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A unique ecological niche fosters hybridization of oak-tree and vineyard isolates of *Saccharomyces cerevisiae*

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Abstract

Differential adaptation to distinct niches can restrict gene flow and promote population differentiation within a species. However, in some cases the distinction between niches can collapse, forming a hybrid niche with features of both environments. We previously reported that distinctions between vineyards and oak soil present an ecological barrier that restricts gene flow between lineages of *Saccharomyces cerevisiae*. Vineyard isolates are tolerant to stresses associated with grapes while North American oak strains are particularly tolerant to freeze-thaw cycles. Here, we report the isolation of *Saccharomyces cerevisiae* strains from Wisconsin cherry trees, which display features common to vineyards (e.g. high sugar concentrations) and frequent freeze-thaw cycles. Genome sequencing revealed that the isolated strains are highly heterozygous and represent recent hybrids of the oak x vineyard lineages. We found that the hybrid strains are phenotypically similar to vineyard strains for some traits, but are more similar to oak strains for other traits. The cherry strains were exceptionally good at growing in cherry juice, raising the possibility that they have adapted to this niche. We performed transcriptome profiling in cherry, oak, and vineyard strains and show that the cherry-tree hybrids display vineyard-like or oak-like expression, depending on the gene sets, and in some cases the expression patterns linked back to shared stress tolerances. Allele-specific expression in these natural hybrids suggested concerted *cis*-regulatory evolution at sets of functionally regulated genes. Our results raise the possibility that hybridization of the two lineages provides a genetic solution to the thriving in this unique niche.

Keywords

ecological divergence; hybridization; allele-specific expression; environmental stress

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DATA ACCESSABILITY

Raw DNA and RNA sequencing data was deposited in NIH SRA database under project number PRJNA287022 and SRP059499. Sanger sequencing, phylogenetic trees, vcf files, read counts, and edgeR results have been archived on Dryad. (doi:10.5061/dryad.bt5v6).

AUTHOR CONTRIBUTIONS

JLW isolated cherry strains from Door County cherry and soil samples and analyzed aquaporin sequences. KJC performed all phenotyping, genotyping, and sequencing analyses, KJC and APG wrote the manuscript.

INTRODUCTION

Adaptation to distinct ecological niches can promote genetic differentiation, and in turn speciation, between populations (Nosil *et al.* 2005; Sexton *et al.* 2014; Wang & Bradburd 2014). Ecological barriers can restrict gene flow when immigrants to an ecological niche are less fit than the native inhabitants that have adapted to that environment (Nosil *et al.* 2005; Foll & Gaggiotti 2006; Wang & Bradburd 2014). For example, reduced gene flow between walking sticks can arise due to divergent cryptic-color adaptation that affects predation of immigrants not adapted to that ecology (Nosil 2004). Another study found almost complete reproductive isolation between populations of yellow monkey flowers adapted to coastal versus inland habitats, due to selection against immigrants invading their non-native niche (Lowry *et al.* 2008). These studies highlight how differential ecological adaptation can lead to restriction of gene flow and population differentiation.

But one question that emerges from these studies regards the consequence of niche consolidation, when an environment displays combined features of two ecological niches to which different populations have adapted? In this situation, the ecological barriers that separate populations may be removed. One possibility is that one population will out-compete the other, if it is more fit under the conditions at hand. Another possibility is that the now co-mingling populations adapt to the new environment, perhaps producing a new lineage that combines the beneficial alleles of the original populations.

We set out to address this question by studying a unique niche for the budding yeast, *Saccharomyces cerevisiae*. *S. cerevisiae* grows in diverse environments from across the globe (Naumov *et al.* 1998, 2006; Mortimer 2000; Sniegowski *et al.* 2002; Fay & Benavides 2005a; Sampaio & Gonçalves 2008; Liti *et al.* 2009; Ezeronye & Legras 2009; Libkind *et al.* 2011; Wang *et al.* 2012; Cromie *et al.* 2013; Hyma & Fay 2013). In some cases, ecological barriers have likely been important in keeping populations separate. Population structure within the species correlates with both niche and geography (Fay & Benavides 2005a; Diezmann & Dietrich 2009; Liti *et al.* 2009; Sicard & Legras 2011; Warringer *et al.* 2011; Wang *et al.* 2012; Cromie *et al.* 2013; Hyma & Fay 2013). The correlation with niche is especially true for the oak and vineyard populations, which thrive in different environments within the same geographical locations (Hyma & Fay 2013; Clowers *et al.* 2015). The two lineages are distinguished by several phenotypes that relate to their respective niches (Gerke *et al.* 2006; Kvitek *et al.* 2008; Will *et al.* 2010; Magwene *et al.* 2011a; Warringer *et al.* 2011; Clowers *et al.* 2015). For instance, vineyard strains are better at surviving copper sulfate, tartaric acid, and high osmotic conditions (Fay *et al.* 2004; Kvitek *et al.* 2008; Warringer *et al.* 2011; Clowers *et al.* 2015), stresses commonly found in the vineyard environment. The oak environment is less well characterized, but it is likely distinguished by different or more variable carbon sources and resource patchiness in which nutrients are harder to come by (Magwene *et al.* 2011). As a result, oak strains are better at surviving in carbon-depleted conditions and on non-fermentable carbon sources (Clowers *et al.* 2015). Oak strains are also more tolerant to heat stress, cell wall and membrane stresses, freeze-thaw (FT) stress, and they are better sporulators than vineyard strains (Gerke *et al.* 2006; Kvitek *et al.* 2008; Clowers *et al.* 2015). Past work from our lab suggests that ecological

