215	Baking	
			CLIB324	Baking	
XIV	8	2	RM11-1a	Vineyard	
			AWRI796	Wine	Borneman *et al.* (2011)
			Kyokai No7	Sake	Akao *et al.* (2011)
			M3707	Biofuel	
IV, VI	<1	1 (*YJMGNAT*)	YJM789	Human	Wei *et al.* (2007)
			Kyokai No7	Sake	Akao *et al.* (2011)
			UC5	Sake	

(continued)

Table 1, *continued*

Locus chromosomal location(s)	Locus size (kb)	No. predicted ORF(s)	Strain(s)	Strain type	Source
			FostersO	Ale	Borneman *et al.* (2011)
			FostersB	Ale	Borneman *et al.* (2011)
			T7	Oak	
			YPS163	Vineyard	
			YJSH1	Biofuel	
			M3707	Biofuel	Brown *et al.* (2013)
			EC9-8	Soil	
IX	<1	1 (*KHR* killer toxin)	YJM789	Human	Wei *et al.* (2007)
			EC1118	Wine	Borneman *et al.* (2011)
			VL3	Wine	Borneman *et al.* (2011)
			Vin13	Wine	Borneman *et al.* (2011)
			FostersO	Ale	Borneman *et al.* (2011)
			FostersB	Ale	Akao *et al.* (2011)
			Kyokai No7	Sake	Brown *et al.* (2013)
			M3707	Biofuel	
XV	5	1 (*AWA1*)	Kyokai No7	Sake	Akao *et al.* (2011)
I, II, VIII, IX, XII, XVI	<1	1 (*BIO6*)	Kyokai No7	Sake	Akao *et al.* (2011)
			UC5	Sake	
			CEN.PK	Laboratory	Otero *et al.* (2010)
			Σ1278b	Laboratory	Dowell *et al.* (2010)
			YJM269	Wine grapes	
			YJSH1	Biofuel	
			ZTW1	Biofuel	
			M3707	Biofuel	Brown *et al.* (2013)
I, II, VIII, IX	<1	1 (*BIO1*)	Kyokai No7	Sake	Akao *et al.* (2011)
			UC5	Sake	
			CEN.PK	Laboratory	Otero *et al.* (2010)
			Σ1278b	Laboratory	Dowell *et al.* (2010)
			YJM269	Wine grapes	
			YJSH1	Biofuel	
			ZTW1	Biofuel	
			M3707	Biofuel	Brown *et al.* (2013)
			UWOPS05_227_2	Palm	Liti *et al.* (2009)
			UWOPS05_217_3	Insect	Liti *et al.* (2009)
			NCYC110	Ginger beer	Liti *et al.* (2009)
			DBVPG6044	Bili wine	Liti *et al.* (2009)
I, XI	1	1 (*EHL*)	Kyokai No7	Sake	Akao *et al.* (2011)
			UC5	Sake	
			YJSH1	Biofuel	
			ZTW1	Biofuel	
			M3707	Biofuel	Brown *et al.* (2013)
			UWOPS05_227_2	Palm	Liti *et al.* (2009)
			UWOPS05_217_3	Insect	Liti *et al.* (2009)
			NCYC110	Ginger beer	Liti *et al.* (2009)
			DBVPG6044	Bili wine	Liti *et al.* (2009)
VI	6	1 (*IRC7* paralog)	Y10	Coconut	
			YJM450	Human	Roncoroni *et al.* (2011)
IV	8	3	Kyokai No7	Sake	Akao *et al.* (2011)
			UC5	Sake	
			CEN.PK	Laboratory	Otero *et al.* (2010)
			Σ1278b	Laboratory	Dowell *et al.* (2010)
			YJM269	Wine grapes	
			YJSH1	Biofuel	
			ZTW1	Biofuel	
			M3707	Biofuel	Brown *et al.* (2013)
			PW5	Palm wine	
			T7	Oak	
Unknown	6	3	Y10	Coconut	

[a] Left arm.
[b] Right arm.

The second industry-defining locus of *S. cerevisiae* was initially identified as one of several genomic fragments that were present in the wine strain EC1118 (Novo *et al.* 2009). Subsequent detailed analysis of several industrial *S. cerevisiae* genomes by Borneman *et al.* (2011) showed that while this cluster of five genes was specific to wine strains (with the exception of the biofuel strain JAY291), it displayed strain-specific differences in copy number, genomic location, and gene order. Diversity in the cluster was shown to be consistent with mobilization into, and throughout, the wine yeast genome as a circular intermediate via an unknown process that has since been proposed to also occur in both mammals and fish (Borneman *et al.* 2011; Fujimura *et al.* 2011; Durkin *et al.* 2012). Subsequent to this work, this genomic feature has been located in the genomes of several additional strains of *S. cerevisiae* that all seemingly reside in the same wine-specific phylogenic clade (Figure 2A).

The final industry-specific locus involves the evolution of biotin prototrophy in a subset of strains of *S. cerevisiae*. While the majority of *S. cerevisiae* isolates, including those used in winemaking and brewing, are biotin auxotrophs, some, such as those used for the production of sake, are able to synthesize biotin *de novo*, presumably due to the very low biotin content of sake mash (Wu *et al.* 2005). This conversion to biotin prototrophy is due to the reacquisition of two ORFs, *BIO1* and *BIO6*, that encode the enzymatic steps that are missing in the biotin pathway of most other strains (Wu *et al.* 2005; Hall and Dietrich 2007). As for many of these species-specific ORFs, the donor species of these DNA sequences is also not clear; however, suggestions hint at a *de novo* origin in *S. cerevisiae*, rather than horizontal acquisition, through duplication and neofunctionalization of *BIO3* (*BIO6*) and YJR154W (*BIO1*) (Hall and Dietrich 2007).

