Evolution of Ecological Dominance of Yeast Species in High-Sugar Environments

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Evolution of Ecological Dominance of Yeast Species in High-Sugar Environments

by

Kathryn Marie Williams

A dissertation presented to the
Graduate School of Arts and Sciences
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

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St. Louis, Missouri
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Kathryn Marie Williams

Washington University in St. Louis

December 2014
For Sarah
ABSTRACT OF THE DISSERTATION

Evolution of Ecological Dominance of Yeast Species in High-Sugar Environments

by

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Doctor of Philosophy in Evolution, Ecology, and Population Biology

Washington University in St. Louis, 2014

Professor Justin Fay, Chair

Two challenging goals of evolutionary biology are to understand how evolutionary innovations evolve and how they contribute to the success of lineages. Evolutionary innovations may arise following whole genome duplication (WGD) events and they are suspected to contribute to the success of lineages by creating ecological opportunity. However, direct evidence for duplicated genes involved in evolutionary innovations remains rare, and the relationship between evolutionary innovations and the success of lineages may be very complex. In this study, I explore the relationship of evolutionary innovation, WGD, and the ecological dominance of yeast species in high-sugar environments. In budding yeast, a major evolutionary transition occurred around the time of a WGD that dramatically changed the way yeast species harness energy. Whereas most yeast species acquire energy through aerobic respiration, post-WGD yeast species such as *Saccharomyces cerevisiae* acquire most of their energy via fermentation. Evolution of the fermentative lifestyle may have required duplicated genes and is suspected to contribute to the ecological dominance of yeast species in high-sugar environments. Direct evidence for the role of duplicated genes involved in this evolutionary innovation remains rare, and it is difficult to know whether dominance in high-sugar environments was a direct consequence of this evolutionary transition or depends upon the acquisition of additional traits.
The objectives of this research were to obtain direct evidence that duplicated genes contribute to the fermentative lifestyle, determine when ecological dominance in high-sugar environments evolved in the yeast lineage, and to identify traits that contribute to the dominance of *S. cerevisiae* in high-sugar environments. In Chapter 1, I provide direct evidence that the duplicated genes *TOM70* and *TOM71* are both required for a trait that evolved during the transition to a fermentative lifestyle. In Chapter 2, I determine that ecological dominance evolved very recently in the yeast lineage and identify multiple fitness traits related to pH, nutrients, and ethanol that contribute to the dominance of *S. cerevisiae* in high-sugar environments. Overall, the findings from this study advance the field of evolutionary biology by providing direct evidence that duplicated genes retained following WGD contribute to an evolutionary innovation and showing that the ecological success of some lineages may not be an immediate consequence of evolutionary innovation but involves the acquisition of multiple fitness traits.
“I am no longer concerned with sensation and innovation, but with the perfection of my style.” – Yves Saint Laurent
Dissertation Introduction
A challenging goal of evolutionary biology is to understand how major evolutionary transitions occur. How did vision evolve during the evolution of vertebrates? How did pollinator-mediated reproduction evolve in plants? What evolutionary changes permitted ground-dwelling dinosaurs, the ancestors of modern birds, to take flight? Certainly, the evolution of certain key traits, such as eyes, flowers, and wings, contributed to these evolutionary feats. However, the fascination with these transitions arises not only from the complexity of the morphological and physiological structures involved, but also from the qualitatively new modes of existence they represent. While the utility of eyes, flowers and wings for extant taxa may be easy to comprehend, the utility of the earliest forms of these traits remains less clear. What type of ecological opportunity did these evolutionary innovations create during evolutionary history?

Exploring the causes and consequences of major evolutionary transitions involves two major questions: First, how do evolutionary innovations evolve? And second, how do evolutionary innovations contribute to the ecological and evolutionary success of lineages? In the following sections I discuss these questions in more detail, introduce the *Saccharomyces* complex of yeast as a study system to address these questions, and then provide a review of what is known about the causes and consequences of a major evolutionary transition that dramatically changed the way yeast species harness energy.

**How do evolutionary innovations evolve?**

The primary issue regarding the emergence of evolutionary innovations is the inherent challenge of introducing major functional modifications that do not reduce organismal fitness. Not only does the emergence of new morphological and physiological traits involve coordinated interactions between many structures, but also these new morphological and physiological traits
must evolve in the context of a complex system that is subject to natural selection, an individual organism. Fisher (1930) showed that as complexity increases, any modification to the system is more likely to disrupt function than be beneficial. Orr (2000) extended this theoretical work and found that complex systems undergo adaptive evolution much more slowly than simple systems, the so-called ‘cost of complexity’. Nevertheless, evolutionary innovations do evolve, and one answer to this complexity quandary lies in the potential redundancy of biological systems.

Functional redundancy is one mechanism that may facilitate evolutionary innovations. In order for new morphological and physiological traits to evolve, at some point during evolutionary history, the prevailing system must exhibit multiple functions. The prevailing system may exhibit multiple functions through one of two mechanisms: either existing structures must possess two roles, the ancestral role and the new role, or multiple structures must possess redundant functions such that one of them can evolve a new function without hindering the ancestral function (Brigandt and Love 2012). Although functional redundancy may arise at different biological levels, the primary way that functional redundancy evolves at the level of genes is through duplication events.

Gene duplication events create genetic redundancy that may facilitate evolutionary innovation. These events primarily occur during meiosis and can duplicate single genes or an entire genome. Whole genome duplication (WGD) events, in particular, are considered a key mechanism in the origin of evolutionary innovations (Ohno 1970). WGD events are associated with evolutionary innovations in many groups of eukaryotes, including the origin of jawed vertebrates and the origin of flowering plants (Holland et al. 1994; De Bodt et al. 2005; Otto 2007). Massive genetic redundancy created during WGD is suspected to relieve constraints imposed by pleiotropy and facilitate the complex morphological and physiological modifications
associated with major evolutionary innovations (Ohno 1970). However, what specific genes are involved in these evolutionary innovations remains largely unknown.

Direct evidence for duplicated genes that contribute to evolutionary innovation remains rare. Although theory predicts a high proportion of duplicated genes will evolve new functions given a sufficiently large population size (Walsh 1995), and both coding sequence analyses and expression studies suggest duplicated genes acquire new functions (Nembaware et al. 2002; Conant and Wagner 2003; Kellis et al. 2004; Gu et al. 2005; Cliften et al. 2006; Hittinger and Carroll 2007; Scannell and Wolfe 2008; Des Marais and Rausher 2008; Kassahn et al. 2009; Guo et al. 2013; Qiu et al. 2014), most studies do not provide direct evidence for functional changes or show how sequence and expression changes affect organismal fitness (but see Hittinger and Carroll 2007). Part of the challenge in providing direct evidence for duplicated genes involved in evolutionary innovations comes from the number of genes involved and the fact that very few eukaryote systems are amenable to genetic analysis. One notable study that provided direct evidence for duplicate genes involved in a fitness trait used the tools available for the model yeast *Saccharomyces cerevisiae* to show that duplication of the galactose-metabolism pathway genes during WGD resolved an adaptive conflict and facilitated their evolution (Hittinger and Carroll 2007). However, this study was limited to a single duplicated gene-pair not known to be involved in a major evolutionary transition. Thus, while WGD is considered a key mechanism in major evolutionary transitions, direct evidence that duplicated genes contribute to evolutionary innovation remains relatively rare.