barriers, along with genetic barriers and differences in life-history traits, are likely working to keep oak and vineyard populations differentiated in nature (Clowers *et al.* 2015).

One set of antagonistic phenotypes that is particularly interesting relates to balancing selection across these different populations. We previously showed that cryotolerant oak-soil strains, mostly isolated from wintry climates in North America, harbor functional alleles of the aquaporins *AQY1* and *AQY2* that give rise to FT tolerance (Will *et al.* 2010). In contrast, osmotolerant vineyard strains have undergone positive selection to lose aquaporin function (and to lose the requirement for aquaporins during sporulation), in order to increase tolerance to a high-sugar environment. The antagonistic pleiotropy of aquaporin function therefore underlies local adaptation to distinct niches. These findings led us to ask: What happens in an environment that experiences both FT cycles and high osmolarity?

To address this question, we isolated *S. cerevisiae* strains from a hybrid niche that includes features of both environments: cherry trees in Wisconsin harbor high-sugar cherry fruits and experience frigid FT cycles. This environment provides a perfect model to study how strains adapted to otherwise separate niches cope with having to survive stresses from both environments. We isolated *S. cerevisiae*, including strains from soil and from fallen fruit, from a Wisconsin cherry orchard in Door County, WI. Surprisingly, genome-wide sequencing revealed that the isolated strains are hybrids between oak and vineyard lineages, which have not been reported before. We phenotyped these natural hybrids, as well as synthetic (lab-created) oak x vineyard hybrid strains and strains of pure oak and vineyard genotypes, in terms of stress tolerance and transcriptome variation. We show that the cherry-tree strains are phenotypically similar to vineyard strains for some traits, but are more similar to oak strains for other traits. Interestingly, while the cherry strains do not grow well in grape juice (similar to oak isolates), they outgrow most other strains in cherry juice. Our results provide an example where hybridization may provide a solution to thrive in a hybrid niche.

MATERIALS AND METHODS

Cherry Strain Isolation

Cherry strains were isolated from a cherry orchard in Fish Creek, Door County, Wisconsin in September, 2009 using methods in (Sniegowski *et al.* 2002). Cherry orchard soil or vortexed cherry samples were added to 40mL of filter sterilized Yeast Enrichment Media (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% sucrose, 8% ethanol, 0.001mg/mL chloramphenicol, 2.5mM HCl) in 50mL conical vials. Vials were closed loosely to allow gas escape and stored at room temperature in the dark undisturbed. After 3 and 7 days in enrichment media, 10uL samples were plated onto Yeast Collection Media (Synthetic Complete (SC) agar media + methyl- α -D-glucopyranoside and 8mM HCl) for 4 days at 30°C. Glossy, off-white colonies were picked and re-streaked to obtain individual colonies. Single isolates were saved as frozen stocks. Putative *Saccharomyces* strains were regrown and sporulated in 1% potassium acetate for test mating to *S. cerevisiae*. Of the isolates that sporulated, tetrads were dissected and spores were isolated for mating with a S288C tester strain: MATa his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0 mal33::Kan. Successfully mated colonies were selected on SC-ura+G418 plates, regrown, and sporulated in 1% potassium

acetate. At least 10 tetrads/strain were dissected to determine spore viability. Strains with high spore viability indicate within species mating event, and thus are determined to be *S. cerevisiae*. Five strains survived *S. cerevisiae* validation.

Intergenic and aquaporin sequence analysis of wild strains and DC spores

Intergenic regions of wild strains and the *S. paradoxus* outgroup on chromosomes 4 (812bp), 9 (518bp), and 10 (452bp) were previously sequenced (Kvitek *et al.* 2008). Additional Sanger sequencing of dissected spore clones of 5 DC strains was performed using the same primers. Single consensus sequence of each spore clone was attained in SeqMan (DNASTAR Inc., Madison, WI, USA), and alignments were performed using ClustalW in MegAlign (DNASTAR Inc., Madison, WI, USA). Phylogenetic trees were created using MrBayes (Huelsenbeck & Ronquist 2001) and edited for publication in Mesquite (Maddison & Maddison 2015). Sanger sequencing of the aquaporin loci was completed as in (Will *et al.* 2010).