In addition to these potentially industry-defining loci there are also several strain-specific ORFs for which important phenotypes can be attributed. *FSY1* was first identified as a member of the large multigenic strain-specific locus present in the EC1118 group of *S. cerevisiae* wine strains (Novo *et al.* 2009) (Table 1). Based on homology to an ORF from *S. pastorianus*, *FSY1* was predicted to encode a H^{+}/fructose symporter that is proposed to have been horizontally transferred into *S. cerevisiae* from an unidentified relative (Galeote *et al.* 2010). The presence of this protein is thought to enable active transport of fructose into the cell, a phenotypic trait that is lacking from the majority of *S. cerevisiae* strains and is expected to provide a selective advantage in the highly concentrated 1:1 mixture of glucose and fructose that is present during wine fermentation.

MPR1 and *MPR2* were first identified as almost identical paralogous ORFs specific to the *S. cerevisiae* strain Σ1278b and were responsible for providing resistance to L-azetidine-2-carboxylic acid (Takagi *et al.* 2000). Subsequent studies have shown that this gene family provides general stress resistance by decreasing the toxic effects of reactive oxygen species (Nishimura *et al.* 2010; Sasano *et al.* 2010). Like *RTM1*, *MPR*-family paralogs are also found in the telomeric regions and can be present in multiple copies within a strain. While they are absent from the laboratory strain S288c, sequencing has identified *MPR*-family ORFs in many industrial strains, including those from winemaking, baking, and biofuel backgrounds, where they presumably provide resistance to stresses imposed by industrial fermentation (Table 1).

The *IRC7* gene encodes a β-lyase that is responsible for the release of volatile thiols that are especially important during winemaking (Thibon *et al.* 2008; Roncoroni *et al.* 2011). While the genomes of all strains of *S. cerevisiae*, including S288c, appear to contain *IRC7*, a highly diverse homolog of this gene (88% DNA identity to S288c *IRC7*) was identified in the human clinical isolate YJM540. This new *IRC7*-family member was subsequently shown to be highly active at thiol release, providing YJM450 with the ability to produce enhanced levels of these aroma compounds compared to other yeast strains (Roncoroni *et al.* 2011). Subsequent genome sequencing has identified this ortholog in only one other strain of *S. cerevisiae* (Y10), isolated from coconut in the Philippines. During its initial characterization, this divergent ortholog was suggested to have been introgressed from *S. paradoxus*. However, given that this gene has been identified only in one strain of *S. paradoxus* (UWOPS91-917.1 isolated in Hawaii), the actual origin of this particular version of *IRC7* may lie outside of both of these species or be a result of rapid sequence divergence of the common *S. cerevisiae* gene (Liti *et al.* 2009; Roncoroni *et al.* 2011).

S. paradoxus and *S. cariocanus*

S. paradoxus represents the closest known relative to *S. cerevisiae* (Figure 1A). Despite this phylogenetic relationship, while *S. cerevisiae* is intimately associated with human industry, there is very little, if any, evidence of an industrial role for *S. paradoxus*, which is instead generally limited to environmental niches where it is associated with trees of the *Quercus* (Oak) genus (and possibly related genera).

Genomic data for *S. paradoxus* suggest that the species comprises two very distinct populations, represented by the Americas and Eurasia, with strains of European and Asian origin also being readily separated into subpopulations within this larger clade (Liti *et al.* 2006, 2009). Across these subpopulations, the levels of nucleotide divergence between the most distant clades (∼4.6%) are far higher than has been observed in *S. cerevisiae* and may be due to an apparent lack of interbreeding in *S. pastorianus* (Liti *et al.* 2009). This lack of interbreeding even extends to populations found on the same tree branches, with no evidence of heterozygous offspring between genetically distinct neighbors observed (Koufopanou *et al.* 2006). This high level of sequence variation has also led to the development of partial reproductive barriers between the strains, with spore viability approaching as little as 30% for interclade crosses and possibly representing the early stages of biological concept speciation for the three subpopulations (Sniegowski *et al.* 2002; Liti *et al.* 2006).

In addition to reproductive isolation imposed by sequence divergence between *S. paradoxus* populations, examples of

reciprocal translocations that affect reproductive success between strains have also been recorded for this species. This is highlighted by the designation of *S. cariocanus* as a separate *Saccharomyces* spp. due to its extremely low spore viability (~5%) when mated to *S. paradoxus* (Naumov *et al.* 2000). However, subsequent genomic analysis has shown that the level of sequence divergence between *S. cariocanus* and *S. paradoxus* strains of the Americas is within the range observed across *S. paradoxus*, with the ultimate cause of the reproductive isolation being due to four reciprocal translocations (II and XVI, IX and XV, XII and XIV, and IV and XI) present in the genomes of the *S. cariocanus* strains (compared to both *S. paradoxus* and *S. cerevisiae* that are colinear) (Fischer *et al.* 2000).