**How do evolutionary innovations contribute to the evolutionary and ecological success of lineages?**
Simpson (1953) was among the earliest evolutionary biologists to recognize the importance of evolutionary innovations in the evolutionary and ecological success of lineages. He argued that certain ‘key mutations’ create the possibility of occupying a new adaptive zone in which a lineage would diversify. In other words, key mutations, or evolutionary innovations, contribute to the evolutionary and ecological success of lineages by creating ecological opportunity.

Ecological opportunity, in its most basic sense, refers to the resource opportunities available for an organism. Evolutionary innovations may create ecological opportunity by enabling organisms to access new or additional resources and liberating them from competition imposed by other lineages (Schluter 2000). Although other mechanisms external to an organism may create ecological opportunity, such as migration to species-poor environments, mass extinction of competitor species, or the emergence of new resources (Simpson 1953), ecological opportunity as a result of evolutionary innovation arises from the morphological and physiological features intrinsic to an organism.

Although most recent studies focus on the potential for evolutionary innovation to increase lineage diversification (Schluter 2000), the causal relationship between evolutionary innovation and the species richness of some clades remains difficult to test, either because of the rarity of some innovations or the lack of appropriate comparisons (Galis 1995). Furthermore, an association between increased species richness and innovation does not necessarily explain how an evolutionary innovation facilitated lineage diversification. Although increased lineage diversification following an evolutionary innovation implies that this transition increased ecological opportunity, few studies explicitly test this hypothesis.
Even when an evolutionary innovation is known to create ecological opportunity in certain environments, such as with the origin of antifreeze proteins in the notothenoid fish lineage (Eastman 1993) or the origin of C₄ photosynthesis in certain grass lineages (Ehleringer et al. 1997; Sage 2004), phylogenetic analyses reveal that the relationship between these evolutionary innovations and the ecological success of descendant lineages may be very complex. Indeed, recent phylogenetic analyses reveal that both the dominance of notothenoid fishes in the coastal waters of Antarctica and the dominance of C₄ grasses in tropical savannah habitats lags behind the emergence of the evolutionary innovations suspected to underlie their ecological success (Edwards et al. 2010; Near et al. 2012; Bouchenak-Khelladi et al. 2014; Spriggs et al. 2014). Other studies have disclosed similar patterns in other systems as well (Wing and Boucher 1998; Alfaro et al. 2009; Schranz et al. 2012). This lag between evolutionary innovation and the ecological success of lineages may occur because ecological success depends on certain environments, communities, or the acquisition of additional traits. However, the specific factors involved remain unknown for many lineages. Thus, there is much to learn about the relationship between evolutionary innovations and the ecological success of lineages.

**Study System**

In order to learn more about how evolutionary innovations evolve and how they contribute to the ecological and evolutionary success of lineages, I chose to explore the relationship of WGD, evolutionary innovation and the ecological success of yeast species in the *Saccharomyces* complex of yeast. This group includes more than 75 yeast species, including the model yeast *Saccharomyces cerevisiae*, which phylogenetic analysis has resolved into 14 clades (Kurtzman and Robnett 2003). Like many groups of eukaryotes, a WGD in this lineage is
associated with a major evolutionary transition that is suspected to facilitate the ecological success of descendent lineages (Wolfe and Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006; Conant and Wolfe 2007). However, unlike most other eukaryotes, the *Saccharomyces* complex of yeast possesses the genomic and genetic tractability needed to determine the genes involved in evolutionary innovation.

Multiple genetic and genomic resources available for the *Saccharomyces* complex of yeast facilitate direct tests of the genes involved in evolutionary innovations. Not only do the laboratory strains and techniques developed for *S. cerevisiae* enable genetic analysis, but the genomic sequence data available for nearly 30 yeast species within this group further facilitates genomic comparisons (Cherry et al. 2012). Two resources in particular help to identify duplicated genes involved in evolutionary innovation, the Yeast Knock-Out (YKO) collection (Giaever et al. 2002) and the Yeast Gene Order Browser (YGOB) (Byrne and Wolfe 2005). The YKO collection consists of a near-comprehensive set of viable gene deletion strains for *S. cerevisiae* that can be used to identify candidate genes for traits, and the Yeast Gene Order Browser (YGOB) consists of many lists of genes duplicated during WGD and their syntenic relationships. These genetic and genomic tools available for yeast help to identify the genetic basis of evolutionary innovation.

**WGD, evolutionary innovation and the ecological success of yeast species**

In the *Saccharomyces* complex of yeast, a major evolutionary innovation occurred around the time of a WGD that dramatically changed the way yeast species harness energy. Whereas most yeast species acquire energy through aerobic respiration, post-WGD yeast species such as *Saccharomyces cerevisiae* acquires most of their energy via fermentation (Pronk et al. 1996;
Evolution of this fermentative lifestyle likely involved multiple steps both before and after the WGD, including the ability to grow without mitochondrial DNA and the transcriptional rewiring of carbon metabolizing enzymes (Ihmels et al. 2005; Merico et al. 2007; Field et al. 2009; Hagman et al. 2013; Lin et al. 2013). While the evolutionary transition to a fermentative lifestyle began prior to the WGD, yeast lineages that diverged after the WGD show a clear preference for fermentation in the presence of oxygen (Merico et al. 2007; Hagman et al. 2013). The evolutionary transition to a fermentative lifestyle in post-WGD yeast species likely involved changes in many genes in multiple pathways.

Duplicated genes retained following WGD in the yeast lineage may underlie the dramatic metabolic changes necessary for the fermentative lifestyle to evolve. Not only do genome-wide analyses of expression patterns and protein evolution reveal evidence for functional changes among duplicated genes following WGD (Gu et al. 2002; Conant and Wagner 2003; Cliften et al. 2006; Scannell and Wolfe 2008), but also duplicated genes are known to be involved in pathways required for the fermentative lifestyle (Thomson et al. 2005; Piskur et al. 2006; Conant and Wolfe 2007). Although direct evidence that functional changes in duplicated genes contributed to the evolution of a fermentative lifestyle remains rare, the functional divergence of alcohol dehydrogenase \((ADH)\) genes, \(ADH1\) and \(ADH2\), is known to enable \textit{S. cerevisiae} to produce and to accumulate ethanol (Thomson et al. 2005). However, the duplication history of these genes is not entirely clear and whether their functional divergence correlates with the WGD remains uncertain. As such, more direct tests are needed to determine whether duplicated genes from the WGD are required for the evolution of the fermentative lifestyle.

Evolution of the fermentative lifestyle may have enabled post-WGD yeast species to dominate high-sugar environments like grape juice. The fermentative lifestyle can yield a growth
advantage in high-sugar environments due to a higher rate of sugar consumption and energy recovery (Pfeiffer et al. 2001; MacLean and Gudelj 2006; Conant and Wolfe 2007), as well as through the production of bulk ethanol that is suspected to inhibit the growth of competitor species (Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006). Additionally, during winemaking the post-WGD yeast species *S. cerevisiae* is known to dominate grape juice (Fleet 2003, 2008), which possesses very high sugar (~120 g/l; Rodicio and Heinisch 2009) and contains hundreds of yeast species (Pretorius 2000; Fleet 2003, 2008; Jolly et al. 2006; Bokulich et al. 2012; Pinto et al. 2014).