Genomic sequencing and analysis

DNA was isolated from cell collections of DC6 and DC16, using Qiagen Genomic Tip/100G Kit. Libraries were prepared using Illumina TrueSeq DNA LT Sample Prep Kit v2 and sequencing on the Illumina HiSeq2000 (100bp paired-end reads) at 16–20x coverage. Raw data was deposited in NIH SRA database under project number PRJNA287022. Paired-end reads were aligned to the reference S288C genome (version R64) using BWA-mem (Li & Durbin 2010) using default settings. SNPs and indels were identified with GATK using base quality score recalibration, indel realignment, duplicate removal, depth of coverage analysis (McKenna *et al.* 2010; DePristo *et al.* 2011). Default parameters were used except –mbq 25 to reduce false positive calls. Variants were filtered using the following suggested GATK criteria: QD < 2, FS > 60, MQ < 40.

Loss of heterozygosity analysis

To analyze the heterozygosity patterns in the cherry strains, the average number of heterozygous SNPs was plotted along 1kb sliding windows with step size of 100bp using custom Perl and R scripts. A custom Perl script was used to identify the number of homozygous genes in each strain. For comparison, an *in silico* hybrid was created by merging oak (YPS163) and vineyard (KEH02580) BAM files (sequenced in (Clowers *et al.* 2015)) and analyzed as above.

Population structure analysis

To demonstrate the DC strains are oak x vineyard hybrids at a genome-wide level, previously published sequencing data (Skelly *et al.* 2013; Bergström *et al.* 2014; Hose *et al.* 2015) and our unpublished sequencing data on isolates from well documented *S. cerevisiae* lineages (Liti *et al.* 2009; Wang *et al.* 2012; Cromie *et al.* 2013; Strobe *et al.* 2015) were gathered for population structure analysis (strains listed in Table S1). All strains were aligned to the S288C reference genome (R64) using BWA-mem (Li & Durbin 2010) and subjected to variant analysis in GATK (McKenna *et al.* 2010; DePristo *et al.* 2011) as above. For PCA of population genetic structure, we used the R package adegenet (Jombart 2008;

Jombart & Ahmed 2011). Data files for PCA were generated using VCFtools (Danecek *et al.* 2011) and PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>) (Purcell *et al.* 2007). We selected 4 axes to represent the variation within the data (Fig. S1).

Phenotyping analysis

Crosses to create artificial hybrids were created previously (YPS163 x KEH02580 and YPS1000 x M14) (Clowers *et al.* 2015) or created as in (Clowers *et al.* 2015) (DY8 x KEH02884 and KEH01267 x KEH02884). All phenotypic analyses were performed in biological duplicate on separate days. For YPD (1% Yeast extract, 2% Peptone, 2% Dextrose) + 1.7M sorbitol, YP + 7% potassium acetate, and YPD + 0.3 mg/mL Congo red stresses, mid-log phase YPD cultures were diluted to optical density (OD₆₀₀) of 0.25, serially diluted onto agar plates containing appropriate stress as well as control non-stress (YPD) plates. A semi-quantitative growth score was calculated for each dilution by comparing growth on stress to non-stress conditions as in (Kvitek *et al.* 2008). FT growth scores were calculated as in (Kvitek *et al.* 2008; Will *et al.* 2010). Briefly, mid-log phase YPD cultures were diluted to an OD₆₀₀ of 0.25 and frozen in ethanol dry ice bath for 2 hours and then thawed before serial diluting on YPD. Growth scores were calculated by comparing growth of serial dilution of frozen and thawed cells compared to ice treated control cells. For liquid 40% white grape juice concentrate (Global Vinters Inc) and 30% tart cherry juice concentrate (Orchard Country Winery and Market, Door County, Wisconsin), log phase YPD cultures were spun down, washed and inoculated into appropriate medium and grown shaking for 24 hours at 30°C. Growth score was calculated as the change in OD₆₀₀ over the 24 hours. For sporulation efficiency, cultures were grown in liquid YPD, shaking for 24 hours at 30°C. Saturated cultures were then diluted to OD₆₀₀ of 1 in 3mL sterile water, spun down, re-suspended in 3mL 1% potassium acetate. Sporulations were carried out for 48 hours, shaking at 25°C. Sporulation efficiency was calculated as the percent of cells that had 2, 3, or 4 spores compared to the total number of cells by counting at least 200 cells/strain on a hemacytometer. Strains and phenotype scores are listed in Table S2.

Because of the variation in range of growth scores, phenotypic data was normalized so that each stress had the same maximum and minimum value for cluster analysis using Cluster 3.0 (de Hoon *et al.* 2004) to produce the heat map Fig. 3. The remaining plots in Fig. 3 represent the average raw scores across strain groups. The T-statistic distribution was calculated by randomly sampling from the phenotype data for each condition 10,000 times.