Despite *S. paradoxus* displaying levels of genetic variation that are far greater than those observed for *S. cerevisiae*, there appears to be considerably less gene content variation within this species (Bergström *et al.* 2014). This difference in SNP *vs.* gene content variation may reflect the different selective pressures observed between the natural ecological niches of *S. paradoxus* in contrast to the potentially sudden and disruptive pressures imposed upon *S. cerevisiae* during its transition toward "domestication." This is supported by widespread phenotype comparisons that show *S. cerevisiae* to display higher intraspecies trait variability than *S. paradoxus* in spite of its lower SNP diversity (Warringer *et al.* 2011).

However, despite these generalizations "atypical" strains of *S. paradoxus*, such as the Hawaiian isolate UWOPS91-917.1, have been identified that do contain significant numbers of novel genes (*e.g.*, *MEL1* and the variant *IRC7*) that impart important phenotypic characteristics. One other key difference in gene content between *S. paradoxus* populations is the presence of an 18-kb element that has introgressed from *S. cerevisiae* into the genomes of European isolates of *S. paradoxus* relative to their American and Far Eastern counterparts. This element has resulted in the replacement of at least 12 *S. paradoxus* ORFs in these strains with equivalent genes from *S. cerevisiae* (Liti *et al.* 2006).

S. mikatae

Despite being included in the first genomic comparisons of the *Saccharomyces sensu stricto* clade, only IFO1815, the type strain of *S. mikatae*, has been sequenced to date (Cliften *et al.* 2003; Kellis *et al.* 2003; Scannell *et al.* 2011). While IFO1815 has been shown to harbor two translocations compared to *S. cerevisiae* (VI and VII, VII and XVI), data suggest that this may be variable across strains as the closely related *S. mikatae* strain IFO1816 appears to contain only a single translocation event (VI and VII) (Fischer *et al.* 2000; Scannell *et al.* 2011).

Given that there is only one strain of *S. mikatae* for which genomic data are available, the levels of interstrain variation within this species remain to be resolved. However, given the ease of current genome sequencing, obtaining a wider understanding of *S. mikatae* genomic variation will likely be limited by the very small number of strains that are currently available for this particular species (with the largest collection of *S. mikatae* strains being limited to a total of only 14, all which are from Japan).

S. arboricolus

S. arboricolus is the newest addition to the *Saccharomyces sensu stricto* clade (Wang and Bai 2008; Naumov *et al.* 2010) with the genome of the *S. arboricolus* type strain (CBS 10644) being recently completed (Liti *et al.* 2013). Like *S. mikatae*, the single representative sequence provides no insight into the diversity within the species, but provides an additional point of comparison to the other *Saccharomyces sensu stricto* species. When compared to the genome of *S. cerevisiae* S288c, the *S. arboricolus* genome harbors one reciprocal translocation between the right arms of chromosomes IV and XIII that is unique to this species, as well as two small inversions (chromosome VI encompassing YFR008W through YFR017C and chromosome XIV from YNL034W through YNL041C) that are shared with *S. uvarum* and *S. kudriavzevii* (Liti *et al.* 2013).

Liti *et al.* (2013) have estimated *S. arboricolus* contains at least 44 and up to 210 genes that are not found in *S. cerevisiae*. However, for some of these ORFs, including *MEL1*, *BIO1*, and *BIO6* and two ancestral paralogs of the *S. cerevisiae SIR1* gene, this is due to widespread loss specifically in *S. cerevisiae* rather than gain in *S. arboricolus*, as they are also found in *S. uvarum* and *S. kudriavzevii* (Hall and Dietrich 2007; Zill *et al.* 2010; Warringer *et al.* 2011).

S. kudriavzevii

Like *S. mikatae*, *S. kudriavzevii* was first isolated in Japan from decaying leaves (Naumov *et al.* 2000). However, unlike *S. mikatae*, in which the limited number of strains are all from Japan, studies have also isolated *S. kudriavzevii* from Europe (from the bark of *Quercus* spp. in Portugal). In the European environment *S. kudriavzevii* is found in sympatric association with both *S. cerevisiae* and *S. paradoxus*, but displays a more cryotolerant phenotype than either of these other species, thereby providing *S. kudriavzevii* with a competitive niche (Sampaio and Gonçalves 2008).

While the first *S. kudriavzevii* genome (IFO 1802) was produced in 2003 by two independent groups (Cliften *et al.* 2003; Kellis *et al.* 2003), additional refinement of the IFO 1802 genome, as well as *de novo* sequencing and assembly of a representative of the Portuguese *S. kudriavzevii* population (ZP591), has now also been completed (Scannell *et al.* 2011, p. 3). Like *S. paradoxus*, the genomes of both *S. kudriavzevii* IFO 1802 and ZP591 are colinear with *S. cerevisiae* (Fischer *et al.* 2000; Scannell *et al.* 2011)

Interestingly, comparative resequencing of 18 currently available *S. kudriavzevii* isolates (4 Japanese and 14 European) showed that while all the Japanese isolates of *S. mikatae* were incapable of assimilating galactose due to the concerted degeneration of the entire multigenic galactose utilization (*GAL*) pathway, all of the European strains carry a fully functional metabolic route and an associated galactose positive phenotype (Hittinger *et al.* 2004, 2010). The origin or selective advantage provided by this balanced polymorphism

between the two geographically isolated groups remain to be determined.

