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Yeast Interspecies Hybrids

The hybrid genomes of *Saccharomyces pastorianus*: A current perspective

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Abstract

***Saccharomyces pastorianus* is a recently evolved interspecies hybrid of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus* used in the production of lager-type beers and has a long-standing history with the brewing industry. At least two distinct types of lager yeasts (Groups I and II) have been identified based on chromosome content and structure. One important feature of the genomes of lager yeasts is the presence of a set of hybrid chromosomes that emerged as a result of homeologous recombination events between the parental chromosomes. The unique genetic composition of the hybrid genomes of *S. pastorianus* affords interesting opportunities for evolution, adaptation and survival of the hybrids. The co-expression of *S. eubayanus*, *S. cerevisiae* and hybrid gene alleles, together with gene dosage effects resulting from the presence of multiple copies of individual genes, creates a complex algorithm for gene expression, cellular biochemistry and physiology. The recent availability of genome sequences for three Group I and ten Group II lager yeast strains provides an opportunity to decipher this complex algorithm and understand how it impacts on the final fermentation product: flavoursome beer. Copyright © 2017 John Wiley & Sons, Ltd.**

Keywords: ***S. pastorianus*; lager yeasts; evolution; hybrid chromosomes; hybrid genes**

Introduction

Saccharomyces pastorianus, often referred to as lager yeasts, emerged approximately 500–600 years ago as a result of hybridization events between two yeast species: *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. The emergence of *S. pastorianus* was directly influenced by social and cultural developments in human societies in Central Europe during the Middle Ages but the anthropogenic relationship with brewing dates back several millennia. The production of alcoholic beverages by fermentation of natural sugars such as honey, fruits, rice and barley by yeasts was recorded in China as far back as 7000 BC (McGovern *et al*., 2004) and in Mesopotamia by 3900 BC (Damerow, 2012). However, it was the convergence of several important environmental and societal factors in the Early and Middle Ages that made the regions of Bohemia and Bavaria in Central Europe the focal point for the development of modern brewing. The temperate climate of the region favoured the growth of barley, wheat and oats – stable carbohydrate sources for extraction of sugars for fermentation – as well as hops, which were added to fermentations to provide flavour as well as stability to the beer due to its antibacterial properties.

By far the most important human intervention in the evolution of lager yeasts was the introduction of the Reinheitsgebot edict, commonly referred to as the Beer Purity Law, in 1516 in Bavaria, which restricted the ingredients of beer to barley, hops and water. Yeasts are not mentioned as an ingredient as the microorganism responsible for converting sugars to alcohol in fermentations

would not be discovered for a further 300 years. Additionally, the brewing of beer was restricted to between St Michael's Day (29 September) and St George's Day (23 April). Beer produced in the winter months was more stable and less likely to 'go off' due to bacterial contamination. At the same time, brewers in Bohemia, who were mainly monks at the time, experimented with storing beer in cool mountain caves, which allowed the beer to gain a rich, full-bodied texture and taste.

The restriction of brewing to the winter months, together with the practice of ageing beer in cool caves, enforced a cooler temperature fermentation regime. The happenstance of interspecies hybridization event(s) between mesophilic *S. cerevisiae* isolates, with high fermentative capacity, and the cryotolerant *S. eubayanus* created an ideal new yeast capable of adapting to the new conditions of brewing.

'Enter *S. pastorianus* stage left'

At least two distinct types of lager yeasts have been identified based on chromosome content and structure (Bond, 2009; Dunn and Sherlock, 2008; Querol and Bond, 2009). Saaz-type (Group I) lager yeasts display a general triploid DNA content with chromosomal aneuploidy. The *S. eubayanus* gene content is higher than the *S. cerevisiae* content in Group I strains. Frohberg-type (Group II) lager yeasts are generally tetraploid in DNA content, containing approximately equal DNA content from *S. eubayanus* and *S. cerevisiae* (Monerawela and Bond, 2017a). As with Group I strains, the Group II strains display chromosomal aneuploidy.

To date, genome sequences for 13 (three Group I and ten Group II) lager yeast strains have been published and are available as Whole Genome Shotgun assembled contigs or as raw sequence reads (De León-Medina *et al*., 2016; Hewitt *et al*., 2014; Kvasnicka *et al*., 2012; Nakao *et al*., 2009; Okuno *et al*., 2016; van den Broek *et al*., 2015; Walther *et al*., 2014). Several of the strains have been sequenced independently by different research groups. Based on genome analyses, the chromosome numbers in Group I and Group II strains range from 45 to 52 and from 42 to 84, respectively (Table 1). The estimated number of chromosomes per genome for the same strain often varies from database to database. These differences result from (i) different methods used for estimating whole genome chromosome copy numbers and/or (ii) evidence of ongoing evolution of strains held in different repositories (Table 1).

Hybrid chromosomes and hybrid genes

In addition to the parental chromosomes, both Group I and II lager yeasts possess hybrid chromosomes resulting from homeologous recombination between the parental chromosomes (Bond *et al*., 2004; Dunn and Sherlock, 2008; Hewitt *et al*., Monerawela and Bond, 2017a, 2017b). The majority of these recombination events occur at specific chromosomal locations on up to 13 different chromosomes. Genome analysis of Group I and Group II lager yeasts identified up to 36 unique recombination sites and defined the recombination epicentres at a nucleotide level (Bond *et al*., 2004; Dunn and Sherlock, 2008; Hewitt *et al*., 2014; Monerawela and Bond, 2017a, 2017b). These analyses identified recombination sites that are common to all lager yeasts as well as sites unique to either group or to individual strains. Two recombination sites – *YGL173C Hyb1* and *YGR285C* (Table 2) – are conserved at a nucleotide level between *all* Group I and II lager yeasts. Four sites (*YER164W*, *YKL203C*, *YKL080W* and *YMR306W*) are common to Group I strains CBS 1503 and CBS 1538 but these sites are not found in the other Group I strain CBS 1513. The latter strain has ten unique recombination sites not shared with the other Group I strains or the Group II strains (Monerawela and Bond, 2017a, 2017b). The Group II strains share five common recombination sites at *YJR009C Hyb 1*, *YMR302C*, *YPL240C*, *YPR160W* and *YPR191W*. Interestingly, several genes (*YDR324C*, *YGL173C*, *YHR165C*, *YJR009C* and *YPR160W*) contain more than one recombination site and different sites are used in different strains (Table 3).