RNA-sequencing and analysis

Two biological replicates (collected on different days) were harvested during log phase growth in YPD (OD₆₀₀ ~0.4), flash frozen in liquid nitrogen and stored at -80°C until RNA extraction. Total RNA was extracted from yeast cells by the hot phenol method as previously described (Gasch 2002). Total RNA was DNase-treated at 37°C for 30 min with TURBO DNase (Life Technologies, Carlsbad, CA), after which RNA was precipitated at -20°C in 2.5M LiCl for 30 min. rRNA depletion of the DNase-treated total RNA and subsequent cDNA library preparation were performed with ScriptSeq Complete Kit H/M/R (Epicentre, Madison, WI), Index PCR Primers (Epicentre) and FailSafe PCR Enzyme Mix (Epicentre). rRNA-depleted RNA was purified with a RNeasy MinElute Cleanup Kit (Qiagen), and

cDNA was purified with Axy Prep MAG PCR Cleanup beads (Corning). cDNA libraries were sequenced on the Illumina HiSeq2000, generating single-end 100bp reads. Raw data was deposited in NIH SRA database under project number PRJNA287022.

Reads were processed with Trimmomatic (Bolger et al. 2014) and mapped to parent-specific reference genomes created by swapping S288C alleles with parental SNPs using BWA-mem (Li & Durbin 2010) with default settings. For the heterozygous cherry strains, parent-specific mapping genome was created by changing all homozygous SNPs to the appropriate allele and masking all heterozygous SNPs to an allele not present in the cherry strain at that position to reduce mapping bias of different alleles. HTseq version 5.5 was used (Anders et al. 2014) to calculate read counts for each gene. The Bioconductor package, edgeR (Robinson *et al.* 2010) was used to determine differential expression between groups of strains (vineyard, oak, and cherry) using a general linearized model with replicate as a blocking factor. Enrichment analysis was performed using FunSpec (Robinson *et al.* 2002).

Allele specific expression analysis

ASE analysis was performed using MBASED (Mayba *et al.* 2014). Pileup files of RNA-sequencing data at heterozygous SNPs was created using SAMtools mpileup (Li *et al.* 2009). A custom Perl script was used to count parental alleles and create input data file for MBASED. MBASED was implemented with is PHASED set to FALSE and numSIM set to 10^6 . The resulting p-values were subject to p-value adjustment in R. ASE genes were identified as those with $FDR < 0.05$ in both biological sequencing replicates. To identify ASE genes with at least 1.5x allelic skew, genes were further filtered for those with major allele frequency > 0.6 . To identify ASE genes with oak versus vineyard higher expression, RNA-sequencing data at ASE genes were clustered using Cluster 3.0 (de Hoon *et al.* 2004). As an alternative approach, custom Perl scripts were used to assign SNPs as vineyard alleles by comparing SNPs to the sequenced vineyard strains used in PCA above. We identified vineyard highly expressed genes as those with over half of the vineyard-assigned SNPs showing higher expression in both biological replicates. Enrichment analysis of vineyard versus oak highly expressed genes was performed using FunSpec (Robinson *et al.* 2002).

RESULTS

Sampling of yeast from a hybrid niche

We isolated *S. cerevisiae* from soil and cherry samples collected from a cherry orchard in Fish Creek, Door County, WI, using standard sampling protocol (see Methods). Five independent samples from the same orchard produced five strains that were confirmed to be *S. cerevisiae* via mating with an *S. cerevisiae* tester strain. Two of the strains were isolated from cherry orchard soil surrounding the trees (DC16 and 21) and three strains were isolated from rotting cherries on the ground (DC1, 6, and 12). All but DC1 had spore viability between 88–95% after mating to the *S. cerevisiae* tester strain. DC1 had reduced spore viability (43%), suggesting it may have acquired mutations or chromosomal alterations that reduce meiotic fidelity and spore viability.

Cherry strains represent hybrids of oak and vineyard isolates

Sequencing of individual alleles suggested that DC6 and 12 were genetically distinct from DC 16 and 21. We therefore chose DC6 and DC16 as representatives for short-read whole-genome resequencing. Compared to the S288C reference genome, DC6 has 64,907 single nucleotide polymorphisms (SNPs) and DC16 has 77,136 SNPs. The two strains are very similar to each other with only 0.22% sequence polymorphism. We found that both strains are highly heterozygous (Fig. 1), suggesting that they may be recent hybrids.

