

Geographic Origin and Diversity of Wine Strains of *Saccharomyces*

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Abstract: The availability of genome sequence information from a large collection of strains of *Saccharomyces* isolated from a variety of geographic regions and ecological niches has enabled a detailed analysis of genome composition and phenotype evolution, the two components of strain diversity. These analyses have also provided a relatively complete depiction of the origins of wine strains. In population genomic analysis, wine strains of *S. cerevisiae* cluster as a highly related group, but one that shows a greater level of phenotypic differentiation than would be predicted based on the level of genomic similarity. Natural and human selection and genetic drift have played roles in the evolution of wine strain diversity. Phenotypic diversity is so extensive that no one strain accurately represents all wine strains with respect to biological properties and fermentation performance. In addition, both commercial and native isolates have been found to carry introgressions, regions of DNA derived from nonhomologous organisms, suggestive of cell fusion events with yeast of different genera and species. Comparative sequence analysis has thus refined our knowledge of yeast lineages and offers explanation for the evolution of phenotypic diversity observed in winery and vineyard populations.

Key words: Yeast diversity, *Saccharomyces*, genomic analysis

The yeast *Saccharomyces cerevisiae* was identified as the agent of beverage fermentation by Louis Pasteur in 1860 (Pasteur 1860). After roughly 150 years of research, our understanding of

27 the biology of this important organism is extensive. The completion of the sequence of the
28 genome of a laboratory strain, S288C, facilitated intensive study of the numbers of genes
29 present in this organism, their functions, and mechanisms of regulation (Oliver et al. 1998). The
30 subsequent sequencing of the genomes of other strains of *Saccharomyces* from diverse
31 ecological niches and of related and unrelated yeast species has generated novel insights into
32 the origin of the modern wine strain lineage, the evolution of phenotypes and genetic diversity,
33 and the biological plasticity of this important industrial organism (Bullard et al. 2010, Carreto et
34 al. 2008, Cavalieri 2009, Cubillos et al. 2011, Diezmann and Dietrich 2009, Ezeronye and
35 Legras 2009, Fay and Benavides 2005a, 2005b, Legras et al. 2007, Lelandais et al. 2011, Liti et
36 al. 2009, Muller and McCusker 2011, Roberts and Oliver 2010, Schacherer et al. 2009, Spor et
37 al. 2009, Warringer et al. 2011).

38 Even though different types of tools and analyses have been applied to the investigation of
39 genetic diversity within *S. cerevisiae*, the single truth that has emerged is that no one strain
40 phenotypically portrays the entire species (Kvitek et al. 2008, Liti and Schacherer 2011).
41 Concerns with use of the strain S288C as characteristic of the species have arisen because this
42 strain derives from a mosaic lineage constructed in the laboratory (Mortimer and Johnston
43 1986) that has never been exposed to native environments or to natural selection (Liti and
44 Schacherer 2011, Warringer et al. 2011). This strain shows genetic variation and growth and
45 expression profiles not observed in wild isolates and rarely clusters with other lineages
46 (Warringer et al. 2011). A second important overall conclusion of these studies is that the
47 diversity that exists is well explained by the genetic phenomena that have been characterized in
48 laboratory strains suggesting that genetic cycles, DNA exchange, cell fusion, and spontaneous
49 mutagenesis that have been so successful in the exploitation of *Saccharomyces* as a model
50 system for research also occur in the wild (Dequin and Casaregola 2011, Lelandais et al. 2011,
51 Sipiczki 2011).

52 Comprehensive analysis of isolates from diverse geographical areas and from different
53 ecological environments within those areas identified five distinct lineages of *Saccharomyces*
54 strains: West African, Malaysian, North American, Sake, and European (Liti et al. 2009, Liti and
55 Schacherer 2011). Strains also group by technological origin (Legras et al. 2007). Analysis of
56 isolates of *Saccharomyces* from throughout the world found that 95% of the wine, winery, and
57 vineyard isolates were grouped in the same cluster with cider strains (Legras et al. 2007). Bread
58 yeasts in contrast were divided into two different groups (Legras et al. 2007). Among the wine
59 yeast distinct subgroupings could be identified. The majority of strains belonged to the Central
60 European group but other clusters, Champagne yeast and the UCD522 group, were also
61 evident (Legras et al. 2007). The UCD522 and Champagne clusters cross geographic origin and
62 are due to the influence of human selection in wine production, as progenitors of these strains
63 were likely introduced into these diverse winemaking regions. These analyses have led to the
64 conclusion that lineage or parentage and technological use have as much of an influence on
65 strain diversity as geographical region. In contrast, strains of *Saccharomyces* from
66 nontechnological origins (fruit, soil, oak, and insects) and other species such as *S. paradoxus*
67 that have not undergone rounds of domestication show a stronger clustering by region of
68 isolation (Liti et al. 2009, Liti and Schacherer 2011).

69 The striking genetic similarity of winery and vineyard isolates has been taken as evidence of a
70 single domestication event followed by dispersal of the domesticated strains (Legras et al. 2007,
71 Sicard and Legras 2011). The first historical evidence of wine production comes from the
72 discovery of tartaric acid in ancient jars of Mesopotamia and suggests a sophisticated wine
73 industry existed in 5000 to 5400 BC (McGovern et al. 1996). The point of origin of the first wine
74 strain is thought to be in what was Mesopotamia, with strain lineages spread as grapevines
75 were spread (Legras et al. 2007). Grapes were domesticated around the same time, suggesting
76 that yeast domestication occurred simultaneously with that of grapes (Sicard and Legras 2011).

77 Analyses of yeast genomic diversity of strains isolated from different technological sources are
78 most consistent with independent domestication events having occurred for wine, beer, sake,
79 and palm wine fermentations, with subsequent dispersion of those yeasts as the technology for
80 production of these beverages spread from region to region (Sicard and Legras 2011).

81 Fermentations represent very specific ecological niches that do not vary widely geographically,
82 thus constraining the evolution of geographic diversity. However, human activities have clearly
83 played a strong role in both the encouragement and limitation of wine strain diversity.

