

1 **Genomic stability and adaptation of beer brewing yeasts during serial repitching in the
2 brewery**

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21 **Abstract**

22 Ale brewing yeast are the result of admixture between diverse strains of *Saccharomyces*
23 *cerevisiae*, resulting in a heterozygous tetraploid that has since undergone numerous genomic
24 rearrangements. As a result, comparisons between the genomes of modern related ale brewing
25 strains show both extensive aneuploidy and mitotic recombination that has resulted in a loss of
26 intragenomic diversity. Similar patterns of intraspecific admixture and subsequent selection for
27 one haplotype have been seen in many domesticated crops, potentially reflecting a general
28 pattern of domestication syndrome between these systems. We set out to explore the evolution
29 of the ale brewing yeast, to understand both polyploid evolution and the process of domestication
30 in the ecologically relevant environment of the brewery. Utilizing a common brewery practice
31 known as 'repitching', in which yeasts are reused over multiple beer fermentations, we generated
32 population time courses from multiple breweries utilizing similar strains of ale yeast. Applying
33 whole-genome sequencing to the time courses, we have found that the same structural variations
34 in the form of aneuploidy and mitotic recombination of particular chromosomes reproducibly rise
35 to detectable frequency during adaptation to brewing conditions across multiple related strains in
36 different breweries. Our results demonstrate that domestication of ale strains is an ongoing
37 process and will likely continue to occur as modern brewing practices develop.

38

39 **Introduction**

40 *Saccharomyces cerevisiae*, the common budding yeast, occupies a diverse number of
41 environments, from an association with oak and fruit trees, to human-related industries such as
42 baking and fermentation [1]. Modern efforts to characterize the diversity of *S. cerevisiae* through
43 large whole-genome sequencing efforts have found a somewhat discrete population structure, in
44 which strains isolated from a particular fermented beverage or geography are more closely related

45 to other yeasts from that environment [2–5]. Due to a tight association with humans, the genomes
46 of yeast are thought to have been shaped by both historical migrations of humans and the
47 environment in which they are reared. One of the best characterized examples of this human-
48 associated adaptation or domestication is the beer brewing yeasts, which are divided into three
49 large clades across the family tree of yeasts. The largest division is split over species barriers
50 between the *S. cerevisiae* ale yeast and the lager yeasts, which are hybrids between *S. cerevisiae*
51 and *S. eubayanus* [6]. The ale yeasts are further divided into two large groups coined as Beer 1
52 and Beer 2, with several smaller mixed origin groups containing yeast from the bread, wine, and
53 spirits industries. While the Beer 2 group consists primarily of diploid individuals used in traditional
54 Belgian styles, the Beer 1 yeasts (herein called ale yeasts) consist of a diverse group of mostly
55 tetraploid strains from Germany, Belgium, the UK, the USA, and Scandinavia [2,7]. The origin of
56 the ale yeasts is hypothesized to come from a historical admixture between several *S. cerevisiae*
57 subpopulations who have similar genomic signatures as the extant populations of European and
58 Asian wine strains with some beer brewing strains that are no longer in existence [8]. The diversity
59 and structure of these populations has allowed for extensive study of the specific molecular
60 adaptations beer brewing yeasts have to their human-created environment, making them an
61 excellent system in which to study the genetic basis of domestication.

62

63 Using a combination of genotype association and phenotyping in previous works, several
64 specific genetic variations have been linked to traits that are either beneficial for the flavor of a
65 particular beer or for growth in a beer brewing environment. First, in comparison to wild strains,
66 the ale brewing yeasts lack the ability to produce the flavor compound 4-vinylguaiacol (4-VG),
67 which is undesirable in certain beer styles, through the inactivation of two genes, *PAD1* and *FDC1*
68 [9,10]. Interestingly, one lineage of ale yeasts which are specifically used for making wheat beers
69 in which 4-VG is a desirable characteristic, retain functional alleles, highlighting the diversity and
70 specialization of the domesticated strains [2,5]. Second, ale yeasts encode an expansion of genes

71 involved in the uptake and breakdown of maltose and maltotriose, two uniquely important sugar
72 sources for beer brewing [2,5]. Finally, both wine and ale brewing yeasts show evidence for loss
73 of function alleles in *AQY1* and *AQY2* resulting in increased osmotolerance in high sugar content
74 environments [5,11]. However, there is a functional allele of *AQY2* present in some of the ale beer
75 strains, potentially indicating either a lack of selection for this allele or an environment-dependent
76 selective benefit. As most of these putative adaptations have been identified because they are
77 shared among most or all of the ale brewing yeasts (Beer 1), it is unclear to what extent there are
78 additional genetic variations which are resultant from adaptive evolution or domestication within
79 subsets of the beer brewing yeasts.

80

81 Furthermore, these single gene events have simplified the process of connecting them to
82 potential phenotypes while other mutations prominent in the lineage are more difficult to interpret.
83 Likely as a result of the reduced ability of ale brewing yeasts to complete meiosis and the
84 increased mutation rate of both aneuploidy and mitotic recombination in tetraploids [12], tracts of
85 homozygosity have been extensively observed in these yeasts. Similarly, in lager brewing yeasts,
86 extensive aneuploidy and mitotic recombination between and within these two genomes have led
87 to tracts of homozygosity favoring certain *S. cerevisiae* or *S. eubayanus* alleles [6,13–15].
88 Although it is unclear what the consequence of these intragenomic events are in ale and lager
89 yeasts, previous works from our group and others have shown that loss of heterozygosity (LOH)
90 caused by mitotic recombination in a previously heterozygous strain can lead to drastic fitness
91 consequences on the time scale of short-term experimental evolution [16–20]. Furthermore, in
92 other yeasts, such as *Candida albicans* and *Cryptococcus neoformans*, extensive aneuploidy and
93 LOH can lead to diverse phenotypic outcomes such as increased drug resistance and competitive
94 growth (reviewed in [21]). Finally, in mitotically dividing human cells, LOH of a non-functional
95 tumor suppressor allele can lead to an increased risk of cancer progression including, among
96 others, *BRCA1* mediated breast and ovarian cancer [22]. While LOH has been both observed

97 frequently in ale yeasts and has been seen to have phenotypic consequences in other mitotic cell
98 populations, it has yet to be linked conclusively to traits in ale brewing yeasts.

99

100 Additionally, ale yeasts' reduced ability to effectively go through meiosis complicates
101 traditional quantitative trait mapping approaches for interpreting genetic variation. Alternative
102 approaches that do not rely on meiosis, such as experimental evolution and genetic screens,
103 have provided valuable insights into adaptation generally, including the importance of specific
104 mutations, copy number variation [23], and ploidy [24]. Therefore, we decided to study adaptation
105 to the brewery by taking advantage of a form of experimental evolution already being conducted
106 at breweries. Typically, professional brewers serially reuse populations of yeast to brew batches
107 of beer in a practice known as repitching or backslopping to reduce the financial burden of
108 constantly buying yeast and to give the yeast the opportunity to physiologically adapt to the
109 brewery. The process begins when a brewery purchases a batch of a particular yeast strain at
110 scale (population size of $\sim 2 \times 10^{13}$) from a propagation company. These yeasts have commonly
111 been grown from a patch of yeast, derived from a clonal glycerol stock stored at -80°C. When a
112 propagation company sends out these yeasts, they often grow the stock beyond the needs of a
113 single brewery to meet the demand for a particular strain, meaning that there are many
114 generations of yeast growth that occur before the yeast arrive in the brewery (minimum of ~ 50
115 yeast generations). Once the yeasts arrive at the brewery they are inoculated or 'pitched' into a
116 cereal and grain derived beer medium or 'wort'. After the completion of fermentation at 10-14
117 days, the yeasts will flocculate to the bottom of the fermenter and are then collected. The brewer
118 will typically collect approximately a third of the yeast, avoiding the trub that is made up of hop
119 and protein particulates, and repitch the yeast into the next fermentation vessel with fresh wort.
120 The actual number of yeast cells that are transferred varies from brewery to brewery and is often
121 modified to match the starting sugar content of the media and the current viability of the yeasts.

122

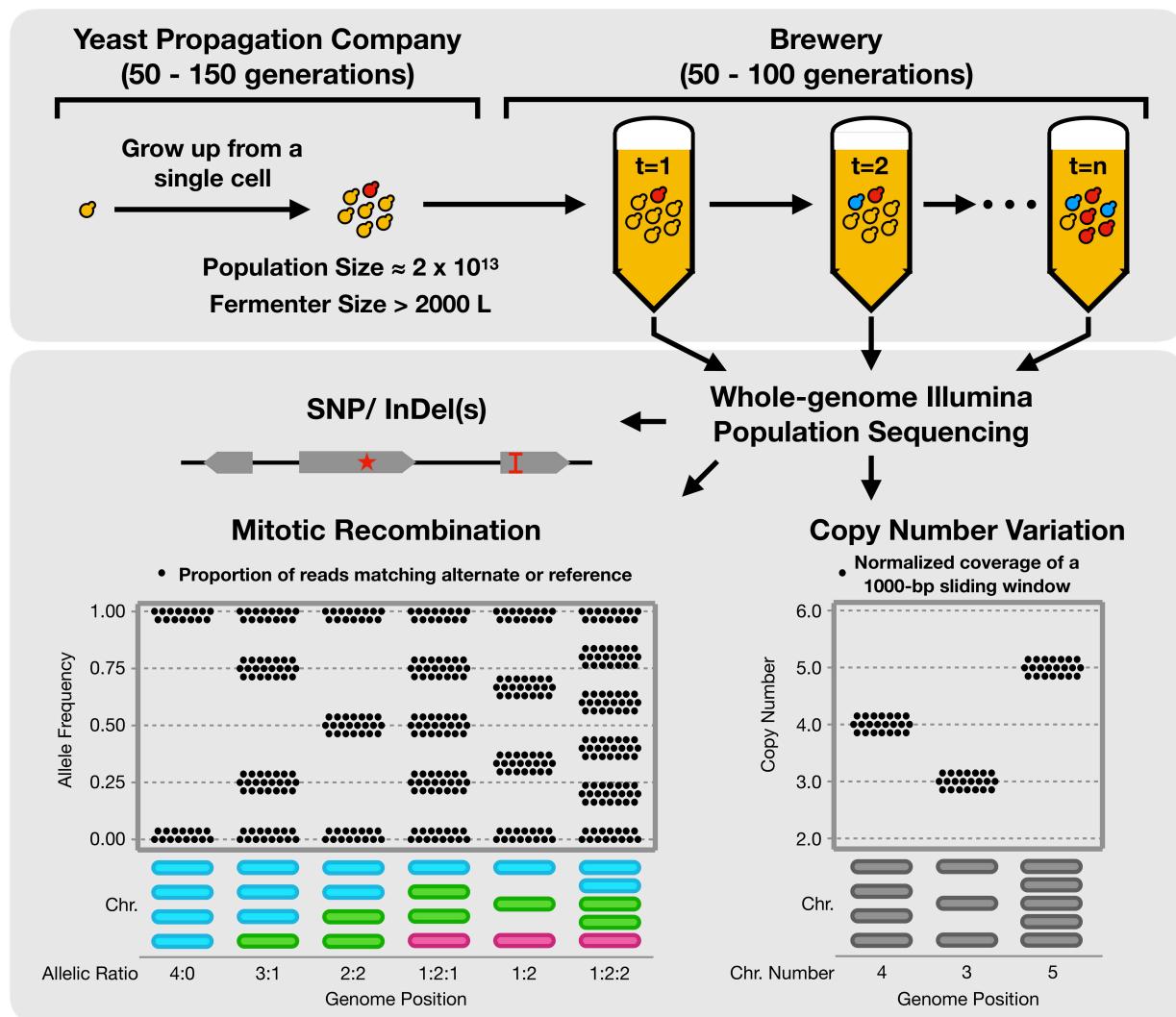
123 Brewers will often limit the number of times that yeast are repitched to ~8 reuses to reduce
124 the possibility of a failed batch by contamination, physiological changes to viability and vitality
125 [25–27], and taste profile changes due to altered physiology [28,29] or genetic mutation. However,
126 there are conflicting results about how long repitching can be continued before beer brewing yeast
127 will undergo a detectable genetic change by evolution. Early research on the genetic consistency
128 of brewing yeasts found the possibility that genetic mutations can affect brewing-relevant
129 characteristics by phenotyping clones isolated from populations of reused yeasts in a continuous
130 use fermenter [30]. Further research by a separate group found changes in flocculation behavior
131 in clone isolates from serially reused yeasts over several years, potentially due to a deletion
132 mutation in a flocculation-related gene [31]. Additionally, one study looking at population samples
133 from serial reuse of yeasts was not able to show any genetic mutation over 135 serial
134 fermentations through the use of gel-electrophoresis based methods [32]. In contrast, some
135 recent works on lager fermentations of buckwheat and quinoa beer have found potential
136 alterations in chromosome length over the course of serial repitching [33]. Despite the recent
137 evidence that genetic based changes in beer characteristics rarely occur over short-term
138 repitching, there are striking phenotypic differences between brewing yeasts that are almost
139 certainly caused by genetic variation. Matching these observations, it is a common practice
140 among professional and home brewers to cultivate a yeast strain for an extended number of yeast
141 pitches to generate a so-called ‘house strain’ with altered brewing characteristics indicating that
142 genetic changes will likely occur over some relatively short time period in the brewery. However,
143 the mutational basis, timing, and consequence of these changes has not been fully documented
144 using modern high-throughput whole genome sequencing.