However, the precise role of the fermentative lifestyle in the ecological success of yeast species in high-sugar environments remains equivocal. Direct competitions between *S. cerevisiae* and several pre-WGD species do not support the role of ethanol but instead implicate different factors besides ethanol (Holm Hansen et al. 2001; Nissen et al. 2003, 2004; Pérez-Nevado et al. 2006; Albergaria et al. 2010; Branco et al. 2014). Also, mono-culture growth rates of various species suggest that temperature may be more important than ethanol concentration (Goddard 2008; Salvadó et al. 2011a). Also, very little is known about the relative fitness of most post-WGD yeast species in high-sugar environments like grape juice, so it is unclear whether *S. cerevisiae's* dominance in wine fermentations reflects certain attributes of the grape juice environment or the other yeast species present within the community, and whether dominance in high-sugar environments is a simple consequence of the fermentative lifestyle or involves the acquisition of additional traits.

**Focus of dissertation**
In order to learn more about how evolutionary innovations evolve and how they contribute to the ecological and evolutionary success of lineages, I explore the relationship of evolutionary innovation, WGD, and the ecological success of diverse yeast species in the *Saccharomyces* complex of yeast. Direct evidence for the role of duplicated genes involved in the evolution of the fermentative lifestyle remains rare, and the lack of fitness data available for many yeast species makes it difficult to know whether ecological dominance of *S. cerevisiae* in high-sugar environments was a direct consequence of this evolutionary transition or depends upon the acquisition of additional traits. The objective of the research presented in subsequent chapters is to identify duplicated genes involved in this evolutionary innovation, to determine when ecological dominance in high-sugar environments evolved in the yeast lineage, and to identify traits that contribute to the dominance of *S. cerevisiae* in high-sugar environments. In Chapter 1, I identify duplicated candidate genes for growth without mtDNA, one of the traits that evolved during the transition to a fermentative lifestyle, and determine whether conserved duplicated genes required for this trait exhibit functional divergence. In Chapter 2, I determine when ecological dominance of high-sugar environments evolved in the yeast lineage and identify multiple traits that confer *S. cerevisiae* with a growth advantage in these environments. Finally, I conclude by providing a summary of the key findings from each of my chapters and discussing the broader implications of my findings in more detail.
Duplicated genes \textit{TOM70} and \textit{TOM71} are both required for growth without mitochondrial DNA in \textit{Saccharomyces cerevisiae}
Abstract

Whole genome duplication (WGD) is believed to facilitate evolutionary innovation. In budding yeast, evolution of the fermentative lifestyle following WGD diminished the role of the mitochondria in energy acquisition. This evolutionary innovation in energy acquisition is associated with the ability of post-WGD yeast species such as Saccharomyces cerevisiae to grow without their mitochondrial DNA (mtDNA). Functional divergence of duplicated genes retained following WGD, called ohnologs, may have contributed to the evolution of this trait, although direct evidence remains rare. The objectives of this study are to identify candidate ohnologs for the evolution of the ability to grow without mtDNA in S. cerevisiae and to determine whether candidate ohnologs for this trait exhibit functional divergence. We identified 18 ohnolog pairs in which one gene is a known or candidate gene for growth without mtDNA. We tested a subset of these genes and confirmed that both TOM70 and its ohnolog TOM71 are required for growth without mtDNA. Our study provides direct evidence that duplicated genes contribute to an evolutionary innovation in the yeast lineage, although this role is likely not due to their functional divergence.
Introduction

Whole genome duplication (WGD) is believed to facilitate evolutionary innovation and is associated with major evolutionary transitions in many groups of eukaryotes (Ohno 1970; Otto 2007), including the origin of jawed vertebrates (Holland et al. 1994) and the origin of flowering plants (De Bodt et al. 2005). These evolutionary transitions involved multiple modifications in morphology and physiology, which likely required functional changes in multiple genes and pathways. Massive genetic redundancy created during WGD is suspected to relieve constraints imposed by pleiotropy and facilitate the evolution of new functions (Ohno 1970). Although WGD frequently correlates with major innovations, the specific genes and changes underlying phenotypic evolution following WGD remain largely unknown.

In budding yeast, a WGD occurred around the time of a major innovation that diminished the role of the mitochondria in energy acquisition. While mitochondria play an essential role in energy acquisition for most eukaryotes, post-WGD yeast species circumvent their mitochondria and instead rely on fermentation for most of their energy acquisition (Pronk et al. 1996; Merico et al. 2007; Hagman et al. 2013). Evolution of the fermentative lifestyle in yeast represents a major evolutionary innovation in energy acquisition suspected to contribute to the ecological success of post-WGD yeast species such as *Saccharomyces cerevisiae* (Wolfe and Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006; Conant and Wolfe 2007). Multiple lines of evidence support that post-WGD yeast species have become autonomous from their mitochondria for energy acquisition, including divergent expression of genes required for rapid growth and genes required for mitochondrial function (Ihmels et al. 2005), relaxed constraints on substitutions in nuclear-encoded mitochondrial genes (Jiang et al. 2008), and most
strikingly, their ability to grow following the loss of their mitochondrial DNA (mtDNA) (Fekete et al. 2007; Merico et al. 2007).

Duplicated genes from the WGD, referred to as ohnologs, may contribute to the diminished role of the mitochondria in energy acquisition in post-WGD yeast species. Ohnologs involved in glycolysis are significantly over-represented among post-WGD species and are suspected to increase glycolytic flux and ethanol fermentation (Conant and Wolfe 2007). Also, of the 20 genes known to contribute to the ability to grow without mtDNA (Cherry et al. 2012), four of these genes, TOM70, SDH3, ICYC1 and SSB1, possess duplicates retained in S. cerevisiae since the WGD (Byrne and Wolfe 2005). Yet, it is not known whether duplication of genes required for growth without mtDNA facilitated the evolution of this trait in post-WGD yeast species. If so, then ohnologs required for growth without mtDNA should exhibit a novel function when compared to their duplicate copy, which is assumed to maintain the ancestral function. Intriguingly, none of the ohnologs of the known or candidate genes for growth without mtDNA genes have been implicated in this trait, which suggest that the known or candidate genes for growth without mtDNA have acquired new functions while their ohnologs maintain the ancestral function. Whether other candidate genes for growth without mtDNA are also duplicated in S. cerevisiae or whether ohnologs required for this trait have diverged in function since the WGD remain unknown.

The objectives of this study were to identify candidate ohnologs in S. cerevisiae for evolution of the ability to grow without mtDNA and to determine whether candidate ohnologs exhibit functional divergence. To identify candidate ohnologs, we synthesized findings from multiple previous studies and identified 18 candidate ohnologs for evolution of the ability to grow without mtDNA. Then, we directly tested the role of multiple candidate genes by
evaluating the growth of corresponding yeast knock-out strains following the loss of their mtDNA. We confirmed that TOM70 is required for growth without mtDNA and also determined, surprisingly, that its ohnolog TOM71 is required for this trait. Our discovery that both TOM70 and TOM71 are required for growth without mtDNA in S. cerevisiae does not support the hypothesis that one of these genes has acquired a new function in post-WGD yeast lineages while the other maintains the ancestral function. Rather, this function was likely also present in the ancestral gene and is now shared by both genes.

