1   **Evolution of ecological dominance of yeast species in high-sugar environments**

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Abstract

In budding yeasts, fermentation in the presence of oxygen evolved around the time of a whole genome duplication (WGD) and is thought to confer dominance in high-sugar environments because ethanol is toxic to many species. While there are many fermentative yeast species, only *Saccharomyces cerevisiae* consistently dominates wine fermentations. In this study, we use co-culture experiments and intrinsic growth rate assays to examine the relative fitness of non-WGD and WGD yeast species across environments to assess when *S. cerevisiae*'s ability to dominate high-sugar environments arose. We show that *S. cerevisiae* dominates nearly all other non-WGD and WGD species except for its sibling species *S. paradoxus* in both grape juice and a high-sugar rich medium. Of the species we tested, *S. cerevisiae* and *S. paradoxus* have evolved the highest ethanol tolerance and intrinsic growth rate in grape juice. However, the ability of *S. cerevisiae* and *S. paradoxus* to dominate certain species depends on the temperature and the type of high-sugar environment. Our results indicate that dominance of high-sugar environments evolved much more recently than the WGD, most likely just prior to or during the differentiation of *Saccharomyces* species, and that evolution of multiple traits contributes to *S. cerevisiae*'s ability to dominate wine fermentations.

Introduction

Evolutionary innovation can promote the ecological dominance of some lineages by enabling them to occupy new niches. While conservation of an innovation among descendent taxa reflects its contribution to their ecological success, ecological dominance may not be an immediate consequence of evolutionary innovation. Phylogenetic studies indicate that the current dominance of some lineages may result from events temporally distinct from major evolutionary transitions (Wing and Boucher 1998; Alfaro et al. 2009; Edwards et al. 2010; Near et al. 2012; Schranz et al. 2012; Bouchenak-Khelladi et al. 2014). This apparent lag between the evolution of an innovation and the rise to dominance of descendent lineages may occur because dominance depends upon certain environments, ecological communities or the acquisition of additional traits.

In budding yeasts, evolution of the ability to ferment sugar in the presence of oxygen dramatically changed the way some species harness energy. Whereas most species acquire energy through respiration in the presence of oxygen, certain species such as *Saccharomyces cerevisiae* acquire most of their energy via the less efficient process of fermentation (Pronk et al. 1996). Evolution of this fermentative lifestyle likely involved multiple steps both before and after a whole genome duplication (WGD) in the yeast lineage, including the ability to grow without mitochondrial electron transport and the transcriptional rewiring of carbon metabolizing enzymes (Ihmels et al. 2005; Merico et al. 2007; Field et al. 2009; Hagman et al. 2013; Lin et al. 2013). While the evolutionary transition to a fermentative lifestyle began prior to the WGD, lineages that diverged after

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60 the WGD show a clear preference for fermentation in the presence of oxygen (Merico et

61 al. 2007; Hagman et al. 2013).

62   Fermentation in the presence of oxygen is thought to provide WGD yeast species

63 with a fitness advantage in high-sugar environments such as grape juice (Wolfe and

64 Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006;

65 Conant and Wolfe 2007). Theoretical modeling shows that a fermentative lifestyle can

66 yield a growth advantage in high-sugar environments due to a higher rate of sugar

67 consumption and energy production (Pfeiffer et al. 2001; MacLean and Gudelj 2006;

68 Conant and Wolfe 2007). Additionally, ethanol produced during fermentation may inhibit

69 the growth of competitor species (Gause 1934; Piskur and Langkjaer 2004; Thomson et

70 al. 2005; Piskur et al. 2006). Thus, the fermentative lifestyle is expected to enable WGD

71 species to dominate high-sugar environments like grape juice.

72   While *S. cerevisiae* has been shown to dominate competitions with multiple non-

73 WGD species (Holm Hansen et al. 2001; Pérez-Nevado et al. 2006), the importance of

74 the fermentative lifestyle remains equivocal. Competition experiments between *S.*

75 *cerevisiae* and several non-WGD species did not support the role of ethanol but instead

76 implicate different factors depending upon which competitor species was used.

77 Competitions with *Torulaspora delbrueckii* and *Lachancea thermotolerans* demonstrated

78 that low-oxygen and cell-density contribute to *S. cerevisiae*'s dominance (Holm Hansen

79 et al. 2001; Nissen et al. 2003, 2004), while competitions with *Hanseniaspora*

80 *guilliermondii* and *H. uvarum* showed that *S. cerevisiae* produces a toxic metabolite

81 derived from glyceraldehyde 3-phosphate dehydrogenase peptides (Pérez-Nevado et al.

82   2006; Albergaria et al. 2010; Branco et al. 2014). While *S. cerevisiae* exhibits high-

83   ethanol tolerance (Pina et al. 2004; Belloch et al. 2008; Arroyo-López et al. 2010;

84   Salvadó et al. 2011a), mono-culture growth rates of various species indicate that

85   temperature is more important to *S. cerevisiae*'s dominance than ethanol tolerance

86   (Goddard 2008; Salvadó et al. 2011a).

87   Within the vineyard environment, grapes and wine musts contain hundreds of

88   yeast species, including a number of fermentative species (Pretorius 2000; Fleet 2003,

89   2008; Jolly et al. 2006; Bokulich et al. 2012; Pinto et al. 2014). Yet, even without the

90   introduction of commercial wine yeast, *S. cerevisiae* consistently dominates grape juice

91   as it ferments to wine (Fleet 2003, 2008). Since little is known about the relative fitness

92   of most WGD species in high-sugar environments like grape juice, it is unclear whether

93   *S. cerevisiae*'s dominance in wine fermentations reflects certain attributes of the grape

94   juice environment or the yeast species present within the community, and whether

95   dominance in high-sugar environments is a simple consequence of the fermentative

96   lifestyle or involves the acquisition of additional traits.

97   The objectives of this study were to determine when the ability to dominate high-

98   sugar environments evolved in the yeast lineage and to identify traits that confer *S.*

99   *cerevisiae* with a growth advantage in these environments. To infer when dominance

100   arose and lessen the impact of any potential strain or species outliers, we examined a

101   taxonomically diverse sample of 18 different yeast species spanning the WGD and the

102   evolution of the fermentative lifestyle (Figure 1). Given that the evolution of the

103   fermentative lifestyle spanned the WGD (Hagman et al. 2013), we included non-WGD

104   species that produce small amounts of ethanol (*Lachancea* and *Torulaspora* species) and

105   WGD species that exhibit intermediate levels of fermentation (*Vanderwaltozyma* and

106   *Tetrapisispora* species). To identify when dominance arose we directly competed these

107   species with one another and found that dominance of high-sugar environments evolved

108   much more recently than the evolution of the fermentative lifestyle. To identify traits

109   involved in dominance we compared species' intrinsic growth rates under a variety of

110   conditions and found that both ethanol and the type of high-sugar environment influence

111   fitness.

112

113   **Material and Methods**

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115   Yeast strains

116

117   A total of 20 yeast strains representing 18 non-WGD and WGD species were used

118   for our experiments (Table S1 and Figure 1). We chose a *S. cerevisiae* strain isolated

119   from oak (YPS163) to represent *S. cerevisiae* (Sniegowski et al. 2002). As a control for

120   population variation within *S. cerevisiae*, we also included I14, a *S. cerevisiae* strain

121   isolated from a vineyard in Italy (Fay and Benavides 2005) and BJ20, a *S. cerevisiae*

122   strain isolated from a tree in China (Wang et al. 2012).

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124   Growth media

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126   The primary assay media used were two high-sugar environments: Chardonnay

127   grape juice (Vintners Reserve, Winexpert Inc., Port Coquitlam, B.C., Canada), hereafter

128   referred to as "Grape", and high-sugar rich medium (10 g/l yeast extract, 20 g/l peptone,

129   120 g/l dextrose), hereafter referred to as "HS". We chose HS to reflect the glucose

130   concentration typical of the grape juice environment, ~ 120 g/l (Rodicio and Heinisch

131   2009). Low-pH HS was made by adjusting the pH of HS from 6.7 to 3.7, the pH of our

132   Grape medium, using tartaric acid, the predominant acid present in grape juice (Radler

133   1993). The media used to test for nutrient limitations in Grape included Grape with one

134   of five nutrient supplements: YP (10 g/l yeast extract, 20 g/l peptone), CM (1.3 g/l

135   synthetic complete with amino acids, 1.7 g/l yeast nitrogen base, and 5 g/l ammonium

136   sulfate), AA (1.3 g/l complete amino acids), NB (1.7 g/l yeast nitrogen base), or AS (5 g/l

137   ammonium sulfate). Assay media to test the ethanol tolerance of each yeast species was

138   YPD (10 g/l yeast extract, 20 g/l peptone, 20 g/l dextrose) with ethanol concentrations

139   ranging from 0-10%. Assay media to identify unknown inhibitor compounds produced by

140   *S. cerevisiae* during growth was YPD made using supernatant from 16 other species

141   (Table S1) grown in mono-culture and co-culture with *S. cerevisiae*. We chose YPD with

142   2% dextrose for ethanol tolerance and supernatant assays because ethanol produced

143   during growth by fermenting species should not attain inhibitory concentrations.

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146   Competition experiments

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148   *Growth conditions* – To assess the relative growth of non-WGD and WGD yeast

149   species in high-sugar environments, we performed two competition experiments in which

150   we measured the abundance of representative strains of multiple species relative to a

151   focal species strain of *S. cerevisiae* or *S. paradoxus* after growth in co-culture. In the first

152   competition experiment (Competition 1), we measured the abundances of six non-WGD

153   and seven WGD species (Table S1) relative to a single *S. cerevisiae* strain (YPS163). As

154   controls, we assessed the ability of the conspecific strain, I14, to grow relative to our

155   reference *S. cerevisiae* strain, and grew each species in mono-culture. Competitions were

156   initiated at approximately 10^3 cells of each species per ml of Grape and HS media. One

157   ml cultures were grown in 2 ml 96-well plates that were covered with breathable film and

158   incubated at 30°C with shaking at 400 rpm for 48 hours. To assess the effects of strain

159   and temperature on the relative growth of WGD species in high-sugar environments, we

160   measured the abundance of five WGD species relative to three *S. cerevisiae* strains

161   (YPS163, I14, and BJ20) and a single strain of *S. paradoxus* (YPS152) following co-

162   culture (Competition 2). Cultures were prepared and grown as in Competition 1, except

163   that replicates of each culture were grown at 30°C and 22°C.

164   *Sampling* – For both competition experiments, samples were taken at the

165   beginning and the end of the experiment and frozen at -20°C for later use. For mono-

166   cultures in Competition 1, cells from *S. cerevisiae* mono-culture were mixed in equal

167   volume with cells from each of the other species' mono-cultures at 48 hours and then

168   frozen.