145

146 Herein we describe the effect of long-term repitching on brewing yeasts from samples
147 collected in collaborations with multiple breweries across the United States and Canada who use
148 an American brewing strain, serially-repitched for greater than 10 cycles. From these

149 collaborations we either collected a time course across the pitches and sequenced several
150 representative time points, or sequenced a starting and final population (Fig 1). Using a
151 combination of short and long read (Illumina and Oxford Nanopore) sequencing, we found large-
152 scale chromosomal rearrangements rising to a detectable frequency even within the first several
153 generations of repitching. As well, we discovered a potential link between a specific mitotic
154 recombination event and both growth phenotypes and flavor metabolite production.

155



156

157 **Fig 1. Research strategy to investigate a natural evolution experiment occurring in the**
158 **brewery.** Ale brewing yeast purchased from propagation companies at industrial scale and
159 serially repitched for >15 beers are sequenced with whole-genome Illumina population
160 sequencing to find signatures of evolution during brewery repitching. Mitotic recombination events
161 are detected through allele frequency graphs (represented here), which denote every position of
162 the genome as a point whose height is determined by the frequency of reads supporting either a
163 reference or alternate allele. Copy number variation is detected and displayed as 1000-bp sliding
164 windows, normalized by the average coverage across the genome.

165 **Results**

166 We set out to tap into the natural evolution experiment occurring at modern breweries to
167 test whether domestication is actively occurring in ale yeasts (Fig 1). We established
168 collaborations with four breweries across the USA and Canada: Postdoc Brewing Co., Drake's
169 Brewing Co., Red Circle Brewing Co., and Elysian Brewing Co. All four breweries use a popular
170 family of American yeast strains known as the 'Chico' yeasts and repitch for an extended number
171 of cycles (>15), facilitating direct comparisons. Each brewery collected population samples of
172 serially repitched yeast from independent beer lineages. For Postdoc Brewing Co. and Elysian
173 Brewing Co. respectively we were able to collect two and three replicate beer lineages, plus one
174 lineage each from the other two brewery partners. A complete record of the brewery populations
175 is available in Table 1. Using whole-genome sequencing, we compared the starting genotype
176 assessed from either a clone or population depending on availability with the last time point
177 population sample for each beer lineage. For one replicate from Postdoc Brewing Co. we
178 sequenced multiple population time points, and a number of representative clones isolated from
179 the beginning and end time points for further experimental use. Given the time it takes to fully
180 ferment a beer at an industrial scale, we estimate that the total time encapsulated in our
181 experiments is on the order of four and a half years of yeast evolution.

182

183 **Table 1. Record of strains from brewery collaborations**

Pop. Name	Brewery	Brewery ID	Strain	Rep.	Repitch Number	Variants Filtered By
PDB1	Postdoc Brewing Co.	PDB	Wyeast 1056	1	0	N/A
PDB6	Postdoc Brewing Co.	PDB	Wyeast 1056	1	6	PDB1
PDB15	Postdoc Brewing Co.	PDB	Wyeast 1056	1	15	PDB1
PDB19	Postdoc Brewing Co.	PDB	Wyeast 1056	1	19	PDB1
PDB26	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB1 Rep. 2	Postdoc Brewing Co.	PDB	Imperial A07	2	1	N/A

PDB29 Rep. 2	Postdoc Brewing Co.	PDB	Imperial A07	2	29	PDB1 Rep. 2
E01	Elysian Brewing Co.	Elysian.	BRY-96	1	0	N/A
E03	Elysian Brewing Co.	Elysian	BRY-96	1	15	E01
E08	Elysian Brewing Co.	Elysian	BRY-96	2	15	E01
E09	Elysian Brewing Co.	Elysian	BRY-96	2	17	E01
E05	Elysian Brewing Co.	Elysian	BRY-96	3	1	E07
E07	Elysian Brewing Co.	Elysian	BRY-96	3	0	N/A
E10	Elysian Brewing Co.	Elysian	BRY-96	3	14	E07
DK01	Drake's Brewing Co.	Drakes	White Labs WLP001	1	24	SRR7406282
RCB01	Red Circle Brewing Co.	Red Circle	Escarpment Cali Ale	1	36	SRR7406282
Clone Name	Brewery	Brewery ID	Strain	Rep.	Repitch Number	Variants Filtered By
PDB1 c1	Postdoc Brewing Co.	PDB	Wyeast 1056	1	1	PDB1
PDB1 c2	Postdoc Brewing Co.	PDB	Wyeast 1056	1	1	PDB1
PDB26_c1	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c2	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c3	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c4	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c5	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c6	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c7	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c9	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c10	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c11	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c12	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c13	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c20	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1
PDB26_c23	Postdoc Brewing Co.	PDB	Wyeast 1056	1	26	PDB1

185 As is common with most breweries, the same recipe was not used for each beer pitch,
186 resulting in a potentially changing environment for the yeasts. Although these experiments are
187 less controlled than traditional laboratory evolution experiments they provide a more realistic
188 capture of the brewing environment. For the Postdoc Brewing Co. experiments, the order of
189 different styles that the yeast went through is available in Supp. Table 1. Overall, the yeasts
190 experienced an estimated final alcohol by volume (ABV) of around 5-6%. As well, for the Elysian
191 samples, data collected at the brewery about the fermentation performance of each beer are
192 available in Supp. Table 2 and show no strong deviation over the repitches.

193

194 Attempting to capture the full repertoire of genome variations that can occur and contribute
195 to evolution, we investigated Single Nucleotide Polymorphisms (SNPs), Insertions and Deletions
196 (InDels), copy number variations (CNVs), and changes in allele frequency resulting from mitotic
197 recombination. First, in order to properly identify what variation occurred *de novo* during serial
198 repitching, and how that variation relates to what occurs across the breweries, we established the
199 relationship between the strains in our study cohort using phylogenetics.

200

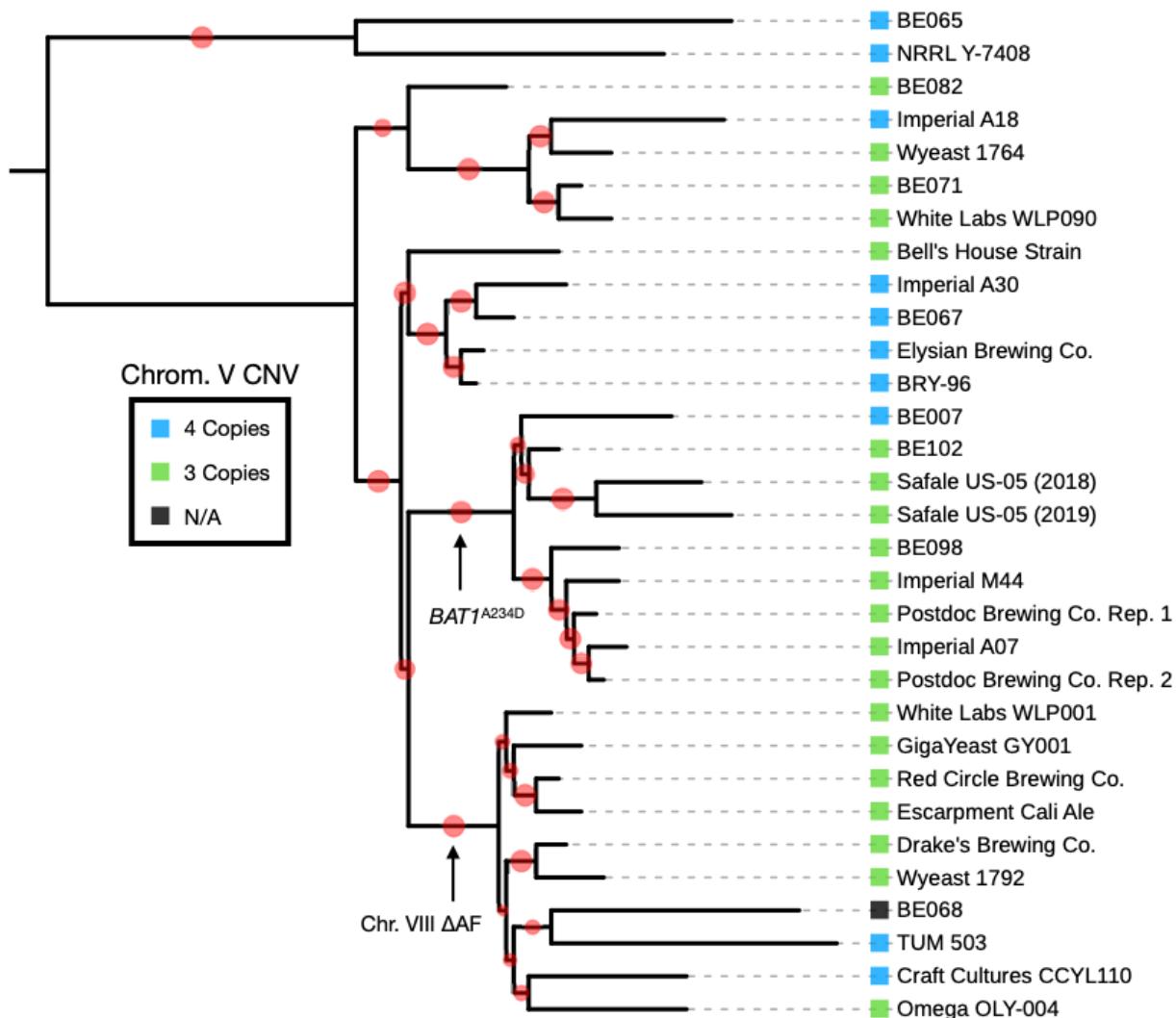
201 **Relationship between strains**

202 The history of the American brewing strains as told by brewers originates from just a
203 handful of breweries. The Chico yeasts are specifically thought to originate from a 'house-strain'
204 of the Sierra Nevada Brewing Company's isolate of BRY-96, which is sold by the Siebel Institute.
205 BRY-96 itself is thought to originate from P. Ballantine and Sons Brewing Company, which started
206 in 1840 in Newark, New Jersey. The strain has since been distributed to a large number of
207 breweries and yeast propagation companies. To provide a fuller picture of the genetic history of
208 the American brewing yeasts, we collected not just the strains used by our brewery partners but
209 also new clone samples of American brewing strains that are available for purchase and not
210 believed to have been previously sequenced. In all, we sequenced 13 American brewing strains,

211 and reanalyzed an additional 17 strains that had previously been sequenced using short-read
212 sequencing (Supp. Table 3). Wanting to confirm the relationships between our study cohort, we
213 applied phylogenetic inference on the strains. From their whole-genome sequence, we built a
214 maximum likelihood tree based on the variation between these strains. However, as mentioned
215 earlier, because there has been extensive mitotic recombination in these yeasts, we suspected
216 that phylogenetic inference could be influenced by large blocks of shared, ancestral variation
217 being lost. To avoid this issue, we filtered the American brewery strains variant calls by the most
218 diverged American strain, BE051, to control for the potential loss of shared variation. As well, to
219 encapsulate the polyploid nature of the beer strains, we encoded heterozygous variation in the
220 genome sequences for phylogenetic inference (see methods for more details).

