Table S1

Nucleotide diversity between lager-brewing yeast genomes and their parental strains

	Saaz			**Frohberg**		
	N*dN	S*dS	all	N*dN	S*dS	all
***S. cerevisiae* (S288c)**	2268.4	4279.7	6548.1	2196.2	4129.6	6325.8
Lager-*S. cerevisiae*	2944.8	4415.6	7360.4	2362.2	3954.4	6316.6
Total changes between lineages	5213.2	8695.3	13908.5	4558.4	8084.0	12642.4
% of bp changed between lineages	0.22%	0.99%	0.43%	0.20%	0.93%	0.40%
% identity between lineages	99.78%	99.01%	**99.57%**	99.80%	99.07%	**99.60%**
changes per 1000 bp	2.2	9.9	4.3	2.0	9.3	4.0
Average # of differences per gene	2.30	3.83	6.13	2.01	3.56	5.57

	Saaz			**Frohberg**		
	N*dN	S*dS	all	N*dN	S*dS	all
***S. eubayanus* (FM1318)**	1232.3	3880.3	5112.6	1224.2	3827.0	5051.2
Lager-*S. eubayanus*	3835.8	5463.8	9299.6	3783.2	5430.6	9213.8
Total changes between lineages	5068.1	9344.1	14412.2	5007.4	9257.6	14265.0
% of bp changed between lineages	0.22%	1.06%	0.45%	0.22%	1.06%	0.45%
% identity between lineages	99.78%	98.94%	**99.55%**	99.78%	98.94%	**99.55%**
changes per 1000 bp	2.2	10.6	4.5	2.2	10.6	4.5
Average # of differences per gene	2.23	4.12	6.35	2.21	4.08	6.29

	Total # nonsynonymous sites	**Total # synonymous sites**	**Total # sites**
Saaz	2324071	881426	3205497
Frohberg	2301842	872845	3174687

Total # of genes: 2268
N*dN : estimated total number of nonsynonymous changes
S*dS: estimated total number of synonymous changes
Saaz: Saaz lineage of lager-brewing yeasts
Frohberg: Frohberg lineage of lager-brewing yeasts
Values discussed in the main text

Maltose utilization genes in *S. eubayanus* and lager-brewing yeasts

Table S2

S. eubayanus (FM1318)

Transporters	***Location***	***Length (aa)***	***Closest S. cerevisiae homolog***	***Identity (%)***[a]
SeubMALT1	ChrII –left arm (subtelomeric)	614	*MAL21*	80.36
SeubMALT2[H]	ChrV – right arm (subtelomeric)	614	*MAL31*	82.19
SeubMALT3[H]	ChrXIII – left arm (subtelomeric)	615	*MAL31*	85.15

Maltases	***Location***	***Length (aa)***	***Closest S. cerevisiae homolog***	***Identity (%)***[a]
SeubIMA-A	ChrII –left arm (subtelomeric)	590	*IMA1*	90.49
SeubIMA-B	ChrV – right arm (subtelomeric)	590	*IMA1* and *IMA4*	92.19
tSeubIMA-C	ChrV – right arm (subtelomeric)	510 (t)	*IMA2*	92.14
SeubMALS	ChrV – right arm (subtelomeric)	586	*MAL32*	84.76
SeubIMA-D	ChrXIII – left arm (subtelomeric)	590	*IMA1*	91
tSeubIMA-E	ChrXVI – left arm (subtelomeric)	181 (t)	*IMA2*	90.61

Regulators	***Location***	***Length (aa)***	***Closest S. cerevisiae homolog***	***Identity (%)***[a]
SeubMAL63A	ChrV – right arm (subtelomeric)	472	*MAL63*	73.83
SeubMAL63B	ChrXIII – left arm (subtelomeric)	471	*MAL63*	84.68
SeubMAL33A	ChrXIII – left arm (subtelomeric)	462	*MAL33*	70.72
SeubMAL33B	ChrXVI – left arm (subtelomeric)	469	*MAL33*	72.44

lager-brewing yeast-Saaz lineage (CBS 1513)