8

169   *DNA extraction* – DNA for Competition 1 was extracted from each sample using

170   a protocol modified from Hoffman (2002) that included adding approximately 200 µl of

171   0.5 mm-diameter glass beads (BioSpec Products, Bartlesville, OK) to each sample and

172   lysing cells in a bead beater (BioSpec Products) on high for 5 minutes at room

173   temperature. For samples grown in Grape, DNA was also column purified to remove an

174   unknown inhibitor of PCR amplification. DNA for Competition 2 was extracted using the

175   ZR-96 Fungal/Bacterial DNA Kit (Zymo Research, Organge, CA).

176   *Pyrosequencing* – To quantify the abundance of *S. cerevisiae* relative to each

177   competitor species in Competition 1, we pyrosequenced species-specific single

178   nucleotide variants (SNVs). Previous studies have shown that pyrosequencing can

179   accurately quantify the frequency of single nucleotide variants in pooled DNA samples

180   (Lavebratt and Sengul 2006). To design pyrosequencing primers, we generated pair-wise

181   sequence alignments of *ACT1* or *CYT1* between *S. cerevisiae* and each of the other

182   species and identified at least one SNV for each pair and designed corresponding primer

183   sets that included (1) forward and reverse primers for PCR and (2) a pyrosequencing

184   primer (Table S2). Pyrosequencing was carried out using a PyroMark Q96 MD

185   Automated pyrosequencer (Qiagen, Valencia, CA) following the protocol described by

186   King and Scott-horton (2007) and the manufacturer's directions.

187   To calibrate each primer set, DNA from samples containing known ratios of cells

188   from *S. cerevisiae* and each of the other species were also pyrosequenced and used to

189   establish standard curves using linear and polynomial regression (Figure S1). For two

190   species (*Nakeseomyces bacillisporus* and *Tetrapisispora blattae*), we were not able to

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191 design sets of primers, and for three species (*Kazachstania lodderae*, *K. martiniae*, and

192 *Kluyveromyces lactis*), we were not able to quantify abundance due to severely biased

193 PCR or pyrosequencing identified by our control calibrations.

194

195   *High-throughput sequencing* – To quantify the abundance of each *S. cerevisiae* or

196 *S. paradoxus* strain relative to each competitor species in Competition 2, we used high-

197 throughput sequencing due to lower cost, larger sample capacity and a change in the

198 availability of pyrosequencing. The internal transcribed sequence (ITS1) between the

199 5.8S and 18S ribosomal genes was amplified using 24 barcoded primers (Bokulich et al.

200 2012). Samples were further multiplexed using 18 indexed adaptors (Table S3).

201 Following quantification and pooling at equal concentrations, the sample library was

202 sequenced on a single 1x250 bp run of an Illumina MiSeq. Sample sequences were de-

203 multiplexed allowing a single mismatch to each barcode or index followed by adaptor

204 trimming and clipping of any low quality bases using ea-utils (Aronesty 2011). Species'

205 counts in each sample were obtained by blastall (v2.2.25) against 287,101 sequences

206 from culturable species in the UNITE+INSD database (Kõljalg et al. 2013). Out of 11.6

207 million raw reads, 8.0 million were included in the analysis with a median of 4,346

208 species tags across 578 samples.

209   ITS1 species counts were calibrated using DNA from samples with known ratios

210 of cells from *S. cerevisiae* or *S. paradoxus* and each of the competitor species. Standard

211 curves were generated using linear regression (Figure S2). For three species with non-

212   linear calibration curves, *Naumovozyma castellii*, *K. lodderae* and *Candida glabrata*, we

213   used a modified regression:

214

215          $y = bx/(1+x(b-1))$

216

217   where b is a bias parameter, x is the observed frequency and y is the known frequency

218   (Moskalev et al. 2011). For two species with particularly strong bias, *N. castellii* and *K.*

219   *lodderae*, we also generated calibration curves for *ACT1* PCR fragments. *ACT1* PCR

220   fragments showed a weaker bias, and so we used these fragments for all competitions

221   between *S. cerevisiae* and either *N. castellii* or *K. lodderae*.

222   *Assessing dominance* – To determine the abundance of *S. cerevisiae or S.*

223   *paradoxus* relative to each species, we adjusted the percentage of species-specific SNVs

224   sequenced during pyrosequencing (Competition 1) or the number of species-specific

225   counts from high-throughout sequencing (Competition 2) using our standard curves. If

226   values were negative after this adjustment, we conservatively adjusted them to 0.005. To

227   calculate the relative fitness of each competitor species, we used:

228

229          $\ln(C_t/R_t) - \ln(C_0/R_0) = t\ln(w_c/w_r)$

230

231

232   where C_t and R_t are the frequencies of the competitor and reference at generation t and w

233   is fitness. For all competitions we assumed $t = 14$ based on changes in cell density.

234   To identify significant growth differences between species, we either used a one-tailed

235   paired Welch's *t* test (Competition 1), or a mixed effect model with random effect for

236   strain and a fixed effect for the competitor's fitness (Competition 2). To correct for

237   multiple comparisons, we used the method of Benjamini and Hochberg (1995) and a false

238   discovery rate (FDR) cutoff of less than 0.01 for Competition 1 and 0.05 for Competition

239   2, since fewer species were competed in Competition 2.

240

241   Intrinsic growth rate experiments

242

243   *Growth assays* – Cultures were inoculated at an optical density (OD) at 600 nm of

244   approximately 0.25 in 1 ml of growth medium and were grown in 2 ml 96-well plates that

245   were covered with breathable film and incubated at 30°C with shaking at 400 rpm for up

246   to 48 hours. Cell density was measured by OD at 620 nm at 0, 4, 8, 12, 18, 24, 36, and 48

247   hours using an iEMS microplate reader (Thermo Lab Systems, Helsinki, Finland).

248   *Analysis* – The intrinsic growth rate (*r*) of each species was calculated for each

249   time interval using the equation:

250

251        $N_t = N_0 e^{rt}$

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253   where N_t is final cell density, N_0 is initial cell density and *t* is time in hours. The average

254   intrinsic growth rate of each species across all sampling intervals for a given medium was

255   then used to evaluate the effect of a treatment (*i.e.*, low-pH or a nutrient supplement) on

12

256   the growth of each species, ($\Delta r_{Treatment} = r_{Treatment} - r_{Control}$), or the effect of the species in a

257   given environment ($\Delta r_{Species} = r_{non\text{-}S.\ cerevisiae} - r_{S.\ cerevisiae}$). While the average growth rate does

258   not distinguish between differences in lag phase, exponential growth rate and carrying

259   capacity, we used it because it had a smaller error among replicates.

260   For two sets of experiments, we only used OD up to and including 24 hours in our

261   analysis: ethanol tolerance experiments and supernatant experiments. For ethanol

262   tolerance experiments we observed flocculation that increased the variability of OD

263   measurements beginning at 36 hours. For inhibitor compound experiments we observed

264   that un-inoculated control samples registered noticeable effects on OD measurements

265   beginning at 36 hours.

266   Ethanol tolerance among species was measured by the ethanol concentration that

267   inhibited growth by 50% (IC_{50}). IC_{50} estimates were obtained by fitting dose response

268   curves using a three-parameter Weibull function in R (R Development Core Team 2013)

269   using the 'drc' package (Ritz and Streibig 2005). Statistical comparisons between the

270   estimated IC_{50} for *S. cerevisiae* and each species were made using the 'comped' function

271   (Ritz and Streibig 2005; R Development Core Team 2013) followed by the Altman and

272   Bland method to calculate *P* values from confidence intervals (Altman and Bland 2011).

273   To correct for multiple comparisons, we used the method of Benjamini and Hochberg

274   (1995) and a FDR cutoff of less than 0.01 for significance.

275

276   **Results**

277   Ecological dominance of yeast species in grape juice evolved more recently than the

278   fermentative lifestyle

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280   To determine when ecological dominance of high-sugar environments evolved,

281   we grew representative strains of multiple non-WGD and WGD yeast species in two

282   high-sugar environments: Chardonnay grape juice (Grape) and a high-sugar rich medium

283   (HS). If dominance in high-sugar environments evolved along with the evolution of

284   fermentation in the presence of oxygen, we expect *S. cerevisiae* will consistently exhibit

285   higher relative fitness than all non-WGD species but not WGD species in both high-sugar

286   environments. Grape was chosen to represent a natural high-sugar environment, and HS

287   was chosen to replicate the sugar concentration typical of the grape juice environment

288   while limiting the potential influence of nutrient content and low-pH. Since the ability to

289   dominate is inherently a relative trait, we assessed the growth of each non-WGD and

290   WGD species relative to a representative *S. cerevisiae* strain isolated from an oak tree

291   (YPS163) using co-cultures. As a control, we also grew a *S. cerevisiae* strain isolated

292   from a vineyard (I14) in co-culture with our reference *S. cerevisiae* strain. If the relative

293   abundance of *S. cerevisiae* was significantly higher at the end compared to the start of the

294   experiment, indicating a higher relative fitness, it was considered "dominant".

295   We find that *S. cerevisiae* dominates nearly all non-WGD and WGD yeast species

296   in Grape and HS co-cultures (Figure 2A and 2B). In both Grape and HS, *S. cerevisiae*

297   increased in abundance relative to 10/12 yeast species (FDR < 0.01, Table S4). In the

298   majority of these co-cultures, *S. cerevisiae* was greater than 90% of the population at 48

299   hours. Notably, *S. cerevisiae* remained a significant proportion of the population even

300   when it did not dominate. These data show that *S. cerevisiae* is able to dominate in

301   multiple high-sugar environments, and they suggest that the ability to dominate high-

302   sugar environments arose recently in yeast evolution.

303   In support of a more recent evolution of ecological success in high-sugar

304   environments, *S. paradoxus* is the only species that persists along with *S. cerevisiae* in

305   Grape and HS co-cultures. Two other strains, *S. cerevisiae* (I14) and *C. glabrata,* also

306   competed well with our *S. cerevisiae* reference. However, their persistence depended

307   upon the environment: *S. cerevisiae* dominated *C. glabrata* in Grape (FDR = 0.0072) but

308   not in HS, whereas it dominated I14 in HS (FDR = 0.0010) but not in Grape. Thus, *S.*

309   *paradoxus* was the only species able to compete well with *S. cerevisiae* in both high-

310   sugar environments.

311   One explanation for *S. cerevisiae*'s dominance in our Grape and HS co-cultures is

312   that it has a greater carrying capacity than other species in these environments, even

313   when they are grown individually. As a control for our co-culture experiments, we also

314   measured the density of each species grown in mono-culture by mixing it with a *S.*

315   *cerevisiae* mono-culture after 48 hours and quantifying the proportion of each species.