221

222 Matching with oral history, we found from our constructed phylogeny that Wyeast 1056
223 (Postdoc Brewing Co.), Imperial A07 (Postdoc Brewing Co.), White Labs WLP001 (Drake's
224 Brewing Co.) and Escarpment's Cali Ale (Red Circle Brewing Co.), and other Chico yeasts are all
225 closely related and form two large clades (Fig 2). As well, we found that the WLP001 and Wyeast
226 1056 clades are likely derived from BRY-96 (Elysian Brewing Co.), as there is only an 11 SNP
227 difference between a reconstructed common ancestor of the two Chico strains and an isolate of
228 what is thought to be the original BRY-96 (kindly donated by Lallemand Inc.). Additionally, from a
229 sequenced isolate of a strain from P. Ballantine and Sons Brewing Company that was deposited
230 in a strain repository in 1972 (NRRL Y-7408), we found that this strain groups outside of the rest
231 of the American brewing strains, indicating that it is indeed a diverged American brewing strain.
232 However, because large segments of variation are lost from NRRL Y-7408 that exist in the internal
233 American brewing strains, we suspect that the Ballantine strain is not the literal genetic ancestor.



234

235 **Fig 2. A maximum-likelihood phylogeny of the American brewing strains reveals several**
236 **large clades.** Specifically, two Chico yeast groups, and their presumed genetic ancestor, BRY-
237 96 were found to group with other commercially available strains. The branch support bootstrap
238 values are displayed in red on the adjoining branch, with smaller values corresponding to less
239 support.

240

241 ***De novo* Single Nucleotide Polymorphisms, Insertions, and Deletions**

242 With the ancestral strain sequences in hand, we next called *de novo* mutations that
243 occurred during each repitching time course. Utilizing multiple SNP and InDel variant callers on
244 the first replicate of the Postdoc Brewing Co. populations we did not find any *de novo* SNP or
245 InDel that occurred during the course of the repitching experiment and reached a detectable
246 frequency (estimated detection limit of ~2% of alternate reads). Using sequencing of clones
247 isolated from the first time point to filter the variant calls from the populations, we found 11
248 mutations that were shared by all of the Postdoc Brewing Co. time points and had occurred in the
249 population before entering the brewery based on the sequences from the clone isolates, the
250 population from the second Postdoc Brewing Co. replicate, and the Imperial A07 clone isolate.
251 Calculating the change in frequency of these mutations over the time course, we found that the
252 only mutation that changed by more than a 1% increase in the population was a synonymous
253 mutation in *PTC6* (which increased from 25.2% to 44.4% in the population, Supp. Table 4). While
254 it is known that synonymous mutations can impact traits, it's more likely that this is a passenger
255 mutation, particularly since the mutation affects only one allele in a pentaploid region of the
256 genome. We additionally observed a number of private SNPs and InDels within clones from both
257 the first and last time points, with an average of 11.9 mutations per clone and a total of 177 unique
258 mutations (Supp. Table 5 and Supp. Fig 1).

259

260 Expanding our analysis to the samples from the other collaborations, we found a total of
261 106 mutations, with an overall average of 15.1 mutations observed in each population (Supp.
262 Table 5). Looking for evidence of adaptive evolution through convergence of mutations, we found
263 that between experiments, there were 5 genes wherein multiple mutations were observed in the
264 coding sequence between experiments (Table 2). We note that mutations in *UBP1*, which
265 encodes a ubiquitin protease, were previously identified in experimental evolution of a lager strain
266 [34], and mutations in *TFB1*, a nucleotide excision repair factor and subunit of TFIIH, were found

267 in strains that had survived for two years in a sealed beer bottle [35]. However, in neither of these
268 cases were phenotypic consequence proven. Further experiments recreating these mutations in
269 clean genetic backgrounds will be necessary to determine their impact.

270

271 **Table 2. The *de novo* mutations within the same genes between brewery populations**

Sample	Tool	Chromosome	Location	Ref	Alt	MutationType	Gene	Gene	Effect	FinalAF
PDB29 Rep. 2	freebayes	chrIV	244970	G	T	coding-nonsynonymous	YDL122W	UBP1	D807Y	0.103
Red Circle Brewing	lofreq	chrIV	244223	G	T	coding-nonsynonymous	YDL122W	UBP1	E558Stop	0.040
Elysian09	lofreq	chrIV	188256	G	C	coding-nonsynonymous	YDL148C	NOP14	Y777Stop	0.101
Elysian10	lofreq	chrIV	188256	G	C	coding-nonsynonymous	YDL148C	NOP14	Y777Stop	0.129
Elysian10	lofreq	chrIV	1085392	C	T	coding-nonsynonymous	YDR311W	TFB1	R110W	0.108
Drake's Brewing Co.	lofreq	chrIV	1085083	G	T	coding-nonsynonymous	YDR311W	TFB1	A7S	0.077
PDB26 Rep. 1	freebayes	chrXI	245205	C	G	coding-nonsynonymous	YKL104C	GFA1	G57R	0.077
PDB29 Rep. 2	freebayes	chrXI	245205	C	G	coding-nonsynonymous	YKL104C	GFA1	G57R	0.117
Elysian03	lofreq	chrXII	905505	G	A	coding-synonymous	YLR392C	ART10	N267N	0.176
Elysian08	lofreq	chrXII	905987	G	A	coding-nonsynonymous	YLR392C	ART10	P107S	0.049

272

273

274 **De novo chromosome copy number variation**

275 We next investigated whether there were any large scale genomic changes by plotting the
276 read coverage across the genome for the Postdoc Brewing Co. time course. In line with previous
277 work, we observed that the 'Chico' yeasts are largely tetraploid, and have had several whole
278 chromosome and segmental copy number changes (CNVs) that occurred at some point in its
279 recent past (Fig 3A). We observed that during the Postdoc Brewing Co. time course there was a
280 copy number chance of chromosome V which led to an increase from 3 to 4 chromosomal copies
281 at a final estimated frequency in the population of 48.2% (Fig 3B). The second Postdoc Brewing
282 experiment also replicated the increase in copy number on chromosome V (Fig 3C). Interestingly
283 though, and unlike in the first Postdoc Brewing Co. replicate, the mutation entered the brewery at
284 an estimated frequency of 27.6% and reached a frequency of 94.9% by the end of the experiment.
285 For the population from Drake's Brewing Co., we observed an estimated frequency of 27.9% for
286 the chromosome V increase in copy number, indicating another replication of the same mutation.
287 While no starting population was available for the sample, two separate sequences of WLP001

288 were uploaded two years apart by different groups and both shared 3 copies of chromosome V,
289 indicating that the starting strain likely had 3 copies. Additionally, the strain from Red Circle
290 Brewing Co., which is of similar origin, maintained 3 copies of chromosome V.

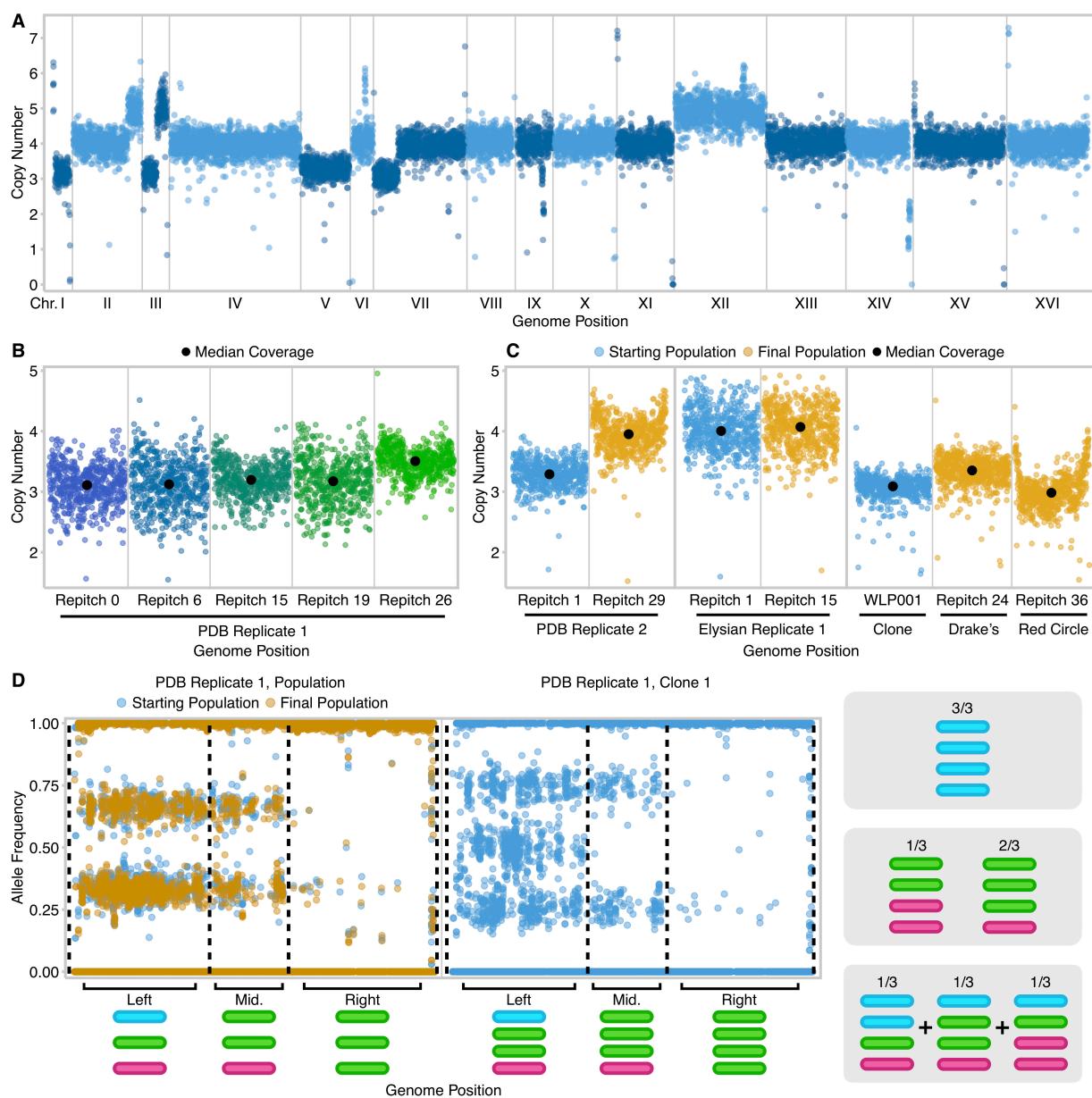
291
292 Wanting to determine whether the potential benefit of the aneuploidy was due to an
293 increase in copy number of a particular haplotype or a restoration of a euploid copy number for
294 dosage balance, we investigated whether one particular copy of chromosome V was recurrently
295 amplified between populations. Our expectation is that gaining a chromosome copy will change
296 the allele frequency of heterozygous variants by a change in the proportion of haplotypes.
297 Through investigation of the direction that variants change allele frequency, we can determine
298 which chromosome is amplified (See Fig 1 for allele frequency plot description). Therefore, we
299 investigated whether the allelic ratio between haplotypes had changed by plotting the allele
300 frequency of variants on chromosome V for the two Postdoc Brewing Co. and Drake's Brewing
301 Co. experiments. However, upon plotting the allele frequency from the first and last time points
302 we found very little to no change had occurred despite the chromosome copy number change
303 (Fig 3D and Supp. Fig 2).

304
305 From a clone isolated from the final population of the Postdoc Brewing Co., replicate 1
306 experiment that had an extra copy of chromosome V, we found that, in a clonal sample, as
307 expected, the allele frequency does change and shows three large chromosomal regions with
308 different allele frequency patterns. The clone helped show that the starting strain has three
309 haplotypes on the left arm, two in the middle in a 2:1 ratio and is homozygous on the right arm.
310 Given these patterns, we expect that depending on which chromosome was amplified, the allele
311 frequency will shift according to the number of haplotypes (Left: 0.33/0.66 to 0.25/0.50/0.75;
312 Center: 0.33/0.66 to 0.25/0.75 or 0.50; Right: No change, summarized on the right of Fig 3D).
313 However, because the allele frequency pattern did not change in a significant manner, we instead

314 concluded that there are likely multiple mutation events, each of which amplified a different
315 chromosome V haplotype. These independent mutations have occurred in separate lineages that
316 have risen in frequency with similar kinetics. Therefore, we suspect that the increase in copy
317 number of chromosome V likely occurred multiple times in both Postdoc Brewing Co. replicates,
318 indicating a haplotype independent fitness benefit.

319

320 The experiment(s) at Elysian Brewing Co. utilized BRY-96, which already contained 4
321 copies of chromosome V and did not show any additional evidence of aneuploidy. It is likely that
322 the ancestral state of chromosome V is euploid based on the phylogenetic relationship between
323 the American brewing strains (Fig 2). Since the chromosome loss event appears to have occurred
324 multiple times in the 'Chico' phylogeny (Fig 2), it's possible that this state could be selectively
325 advantageous in certain environments. An alternative explanation is that when the common
326 ancestor of WLP001 and Wyeast 1056 was clone isolated, the single-cell bottleneck fixed a
327 deleterious mutation for growth, which was then reverted upon serial passaging in brewery
328 conditions.