S. eubayanus

Due to its contribution to the genome of the lager hybrid *S. pastorianus*, the existence of *S. eubayanus* was long predicted without a representative of the species having been identified (Martini and Kurtzman 1985; Rainieri *et al.* 2006; Dunn and Sherlock 2008; Nakao *et al.* 2009; Nguyen *et al.* 2011). This was finally resolved through the isolation and genomic analysis of an entirely new *Saccharomyces* species, *S. eubayanus*, although it remains the only *Saccharomyces* species for which a *de novo* assembly is not available (Libkind *et al.* 2011).

Surprisingly, rather than the *S. eubayanus* parent of *S. pastorianus* being of European origin, as is the case for the *S. cerevisiae* portion of the *S. pastorianus* genome, it appears that *S. eubayanus* may have been imported into Europe. As yet, pure *S. eubayanus* has not been isolated from Europe; however, a diverse number of strains have been readily found associated with southern beech (*Nothofagus* spp.) in Patagonia, where they form at least two distinct and diverse populations (Libkind *et al.* 2011; Peris *et al.* 2014). Furthermore, it appears that, in addition to being imported into Europe, these two distinct types of *S. eubayanus* may have both been imported into North America where they underwent admixture to produce a hybrid population (Peris *et al.* 2014).

In addition to being a parent of *S. pastorianus*, the identification of *S. eubayanus* as a pure species also affected the species definition of *S. bayanus*, as it appears that many members of this species complex are actually hybrids of *S. eubayanus* and *S. uvarum* (Nguyen *et al.* 2011).

S. uvarum and S. bayanus

The classification of the *S. uvarum* and *S. bayanus* species remains one of the more contentious issues in the classification of the *Saccharomyces sensu stricto* group. Initially composed of five species (*S. bayanus*, *S. globosus*, *S. heterogenicus*, *S. inusitatus*, and *S. uvarum*), these were subsequently merged into *S. bayanus* on the basis of DNA:DNA hybridization (Martini and Kurtzman 1985). However, the recent detailed examinations of the *S. pastorianus* and *S. eubayanus* genomes have shown that, from a genomic viewpoint, there are two clearly defined groups within the *S. bayanus* species defined by Martini and Kurtzman that relate back to the original *S. bayanus* and *S. uvarum* subspecies (Libkind *et al.* 2011; Nguyen *et al.* 2011).

In this genome-centric division, *S. uvarum* (*S. bayanus* var. *uvarum*) strains represent a pure lineage that contains very little genetic input from other *Saccharomyces* species (Figure 1B). While strains of this species are readily isolated from natural environments and low-temperature industrial fermentations, a *de novo* assembly exists for only the type strain of *S. uvarum*, CBS7001 (isolated from an insect in Spain and originally identified as *S. bayanus*) (Martini and Kurtzman 1985). This strain differs from *S. cerevisiae* by four translocations (XIII and XV, VI and X, V and VII, and II and IV) (Fischer *et al.* 2000; Scannell *et al.* 2011).

Interspecies comparison has shown that *S. uvarum* is the only *Saccharomyces sensu stricto* species to retain the budding yeast *Dicer* homolog that composes part of the RNAi machinery and the paralog of *S. cerevisiae* *GAL80* that was present following the whole-genome duplication event. Both these genes are found in more distantly related yeast species such as *Naumovozyma* (formerly *S. castelli*) but have been lost from the rest of the *Saccharomyces sensu stricto* lineage (Hittinger *et al.* 2004; Cliften *et al.* 2006; Drinnenberg *et al.* 2009; Scannell *et al.* 2011).

In contrast, *S. bayanus* (*S. bayanus* var. *bayanus*) strains such as CBS380^T (*S. bayanus* type strain) or NBRC1948 represent highly recombined, interspecific hybrids that comprise almost equal genomic contributions from *S. eubayanus* and *S. uvarum*, with a minor (70–80 kb) input from *S. cerevisiae* (Figure 1C). The *S. cerevisiae* portion of the *S. bayanus* genome encodes a number of genes, but the main phenotypic consequence is likely to relate to the ability to of *S. bayanus* to metabolize maltose and maltotriose, a phenotype that is lacking in *S. uvarum*. While the phylogeny of two of these genes suggests that they originated from a European wine strain of *S. cerevisiae* (Libkind *et al.* 2011; Nguyen *et al.* 2011), the presence of the *RTM1* cluster in these strains adjacent to these genes is more consistent with the fragment originating from a European ale or distilling strain of *S. cerevisiae*.

While the formation of *S. bayanus* may have occurred as a result of the environmental sympatric association of *S. eubayanus* and *S. uvarum* (Libkind *et al.* 2011), *S. bayanus* strains have been isolated only from "artificial" brewery environments and may therefore share their origin with *S. pastorianus* in European breweries during the Middle Ages. Furthermore, it has been suggested that CBS380^T-type strains may be the result of additional hybridization events between *S. uvarum* (*e.g.*, CBS7001) and *S. bayanus* (*e.g.*, NBRC1948) as these strains are interfertile and produce progeny with chromosomal content similar to that of CBS380^T (Nguyen *et al.* 2011). This intercrossing ability may therefore have accelerated the recombination and consolidation of parental chromosomes in *S. bayanus* compared to those in *S. pastorianus*, leading to a highly composite genomic arrangement of *S. bayanus* when compared to *S. pastorianus*, which still displays many of the chromosomal hallmarks and copy number effects of the original hybridization event.