Population analysis suggests that the cherry strains are a combination of oak and vineyard lineages, confirming previous RAD-tag analysis of the strains (Cromie *et al.* 2013). We employed principal component analysis (PCA) to compare cherry strains to representatives from five *S. cerevisiae* populations (5 North American oak strains, 6 European/vineyard strains, 3 West African strains, 3 Malaysian strains, and 4 Sake strains, listed in Table S1) for which genome sequence was available (Liti *et al.* 2009; Skelly *et al.* 2011; Bergström *et al.* 2014; Clowers *et al.* 2015; Hose *et al.* 2015). Principal component (PC) 1 clearly distinguished the vineyard strains from the other populations, while PC2 distinguishes the remaining populations from the oak strains (Fig. 2). PC3 distinguishes the Malaysian and West African strains, and PC4 distinguishes one of the oak strains from the rest (Fig. S1). The cherry strains fall right between the oak and vineyard strains along the PC1 axis (Fig. 2). One vineyard strain, I14 (isolated from vineyard soil in Italy (Fay & Benavides 2005b), had a similar demographic pattern as the cherry strains (consistent with (Hyma & Fay 2013)), though it has greatly reduced levels of heterozygosity compared to the cherry strains (with only 2,350 heterozygous SNPs compared to over 44,000 heterozygous SNPs in each of the cherry strains). Together with the high degree of heterozygosity in these strains, this suggests that cherry strains are recent oak x vineyard hybrids, as previously suggested by RAD-tag sequencing (Cromie *et al.* 2013). This was confirmed by sequencing of three intergenic loci from dissected spores of the cherry strains, which shows that in some cases, two different alleles are present within a single strain (Fig. S2).

To evaluate the expected levels of heterozygosity in a nascent oak-vineyard hybrid, we generated an *in silico* hybrid sequence, by simply concatenating the read files for a pure oak and a pure vineyard strain (Clowers *et al.* 2015). The cherry strains showed a slightly lower level of heterozygosity than the *in silico* hybrid (Fig. 1, Table S1). Interestingly, there are clear regions showing loss of heterozygosity (LOH) in the cherry strains. We assessed LOH at the gene level and found that DC6 has 1,628 homozygous genes and DC16 has 2,064 homozygous genes for which there are clear distinguishing polymorphisms in the *in silico* hybrid. We also noted elevated levels of heterozygosity at some chromosomal ends. These data suggest that the cherry strains emerge from recent hybridization of oak and vineyard lineages and that the strains have had time to accumulate some degree of LOH.

We were particularly interested in assessing the status of the *AQY1* and *AQY2* aquaporin alleles. Functional aquaporins are important for FT tolerance, but loss of aquaporin function has been selected for to improve osmotic-stress tolerance in vineyard strains (Will *et al.* 2010). All five cherry strains harbored a non-functional *AQY1* allele, the *ayq1-V121M* polymorphism discussed in (Carbrey *et al.* 2001; Will *et al.* 2010). However, DC16 and DC21 that were isolated from cherry-tree soil are heterozygous for *AQY2*, containing one

nonfunctional vineyard allele and one functional allele found in previously in a Wisconsin oak strain (Will *et al.* 2010). The remaining cherry strains had homozygous non-functional *AQY2* alleles. The results correlated with the ability of these strains to survive FT stress and raise interesting implications about the selective pressure in this niche (see below and Discussion).

Cherry strains share phenotypes with both lineages

Having established that the cherry strains are hybrids, we were curious to characterize their ability to survive stresses that distinguish oak and vineyard strains. We assayed cell viability under a variety of stresses, as well as sporulation efficiency after nutrient starvation, in the cherry strains along with a panel of oak and vineyard strains. As a comparison, we also generated ‘artificial’ laboratory hybrids by crossing oak and vineyard strains in the lab. Conditions were selected based on known phenotypic differences in oak and vineyard strains (Gerke *et al.* 2006; Kvitek *et al.* 2008; Lorenz & Cohen 2012; Clowers *et al.* 2015). We found several interesting trends comparing the cherry strains with oak, vineyard, and artificial hybrids. Supporting previous results, oak strains are better at surviving cell wall stress (Congo red) (Kvitek *et al.* 2008; Clowers *et al.* 2015) and FT stress (Kvitek *et al.* 2008; Will *et al.* 2010), grew better on the non-fermentable carbon potassium acetate (Clowers *et al.* 2015), and sporulated faster than other strains (Gerke *et al.* 2006) (Fig. 3, Table S2). Vineyard strains are better at surviving high osmolarity and growing in grape juice (Fig. 3, Table S2)). We found that the cherry strains are more similar to vineyard strains for some stresses, displaying high osmo-tolerance, low Congo red tolerance and poorer growth on potassium acetate (Fig. 3, Table S2)). In the case of FT stress, cherry strains were vineyard like in that they were more sensitive than both the oak strains and the artificial hybrids; however, the two cherry strains that were heterozygous for the functional *AQY2* allele showed intermediate FT tolerance. In contrast, the cherry strains behaved like oak strains in that they do not grow as well in grape juice compared to vineyard strains and they have higher sporulation efficiency than vineyard strains (Fig. 3, Table S2)). Interestingly, the cherry strains were phenotypically distinct from the artificial hybrids for nearly half the phenotypes. Taking all of the stress phenotypes into account via hierarchical clustering analysis (Fig. 3a), we find that lab-created hybrids are phenotypically closer to oak strains and the natural cherry-tree hybrids are more like the vineyard strains.