316   *S. cerevisiae* has a carrying capacity similar to the majority of yeast species in

317   Grape and HS (Figure 2C and 2D). In Grape, the abundance of *S. cerevisiae* was

318   significantly greater than only 2/12 species after 48 hours of mono-culture (Figure 2C,

319   FDR < 0.01). Species that obtained significantly lower carrying capacities included the

320   non-WGD species *Hanseniaspora vineae* and the WGD species *Vanderwaltozyma*

321   *polyspora*, which were 1% and 3% of *S. cerevisiae*'s abundance after 48 hours in mono-

322   culture. The relative population size of *S. cerevisiae* was also not significantly greater

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323   than 12/12 species tested in HS. These data imply that *S. cerevisiae*'s dominance in

324   Grape and HS co-cultures is not due to differences between species in their individual

325   carrying capacities.

326   Dominance of high-sugar environments appears to be a trait shared by *S.*

327   *cerevisiae* and *S. paradoxus*. However, we only used one representative strain of *S.*

328   *cerevisiae* and did not compete *S. paradoxus* with other species. To test whether *S.*

329   *cerevisiae*'s dominance is a property of the strain used or the species we repeated a subset

330   of the Grape and HS competitions using two additional strains of *S. cerevisiae*: a soil

331   isolate (I14) from Italy that is closely related to other European strains (Fay and

332   Benavides 2005) and a tree isolate from China that is diverged from both the European

333   strain and our reference strain YPS163 from North America (Wang et al. 2012). The

334   fitness of the three *S. cerevisiae* strains together in relation to each competitor species

335   indicates that *S. cerevisiae*: dominates *S. mikatae*, *S. uvarum*, *N. castelli* and *K. lodderae*

336   in both Grape and HS, dominates *C. glabrata* in Grape but not HS, and has no fitness

337   differences in comparison to *S. paradoxus* (FDR < 0.05, Figure 3A and 3B). These

338   results confirm our previous results based on a single *S. cerevisiae* reference strain and

339   add *K. lodderae* to the list of species dominated by *S. cerevisiae*.

340   Using *S. paradoxus* as the reference species we find that *S. paradoxus* dominates

341   the same yeast species as *S. cerevisiae* in Grape (Figure 3A). In HS, *S. paradoxus*

342   dominates fewer species than *S. cerevisiae*; it does not dominate *S. mikatae* and *K.*

343   *lodderae* (Figure 3B). Thus, the results of our competition experiments with *S.*

344   *paradoxus* show that dominance in Grape is shared by both *S. cerevisiae* and *S.*

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345   *paradoxus* but that the ability of *S. paradoxus* to dominate in HS is not as strong as that

346   of *S. cerevisiae*.

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348   Temperature affects ecological dominance of certain yeast species in high-sugar

349   environments

350

351   The *Saccharomyces* species have differentiated in both their optimal and

352   maximum growth temperature (Belloch et al. 2008; Gonçalves et al. 2011; Salvadó et al.

353   2011b; Kurtzman et al. 2011). Because *S. cerevisiae*'s optimal growth temperature

354   (32°C), is higher than that of *S. paradoxus* (30°C), *S. mikatae* (29°C) and *S. uvarum*

355   (26°C) (Salvadó et al. 2011b), its dominance of high-sugar environments could depend

356   on the high temperature (30°C) of our initial competition experiment. To examine this

357   possibility, we competed a subset of species at a lower temperature (22°C).

358   Similar to the results of our competition experiments performed at high

359   temperature, at 22°C both *S. cerevisiae* and *S. paradoxus* dominated *S. uvarum* and *N.*

360   *castellii* in Grape and HS, and dominated *C. glabrata* in Grape but not HS (Figure 3C and

361   3D). However, at the lower temperature neither *S. cerevisiae* nor *S. paradoxus* dominated

362   *S. mikatae* or *K. lodderae* in Grape or HS, and *K. lodderae* dominated *S. cerevisiae* and

363   *S. paradoxus* in HS. Thus, ecological dominance of certain yeast species in high-sugar

364   environments depends on temperature, which implicates the evolution of thermal

365   tolerance among the *Saccharomyces* species in the evolution of ecological dominance.

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367   *S. cerevisiae* has a distinct competitive advantage in grape juice

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369   Our finding that many WGD yeast species compete poorly with *S. cerevisiae* in

370   high-sugar environments suggests that the fermentative lifestyle is not sufficient to confer

371   ecological success in these environments. Since the majority of the species we tested are

372   capable of achieving similar carrying capacities to *S. cerevisiae* in these environments

373   when grown individually, *S. cerevisie*'s dominance in our Grape and HS co-cultures must

374   be related to either differences in intrinsic growth rates or interference competition. To

375   investigate these two modes of ecological dominance, we measured the intrinsic growth

376   rate of each species in mono-culture. If *S. cerevisiae* does not exhibit a greater intrinsic

377   growth rate than other species, then its ability to dominate in these environments can be

378   attributed to interference competition.

379   *S. cerevisiae* has a greater intrinsic growth rate than nearly all yeast species in the

380   grape juice environment (Figure 4). Compared to *S. cerevisiae,* 16/17 species exhibited a

381   significantly lower intrinsic growth rate in Grape (FDR < 0.01, Table S5). The one

382   notable exception to this pattern was *S. paradoxus*, which had a lower growth rate but did

383   not meet our cutoff for significance (FDR = 0.028). In stark contrast to our finding in

384   Grape, when we compared the intrinsic growth rate of *S. cerevisiae* to the intrinsic

385   growth rate of each of the other species in HS, we did not observe any significant

386   difference for 17/17 species (Figure 4B). These results suggest different or multiple

387   mechanisms contribute to the dominance of *S. cerevisiae* in high-sugar environments.

388   Furthermore, they support the recent evolution of traits required for ecological success in

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389   the grape juice environment. In the following sections we examine factors that may

390   contribute to the dominance of *S. cerevisiae* in both HS and Grape.

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392   Evolution of ethanol tolerance and its potential role in interference competition

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394   *S. cerevisiae*'s ability to produce and tolerate ethanol is one way in which it may

395   dominate other species in high-sugar environments. Although previous studies showed

396   that *S. cerevisiae* tolerates higher ethanol concentrations than many yeast species, they

397   only included 4/17 of the species used in this study (Pina et al. 2004; Belloch et al. 2008;

398   Arroyo-López et al. 2010; Salvadó et al. 2011a). To examine the potential impact of

399   ethanol on the growth of each species, we measured the intrinsic growth rate of each

400   species in YPD supplemented with ethanol at concentrations ranging from 0-10% and

401   calculated the ethanol concentration that inhibited growth rate by 50% (IC_{50}) for each

402   species by fitting dose-response curves to the growth rate (see Materials and Methods).

403   *S. cerevisiae* had an IC_{50} greater than 15/17 yeast species (Figure 5 and Table S6).

404   The two exceptions to this pattern were *S. cerevisiae*'s closest relative, *S. paradoxus*

405   (FDR = 0.0502) and *C. glabrata* (FDR = 0.0320). *C. glabrata* grew as well as *S.*

406   *cerevisiae* at moderate ethanol concentrations, and it grew better than *S. cerevisiae* at low

407   ethanol concentrations (Figure S3 and Table S6). However, most of *S. cerevisiae*'s

408   growth advantage occurred at ethanol concentrations at or above 4% (Figure S3 and

409   Table S6). Thus, while all species tolerate low concentrations of ethanol (< 4%), *S.*

19

410 *cerevisiae* exhibits a growth advantage compared to most species at high-ethanol

411 concentrations.

412

413 No evidence for interference competition mediated by other toxic metabolites

414

415   Previous studies found that *S. cerevisiae* produced toxic metabolites other than

416 ethanol that inhibit the growth of competitor species (van Vuuren and Jacobs 1992;

417 Magliani et al. 1997; Musmanno et al. 1999; Pérez-Nevado et al. 2006; Albergaria et al.

418 2010; Rodríguez-Cousiño et al. 2011; Branco et al. 2014). However, other studies either

419 did not find any evidence that *S. cerevisiae* produced an inhibitory compound (Torija et

420 al. 2001; Nissen et al. 2003; Arroyo-López et al. 2011) or found that the ability to

421 produce killer toxins varied among *S. cerevisiae* strains (Gutiérrez et al. 2001; Sangorrín

422 et al. 2007; Maqueda et al. 2012). To determine whether the *S. cerevisiae* strain we used

423 during our assays produces an inhibitor compound, we grew each species in the

424 supernatant obtained from YPD mono-cultures and co-cultures with *S. cerevisiae.* We

425 chose YPD, which contains 2% dextrose, because ethanol concentrations should not

426 attain inhibitory concentrations during growth. In no instance did the supernatant inhibit

427 the subsequent growth of each species (Figure S4 and Table S7).

428

429 Low-pH and nutrient limitations contribute to *S. cerevisiae*'s intrinsic growth rate

430 advantage in grape juice

431

20

432   Grape juice differs from high-sugar rich medium in that it has a lower pH (pH =

433   3.7 vs pH = 6.7) and reduced levels of nutrients, most notably yeast assimilable nitrogen

434   (Henschke and Jiranek 1993). To determine whether *S. cerevisiae*'s higher intrinsic grow

435   rate in grape juice is related to pH or nutrient deficiencies we measured the effects of

436   altered pH of HS and nutrient content of Grape for each species.

437   To test the effect of pH on the intrinsic growth rate of each species, we grew each

438   species in low-pH HS, HS adjusted to the same acidity level as our Grape medium. As a

439   control, we compared each species' growth in low-pH HS to its growth in HS. If *S.*

440   *cerevisiae*'s intrinsic growth rate is greater than other species in Grape due to low-pH,

441   then *S. cerevisiae* should also exhibit a higher intrinsic growth rate than other species in

442   low-pH HS.

443   *S. cerevisiae* has an intrinsic growth rate advantage in low-pH HS (Figure 6).

444   When grown in low-pH HS, 4/18 species exhibited a significantly lower intrinsic growth

445   rate when compared to growth in HS (Figure 6A and Table S8). Notably, only three

446   species, including *S. cerevisiae* were not affected by low-pH at a nominal level of

447   significance ($P < 0.05$) compared to an FDR cutoff of 0.01. Additionally, when we

448   compared the intrinsic growth rate of *S. cerevisiae* in low-pH HS to each of the other

449   species in this environment, *S. cerevisiae*'s intrinsic growth rate was greater than 8/17

450   species (Figure 6B), compared to 0/17 species observed in HS (Figure 4B).