329

330 **Fig 3. Copy number variation of chromosome V occurs multiple times between breweries**
 331 **and within the same brewery.** (A) Whole-genome coverage of Imperial A07, highlighting the
 332 degree of chromosomal and sub-telomeric copy number alterations. (B) A time course of the
 333 Postdoc Brewing Co. replicate 1 is shown in 1000-bp coverage windows. A copy number change
 334 of chromosome V reaches 48.2% of the population by repitch 26. (C) The copy number increase
 335 of the second Postdoc Brewing Co. replicate population, starting at 27.6% of the population and
 336 reaching fixation by the 29th repitch. The strain BRY-96, which is used in Elysian Brewing Co.

337 starts with a euploid copy number and remains constant during repitching. Drake's Brewing Co.,
338 which is from WLP001, has an aneuploid lineage which reaches 94.9% of the population. The
339 sample from Red Circle Brewing Co. showed an increase coverage near the telomeres across its
340 genome, but this is likely a well-documented artifact [2] (D) Allele frequency of the Postdoc
341 Brewing Co. replicate 1 population and a clone isolated from that population showing the number
342 and pattern of haplotypes on chromosome V. The lack of a shift in allele frequency indicates that
343 in the population, multiple lineages likely independently had different haplotypes amplified. The
344 probability of any given haplotype being amplified is displayed on the right.

345

346 ***De novo* changes in heterozygosity**

347 We next investigated whether there were copy number neutral changes in heterozygosity
348 due to mitotic recombination by plotting the allele frequency of all positions in the genome. First,
349 looking at the allele frequency of the Postdoc Brewing Co. populations over the sampled
350 repitches, we observed a marked shift on the right arm of chromosome VIII starting at repitch
351 number 15, angling towards an allele frequency of 0.50 (Fig 4A). Using the allele frequency of
352 positions at the terminal end of the chromosome, we calculated that the allele frequency change
353 reached a frequency of 43.8% by the end of the experiment. From clones isolated from the first
354 replicate of the Postdoc Brewing Co. experiment, we identified that there were numerous, private
355 breakpoints in each clone where the allele frequency changed from a haplotype ratio of 3:1 to 2:2
356 (Fig 4C; full list at Supp. Fig 3). We determined that the signal from the individual clone
357 breakpoints stacked in the population data to create the angled pattern, with all events sharing a
358 2:2 ratio at the most distal segment of the chromosome. From these data, we concluded that there
359 are two chromosomal haplotypes on the right arm of chromosome VIII in a 3:1 major to minor
360 ratio that are broken up by a mitotic recombination event that occurs numerous times
361 independently in the population.

362

363 Expanding our analysis to the other replicate populations, we observed that the second
364 experiment from Postdoc Brewing Co. had a similar angled allele frequency change reaching
365 25.1% in the population, while all the Elysian Brewing Co. experiments showed sharp *de novo*
366 breakpoints with different start locations (Fig 4B). Surprisingly, samples from breweries using the
367 WLP001 strain from White Labs already had a starting fixed allele frequency change before
368 entering the brewery. When we further investigated the rest of the American strains we found that
369 the entire branch leading to WLP001 shared this breakpoint, indicating that it had occurred since
370 its divergence from BRY-96 (Fig 2). Observing the same allele frequency change between
371 multiple replicates at multiple breweries and independently within the American brewing yeasts,
372 we concluded that this allele frequency change likely confers an adaptive benefit. From the
373 Postdoc Brewing Co. replicate 1, for which we sequenced multiple intermediate samples, we
374 estimated the selective benefit of the allele frequency change would be 5.70%, using a value of
375 3 generations per repitch.

376

377 We additionally observed chromosomes XII and XV experiencing convergent mitotic
378 recombination events in 6 and 4 of the other populations respectively (Supp. Fig 4 and 5). After
379 noting the mitotic recombination on chromosome XII in the other populations, we noticed that the
380 first Postdoc Brewing Co. replicate likely had a similar event nearly fix in the population before it
381 entered the brewery as one of the starting clones, Postdoc Brewing Co., timepoint 1 clone 1, did
382 not have the allele frequency change. Using the clone that did not have the allele frequency
383 change, we looked for any variation that experienced a LOH as a result of the mitotic
384 recombination as this is the most likely source of an adaptive benefit for a mitotic recombination.
385 However, through computational and manual inspection, we determined that no variation was lost
386 as a result of the chromosome XII mitotic recombination (though other explanations are possible
387 as well, such as allele copy number changes). Notably, the right arm of chromosome XII has been

388 observed to have the highest amount of homozygosity among natural and industrial strains of
389 yeast, potentially due to the presence of the rDNA locus on chromosome XII [3].

390

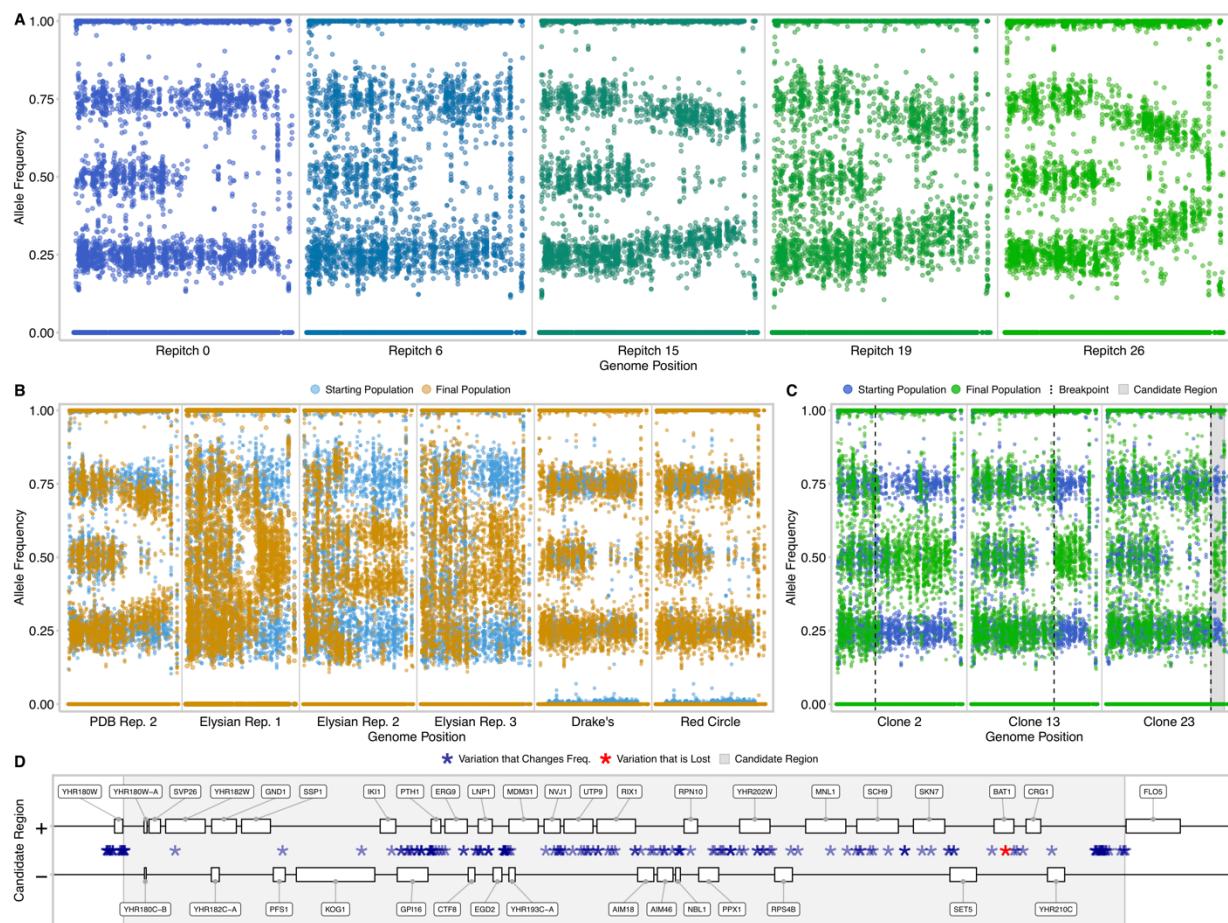
391 **Possible driver genes on chromosome VIII**

392 Wanting to discover the basis of the selective benefit for the chromosome VIII mitotic
393 recombination events, we also investigated whether any variation was eliminated as a result of
394 this allele frequency change. We compared several clones from the first Postdoc Brewing Co.
395 repitch experiment to identify the smallest candidate region in which the allele frequency change
396 occurred, we then filtered for positions inside of the region where variation is lost (Fig 4C). As the
397 SacCer3 reference genome does not capture the genome structure at the end of chromosome
398 VIII and breaks down at the *FLO5* gene (see below), our analysis of lost variation spanned
399 *YHR180W* to *FLO5* (Fig 4D). There was a single nonsynonymous mutation on one haplotype that
400 was eliminated in every clone bearing a known allele frequency change and, notably, two clones
401 without an allele frequency change (PDB26, clones 7 and 12). The mutation (an alanine-to-
402 asparagine substitution) was found at position 234 in the gene *BAT1*, which encodes a
403 mitochondrial branched-chain amino acid (BCAA) aminotransferase Bat1 that is critical in the
404 metabolism of BCAA (valine, leucine, and isoleucine). Due to the importance of Bat1 for BCAA
405 metabolism even beyond the context of this study, we analyzed the function of the A234D variant
406 in a companion study (Jirasin Koonthongkaew et al., submitted [36]). Briefly, we discovered that
407 in an otherwise isogenic background, the *BAT1* variant (*BAT1*^{A234D}) leads to the same phenotype
408 as a null allele in *BAT1*. Specifically, we found that both the null allele and the *BAT1*^{A234D} allele
409 caused a growth defect in minimal medium, reduced levels of intracellular valine and leucine
410 during the logarithmic and stationary phases, respectively, and produced more fusel alcohols.

411

412 Interestingly, we found that in the Elysian Brewing Co. experiments, *BAT1* did not contain
413 the A234D allele, but the allele frequency change on chromosome VIII still occurred. Investigating
414 these populations, we found no additional variation that was lost as a result of the mitotic
415 recombination, leading us to suspect that there was additional gene content at the end of
416 chromosome VIII that could be further driving the benefit of the mitotic recombination. However,
417 the level of structural divergence between the SacCer3 reference genome and the beer strain
418 was too great to be bridged using short read sequencing, especially due to the repetitive and
419 paralogous nature of the flocculin gene. As there are no currently available long-read sequencing
420 data for the American brewing strains, we generated our own using clones isolated from the first
421 Postdoc Brewing Co. experiment.

422



423

424 **Fig 4. Mitotic recombination events spanning the same region recurrently mutated across**
425 **multiple populations (A) Allele frequency plots of serially-repitched populations from the first**
426 **Postdoc Brewing Co. replicate showing an allele frequency change, appearing at the 15th repitch**
427 **and reaching a 43.8% frequency in the population by the 26th repitch. (B) Replicate populations**
428 **from Postdoc Brewing Co., Elysian Brewing Co., Drake's Brewing Co., and Red Circle Brewing**
429 **Co. showing the chromosome VIII allele frequency change. When the sample from Drake's and**
430 **Red Circle were compared to their ancestors, WLP001 and Cali Ale, it was found that the allele**
431 **frequency change had previously occurred and is fixed in the strain. (C) Chromosome VIII of three**
432 **representative clones from the 26th repitch from the first Postdoc Brewing Co. experiment**
433 **showing the different breakpoints of the mitotic recombination event. The region used to detect**
434 **lost variation is highlighted in grey. (D) The minimal region, as detected from clone 23 is displayed**

435 with all known ORFs and variation that is either lost or changes frequency as a result of the mitotic
436 recombination.

