**SUPPLEMENTARY METHODS**

*Sample areas and yeast isolation*

Bark samples from ‘lenga’ (*Nothofagus pumilio*), coigüe (*N.dombeyi*) and ‘ñirre’ (*N. Antarctica*) and *Araucaria araucana* were obtained aseptically from ten sampling sites in Chile (collection date, GPS coordinates, **Figure 1**): National Park Altos de Lircay (January 2018, 35°36’34’’S, 70°57’58’’W), Nahuelbuta National Park (February 2018, 37°47’33’’S, 72°59’53’’W), Villarrica National Park (January 2017, 39°28’52’’S, 71°45’50’’W), Choshuenco National Park (January 2018, 39°50’2’’S, 72°4’57’’W), Antillanca National Park (November 2017, 40°46’23’’S, 72°12’15’’W), Vicente Pérez Rosales National Park (November 2017, 41°6’15’’S, 72°29’45’’W), Coyhaique National Reserve (February 2017, 45°31’23’’S, 71°59’19’’W), Torres del Paine National Park (February 2018, 50°56’32’’S, 73°24’24’’W), Magallanes National Reserve (January 2018, 53°8’45’’S, 71°0’12’’W) and Karukinka Natural Park (January 2018, 54°6’4’’S, 69°21’24’’W). All sampling sites were located at least five km from human settlements.

For each site, at least 25 bark samples of about 1g and 20 x 1 mm were obtained and immediately incubated in a 15 mL tube containing 10 mL of enrichment media. The media contained 2% yeast nitrogen base, 1% raffinose, 2% peptone and 8% ethanol {Sampaio, 2008 #1076}. Overall, 553 samples were collected (**Table S1**). Samples were incubated for two weeks at 20°C without agitation and were subsequently vortexed and plated (5 L) onto YPD agar (1% yeast extract, 2% peptone, 2% glucose and 2% agar). Isolated colonies were stored in glycerol 20% v/v and stored at -80°C in the Molecular Genetics Laboratory yeast collection at Universidad de Santiago de Chile.

*Saccharomyces eubayanus* identification *and FACS analysis*

We amplified and sequenced the internal transcribed spacer region (ITS) to identify colonies to the genus level. For this, ITS1 and ITS4 primers {J White, 1990 #1160} were used and we classified as *Saccharomyces* fragment sizes ranging between 830 and 880 bp {Pham, 2011 #1161}. Species identification was conducted using the polymorphic marker *GSY1* and *RIP1* through amplification and enzyme restriction (see details in {Peris, 2014 #1009}). Then, restriction fragment length polymorphism was performed using the restriction enzymes *HaeIII* and *EcoRI* as previously described {Peris, 2014 #1009}. Colonies were classified based on restriction patterns as either *S. eubayanus*, *S. uvarum* or *S. cerevisiae* {Peris, 2014 #1009}. In many cases, species identification was confirmed by Sanger-sequencing of the ITS region, which was attained using a BLASTN against the Genbank database under 100% identity as threshold.

DNA content was analysed using a propidium iodide (PI) staining assay. Cells were first pulled out from glycerol stocks on YPD solid media and incubated overnight at 30 °C. The following day a small portion of each patch was taken with a pipette tip and transferred in liquid YPD in a 96-well plate and incubated overnight at 30 °C. Then, 3 μl were taken and resuspended in 100 μl of cold 70% ethanol. Cells were fixed overnight at 4 °C, washed twice with PBS, resuspended in 100 μl of staining solution (15 μM PI, 100 μg/ml RNase A, 0.1% v/v Triton-X, in PBS) and finally incubated for 3 h at 37 °C in the dark. Ten thousand cells for each sample were analysed on a FACS-Calibur flow cytometer using the HTS module for processing 96-well plates. Cells were excited at 488 nM and fluorescence was collected with a FL2-A filter. The data collected were analysed in R with flowCore {Hahne, 2009 #1164} and flowViz {Sarkar, 2008 #1166} and plotted with ggplot. The highest density value of FL2-A was associated with the ploidy level of G1 cells, thus cells that are not dividing, and used for inferring the ploidy state of the sample. FL2-A values between 60 and 110 for G1 cells were associated with haploid state, FL2-A values between 120 and 220 were associated with diploid state and FL2-A values between 290 and 400 were associated with a tetraploid state.