451   To test the effect of nutrient deficiency on the intrinsic growth rate of each

452   species, we grew each species in Grape supplemented with one of several different

453   nutrient sources that varied in complexity: YP, CM, NB, AA, and AS. YP is the rich

21

454   nutritive base of the HS environment, CM contains vitamins and minerals, amino acids,

455   and a single good nitrogen source, and NB, AA, and AS are the vitamins and minerals,

456   amino acids, and nitrogen source (ammonium sulfate) components of CM, respectively.

457   As a control, we compared each species' growth in Grape with a nutrient supplement to

458   its growth in Grape without the nutrient supplement. If nutrient limitations contribute to

459   intrinsic growth rate differences between species, then nutrient supplements in Grape

460   should increase each species' growth rate and reduce or eliminate intrinsic growth rate

461   differences between species.

462   Most yeast species are nutrient limited in grape juice. Of the 18 species we

463   assayed, 12 exhibited a significant increase in intrinsic growth rate with the addition of

464   one or more nutrient supplements, including *S. cerevisiae* (Figure 7, Figure S5 A-D, and

465   Table S9). However, which nutrients elicited a significant increase in growth varied by

466   species. For example, *S. uvarum* was positively affected by the addition of YP and NB,

467   whereas *C. glabrata* was positively affected by YP, CM and AA. Overall, YP positively

468   affected the intrinsic growth rate of the most species (11), followed by NB (7), CM (6),

469   and AA (3). However, none of the species we assayed grew significantly better with the

470   addition of AS, a good nitrogen source.

471   Nutrient supplements eliminate the intrinsic growth rate differences between *S.*

472   *cerevisiae* and nearly all other yeast species. Of the 17 species that grew significantly

473   slower than *S. cerevisiae* in Grape (Figure 4A), only two species, *N. castellii* and *V.*

474   *polyspora*, grow significantly slower than *S. cerevisiae* in spite of all of the nutrient

475   supplements used in our experiments (Table S9). Overall, Grape supplemented with CM

22

476   had the fewest number of species that still grew significantly slower than *S. cerevisiae*

477   (4), followed by Grape supplemented with AS (7), NB (12), YP (14), and AA (16).

478

479   **Discussion**

480

481   The diversion of more sugar to fermentation than respiration in the presence of

482   oxygen, i.e. the fermentative lifestyle, provides many yeast species the opportunity to

483   exploit novel environments and ecological strategies. One such species, *S. cerevisiae*,

484   consistently dominates wine fermentations and has become widely used to ferment beer,

485   bread and wine. In this study, we investigated when *S. cerevisiae's* ability to dominate

486   high-sugar environments evolved and whether its dominance is a simple consequence of

487   the fermentative lifestyle. We find that dominance evolved much more recently than the

488   evolution of the fermentative lifestyle and for certain species depends on the temperature

489   and growth medium. Our results suggest that *S. cerevisiae*'s frequently observed

490   dominance of grape juice fermentations is mediated by the evolution of multiple traits

491   that build on an ancient change in metabolism.

492

493   Evolution of dominance in relation to the fermentative lifestyle

494

495   Our study indicates that dominance in high-sugar environments evolved much

496   more recently than the WGD and the transition to the fermentative lifestyle (Hagman et

497   al. 2013). While previous studies showed that *S. cerevisiae* dominates multiple other non-

498 WGD species (Holm Hansen et al. 2001; Fleet 2003, 2008; Pérez-Nevado et al. 2006;

499 Goddard 2008), our findings demonstrate that *S. cerevisiae* and its closest relative, *S.*

500 *paradoxus* also dominate most other WGD yeast species in high-sugar environments and

501 that the temperature and growth medium are also important. The ability of *S. cerevisiae*

502 and *S. paradoxus* to dominate representatives of multiple taxonomically diverse species

503 suggests that dominance arose quite recently. However, the change in dominance

504 relationships across environments implies that: i) multiple traits underlie dominance, ii)

505 these traits have changed on lineages other than that leading to the ancestor of *S.*

506 *cerevisiae* and *S. paradoxus*, and iii) dominance cannot be ascribed to a single lineage.

507   Multiple, distantly-related WGD lineages grow well in nutrient rich, high-sugar

508 environments, but few species grow as well as *S. cerevisiae* in grape juice. While *S.*

509 *cerevisiae* and *S. paradoxus* dominate most WGD yeast species in HS medium, they do

510 not dominate *C. glabrata* in this environment, and depending upon the temperature, they

511 also lose some or all of their competitive advantage over *S. mikatae* and *K. lodderae*.

512 Remarkably, *K. lodderae*'s higher relative fitness in HS compared to Grape contributes to

513 its unique ability to dominate *S. cerevisiae* and *S. paradoxus* in HS at low-temperature.

514 One explanation for the exceptional growth observed for these species is that the ability

515 to compete in nutrient rich, high-sugar environment evolved independently along the

516 lineages that gave rise to *K. lodderae*, *C. glabrata,* and the *Saccharomyces* species.

517 Alternatively, the ability to compete well in nutrient rich, high-sugar environments may

518 have evolved early during the transition to the fermentative lifestyle, followed by

519 multiple, independent losses. However, our findings suggest that the more parsimonious

520   explanation is that the ability to dominate evolved much more recently than the transition

521   to the fermentative lifestyle.

522   Evolution of temperature preferences affects the dominance of certain species.

523   Among the *Saccharomyces* species, *S. cerevisiae*, *S. paradoxus* and *S. mikatae* can grow

524   at 37°C, whereas *S. arboricolus*, *S. uvarum* and *S. kudriavzevii* cannot and the latter two

525   are considered cryophilic (Belloch et al. 2008; Gonçalves et al. 2011; Salvadó et al.

526   2011b). Outside the *Saccharomyces* species, *K. lodderae*, *K. martiniae*, *T. blattae*, *L.*

527   *waltii* and *H. vineae* cannot grow at 37°C (Kurtzman et al. 2011), indicating that growth

528   at high temperature has been gained and/or lost multiple times. Given the species tree

529   (Figure 1), we attribute *S. cerevisiae*'s and *S. paradoxus*' dominance of *S. mikatae* at 30°C

530   but not 22°C to evolution of thermal tolerance. Similarly, *S. cerevisiae*'s dominance of *K.*

531   *lodderae* in Grape at 30°C but not 22°C may also depend on the evolution of thermal

532   tolerance. Although we did not specifically assay dominance below 22°C, the outcome of

533   competitions performed at even cooler temperatures may change. *S. uvarum* dominates

534   some wines at low temperatures (Torriani et al. 1999; Naumov et al. 2000; Sipiczk et al.

535   2001; Rementeria et al. 2003; Demuyter et al. 2004), and *S. kudriavzevii* does not

536   dominate but competes better with *S. cerevisiae* at low temperatures (Arroyo-López et al.

537   2011). However, *S. cerevisiae*'s dominance of *S. uvarum* may not be a simple

538   consequence of thermal tolerance; *S. uvarum*'s growth rate in grape juice increased with

539   supplementation of rich medium (YP) to a rate equivalent to that of *S. cerevisiae*.

540   Furthermore, we did not observe a significant intrinsic growth rate difference between

541   these species in high-sugar rich medium when grown at 30°C (Figure 4).

542   The thermal differentiation of *Saccharomyces* species raises the possibility that

543   the ability to dominate high-sugar environments evolved prior to their differentiation.

544   Dominance of high-sugar environments may have evolved progressively such that WGD

545   yeast species can outcompete non-WGD yeast species even though they lose to *S.*

546   *cerevisiae*. However, more pair-wise competitions between other non-Saccharomyces

547   WGD species and non-WGD species are needed to test that hypothesis. While the

548   evolution of dominance cannot be pinned to any particular lineage, we see no clear

549   progression to higher ethanol tolerance among WGD yeast species or evidence for higher

550   intrinsic growth rate in grape juice outside of the *Saccharomyces* species. Thus, in

551   addition to thermal differentiation, other traits likely to contribute to dominance evolved

552   among the *Saccharomyces* species.

553   One limitation of our ability to make inferences about the evolution of dominance

554   and how the environment influences it is that most species were only represented by a

555   single strain. While the fitness of three diverse strains of *S. cerevisiae* are generally quite

556   similar to one another and to *S. paradoxus*, competitor strains may not always be

557   representative of the species. This possibility places some limits on the interpretation of

558   dominance relationships specific to a single species, e.g. *C. glabrata* and *K. lodderae*, but

559   is unlikely to explain overall patterns of dominance.

560

561   Multiple mechanisms of ecological dominance

562

563   *S. cerevisiae*'s dominance of high-sugar environments cannot be explained by a

564   single mechanism. In addition to thermal tolerance discussed above, we also find

565   evidence for ethanol tolerance and a high intrinsic growth rate in grape juice. Interference

566   competition through the production of ethanol provides one explanation for *S.*

567   *cerevisiae*'s dominance of high-sugar rich medium. Consistent with previous studies

568   (Pina et al. 2004; Belloch et al. 2008; Arroyo-López et al. 2010; Salvadó et al. 2011a), we

569   found that *S. cerevisiae* exhibits greater ethanol tolerance than most species. In support of

570   the role of ethanol tolerance in dominance, *S. paradoxus* and *C. glabrata* exhibited

571   ethanol tolerance similar to *S. cerevisiae* and were the only two species that were not

572   dominated by *S. cerevisiae* in high-sugar rich medium at high temperature.

573   However, previous studies showed that oxygen, cell density and an inhibitory

574   peptide affect *S. cerevisiae*'s dominance of various non-WGD species (Holm Hansen et

575   al. 2001; Nissen et al. 2003, 2004; Pérez-Nevado et al. 2006; Albergaria et al. 2010;

576   Branco et al. 2014). These studies excluded the effects of ethanol because non-WGD

577   species initiated cell death by some other mechanism before ethanol reached inhibitory

578   concentrations. While we only measured competitions with two of the species used in

579   earlier studies, *T. delbrueckii* and *L. thermotolerans*, we cannot exclude the possibility

580   that these species were dominated for reasons other than ethanol inhibition. One

581   difference between our experiments and those of prior studies is that they were carried

582   out with low or no agitation, whereas we performed our competitions under high

583   agitation (400 rpm). Agitation is expected to increase dissolved oxygen and might

27

584   eliminate cell density and confinement effects (Nissen et al. 2003, 2004; Arneborg et al.

585   2005).