**Materials and Methods**

Identification of candidate ohnolog pairs

To identify candidate ohnolog pairs for the evolution of growth with mtDNA, we annotated a list of 551 ohnolog pairs (1,102 genes) that are conserved in S. cerevisiae (Byrne and Wolfe 2005) with phenotype data we obtained from the Saccharomyces Genome Database (SGD) (Cherry et al. 2012) and a previous genetic screen (Dunn et al. 2006). We considered an ohnolog pair a candidate for evolution of growth without mtDNA if at least one gene within the pair met one of the following criteria: it was associated with a ‘petite-negative’ phenotype according to SGD or if a null mutation caused a very slow growth rate relative to other null mutations in ethidium bromide. We chose these selection criteria because yeast strains that do not grow without their mtDNA are referred to as ‘petite-negative’ (yeast strains that do grow without their mtDNA form small colonies called ‘petites’), and slow growth in ethidium bromide may reflect an inability to tolerate the loss of mtDNA because ethidium bromide prevents mtDNA from replicating (Slonimski et al. 1968). Using these criteria, we selected the top 103 slowest growing genes identified by the previous genetic screen (Dunn et al. 2006) and 27 genes
that were associated with a petite-negative phenotype according to the SGD (Cherry et al. 2012). After removing redundant listings, our list included 118 genes, including 20 genes required to form petites known from previous studies (Cherry et al. 2012). We searched the list of 551 ohnolog pairs (1,102 genes) in *S. cerevisiae* for the 118 known or candidate genes for growth without mtDNA and identified 18 ohnolog pairs in which one gene was a known or candidate gene for growth without mtDNA (Table 1.1). Notably, only one gene in each ohnolog pair met our initial search criteria, which suggest that these genes may have acquired new functions required for grow without mtDNA in post-WGD yeast lineages.

**Media**

Media used to assay the ability to grow without mtDNA included YPD (1% yeast extract, 2% peptone, 2% dextrose) and YPD with 25 ng per ml of ethidium bromide (YPD with EtBr). YPD with EtBR was used because ethidium bromide is known to prevent replication of mtDNA thereby eliminating mtDNA in subsequent generations (Slonimski et al. 1968), and YPD was used as a control. Following treatment in YPD and YPD with EtBr, all strains were grown on YPD plates (YPD with 2% agar) to evaluate growth. To ensure the loss of mtDNA, we also used YPEG (1% yeast extract, 2% peptone, 2% ethanol, 3% glycerol). Strains that have lost their mtDNA will not be able to respire and therefore will be unable to grow on YPEG because it only contains non-fermentable carbon sources (ethanol and glycerol).

**Construction of gene deletion strains**

To construct deletion strains, gene-specific primers and PCR were used to amplify KanMX deletion cassettes from the Yeast-Knock-Out (YKO) collection (Giaever et al. 2002).
The lithium acetate method described by Becker and Lundblad (2002) was then used to transform KanMX deletion cassettes into the *S. cerevisiae* laboratory strain YJF173 (S288c background: MATa ho ura3-52). Deletion of each candidate gene was confirmed using selection for resistance to G418 and PCR.

**Phenotypic analysis of growth without mtDNA**

To test growth without mtDNA, deletion strains were pre-cultured for 18-20 hours in liquid YPD at 30°C with shaking at 300 rpm, and then diluted 1:1000 into 1 ml of liquid YPD and YPD with EtBr in 14 ml tubes. Cells were grown at 30°C with shaking at 300 rpm for either 7 hours (YPD treatment) or 22 hours (YPD with EtBr treatment), and then diluted to an optical density at 600nm of 0.02. Diluted cultures were then plated in 10-fold dilutions onto YPD plates and grown at 30°C for 48 hours. Deletion strains of genes required for growth without mtDNA should not grow on YPD following treatment with YPD with EtBr. For controls, we also evaluated the phenotype of YJF173, which should grow following treatment with YPD with EtBr, and a representative strain of the pre-WGD yeast species, *Kluyveromyces lactis* (NRRL Y-8279), which should not grow following treatment with YPD with EtBr. To ensure that strains that grew following treatment with YPD with EtBr had lost their mtDNA, we also assayed them for their ability to grow without a fermentable carbon source using YPEG plates. If mtDNA was sufficiently lost following treatment with YPD with EtBr, deletion strains will not be able to respire and therefore will be unable to grow on non-fermentable medium.

**Results**

18 known or candidate genes for growth without mtDNA are duplicated in *S. cerevisiae*
Duplicated genes retained following WGD, referred to as ohnologs, in the yeast lineage may contribute to the evolution of the ability to grow without mtDNA. We identified known or candidate genes for growth without mtDNA that possess ohnologs by searching a list of 551 ohnolog pairs in *S. cerevisiae* (Byrne and Wolfe 2005) with a list of 118 known or candidate genes for growth without mtDNA (Dunn et al. 2006; Cherry et al. 2012) (see Materials and Methods for details). We identified 18 ohnolog pairs present in *S. cerevisiae* in which one gene was a known or candidate gene for growth without mtDNA (Table 1.1). Notably, only one gene in each ohnolog pair met our initial search criteria, which suggests that these genes may have acquired new functions required for grow without mtDNA in post-WGD yeast lineages.

**TOM70** is required for growth without mtDNA

Several of the candidate genes in our list of 18 ohnolog pairs were identified in a previous study via a high-throughput assay (Dunn et al. 2006). High-throughput studies can be imprecise and will sometimes lead to spurious findings. To confirm that candidate genes were required for growth without mtDNA, we constructed deletion strains for a subset of candidate genes, **TOM70**, **TPK1**, **SSB1**, **ISU1**, and **YIA6**, and evaluated their phenotype following the loss of their mtDNA. We used ethidium bromide treatment (YPD with EtBR) to eliminate mtDNA and YPD to evaluate growth. If a gene is required for growth without mtDNA, then its corresponding deletion strain should not be able to grow following growth in YPD with EtBR. Of the five candidates we directly tested, only **tom70Δ** did not grow following YPD with EtBR treatment (Figure 1.1). This finding demonstrates that **TOM70** is required for growth without mtDNA, which confirms those from an earlier study (Dunn and Jensen 2003). Deletion strains for the
other candidates we tested grew following ethidium bromide treatment, and so these genes are not required for growth without mtDNA.

Ohnologs *TOM70* and *TOM71* are both required for growth without mtDNA

Evolution of the ability to growth without mtDNA following WGD in the yeast lineage may result from functional divergence of ohnologs. To determine whether *TOM70* and its ohnolog, *TOM71*, have diverged in function, we constructed a deletion strain for *TOM71* and evaluated its phenotype relative to *tom70Δ* following treatment with YPD with EtBr. If these ohnologs have diverged in function, then we expect that *tom70Δ* will not grow following treatment with EtBr, but that *tom71Δ* will. Neither *tom70Δ* nor *tom71Δ* grew following treatment with EtBr (Figure 1.2). Thus, both *TOM70* and *TOM71* are required for growth without mtDNA, which does not support the hypothesis that these genes have acquired new functions required for grow without mtDNA in post-WGD yeast lineages.

**Discussion**

The ability to grow without mitochondrial DNA demonstrates the markedly reduced role of mitochondria during energy production by post-WGD yeast species. Functional divergence of duplicated genes retained following WGD, referred to as ohnologs, may contribute to the evolution of this trait. In this study, we identified 18 ohnolog pairs in which one gene is a known or candidate gene for growth without mtDNA and directly tested a subset of these genes. We confirmed that both *TOM70* and its ohnolog *TOM71* are required for growth without mtDNA in *S. cerevisiae*. Our study provides direct evidence that duplicated genes contribute to an
evolutionary innovation in the yeast lineage, although this role is likely not due to their functional divergence.