Transporters	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
Lager – MTT1[M, H] *(tSaazMAL61-C)*	370	*inconclusive*	*MAL61*	92.14
Lager – AGT1 [M, H] *(SaazMAL11-CA)*	611	*inconclusive*	*MAL11*	85.9
SaazMALT1-E	595	*inconclusive*	*SeubMALT1*	87.88
SaazMALT2-E[i, H]	612	*S. eubayanus*[S]	*SeubMALT2*	89.18
SaazMAL11-CB[i, H]	617	*S. cerevisiae*	*MAL11*	96.45

Maltases	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
tSaazMAL32-C	542	*S. cerevisiae*	*MAL32*	99.63
SaazIMA-CA	590	*S. cerevisiae*	*IMA2*	99.83
SaazIMA-EC	596	*S. eubayanus*[S]	*tSeubIMA-C*	98.62
SaazMALS-E	586	*S. eubayanus*[S]	*SeubMALS*	95.21
tSaazIMA-CB	575	*inconclusive*	*IMA5*	87.63
SaazIMA-CC	590	*S. cerevisiae*[S]	*IMA2*	99.49
SaazIMA-CD	582	*S. cerevisiae*[S]	*IMA5*	97.42
tSaazIMA1-C	166	*S. cerevisiae*	*IMA1*	100
tSaazIMA-EA	166	*S. cerevisiae*	*SeubIMA-A* (Ale)	83.03 (>99)[A]

Regulators	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
tSaazMAL63-C	181	*S. cerevisiae*	*MAL63*	96.13
SaazMAL13-C[i]	474	*S. cerevisiae*	*MAL13* (Ale)	99.58 (100)[A]
SaazMAL63-EA	478	*inconclusive*	*SeubMAL63A*	71.49
SaazMAL33B-EB[i]	499	*S. eubayanus*[S]	*SeubMAL33B*	86.75

lager-brewing yeast-Frohberg lineage (W34/70)

Transporters	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
Lager – MTT1[M, H] *(WeihenMAL31-CA)*	616	*inconclusive*	*MAL31*	90.72
Lager – AGT1[M, H] *(WeihenMAL11-CA)*	611	*inconclusive*	*MAL11*	85.9
WeihenMALT1-E	595	*inconclusive*	*SeubMALT1*	87.88
WeihenMPH3-C[H]	603	*S. cerevisiae*	*MPH3*	99.34
tWeihenMAL31-CB[H]	363	*S. cerevisiae*	*MAL31*	96.69
WeihenMALT2-E[i,H]	612	*S. eubayanus*[S]	*SeubMALT2*	89.18
WeihenMAL11-CB[i,H]	617	*S. cerevisiae*	*MAL11*	96.45

Maltases	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
tWeihenMAL32-C	573	*S. cerevisiae*	*MAL32*	99.48
tWeihenIMA-EB	338	*S. eubayanus*	*SeubIMA-B*	97.92
WeihenIMA-CA	582	*S. cerevisiae*	*IMA5*	97.25
WeihenMALS-E	586	*S. eubayanus*[S]	*SeubMALS*	95.21
WeihenIMA-CB	582	*inconclusive*	*IMA5*	87.78
tWeihenIMA-CC	198	*S. cerevisiae*	*IMA1*	99.49
tWeihenIMA-CD	198	*S. cerevisiae*	*IMA1*	99.49
tWeihenIMA-CE	198	*S. cerevisiae*	*IMA1* (Ale)	86.8 (>97)[A]
tWeihenIMA-EA	264	*S. eubayanus*[S]	*SeubIMA-A*	96.98