586   Multiple lines of evidence suggest that *S. cerevisiae*'s dominance in grape juice is

587   influenced by its high intrinsic growth rate in this environment. *S. cerevisiae* exhibited

588   the highest rate of growth in grape juice, significantly higher than all species except *S.*

589   *paradoxus*. The absence of any difference in growth rate in high-sugar rich medium

590   implies that *S. cerevisiae*'s intrinsic growth advantage in grape juice is specific to grape

591   juice or similar environments. Furthermore, lowering the pH of high-sugar rich medium

592   did not affect *S. cerevisiae* but affected the growth of other species, and supplementation

593   of nutrients to grape juice increased the growth of many species but had little to no effect

594   on *S. cerevisiae*. Notably, *S. uvarum*, *C. glabrata* and *H. vineae* grew as well as *S.*

595   *cerevisiae* in low-pH medium and in grape juice supplemented with rich nutrients (YP),

596   indicating that low nutrients alone may explain their slow growth in grape juice.

597   The relative importance of temperature, intrinsic growth rate and ethanol

598   inhibition to *S. cerevisiae*'s dominance of grape juice is uncertain since their effects are

599   difficult to disentangle from one another. In support of temperature, fermentation is

600   exothermic and can increase the temperature of wine must by as much as 10°C (Boulton

601   1979; Goddard 2008). However, we found that nutrient supplementation increased most

602   species' intrinsic growth rate in grape juice at 30°C. Ethanol inhibition is not likely to be

603   important until the later stages of fermentation because most species were not

604   significantly inhibited by ethanol concentrations below 5%, similar to previous reports

28

605   (Goddard 2008; Salvadó et al. 2011a). As such, we favor intrinsic growth rate in grape

606   juice as a driver of dominance as it likely acts earlier and throughout the competition.

607   Interactions between factors may also contribute to *S. cerevisiae*'s dominance.

608   Ethanol and high-temperature act synergistically to decrease growth due to their

609   overlapping effects on lipid membrane integrity (Piper 1995). Lipid membrane integrity

610   importantly affects proton (H+) transport across the cell membrane, and the combined

611   effects of ethanol and high-temperature increase the lipid membrane's H+ permeability

612   (Madeira et al. 2010). Increased H+ permeability can also result in reduced intracellular

613   pH, particularly in acidic environments such as grape juice. While we did not measure

614   any interaction effects, Goddard (2008) found interactions between the effects of

615   temperature, ethanol and media, including grape juice, on the growth rate of

616   *Saccharomyces* versus non-*Saccharomyces* species.

617

618   Ecology of high-sugar environments

619

620   The fermentative lifestyle is hypothesized to coincide with the evolution of

621   flowering plants due to the abundance of diverse high-sugar environments (Wolfe and

622   Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Conant and Wolfe 2007).

623   While the ecology of fermentative species is not well known, many have been isolated

624   from insects and may be transported to high-sugar environments (Kurtzman et al. 2011).

625   However, the recent evolution of traits that contribute to *S. cerevisiae*'s dominance of

626   high-sugar environments is perplexing. Although both *S. cerevisiae* and *S. paradoxus* can

29

627   be found in vineyards (Redzepović et al. 2002; Hyma and Fay 2013), these and other

628   *Saccharomyces* species are commonly associated with tree bark, soil and decaying leaves

629   (Naumov et al. 1998; Sniegowski et al. 2002; Zhang et al. 2010; Wang et al. 2012; Hyma

630   and Fay 2013). Given their abundance in arboreal habitats, it seems unlikely that their

631   exceptional fitness in grape juice is due to adaptation to grape juice fermentations and

632   thereby provides evidence for exaptation (Larson et al. 2013). One way in which these

633   species may have become adapted to high-sugar but low-nutrient environments is through

634   associations with insect-honeydew, which is high in sugar (>10 g/l) but low in amino

635   acids (Douglas 1993; Fischer and Shingleton 2001; Fischer et al. 2002). While a variety

636   of insects and other animals exploit honeydew for its sugar resources (Beggs and Wardle

637   2006), and a recent investigation revealed that many taxonomically diverse fungi

638   compete for honeydew (Dhami et al. 2013), no *Saccharomyces* species were found

639   associated with aphid honeydew from Black Beach trees in New Zealand (Serjeant et al.

640   2008). It is also possible that *S. cerevisiae* is not adapted to a particular niche but is a

641   generalist that happens to be particularly fit in high-sugar environments (Goddard and

642   Greig 2015).

643   In addition to dominance relationships we studied, a variety of other factors may

644   contribute to *S. cerevisiae*'s observed dominance of grape juice fermentations in

645   vineyards. Many WGD species may not be present within vineyard environments. Of the

646   non-*Saccharomyces* species used in this study, only the non-WGD species *Z. rouxii*, *T.*

647   *delbrueckii*, *L. thermotolerans*, and *H. vineae* have been reportedly isolated from grapes

648   or wine must (Kurtzman et al. 2011). Furthermore, sulfites are frequently added to wine

649   must, and wine strains of *S. cerevisiae* are known to exhibit higher levels of sulfite

650   resistance than non-wine strains (Pérez-Ortín et al. 2002; Yuasa et al. 2004). Interspecific

651   differences in sulfite resistance (Engle and Fay 2012), copper resistance (Warringer et al.

652   2011) and other environmental conditions that we did not examine may thus contribute to

653   *S. cerevisiae*'s observed dominance of wine fermentations. Although we find *S.*

654   *paradoxus* to be competitive with *S. cerevisiae* in controlled laboratory settings, it has

655   been reported to be the dominant yeast species in only a few fermentations (Redzepović

656   et al. 2002; Valero et al. 2007). Complicating such comparisons, however, is the

657   possibility that *S. paradoxus* may not have always been distinguishable from *S.*

658   *cerevisiae*, particularly in earlier studies of dominant yeast species from spontaneous

659   wine fermentations.

660

661   **Acknowledgements**

662

666

667   **Literature Cited**

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941 **Figure Legends**

942

943 **Figure 1. Phylogenetic relationship of yeast species used in this study.** The phylogeny

944 is based on two previous studies (Kurtzman and Robnett 2003; Salichos and Rokas 2013)

945 and the placement of the *Nakaseomyces* (*C. glabrata* and *N. bacillisporus*) using

946 chromosome rearrangements (Scannell et al. 2006). The whole genome duplication

947 (WGD) event is shown by a star.

948

949 **Figure 2. Fitness differences of *S. cerevisiae* relative to WGD and non-WGD yeast**

950 **species after co-culture or mono-culture in two high-sugar environments.** The

951 fitness of each species in Grape (A) and HS (B) co-cultures and Grape (C) and HS (D)

952 mono-cultures. Bars and whiskers represent the mean and standard error (n = 3) of the

953 difference between the competitor fitness (w_c) and the *S. cerevisiae* (YPS163) reference

954 fitness (w_r), which is set to one. Species labels are Scer (*S. cerevisiae*), Spar (*S.*

955 *paradoxus*), Smik (*S. mikatae*), Suva (*S. uvarum*), Cgla (*C. glabrata*), Ncas (*N. castellii*),

956 Vpol (*V. polyspora*), Tdel (*T. delbruekii*), Lthe (*L. thermotolerans*), Lwal (*L. waltii*), Lklu

957 (*L. kluyveri*), Hvin (*H. vineae*) and WGD and non-WGD species are indicated. Fitness

958 significantly different from the reference is shown for FDR < 0.01 (*).

959

960 **Figure 3. Fitness differences depend on temperature and growth medium.** Fitness

961 differences between each competitor species and a reference strain are shown for

962 competitions at 30°C in Grape (A) or HS (B) and at 22°C in Grape (C) or HS (D)

47

963 medium. Reference strains are three *S. cerevisiae* strains (YPS163, BJ20 and I14) and *S.*

964 *paradoxus* (Spar). Bars and whiskers represent the mean and its standard error (n = 3).

965 Significant differences in fitness are shown for FDR < 0.05 (*). Species abbreviations are

966 the same as in Figure 2.

967

968 **Figure 4. Intrinsic growth rate differences in high-sugar environments.** The intrinsic

969 growth rate of each species in Grape (A) and HS (B). WGD and non-WGD species are

970 indicated. Bars and whiskers represent the mean and standard deviation of the growth rate

971 (n = 3). Species that did not differ significantly from *S. cerevisiae* at an FDR cutoff of

972 0.01 are indicated (NS).

973

974 **Figure 5. Species differences in ethanol tolerance.** Mean (bars) and standard error

975 (whiskers) of the concentration of ethanol (%) that inhibits growth by 50% (IC_{50}) of each

976 yeast species (n = 3). WGD and non-WGD species are indicated. Species with an IC_{50} that

977 did not differ significantly from *S. cerevisiae* at an FDR cutoff of 0.01 are indicated (NS).

978

979 **Figure 6. Intrinsic growth differences in response to low-pH.** (A) The effect of low-

980 pH treatment on the intrinsic growth rate (*r*) of each species in HS ($\Delta r_{Treatment} = r_{Treatment} -$

981 r_{HS}), and (B) the difference in the intrinsic growth rate between *S. cerevisiae* and each

982 species in low-pH HS ($\Delta r_{Species} = r_{non\text{-}S.\ cerevisiae} - r_{S.\ cerevisiae}$). WGD and non-WGD species are

983 indicated. Whiskers for each bar show 95% confidence intervals (n = 3). Significant

984 differences in the growth rate of each species with or without low-pH and differences

48

985   between *S. cerevisiae* and each species in low-pH are shown for FDR < 0.01 (*) and FDR

986   < 0.001 (**).

987

988   **Figure 7. Intrinsic growth rate in Grape supplemented with nutrients.** The mean

989   intrinsic growth rate of each species in Grape supplemented with nutrients (YP). WGD

990   and non-WGD species are indicated. Bars and whiskers represent the mean and standard

991   deviation of the growth rate (n = 3). Diamonds represent the mean growth rate in Grape

992   without YP. Significant differences in the growth rate of each species with or without YP

993   (a) and differences between *S. cerevisiae* and each species in YP (b) are labeled above

994   each bar for FDR < 0.01.

995

996   **Supplemental Information**

997

998   **Table S1. Yeast strains used in this study.**

999

1000   **Table S2. PCR and pyrosequencing primers.**

1001

1002   **Table S3. PCR primers, barcodes and adaptors used for high-throughput**

1003   **sequencing.**

1004

1005   **Table S4. Significance of changes in the relative abundance of *S. cerevisiae* com-**

1006   **pared to other yeast species in high-sugar environments.**

49

1007

Table S5. Significance of intrinsic growth rate differences between *S. cerevisiae* and other yeast species in high-sugar environments.

1010

Table S6. Significance of IC$_{50}$ and intrinsic growth rate differences between *S. cerevisiae* and other yeast species in ethanol (%).

1013

Table S7. Significance of intrinsic growth rate differences between each species' own supernatant and supernatant from *S. cerevisiae* mono-culture and co-culture.