The ability to grow without mitochondrial DNA depends on many genes.

Many duplicated and non-duplicated genes are required for growth without mtDNA in *S. cerevisiae*. Classical genetic studies have identified 20 genes in *S. cerevisiae* related to the ability of *S. cerevisiae* to grow without mtDNA (Cherry et al. 2012), four of which possess ohnologs, including *TOM70, SDH3, ICYC1* and *SSB1* (Byrne and Wolfe 2005). A high-throughput genetic screen using the Yeast Knock-Out collection (Giaever et al. 2002) identified ~100 additional candidate genes for growth without mtDNA (Dunn et al. 2006) that we determined using the YGOB (Byrne and Wolfe 2005) included an additional 14 candidate genes that also possess ohnologs. Overall, more than 100 genes have been identified as known or candidate genes for growth without mtDNA, including 18 that are retained in duplicate in *S. cerevisiae* since the WGD. While the 18 candidate ohnologs for growth without mtDNA comprise a small proportion of the 551 ohnologs present in *S. cerevisiae* (Byrne and Wolfe 2005), functional changes within these genes may have been required for the evolution of this trait given its association with the WGD in the *Saccharomyces* complex of yeast (Fekete et al. 2007; Merico et al. 2007) and the suspected role of WGD in the evolution of new traits (Ohno 1970).

The requirement of some candidate genes and their ohnologs for growth without mtDNA remains uncertain. A previous study (Dunn and Jensen 2003) showed that *ICYC1* and *SSB1* exhibited reduced growth in response to ethidium bromide treatment, although *ICYC1* was associated with a more subtle phenotype than other genes and *SSB1* did not appear to be required for this trait in our experiments (Figure 1.1). This difference could be due to differences in strain
background or in the sensitivity of our assay. Because subtle phenotypes are difficult to work with, we decided not to pursue these genes further. The requirement for \textit{SDH3} for growth without mtDNA is a relatively recent discovery (Gebert et al. 2011) and the function of its ohnolog, \textit{SHH3}, is unknown. As such, we decided to focus our efforts on genes that were better characterized. Of the 18 candidate ohnologs we identified, only one member of each ohnolog pair was identified as being involved in growth without mtDNA by previous studies (Dunn et al. 2006; Cherry et al. 2012), either because they were not evaluated or they did not meet the minimum threshold for inclusion in the candidate gene list. We only tested both genes in a single ohnolog pair, \textit{TOM70} and \textit{TOM71}, because TOM70 was the only gene we confirmed as being required for growth without mtDNA in our initial experiments. Additional direct tests will be required to determine whether both genes in other ohnolog pairs are required for growth without mtDNA, or whether they exhibit functional divergence.

Ohnologs required for growth without mitochondrial DNA possess overlapping functions.

\textit{TOM70} and \textit{TOM71} possess overlapping functions. \textit{TOM70} is known to be required for growth without mtDNA (Dunn and Jensen 2003), although our study is the first to report that \textit{TOM71} is also required for this trait. We constructed knock-out strains for each of these genes and confirmed that neither of these knock-out strains tolerates the loss of their mtDNA, indicating that both genes are required for this trait.

Both \textit{TOM70} and \textit{TOM71} encode integral proteins of the mitochondrial membrane responsible for the recognition and import of proteins directed to the mitochondrion. \textit{TOM70} is part of the translocase outer membrane (TOM) complex of the mitochondrion and \textit{TOM71}, though poorly characterized compared to \textit{TOM70}, is also a known component of the
mitochondrial membrane (Hines et al. 1990; Söllner et al. 1990; Schlossmann et al. 1996). Although findings from one study indicate that these genes exhibit non-overlapping functions and they only share 53% amino acid identity (Schlossmann et al. 1996), over-expression of \textit{TOM71} is known to recover mitochondrial import of certain proteins in \textit{TOM70} null mutants (Koh et al. 2001).

Maintaining mitochondrial import helps cells tolerate the loss of their mitochondrial DNA. \textit{TOM70} and \textit{TOM71} may be required for growth without mtDNA because they facilitate mitochondrial import. Loss of mtDNA eliminates a cell’s ability to generate an electrochemical potential across the mitochondrial membrane via the electron transport chain (Tzagoloff 1982). The electron transport chain not only facilitates energy acquisition, but also helps ensure protein transport across the mitochondrial membrane. Approximately 1000 proteins are imported into yeast mitochondria for a variety of biological processes (Sickman 2003; Jensen et al. 2004), which means that mitochondrial membrane transport is important for many cell functions besides energy function, e.g., aging and apoptosis (Green and Kroemer 2004; Trifunovic et al. 2004). \textit{TOM70} and \textit{TOM71} may facilitate growth without mtDNA by maintaining mitochondrial import following the loss of mtDNA and ensuring that other important mitochondrial functions continue. Notably, many of the other genes in our list of candidate ohnologs for growth without mtDNA are also associated with the mitochondria (Table 2.1).

Evolution of \textit{TOM70} and \textit{TOM71} likely did not contribute to ability to grow without mitochondrial DNA.
Although both *TOM70* and *TOM71* are required for growth without mtDNA, evolution of these genes following WGD likely did not contribute to this trait. While evolution of duplicated genes may lead to the acquisition of new functions, duplicated genes may also undergo ‘subfunctionalization’ in which they evolve complementary functions equivalent to that of the single-copy ancestral gene (Force et al. 1999; Lynch 2000). One explanation for why *TOM70* and *TOM71* are both required for growth without mtDNA is that these genes have diverged due to subfunctionalization. In this case, the requirement of both genes in the ability to grow without mtDNA did not result from their divergence because the function was already present in the ancestral gene. While it is possible that both of these genes have evolved a new function since the WGD, the acquisition of new functions in both genes is not required to explain why both are required for growth without mtDNA, and it is more parsimonious to assume that the ancestral gene was also required for this trait. Furthermore, these genes do exhibit some degree of divergence in their coding regions (53% amino acid identity) and are known to exhibit partially-overlapping functions during mitochondrial import (Schlossmann et al. 1996; Koh et al. 2001), and so subfunctionlization of the ancestral function most likely explains their contribution to the ability to tolerate the loss of mtDNA. Overall, our findings provide direct evidence that *TOM70* and *TOM71* play an important role in the ability to grow without mtDNA, but that this role is not likely a result of their functional divergence. One possibility is that the ability to grow without mtDNA depends upon changes in some unknown gene, whose product depends on *TOM70* and *TOM71*, e.g., for import into the mitochondria.
Figure 1.1 **TOM70 is required for growth without mitochondrial electron transport.** Growth of strains on YPD plates following treatment with either YPD (-EtBr) or YPD with ethidium bromide (+EtBr) is shown. Strains are listed and include wild-type YJF173 (WT), *K. lactis* (Y-8279), and six deletions strains in the YJF173 background, *tom70Δ, tpk1Δ, phb1Δ, ssb1Δ, isu1Δ*, and *yia6Δ*. Since ethidium bromide prevents replication of mtDNA, strains that require mtDNA for growth do not grow on YPD plates following treatment with ethidium bromide. Like *K. lactis*, which requires mtDNA for growth, *tom70Δ* does not grow following treatment with ethidium bromide.
**Figure 1.2** **TOM70** and **TOM71** are both required for growth without mtDNA. Growth of strains following treatment with YPD (-EtBr) or YPD with ethidium bromide (+EtBr) is shown. Strains are listed and include wild-type YJF173 (WT), *K. lactis* (Y-8279), and two deletion strains in the YJF173 background, *tom70Δ* and *tom71Δ*. (A) Growth on YPD plates. Since ethidium bromide prevents replication of mtDNA, strains that require mtDNA for growth do not grow on YPD plates following treatment with ethidium bromide. Like *K. lactis*, which requires mtDNA for growth, neither *tom70Δ* nor *tom71Δ* grow on YPD plates following treatment with ethidium bromide. (B) Growth on YPEG plates. Strains that lack mtDNA must
rely on fermentation to grow and will not grown on YPEG plates because they lack a fermentable carbon source. All strains were unable to grow on YPEG plates following treatment with ethidium bromide.
Table 1.1 *Saccharomyces cerevisiae* known and candidate genes for growth without mtDNA and their ohnologs.