84 There is clear evidence in wine strain lineages of the imposition of evolutionary bottlenecks
85 (Sicard and Legras 2011). An evolutionary bottleneck occurs when there is a strong selection
86 against the majority of the strain diversity and in favor of one or a few traits in an ecological
87 niche. In the case of wine strains, a bottleneck appears to have been introduced due to the use
88 of sulfite as an antimicrobial agent during wine production (Pérez-Ortín et al. 2002, Yuasa et al.
89 2004). A rare chromosomal rearrangement occurred in one yeast lineage that resulted in the
90 creation of a dominant allele of the sulfite pump *SSU1-R* that confers a high level of resistance
91 to sulfite (Pérez-Ortín et al. 2002, Yuasa et al. 2004). Roughly 50% of the modern day wine
92 isolates examined carry this specific chromosomal rearrangement (Yuasa et al. 2004). The
93 widespread occurrence in nature of this specific chromosomal rearrangement suggests that the
94 pervasive use of sulfite conferred such a growth advantage to strains carrying *SSU1-R* that the
95 allele became fixed in the population. Similarly copper resistance is also predominant in wine
96 strains, suggesting the use of copper in the vineyard strongly selected against strains that were
97 copper sensitive (Kvitek et al. 2008).

98 Although analyses of genome structure suggest that wine strains are a highly related group
99 when compared to the diversity of other members of the genus and species, there is significant
100 phenotypic variation among wine strains. Interestingly, vineyard and winery strains do not
101 segregate as separate clusters regardless of region of isolation, suggesting that these two

102 populations mix freely. The discussion of whether yeast originates in the vineyard or the winery
103 is moot at this point as there is too much exchange between these anthropic environments,
104 likely mediated by insect transfer. It is also clear from these studies that the introduction of
105 commercial strains will add to the phenotypic mix of the overall indigenous population and may
106 provide genetic diversity that would not have arisen if these strains had not been introduced.
107 The widespread penetration of the UCD522 genome across diverse wine-producing regions
108 demonstrates that introduction of a genotype that is more fit for a technological environment will
109 lead to spread of that genotype in the formation of mosaic lineages (Legras et al. 2007). Yeast
110 strain diversity arises from a combination of genetic phenomena: spontaneous mutation and
111 genetic drift, positive and negative trait selection imposed by ecological niche, inherent
112 chromosomal instability, high rates of homologous recombination, and presence of
113 nonhomologous chromosomal rearrangements.

114 **Genetic Mechanisms Underlying the Evolution of Genetic Diversity in *Saccharomyces***

115 There are several genetic mechanisms by which genomes may change and phenotypic diversity
116 emerge. Mutations or alterations of the coding sequence of the parental DNA can arise either
117 due to DNA damage caused by external agents or more commonly by the oxidative
118 intermediates of metabolism or errors in DNA replication or repair. Mutations may be beneficial,
119 neutral, or detrimental to the cell depending upon its environment. Thus heterozygosity, the
120 occurrence of allele diversity for a specific gene, happens naturally in yeast populations of
121 individuals giving rise to genomic variation. Both sexual and asexual reproduction contribute to
122 further population diversity. Recombination between chromosomes and loss of chromosome
123 integrity can occur during asexual reproduction. Chromosomal repair mechanisms may
124 introduce further genetic change or lead to the loss of one of a pair of alleles, termed *gene*
125 *conversion*. Sexual reproduction by design leads to a reshuffling of genetic material across
126 homologous chromosomes and also yields haploid progeny that will then self-mate

127 (homodiploidization) or mate with a nonrelated haploid (heterodiploidization or outcrossing).
128 Analysis of strain diversity suggests that reproduction in the wild is primarily asexual but that
129 sexual reproduction occurs on average about every 1000 asexual generations (Warringer et al.
130 2011). However, the majority of sexual reproduction occurs between haploids derived from the
131 same parent or from self-fertilization, with outcrossing a rarer event, one in every 50,000
132 divisions (Ruderfer et al. 2006, Tsai et al. 2008, Warringer et al. 2011).

133 Differences in chromosome number (aneuploidy) or numbers of chromosome sets (polyploidy)
134 also occur as a consequence of natural selection and abortive cell cycles or aberrant cell fusion
135 events. Genetic material can also be transferred across unrelated genera and species either
136 due to cell fusion events (hybrid formation) or uptake and stabilization of DNA from the
137 environment (lateral gene transfer). Analysis of wine strain diversity indicates that hybridization,
138 the fusing of the genomes of two unrelated yeasts, as well as introgression, the incorporation of
139 DNA from a different genus or species into the genome of the recipient, have occurred in the
140 wild, creating strains that were more fit for localized vineyard or winery niches (Belloch et al.
141 2009, Dequin and Casaregola 2011, Gonzalez et al. 2007, 2008, Masneuf et al. 1998, Naumova
142 et al. 2005).

143 Wine strains are typically homothallic diploids (Dequin and Casaregola 2011, Mortimer et al.
144 1994), meaning that they contain two sets of chromosomes and are able to undergo sporulation
145 producing four haploid spores. The term *homothallic* refers to the trait of being self-fertile, which
146 in the case of yeast means an isolated haploid cell has the ability to become diploid. Haploid
147 spores may mate with each other following germination reproducing the diploid state. Alternately
148 in homothallic strains, a haploid population displays switching of mating type, allowing mothers
149 and daughter cells to mate regenerating a diploid phase. Extensive crossing over between
150 homologous chromosomes occurs during sporulation. The resolution of crossover regions leads
151 to a reshuffling of the composition of the chromosomes. Recessive mutations can then be

152 expressed in the haploid state, and if these mutations make the strain more fit for its
153 environment, then they may confer a positive growth advantage. Thus wine strains are able to
154 undergo sporulation and quickly regenerate the diploid state from haploid progeny. The genomic
155 composition of the spores will be the same as in the original parental strain but the spores will
156 carry different arrangements of alleles that lead to the expression of different phenotypes. Any
157 recessive mutations that arose during asexual or vegetative growth can then become expressed
158 in the population and leads to the rapid evolution of diverse genotypes and ensures the survival
159 of the species (Sipiczki 2008, Warringer et al. 2011). Cycles of sporulation and mating allow not
160 only for the evolution of novel genomes with selective advantage in an ecological niche but also
161 for the loss of detrimental alleles from the population, a process referred to as genome renewal
162 (Mortimer et al. 1994).