437

438 **Oxford Nanopore Technology (ONT) based long-read analysis**

439 Using a MinION sequencer, we generated ONT reads from a clone isolated from the first
440 time point and three clones from the last time point (each with an allele frequency change). From
441 these reads we generated individual assemblies from each of the sequencing runs and polished
442 them for quality using both the ONT and Illumina reads (see methods). We found that multiple
443 larger scale genome rearrangements had occurred at the telomeres of the beer strains. In
444 particular, the right end of chromosome VIII had two separate rearrangements versus the
445 SacCer3 reference that had occurred sometime in the ale brewing yeast past, one matching the
446 left arm of chromosome I and the other the left end of chromosome IX. Furthermore we found that
447 the sequence found at the left end of chromosome I had also transferred to the right arm of
448 chromosome I. Based on previous literature we suspect that these two intragenomic
449 recombination events have been referred to as Lg-*Flo1* (chimera between *FLO5* and *YAL065C*
450 originally discovered in lager yeast) and *ILF1* (chimera between *FLO5* and *YIL169C*) [37]. Beyond
451 the chimeric flocculins, we also discovered that additional gene content, extending to the
452 telomere, was transferred. From the chromosome IX segment, *HXT12*, *IMA3*, *VTH1*, and *PAU14*
453 were duplicated to chromosome VIII. From the chromosome I segment, *SEO1* and *PAU8* were
454 duplicated.

455

456 Using alignments of the ONT reads back to polished assemblies, we established what
457 variation and haplotypes were attached to which telomeric ends. Specifically, we found that the
458 chromosome encoding the *BAT1*^{A234D} allele is connected to the fragment from chromosome IX.
459 Additionally, the minor haplotype is connected to the fragment from chromosome I, while the
460 remaining two chromosomes are connected to the content from chromosome IX. While these

461 observations were confirmed using ONT reads from clone isolates from the Postdoc Brewing Co.
462 experiment, we have further found that the copy number of the chromosome I fragment containing
463 *SEO1* increases in both Postdoc Brewing Co. populations and the three Elysian Brewing Co.
464 populations by the final timepoint, meaning that the copy number of *Lg-FLO1* and *SEO1* likely
465 both increased in all populations that experienced a chromosome VIII mitotic recombination
466 (Supp. Fig 6).

467

468 **Flocculation**

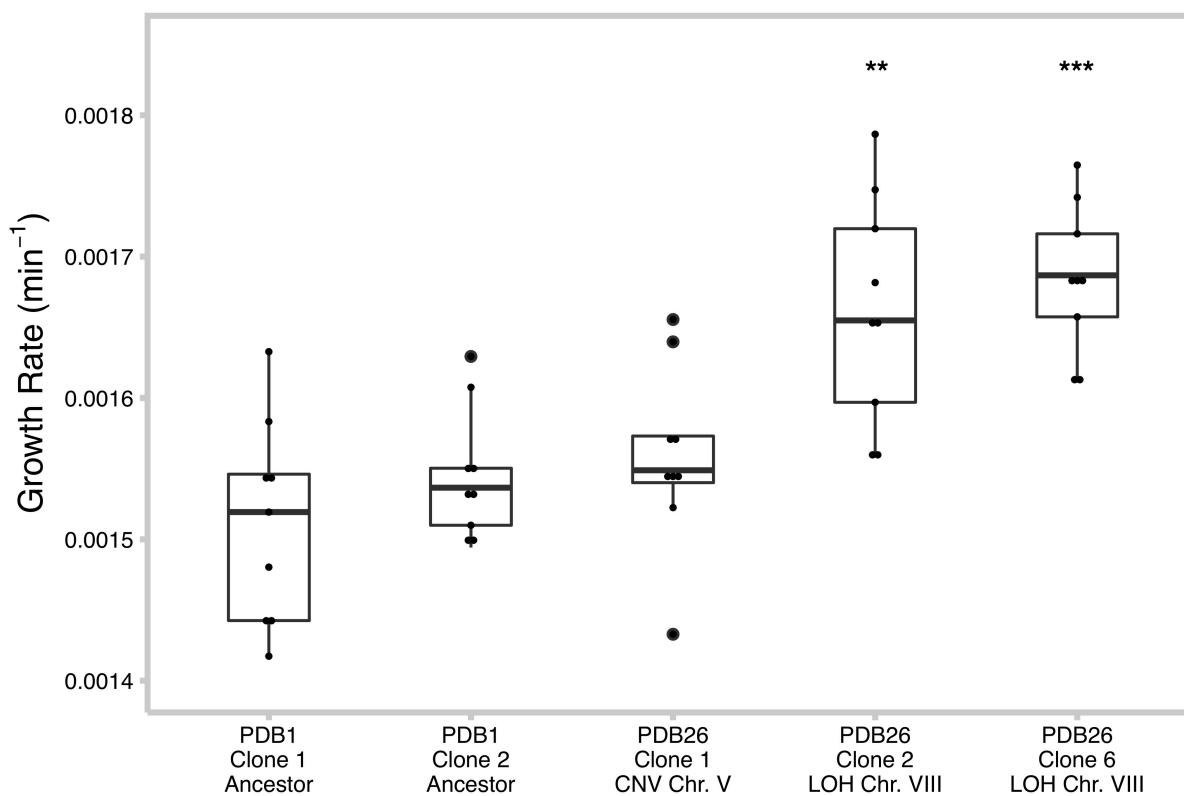
469 As there was a change in the copy number of *Lg-FLO1*, we tested whether there were any
470 changes in flocculation rate of clones isolated from the first versus the last time point of the
471 Postdoc Brewing Co. first replicate experiment. We found that there are no substantial shifts
472 between clones bearing the chromosome VIII allele frequency change and the clones that do not
473 (Supp. Fig 7). However, because the experiments were conducted in small scale laboratory
474 conditions in non-optimal media conditions to test for flocculation of beer brewing strains, more
475 experimentation is required to conclusively eliminate the possibility that there are differences in
476 flocculation speed or strength between the clones.

477

478 **Growth Phenotypes**

479 Given the potential of an evolutionary benefit of the allele frequency change on
480 chromosome VIII and the aneuploidy of chromosome V, we tested for any growth changes in
481 brewers wort of clones bearing these mutations. Fitting growth curves of these yeasts with a linear
482 model on the period of exponential growth, we analyzed whether there were any changes in the
483 growth rate or lag time of the clones using a Kruskal-Wallis statistical test. Finding a difference in
484 the growth rates (p -value = 3.718×10^{-5}), we further probed for differences between clones using
485 a Mann-Whitney U test. Consequentially, we found that the growth rate of the two clones with the

486 chromosome VIII allele frequency change had a significantly increased growth rate versus the
487 two clones isolated from the first timepoint (Fig 5).
488



489
490 **Fig 5. Clones bearing a Chr. VIII mitotic recombination grew significantly faster in brewers
491 wort.** Growth rates of clones isolated from the first Postdoc Brewing replicate were measured
492 over 24 hours of growth in brewers wort using a plate reader and compared using a Kruskal-
493 Wallis test (p -value = 3.718×10^{-5}). Comparisons using a Mann-Whitney U test revealed
494 significant differences between clones bearing the mitotic recombination on chromosome VIII (**
495 p -value < 0.001; *** p -value < 0.0001).
496

497 **Changes in Sensory Profiles**

498 More than anything, the phenotype that is most important for brewers is the taste of their
499 beer. To assay for any changes in flavor, we brewed beer with the ancestral strain and two of the
500 clones described above, and measured both the molecular profile of the beer and the sensory
501 profile with a crowd-sourced panel from attendees at a Homebrewing Convention. Among the two
502 fermentation replicates that we tested with chemical profiling, we found that there was an
503 increased quantity of isobutanol isoamyl acetate and amyl alcohols in the clone lacking the
504 *BAT1*^{A234D} allele (Table 3). Notably, our prior investigation of the A234D allele in a laboratory
505 strain background conclusively found this same pattern, potentially indicating *BAT1* as the driver
506 of the metabolite differences (Jirasin Koonthongkaew et al., submitted [36]).

507

508 Additionally, we observed from the specific gravity measurements that the fermentations
509 with the clone that experienced the mitotic recombination on chromosome VIII (PDB26 clone 6)
510 potentially did not go to completion when compared to the fermentations from the clones
511 containing the *BAT1*^{A234D} allele (PDB1 clone 1, and PDB26 clone 1). While we don't know if this
512 is linked to this specific allele or another mutation that the clone has, this feature overwhelmed
513 the sensory panel, who found the beer to be different in both its maltiness and sweetness (Supp.
514 Table 6). As well, we found an increase in the production of diacetyl, total and otherwise, but we
515 do not know definitively whether this was because of reduced ability to clean up the fermented
516 product due to a fermentation delay. Further replicates and additional testing of clones genetically
517 manipulated to alter the *BAT1* allele identity are warranted to conclusively test the impact of the
518 chromosome VIII allele frequency change, with and without the *BAT1*^{A234D} allele, on beer
519 characteristics.

520

521 **Table 3. Sensory analysis of beers brewed with clones from first Postdoc Brewing replicate**

Sample Name	BAT1 Status	Ferment	Alcohol (%v/v)	Specific Gravity	Alcohol (%w/w)	pH Value	Acetaldehyde (ppm)	1-Propanol (ppm)	Isobutanol (ppm)	Amyl Alcohols (ppm)	Iso-Amyl Acetate (ppm)	Diacetyl (ppb)	Total Diacetyl (ppb)	2,3-Pentanedione (ppb)	Total 2,3-Pentanedione (ppb)
PDB1 Clone 1	A234D	1	5.1	1.007552	4	4.54	5.89	28.78	18.12	66.29	0.42	32.35	44.32	<10	<10
PDB26 Clone 6	+	1	4.72	1.010501	3.69	4.65	6.64	17.82	8.26	39.82	0.28	74.83	116.86	11.96	18.83
PDB26 Clone 1	A234D	1	5.1	1.007233	4	4.63	3.55	24.77	15.77	63.9	0.89	24.06	49.47	<10	<10
PDB1 Clone 1	A234D	2	4.56	1.015936	3.55	4.12	3.03	24.8	43.28	93.42	0.18	73.55	89.61	<10	<10
PDB26 Clone 6	+	2	4.5	1.016369	3.5	3.96	3.84	23.28	29.6	75.36	0.06	81.78	121.92	<10	10.12
Wort Control	N/A	2	0	1.050506	0	4.41	0.65	5.28	0	0	0	37.83	41.66	<10	<10

522

523

524 **Discussion**

525 Using whole-genome sequencing on yeast serially repitched across four breweries, seven
526 populations, and three different strains, we observed the repeated occurrence of convergent
527 mutations rising to high frequency in the populations. Notably, we observed multiple types of
528 structural variation impacting chromosomes V, VIII, XII, and XV across multiple replicates.
529 Through subsequent phenotyping of clones bearing some of these mutations, we have found a
530 growth rate benefit in strains carrying the mitotic recombination event on chromosome VIII when
531 grown in brewers wort. From these data we have concluded that these mutations are likely
532 beneficial and selected for in the brewery, indicating that despite centuries of growth in the
533 brewery, ale yeasts continue to show signatures of new adaptations.

534

535 Given the few number of convergent mutations, we sought to determine the driving force
536 behind the potential adaptive benefit of the chromosome V copy number change and the
537 chromosome VIII mitotic recombination. First, from clone and population sequencing, we were
538 able to determine that the copy number change was not haplotype dependent, meaning that the
539 benefit likely originated from a dosage balance with the rest of the genome. Second, we found
540 that as a result of the mitotic recombination on chromosome VIII a point mutation in *BAT1* in the

541 Postdoc Brewing Co. populations was recurrently lost. Creating the *BAT1*^{A234D} allele in a lab strain
542 and comparing to an isogenic wild-type, we discovered that the mutation led to a number of
543 phenotypes including a sensitivity to osmotic stress, reduced fermentation ability when grown in
544 20% glucose, and a growth defect in minimal media (Jirasin Koonthongkaew et al., submitted
545 [36]). We similarly found that the ale strain clones bearing the *BAT1*^{A234D} allele have a growth
546 defect, indicating its likely influence on the brewing yeast growth and the origin of at least part of
547 the mitotic recombination benefit. However, the populations from Elysian Brewing Co. did not
548 have the *BAT1*^{A234D} allele, indicating there is likely to be additional adaptive consequences from
549 the mitotic recombination event. Therefore, we applied long-read sequencing and *de novo*
550 assembly on the clones from the first Postdoc Brewing Co. replicate to resolve the structure of
551 the telomeric regions. As a result we found that the mitotic recombination led to a copy number
552 increase of *Lg-FLO1* and *SEO1* from the minor haplotype and reduction of *ILF1*, *HXT12*, *IMA3*,
553 *VTH1*, and *PAU14* from the major haplotype. Given the alteration in copy number of multiple
554 flocculation associated genes, we tested several clones with and without the mitotic
555 recombination and found no strong difference in the rate that they settled. Considering the lack of
556 an obvious flocculation difference, we suspect that either our methods to measure flocculation
557 were not sufficient to detect a significant change or that the benefit was derived from either the
558 loss of the major haplotype gene copies, or the gain of *SEO1*, whose function is presumed to be
559 related to nitrogen uptake [38]. Notably, there are six copies of *SEO1* in the genome; however,
560 this might be coincidental due to its linkage to flocculin-associated gene sequences.