<table>
<thead>
<tr>
<th>Name</th>
<th>Systematic</th>
<th>Function</th>
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<th>Source</th>
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</thead>
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<td>YBR024W</td>
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</tr>
<tr>
<td>IRA2</td>
<td>YOL081W</td>
<td>GTPase-activating protein</td>
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<td>Dunn et al. 2006</td>
</tr>
<tr>
<td>PET9</td>
<td>YBL030C</td>
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<td>Candidate</td>
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</tr>
<tr>
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<td>Candidate</td>
<td>Dunn et al. 2006</td>
</tr>
<tr>
<td>RSP24B</td>
<td>YIL069C</td>
<td>Component of the small (40S) ribosomal subunit</td>
<td>Candidate</td>
<td>Dunn et al. 2006</td>
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<td>AP4A phosphorylase</td>
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<td>This study, SGD</td>
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<td>not required</td>
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Ohnologs were identified using resources available via the Yeast Gene Order Browser (Byrne and Wolfe 2005). Functional information was obtained from SGD. SGD is the *Saccharomyces* Genome Database (Cherry et al. 2012).
<table>
<thead>
<tr>
<th>Name</th>
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<th>Status</th>
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<td>YER074W</td>
<td>Protein component of the small (40S) ribosomal subunit</td>
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<td>APA2</td>
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<td>SSB2</td>
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<td>ISU2</td>
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<td>cAMP-dependent protein kinase catalytic subunit</td>
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Chapter 2

Evolution of ecological dominance of yeast species in high-sugar environments
Abstract

Evolutionary innovation can lead to the ecological dominance of descendent lineages. In budding yeasts, fermentation in the presence of oxygen evolved around the time of a whole genome duplication (WGD) and caused a dramatic shift in the mode of energy production. A fermentative lifestyle is thought to confer dominance in high-sugar environments because ethanol, a product of fermentation, is toxic to many species. While there are many fermentative yeast species, only *Saccharomyces cerevisiae* consistently dominates wine fermentations. In this study, we use co-culture experiments and intrinsic growth rate assays to assess the relative fitness of pre- and post-WGD yeast species across environments to determine when *S. cerevisiae*’s ability to dominate high-sugar environments arose and to identify what other traits may contribute to dominance. We show that *S. cerevisiae* dominates nearly all other pre- and post-WGD species except for its sibling species *S. paradoxus* in both grape juice and a high-sugar rich medium and that *S. cerevisiae*’s greater relative fitness in ethanol, low-pH and poor nutrient conditions contribute to its dominance. Our results suggest that dominance of grape juice fermentations evolved recently in the *Saccharomyces* species through the acquisition of multiple traits, only one of which depends on the fermentative lifestyle.
Introduction

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

In budding yeasts, evolution of the ability to ferment sugar in the presence of oxygen dramatically changed the way some yeast species harness energy. Whereas most yeast species generate energy through respiration in the presence of oxygen, certain species such as *Saccharomyces cerevisiae* generate most of their energy via the less efficient process of fermentation in the presence of oxygen (Pronk et al. 1996). Evolution of this fermentative lifestyle likely involved multiple steps both before and after a whole genome duplication (WGD) in the yeast lineage, including the ability to grow without mitochondrial electron transport and the transcriptional rewiring of carbon metabolizing enzymes (Ihmels et al. 2005; Merico et al. 2007; Field et al. 2009; Hagman et al. 2013; Lin et al. 2013). While the evolutionary transition to a fermentative lifestyle began prior to the WGD, yeast lineages that diverged after the WGD show a clear preference for fermentation in the presence of oxygen (Merico et al. 2007; Hagman et al. 2013).
Fermentation in the presence of oxygen is thought to provide post-WGD yeast species with a fitness advantage in high-sugar environments such as grape juice (Wolfe and Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006; Conant and Wolfe 2007). Theoretical modeling shows that a fermentative lifestyle can yield a growth advantage in high-sugar environments due to a higher rate of sugar consumption and energy acquisition (Pfeiffer et al. 2001; MacLean and Gudelj 2006; Conant and Wolfe 2007). Additionally, ethanol produced during fermentation may inhibit the growth of competitor species (Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006). Thus, the fermentative lifestyle is expected to enable post-WGD yeast species to dominate high-sugar environments like grape juice.

While *S. cerevisiae* has been shown to dominate competitions with multiple pre-WGD species (Holm Hansen et al. 2001; Pérez-Nevado et al. 2006), the importance of the fermentative lifestyle remains equivocal. Competition experiments between *S. cerevisiae* and several pre-WGD species did not support the role of ethanol but instead implicate different factors depending upon which competitor species were used. Competitions with *Torulaspora delbrueckii* and *Lachancea thermotolerans* demonstrated that low-oxygen and cell-density contribute to *S. cerevisiae*’s dominance (Holm Hansen et al. 2001; Nissen et al. 2003, 2004), while competitions with *Hansenaspora guillermondii* and *H. uvarum* showed that *S. cerevisiae* produces a toxic metabolite derived from glyceraldehyde 3-phosphate dehydrogenase peptides (Pérez-Nevado et al. 2006; Albergaria et al. 2010; Branco et al. 2014). While *S. cerevisiae* exhibits high-ethanol tolerance (Pina et al. 2004; Belloch et al. 2008; Arroyo - López et al. 2010; Salvadó et al. 2011a), mono-culture growth rates of various species indicate that temperature is
more important to *S. cerevisiae's* dominance than ethanol tolerance (Goddard 2008; Salvadó et al. 2011a).

Within the vineyard environment, grapes and wine must contain hundreds of yeast species, including a number of fermentative species (Pretorius 2000; Fleet 2003, 2008; Jolly et al. 2006; Bokulich et al. 2012; Pinto et al. 2014). Yet, even without the introduction of commercial wine yeast, *S. cerevisiae* consistently dominates grape juice as it ferments to wine (Fleet 2003, 2008). Since little is known about the relative fitness of most post-WGD yeast species in high-sugar environments like grape juice, it is unclear whether *S. cerevisiae's* dominance in wine fermentations reflects certain attributes of the grape juice environment or the yeast species present within the community, and whether dominance in high-sugar environments is a simple consequence of the fermentative lifestyle or involves the acquisition of additional traits.

The objectives of this study were to determine when the ability to dominate high-sugar environments evolved in the yeast lineage and to identify traits that confer *S. cerevisiae* with a growth advantage in these environments. To infer when dominance arose and lessen the impact of any potential strain or species outliers, we examined a taxonomically diverse sample of 18 different yeast species spanning the WGD and the evolution of the fermentative lifestyle (Figure 1.1). We find that dominance of high-sugar environments evolved recently along the lineage leading to *S. cerevisiae* and its sibling species *S. paradoxus* and that multiple traits increase *S. cerevisiae's* intrinsic growth rate in grape juice.