163 Genetic differences or heterozygosities that lead to altered phenotypes are commonly thought
164 of as being due to the appearance of a mutation in a coding sequence creating a protein of
165 modified function or activity. Mutations in *cis* regulatory regions can also occur, changing the
166 timing or level of production of a gene product, which may also lead to the appearance of a
167 novel biological trait. These differences are referred to as single nucleotide polymorphisms,
168 meaning that a single base pair in the DNA has changed. Many such mutations are neutral, that
169 is, do not change either the primary sequence of a protein or its activity or regulation. However,
170 changes in sequence that lead to a nonconservative change in the amino acid composition of
171 the encoded protein may impact the functionality of the protein and lead to the appearance of an
172 altered or new trait in the organism. In some cases these changes may be small insertions or
173 deletions (indels) that alter the protein in some fundamental way or that may lead to loss of
174 function altogether (Dowell et al. 2010). Such mutations arise naturally during DNA replication if
175 a mismatch occurs that is not detected or correctly repaired. If the mutation occurs in a
176 regulatory gene or complex, the impact can be profound and affect the expression of numerous

177 genes. A lack of absolute fidelity in DNA replication ensures population genomic variation will
178 occur and confers a rapid adaptive evolution capability to the population (Sipiczki 2008).

179 A change in phenotype of a cell may lead to selection for subsequent compensatory changes
180 elsewhere in the genome (Sipiczki 2008, Warringer et al. 2011). The initiating mutation may
181 lead to the evolution of subsequent changes that modify the impact of that mutation. These
182 secondary mutations may enhance or suppress the original phenotype or modify its expression
183 in the population. Specific trait variation shows a strong effect of population history (Warringer et
184 al. 2011), meaning that the starting composition of a genome sets the stage for subsequent
185 evolutionary and adaptive changes.

186 Alteration of gene copy number can also lead to the appearance of phenotypic diversity and
187 drive the selection for compensatory mutation. Analysis of a set of deletion mutants of
188 *Saccharomyces* showed that the appearance of aneuploidy was a common occurrence in such
189 strains with entire chromosomes or parts of chromosomes amplified (Hughes et al. 2000). The
190 loss of a gene conferring a selective disadvantage was compensated for by the amplification of
191 a homolog of that gene from another chromosome (Hughes et al. 2000). These rare events
192 conferred such a growth advantage that these cells eventually dominate the population. Even in
193 diploid organisms loss of one allele can lead to haploinsufficiency, the situation in which the loss
194 of one copy of a gene cannot be compensated for by the level of expression of the remaining
195 copy, which can drive amplification of the remaining good copy of the gene (Deutschbauer et al.
196 2005). Such amplifications generally are not precise and result in amplification of a larger
197 portion of the genome. Over-expression of some genes can also be deleterious in yeast and
198 would lead to compensatory mutations. There is some debate in these situations as to whether
199 the rate of mutation itself is increased by the biotic stress imposed by the initiator mutation or
200 whether the domination by the modified population is simply due to the strong growth advantage
201 that it confers to the cells.

202 In addition to this classical view of the origins of genetic diversity, other types of genome
203 rearrangements can also occur that will modify gene expression and therefore lead to the
204 development of altered phenotypes. *Saccharomyces* contains mobile repetitive elements called
205 Ty elements that can hop around the genome (reviewed in Sipiczki 2011). Insertion of a Ty
206 element into a gene may lead to a loss of function if a disruption of the coding sequence occurs.
207 Insertion in a regulatory region can result in an altered expression profile for the gene, which
208 can again lead to an altered phenotype. The presence of regions of homology created by the Ty
209 elements can lead to recombination events across unrelated chromosomes, moving large
210 regions of DNA from one chromosome to another (Sipiczki 2011). Significant diversity in the
211 number and insertional positions of Ty elements has been observed in wine strains (Carreto et
212 al. 2008).

213 Changes in the position and location of genes within chromosomes can arise via other
214 mechanisms and also alter gene expression (Field et al. 2009, Tirosh et al. 2010).

215 Chromosomal DNA is ordered in the nucleus by being woven around protein complexes called
216 nucleosomes that are formed by histones and other chromatin-associated proteins (Jansen and
217 Verstrepen 2011). The position of a gene with respect to nucleosome binding can affect access
218 to RNA polymerase and therefore levels of expression (Lelandais et al. 2011).

219 The chromosomes of wine strains also show a diversity in size and on rare occasion in number
220 (Bidenne et al. 1992, Briones et al. 1996, Carreto et al. 2008, Codon et al. 1998, Infante et al.
221 2003, Izquierdo Canas et al. 1997, Johnston et al. 2000, Vezinhet et al. 1990, Yamamoto et al.
222 1991). Chromosomes are attached to the nuclear membrane via telomeric regions and must
223 deattach during replication as the centromeres become attached to the spindle pole bodies for
224 segregation. On occasion this system fails and the tension on two anchored points of the same
225 chromosome can result in chromosome breakage. Repair mechanisms save both ends of the
226 chromosome but often they are not put back together precisely as they were before the break.

227 This breakage and repair can lead to the significant chromosome or karyotype diversity that has
228 been observed in *Saccharomyces* (Bidenne et al. 1992, Briones et al. 1996, Izquierdo Canas et
229 al. 1997, Johnston and Mortimer 1986, Vezinhet et al. 1990, Yamamoto et al. 1991). The
230 variation in karyotype in wine populations can be significant (Vezinhet et al. 1990, Yamamoto et
231 al. 1991). In addition, given the dynamic nature of telomeric regions, genes located in these
232 regions can be readily amplified during chromosome breakage and repair or due to normal
233 recombination mechanisms. Once multiple copies of a gene exist, those individual copies may
234 then diverge from each other, leading to the evolution of altered functions within the cell. The
235 frequency of chromosomal rearrangements is strain dependent (Carro et al. 2003, Nadal et al.
236 1999). Multiple subpopulations with differing karyotypes may exist in the same fermentation
237 (Longo and Vezinhet 1993, Miklos et al. 1997).

238 Entire sets of chromosomes or changes in cell ploidy also occur (Sipiczki 2011). Modern strains
239 of *Saccharomyces* arose from an ancient genome duplication event that subsequently allowed
240 divergence of duplicated genes to alternative or specialized functions (Kellis et al. 2004). The
241 creation of two copies of a gene allows both to then undergo the process of natural mutation
242 and selection and leads to diversification of cell functions. Increases in ploidy arise either as a
243 failure of cell division or chromosomal segregation but can also arise due to abortive mating or
244 cell fusion events within a species (Sicard and Legras 2011).