561

562 Considering the long history of beer brewing, one might presume that the yeast
563 specialized in malt fermentation would already be pre-adapted to the brewery environment.
564 Especially given that repitching is not a new phenomenon with Louis Pasteur commenting in 1876
565 on the practice of passaging yeast within and between breweries:

566

567 [T]he wort is never left to ferment spontaneously, the fermentation being invariably
568 produced by the addition of yeast formed on the spot in a preceding operation, or procured
569 from some other working brewery, which, again, had at some time been supplied from a
570 third brewery, which itself had derived it from another, and so on, as far back as the oldest
571 brewery that can be imagined. ... [T]he interchange of yeasts amongst breweries is a time-
572 honoured custom, which has been observed in all countries at all periods, as far back as
573 we can trace the history of brewing. ([39], p. 186)

574

575 However, within our experiments we see multiple mutations overtaking the population in
576 a relatively short period of time. The most parsimonious answer is that the brewing process has
577 somehow changed in a way that creates new selective pressures, allowing for novel, highly
578 beneficial mutations to evolve. One possibility is that the breweries which we partnered with utilize
579 styles that the 'Chico' yeasts had not been extensively exposed to. Specifically, in the United
580 States, styles of beer that are high in both final alcohol and hop content have become popular
581 and are utilized extensively by Postdoc Brewing Co., Drake's Brewing Co. and Elysian Brewing
582 Co. Seeing as high hop and alcohol content can be stressors, we hypothesize that this could be
583 one of the contributors to new adaptations. Further experimentation using defined media, varied
584 in both hop and sugar content, will be able to test this hypothesis. Another possibility is that
585 industry has shifted in the last several decades towards the use of pure clonal strains and
586 propagation companies for yeast maintenance versus keeping yeast at scale in the brewery
587 constantly through continual reuse. Often, to create a stock of a brewing strain the population is
588 bottlenecked down to a very small size, making a single representation of the population. Through
589 this process it is likely that mutations that aren't representative of the population and are
590 potentially deleterious become fixed. Further exacerbating the problem, this process repeats
591 every time a new propagation company creates their own version of a strain. When the strain is

592 then grown to a massive population size in stressful brewing conditions, there is then selection
593 for *de novo* reversions of the deleterious mutations. Within our brewing experiments, we have
594 seen two examples that potentially fit this explanation. First, the copy number of chromosome V
595 returned to a euploid copy number, which was the hypothesized ancestral state. Second, the
596 nonsynonymous mutation in *BAT1* was reverted by mitotic recombination. While both of these
597 hypotheses would potentially explain the source of new adaptations, further work is required to
598 rigorously test their veracity.

599

600 Another interesting complication for creating a single strain representation of a population
601 was the occurrence of multiple lineages in the Postdoc Brewing Co. experiments that had the
602 same or similar mutations within the population. This phenomenon, called clonal interference,
603 occurs when a new beneficial mutation is unable to completely overtake the population before
604 another beneficial mutation occurs. This creates competition between the new and old beneficial
605 mutation, preventing a single lineage from taking over the population. Typically the parameters
606 that are thought to control the degree of clonal interference during adaptive evolution are the
607 mutation rate of beneficial mutations, the selective benefit of those mutations, and the population
608 size [40]. As mentioned above, breweries have an immense population size, creating an ideal
609 environment for clonal interference. However, it is unclear why in the non-Postdoc Brewing Co.
610 populations we did not see the same degree of clonal interference. We suspect that the euploid
611 nature of chromosome V of BRY-96 and the preexisting mitotic recombination in WLP001 may
612 have allowed for a different population dynamic that led to a single lineage dominating the
613 population. The other possibility is that the beneficial mutations observed in the non-Postdoc
614 Brewing Co. populations occurred earlier in their outgrowth, leading to a single lineage dominating
615 the population.

616

617 Matching with this hypothesis, we found that on multiple instances the yeast entering the
618 brewery already had undergone some amount of genetic divergence from the stock's genotype.
619 This is likely due to the number of generations required for a stock of yeast to be grown to a
620 population size needed by professional brewers. For example, given a 20 hectoliter batch of wort,
621 the recommendation by White Labs, a prominent propagation company, is to add 2.4×10^{13} cells
622 of yeast. The absolute minimum number of generations required to reach this number of yeast
623 from a single cell, assuming a doubling per generation with no death, is 44.4 generations of yeast
624 growth, which is almost certainly an underestimate. The number of generations occurring in the
625 brewery, assuming 3 generations per beer fermentation, is 45 generations for 15 serial repitches
626 and 78 for 26 serial repitches, meaning the growth period at the propagation company constitutes
627 anywhere from half to a quarter of the yeasts growth in our experiments. As well, because
628 mutations enter the population at a proportion of one over the total population size, beneficial
629 mutations that occur earlier in the outgrowth have a higher probability of reaching a high
630 frequency. Given the number of yeast cells needed by a brewer, it is likely inevitable that some
631 amount of detectable evolution will occur prior to a pitch even entering the brewery.

632

633 Typically, the professional brewer wants to know how long they can reuse their yeast
634 before they will start to notice considerable difference in the characteristics of the yeast or beer.
635 Perhaps the most accurate but somewhat unsatisfying response is that it depends on a number
636 of factors. Specifically, the timing might be different given the spectrum of adaptive mutations that
637 a particular strain has access to, the individual mutation rate of that isolate, and the number of
638 generations that the population was grown at the propagation company. Even given replicates
639 using the same strain, there is an element of stochastic mutation that can potentially drastically
640 change how a brewery population evolves. Looking to the future and at methods to have serially
641 repitched populations with fewer impactful mutations may begin with sequencing more
642 populations and finding isolates that are better preadapted to the modern brewery. However, this

643 strategy assumes these mutations do not have undesirable tradeoffs on other aspects of
644 performance, such as on flavor profile. As well, if the possible spectrum of adaptive mutations is
645 determined for a given strain it may be possible for a commercial service to track the frequency
646 of these mutations over time and identify when they start impacting the beer. We note that due to
647 the variability in beer styles employed during most of the time courses analyzed here, we were
648 unable to rigorously track changes in fermentation characteristics and/or beer quality that may
649 have happened in tandem with the rise of these mutations.

650

651 In conclusion, we observed multiple independent brewing yeast populations with high-
652 frequency structural mutations that likely contributed to a change in growth characteristics.
653 Discovering the likely adaptive benefit of mitotic recombination events in the brewery raises the
654 possibility that historical ale brewing yeast adaptation was due in part to these kinds of structural
655 mutations. Notably, the ale yeasts are thought to have originated from an admixture event which
656 introduced intragenomic variation into the ancestor of the modern brewing strains [8]. Potentially,
657 ale yeasts have the capacity to adapt to new conditions using mitotic recombination on existing
658 variation to eliminate or fix deleterious or adaptive alleles respectively. Such events have been
659 observed within the lager brewing yeasts wherein similar patterns of structural variation have been
660 linked to phenotypic outcomes [41,42]. Furthermore, multiple mitotic recombination events were
661 shown to lead to changes in both sugar utilization and flocculation when *de novo* hybrids
662 between *S. cerevisiae* and *S. eubayanus* were evolved in simulated brewing conditions [20].
663 Given the prevalence of structural mutations in the history of the genome of brewing yeasts, and
664 their link to adaptive phenotypic outcomes, further investigation into the consequences of this
665 variation will likely provide additional insights into their domestication.

666

667 **Materials and Methods**

668 **Evolution in the Brewery**

669 Depending on the brewery, yeast cells were ordered from Wyeast, Imperial Yeast, White
670 Labs, Escarpment Laboratories, or an internal propagation service (in the case of Elysian Brewing
671 Co.). For some of the experiments, starting samples were collected either from the shipment or
672 from the first beer brewed with the yeast. Otherwise these yeast cells were commonly grown for
673 several generations in low density wort, then transferred into a cycle of several ale beer recipes
674 ranging from barley wine to double IPAs. The precise recipe and conditions are proprietary for
675 some of the breweries, however Postdoc Brewing Co. has provided the style in which the yeast
676 were passaged through (Supp. Table 1). For the Postdoc Brewing Co. samples, they were
677 collected from the middle of the flocculated yeast after the runnings of hop and protein particulate
678 was disposed of. Once samples were collected, they were stored at 4°C in a sterile, airtight
679 container until transfer to the laboratory was possible. Upon arrival in the laboratory, the samples
680 were thoroughly mixed and 1 mL was transferred to a 25% glycerol stock that was subsequently
681 frozen at -70°C.

682

683 **Short-read genome sequencing**

684 Populations of yeast cells, previously stored in 25% glycerol at -70°C were transferred to
685 deionized water (diH₂O) and measured for cell density using a hemocytometer. Based on cell
686 density counts in the diH₂O, the cell suspensions were diluted and plated to collect approximately
687 1,000 independent yeast colonies, grown for 4 days on yeast extract peptone dextrose (YE PD)
688 plates with 2% glucose and 1.7% agar at room temperature. These plates were scraped for cells
689 with a sterile glass rod, concentrated by centrifugation, and washed in diH₂O. DNA was then
690 extracted from the cell pellets using a modified Hoffman-Winston preparation [43].

691

692 Single clone isolates were generated from a population glycerol stock. In short, the
693 brewery populations were streaked onto a YEPD plate and grown at room temperature. A single
694 colony was isolated and grown overnight in 5 mL of YEPD liquid medium with rotation. A portion
695 of the overnight culture was stored in a 25% glycerol stock for archiving and subsequent analysis.
696 The remaining cells were concentrated, washed with diH₂O and had their DNA extracted with a
697 modified Hoffman-Winston preparation [43]. Clones 13 through 23 were selected for sequencing
698 based on their likelihood for bearing a chromosome VIII allele frequency change from genotyping
699 using PCR and Sanger sequencing for a SNP frequency within a variable region on the end of
700 the chromosome.

701

702 After measuring the concentration of DNA using a Qubit Fluorometer (Thermo Fisher
703 Scientific), dual-indexed Illumina libraries were generated using a Nextera sample preparation kit
704 (Illumina, Inc.) with 50 ng of input DNA. The genomic libraries were sequenced using 150-bp
705 paired end sequencing on an Illumina NextSeq 500 using the manufacturer's recommended
706 protocols.

707

708 **Whole genome analysis**

709 The Illumina reads were demultiplexed using bcl2fastq with default parameters. The reads
710 were then aligned to the SacCer3 reference genome (R64-2-1) using BWA-mem (version 0.7.15)
711 [44]. The alignments, after being sorted and indexed with SAMtools [45] (version 1.9) were
712 marked for duplicates using Picard Tools (version 2.6.0). When libraries were sequenced on
713 multiple lanes or runs, the alignments were combined using SAMtools. Afterwards, the alignments
714 had their InDels realigned using GATK (version 3.7).

715

716 Short mutations such as SNPs and InDels were then called using three separate variant
717 calling software packages. First, BCFtools call using modified input parameters was used.

718 Second, FreeBayes (version 1.0.2-6-g3ce827d) [46] using input parameters (–pooled-discrete –
719 pooled-continuous –report-genotype-likelihood-max –allelebalance-priors-off –min-alternate-
720 fraction 0.1) were used to call both SNPs and InDels. Finally, in a paired mode with the sample's
721 ancestor, LoFreq was used to call SNPs [47]. For all of the variant callers, BEDtools was used to
722 filter the variants called for a sample versus its ancestor [48]. Each variant file was subsequently
723 filtered using standard parameters that are listed in Supp. Table 7. The three variant files were
724 then filtered to exclude overlaps of the same variant and combined into one file using a custom
725 script. Afterwards, the annotation and impact of the variants were determined using a script
726 previously published in [49]. Finally, each variant that passed all filters was manually checked for
727 its authenticity in the Integrative Genomics Viewer (IGV) [50]. When variant calls from BCFtools
728 call exceeded 300 variants, these files were ignored, as they were found to contain primarily false-
729 positives through manual inspection and comparisons with other software.