1016

Table S8. Significance of low-pH on the intrinsic growth rate of *S. cerevisiae* and other species in HS.

1019

Table S9. Significance of nutrient supplements on the intrinsic growth rate of S. *cerevisiae* and other species in Grape.

1022

1023

1024

Figure S1. Pyrosequencing calibration. Relationship between the known frequency (%) of the reference species based on cell density and the estimated frequency (%) of the reference species based on pyrosequencing relative to *S. cerevisiae* (YPS163). Reference

1028   species include *S. cerevisiae* (I14) (A), *S. paradoxus* (B), *S. mikatae* (C), *S. uvarum* (D),

1029   *C. glabrata* (E), *N. castellii* (F), *V. polyspora* (G), *Z. rouxii* (H), *T. delbrueckii* (I), *L.*

1030   *thermotolerans* (J), *L. waltii* (K), *L. kluyveri* (L), and *H. vineae* (M). Calibration

1031   equations based on linear or polynomial regression analysis and *R*-squared values for

1032   each model are indicated.

1033

1034   **Figure S2. High-throughput sequencing calibration.** Relationship between the known

1035   frequency (%) of the reference species based on cell density and the estimated frequency

1036   (%) of the reference species based on high-throughput sequencing. Panels show the

1037   relative abundance of the *S. cerevisiae* reference (YPS163) in comparison to *S.*

1038   *paradoxus* (A), *S. mikatae* (B) *S. uvarum* (C), *K. lodderae* (D), *C. glabrata* (E) and *N.*

1039   *castellii* (F); and the relative abundance of *S. paradoxus* (YPS152) in comparison to *S.*

1040   *mikatae* (G), *S. uvarum* (H), *K. lodderae* (I), *C. glabrata* (J) and *N. castellii* (K). *K.*

1041   *lodderae* and *N. castellii* calibrations with *S. cerevisiae* are based on ACT1; all others are

1042   based on ITS1. Calibration curves (blue lines) and *R*-squared values are shown in each

1043   panel.

1044

1045   **Figure S3. The effect of ethanol on the intrinsic growth rate of each species.** Each

1046   line shows mean intrinsic growth rate (*r*) of an individual species based on three

1047   replicates as a function of ethanol (%) added at the beginning of growth. Significant

1048   differences in the growth rate of each species and *S. cerevisiae* are shown for FDR < 0.01

1049   (shaded region). Error bars have been omitted for clarity.

1050

1051

1052 **Figure S4. Intrinsic growth rate in YPD made with supernatant.** The intrinsic growth

1053 rate of each species (A-O) in YPD made with the supernatant from each species' own

1054 supernatant when grown in mono-culture (red), the supernatant from *S. cerevisiae*

1055 (YPS163) grown in mono-culture (blue), and the supernatant from co-culture with *S.*

1056 *cerevisiae* (green). Species are *S. cerevisiae* (I14) (A), S. *paradoxus* (B), *S. mikatae* (C),

1057 *S. uvarum* (D), *K. lodderae* (E), *K. martiniae* (F), *N. castellii* (G), *C. glabrata* (H), *V.*

1058 *polyspora* (I), *Z. rouxii* (J), *T. delbrueckii* (K), *L. thermotolerans* (L), *L. waltii* (M), *K.*

1059 *lactis* (N), and *H. vineae* (O). Bars and whiskers represent the mean and standard

1060 deviation of the growth rate.

1061

1062 **Figure S5. Intrinsic growth rate in Grape supplemented with various nutrients.** The

1063 mean intrinsic growth rate of each species in Grape supplemented with CM (A), NB (B),

1064 AA (C) and AS (D). Names of WGD (blue) and non-WGD (red) species are colored.

1065 Bars and whiskers represent the mean and standard deviation of the growth rate.

1066 Significant differences in the growth rate of each species with or without the added

1067 nutrient (a) and differences between *S. cerevisiae* and each species with the added

1068 nutrient (b) are labeled above each bar for FDR < 0.01.

Figure 1
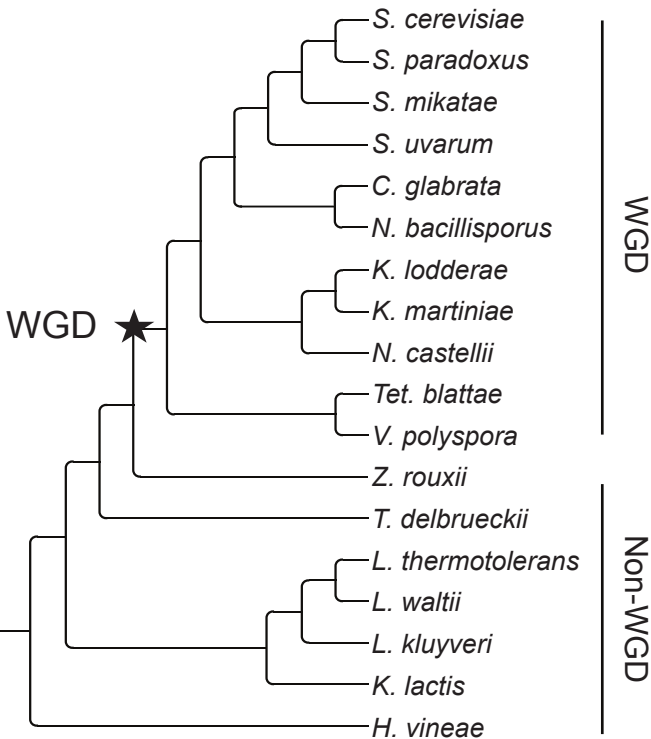
Figure 2

Figure 3

Figure 4

A

Grape mono-culture

B

HS mono-culture

Figure 5

Figure 6

Figure 7

Figure S1 A-F

Figure S1 G-L

G

V. polyspora

$R^2 = 0.994$

$y = 0.9666\,x + 4.5497$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

H

Z. rouxii

$R^2 = 0.996$

$y = 3.4147 + 1.5801\,x - 0.0066x^2$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

I

T. delbrueckii

$R^2 = 0.996$

$y = 0.6496 + 0.3513x + 0.006\,x^2$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

J

L. thermotolerans

$R^2 = 0.999$

$y = -1.7112 + 0.8765\,x + 0.0014\,x^2$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

K

L. waltii

$R^2 = 0.983$

$y = 7.033 + 1.1548\,x - 0.0025\,x^2$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

L

L. kluyveri

$R^2 = 0.996$

$y = 0.6496 + 0.3513\,x + 0.006\,x^2$

Known frequency (%) of reference (cell density)

Estimated frequency (%) of reference (pyrosequencing)

Figure S1 M

M

H. vineae

$R^2 = 0.99$

$y = 0.3413 + 1.814\,x - 0.0082\,x^2$

Estimated frequency (%) of reference (pyrosequencing)
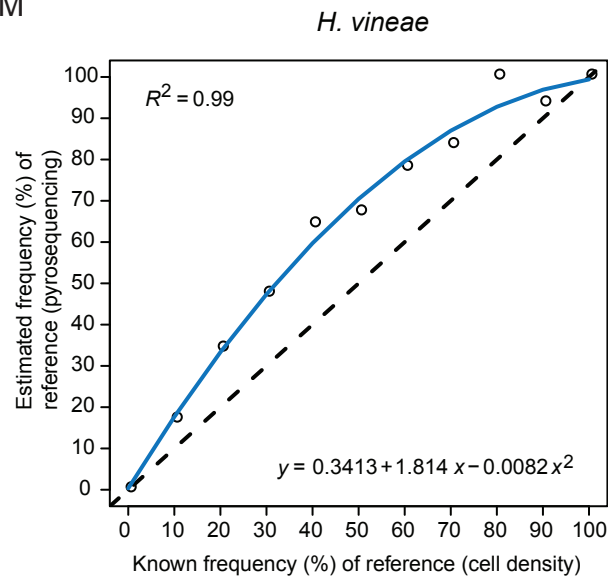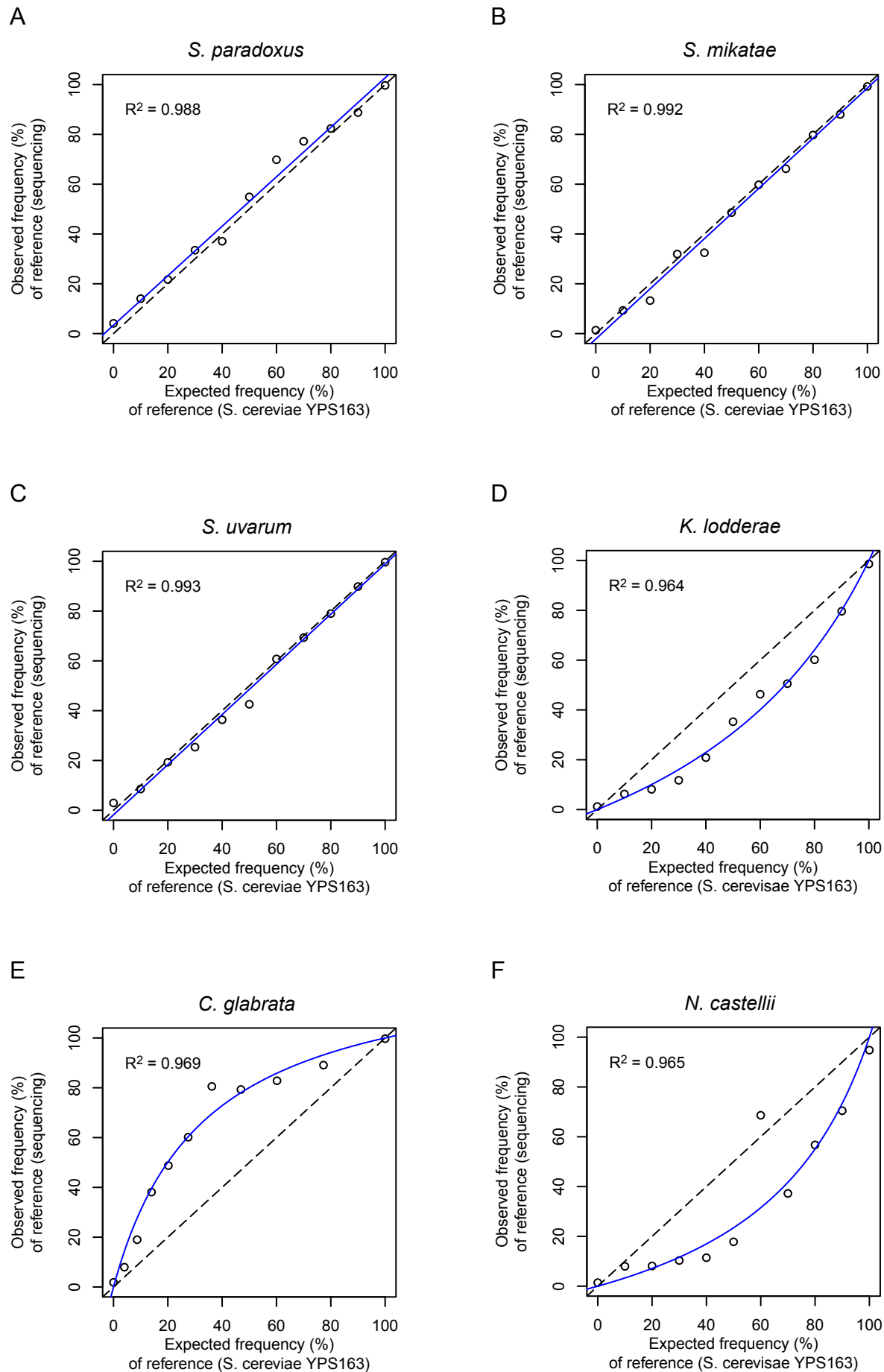
Known frequency (%) of reference (cell density)