*Sequencing, Reads processing and Mapping*

DNA was obtained using a Qiagen Genomic-tip 20/G kit (Qiagen, Hilden, Germany). The library prep reaction used was a 100x miniaturized version of the Illumina Nextera method. In this prep, 1.6 ng of total DNA mass is tagmented in a 5X diluted Tagmentation reaction. The 0.5 L reaction was quenched by 0.5% SDS(0.125% final concentration) at room temperature for 5 minutes. After quenching, 125 nL of a P5 sequencing barcode and 125 nL of a P7 sequencing barcode were added to the 0.625 nL reaction. In order to amplify the library of inserts, 24.125 L of 1X KAPA Library Amplification Master Mix were added to the reaction. The library went through 15 cycles of PCR to add the barcodes to then amplify the library to a concentration >4 nM. The libraries were then normalized and pooled according to the Illumina standard operating procedure and sequenced on a NextSeq 500/550 High Output Kit v2.5 (300 Cycles) flow cell.

Read quality and summary statistics were examined using FastQC 0.11.8 {Andrews, 2014 #1167}. Reads were processed with fastp 0.19.4 (low quality 3’ end trimming, 37 bp minimum read size) {Brickwedde, 2018 #1126;Chen, 2018 #1168}. We also obtained publicly available sequencing reads of *S. eubayanus* {Brickwedde, 2018 #1126;Peris, 2016 #956;Bing, 2014 #957;Gayevskiy, 2016 #955} and *S. pastorianus* (Baker et al, 2015} from the SRA database, which were processed similarly, i.e. visual inspection with FastQC and processing adaptors, low quality 3’ ends, and read size, with fastp. Processed reads were aligned against the *Saccharomyces eubayanus* CBS12357T reference genome {Brickwedde, 2018 #1126} using BWA-mem (options: -M -R){Li, 2013 #1169}. Mapping quality and overall statistics were collected and examined with Qualimap {García-Alcalde, 2012 #1254}. Summary statistics are shown in **Table S2**. Sorting and indexing of output bam files were performed using SAMTOOLS 1.9 {Li, 2009 #803}. A *S. uvarum* isolate (CL1105) isolated from Nahuelbuta was also mapped to the *S. eubayanus* and *S. uvarum* CBS7001 genome {Almeida, 2014 #459;Scannell, 2011 #1059} for phylogenetic analysis. In addition, for Treemix analysis, *S. cerevisiae* reads (PRJNA340312) were mapped to *S. eubayanus* genome.

*Variant calling*

Mapping files were tagged for duplicates using MarkDuplicates of Picard tools 2.18.14 (http://broadinstitute.github.io/picard/). Variant calling and filtering was done with GATK version 4.0.10.1 {DePristo, 2011 #1172}. More specifically, variants were called per sample and chromosome using HaplotypeCaller (default settings), after which variant databases were build using GenomicsDBImport. Genotypes for each chromosome were called using GenotypeGVCFs (-G StandardAnnotation). Variant files were merged into one genome-wide file using MergeVcfs. This file was divided by SNP calls and INDEL calls using SelectVariants. We applied GATK recommended filters to both variant files, i.e. for SNPs “QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0”, and for INDELS “QD < 2.0 || FS > 200.0 || ReadPosRankSum < -20.0". Furthermore, we applied a stricter criteria to filter heterozygous calls using bcftools view (-e 'GT="0/1" & QUAL<7000 & AC=1') version 1.9 {Li, 2009 #803}}. This VCF file was further filtered, depending of what was required for the given analysis, using vcftools {Van der Auwera, 2013 #1173}. For all datasets, we only considered SNPs that had no missing data using vcftools option –max-missing 1. Furthermore the effect of each variant was assessed and annotated with SnpEff version 4.3t {Cingolani, 2012 #1174}, using an updated version of *S. eubayanus* gene annotations {Brickwedde, 2018 #1126}