How these hybrid chromosomes were formed in *S. pastorinaus* is still unclear; however, an analysis of the DNA sequences at the recombination epicentres identified two common sequence motifs. The first consists of sequential runs of thymidines flanking a short purine-rich sequence, on one strand of the DNA, while the second contains

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Table 1. Chromosome content of selected Group I and II lager yeasts

—	Group I									Group II								
Chr.	CBS1513			CBS1503			CBS1538			WS 34/70			CBS1260			CBS1483		
Type	Sc	Se	Hy	Sc	Se	Hy	Sc	Se	Hy	Sc	Se	Hy	Sc	Se	Hy	Sc	Se	Hy
I	1	1	0	2–3	1	0	0	1	1	2–3	1–3	0	1	2	0	2–3	1–2	0
II*	0–1	2	0–1	1	2	0	0	3	0	2–3	2	0	2	1	0	3	1	0
III	2	0	1	0	3	1	0	3	0	1–2	0	3–4	0–2	0	1–2	0	0	4
IV#	0	2	1	0	2	1	0	3	0	2–3	1–2	0	0–2	1	0–2	3	1	0
V	1	2	0	0	0	3	0	2	1	2–3◆	2	0	0	0	3	3	2	0
VI	0	3	0	0	3	0	0	3	0	1–3	2–3	0	1	1–2	0	1	2–3	0
VII	0	0	3	0	0	3	0	0	3	2–3	0	2	2	0	1	1	0	3
VIII^	0-1	2	0-1	1	2	0–1	0	3	0	1–4	2	0–1	2	1	0	5	1	0
IX	1	2	0	2	1	0	1	2	0	3–5	1	0	0	0	3	3	2	0
X	1	2	0	1	2	0	0	2	1	1	1	2	1–2	1	1	1	1	1
XI	0	3	0	0	2	1	0	2	1	2–3	1	1	1	1–2	2	2	2	0
XII	0	3	0	0	3	0	0	3	0	2–3	2	0	1	2	0	2	2	0
XIII	0	2	1	0	1	2	0	3	0	2–3	0	2	0–1	0	2–3	2	0	2
XIV	1	2	0	0	3	0	1	2	0	2–3	2	0	2	0	1	2	2	0
XV§	0	2	1	0–1	1–2	0–1	0	3	0	2–3	1–2	0	2	1	0	3	1	0
XVI	0	0	3	0	0	3	0	3	0	0	0	4	0	0	3	0	0	4
Sub-total	7–9	28	10–12	7–9	26–27	14–16	2	38	7	27–45	19–23	14–16	14–21	11–13	17–21	33–34	19–21	14
Total	45–49			47–52			47			60–84			42–55			66–69		

Chr., chromosome number; Sc, *S. cerevisiae*; Se, *S. eubayanus*; Hy, hybrid. Translocations: *S. *eubayanus* chr. II–IV, #S. *eubayanus* chr. IV–II; ^S. *eubayanus* chr. VIII–XV; §S. *eubayanus* chr. XV–VIII. ◆WS34/70 contains a translocation between *S. cerevisiae* chr. V and chr. XI present in one copy. Data for table are extracted from Hewitt *et al.* (2014), Okuno *et al.* (2016), van den Broek *et al.* (2015) and Walther *et al.* (2014). Chromosome estimates for WS 34/70 from Nakao *et al.* (2009) were excluded.

a pyrimidine stretch adjoining a purine-rich sequences. Thirty of the 36 recombination epicentres contain one or other of the motifs, indicating that the recombination events may have occurred by a common molecular mechanism (Monerawela and Bond, 2017a, 2017b). Generally, recombination events are initiated by double-strand breaks (DSBs) at a chromosomal site and can occur as a result of replication fork stalling or stuttering during replication of repetitive DNA sequences and also at crossover events during meiosis or as a result of exposure of cells to radiation or chemical agents. Homeologous recombination events may also arise through a process referred to as 'returned to growth', in which polyploid cells enter the process of meiosis but return to mitotic growth prior to spore formation (Laureau *et al.*, 2016). There appears to be no correlation between the recombination epicentres and the location of replication initiation sites, meiosis-induced DSB sites or repetitive elements in the genome of *S. pastorianus*; however, interestingly, recombination at several of the sites was induced when lager yeasts were exposed to high temperature and high sugar concentrations (James *et al.*, 2008; Monerawela and Bond, 2017a, 2017b). Furthermore, recombination at at least one of the sites, *YGR285C*, has been identified in other hybrids of *S. eubayanus* (Libkind *et al.*, 2011). Thus the recombination sites may represent fragile chromosomal locations that are prone to DSBs, possibly induced as a response to environmental stress.

A consequence of the formation of hybrid chromosomes in lager yeasts is the emergence of hybrid genes that are truly unique to the species (Hewitt *et al.*, 2014). Of the 36 identified recombination epicentres, 30 are intragenic (Table 2). The locations of recombination sites within the hybrid genes are not generally conserved and recombinations occur at the 5′ end, in the middle and at the 3′ end of genes (Table 3). With the exception of one hybrid gene, *YPR160W*, found in the Group II strain CMBS (Usher and Bond, 2009) and the hybrid gene *YOR109W*, which encodes for a truncated open reading frame (ORF), all hybrid genes encode putative uninterrupted ORFs, part *S. cerevisiae*-like and *S. eubayanus*-like, and encode for proteins of diverse functions (Table 2).

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Table 2. Hybrid genes in *S. pastorianus*

Chr.	Gene	ORF	Activity; function
I	*YAL054C*	*ACS1*	Acetyl-CoA synthetase; catalyses formation of acetyl-CoA from acetate and CoA
II	*YBR289W*	*SNF5*	Component of SWI/SNF complex; chromatin structure and transcription
IV	*YDR324C Hyb1*	*UTP4*	Subunit of U3-containing pre-ribosome and SSU processome complexes; assembly
IV	*YDR324C Hyb2*		of small ribosomal subunit and production of 18S rRNA
V	*YER164W*	*CHD1*	Chromatin remodeller; regulates numerous aspects of transcription
VII	*YGL173C Hyb1*	*XRN1*	5′ to 3′ exoribonuclease; mRNA degradation
VII	*YGL173C Hyb2*		
VII	*YGR285C*	*ZUO1*	Ribosome-associated chaperone; also functions in ribosome biogenesis
VIII	*YHR165C Hyb1*	*PRP8*	Component of U4/U6–U5 snRNP complex; mRNA splicing
VIII	*YHR165C Hyb2*		
X	*YJL197W*	*UBP12*	Ubiquitin-specific protease; cleaves ubiquitin from ubiquitinated proteins
X	*YJR009C Hyb1*	*TDH2*	Glyceraldehyde-3-phosphate dehydrogenase; glycolysis and gluconeogenesis
X	*YJR009C Hyb2*		
XI	*YKL203C*	*TOR2*	Component of TORC1 and TORC2 subunit; phosphatidylinositol-kinase-related;protein kinase, cell-cycle dependent polarization of the actin cytoskeleton
XI	*YKL080W*	*VMA5*	Subunit C of the V1 peripheral membrane domain of V-ATPase; electrogenic proton pump
XI	*YKL045W*	*PRI2*	DNA primase and DNA polymerase α subunit; DNA replication
XII	*YLR410W*	*VIP1*	Inositol hexakisphosphate and inositol heptakisphosphate kinase; inositol phosphate biosynthesis
XIII	*YML073C*	*RPL6A*	Ribosomal 60S subunit protein L6A
XIII	*YML051W*	*GAL80*	Transcriptional regulator; repressor of GAL genes transcription
XIII	*YMR287C*	*DSS1*	3′ to 5′ exoribonuclease; component of the mitochondrial degradosome
XIII	*YMR302C*	*YME2*	Inner mitochondrial membrane protein; involved in maintaining mitochondrial nucleoid structure
XIII	*YMR306W*	*FKS3*	Involved in spore wall assembly
XV	*YOR092W*	*ECM3*	Unknown function
XV	*YOR109W*	*INP53*	Polyphosphatidylinositol phosphatase; trans Golgi network-to-early-endosome pathway
XV	*YOR133W*	*EFT1*	Elongation factor 2; catalyzes ribosomal translocation during protein synthesis
XVI	*YPL240C*	*HSP82*	HSP90 chaperone; protein chaperone, stress response
XVI	*YPL036W*	*PMA2*	Plasma membrane H^+-ATPase; pumps protons out of cell and regulates cytoplasmic pH
XVI	*YPR160W Hyb1*	*GPH1*	Glycogen phosphorylase; mobilizes glycogen to glucose-1-P
XVI	*YPR160W Hyb2*		
XVI	*YPR191W*	*QCR2*	Subunit 2 of ubiquinol cytochrome-c reductase; component of the mitochondrial inner membrane electron transport chain