Other Interspecific Hybrids

The integrity of species within the *Saccharomyces sensu stricto* complex is the result of postzygotic reproductive barriers ($<1\%$ viable meiotic spores) that appear to be driven primarily by sequence divergence, rather than chromosomal rearrangements, as engineering colinear genomes between divergent species does not produce efficient intraspecific fertility (Naumov 1987; Chambers *et al.* 1996; Hunter *et al.* 1996; Delneri *et al.* 2003; Greig *et al.* 2003). However,

as this barrier is postzygotic, diploid or polyploid hybrids that do form via interspecific mating events are able to reproduce indefinitely via mitotic division. This phenomenon is not limited to laboratory experimentation and there are numerous reports of *Saccharomyces* interspecific hybrids being associated with cold fermentative environments, such as those observed in winemaking and beer brewing in Northern European countries (Sipiczki 2008).

S. pastorianus

As early as the 1980s the lager yeast *S. pastorianus* was suggested to be the result of a relatively recent (15th–16th century) interspecific hybridization event between *S. cerevisiae* and at least one other *Saccharomyces* spp. (Martini and Kurtzman 1985). Initial genomic analysis of several lager yeast genomes, using microarray-based comparative genome hybridization (aCGH), subsequently confirmed via genome sequencing, suggested that there were two distinct *S. pastorianus* sublineages, which could be roughly categorized by their geographic origin and Saaz and Frohberg types (Dunn and Sherlock 2008; Nakao *et al.* 2009; Walther *et al.* 2014), and that the non-*S. cerevisiae* parent of both types of *S. pastorianus* was more similar to but not entirely the same as *S. bayanus* (*S. bayanus* var. *bayanus*), which itself was considered to be a possible hybrid strain (see below) (Nakao *et al.* 2009; Walther *et al.* 2014).

The mystery surrounding the non-*S. cerevisiae* parent of *S. pastorianus* was finally resolved, as discussed above, by the work of Libkind *et al.* (2011) via the isolation and identification of an entirely new *Saccharomyces* species, *S. eubayanus* (Figure 1). The genomic sequence of *S. eubayanus* was highly homologous to the non-*S. cerevisiae* portions of the *S. pastorianus* genome, suggesting that the hybridization event that gave rise to *S. pastorianus* occurred between *S. cerevisiae* and *S. eubayanus*, presumably following the incidental importation of *S. eubayanus* into Europe, although the exact source of the *S. eubayanus* parent remains controversial (Libkind *et al.* 2011; Bing *et al.* 2014).

Regardless of the true geographical origin of the parental strain, the increased data afforded by genome sequencing also accurately showed that the Saaz-type strains of *S. pastorianus* (*e.g.*, former *S. carlsbergensis* strains) are generally triploid (2n *S. eubayanus*, 1n *S. cerevisiae*), but with ~3.5 Mb of DNA missing from the *S. cerevisiae* contribution (including the entirety of chromosomes VI, XI, and XII), while the Frohberg-type *S. pastorianus* strains (*e.g.*, Weihenstephan strain WS34/70) are primarily tetraploid (2n, 2n) with limited loss of contributions from either parent.

In addition, despite the different chromosomal content of the two *S. pastorianus* groups suggesting independent origins, the analysis of the genetic variation present across the *S. eubayanus* portion of the genome, combined with the presence of several common genetic rearrangements between *S. cerevisiae* and *S. eubayanus* chromosomes in the Saaz and Frohberg lineages, suggests that both these groups arose from a single, common hybridization event (Dunn and Sherlock 2008; Peris *et al.* 2014; Walther *et al.* 2014). Under this model, differential mitotic recombination, unequal parental chromosomal loss, and recombination between homeologous parental chromosomes are proposed to have divergently acted on the same common ancestor to produce the two *S. pastorianus* groups, with differences in cryotolerance being a suspected phenotypic selective driver (Rainieri *et al.* 2006; Dunn and Sherlock 2008; Nakao *et al.* 2009; Libkind *et al.* 2011; Walther *et al.* 2014).

Wine yeast hybrids

Like the situation observed in brewing, wine fermentations performed at warm temperatures (>20°) are naturally dominated by *S. cerevisiae*. However, it is becoming increasingly evident that wine fermentations performed at lower temperature ranges are readily dominated by naturally occurring interspecific hybrids, including those formed between *S. cerevisiae* and either *S. uvarum* (González *et al.* 2006; Le Jeune *et al.* 2007) or *S. kudriavzevii* (González *et al.* 2006; Erny *et al.* 2012). In addition to naturally occurring interspecific hybrids, hybrids of *S. cerevisiae* and either *S. paradoxus*, *S. kudriavzevii*, or *S. mikatae* have been artificially induced for commercialization purposes (Bellon *et al.* 2011, 2013). Like the situation observed for *S. pastorianus*, these hybrid strains are often not complete and contain varying amounts of each parental genome (Dunn *et al.* 2012; Erny *et al.* 2012).

The only assembled genome sequence available for a hybrid wine yeast strain is that of the commercial strain VIN7 (Borneman *et al.* 2012). Analysis showed that VIN7 was an allotriploid, resulting from hybridization between a heterozygous diploid wine-like strain of *S. cerevisiae* and a haploid, European isolate of *S. kudriavzevii* (Figure 1C). Unlike lager yeast, and many other hybrid wine strains, VIN7 appears to be an almost complete hybrid and with limited genetic rearrangement between the two parental genomes (as few as three cases of recombination between homeologous chromosome pairs).