730

731 As noted earlier, there are multiple haplotypes containing varying degrees of shared
732 variation between homologous chromosomes. To quantify and observe the degree that this
733 variation has been altered through mitotic recombination, allele frequency was calculated and
734 plotted for all genomic coordinates. Briefly, from the previously generated alignments, variant calls
735 were generated using the GATK (version 3.7) HaplotypeCaller. These variant calls were passed
736 to GATK VariantToTable and modified using an in-house java script into a per base allele ratio
737 between a reference and alternate allele. Subsequently, the allele frequency was plotted using
738 an R script with ggplot2. Changes in the ratios between haplotypes were visually determined
739 through inspection of these plots. Precise values on the proportion of the allele frequency change
740 of chromosome VIII in the population were generated using an average of the change in allele
741 frequency of a set of SNPs that were highly representative of the mitotic recombination events in
742 the clones at the end of chromosome VIII. These values were then used to calculate the selective
743 benefit of the chromosome VIII allele frequency.

744

745 Using the alignments listed above, the copy number of the genome was determined and
746 plotted using an in-house script. Briefly, the total genome coverage was calculated using GATK
747 (version 2.6.5) DepthOfCoverage. Next, the per window average coverage across the genome
748 was calculated using the command-line tools version of IGVtools. These files were combined
749 using a python script to generate a normalized coverage measure. As many of the samples
750 included a ‘wavy’ coverage in which the coverage varied across the genome in an inconsistent
751 and seemingly random pattern, the per ORF coverage was unable to be accurately determined.
752 In the cases that the coverage was too ‘wavy’ to accurately determine the coverage, the allele
753 frequency plots that are described above were used to determine the copy number as the per
754 allele coverage remained unchanged by the ‘wavy’ sequencing artifact.

755

756 **Phylogenomic analysis**

757 In order to properly understand the diversity and previous evolutionary history of the
758 American brewing strains, all publicly available brewing strains whole genome sequencing were
759 processed into a phylogenetic representation. Capturing the most amount of American diversity
760 possible, some strains that had not previously been sequenced, but suspected to be part of the
761 American yeast group (due to tips from professional and amateur brewers) were ordered and
762 kindly donated from a variety of yeast propagation companies. As described above, the strains
763 had their DNA extracted and sequenced using the paired-end Illumina technology. All of the
764 sequencing reads were aligned using a similar strategy as previously described above with slight
765 modifications, and called for variants in the GVCF mode using GATK (version 4.1.1.0)
766 HaplotypeCaller on regions of high confidence (excluding the first and last 50 kb of each
767 chromosome to avoid poorly assembled telomeric sequences). Individual variant calls were
768 collected and jointly genotyped using GATK GenomicsDBImport and GenotypeGVCFs and
769 filtered with GATK (version 4.1.3.0). Removing the influence of ancestral variation lost by mitotic

770 recombination, the SNPs from the sample excluding BE051 were then filtered by SNPs called
771 from BE051 using BEDtools [48]. The SNP calls were then converted into two concatenated fasta
772 files wherein the first fasta was the SacCer3 reference genome with the reference allele as listed
773 if the strain was either heterozygous or homozygous for the reference allele. The second fasta
774 also contained the SacCer3 allele unless a heterozygous or homozygous variant position was
775 detected in which case the alternate allele was outputted. This task was done using BCFtools.
776 The concatenated fastas from all of the American brewing strains were passed to IQTree2 to
777 generate a maximum-likelihood tree using GTR₄ + gamma model [51]. The tree was then modified
778 for aesthetics and annotation using iTOL [52].

779

780 Comparisons between the 'Chico' yeasts and BRY-96 for determination of the ancestry of
781 the 'Chico' yeasts was done using the aforementioned SNP calls. First, the union of the SNPs
782 called in WLP001 and Wyeast 1056 was generated using BEDtools. Second, the mutations
783 unique to BRY-96 when compared with that union were generated. Finally, the remaining SNPs
784 from BRY-96 were manually inspected for veracity using IGV.

785

786 **Flocculation**

787 The rate of flocculation was quantitatively measured similar to previously reported [53].
788 Briefly, yeast of the appropriate genotype, plated on a 2% YPD plate were grown from a single
789 colony in 5 mL of 2% YPD liquid medium for 72 hours at 30°C with rotation. The yeast cultures
790 were then vortexed for a minimum of 5 seconds to ensure complete resuspension. Photographs
791 were then taken of the yeast after 60 minutes while the culture tubes remained undisturbed.
792 Afterwards, using a semi-automated script written for ImageJ [54], the images were converted to
793 black and white, and the plot profiles of the culture tube's grey intensity were collected from the
794 bottom of the tube to the meniscus. Next, to determine the degree of settling in an unbiased
795 manner, an automated script written in python was used to find the point in the culture that the

796 intensity reached half of the maximum grey value. The point at which the yeast had flocculated to
797 in the culture tube after 60 minutes was used to create a ratio based on the total length of the
798 culture. Three measurements were taken per culture and the average of these measurements
799 was reported. Two biological replicates were conducted from independent colonies.

800

801 **Brewers wort media**

802 The brewers wort media, utilized for the growth phenotyping and fermentation analysis
803 was made as previously mentioned in [55] with slight modification. Briefly, 320 grams of amber
804 liquid malt extract from Breiss Malt and Ingredients Co were mixed with 1.5 liters of distilled water
805 and boiled for an hour. Fifteen minutes before the boil finished, 0.2 gram of the Wyeast Beer
806 Nutrient Blend was added to the mixture according to the manufacturer's guidelines. After the wort
807 had been chilled to a workable temperature, the specific gravity was measured using a
808 hydrometer (and the value read was corrected based on the temperature), and the media was
809 passed through fresh Melitta filters to remove any large coagulants. Next, the media was passed
810 through a 0.45 micron filter (Nalgene 500mL Rapid-Flow Bottle Top Filters) to completely sterilize
811 the media. The specific gravity of all batches used herein were found to be the same value of
812 1.050.

813

814 **Growth phenotypes**

815 The growth characteristic of clones isolated from the first replicate population from
816 Postdoc Brewing Co. was measured. First, single yeast colonies from a 2% YPD plate were grown
817 in wort medium for 48 hours with rotation. Next the optical density of the cultures was measured
818 at 600 nm (OD600). Each culture was then diluted in an appropriate amount of wort to reach a
819 final OD600 of 0.1. The back-diluted cultures were further transferred to a 96-well plate at a
820 volume of 200 microliters per well. Using a Biotek Synergy H1 plate reader, the OD600 of the 96-
821 well plate was measured every 15 minutes for 24-48 hours while shaking in a double orbital

822 pattern at room temperature. Utilizing a script written in the R programming language, the growth
823 data from the plate reader were analyzed using the growthrates package. Employing the
824 growthrates implementation of fitting linear models to the exponential growth period outlined in
825 [56], we extracted the maximum growth rate of the clones and the length of the lag growth period.
826 To determine whether there was a difference in the growth rates between clones, we first
827 conducted a Kruskal Wallis rank sum test. Further testing of differences between clone growth
828 rates was done using Mann-Whitney tests.

829

830 **Sensory profiling**

831 The isolated clones from the first Postdoc Brewing replicate were tested for differences in
832 the production of flavor compounds and the effect these compounds had on the beer sensory
833 profile. First, two separate beer batches (beer batches 1 and 2) were generated from
834 fermentations carried out either at Postdoc Brewing (using an all grain pale ale recipe) or in the
835 laboratory (using the malt extract wort mentioned earlier). The yeast that fermented the beer were
836 grown in the laboratory from single colonies to the desired cell count in 2% YEPD liquid medium
837 with shaking. For the first and second beer batch, the yeast were concentrated with centrifugation
838 and pitched into the wort at a rate of 3.5×10^5 and 1.0×10^6 cells per degree of plato respectively.

839

840 Second, both beer batches were submitted to White Laboratories for analytical services
841 including gas chromatography measurements of a number of flavor compounds and
842 measurements of alcohol percentage and specific gravity. Next, the beers from batch 1 were
843 submitted to an untrained judging panel (n=95) at the Homebrew Con 2018, who used a
844 standardized beer scoresheet from the Beer Judge Certification Program to analyze the profile of
845 the beer. The identity of the beers were kept masked from the participants while they filled out
846 their analysis. Afterwards, the scoresheets were aggregated and analyzed for differences using
847 a Kruskal Wallis rank sum statistical test.

848

849 **Oxford nanopore sequencing**

850 Yeast cell cultures were grown overnight at 30°C in 20 mL of YPD medium to early
851 stationary phase before cells were harvested by centrifugation. Total genomic DNA was then
852 extracted using the QIAGEN Genomic-tip 100/G according to the manufacturer's instructions. The
853 extracted DNA was barcoded using the EXP-NBD104 native barcoding kit (Oxford Nanopore
854 Technologies) and the concentration of the barcoded DNA was measured with a Qubit 1.0
855 fluorometer (Thermo Fisher Scientific). The barcoded DNA samples were pooled with an equal
856 concentration for each strain. Using the SQK-LSK109 ligation sequencing kit (Oxford Nanopore
857 Technologies), the adapters were ligated on the barcoded DNA. Finally, the sequencing mix was
858 added to the R9.3 flowcell for a 48 hour run.

859

860 **Assembly generation and polishing**

861 The ONT reads were demultiplexed using Guppy with default parameters. The adapters
862 on the raw reads were removed using Porechop. Afterwards, each sample was independently run
863 through SMARTdenovo with default parameters to generate a draft genome assembly. To
864 improve the quality of the assembly, the draft sequences were first run through racon then
865 medaka. Next they were refined using pilon and the Illumina reads previously generated for the
866 four clones sequenced on the MinION. The identity of the contigs was determined through
867 pairwise alignment of the contigs (masked with RepeatMasker) to the SacCer3 reference genome
868 using Minimap2 and plotted using an R package called DotPlotly. Confirmation of the contig
869 identity, and the inferred identity of the ancestor was done using a combination of Minimap2
870 alignments of the SacCer3 reference ORFs, SacCer3 reference sequence, ONT reads, and
871 Illumina reads, all visualized in IGV.

872

873 **Data availability**

874 All whole-genome sequencing data was uploaded to <https://www.ncbi.nlm.nih.gov/sra> with the

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876

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896

897 **Competing Interests**

898 We declare a financial interest in the success of the breweries associated with the authors of this
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901

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1072

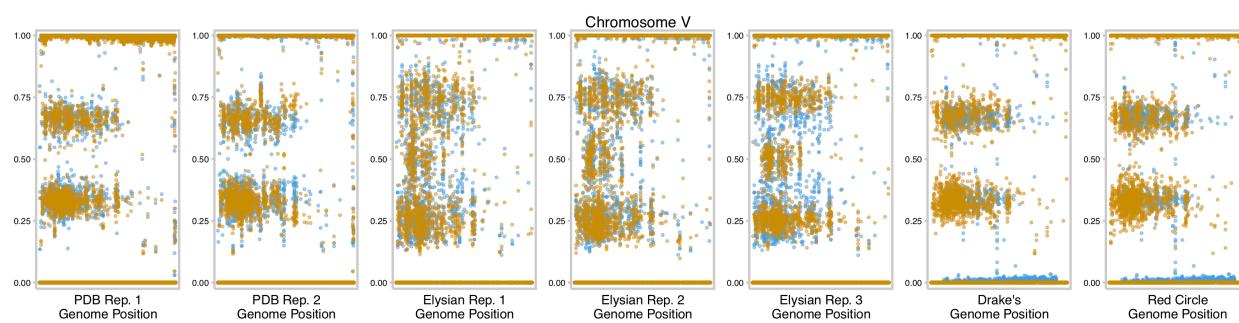
1073 **Supplementary Figures**

		PDB1		PDB26														
		Clone 1	Clone 2	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6	Clone 7	Clone 9	Clone 10	Clone 11	Clone 12	Clone 13	Clone 20	Clone 23	
PDB26	Clone 1	10	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	
	Clone 2	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Clone 1	0	0	20	1	0	0	0	0	0	1	0	0	0	0	0	0	
	Clone 2	0	0	1	10	0	0	0	0	0	1	0	0	0	0	0	1	
	Clone 3	1	0	0	0	14	0	0	1	0	0	0	0	0	0	0	0	
	Clone 4	0	0	0	0	0	6	0	0	0	0	0	0	0	1	0	0	
	Clone 5	0	0	0	0	0	0	13	0	1	0	0	0	0	0	0	0	
	Clone 6	1	0	0	0	1	0	0	8	0	0	0	0	0	0	0	0	
	Clone 7	0	0	0	0	0	0	1	0	8	0	0	0	0	0	0	0	
	Clone 9	0	0	1	1	0	0	0	0	0	18	0	0	0	1	2	1	
	Clone 10	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	
	Clone 11	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	
	Clone 12	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	
	Clone 13	0	0	0	0	0	1	0	0	0	1	0	0	0	12	0	0	
	Clone 20	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	26	
	Clone 23	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	15	

1074

1075 **Supplementary Fig 1.** The number of mutations shared between clones isolated from the first
 1076 Postdoc Brewing replicate experiment. The orange highlight indicates mutations that were also
 1077 observed in the first or last timepoint populations from the first Postdoc Brewing replicate.