Chr, chromosome number; ORF, open reading frame.

The hybrid proteins encoded by hybrid genes share between 86% and 100% sequence identities with the respective proteins of the parental species (Table 3).

The presence of a unique set of hybrid genes in the genome of lager yeasts raises interesting cellular and molecular consequences for the yeasts. Firstly, in addition to the hybrid alleles, the cells contain allelic variants encoded by the parental *S. cerevisiae* and *S. eubayanus* chromosomes. A second layer of complexity is added by the fact that the copy number and ratio of the allelic variants may differ from strain to strain, depending on the chromosome copy number (Table 1). Allelic variants may possess different cellular activities: for example, have different substrate affinities, compete for substrate binding, possess different enzymatic activities or catalytic rates or have altered protein–protein interactions within multi-protein complexes. Thus the co-expression of allelic variants, together with copy number variations (gene dosage), creates a metabolic landscape unique to lager yeasts.

The identification of allelic variants through genome analysis has laid the groundwork for future phenotypic analysis of these unique hybrid genes. Of the 25 different hybrid genes with uninterrupted ORFs, gene ontology analysis identifies 11 as encoding for proteins with hydroylase activity. Six of the 30 hybrid genes encode for proteins involved in RNA metabolism (*UTP4*, *XRN1*, *ZUO1*, *PRP8*, *RPL6A* and *DSS1*), three are involved in chromatin/transcription regulation (*SNF5*, *CDH1*, *GAL80*), three are electron/proton transporters (*VMA5*, *PMA2*, *QCR2*) and three are involved in carbohydrate metabolism (*TDH2*,

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Table 3. Hybrid genes: amino acid identities to *S. cerevisiae* and *S. eubayanus* isoforms

Chr.	Gene (strain)	Structure of ORF	% Amino acid identity to Sc	% Amino acid identity to Se
I	*YAL054C*		97.76	96.35
II	*YBR289W*		88.15	86.35
IV	*YDR324C Hyb1* (CBS1503)		98.01	91.22
IV	*YDR324C Hyb2* (CBS1513)		98.94	90.29
V	*YER164W*		94.75	94.2
VII	*YGL173C Hyb1*		99.28	88.18
VII	*YGL173C Hyb2* (CBS1513)		87.50	99.67
VII	*YGR285C*		94.23	97.69
VIII	*YHR165C Hyb1* (CBS1513)		94.30	96.98
VIII	*YHR165C Hyb2* (WS 34/70)		93.05	98.3
X	*YJL197W*		86.02	92.21
X	*YJR009C Hyb1*		98.19	98.80
X	*YJR009C Hyb2*		97.90	97.80
XI	*YKL203C*		96.40	97.41
XI	*YKL080W*		100	95.82
XI	*YKL045W*		98.30	96.03
XII	*YLR410W*		99.13	91.47
XIII	*YML073C*		98.30	95.45
XIII	*YML051W*		96.78	94.94
XIII	*YMR287C*		85.86	92.67
XIII	*YMR302C*		97.41	92.6
XIII	*YMR306W*		97.03	94.4
XV	*YOR092W*		99.84	86.4
XV	*YOR109W**		98.37	90.79
XV	*YOR133W*		98.81	98.46
XVI	*YPL240C*		96.62	94.20
XVI	*YPL036W*		97.04	95.61
XVI	*YPR160W Hyb1* (CMBS)		ORF Interrupted	ORF Interrupted
XVI	*YPR160W Hyb 2* (WS 34/70)		97.12	98.23
XVI	*YPR191W*		95.11	91.58

Chr., chromosome number; hybrid genes unique to specific strains: strain shown in brackets. *Stop codon introduced into *YOR109W*, protein truncated by 94 residues. Sc, *S. cerevisiae*; Se, *S. eubayanus*.
Grey, *S. cerevisiae*-like; black, *S. eubayanus*-like.

GPH1, *ACS1*) (Table 2). Thus the encoded hybrid proteins may impact several essential and diverse cellular biological processes.

To date, the only hybrid gene whose activity has been evaluated is the hybrid *YPR160W/GPH1* allele found in the Group II *S. pastorianus* strain CMBS-33 (Usher and Bond, 2009). *YPR160W/GPH1* encodes for glycogen phosphorylase, which converts glycogen to glucose 1-phosphate, an enzymatic step required for the mobilization of stored glycogen. The strain CMBS contains three copies of the hybrid gene in addition to one *S. eubayanus* allele residing on chromosome XVI (Bond *et al*., 2004). DNA sequence analysis of the hybrid alleles revealed the presence of a stop codon within the ORF, which abrogates the production of a functional hybrid protein. Quantification of glycogen levels in the cells suggests that the *S. eubayanus* allele of *GPH1* is functional (Usher and Bond, 2009). Interestingly, the Group II *S. pastorianus* strain WS 34/70 also contains a hybrid *YPR160W* gene; however, in this strain, the recombination site differs from that found in strain CMBS and created an uninterrupted ORF (Table 3). Molecular and phenotypic analyses of the *YPR160W* allele in WS 34/70 have not yet been investigated.