Interestingly, the cherry strains grew extremely well in cherry juice produced in Door County, WI (Fig. 3, Table S2), a condition that closely mimics the environment from which they were isolated. Cherry strains grew significantly better than the oak strains and better than two of the three vineyard strains. The difference between the cherry and vineyard strains was not statistically significant, and exploration of a broader set of vineyard strains showed wide variation in this phenotype in the vineyard population (data not shown). The cherry strains grow much better in Door County cherry juice than artificial oak x vineyard hybrids, once again distinguishing them from the lab-generated hybrids (Fig. 3, Table S2) (see Discussion).

Gene expression in cherry strains shows both oak-like and vineyard-like features

Given the phenotypic findings, we were interested to investigate trends in gene expression: are the cherry strains more oak-like or more vineyard-like? To answer this question, we profiled the transcriptomes in two oak, two vineyard, and the two cherry strains growing exponentially in rich lab medium. We identified 2,072 genes as differentially expressed between the two oak strains *versus* the two vineyard strains (FDR < 0.05). Cherry strains had similar (statistically indistinguishable, FDR > 0.05) expression to vineyard strains at 617 (29.8%) of these genes (Fig. 4). Of this subset of genes, 298 were higher expressed (enriched for genes encoding site-specific transcription factors, chromatin-binding proteins, proteins localized to the nucleus or nucleolus, and proteins involved in meiosis or DNA damage repair) and 319 were lower expressed compared to oak strains (enriched for genes involved in respiration, transport, translation/ribosomal proteins, and proteins localized to the ER and Golgi apparatus (Fig. 4, Table S3)). We found that cherry strains have similar (statistically indistinguishable, FDR > 0.05) expression to oak strains at 496 (23.8%) of the 2,072 genes whose expression differentiates oak and vineyard populations (Fig. 4). Within this set of genes, 187 were expressed more highly (enriched for ribosomal proteins and iron ion binding proteins) and 306 genes were lower expressed compared to vineyard strains (involved in amino acid biosynthesis, sulfate assimilation, morphology, oxido-reductase activity, major facilitator superfamily proteins, and NAD/NADP binding (Fig. 4, Table S3)).

Interestingly, there were 330 genes that showed cherry-specific expression, where expression was distinct from both oak and vineyard strains. At 125 of these genes, expression was intermediate to the oak and vineyard groups – these genes were enriched for genes linked to catalytic activity, cellular amino acid biosynthetic process, and morphology (Fig. 4, Table S3). There were an additional 125 genes whose expression was lower, and 80 genes whose expression was higher, than both the oak and vineyard groups. The lower-expressed genes were enriched for oxido-reductases, hexokinases, and glycolysis enzymes while the higher expressed genes were enriched for intron homing, DNA restriction, and DNA integration (Fig. 4, Table S3).

Several of the affected functional categories were related to the phenotypes reported in Fig 3. For example, the ability to grow in grape juice is linked to sulfite metabolism and the elevated expression of the sulfite pump *SSU1* (Yuasa *et al.* 2005; Nardi *et al.* 2010; Brion *et al.* 2013; Zimmer *et al.* 2014; Clowers *et al.* 2015). Here we find that vineyard strains have higher expression of sulfur-metabolism genes, including *SSU1*, compared to oak and cherry strains that grow poorly in grape juice. Of these genes, 11 were found to be important for growth in grape juice (Clowers *et al.* 2015); 8 of these 11 showed higher expression in vineyard strains. In contrast, oak strains grow better than vineyard and cherry isolates on non-fermentable potassium acetate. We found that oak strains show considerably higher expression of respiration genes, including at 37 genes identified in the lab strain as important for efficient respiration (Dimmer *et al.* 2002; Luban *et al.* 2005; Merz & Westermann 2009). These results raise the possibility that differences in gene expression may contribute to the different phenotypes observed across strain groups.

Allelic-specific expression is associated with functional gene groups

The transcriptome sequencing in the hybrid strains allowed us to assess allele-specific expression (ASE) in these natural hybrids. Because expression of the two alleles occurs in the same cellular milieu, allelic differences in expression are taken to reflect polymorphisms that act *in cis* on the affected DNA molecule. We used the program MBASED (Mayba *et al.* 2014), which aggregates information across adjacent SNPs to obtain a single measure of ASE for each gene using unphased transcriptome data (Mayba *et al.* 2014). We identified 364 genes in DC6 and 273 genes in DC16 with significant ASE in both biological replicates (FDR < 0.05). The ability to detect very small but significant differences in ASE increases for highly expressed genes (Mayba *et al.* 2014). We therefore also scored the subset of genes for which the allelic skew was at least 1.5x. This restriction identified 146 ASE genes in DC6 and 132 ASE genes in DC16.