However, rather than providing only a cold-tolerant growth advantage, it appears that the presence of the *S. kudriavzevii* genome may have fortuitously allowed for VIN7 to release much larger amounts (often over double) of the fruity volatile thiol 4-mercapto-4- methylpentan-2-one (4MMP) from grape-derived, nonvolatile precursors during fermentation than *S. cerevisiae* wine yeast strains, providing a basis for ongoing genetic selection in a winemaking environment via human intervention (González *et al.* 2007; King *et al.* 2008; Swiegers *et al.* 2009).

Concluding Remarks

The wealth of genomic data that are available for the *Saccharomyces* genus provides an unprecedented insight into the evolution of this important group of microorganisms. However, advances in long-read genome sequencing assembly techniques are set to allow for even greater ease of *de novo* assembly, rather than genomic resequencing, of large numbers of strains. This will provide a detailed estimation of the breadth of the pan-genome of the *Saccharomyces sensu*

stricto clade and how this relates to the high levels of diversity that are observed across the many varied phenotypic characteristics inherent in the *Saccharomyces sensu stricto* group.

Acknowledgments

Research at The Australian Wine Research Institute (AWRI) is supported by Australia's grape growers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. The AWRI is part of the Wine Innovation Cluster in the Waite Precinct, South Australia. I.S.P. is supported by an internal grant from Macquarie University.

Literature Cited

Akao, T., I. Yashiro, A. Hosoyama, H. Kitagaki, H. Horikawa *et al.*, 2011 Whole-genome sequencing of sake yeast *Saccharomyces cerevisiae* Kyokai no. 7. DNA Res. Int. J. Rapid Publ. Rep. Genes Genomes 18: 423–434.

Argueso, J. L., M. F. Carazzolle, P. A. Mieczkowski, F. M. Duarte, O. V. C. Netto *et al.*, 2009 Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production. Genome Res. 19: 2258–2270.

Bellon, J. R., J. M. Eglinton, T. E. Siebert, A. P. Pollnitz, L. Rose *et al.*, 2011 Newly generated interspecific wine yeast hybrids introduce flavour and aroma diversity to wines. Appl. Microbiol. Biotechnol. 91: 603–612.

Bellon, J. R., F. Schmid, D. L. Capone, B. L. Dunn, and P. J. Chambers, 2013 Introducing a new breed of wine yeast: interspecific hybridisation between a commercial *Saccharomyces cerevisiae* wine yeast and *Saccharomyces mikatae*. PLoS ONE 8: e62053.

Bergström, A., J. T. Simpson, F. Salinas, B. Barré, L. Parts *et al.*, 2014 A high-definition view of functional genetic variation from natural yeast genomes. Mol. Biol. Evol. 31: 872–888.

Bing, J., P.-J. Han, W.-Q. Liu, Q.-M. Wang, and F.-Y. Bai, 2014 Evidence for a Far East Asian origin of lager beer yeast. Curr. Biol. 24: R380–R381.

Borneman, A. R., A. H. Forgan, I. S. Pretorius, and P. J. Chambers, 2008 Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. FEMS Yeast Res. 8: 1185–1195.

Borneman, A. R., B. A. Desany, D. Riches, J. P. Affourtit, A. H. Forgan *et al.*, 2011 Whole-genome comparison reveals novel genetic elements that characterize the genome of industrial strains of *Saccharomyces cerevisiae*. PLoS Genet. 7: e1001287.

Borneman, A. R., B. A. Desany, D. Riches, J. P. Affourtit, A. H. Forgan *et al.*, 2012 The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. FEMS Yeast Res. 12: 88–96.

Brown, S. D., D. M. Klingeman, C. M. Johnson, A. Clum, A. Aerts *et al.*, 2013 Genome sequences of industrially relevant *Saccharomyces cerevisiae* strain M3707, isolated from a sample of distillers yeast and four haploid derivatives. Genome Announc. 1: e00323-13.

Chambers, S. R., N. Hunter, E. J. Louis, and R. H. Borts, 1996 The mismatch repair system reduces meiotic homeologous recombination and stimulates recombination-dependent chromosome loss. Mol. Cell. Biol. 16: 6110–6120.

Cliften, P., P. Sudarsanam, A. Desikan, L. Fulton, B. Fulton *et al.*, 2003 Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. Science 301: 71–76.

Cliften, P. F., R. S. Fulton, R. K. Wilson, and M. Johnston, 2006 After the duplication: gene loss and adaptation in *Saccharomyces genomes*. Genetics 172: 863–872.

Delneri, D., I. Colson, S. Grammenoudi, I. N. Roberts, E. J. Louis *et al.*, 2003 Engineering evolution to study speciation in yeasts. Nature 422: 68–72.

Dowell, R. D., O. Ryan, A. Jansen, D. Cheung, S. Agarwala *et al.*, 2010 Genotype to phenotype: a complex problem. Science 328: 469.

Drinnenberg, I. A., D. E. Weinberg, K. T. Xie, J. P. Mower, K. H. Wolfe *et al.*, 2009 RNAi in budding yeast. Science 326: 544–550.

Dunn, B., and G. Sherlock, 2008 Reconstruction of the genome origins and evolution of the hybrid lager yeast *Saccharomyces pastorianus*. Genome Res. 18: 1610–1623.