The over-representation of genes involved in RNA metabolism in the pool of hybrid genes has the potential for overarching influences on the RNA landscape and subsequent proteome of the lager yeast cell. One significant gene, central to the establishment of the steady state pool of cellular RNA, is *YGL173C*, which encodes for *XRN1*, an exonuclease required for cytoplasmic RNA degradation (Braun and Young, 2014;

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Nagarajan *et al*., 2013; Parker, 2012). In addition to its role in RNA degradation, *XRN1* has also been shown to activate transcription of a selected group of genes (Haimovich *et al*., 2013; Medina *et al*., 2014). Thus alterations in the expression of *XRN1* or the activities of its encoded protein may have global effects on cellular metabolism. Group I and II lager yeasts possess different *XRN1* alleles: Group I strains CBS1503 and CBS1538 have three copies of the *XRN1 Hyb1* (Hybrid 1) allele (Table 3). The Group I lager yeast, CBS1513, contains two copies of the *XRN1 Hyb 1* allele and one copy of the *XRN1 Hyb 2* allele (Table 3). Group II lager yeasts contain two copies of an *S. cerevisiae* allele and two copies of the *XRN1 Hyb1* allele. The hybrid genes contain different 5′ untranslated regions (UTRs): *Hyb 1* is *S. eubayanus*-like whereas *Hyb 2* is *S. cerevisiae*-like (Table 3). The co-expression of different *XRN1* alleles in Group I and II lager yeasts, together with the presence of different 5′ UTRs on the hybrid genes, could potentially lead to quite distinctive RNA landscapes in the two types of yeasts.

The evolutionary pathway of lager yeasts

With the availability of genome sequences for several hundreds of *Saccharomyces* spp., it is possible to chart putative evolutionary pathways that led to the current-day strains of *S. pastorianus*.

Sailing the oceans and spinning silk: the *S. eubayanus* lineages

The cryotolerant species *S. eubayanus* was originally discovered in Patagonia, South America (Libkind *et al*., 2011), and shares 99.5% sequence identity with the *S. eubayanus* sub-genome of *S. pastorianus*. It is associated with natural fermentations of sugar-rich galls formed on *Nothofagus* trees by the fungus *Cytarria*. At the time of this discovery, isolates of *S. eubayanus* had only been found in Patagonia; Libkind *et al*. (2011) therefore proposed that the species might have been brought to Europe on the ships of conquistadors during the exploration of the New World. Since this initial discovery, isolates of *S. eubayanus* have also been found in China, Tibet, North America and New Zealand (Bing *et al*., 2014; Gayevskiy and Goddard, 2016; Peris *et al*., 2014; Rodriguez *et al*., 2014) but to date have still not been found in Europe (in either the wild or other reservoirs) despite the fact that the presumed original interspecific hybridization event(s) occurred in Europe. Genome analysis of *S. eubayanus* isolates uncovered a complex lineal relationship between isolates from the different geographical locations (Peris *et al*., 2014, 2016). This analysis also revealed that the Tibetan isolates most closely resembled the *S. eubayanus* sub-genome in *S. pastorianus* (Baker *et al*., 2015; Bing *et al*., 2014; Peris *et al*., 2016), thus postulating a rival hypothesis that *S. eubayanus* arrived in Europe via the Silk Route from China or Tibet. However, no sole isolate appears to be the lowest common ancestor (LCA) of the *S. eubayanus* parent of lager yeasts as gene alleles from at least two geographically distinct isolates are present in the lager yeasts. It appears that strains of Tibetan origin contribute 66% of the lager *S. eubayanus* sub-genome whereas the remainder of the sub-genome (34%) is most closely related to an isolate from North Carolina, USA (Peris *et al*., 2016). The presence of different allelic variants of *S. eubayanus* in the genome of *S. pastorianus* raises the possibility that more than one isolate of *S. eubayanus* may have contributed to the genetic make-up of *S. pastorianus*.

What ales the *S. cerevisiae* parent(s)?

The yeasts used in the production of beer in the Middle Ages were probably *S. cerevisiae* isolates. The yeasts were described as top fermenters as they floated to the top of the open vats at the end of fermentation. The foamy material, which contained the yeast, was scooped off and used to start the next fermentation. Genome analysis confirms that the second parent of *S. pastorianus* shares 99% sequence identity with *S. cerevisiae*. The recent genome analysis of several hundreds of strains/isolates of *S. cerevisiae* has shed some light on the possible *S. cerevisiae* ancestor of the lager yeasts. In general, yeasts associated with brewing segregate into two to three distinct sub-clades of *S. cerevisiae* isolates (Gallone *et al*., 2016; Gonçalves *et al*., 2016). One sub-clade (named Beer 1) contains isolates from the British Isles (Britain and Ireland), the USA and from Germany and Belgium. The second sub-clade

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(Beer 2) is more closely related to wine *S. cerevisiae* but contains approximately 20% of brewing strains. In a separate study, Gonçalves *et al*. (2016) subdivided beer isolates into three clades, namely wheat, English–Irish ales and German Alt-Kolsch beers, respectively (Gonçalves *et al*., 2016). The latter study, which included an analysis of over 90 *S. cerevisiae* strains including bread, beer, sake, wine and wild yeasts from different geographical locations, placed the *S. cerevisiae* sub-genome of Group II lager yeasts in a separate sub-clade within the English–Irish beer clade (Gonçalves *et al*., 2016). Based on these analyses, as well as previous phylogenetic studies (Legras *et al*., 2007; Nguyen *et al*., 2011), as indeed what was expected, ale-like yeasts appear to be the closest ancestors of the *S. cerevisiae* sub-genome of the lager yeasts.

Follow the tails: lineages of Group II lager yeasts

Identifying the LCA of the *S. cerevisiae* parent of *S. pastorianus* is hampered by the fact that genetic variation between *S. cerevisiae* strains/isolates is relatively low. As most variation is accounted for by changes in sub-telomeric regions of chromosomes (Bergström *et al*., 2014; Borneman and Pretorius, 2015), Monerawela *et al*., 2015 conducted a comparison of the sub-telomeric regions of representative Group I and II strains of *S. pastorianus* and the sub-telomeric regions of 31 *S. cerevisiae* strains, from different industrial, laboratory, clinical or environmental sources. This study revealed that Group I lager yeasts have lost substantial genetic information from the sub-telomeric regions of chromosomes that is retained in the Group II lager yeasts. Specifically, genetic information at the left and right sub-telomeres (LST/RST) of chromosomes IV, VI, XI, XII, XIII as well as RSTs XV and XVI of the Group II lager yeasts is absent in the Group I strain CBS1513. Genetic information at RST XIII of the Group II strain was also lost in 26 of the 31 *S. cerevisiae* strains, while the other sub-telomeric regions that are absent in the Group I lager yeasts were also lost to varying degrees in the *S. cerevisiae* strains examined. The region LST XIV, which is present in both Group I and II lager yeasts, is also extensively lost in *S. cerevisiae* strains (Monerawela *et al*., 2015).