Figure S1. Pyrosequencing calibration. Relationship between the known frequency (%) of the reference species based on cell density and the estimated frequency (%) of the reference species based on pyrosequencing relative to *S. cerevisiae* (YPS163). Reference species are *S. cerevisiae* (I14) (A), *S. paradoxus* (B), *S. mikatae* (C), *S. uvarum* (D), *C. glabrata* (E), *N. castellii* (F), *V. polyspora* (G), *Z. rouxii* (H), *T. delbrueckii* (I), *L. thermotolerans* (J), *L. waltii* (K), *L. kluyveri* (L), and *H. vineae* (M). Calibration equations based on linear or polynormial regression analysis and R-squared values for each model are shown.

Figure S2 A-F

A — *S. paradoxus*, $R^2 = 0.988$
B — *S. mikatae*, $R^2 = 0.992$
C — *S. uvarum*, $R^2 = 0.993$
D — *K. lodderae*, $R^2 = 0.964$
E — *C. glabrata*, $R^2 = 0.969$
F — *N. castellii*, $R^2 = 0.965$
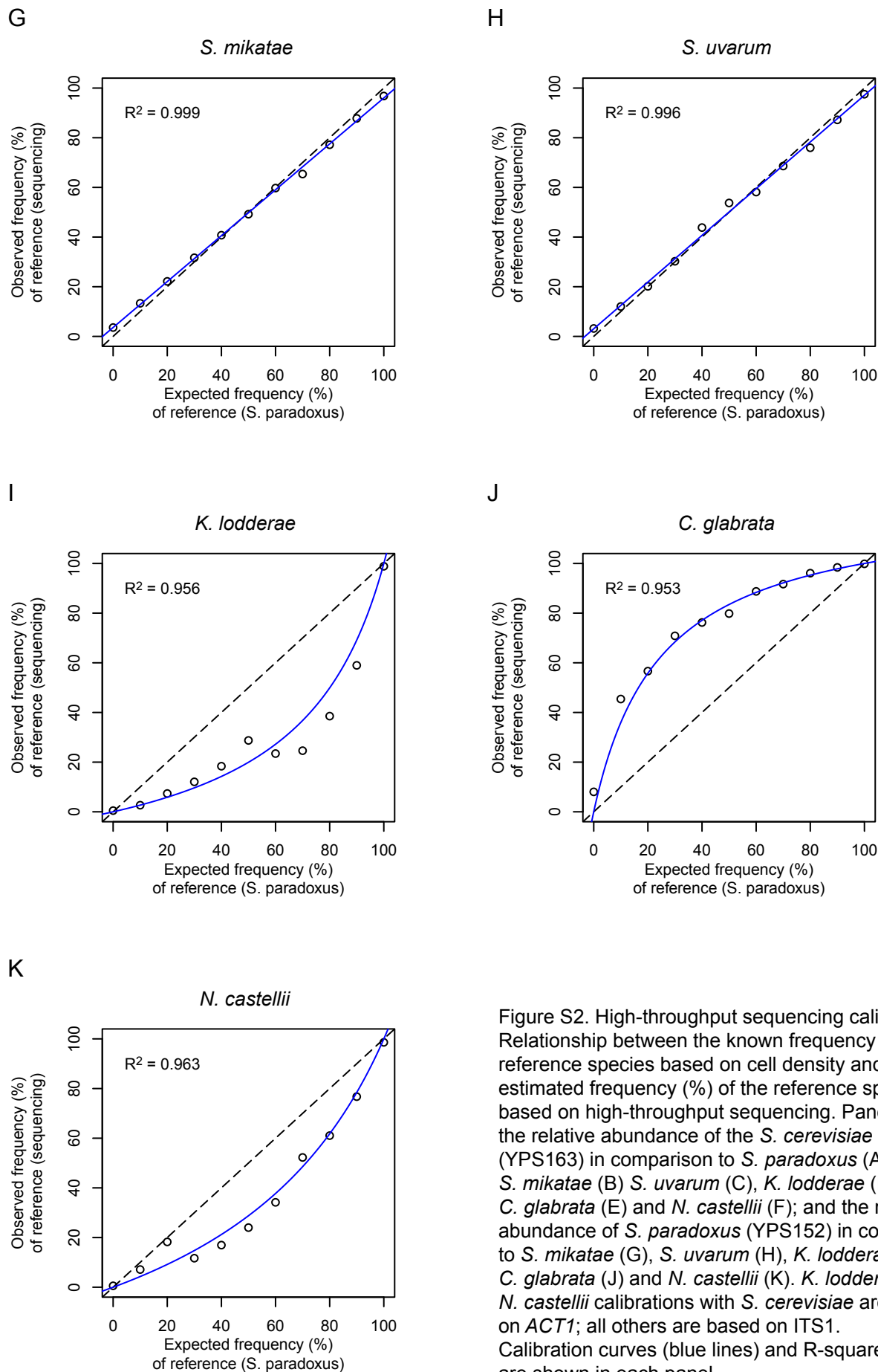
Figure S2 G-K

Figure S2. High-throughput sequencing calibration. Relationship between the known frequency (%) of the reference species based on cell density and the estimated frequency (%) of the reference species based on high-throughput sequencing. Panels show the relative abundance of the *S. cerevisiae* reference (YPS163) in comparison to *S. paradoxus* (A), *S. mikatae* (B) *S. uvarum* (C), *K. lodderae* (D), *C. glabrata* (E) and *N. castellii* (F); and the relative abundance of *S. paradoxus* (YPS152) in comparison to *S. mikatae* (G), *S. uvarum* (H), *K. lodderae* (I), *C. glabrata* (J) and *N. castellii* (K). *K. lodderae* and *N. castellii* calibrations with *S. cerevisiae* are based on *ACT1*; all others are based on ITS1. Calibration curves (blue lines) and R-squared values are shown in each panel.
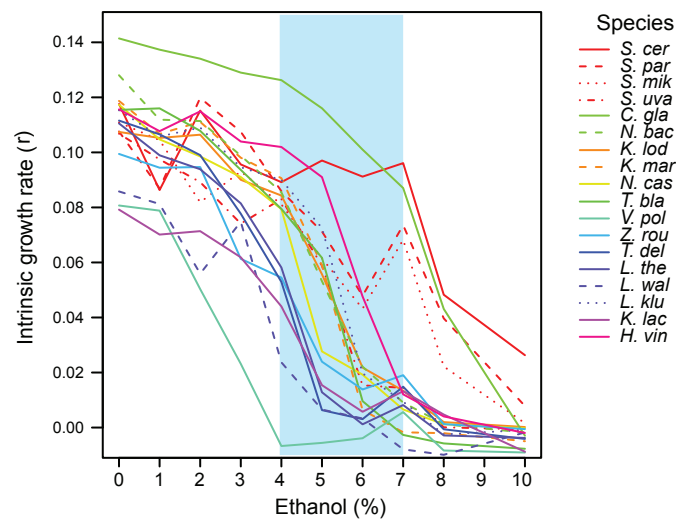
Figure S3