245 Hybrid yeast strains may be formed as a consequence of abortive attempts at sexual
246 reproduction between different species of yeast. In this case, cell membranes fuse followed by
247 fusion of the nuclei of the two organisms, creating an unstable number of chromosomes some
248 of which will then be lost (Ramirez et al. 2004). Chromosomal rearrangements also occur,
249 leading to the formation of chimeric chromosomes. Several analyzed wine strains appear to
250 have arisen from natural hybrid formation in nature (Belloch et al. 2009, Gonzalez et al. 2007,
251 2008, Masneuf et al. 1998, Naumova et al. 2005). In some cases hybridization occurred

252 between haploid cells, in other cases diploid cells seem to have been involved or a
253 haploid/diploid cell fusion event. Following hybridization chromosomal loss, rearrangements and
254 chimeric chromosome formation may occur (Belloch et al. 2009) upon which natural selection
255 will operate to enrich for the most-fit outcomes of the hybridization. The surviving populations of
256 these events are stable mitotically, although often not able to engage in sexual reproduction
257 with other strains.

258 In addition to genetic changes within a species another source of genetic diversity can be the
259 introduction of nonnative DNA from other organisms present in the population. This
260 phenomenon is often referred to as lateral gene transfer and is observed in many population
261 studies. In wine strains of *Saccharomyces* introgression of DNA segments from unrelated
262 organisms has also been observed (Novo et al. 2009). It is not clear that introgression arose
263 due to lateral gene transfer, the pickup of DNA from the environment, rather than the residual
264 effects of abortive hybrid formation between unrelated yeasts. Sequence analysis of the
265 commercial strain EC1118 revealed that this strain had undergone three independent
266 introgression events and carries three large blocks of genetic information not typically found in
267 other wine strains of *Saccharomyces*. These introgressions carried a total of 34 functional
268 genes and have resulted in the expression of three relatively unique phenotypic traits (Dequin
269 and Casaregola 2011, Novo et al. 2009).

270 Genetic changes may be neutral, beneficial, or detrimental. Neutral changes would confer no
271 selective disadvantage in the immediate environment but may be useful as conditions change or
272 may become detrimental. Beneficial genome changes confer some selective advantage to the
273 current environment, generally leading to a dominance of that environment by that genotype.
274 Detrimental genome changes can be severe and lead to cell death or may simply make cells
275 less fit for the current environment, relegating those genotypes to a minor role. As with neutral
276 and beneficial mutative changes, detrimental is likewise defined by the environment and what is

277 detrimental under one condition may become beneficial under another. Often in fermentation
278 multiple genotypes exist and the dominating genotype may change during fermentation as
279 alternate genotypes become more favored. The development of a novel trait may be caused by
280 a single mutation or recombination event or by the combinatorial effects of multiple mutations, a
281 phenomenon referred to as quantitative, polygenic, or multilocus inheritance. Several complex
282 genetic traits in wine strains of *Saccharomyces* are polygenic and it is a combination of alleles
283 of divergent genes that leads to greater fitness (Bullard et al. 2010, Cubillos et al. 2011).

284 Genetic diversity arises from the operation of multiple mechanisms of DNA sequence
285 divergence and genome alteration. The underlying processes are random events that are then
286 selected according to environmental constraints. The ultimate selection is for the strain that is
287 the most fit for the environment, that is, that can grow the fastest and attain the highest
288 population density (Warringer et al. 2011). Modification of lag time in adaptation to a new
289 environment seems less important of a selective factor than rate of growth and final cell
290 biomass (Warringer et al. 2011). Subtle changes in an environment can tip the balance of
291 fitness toward other genotypes present as subpopulations, allowing those populations to grow
292 and displace the original dominant genotype. Genotypic change in wine yeast is a dynamic and
293 recurrent process.

294 **Analytical Tools for Assessment of Yeast Diversity**

295 The genomic diversity of wine strains has been assessed in a variety of ways. There are two
296 main features that are used to determine relatedness of genomes: sequence conservation, the
297 differences in primary sequence of coding and non-coding regions, and gene synteny, the
298 maintenance of the relative positioning of genes on a linear chromosome. Often differences in
299 coding regions or in genes with highly conserved gene function may be weighted differently (to
300 define genus) than in those regions or genes that are more free to evolve (to define species or

301 strains). Although yeast do not possess the classic operon structure of bacteria, the
302 colocalization of genes with related functions under the control of a single promoter, unrelated
303 genes that are adjacent or show linkage can be used to define strain relatedness. Rare
304 recombination and crossover events can disrupt gene linkages. If the physical colocalization of
305 genes shows similarity between strains, then those strains have not been separated
306 evolutionarily long enough to demonstrate synteny divergence and are therefore related. Even if
307 the primary sequence has diverged between two strains, a reflection of rates of spontaneous
308 mutagenesis, selection, and adaptation, the conservation of the relative physical positions of the
309 genes can be used to define the relationship of the two strains. Localized positioning also allows
310 mapping of chromosomal rearrangements, the exchange of large regions of DNA between
311 nonhomologous chromosomes, as the large region will maintain the gene positional orientation
312 of the originating chromosome. Primary sequence divergence and loss of gene synteny are
313 caused by different factors in evolution and occur at different rates in populations and thus
314 provide complementary evidence in the delineation of strain lineages and relationships.

315 Two other types of evidence are also useful in determining strain relatedness, the number and
316 position of mobile or repeated sequences, such as the yeast Ty elements and microsatellites,
317 and assessment of the sequence of the mitochondrial genome of yeast (reviewed in Liti and
318 Schacherer 2011). The mitochondrial DNA shows similar properties of primary sequence
319 divergence and is inherited independently of the nuclear genome. The number and positioning
320 of Ty elements can be used to define strain similarity as even though these sequences are able
321 to move within the genome such movement is rare enough that the positional location of these
322 elements can define relatedness of the genomes under investigation (Ness et al. 2006, Rachidi
323 et al. 1999). Microsatellites are regions of repetitive DNA also found in yeast. These regions can
324 serve as sites of the initiation of homologous recombination among nonhomologous
325 chromosomes and thus can be dispersed throughout the genome. Like Ty elements the

326 number, position, and conservation of sequence of microsatellite regions have diagnostic value
327 in defining strain relatedness. Strain differences can also be compared using RiboPrinter
328 technology to assess sequence diversity (Arvik et al. 2005).