*Phylogeny and population structure analyses*

We obtained a phylogenetic tree using 590,909 bialllelic SNPs. VCF files were imported to R (version 3.5.2){Development Core Team, #1238} and converted to genlight objects with vcfR version 1.8.0 {Knaus, 2017 #1175}. A bitwise distance matrix was calculated with the package poppr version 2.8.1 {Kamvar, 2014 #1176}, and a neighbour-joining tree was built using the function aboot, using 1000 bootstraps. Trees were visualized in the iTOL website (http://itol.embl.de). For STRUCTURE analysis, a thinned version of the VCF file was generated with vcftools 0.1.15 (--thin 1000){Danecek, 2011 #1178}, containing 9,885 similarly-spaced SNPs, while including only *S. eubayanus* strains. Structure was run on this dataset five times for K values ranging from 3 to 7, with 10,000 burn-in and 100,000 replications for each run and using admixture model, infer alpha, lambda =1, fpriormean =1, unifprioralpha 1, alpha max 10. The structure-selector website was used to obtain the optimal K values (http://lmme.qdio.ac.cn/StructureSelector/) {Li, 2018 #1179} according to the Evanno method {Evanno, 2005, 2005} and to obtain the final results for each K, which were plotted using CLUMPAK {Kopelman, #1181}. The resulting diagrams were visualised using structure plot (http://omicsspeaks.com/strplot2/){Ramasamy, 2014 #1182}. In addition, we performed clustering analyses of the same samples by using SMARTPCA without outlier removal {Patterson, 2006 #1289}. For fineSTRUCTURE analysis {Lawson, 2012 #1290}, a VCF file that included all SNPs called among *S. eubayanus* strains was phased using BEAGLE 3.0.4 {Browning, 2007 #1291}. As we lacked a *S. eubayanus* recombination map, we used a constant recombination rate between consecutive SNPs based on *S. cerevisae* average recombination rate (0.4 cM/kbp, {Cubillos, 2011 #225}). All versus all chromosomal painting was performed with Chromopainter V2, and its output was further analysed with fineSTRUCTURE (-x 100000 -y 100000 -z 1000). Plotting of the ancestry matrix was done using fineSTRUCTURE R scripts.

*Analyses of ancient and recent admixture*

Ancient admixture between populations of *S. eubayanus* was tested with Treemix {Pickrell, 2012 #1292} and ADMIXTOOLS {Patterson, 2012 #1296}. For Treemix we analysed only *S. eubayanus* individuals that did not show any sign of recent admixture according to STRUCTURE results, plus the *S. uvarum* and a *S. cerevisiae* individual were kept as outgroups. In addition, we pruned out SNPs that were in linkage disequilibrium using PLINK (--indep-pairwise 50 10 0.2). We dissected the five *S. eubayanus* populations into subpopulations according to geographical locality and clusters obtained with fineSTRUCTURE as criteria. Treemix was first run ten times for each value of m (migration events) ranging from 1 to 6 (-noss –k 500) and two optimal m values (2 and 4) were estimated using the optM R package (<https://cran.r-project.org/web/packages/OptM/index.html>) (Table S3a). Treemix was subsequently run 100 times (2 and 4 migrations, -noss –k 500) after which a consensus tree and bootstrap values were obtained using the BITE R package {Milanesi, 2017 #1293}. We calculated f4 statistics between PA, PB-1, PB-2, PB-3, and HOL populations using the r package admixr {Petr, 2019 #1294}. Admixture graph fitting of the calculated f4 statistics was done using the R package admixturegraph {Leppälä, 2017 #1295}. Seven models were tested which were ranked according to their minimal error values.

The variants of the mosaic *S. eubayanus* strains were split to bins of 100 SNPs (on average ~5kb windows) and each bin was assigned to either of the populations (i.e. PB-1, PB-2, PB-3, or PA) using adegenet’s hyb.pred function {Jombart, 2008 #1183} . This algorithm uses DAPC to estimate membership probability of a hybrid dataset to a known cluster (populations). Another sliding window analysis was used to calculate nucleotide divergence across the genome of the mosaic strains against their most likely parental subpopulations by using PopGenome’s “diversity stats between” analysis on bins of 100 SNPs (version 2.6.0) {Pfeifer, 2014 #1013}. We used GLOBETHROTTER {Hellenthal, 2014 #1297} to estimate the most likely parent subpopulations and the generations since admixture of the mosaic strains. GLOBETHROTTER was run using the output of Chromopainter V2 which this time was performed using the target and candidate donor subpopulations (NULL IND = 0). Mosaic strains of Karukinka and North America were grouped as populations which allowed us to bootstrap the value of their admixture event date (20 bootstraps). TREEMIX was also ran using a LD-filtered version of this dataset while allowing for 8 migration events. For all gene flow analyses, we excluded individuals that had more than 5% of missing data, which included the lager strains, and PB strains from Argentina.

*Population Genetics*

We estimated π and Tajima’s D using the R packages PopGenome. Values of *F*st were calculated with StAMPP 1.5.1 Weir and Cockerham's unbiased estimator {Weir and Cockerham 1984}{Pembleton, 2013 #1187} to obtain 95% confidence intervals by performing 5,000 bootstraps. LD decay was estimated by calculating R2 values using vcftools (----geno-r2 --ld-window-bp 100000), which were imported into R to calculate a regression according to {Hill, 1988 #1185}, for which the half decay was estimated (Ldmax/2).