Dunn, B., C. Richter, D. J. Kvitek, T. Pugh, and G. Sherlock, 2012 Analysis of the Saccharomyces cerevisiae pan-genome reveals a pool of copy number variants distributed in diverse yeast strains from differing industrial environments. Genome Res. 22: 908–924.

Durkin, K., W. Coppieters, C. Drögemüller, N. Ahariz, N. Cambisano *et al.*, 2012 Serial translocation by means of circular intermediates underlies colour sidedness in cattle. Nature 482: 81–84.

Erny, C., P. Raoult, A. Alais, G. Butterlin, P. Delobel *et al.*, 2012 Ecological success of a group of *Saccharomyces cerevisiae/Saccharomyces kudriavzevii* hybrids in the Northern European wine-making environment. Appl. Environ. Microbiol. 78: 3256–3265.

Fischer, G., S. A. James, I. N. Roberts, S. G. Oliver, and E. J. Louis, 2000 Chromosomal evolution in *Saccharomyces*. Nature 405: 451–454.

Fujimura, K., M. A. Conte, and T. D. Kocher, 2011 Circular DNA intermediate in the duplication of Nile *Tilapia vasa* genes. PLoS ONE 6: e29477.

Galeote, V., M. Novo, M. Salema-Oom, C. Brion, E. Valério *et al.*, 2010 FSY1, a horizontally transferred gene in the *Saccharomyces cerevisiae* EC1118 wine yeast strain, encodes a high-affinity fructose/H+ symporter. Microbiology 156: 3754–3761.

Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon *et al.*, 1996 Life with 6000 genes. Science 274(546): 563–567.

González, S. S., E. Barrio, J. Gafner, and A. Querol, 2006 Natural hybrids from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Res. 6: 1221–1234.

González, S. S., L. Gallo, M. A. D. Climent, E. Barrio, and A. Querol, 2007 Enological characterization of natural hybrids from *Saccharomyces cerevisiae* and *S. kudriavzevii*. Int. J. Food Microbiol. 116: 11–18.

Greig, D., M. Travisano, E. J. Louis, and R. H. Borts, 2003 A role for the mismatch repair system during incipient speciation in *Saccharomyces*. J. Evol. Biol. 16: 429–437.

Hall, C., and F. S. Dietrich, 2007 The reacquisition of biotin prototrophy in *Saccharomyces cerevisiae* involved horizontal gene transfer, gene duplication and gene clustering. Genetics 177: 2293–2307.

Hittinger, C. T., A. Rokas, and S. B. Carroll, 2004 Parallel inactivation of multiple *GAL* pathway genes and ecological diversification in yeasts. Proc. Natl. Acad. Sci. USA 101: 14144–14149.

Hittinger, C. T., P. Gonçalves, J. P. Sampaio, J. Dover, M. Johnston *et al.*, 2010 Remarkably ancient balanced polymorphisms in a multi-locus gene network. Nature 464: 54–58.

Hunter, N., S. R. Chambers, E. J. Louis, and R. H. Borts, 1996 The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. EMBO J. 15: 1726–1733.

Le Jeune, C., M. Lollier, C. Demuyter, C. Erny, J.-L. Legras *et al.*, 2007 Characterization of natural hybrids of *Saccharomyces*