329 Three comprehensive studies of yeast diversity used different methodologies. The first and
330 broadest study used microsatellite typing of 651 strains (Legras et al. 2007). As this method is
331 relatively easy to perform, a large collection of isolates can be processed. The clear relatedness
332 of wine strains as a group independent of geographic origin or location (vineyard or winery) was
333 demonstrated in this investigation (Legras et al. 2007). In a second study, oligonucleotide arrays
334 were used to identify regions of sequence dissimilarity across a collection of 63 yeast strains
335 (Schacherer et al. 2007, 2009). Oligonucleotide arrays consist of small roughly 10 to 20mer
336 oligos representing the genetic composition of the sequenced strain S288C (Gresham et al.
337 2006). If a mismatch occurs, meaning that a tested strain has a sequence that differs from that
338 of the template reporter organism, the tested strain will not show hybridization to that spot. This
339 method can be used to identify conserved and nonconserved regions across populations of
340 organisms. However the absence of a signal could mean the loss of a gene or the retention of a
341 modified version of that gene in the tested strain. Therefore, a subsequent analysis of strain
342 diversity combined the use of these tiling arrays to identify divergent readings, followed by
343 analysis of the region of the DNA via PCR amplification to determine if the absence of
344 hybridization was due to deletion of that region of the chromosome or to areas of large
345 sequence polymorphisms (Muller and McCusker 2011). In the majority of cases the absence of
346 signal was confirmed to be due to sequence divergence and not loss of the gene. These
347 researchers were able to then examine the level of sequence divergence across physically
348 colocalized genes to define large regions of sequence polymorphisms, that is, long stretches of
349 DNA that were highly diverse between the tested and template strains. These large sequence

350 polymorphisms were biased toward subtelomeric regions and also revealed the existence of
351 introgressions in wine strains (Muller and McCusker 2011).

352 Another wide-ranging study of yeast diversity used a low-fold coverage of genome sequencing
353 of over 70 strains to identify regions of differences (Liti et al. 2009). Direct sequencing provides
354 a more comprehensive picture of genome variability but is also not biased by use of tiling arrays
355 based on the S288C genome. This analysis also led to the conclusion that wine strains are a
356 more homologous group than would be expected, indicating that a single domestication event
357 likely gave rise to the current diverse population of vineyard and winery isolates, consistent with
358 previous analyses (Fay and Benavides 2005a). Finally, whole organism sequencing has been
359 performed on several yeast strains (Argueso et al. 2009, Borneman et al. 2008, Liti et al. 2009,
360 Novo et al. 2009, Wei et al. 2007). The availability of entire genomes provides the most robust
361 assessment of relatedness and allowed identification of introgressions not only from other
362 species of *Saccharomyces* but also from other yeast genera, providing a more complete
363 depiction of the genomic constitution and population diversity of *Saccharomyces*.

364 The occurrence of linkage disequilibrium in wine strains has also been investigated (Schacherer
365 et al. 2009). Linkage disequilibrium refers to the nonrandom association of alleles of distinct
366 genes and can be thought of as an estimate of the conservation of mutated adjacent alleles
367 within a chromosome. In other words, if a strain inherits allele A of gene A, how likely is it that it
368 will also carry allele B of gene B versus another version of gene B. This analysis showed that
369 linkage disequilibrium was greater in clinical, distillery and laboratory isolates than in wine
370 isolates, indicating that the wine strain lineage is older than that of the other groups of strains
371 (Schacherer et al. 2009).

372 Often, however, the mere existence of a sequence and cataloging of the genetic differences
373 does not provide a complete picture of the phenotypic diversity of strains. Mutations may be

374 neutral and some regions of the genome may tolerate variability without loss of function more
375 than other regions so other tools must be employed to evaluate the impact of the genetic
376 changes defined on the phenotype of the organism (Fay and Benavides 2005b). Neutral
377 changes by definition reflect random genetic drift while those that confer a trait or phenotype
378 can be selected for or against by the environment. The phenotype is the expression of the
379 genotype and seemingly small changes in genotype may have a profound impact on phenotype.
380 Two types of studies have been undertaken to define phenotypes in a comprehensive way to be
381 compared to genotypic characterization. In one of these studies a host of phenotypic traits,
382 growth rates, growth requirements, resistances and sensitivities, to characterize the relationship
383 between genotype and trait variation (Warringer et al. 2011). Such massive screens are
384 invaluable but time-consuming to conduct.

385 Other researchers have relied on comparative transcript profiling (DeRisi et al. 1997,
386 Kuthan et al. 2003, Lashkari et al. 1997, Lockhart et al 1996, Lockhart and Winzeler 2000,
387 Schena et al. 1995, Velculescu et al. 1995) to infer phenotype from the pattern of expression of
388 genes under defined conditions and to define the relationships among strains (Bullard et al.
389 2010, Cavalieri 2009, Kvitek et al. 2008, Lelandais et al. 2011, Rossouw et al. 2009, 2010).
390 Conservation of a transcriptional response would indicate functional relatedness of the
391 organisms under investigation (reviewed in Lelandais et al. 2011). The occurrence of two types
392 of regulatory mutations have been investigated: those acting in *trans*, genes involved in signal
393 generation, signal transduction, or regulated gene expression, and in *cis*, those occurring within
394 the promoter or regulatory region of a gene or group of genes (Cavalieri et al. 2000, Cavalieri
395 2009). Changes in promoter regions that confer altered binding properties for regulatory factors
396 can be found but do not appear to be drivers of diversity (Cavalieri 2009). In contrast mutations
397 that impact the binding capacity or regulatory functionality of *trans* factors have been identified
398 in wild populations and do seem to underlie a component of strain diversity (Cavalieri 2009). A
399 classic example of the impact of *trans* mutations in wine populations is the discovery of mutation

400 of the *SSY1* gene that is involved in sensing external amino acids (Brown et al. 2008, Cavalieri
401 2009). The loss of *SSY1* results in cells unable to detect or respond to external amino acids and
402 was identified in a vineyard population (Brown et al. 2008, Cavalieri et al. 2009). This single
403 mutation impacted the expression of roughly 400 genes (Brown et al. 2008, Cavalieri et al.
404 2009). A change in a transcription factor can result in the appearance of multiple compensatory
405 *cis* changes throughout the genome (Bullard et al. 2010). Proteomic and metabolite profiling has
406 also been used to assess strain differences solely and in comparison to the transcriptome
407 (Rossouw et al. 2008, 2009, 2010). These analyses indicate that, depending upon gene
408 function, transcript profiles have predictive value for protein and metabolic activity. Further,
409 changes in proteome and metabolome reflect changes in the genome and the population history
410 of the strain and have value in defining strain relatedness in spite of the fact that a single
411 mutation can have a broad impact on expression profiles.