Regulators	***Length (aa)***	***Likely Origin***	***Closest homolog***	***Identity (%)***[a]
tWeihenMAL63-C	460	*S. cerevisiae*	*MAL63* (Ale)	81.96 (>98)[A]
WeihenMAL33-C	471	*S. cerevisiae*	*MAL33*	91.24 (>99)[A]
WeihenMAL33B-EA	478	*inconclusive*	*SeubMAL33B*	71.79
WeihenMAL13-C[i]	474	*S. cerevisiae*	*MAL13*	100
WeihenMAL33B-EB[i]	499	*S. eubayanus*[S]	*SeubMAL33B*	86.75

t – truncated sequence

[i] likely to be inactivated

[M] previously identified as a maltotriose transporter

[H] *S. cerevisiae* homolog can transport maltotriose

[S] origin supported by synteny

[a] based on amino acid and corrected for frameshift if necessary

[A] based on comparison to Foster's O and Foster's B base pair sequence

Table S3

WU-BLAST results and amino acid alignment of *MTT1*-like sequences from non-S288c strains of *S. cerevisiae*

		From WU-BLAST						
S. cerevisiae strain	amino acid ID to *MTT1*[a]	E-value	ID (%)	Location (contig/scaffold accession #)	start	end	Strain Description[b]	Notes
T7	96.42	0	95	AFDE01000045.1	83091	84938	Missouri oak tree exudate isolate.	Extracted sequence is reverse complement of *MTT1*.
DBVPG6044	96.09	0	95	JRIG01000448.1	8583	10430	Haploid derivative of West African bili wine isolate.	Extracted sequence is reverse complement of *MTT1*. In order to put the sequence into frame what is likely an extra A was removed from a poly A tract and a likely missing T in a poly T tract put in. This modified sequence still has an early stop codon.
YPS163	91.38 (overall)	0	95	JRIC01000169.1	81	1964	Pennsylvania woodland isolate.	Part of the end of this sequence is obscured by poor sequence (a tract of N's). When the part before this is translated and aligned to *MTT1* the percent ID is 96.32%, while for the remaining sequence after the obscured portion the percent ID for the translated sequence is 92.86%.
Sigma1278b	95.12	0	94	ACVY01000024.1	161442	163289	Laboratory strain.	Extracted sequence is reverse complement of *MTT1*.
SK1	96.31	0	95	JRIH01000332.1	1265	2975	Laboratory strain.	Sequence is incomplete because the contig ends. Like DBVPG6044, in order to put the sequence into frame what is likely an extra A in a poly A tract was removed and a likely missing T from a poly tract put in. This modified sequence also still has an early stop codon.
FL100	93.2	0	94	JRIT01000039.1	100613	102381	Laboratory strain.	Extracted sequence is reverse complement of *MTT1*. Part of the end of this sequence is obscured by poor sequence (a tract of N's). When the translated sequences before the poor sequence is aligned to *MTT1* the percent ID is 94.69%, while for the remaining sequence after the obscured portion the percent ID for the translated sequence is 92.86%.

Nucleotide sequence of *MTT1* from lager yeast of the Frohberg lineage (strain W34/70) used as WU-BLAST query sequence against "OTHER S. cerevisiae STRAINS: GENOMIC SEQUENCE" database in *Saacharomyces* Genome Database (www.yeastgenome.org) on 5-12-2015.

[a] pairwise alignment with *MTT1* WS34/70 in MUSCLE

[b] description from *Saccharomyces* Genome Database (http://www.yeastgenome.org/download-data/sequence)

Table S4

Number of synonymous substitutions in the *S. eubayanus* vs. *S. cerevisiae* subgenomes of lager-brewing yeasts

		sites with synonymous substitutions in *S. eubayanus* subgenome of lager-brewing yeasts			sites with synonymous substitutions in *S. cerevisiae* subgenome of lager-brewing yeasts		
Lineage	**Total # of synonymous sites**	**total # of substitutions**	**proportion of sites**	**99% binomial confidence interval**	**total # of substitutions**	**proportion of sites**	**99% binomial confidence interval**
Saaz	2310707	194	8.40E-05	(6.924e-5, 0.0001008)	2240	9.69E-04	(0.0009175, 0.001023)
Frohberg	2310707	191	8.27E-05	(6.807e-5, 9.935e-5)	1919	8.31E-04	(0.0007825, 0.0008805)
combined	2310707	385	1.67E-04	(0.0001456, 0.0001898)	4159	1.80E-03	(0.001729, 0.001873)