Interestingly, the genes subject to ASE in each strain were enriched for functional groups. Overall, the genes subject to at least 1.5X ASE in DC6 and DC16 were enriched for fungal-type cell wall. Assessing the directionality of the effect is somewhat challenging, because we do not have phased SNPs for the two haplotypes. However, we attempted to distinguish the favored allele in two ways. First, we simply clustered the ASE genes based on the RNA-seq data measured in oak, vineyard, and cherry strains. We then identified genes in which the oak-strain expression was higher than the vineyard-strain expression and for which the cherry strain expression was intermediate. These genes were enriched for those linked to fungal-type cell wall, plasma membrane, oxidation-reduction process, ER, and vacuole annotations (Table 1). In a second approach, we attempted to assess which allele was expressed higher in the hybrid by assaying SNPs associated with the vineyard population. Through this approach, we could assign the higher-expressed allele in 199 and 163 ASE genes in DC6 and DC16, respectively. Of the genes inferred to have higher expression in the oak strain, they were significantly enriched for genes linked to the fungal-type cell wall (Table 1, asterisks). The over-abundance of functionally related genes in those that display ASE suggests step-wise *cis*-regulatory evolution in cell wall genes of oak versus vineyard strains.

DISCUSSION

We set out to investigate how a species that displays separate populations adapted to different niches thrives in an environment that combines components of the different environments. Oak and vineyard populations of *S. cerevisiae* represent distinct lineages that have adapted to their respective niches in nature. We isolated strains from a hybrid niche that combines the high osmotic environment of vineyards with freezing and thawing cycles of the oak environment in a cherry orchard in Wisconsin. In this particular case, our results reveal that hybridization is an effective solution that produces unique strains with a combination of beneficial alleles from the original populations.

The cherry strains are highly heterozygous and harbor both oak and vineyard alleles, strongly suggesting that they are recent hybrids of the two lineages – yet the cherry strains are phenotypically unique compared to the artificial hybrids created in the lab. In contrast to the artificial hybrids that typically showed an intermediate oak-vineyard phenotype, the

cherry strains shared more phenotypes with the vineyard strains, but in some cases displayed the oak traits and in others presented an intermediate phenotype. Interestingly, the cherry strains showed lower FT tolerance than oak strains and the artificial hybrids; two of the strains had lost all functional copies of the aquaporins through LOH, while two of the strains isolated from soil were heterozygous for *AQY2*. This intriguing result suggests that the cherry niche provides a stronger pressure to lose aquaporin function (likely to survive the high osmolarity of fruit) than to keep it for FT survival. The cherry strains outperformed most other strains in Door County cherry juice (although some vineyard strains can also grow in this medium). Together, these results suggest that the recent hybridization event produced unique strains that have likely adapted to the North American cherry environment.

In several cases, the expression of functionally important genes in cherry versus oak or vineyard strains paralleled their phenotypic differences, raising the possibility that these expression differences are causal. We had the unique opportunity to analyze ASE in a natural, heterozygous hybrid between oak and vineyard strains. Remarkably, we found significant enrichment for functionally related genes among those subject to ASE; in several cases – namely genes linked to the cell wall – expression was consistently higher from the oak allele. This result suggests that there has been concerted *cis*-regulatory changes in the genes related to cell wall metabolism; coupled with the higher tolerance to cell-wall damaging drugs (Fig 3 and (Clowers *et al.* 2015)), this raises the possibility of positive selection in the oak strains.

Natural hybridization is considered to be an important force behind evolution and speciation (Anderson 1948; Anderson & Stebbins 1954; Lewontin & Birch 1966). The role of ecological factors in the emergence of natural hybrids is of great interest, especially in face of climate change, as hybridization may help organisms acquire adaptive traits and respond to environmental change (Arnold 2004; Hoffmann & Sgrò 2011; Becker *et al.* 2013). The low amount of gene flow between oak and vineyard hybrids despite close proximity and some migration between niches (Hyma & Fay 2013) suggests that ecological barriers may limit the ability of these populations to mate, or that hybrids are unfit in oak or vineyard niches. However, we find that in a very specific niche that combines components of each population's niche, hybridization could play a key role in the species ability to thrive in this environment. Our results support the idea that hybridization plays an important role in providing a source of variation for adaptation to new or changing environments (Anderson 1948; Anderson & Stebbins 1954; Lewontin & Birch 1966; Arnold 2004; Hoffmann & Sgrò 2011; Becker *et al.* 2013) and reviewed in (Arnold 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cherry strains are highly heterozygous

The number of heterozygous SNPs/kb was calculated in sliding windows of 1kb with a 100bp step size. The average number of heterozygous SNPs/kb (y-axis) is plotted across the 16 chromosomes in the genome (x-axis) for A) DC6, B) DC16, and C) *in silico* hybrid. The *in silico* hybrid sequence was generated by concatenating the read files of strains YPS163 and KEH02580.