Several genes, originally identified as being unique to *S. pastorianus*, lie within or proximal to RST XIII and LST XIV. Specifically the genes *AMD*, *TYP* and *TRR*, originally curated as 'lager specific', are found at RST XIII, RST XIII and LST XIV respectively (Monerawela *et al*., 2015; Nakao *et al*., 2009). These three genes putatively encode for an amidase, a tyrosine permease and a transcriptional regulator, respectively. One additional gene, *HYPO*, encoding a hypothetical ORF, was also originally designed as 'lager specific' (Nakao *et al*., 2009). This gene lies centromere-proximal to LST XVI and is lost in 19 of the 31 *S. cerevisiae* strains examined by Monerawela *et al*. (2015). Thus, while the 'lager-specific' genes can no longer be considered unique to *S. pastorianus*, these genes, together with RST XIII, represent genetic information retained in Group II lager yeasts and thus can act as genetic markers to identify nearest neighbours and possible LCAs (Table 4). Using these markers, together with LST IV, which distinguishes Group I and II lager yeasts, Monerawela *et al*. (2015) categorized 78 *S. cerevisiae* strains based on the patterns of the presence or absence of the markers, either through genome analysis or by polymerase chain reaction (PCR). In total, just seven strains retained the six genetic markers of Group II lager yeasts (Table 4). These include four *S. cerevisiae* strains used in stout production in Britain and Ireland, two isolates from the Malabar Coast in southwest India and one isolate identified as a wine yeast (PW5). PW5 was originally isolated from a natural fermentation of the sap of the *Raphia* palm in Aba, Nigeria, in 2002. Interestingly, the isolates from the Malabar Coast of southwest India are used in the production of toddy (palm wine) from coconut palms. Whether this relationship between the *S. cerevisiae* sub-genome of Group II lager yeasts, stout yeasts and Indian/Africa yeasts is serendipitous or reflects a true evolutionary link remains to be uncovered.

Working on a hypothesis that the six genetic markers found in the *S. pastorianus* Group II *S. cerevisiae* sub-genome represent 'additional' genetic information lost in most *S. cerevisiae* strains, we examined the genomes of the several hundred *S. cerevisiae* isolates/strains currently deposited in Genbank (www.ncbi.nlm.nih.gov/genbank) (Bergström *et al*., 2014; Borneman *et al*., 2011, 2016; Gallone *et al*., 2016; Gonçalves *et al*., 2016; Liti *et al*., 2009; Strope *et al*., 2015; Wang *et al*., 2012; Zhu *et al*., 2016) for the presence of RST XIII, the largest chromosomal region

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Table 4. Presence or absence of sub-telomeric regions and associated genes

Strain	RST XIII	LST IV	*TYP*(RST XIII)	*AMD*(RST XIII)	*TRR*(LST XIV)	*Hypo*(LST XVI)
S. pastorianus Group II (10)	+	+	+	+	+	+
S. cerevisiae / stout (4)	+	+	+	+	+	+
S. cerevisiae / palm tree isolates (3)	+	+	+	+	+	+
S. cerevisiae / Schneider Weisse	+	–	+	+	+	+
S. pastorianus Group I (CBS 1503, 1513)	–	–	–	–	+	+
S. pastorinaus Group I (CBS 1538)	–	–	–	–	+	–
S. cerevisiae / ales (8)	–	–	–	–	+	+
S. cerevisiae / ales (2)	–	+	–	–	+	+

+, presence; –, absence. The absent regions are also shaded grey. Number of strains/isolates are shown in brackets. Adapted from Supplementary Table 4 in Monerawela *et al.* (2015).

amongst the six markers (Supporting information, Table S1). A total of 201 *S. cerevisiae* strains were identified as containing RST XIII (Table S1, column RST XIII). The query coverage ranged from 12% to 100%, with 79 strains displaying >80% coverage. The sequence identity ranged from 86% to 99%. Next, this set of strains was screened for the presence of *TYP*, a gene on RST XIII which appears can be lost independently of RST XIII: in the 78 *S. cerevisiae* strains examined by PCR and/or bioinformatics analysis of genomes, *TYP* was absent in 53 strains whereas RST XIII was absent in 39 strains (Monerawela *et al*., 2015). This screen narrowed the list of the most likely nearest neighbours to the Group II lager yeasts to 25 *S. cerevisiae* strains (Supporting information, Table S2, column TYP). This subset of strains was then used in a BLAST analysis using a query sequence compiled of sub-telomeric sequences that distinguishes Group I and II lager yeasts (Table S2). From this analysis, three beer strains (BE072, BE074 and BE093) used in the production of Hefeweizen beer (a wheat beer produced from malted wheat) were identified as the most likely nearest neighbour(s) of Group II *S. pastorianus* (Figure 1).

The history of Hefeweizen provides yet another story of possible human interference in the evolution of yeasts. In 1520, in a bid to get around the Reinheitsgebot, the Duke of Wittlesbach provided a right to a single brewery in the village of Schwarzach near the Czech Republic to use wheat to produce 'Weissbier'. This exclusive arrangement ensured that yeasts in the production of Weissbier were subjected to different environmental and physiological conditions, which may have impacted on their evolution. This arrangement remained in place until 1605 when Duke Maximillian I expanded the rights to brew Weissbier to other towns and villages across Bavaria. In 1856, George Schneider I bought the brewery 'Weisses Bräuhaus', which still produces Hefe-Weizenbier under the name Schneider Weisse. Interestingly, in the initial six-marker analysis conducted by Monerawela *et al*. (2015), yeast isolated from a bottle of Schneider Weisse contained five of the six DNA markers but lacked the region LST IV (Table 4). Also, strain PW5, which was shown to contain all six Group II markers (Table 4), is more distant from *S. pastorianus* Group II yeast on the phylogenetic tree. Genome data on the other two palm tree isolates (Table 4) are not yet available. Thus further analysis will be required to tease out the phylogenetic relationship between the palm tree isolates and the Weissbier isolates and to reconcile the presence or absence of sub-telomeric regions with the nearest-neighbour phylogenetic analysis.