Table S5

BOWTIE2 mapping for placement of unplaced scaffolds

unplaced scaffold	**unplaced contig**	**scaffold-possible placement**	**contig-possible placements**	**mate support count>100**
MALψ cluster scaffold_18	contig_140	chrII-L	contig_1	450
		chrII-L	contig_2	350
		chrXIII-R	contig_40	292
		chrXIII-R	contig_42	230
		self	contig_140	7248
		FRE cluster(scaffold_19)	contig_141	506
		fully contained in conitg_141	contig_142	496
FRE cluster scaffold_19	contig_141	chrXV-R	contig_62	571
		MAL cluster(scaffold_18)	contig_140	506
		self	contig_141	2899
		fully contained in conitg_141	contig_142	112
PEP1 scaffold_21	contig_143	chrII	contig_3	292
		chrII	contig_4	266
		chrXIV	contig_67	231
		chrXIV	contig_68	420
		self	contig_143	1994
PPH22 scaffold_25	contig_147	chrIV	contig_37	402
		chrIV	contig_38	343
		self	contig_147	1251
GAL2 scaffold_28	contig_150	chrXII	contig_109	321
		chrXII	contig_111	360
		self	contig_150	1035

Table S6

Mate-pair support matrix for unplaced contigs and their possible placements

	contig_1	contig_2	contig_3	contig_4	contig_37	contig_38	contig_40	contig_42	contig_62	contig_67	contig_68	contig_109	contig_111	contig_140	contig_141	contig_142	contig_143	contig_147	contig_150
contig_1	3835	456	0	0	0	0	4	9	1	0	0	0	0	450	25	8	0	0	0
contig_2	456	51420	1008	0	3	6	0	0	7	0	6	9	2	350	12	1	0	0	0
contig_3	0	1008	20263	98	2	1	0	0	0	43	72	3	4	0	0	0	292	0	0
contig_4	0	0	98	5645	0	0	0	0	2	86	85	0	1	0	0	0	266	0	0
contig_37	0	3	2	0	39615	596	3	0	8	0	5	2	1	0	1	0	0	402	0
contig_38	0	6	1	0	596	32829	0	0	1	0	8	3	5	1	0	0	0	343	0
contig_40	4	0	0	0	3	0	11711	2	0	0	0	3	0	292	1	0	0	0	0
contig_42	9	0	0	0	0	0	2	3660	0	0	0	0	1	230	0	0	0	0	0
contig_62	1	7	0	2	8	1	0	0	46169	1	9	6	3	10	571	604	0	0	0
contig_67	0	0	43	86	0	0	0	0	1	3835	251	0	0	0	0	0	231	0	0
contig_68	0	6	72	85	5	8	0	0	9	251	80556	15	8	0	1	0	420	0	0
contig_109	0	9	3	0	2	3	3	0	6	0	15	71823	288	1	0	0	0	0	321
contig_111	0	2	4	1	1	5	0	1	3	0	8	288	53586	0	1	0	0	0	360
contig_140	450	350	0	0	0	1	292	230	10	0	0	1	0	7248	506	496	0	0	0
contig_141	25	12	0	0	1	0	1	0	571	0	1	0	1	506	2899	112	0	0	0
contig_142	8	1	0	0	0	0	0	0	604	0	0	0	0	496	112	2901	0	0	0
contig_143	0	0	292	266	0	0	0	0	0	231	420	0	0	0	0	0	1994	0	0
contig_147	0	0	0	0	402	343	0	0	0	0	0	0	0	0	0	0	0	1251	0
contig_150	0	0	0	0	0	0	0	0	0	0	0	321	360	0	0	0	0	0	1035

self pairing
0 mate-pair support
0 < mate-pair support < 100
mate-pair support > 100