The R package hierfstat {GOUDET, 2005 #1162} was used to calculate *F*is, Hs, and Ho by using the basic.stats function. Bootstrapping per loci on each population’s *F*is was done using hierfstat’s boot.ppfis, obtaining the 50th and 97.5th quantiles after 50000 boostraps. To perform a Mantel test, first the Nei’s genetic distances between subpopulations (considering localities) was calculated with the R package StAMPP {Pembleton, 2013 #1187}. Euclidean distance between localities was calculated using latitude and distances coordinates with R ‘dist’ function. Randel test was performed using the ade4 R package {Dray, 2007 #1163}.

*Pangenome*

Isolates were assembled with Spades using k from 21 to 67. To detect the non-reference material, we used the custom pipeline based on the method described in {Peter, 2018 #1053}. LRSDAY software {Yue, #1188} was used to annotate the non-reference material. The newly annotated ORFs were added to the reference ORFs and a custom pipeline, also based on methods from {Peter, #1053} was used to collapse ORFs with identity percentage over 95, selecting an unique reference for each groups of allelic variants to obtain a list of non-redundant pangenomic ORF sequences. Confirmation of presence of these ORFs was obtained by mapping the reads of each strain to the set of pangenomic ORFs using BWA mem with the option – U 0. Filtering was performed with samtools with options –bSq 20 –F260. To identify potential lateral transferred ORFs we blast searched against an in-house database of 57 yeasts ORFeomes and to the currently available genomes from the yeast1000+ genome project (https://y1000plus.wei.wisc.edu/). To be robust, a LGT hit should cover over 75% of the query with an id >90%. SMART (Simple Modular Architecture Research Tool), used in GENOMIC mode, was used to identify known PFAM protein domains and homologies {Bateman, 2004 #1305} using all the optional features: Outlier homologues, PFAM domains, signal peptides and internal repeats.

*Strains Phenotyping* *and Fermentations*

The microcultivation phenotyping assay of the *S. eubayanus* strains was performed as previously described {Kessi-Perez, 2016 #585}. Briefly, isolates were pre-cultivated in 200 L of YNB medium supplemented with glucose 2% for 48h at 25°C. For the experimental assay, strains were inoculated to an optical density (OD) of 0.03–0.1 (wavelenght 630 nm) in 200 uL of media and incubated without agitation at 25°C for 24 h (YNB control) and 48 h for other conditions in a Tecan Sunrise absorbance microplate reader. OD was measured every 20 minutes using a 630 nm filter. Each experiment was performed in quadruplicate. Maximum growth rate, lag time and OD max for each strain were calculated using GrowthRates software with default parameters {Hall, 2014, PMID: 24170494}.

*Fermentation in beer wort and HPLC analysis*

Fermentations were conducted using a 12°P high-gravity wort at 12°C in 50 mL (micro-fermentations). The 12 °P wort was prepared from a Munton's Connoisseurs Pilsner Lager kit (Muntons plc, England). The worts were oxygenated to 15 mg/L prior to pitching. For the micro-fermentations, the strains were initially grown with constant agitation in 5 mL of wort for 48 hours at 15°C. Following this, 50 mL of fresh wort were inoculated to a final concentration of 15 × 106 viable cells/mL and fermentations were maintained for seven days. Fermentations were weighed every day to calculate the CO2 output. The fermentations were maintained until no-CO2 lost was observed. At the end of the fermentation, the fermented worts were centrifuged at 9,000xg for 10 min and the supernatant was collected. From this, the concentration of extracellular metabolites was determined using HPLC. Specifically, 20 L of filtered wort were injected in a Shimadzu Prominence HPLC (Shimadzu, USA) with a Bio-Rad HPX –87H column (Nissen et al., 1997). In this way, the concentrations of glucose, fructose, maltose, maltotriose, ethanol, and glycerol were estimated.

*Data Analysis*

Multiple comparisons across localities were performed utilising a non-parametric Kruskal-Wallis test and Dunn's Multiple Test Comparison. Genomewide *F*is and *F*st data across lineages was compared using paired Student t-test. Spearman rank correlation test and Pearson test were performed to determine correlations between variables. Finally, all analyses were performed utilising GraphPad Prism Software 5.2, except for correlation analysis which were performed in R {Development Core Team, #1238}. In all cases *p*-values < 0.05 were considered as significant.