Keep it in the brewery: Lineage of group I lager yeasts

To date, genome sequences for just three Group I isolates (CBS 1513, CBS 1538 and CBS 1503) have been generated (Table 1). The three strains were originally isolated by Emil Hansen at the Carlsberg brewery. These yeasts had the property of depositing at the bottom of the tank at the end of fermentation and were termed 'Unterhefe' (bottom yeast). CBS 1513 is a descendent of a strain designated as Unterhefe Nr. I, isolated in the brewery by Hansen in 1883. The strain was later renamed *S. carlsbergensis* in 1908. Two other beer yeast isolates were identified by Hansen:

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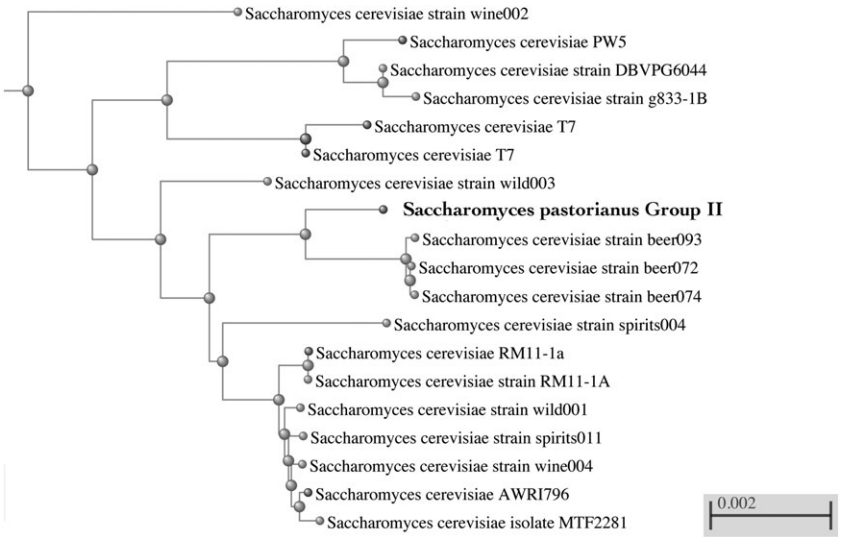

Figure 1. Phylogenetic tree of Group II *S. pastorianus*. *S. cerevisiae* strains containing the genomic region, RST XIII, and the gene *TYP*, which also resides on chromosome XIII, were filtered from the *S. cerevisiae* genome databases at NCBI (www.ncbi.nlm.nih.gov). A nearest-neighbour phylogenetic tree was assembled from this subset of strains using sub-telomeric regions of the prototypic Group II lager WS 34/70 as a query sequence. The clades clustering closest to *S. pastorianus* Group II (in bold) are shown. The three wheat beer strains (beer072, beer074 and beer093) are present in a sub-clade along with the *S. pastorianus* Group II WS 34/70 strain. Scale bar: base substitution per site.

Unterhefe Nr. II, which was designated as *S. monacensis* (CBS 1503); and CBS 1538, which was named *S. pastorianus* Reess *ex* Hansen 1904 when it was deposited into the Centraalbureau voor Schimmelcultures (CBS) in 1935. The species name *pastorianus* was assigned to the latter in deference to the original classification by Max Reese of lager yeasts as *S. pastorianus* in 1870 (Barnett, 2000).

The lineages of the Group I strains have been examined as part of a larger phylogenetic analysis of *S. cerevisiae* strains from diverse geographic locations using 12 microsatellite gene loci as comparators (Nguyen *et al*., 2011). Based on this analysis, strains CBS1513 and 1503 clustered with two beer strains CLIB277 and 276 into an *S. monacensis* group. This group of strains are defined as having a low *S. cerevisiae* gene content as only five to six of the 12 microsatellite loci were amplified in these strains. CBS1513, clustered with rum and distillery strains, suggesting a different evolutionary lineage for this Group I strain. The divergent lineage of CBS1513 is also evidenced by its unique set of hybrid chromosomes that are distinct to those found in strains CBS1503 and 1538 (Monerawela and Bond, 2017a, 2017b). Phylogenetic analysis of the Group I strains using both sub-telomeric and non-telomeric sequences as comparators reveals very low levels of sequence diversity between the three Group I strains (0.07–0.11 SNPs kbp^{-1}) suggestive of a common ancestor (Monerawela, unpublished).

Multiple meetings

Several hypotheses have been proposed to account for the difference in genetic make-up of the Group I and II lager yeasts. First, the presence of shared recombination sites suggests that Group I and II yeasts share a common ancestor (Okuno *et al*., 2016; Wendland, 2014). However, Group II yeasts contain substantial genetic material at the ends of *S. cerevisiae* chromosomes that has been lost in the Group I yeasts (Monerawela *et al*., 2015). The genetic material, specifically at RST XIII, which is absent in Group I lager yeasts, is also absent in the majority of current-day ale yeasts (Table 4). As it is highly unlikely that ale and Group I lager yeasts would independently lose the same genetic material, Monerawela *et al*. (2015) proposed that Group I and II yeasts arose by independent hybridizations with *S. eubayanus*.

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This hypothesis was supported by the fact that the rate and estimated number of synonymous substitutions was over ten times higher in the *S. cerevisiae* sub-genomes of Group I and II lager yeasts compared to the *S. eubayanus* sub-genome, indicative of divergence of the *S. cerevisiae* sub-genomes prior to the hybridization event. Single nucleotide polymorphism (SNP) analysis reveals an average of 3.3 SNPs kbp^{-1} difference between the prototypic Group I and II strains, CBS1513 and WS 34/70 respectively. This rate of SNP accumulation is over 10-fold higher than expected from divergence of the two groups following a single hybridization event that occurred some 500–600 years ago (Baker *et al*., 2015; Monerawela *et al*., 2015). A recent genome-wide SNP analysis of the *S. cerevisiae* sub-genome of Group I and II lager yeasts provides further insight into the evolution of lager yeasts (Okuno *et al*., 2016). This new analysis reveals that the Group II lager yeasts appears to contain two *S. cerevisiae* sub-genomes (Okuno *et al*., 2016). The SNPs identified in one of these sub-genomes are shared with the *S. cerevisiae* sub-genome of Group I lager yeasts, indicating that Group I and II lager yeasts may share one *S. cerevisiae* sub-genome but Group II contains an additional *S. cerevisiae* sub-genome. This finding is consistent with the data showing that Group II lager yeasts contain 'additional' genetic information at the sub-telomeres not found in Group I strains (Monerawela *et al*., 2015). Based on these data, Okuno *et al*. (2016) proposed that at least one hybridization event is shared between the Group I and II lager yeasts and proposed two possible evolutionary routes: (i) an initial hybridization of a diploid ale yeast with a diploid *S. eubayanus*, followed by substantial loss of the *S. cerevisiae* gene content in Group I yeasts; or (ii) a hybridization between a haploid *S. cerevisiae* and a diploid *S. eubayanus*, followed by a second round of hybridization between this early hybrid with a haploid *S. cerevisiae*.