412 **Functional Diversity of Wine Strains**

413 The analysis of wine strain diversity begins with the study of genomic relatedness within
414 the genus *Saccharomyces*. There are currently eight recognized species within
415 *Saccharomyces*: *S. arboricolus*, *S. bayanus*, *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S.*
416 *mikatae*, *S. paradoxus*, and *S. pastorianus* (Kurtzman 2003, Kurtzman and Robnett 2003,
417 Naumov 1996, Wang and Bai 2008). *Saccharomyces pastorianus* is a hybrid species thought to
418 have derived from a fusion event of *S. cerevisiae* and *S. bayanus* (reviewed in Dequin and
419 Casaregola 2011). Analysis of trait variation suggests that *S. cerevisiae*, *S. mikatae*, and *S.*
420 *paradoxus* form a cluster of strains with *S. arboricolus*, *S. bayanus*, and *S. kudriavzevii* forming
421 a second cluster (Warringer et al. 2011). *Saccharomyces pastorianus*, *S. cerevisiae*, and *S.*
422 *bayanus* are found associated with fermentations and anthropic environments and *S. paradoxus*
423 and *S. kudriavzevii* have been found in vineyards. That these species inhabit similar ecological
424 niches is confirmed by the discovery of interspecies hybrids. Wine isolates that are hybrids of *S.*

425 *cerevisiae* and *S. kudriavzevii*, *S. cerevisiae* and *S. bayanus*, and a triple hybrid of *S.*
426 *cerevisiae*, *S. kudriavzevii*, and *S. bayanus* have been identified in wine populations (Belloch et
427 al. 2009, González et al. 2006, 2007, 2008, Lopandic et al. 2007, Masneuf et al. 1998, 2002).
428 The majority of vineyard and winery isolates appear to be homothallic diploids and the main
429 mode of cellular reproduction appears to be clonal rather than sexual (Legras et al. 2007).
430 Roughly 28% of the over 600 wine and vineyard isolates examined were homozygous,
431 suggesting that sporulation and self-diploidization occur in the wild (Legras et al. 2007).

432 Two primary species of *Saccharomyces* are found during the alcoholic fermentation: *S.*
433 *cerevisiae* and *S. bayanus* (formerly *S. uvarum*), with *S. cerevisiae* being the more prevalent
434 (Sipiczki 2002). Occasionally, *S. pastorianus* can be found as can the hybrids of these yeasts
435 (Naumov 1996). Sequence comparisons between *S. cerevisiae* and *S. bayanus* indicate ~80%
436 identity of coding sequences and ~74% identity of noncoding sequences (Cliften et al. 2003).

437 *Saccharomyces* can be found in vineyards and winery environments. It is a common resident of
438 winery flora and can be found in the vineyard albeit at a much lower frequency in the overall
439 yeast flora population. Whether or not *Saccharomyces* is a true vineyard resident or just
440 repeatedly introduced to the vineyard by winery operations has been much debated.

441 *Saccharomyces cerevisiae* can be isolated from vineyards even in cases where the practice of
442 placing spent yeast lees in the vineyard as fertilizer has not occurred (Clemente-Jimenez et al.
443 2004, Martini et al. 1996, Torok et al. 1996, Valero et al. 2005). *Saccharomyces* is commonly
444 isolated from heavily damaged grapes (Mortimer and Polsinelli 1999) regardless of the nature of
445 the damage. Mechanical damage to clusters leads to a bloom of yeast residents on the surface
446 of the fruit and an increase in the population size of *S. cerevisiae*. Damage may also occur
447 because of mold-induced cluster rot and *Saccharomyces* can be isolated from these
448 environments as well. However since rotting clusters attract insects and insects are vectors of
449 *Saccharomyces* it is not clear if the *S. cerevisiae* strains present arose from the surface of the

450 fruit or not. Direct inoculation of vineyards with commercial yeasts did not lead to their
451 establishment among the vineyard flora (Comitini and Ciani 2006, Valero et al. 2005), even
452 when damaged berries were inoculated (Comitini and Ciani 2006). Since the level of inoculation
453 in these studies was much higher than would be predicted from insect vectors, these data
454 support the view that *Saccharomyces* can be considered a minor resident of the surface of a
455 grape with conditions leading to the seepage of berry components selecting for *Saccharomyces*
456 proliferation. With creation of fermentative conditions upon crushing of the fruit, even more
457 strongly enriching conditions are presented to this microbe. *Saccharomyces*, initially present
458 below detectable levels in spontaneous grape juice fermentations, will often be found as the
459 dominate species at the end of fermentation, even under aseptic grape cluster harvesting
460 conditions (Comitini and Ciani 2006, Valero et al. 2005). The population density of
461 *Saccharomyces* in a winery is often higher than the density in the vineyard, and in the absence
462 of selective pressure the greater number of winery yeast relative to vineyard yeast will lead to a
463 greater contribution to the fermentation itself by the winery residents. In essence,
464 *Saccharomyces* can be found in both the vineyard and the winery and which strains dominate a
465 fermentation depend upon trait variation and selection imposed by the fermentation process.