Figure S3. The effect of ethanol on the instrinsic growth rate of each species. Each line shows mean intrinsic growth rate (r) of an individual species based on three replicates as a function of ethanol (%) added at the beginning of growth. Significant differences in the growth rate of each species and *S. cerevisiae* are shown for FDR < 0.01 (shaded region). Error bars have been omitted for clarity.
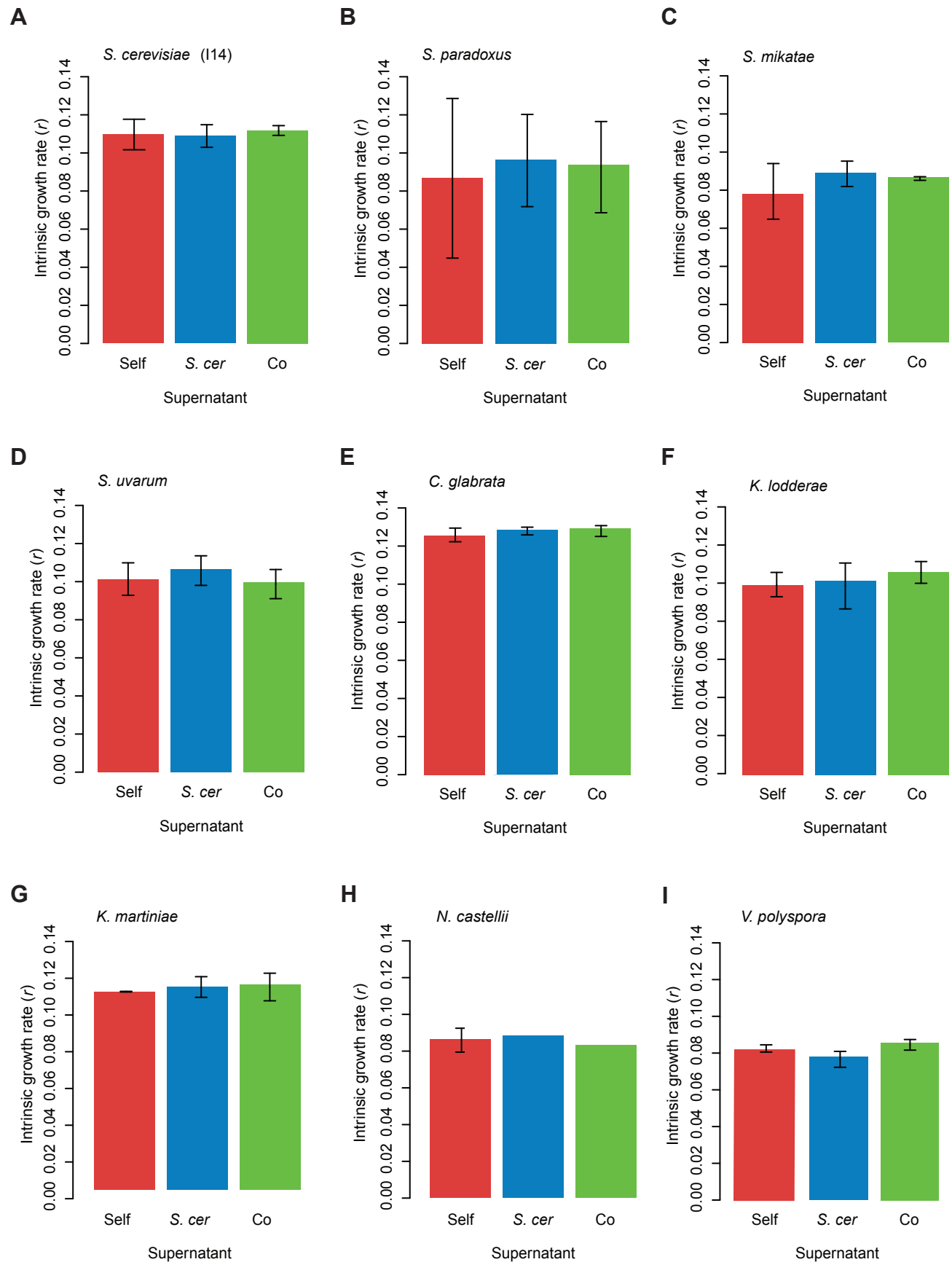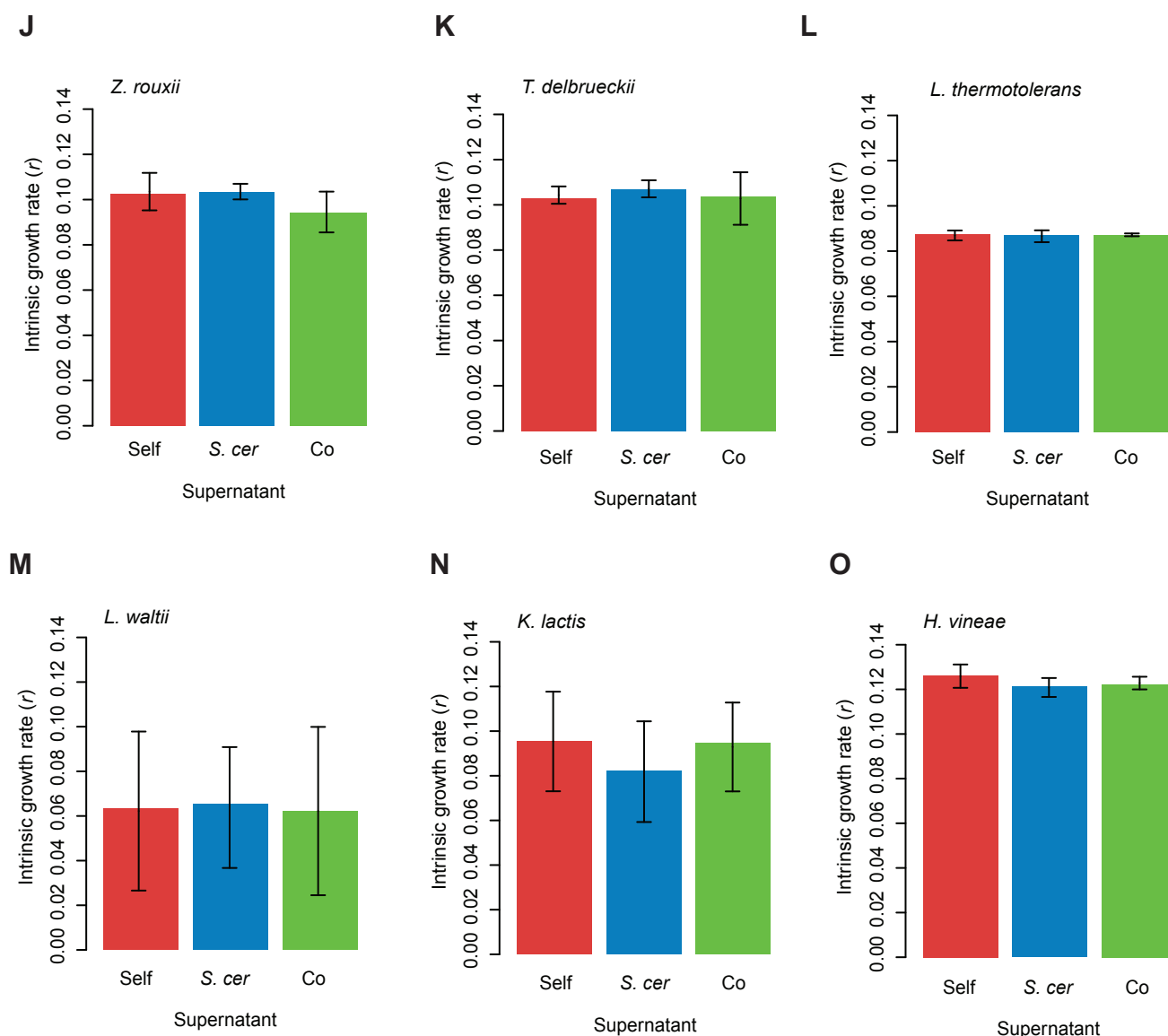
Figure S4 A-I

Figure S4 J-O

Figure S4. Intrinsic growth rate in YPD made with supernatant. The intrinsic growth rate of each species (A-O) in YPD made with the supernatant from each species' own supernatant when grown in mono-culture (red), the supernatant from *S. cerevisiae* (YPS163) grown in mono-culture (blue), and the supernatant from co-culture with *S. cerevisiae* (green). Species are *S. cerevisiae* (I14) (A), *S. paradoxus* (B), *S. mikatae* (C), *S. uvarum* (D), *K. lodderae* (F), *N. castellii* (G), *C. glabrata* (H), *V. polyspora* (I), *Z. rouxii* (J), *T. delbrueckii* (K), *L. thermotolerans* (L), *L. waltii* (M), *K. lactis* (N), and *H. vineae* (O). Bars and whiskers represent the mean and standard deviation of the growth rate.
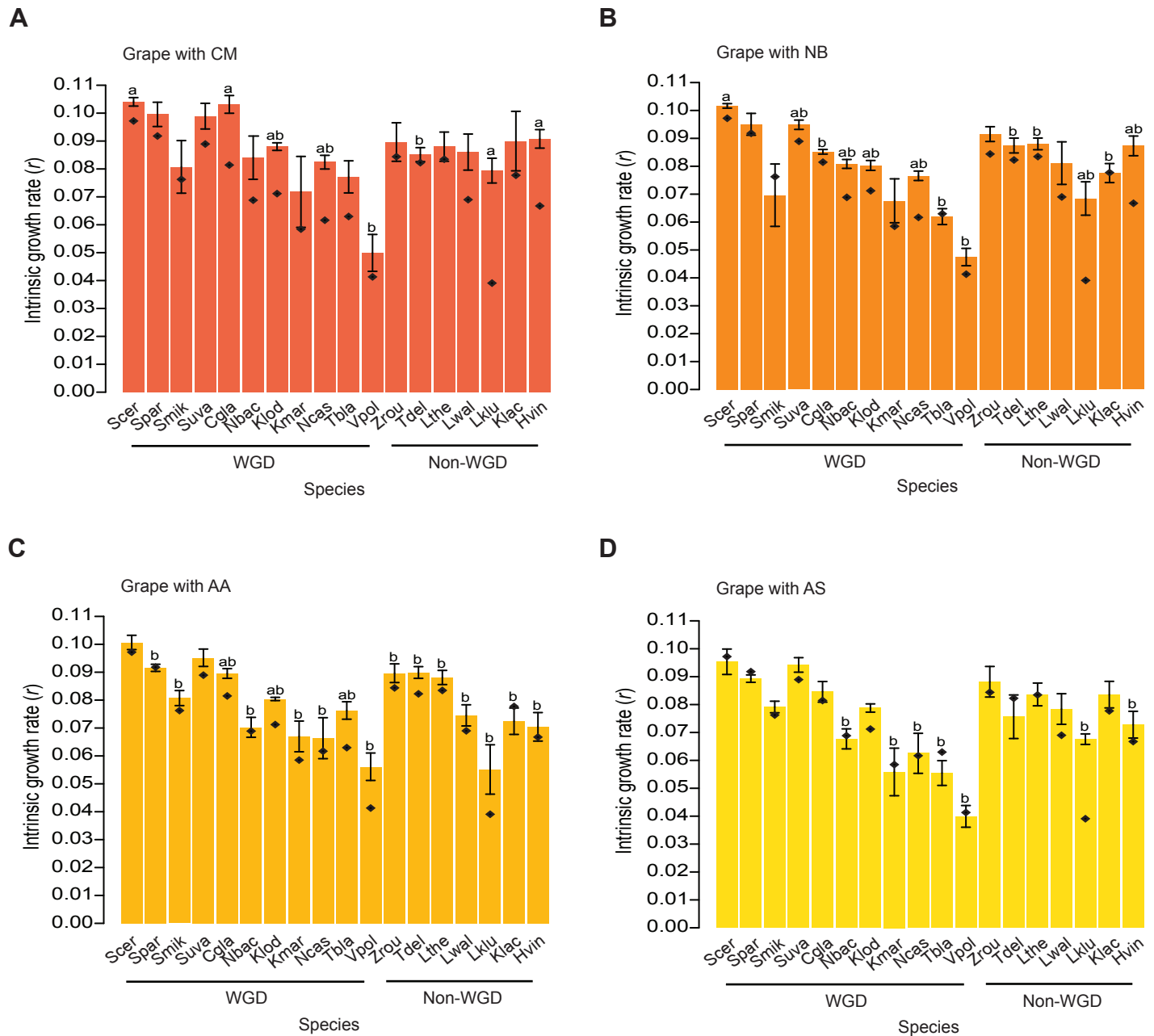
Figure S5

Figure S5. Intrinsic growth rate in Grape supplemented with various nutrients. The mean instrinsic growth rate of each species in Grape supplemented with CM (A), NB (B), AA (C) and AS (D). WGD and non-WGD species are indicated. Bars and whiskers represent the mean and standard deviation of the growth rate. Diamonds represent the average growth rate in Grape without supplements. Significant differences in the growth rate of each species with or without the added nutrient (a) and differences between *S. cerevisiae* and each species with the added nutrient (b) are labeled above each bar for FDR < 0.01.