Figure 2. Cherry strains are oak x vineyard hybrids

Population genetic structure analysis of 25 *S. cerevisiae* strains performed using PCA. PCA analysis was performed using variant information for 25 strains at 207,960 SNPs. The 25 strains are colored according to PC1, 2, and 3 and plotted across PC1 and PC2. Vineyard strain, I14, shows a similar genetic background to the cherry strains.

Figure 3. Cherry strains are more phenotypically similar to vineyard strains

(A) Hierarchical clustering of strains based on their phenotype scores in a variety of conditions (see Materials and Methods). Blue indicates robust growth and grey represents poor growth under the denoted conditions. Conditions are labeled across the top (SB= 1.7M sorbitol, FT= freeze-thaw, GJ= grape juice, CJ= cherry juice, SP= sporulation efficiency, CR= 0.3mg/mL Congo red, PA= 7% potassium acetate). Strains are labeled on the right and colored by group. (B) Bar graphs show average and standard error of population mean of the data shown in (A), for cherry ('DC', grey), artificial hybrids ('HYB', purple), oak (green), and vineyard ('VIN', red) strains. Asterisks denote statistical significance, two-tailed T-test with permutation (See Materials and Methods). (*) $p < 0.05$, (**) $p < 0.005$, (***) $p < 0.0005$.

Figure 4. Cherry strains show vineyard-like and oak-like expression at different subsets of genes
 Circles represent the number of genes in cherry strains whose expression was similar to vineyard strains (left of axis) or oak strains (right of axis). Circles above the axis represent more highly expressed genes, circles below represent lowly expressed genes in that subgroup. Purple circles represent genes that were expressed higher (top), intermediate (middle), or lower (bottom) than both the oak and vineyard strain groups. The word plot represents functional enrichments observed for genes displayed in the different sectors.

Table 1**Heterozygosity in cherry strains and artificial hybrid**

Comparison of the number of heterozygous versus the number of total SNPs for each chromosome in DC6, DC16, and the *in silico* hybrid.

Chr	DC 6			DC 16			<i>in silico</i> Hybrid		
	# of Het SNPs	# of all SNPs	% Het SNPs	# of Het SNPs	# of all SNPs	% Het SNPs	# of Het SNPs	# of all SNPs	% Het SNPs
1	1197	1933	61.92	1121	1879	59.66	1401	2053	68.24
2	4027	5832	69.05	3013	5498	54.80	5144	6781	75.86
3	1416	2194	64.54	1635	2213	73.88	2250	2501	89.96
4	5182	8357	62.01	6687	9842	67.94	9807	11695	83.86
5	2050	3553	57.70	2091	3946	52.99	3684	4615	79.83
6	1064	2016	52.78	669	1901	35.19	2172	2832	76.69
7	4967	7176	69.22	4549	7123	63.86	7188	8702	82.60
8	2017	3027	66.63	2368	3601	65.76	3488	4413	79.04
9	1618	3122	51.83	2958	4342	68.13	2948	3927	75.07
10	2941	4932	59.63	3207	4836	66.32	5042	6488	77.71
11	1960	4187	46.81	1371	3904	35.12	4265	5545	76.92
12	4321	6856	63.03	3578	6453	55.45	6421	7988	80.38
13	3405	5738	59.34	3095	5653	54.75	5354	6625	80.82
14	2954	4296	68.76	2856	4464	63.98	4999	5825	85.82
15	3648	6925	52.68	3880	7583	51.17	6751	9121	74.02
16	2767	4119	67.18	1171	3263	35.89	6260	7175	87.25
Total	45534	74263	61.31	44249	76501	57.84	77174	96286	80.15

Table 2
Functional enrichment in ASE genes

Gene enrichments found from clustering analysis are displayed. Asterisks denote enrichments found from allele assignment analysis and italicized enrichments were found after the 1.5x allelic skew cutoff.

DC6 ASE genes		DC16 ASE genes	
Functional Group	P-value	Functional Group	P-value
Oak > Vineyard (Clustering)		Oak > Vineyard (Clustering)	
<i>fungus-type cell wall **</i>	<i>9.68E-13</i>	<i>fungus-type cell wall **</i>	1.10E-12
sugar, glucoside, polyol and carboxylate catabolism	7.41E-11	ER	1.06E-08
plasma membrane **	1.81E-10	vacuole	1.67E-07
ER	1.23E-07	polysaccharide metabolism **	6.56E-07
oxidation-reduction process	1.67E-07	membrane	1.33E-06
ribosome	3.61E-07	glucosidase activity **	1.61E-06
vacuole	7.77E-07	hydrolase activity, hydrolyzing O-glycosyl compounds	3.67E-06
tricarboxylic-acid pathway	2.59E-06		
		Vineyard > Oak (Clustering)	
		cellular amino acid biosynthetic process	1.32E-08