466 Significant genetic diversity exists among wine strains of both *S. cerevisiae* whether
467 isolated from the vineyard or winery (Baleiras Couto et al. 1996, Briones et al. 1996, Gallego et
468 al. 2005, Khan et al. 2000, Lopes et al. 2002, Sabate et al. 1998, Schuller et al. 2005, Schütz
469 and Gafner 1993, 1994, Valero et al. 2006, Van der Westhuizen et al. 2000a, 2000b, Versavaud
470 et al. 1995) and *S. bayanus* (Sipiczki 2002). Analysis of over 1,600 isolates of *S. cerevisiae* from
471 54 spontaneous fermentations demonstrated the existence of 297 unique strains (Schuller et al.
472 2005). In a more limited study, 13 out of 16 isolates (81%) were determined to be unique strains
473 and the four identical strains were isolated from the same location (Baleiras Couto et al. 1996).
474 Even higher ratios of unique genotypes have been found—87.5% (Valero et al. 2006), 81 to

475 91% (Gallego et al. 2005), and 91 to 96% (Schuller et al. 2005)—depending on the technique
476 used. The greatest numbers of genotypes in all of these studies are represented by a single
477 isolate, indicating that the true extent of the diversity present in the wild is still being
478 underestimated. Significant strain diversity exists within the same vineyard environment,
479 suggesting the importance of localized conditions for the selection of genetically modified strains
480 or, alternately, the existence of factors driving genetic change. One such factor may be
481 exposure to ultraviolet light. Metabolites produced either by the plant or by other microbes in the
482 environment, such as mycotoxins, may also serve to accelerate the appearance of genetic
483 differences in the absence of any direct selective pressure. Interestingly in studies with large
484 enough populations of both vineyard and winery isolates, strains do not cluster genomically
485 based upon site of origin (Legras et al. 2007), again indicating that there is significant flux and
486 comingling between the vineyard and winery populations.

487 Not surprisingly, the fitness of strains for specific fermentation niches also has been
488 found to vary. In some cases, only one or a few strains dominate throughout fermentation
489 (Versavaud et al. 1995). In contrast, other researchers have found that different strains
490 dominate at different stages of the fermentation (Egli et al. 1998, Sabate et al. 1998, Schütz and
491 Gafner 1993) or that several strains of *Saccharomyces* appear to be simultaneously present in
492 equivalently high numbers (Torija et al. 2001, Vezinhet et al. 1992). Presumably, the biodiversity
493 of wine strains in the environment results in these different patterns of dominance in
494 fermentations. Strains that are dominant in one environment may not show the same degree of
495 dominance in another, because the strain attributes conferring dominance may be best suited to
496 the fermentation conditions of a specific winery or vintage. As conditions of production change,
497 different strains may become dominant. Assessment of strain diversity across vintages has
498 shown that different strains are present each year (Gutierrez et al. 1999, Schuller et al. 2005).

499 The genetic diversity of wine yeasts has also been documented using genomic sequence
500 comparisons and functional genomic analysis of transcript profiles (Borneman et al. 2008, Dunn
501 et al. 2005, Fay et al. 2004, Gresham et al. 2006, Legras et al. 2007, Liti et al. 2006, 2009,
502 Schacherer et al. 2009, Townsend et al. 2003, Tsai et al. 2008, Winzeler et al. 2003). Strains
503 that are undistinguishable from each other by genomic or mitochondrial DNA profiling may carry
504 mutations leading to changes in important enological phenotypes, particularly if the genetic
505 differences are targeted to high-impact genes (such as transcription factors) or genes involved
506 in flavor modification or production. Indeed, analyses of the presence of single nucleotide
507 polymorphisms (SNP) suggest that they exist across populations of *Saccharomyces* with a
508 frequency of ~2.8 SNPs per kilobase of DNA (Schacherer et al. 2009). In a sequence
509 comparison of wine strain AWRI1631 to S288c, a SNP frequency of 1 per 150 base pairs, or
510 roughly 7 SNPs per kilobase, was found (Borneman et al. 2008). SNPs occur less frequently in
511 genes located near the centromere and more frequently for genes located in subtelomeric
512 regions (Schacherer et al. 2009). Deletions of genetic material also occur (Schacherer et al.
513 2009) but are found at a very low frequency in the essential genes. Thus, there is the potential
514 for significant variation in gene expression profiles as a consequence of underlying genetic
515 differences across strains, making comparisons of strains grown under different conditions
516 challenging.

517 The sequence of a commercial isolate of EC1118 revealed that this strain contains large
518 stretches of DNA not normally found in wine yeast and not thought to originate from *S.*
519 *cerevisiae* (Novo et al. 2009). If genetically modified organism (GMO) is defined as the
520 presence of nonnative DNA, then EC1118 would be considered to be a naturally arising GMO.
521 The genes of these regions contained by EC1118 carry functional genes that impact the
522 fermentation phenotypes of this strain. EC1118 possesses an analog of the *FSY1* gene also
523 found in *S. pastorianus* that encodes a fructose proton symporter (Galeote et al. 2010). If

524 functional as such in EC1118, this transporter would couple fructose movements to those of
525 protons and lead to more efficient transport of fructose into the cell. This transporter has a high
526 affinity for fructose in contrast to the native *HXT* hexose transporter genes and is thought to be
527 responsible for the elevated use of fructose late in fermentation seen in EC1118. EC1118 also
528 carries a gene encoding a protein similar to the peptide transporters found in fungi and can
529 therefore perhaps transport a wider array of peptides to be broken down internally and used as
530 nitrogen source (Damon et al. 2011). The source of this introgression is unknown. The third
531 introgression carries genes with close relatives in *Zygosaccharomyces bailii* (Novo et al. 2009),
532 suggesting it may have arisen from this yeast. This particular region is not unique to EC1118
533 and is found in other wine yeast strains (Dequin and Casaregola 2011, Galeote et al. 2011).
534 This region contains an autonomously replicating sequence, suggesting that it may be able to
535 amplify in the genome. In support of this, some wine strains have been found to carry multiple
536 regions of this DNA (Galeote et al. 2011). The function(s) of this region are not yet known, but
537 its appearance in many wine strains and amplification therein suggest a positive selection for
538 the presence of this region in wine strains. Some of the winemaking traits that have made
539 EC1118 a popular selection for wine production may have indeed arisen from natural
540 introgression from other species and genera of yeast.

541 Genomic analyses have revealed that many commercial strains have acquired altered
542 signaling properties (Verstrepen et al. 2004) and these signaling differences may be important
543 for differential tolerance to various stressors. It is likely that increased basal levels of expression
544 of genes involved in tolerance to stressful conditions allows more rapid adaptation to those
545 conditions and, therefore, enhance survival (Bisson et al. 2007). However, high basal levels of
546 expression of these genes may result in slower initial growth rates and lack of an ability to
547 dominate fermentations (Bisson et al. 2007). There appears to be a dynamic interplay between
548 expression of genes associated with stress tolerance and those associated with rapid growth

549 (Bisson et al. 2007). Given the existence of multiple stressors in the environment and the feast
550 or famine atmosphere of growth on the surface of fruits, it is not surprising that vast biodiversity
551 of both genetic composition and gene expression profiles is observed in native *Saccharomyces*
552 isolates.