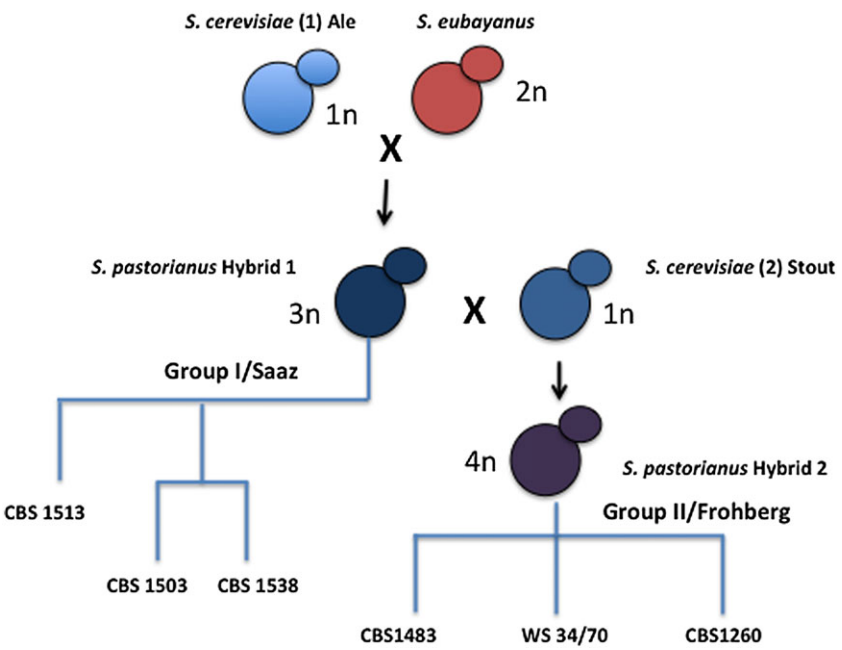

Figure 2. Origins of Group I and II *S. pastorianus* through multiple hybridization events. A possible evolutionary route leading to the Group I and II *S. pastorianus* strains is depicted. The first hybridization event took place between an *S. cerevisiae* ale yeast lacking RST XIII (*S. cerevisiae* (1) ale) and *S. eubayanus*. This initial lager yeast hybrid is designated as *S. pastorianus* Hybrid 1. *S. pastorianus* Hybrid 1 is the progenitor strain of Group I lager yeasts. Within Group I lager yeasts CBS1513 appears as an out-group due to its unique recombination events, while strains CBS1503 and 1538 share common recombination sites. Group II lager yeasts emerged following a second hybridization event between *S. pastorianus* Hybrid 1 and an *S. cerevisiae* strain similar to yeasts used in stout production (*S. cerevisiae* (2) Stout) to create *S. pastorianus* Hybrid 2, the progenitor of Group II lager yeasts. The Group II lager yeasts have diverged further as a result of subsequent unique recombination events between the parental chromosomes as depicted by the divergence of three Group II strains CBS1483, WS 34/70 and CBS1260

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A recent meta-analysis of recombination sites in Group I and II lager yeasts sheds additional light on the possible evolutionary routes leading to the Group I and II lager yeasts (Monerawela and Bond, 2017a, 2017b). This analysis confirms that two of the recombination events leading to hybrid chromosomes are shared between *all* Group I and II lager yeasts (Monerawela and Bond, 2017a, 2017b; Okuno *et al*., 2016; Walther *et al*., 2014). Thereafter, the two groups appear to have evolved independently through further recombination events between the parental chromosomes, yielding a set of hybrid chromosomes unique to each group. Furthermore, divergent evolution as a result of additional recombination events unique to each strain is observed within each group (Monerawela and Bond, 2017a, 2017b). This latter observation suggests that the lager yeasts are continually evolving.

Taken together, we propose that the accumulated data favour an evolutionary model involving sequential rounds of hybridization between *S. eubayanus* and different *S. cerevisiae* isolates (Figure 2). The first hybridization event may have occurred between an ale-like *S. cerevisiae*, which had already lost RST XIII, and a diploid *S. eubayanus*, giving rise to the progenitor of Group I/Saaz-type yeasts. The assumption that the *S. eubayanus* parent was diploid is based on the fact that the *S. eubayanus*-type strain PYCC6148 is reported to be diploid (Baker *et al*., 2015; Hebly *et al*., 2015). However, the finding that the *S. eubayanus* sub-genome of lager yeasts contains allelic variants found in different *S. eubayanus* isolates suggests that genetic admixture of *S. eubayanus* strains may have occurred prior to the initial hybridization with *S. cerevisiae* (Peris *et al*., 2016). Following this initial hybridization event between *S. eubayanus* and *S. cerevisiae*, a subsequent second hybridization event may have occurred between this progenitor strain and a 'stout-like' yeast (bearing additional genetic material on RST XIII), resulting in the Group II/Frohberg lineage (Figure 2).

In conclusion, the complexity of the hybrid genomes found in *S. pastorianus* strains affords interesting pathways for evolution, adaptation and survival strategies of this important group of interspecific hybrids. The polyploid genome provides buffering against lethal gene mutations through redundancy and can afford the opportunity to lose large regions of one species' genome or the other, resulting in quite different copy numbers of various genome regions. Redundancy also allows for divergence in function of duplicate copies of genes. Additionally, the co-expression of *S. eubayanus*, *S. cerevisiae* and hybrid gene alleles, together with gene dosage effects resulting from the presence of multiple copies of individual genes, creates a complex algorithm for gene expression, cellular biochemistry and physiology. Much remains to be done to decipher this complex algorithm, which impacts the final fermentation product: flavoursome beer.

Conflict of interest

The authors declare no conflicts of interest.

References

Baker E, Wang B, Bellora N, *et al*. 2015. The genome sequence of *Saccharomyces eubayanus* and the domestication of lager-brewing yeasts. *Mol Biol Evol* **32**: 2818–2831.

Barnett JA. 2000. A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850–1880. *Yeast* **16**: 755–771.

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

Bing J, Han P-J, Liu W-Q, Wang Q-M, Feng-Yan B. 2014. Evidence for a Far East Asian origin of lager beer yeast. *Curr Biol* **24**: R380–R381.

Bond U. 2009. The genomes of lager yeasts. *Adv Appl Microbiol* **69**: 159–182.

Bond U, Neal C, Donnelly D, James T. 2004. Aneuploidy and copy number breakpoints in the genome of lager yeasts mapped by microarray hybridisation. *Curr Genet* **45**: 360–370.

Borneman AR, Pretorius IS. 2015. Genomic insights into the *Saccharomyces sensu stricto* complex. *Genetics* **199**: 281–291.

Borneman A, Desany B, Riches D, *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 AR, Forgan AH, Kolouchova R, Fraser JA, Schmidt SA. 2016. Whole genome comparison reveals high levels of inbreeding and strain redundancy across the spectrum of commercial wine strains of *Saccharomyces cerevisiae*. *G3: Genes Genomes Genet* **6**: 957–971.