cerevisiae and *Saccharomyces bayanus var. uvarum*. FEMS Yeast Res. 7: 540–549.
Kellis, M., N. Patterson, M. Endrizzi, B. Birren, and E. S. Lander, 2003 Sequencing and comparison of yeast species to identify genes and regulatory elements. Nature 423: 241–254.
King, E. S., J. H. Swiegers, B. Travis, I. L. Francis, S. E. P. Bastian *et al.*, 2008 Coinoculated fermentations using Saccharomyces yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. Sauvignon Blanc wines. J. Agric. Food Chem. 56: 10829–10837.
Koufopanou, V., J. Hughes, G. Bell, and A. Burt, 2006 The spatial scale of genetic differentiation in a model organism: the wild yeast *Saccharomyces paradoxus*. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361: 1941–1946.
Libkind, D., C. T. Hittinger, E. Valério, C. Gonçalves, J. Dover *et al.*, 2011 Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc. Natl. Acad. Sci. USA 108: 14539–14544.
Liti, G., D. B. H. Barton, and E. J. Louis, 2006 Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. Genetics 174: 839–850.
Liti, G., D. M. Carter, A. M. Moses, J. Warringer, L. Parts *et al.*, 2009 Population genomics of domestic and wild yeasts. Nature 458: 337–341.
Liti, G., A. N. Nguyen Ba, M. Blythe, C. A. Müller, A. Bergström *et al.*, 2013 High quality *de novo* sequencing and assembly of the *Saccharomyces arboricolus* genome. BMC Genomics 14: 69.
Martini, A. V., and C. P. Kurtzman, 1985 Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces sensu stricto*. Int. J. Syst. Bacteriol. 35: 508–511.
Nakao, Y., T. Kanamori, T. Itoh, Y. Kodama, S. Rainieri *et al.*, 2009 Genome sequence of the lager brewing yeast, an interspecies hybrid. DNA Res. 16: 115–129.
Naumov, G. I., 1987 Genetic basis for classification and identification of the *Ascomycetous* yeasts. Stud. Mycol. 30: 469–475.
Naumov, G. I., S. A. James, E. S. Naumova, E. J. Louis, and I. N. Roberts, 2000 Three new species in the *Saccharomyces sensu stricto* complex: *Saccharomyces cariocanus*, *Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. Int. J. Syst. Evol. Microbiol. 50(Pt 5): 1931–1942.
Naumov, G. I., E. S. Naumova, and I. Masneuf-Pomarède, 2010 Genetic identification of new biological species *Saccharomyces arboricolus* Wang et Bai. Antonie van Leeuwenhoek 98: 1–7.
Ness, F., and M. Aigle, 1995 RTM1: a member of a new family of telomeric repeated genes in yeast. Genetics 140: 945–956.
Nguyen, H.-V., J.-L. Legras, C. Neuvéglise, and C. Gaillardin, 2011 Deciphering the hybridisation history leading to the Lager lineage based on the mosaic genomes of *Saccharomyces bayanus* strains NBRC1948 and CBS380. PLoS ONE 6: e25821.
Nishimura, A., T. Kotani, Y. Sasano, and H. Takagi, 2010 An antioxidative mechanism mediated by the yeast N-acetyltransferase Mpr1: oxidative stress-induced arginine synthesis and its physiological role. FEMS Yeast Res. 10: 687–698.
Novo, M., F. Bigey, E. Beyne, V. Galeote, F. Gavory *et al.*, 2009 Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. Proc. Natl. Acad. Sci. USA 106: 16333–16338.
Otero, J. M., W. Vongsangnak, M. A. Asadollahi, R. Olivares-Hernandes, J. Maury *et al.*, 2010 Whole genome sequencing of *Saccharomyces cerevisiae*: from genotype to phenotype for improved metabolic engineering applications. BMC Genomics 11: 723.
Peris, D., K. Sylvester, D. Libkind, P. Gonçalves, J. P. Sampaio *et al.*, 2014 Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. Mol. Ecol. 23: 2031–2045.
Rainieri, S., Y. Kodama, Y. Kaneko, K. Mikata, Y. Nakao *et al.*, 2006 Pure and mixed genetic lines of *Saccharomyces bayanus* and *Saccharomyces pastorianus* and their contribution to the lager brewing strain genome. Appl. Environ. Microbiol. 72: 3968–3974.
Roncoroni, M., M. Santiago, D. O. Hooks, S. Moroney, M. J. Harsch *et al.*, 2011 The yeast *IRC7* gene encodes a β-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine. Food Microbiol. 28: 926–935.
Sampaio, J. P., and P. Gonçalves, 2008 Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. Appl. Environ. Microbiol. 74: 2144–2152.
Sasano, Y., S. Takahashi, J. Shima, and H. Takagi, 2010 Antioxidant N-acetyltransferase Mpr1/2 of industrial baker's yeast enhances fermentation ability after air-drying stress in bread dough. Int. J. Food Microbiol. 138: 181–185.
Scannell, D. R., O. A. Zill, A. Rokas, C. Payen, M. J. Dunham *et al.*, 2011 The awesome power of yeast evolutionary genetics: new genome sequences and strain resources for the *Saccharomyces sensu stricto* genus. G3(Bethesda) 1: 11–25.
Schacherer, J., J. A. Shapiro, D. M. Ruderfer, and L. Kruglyak, 2009 Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. Nature 458: 342–345.
Sipiczki, M., 2008 Interspecies hybridization and recombination in *Saccharomyces* wine yeasts. FEMS Yeast Res. 8: 996–1007.
Sniegowski, P. D., P. G. Dombrowski, and E. Fingerman, 2002 *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. FEMS Yeast Res. 1: 299–306.
Swiegers, J. H., R. L. Kievit, T. Siebert, K. A. Lattey, B. R. Bramley *et al.*, 2009 The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiol. 26: 204–211.
Takagi, H., M. Shichiri, M. Takemura, M. Mohri, and S. Nakamori, 2000 *Saccharomyces cerevisiae* sigma 1278b has novel genes of the N-acetyltransferase gene superfamily required for L-proline analogue resistance. J. Bacteriol. 182: 4249–4256.
Thibon, C., P. Marullo, O. Claisse, C. Cullin, D. Dubourdieu *et al.*, 2008 Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. FEMS Yeast Res. 8: 1076–1086.
Walther, A., A. Hesselbart, and J. Wendland, 2014 Genome sequence of *Saccharomyces carlsbergensis*, the world's first pure culture lager yeast. G3 4: 783–793.
Wang, S.-A., and F.-Y. Bai, 2008 *Saccharomyces arboricolus sp. nov.*, a yeast species from tree bark. Int. J. Syst. Evol. Microbiol. 58: 510–514.
Warringer, J., E. Zörgö, F. A. Cubillos, A. Zia, A. Gjuvsland *et al.*, 2011 Trait variation in yeast is defined by population history. PLoS Genet. 7: e1002111.
Wei, W., J. H. McCusker, R. W. Hyman, T. Jones, Y. Ning *et al.*, 2007 Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM789. Proc. Natl. Acad. Sci. USA 104: 12825–12830.
Wu, H., K. Ito, and H. Shimoi, 2005 Identification and characterization of a novel biotin biosynthesis gene in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 71: 6845–6855.
Zill, O. A., D. Scannell, L. Teytelman, and J. Rine, 2010 Co-evolution of transcriptional silencing proteins and the DNA elements specifying their assembly. PLoS Biol. 8: e1000550.

Communicating editor: J. Rine