553 Commercial and native yeast isolates display greater genomic and genetic instability
554 than laboratory strains (Ambrona et al. 2005), and aberrations in the number of some
555 chromosomes are common (Bakalinsky and Snow 1990). Wild strains are generally homothallic
556 and show low sporulation rates and poor spore viability. They also display a high degree of
557 heterozygosity, chromosomal polymorphisms and rearrangements, and karyotype instability
558 (Carro and Pina 2001, Codon et al. 1998, Hughes et al. 2000, Izquierdo Canas et al. 1997,
559 Johnston et al. 2000, Landry et al. 2006a, 2006b, Longo and Vezinhet 1993, Mortimer 2000,
560 Myers et al. 2004, Oshiro and Winzeler 2000). The dynamic nature of the genome likely poses a
561 distinct advantage in the environment, as evidenced by the extensive diversity observed among
562 native isolates from the same site (Hauser et al. 2001). The biodiversity of wine strains of
563 *Saccharomyces* is likely a consequence of both natural selection and random mutagenesis and
564 accumulation of mutations. Wild yeasts show elevated rates of spontaneous mutagenesis
565 which, if followed by sporulation and diploidization, can lead to the rapid creation of significant
566 diversity across a population. The return to a homozygous state has been termed “genome
567 renewal” (Ambrona and Ramierez 2007, Mortimer et al. 1994). Some underlying features of
568 gene expression in wine strains will likely be conserved across this rich biodiversity while others
569 may show striking strain dependence. It is important to note that comparisons of gene
570 expression in recently isolated native strains of *S. cerevisiae* versus those that have been
571 cultivated in laboratories demonstrate clear differences in expression profiles of wild strains and
572 their domesticated derivatives (Kuthan et al. 2003, Palkova 2004). Strains rapidly lose some
573 phenotypes associated with growth in the wild upon laboratory cultivation (Palkova 2004).

574 **Impact of Strain Diversity on Wine Production**

575 Even though constrained by the demands of a highly specialized ecological niche, grape juice
576 fermentation, significant diversity exists among wine isolates of *Saccharomyces*. That no one
577 strain represents the entire spectrum of wine yeast diversity is both beneficial and problematic
578 for wine production. The beneficial impact of such diversity is the ability to obtain different flavor
579 and aroma profiles of the wine depending upon which strain has been used. Of course, astute
580 use of diversity in this manner means understanding the underlying genetic capabilities of the
581 yeast strains themselves as well as the potential for flavor and aroma evolution due to the
582 composition of the grapes at harvest. The challenge of diversity for wine production is that strain
583 nutritional needs, tolerances of stresses such as temperature and ethanol, compatibility with
584 other microbes present, and fermentation characteristics will vary such that one recipe will not
585 apply to all strains.

586 Fermentation conditions may also foster the development of diversity in the population within
587 the fermentation. Whether one strain dominates throughout a fermentation or there is a
588 progressive change in dominant genotypes depends upon the starting genomic composition and
589 phenotypic constitution of the initiator populations, their relative numbers, and the selective
590 potential imposed by fermentation conditions. Temperature has been shown to be an important
591 driver of strain evolution (Salvado et al. 2011). Temperatures of grape processing, must or juice
592 holding, and of fermentation itself pose selections for specific types of strains. Use of nutritional
593 supplements also impacts strain diversity and persistence. Strains carrying the *ssy1* mutation
594 (Brown et al. 2008) clearly detect nitrogen in the fermentation differently than strains not
595 mutated in this gene. The widespread use of sulfite has also imposed a selection in favor of
596 sulfite-resistant genotypes. Thus, the imposition of specific fermentation conditions can directly
597 influence population diversity and persistence.

598 The analysis of a large collection of strains by Legras et al. (2007) illustrates another important
599 facet of wine strain diversity. The discovery of genetic signatures of the UCD522 strain in
600 diverse populations of winery and vineyard yeasts isolated from around the world demonstrates
601 the penetrance and persistence a niche-favored genome can have in wine-producing regions. It
602 further demonstrates that use of commercial strains may impact the genetic composition of the
603 native populations through outcrossing or hybrid formation followed by selection for fitness. The
604 genomes of fermentation strains are in a constant state of flux and evolution. The process of
605 fermentation is thought to impose a strong selective pressure and therefore is a powerful
606 evolutionary force in the generation of diversity (Cubillos et al. 2011, Kvittek et al. 2008, Sipiczki
607 2011, Spor et al. 2009, Warringer et al. 2011).

608 Perhaps the most surprising conclusion from the analysis of wine strain diversity is the
609 existence of interspecies hybrids (Belloch et al. 2009, González et al. 2006, 2007, 2008,
610 Lopandic et al. 2007, Masneuf et al. 1998, 2002) and introgressions from non-*Saccharomyces*
611 genera (Damon et al. 2011, Galeote et al. 2010, Novo et al. 2009). These variants have arisen
612 naturally in native populations and demonstrate the potential for development of novel
613 reproductively isolated organisms. Hybrid genomes appear to be persistent in the wild despite
614 greater reproductive isolation and such hybrids confer a fitness advantage since they seem to
615 have evolved independently. Hybrids display complex patterns of gene expression (Tirosh et al.
616 2010) and set the stage for rapid evolution of further diversity.

617

Conclusions

618 Extensive analyses of strain variation within *S. cerevisiae* have provided a wealth of information
619 on the origin of wine strains and the subsequent evolution of strain diversity. Several studies
620 have independently confirmed the view of a single domestication event having given rise to the
621 modern population of vineyard and winery isolates with the spread of wine strains paralleling the

622 migration of grapevines (Legras et al. 2007). There is simultaneously greater genomic similarity
623 and greater phenotypic variation in the wine strains analyzed. The genomic similarity suggests
624 the presence of evolutionary bottlenecks, the selection for fewer genotypes in a population, with
625 subsequent reamplification of diversity. Sulfite use appears to be one such bottleneck imposed
626 in the Middle Ages (Pérez-Ortín et al. 2002, Sicard and Legras 2011). Diversity arises from the
627 well-described processes of spontaneous mutagenesis, genetic drift, and environmental
628 selection. The knowledge gained from the analysis of wine strain diversity and the correlation
629 between genotype and phenotype can be used in the development and identification of specific
630 strains well-tailored for specific production needs.

631

632

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