Braun KA, Young ET. 2014. Coupling mRNA synthesis and decay. *Mol Cell Biol* **34**: 4078–4087.

van den Broek M, Bolat I, Nijkamp JF, *et al*. 2015. Chromosomal copy number variation in *Saccharomyces pastorianus* is evidence for extensive genome dynamics in industrial lager brewing strains. *Appl Environ Microbiol* **81**: 6253–6267.

Damerow P. 2012. Sumerian beer: the origins of brewing technology in ancient Mesopotamia. *Cineiform Digital Library J* **2**: 1–20.

De León-Medina PM, Elizondo-González R, Damas-Buenrostro LC, *et al*. 2016. Genome annotation of a *Saccharomyces* sp. lager brewer's yeast. *Genomics Data* **9**: 25–29.

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

Gallone B, Steensels J, Prahl T, *et al*. 2016. Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell* **166**: 1397–1410.

Gayevskiy V, Goddard MR. 2016. *Saccharomyces eubayanus* and *Saccharomyces arboricola* reside in North Island native New Zealand forests. *Environ Microbiol* **18**: 1137–1147.

Gonçalves M, Pontes A, Almeida P, *et al*. 2016. Distinct domestication trajectories in top-fermenting beer yeasts and wine yeasts. *Curr Biol* **26**: 1–12.

Haimovich G, Medina DA, Causse SZ, *et al*. 2013. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell* **153**: 1000–1011.

Hebly M, Brickwedde A, Bolat I, *et al*. 2015. *S. cerevisiae* × *S. eubayanus* interspecific hybrid, the best of both worlds and beyond. *FEMS Yeast Res* **15**: fov005.

Hewitt SK, Donaldson IJ, Lovell SC, Delneri D. 2014. Sequencing and characterisation of rearrangements in three *S. pastorianus* strains reveals the presence of chimeric genes and gives evidence of breakpoint reuse. *PloS ONE* **9** e92203.

James T, Usher J, Campbell S, Bond U. 2008. Lager yeasts possess dynamic genomes that undergo rearrangements and gene amplification in response to stress. *Curr Genet* **53**: 139–152.

Kvasnicka J, Mokrejs M, Hajkova J, *et al*. 2012. *Saccharomyces pastorianus* CCY48–91, whole genome shotgun sequencing project. Department of Biotechnology: Institute of Chemical Technology, Prague.

Laureau R, Loeillet S, Salinas F, *et al*. 2016. Extensive recombination of a yeast diploid hybrid through meiotic reversion. *PLoS Genet* **12**: e1005781.

Legras J-L, Merdinoglu D, Cornuet J-M, Karst F. 2007. Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Mol Ecol* **16**: 2091–2102.

Libkind D, Hittinger C, Valério E, *et al*. 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proc Natl Acad Sci U S A* **108**: 14539–14544.

Liti G, Carter D, Moses A, *et al*. 2009. Population genomics of domestic and wild yeasts. *Nature* **458**: 337–341.

McGovern PE, Zhang J, Tang J, *et al*. 2004. Fermented beverages of pre- and proto-historic China. *Proc Natl Acad Sci U S A* **101**: 17593–17598.

Medina DA, Jordan-Pla A, Millan-Zambrano G, *et al*. 2014. Cytoplasmic 5′–3′ exonuclease Xrn1p is also a genome-wide transcription factor in yeast. *Front Genet* **5**: 1.

Monerawela C, Bond U. 2017a. Brewing up a storm: the genomes of lager yeasts and how they evolved. *Biotechnol Adv* **35**: 512–519.

Monerawela C, Bond U. 2017b. Recombination sites on hybrid chromosomes in *S. pastorianus* share common sequence motifs and define a complex evolutionary relationship between Group I and II lager yeasts. *FEMS Yeast Res* (in press).

Monerawela C, James TC, Wolfe KH, Bond U. 2015. Loss of lager specific genes and subtelomeric regions define two different *Saccharomyces cerevisiae* lineages for *Saccharomyces pastorianus* Group I and II strains. *FEMS Yeast Res* **15**: fou008.

Nagarajan VK, Jones CI, Newbury SF, Green PJ. 2013. XRN 5′→3′ exoribonucleases: structure, mechanisms and functions. *Biochim Biophys Acta* **1829**: 590–603.

Nakao Y, Kanamori T, Itoh T, *et al*. 2009. Genome sequence of the lager brewing yeast, an interspecies hybrid. *DNA Res* **16**: 115–129.

Nguyen HV, Legras JL, Neuveglise C, Gaillardin C. 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.

Okuno M, Kajitani R, Ryusui R, *et al*. 2016. Next-generation sequencing analysis of lager brewing yeast strains reveals the evolutionary history of interspecies hybridization. *DNA Res* **23**: 67–80.

Parker R. 2012. RNA degradation in *Saccharomyces cerevisae*. *Genetics* **191**: 671–702.

Peris D, Sylvester K, Libkind D, *et al*. 2014. Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Mol Ecol* **23**: 2031–2045.

Peris D, Langdon QK, Moriarty RV, *et al*. 2016. Complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. *PLoS Genet* **12**: e1006155.

Querol A, Bond U. 2009. The complex and dynamic genomes of industrial yeasts. *FEMS Microbiol Lett* **293**: 1–10.

Rodriguez ME, Perez-Traves L, Sangorrin MP, Barrio E, Lopes CA. 2014. *Saccharomyces eubayanus* and *Saccharomyces uvarum* associated with the fermentation of *Araucaria araucana* seeds in Patagonia. *FEMS Yeast Res* **14**: 948–965.

Strope PK, Skelly DA, Kozmin S, *et al*. 2015. The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. *Genome Res* **25**: 762–774.

Usher J, Bond U. 2009. Recombination between homoeologous chromosomes of lager yeasts leads to loss of function of the hybrid GPH1 gene. *Appl Environ Microbiol* **75**: 4573–4579.

Walther A, Hesselbart A, Wendland J. 2014. Genome sequence of *Saccharomyces carlsbergensis*, the world's first pure culture lager yeast. *G3: Genes Genomes Genet* **4**: 783–793.

Wang QM, Liu WQ, Liti G, Wang SA, Bai FY. 2012. Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity. *Mol Ecol* **21**: 5404–5417.

Wendland J. 2014. Lager yeast comes of age. *Eukaryot Cell* **13**: 1256–1265.

Zhu YO, Sherlock G, Petrov DA. 2016. Whole genome analysis of 132 clinical *Saccharomyces cerevisiae* strains reveals extensive ploidy variation. *G3: Genes Genomes Genet* **6**: 2421–2434.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. List of strains containing RST XIII and TYP

Table S2. Chromosome regions and coordinates of Group II WS 34/70 used for phylogenetic tree analysis