**Material and Methods**

Yeast strains
A total of 19 yeast strains representing 18 pre- and post-WGD yeast species were used for our experiments (Table S2.1 and Figure 2.1). We chose a *S. cerevisiae* strain isolated from oak (YPS163) to represent *S. cerevisiae* in all experiments. As a control, we also included a *S. cerevisiae* strain isolated from the vineyard (I14). P. Sniegowski, E. Louis, M. Johnston, M. Eisen, and C. Kurtzman kindly provided representative strains for each yeast species.

**Growth media**

The primary assay media used were two high-sugar environments: Chardonnay grape juice (Vintners Reserve, Winexpert Inc., Port Coquitlam, B.C., Canada), hereafter referred to as “Grape”, and high-sugar rich medium (10 g/l yeast extract, 20 g/l peptone, 120 g/l dextrose), hereafter referred to as “HS”. We chose HS to reflect the glucose concentration typical of the grape juice environment (~ 120 g/l; Rodicio and Heinisch 2009) while limiting the potential influence of nutrient content and low-pH. The medium used to test the effect of acidity on growth was low-pH HS, made by adjusting the pH of HS from 6.7 to 3.7 using tartaric acid, the predominant acid present in grape juice (Radler 1993). We chose pH 3.7 as our low-pH value because it is the same pH as our Grape medium. The media used to test for nutrient limitations in Grape included Grape with one of five nutrient supplements: YP (10 g/l yeast extract, 20 g/l peptone), CM (1.3 g/l synthetic complete with amino acids, 1.7 g/l yeast nitrogen base, and 5 g/l ammonium sulfate), AA (1.3 g/l complete amino acids), NB (1.7 g/l yeast nitrogen base), or AS (5 g/l ammonium sulfate). We chose YP because it is the nutritive base of our HS environment, and we chose the other nutrient supplements because they are less complex than YP. Assay media to test the ethanol tolerance of each yeast species included YPD (10 g/l yeast extract, 20 g/l peptone, 20 g/l dextrose) with ethanol concentrations ranging from 0-10%. Assay media to
identify unknown inhibitor compounds produced by \textit{S. cerevisiae} during growth included YPD made using supernatant from 16 other species (Table S2.1) grown in mono-culture and co-culture with \textit{S. cerevisiae}. We chose YPD with 2\% dextrose for ethanol tolerance and supernatant assays because ethanol produced during growth by fermenting species should not attain inhibitory concentrations.

Preliminary cultures

All yeast strains were streaked from frozen stocks and maintained on YPD plates grown at 30 °C for two to three days prior to each assay. To prepare strains for each experiment, cells were grown in preliminary cultures with 2-4 ml of YPD with shaking (200-300 rpm) at 30°C for 22-24 hours.

Competition experiments

\textit{Growth conditions} – To assess the ability of pre- and post-WGD yeast species to grow relative to \textit{S. cerevisiae}, we measured the abundance of representative strains of six pre-WGD yeast species and seven post-WGD yeast species (Table S2.1) relative to the \textit{S. cerevisiae} strain (YPS163) after growth in co-culture. As controls, we assessed the ability of the conspecific strain, I14, to grow relative to our reference \textit{S. cerevisiae} strain, and grew each species in mono-culture. To begin each culture, cells from three replicate preliminary cultures of each species were diluted to a final concentration of approximately \(10^7\) cells per ml in 1 ml of Grape and HS media. For mono-cultures, each species was diluted individually. For co-cultures, each species was diluted individually and then mixed in equal volume with \textit{S. cerevisiae}. One ml cultures
were grown in 2 ml 96-well plates that were covered with breathable film and incubated at 30°C with shaking at 400 rpm for 48 hours.

Sampling – Samples were taken at the beginning and the end of the experiment and frozen at -20°C for later use. At the beginning of the experiment, diluted cells from *S. cerevisiae* were mixed in equal volume with diluted cells from each species and then immediately frozen. At the end of the experiment (after 48 hours), we took samples to quantify the ratio of *S. cerevisiae* relative to each species after co-culture and mono-culture. For mono-cultures, cells from *S. cerevisiae* mono-culture were mixed in equal volume with cells from each of the other species’ mono-cultures at 48 hours and then frozen.

DNA extraction – DNA was extracted from each sample using a protocol modified from Hoffman (2002) that included adding approximately 200 μl of 0.5 mm-diameter glass beads (BioSpec Products, Bartlesville, OK) to each sample and lysing cells in a bead beater (BioSpec Products) on high for 5 minutes at room temperature. For samples grown in Grape, DNA was also column purified to remove an unknown inhibitor of PCR amplification.

Pyrosequencing – To quantify the abundance of *S. cerevisiae* relative to each species, we pyrosequenced species-specific single nucleotide variants (SNVs) using pyrosequencing primers designed and calibrated for this study. To design pyrosequencing primers, we generated pairwise sequence alignments of *ACT1* or *CYT1* between *S. cerevisiae* and each of the other species and then manually identified at least one SNV for each pair and designed corresponding primer sets that included (1) forward and reverse primers for PCR and (2) a pyrosequencing primer (Table S2.2). To prepare samples for pyrosequencing, DNA fragments containing a SNV were amplified by PCR and then sequenced using a PyroMark Q96 MD Automated pyrosequencer.
(Qiagen, Valencia, CA) following the protocol described by King and Scott-horton (2007) and the manufacturer's directions.

To calibrate each primer set, DNA from samples containing known ratios of cells from *S. cerevisiae* and each of the other species were also pyrosequenced and used to establish standard curves using linear and polynomial regression (Figure S2.1). For two species (*Nakeseomyces bacillisporus* and *Tetrapisispora blattae*) we were not able to design sets of primers, and for three species (*Kazachstania lodderae*, *Kaz. martiniae*, and *Kluyveromyces lactis*) we were not able to quantify abundance due to severely biased PCR or pyrosequencing identified by our control calibrations.

**Analysis** – To determine the abundance of *S. cerevisiae* relative to each species, we adjusted the percentage of species-specific SNVs sequenced during pyrosequencing using our standard curves. If values were negative after this adjustment, we treated them as zero. To identify growth differences between species, we used one-tailed paired Welch’s *t* test to test whether the relative abundance of *S. cerevisiae* at the end of the experiment was greater than it was at the start of the experiment. To correct for multiple comparisons, we used the method of Benjamini and Hochberg (1995) and a false discovery rate (FDR) cutoff of less than 0.01 for significance. If the relative abundance of *S. cerevisiae* was significantly greater at the end of the experiment, it was considered “dominant.”

Intrinsic growth rate experiments

**Growth assays** – Cells from three replicate preliminary cultures of each species were diluted to an optical density (OD) at 600 nm of approximately 0.25 in 1 ml of growth medium and were grown in 2 ml 96-well plates that were covered with breathable film and incubated at
30°C with shaking at 400 rpm for up to 48 hours. Cell density was measured by OD at 620 nm at 0, 4, 8, 12, 18, 24, 36, and 48 hours using an iEMS microplate reader (Thermo Lab Systems, Helsinki, Finland).