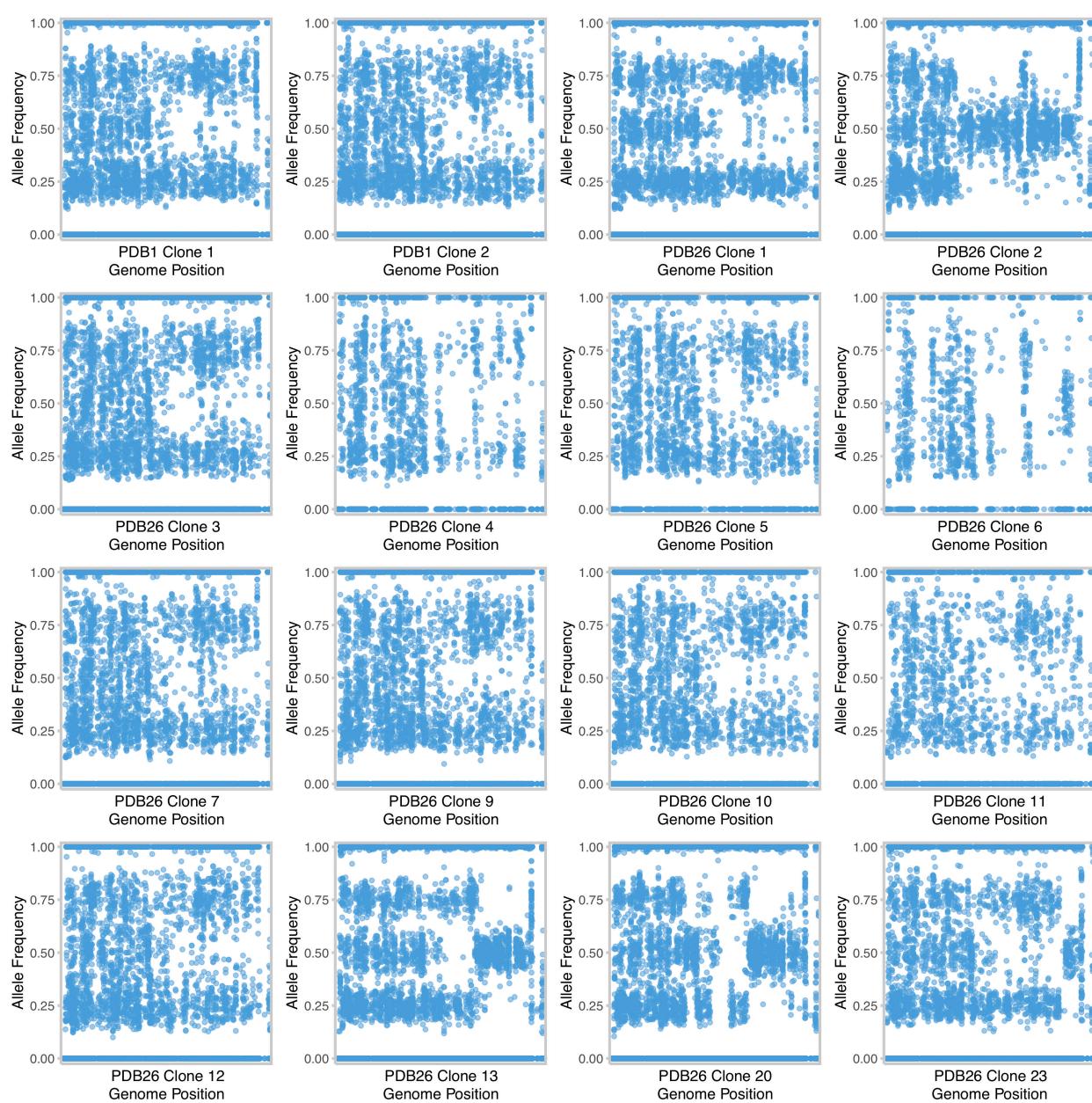
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1080 **Supplementary Fig 2.** Allele frequency of chromosome V with the first timepoint colored in blue
1081 and the final timepoint in orange.

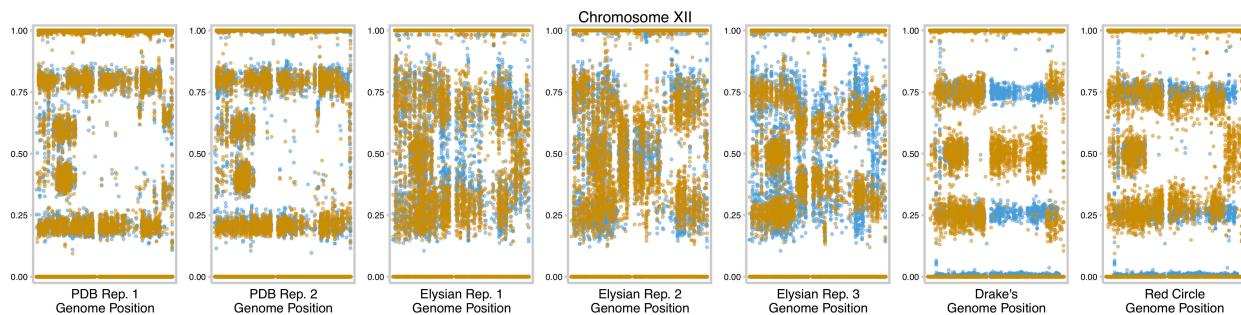
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1084 **Supplementary Fig 3.** Allele frequency of chromosome VIII for every clone isolated from the first
1085 Postdoc Brewing Co. replicate.

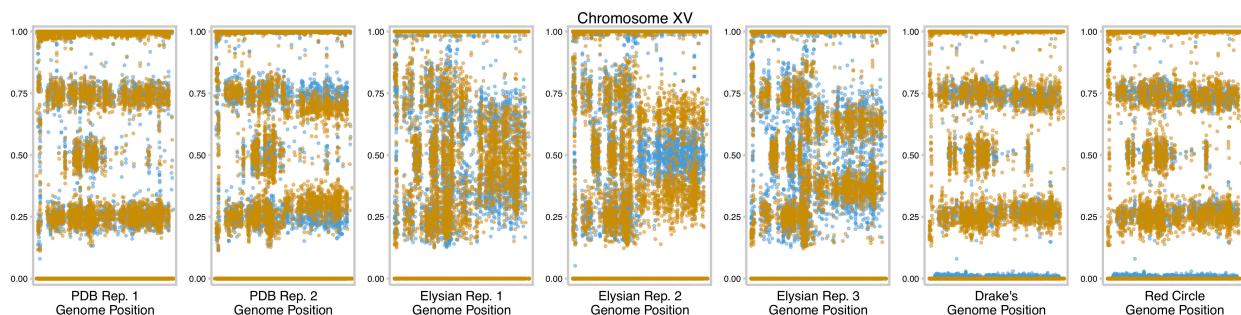
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1088 **Supplementary Fig 4.** Allele frequency of chromosome XII with the first timepoint colored in blue
1089 and the final timepoint in orange.

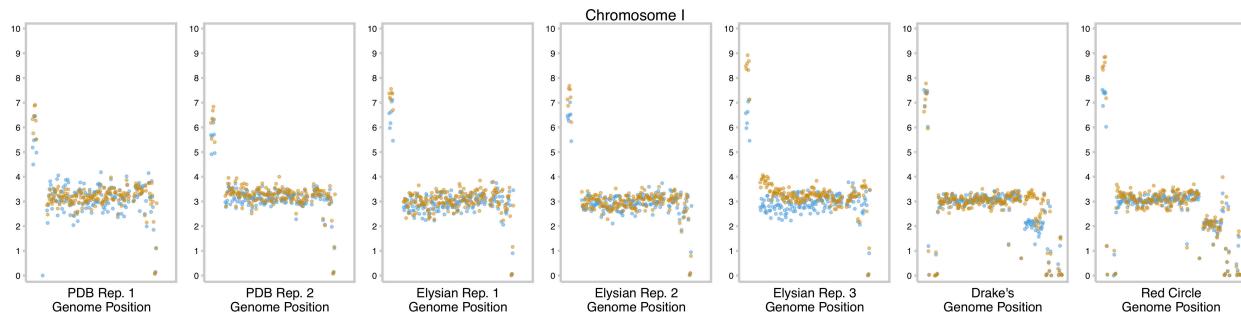
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1091

1092 **Supplementary Fig 5.** Allele frequency of chromosome XV with the first timepoint colored in blue
1093 and the final timepoint in orange.

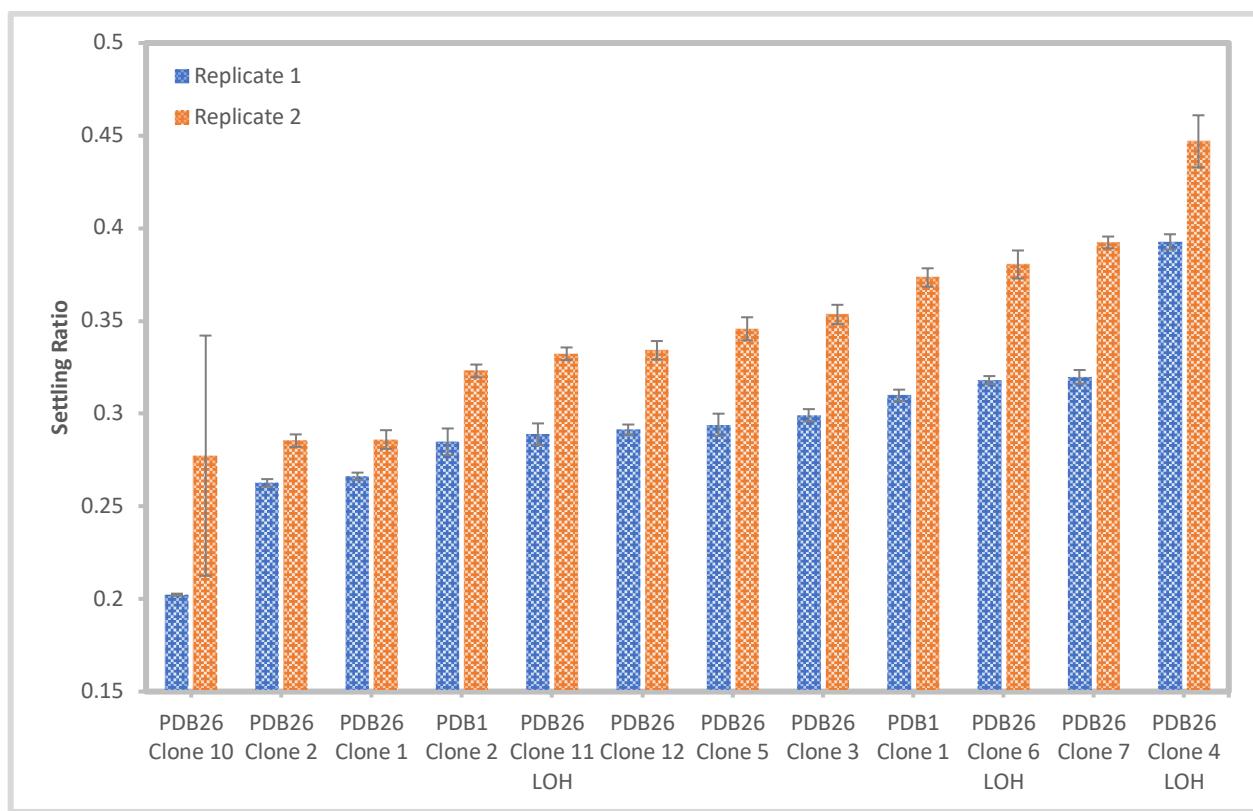
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1096 **Supplementary Fig 6.** Copy number of chromosome I shown as 1000-bp sliding windows with
1097 the first timepoint colored in blue and the final timepoint in orange.

1098



1099

1100 **Supplementary Fig 7.** Settling ratio of clones isolated from the first Postdoc Brewing Co.
1101 replicate.