**Analysis** – The intrinsic growth rate ($r$) of each species was calculated for each time interval using the equation $N_t = N_0 e^{rt}$, where $N_t$ is final cell density, $N_0$ is initial cell density and $t$ is time in hours. The average intrinsic growth rate of each species for a given medium was then used to evaluate the effect of a treatment (i.e., low-pH or a nutrient supplement) on the growth of each species, ($Δr_{Treatment} = r_{Treatment} - r_{Control}$), or the effect of the species in a given environment ($Δr_{Species} = r_{non-S. cerevisiae} - r_{S. cerevisiae}$).

For two sets of experiments, we only used OD up to and including 24 hours in our analysis: ethanol tolerance experiments and supernatant experiments to identify unknown inhibitor compounds. For ethanol tolerance experiments, we observed flocculation in numerous samples that increased the variability of OD measurements beginning at 36 hours. For inhibitor compound experiments, we observed that un-inoculated control samples registered noticeable effects on OD measurements beginning at 36 hours for many samples.

Ethanol tolerance among species was measured by the ethanol concentration that inhibited growth by 50% ($IC_{50}$). $IC_{50}$ estimates were obtained by fitting dose response curves using a three-parameter Weibull function in R (R Development Core Team 2013) using the ‘drc’ package (Ritz and Streibig 2005). Statistical comparisons between the estimated $IC_{50}$ for $S$. cerevisiae and each species were made using the ‘comped’ function (Ritz and Streibig 2005; R Development Core Team 2013) followed by the Altman and Bland method to calculate $P$ values from confidence intervals (Altman and Bland 2011). To correct for multiple comparisons, we
used the method of Benjamini and Hochberg (1995) and a FDR cutoff of less than 0.01 for significance.

Principal Component Analysis

We conducted principal component analysis (PCA) using the intrinsic growth rates from grape juice (Grape and Grape with each of five nutrient supplements), high-sugar (HS and low-pH HS), and each of the ethanol treatments in YPD. Principal components were obtained using the ‘prcomp’ function in R (R Development Core Team 2013) after scaling and centering the intrinsic growth rates.

Results

Evolution of ecological dominance in grape juice evolved recently in the lineage leading to S. cerevisiae and S. paradoxus

To determine when ecological dominance of high-sugar environments evolved in the yeast lineage, we grew representative strains of multiple pre- and post-WGD yeast species in two high-sugar environments: Chardonnay grape juice (Grape) and a high-sugar rich medium (HS). If dominance in high-sugar environments evolved along with the evolution of fermentation in the presence of oxygen, we expect S. cerevisiae to dominate all pre-WGD yeast species but not consistently dominate post-WGD yeast species in both high-sugar environments. Grape was chosen to represent a natural high-sugar environment, and HS was chosen to replicate the sugar concentration typical of the grape juice environment while limiting the potential influence of nutrient content and low-pH. Since the ability to dominate is inherently a relative trait, we assessed the growth of each pre- and post-WGD yeast species relative to a representative S.
cerevisiae strain isolated from an oak tree (YPS163) using co-cultures. As a control, we also grew a S. cerevisiae strain isolated from a vineyard (I14) in co-culture with our reference S. cerevisiae strain. If the relative abundance of S. cerevisiae was significantly greater at the end of the experiment, it was considered “dominant”.

We find that S. cerevisiae dominates nearly all pre- and post-WGD yeast species in Grape and HS co-cultures (Figure 2.2A and 2.2B). In both Grape and HS, S. cerevisiae increased in abundance by 48 hours relative to 11 out of 13 pre- and post-WGD yeast species (FDR < 0.01, Table S2.3). In the majority of these co-cultures, S. cerevisiae was greater than 90% of the population at 48 hours. Notably, S. cerevisiae remained a significant proportion of the population even when it did not dominate. These data show that S. cerevisiae is able to dominate in multiple high-sugar environments, and they suggest that the ability to dominate high-sugar environments arose recently in yeast evolution.

In support of a more recent evolution of ecological success in high-sugar environments, S. paradoxus is the only species that persists along with S. cerevisiae in Grape and HS co-cultures. At 48 hours, the percentage of S. paradoxus present was 58% in Grape and 35% in HS (Figure 2.2A and 2.2B), which was not associated with a significant change in the percentage of S. cerevisiae present in either environment (Table S2.3). Two other strains, S. cerevisiae (I14) and Candida glabrata, also competed well with our S. cerevisiae reference. However, their persistence depended upon the environment: S. cerevisiae dominated C. glabrata in Grape (FDR = 0.0072) but not in HS, whereas it dominated I14 in HS (FDR = 0.0010) but not in Grape. Thus, when competing with S. cerevisiae, S. paradoxus was the only species able to compete well in both high-sugar environments.
One explanation for *S. cerevisiae*’s dominance in our Grape and HS co-cultures is that it has a greater carrying capacity than other species in these environments even when they are grown individually. As a control for our co-culture experiments, we also measured the density of each species grown in mono-culture by mixing it with a *S. cerevisiae* mono-culture after 48 hours and quantifying the proportion of each species by pyrosequencing (see Materials and Methods).

*S. cerevisiae* has a carrying capacity similar to the majority of pre- and post-WGD yeast species in Grape and HS (Figure 2.2C and 2.2D). In Grape, the abundance of *S. cerevisiae* was significantly greater than only 2 out of 13 species after 48 hours of mono-culture (Figure 2.2C, FDR < 0.01). Species that obtained significantly lower carrying capacities included the pre-WGD yeast species *H. vineae* and the post-WGD yeast species *Vanderwaltozyma polyspora*, which were 1% and 3% of *S. cerevisiae*’s abundance after 48 hours in mono-culture. The relative population size of *S. cerevisiae* was also not significantly greater than 13 out of 13 species tested in HS. These data imply that *S. cerevisiae*’s dominance in Grape and HS co-cultures is not due to differences between species in their individual carrying capacities.

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

Our finding that many post-WGD yeast species compete poorly with *S. cerevisiae* in high-sugar environments suggests that the fermentative lifestyle is not sufficient to confer ecological success in these environments. Since the majority of yeast species are capable of achieving similar carrying capacities to *S. cerevisiae* in these environments when grown individually, *S. cerevisiae*’s dominance in our Grape and HS co-cultures must be related to either differences in intrinsic growth rates or interference competition. To investigate these two modes
of ecological dominance, we measured the intrinsic growth rate of each species in mono-culture. If *S. cerevisiae* does not exhibit a greater intrinsic growth rate than other species, then its ability to dominate in these environments can be attributed to interference competition.

*S. cerevisiae* has a greater intrinsic growth rate than nearly all pre- and post-WGD yeast species in the grape juice environment (Figure 2.3). Compared to *S. cerevisiae*, 16/17 yeast species exhibited a significantly lower intrinsic growth rate in Grape (FDR < 0.01, Table S2.4). The one notable exception to this pattern was *S. paradoxus*, which had a lower growth rate but did not meet our cutoff for significance (FDR = 0.0284). In stark contrast to our finding in Grape, when we compared the intrinsic growth rate of *S. cerevisiae* to the intrinsic growth rate of each of the other species in HS, we did not observe any significant difference for 17/17 species (Figure 2.3B). These results suggest different or multiple mechanisms contribute to the dominance of *S. cerevisiae* in high-sugar environments. Furthermore, they support the recent evolution of traits required for ecological success in the grape juice environment. In the following sections we examine factors that may contribute to the dominance of *S. cerevisiae* in both HS and Grape.

Evolution of ethanol tolerance and its potential role in interference competition

*S. cerevisiae*’s ability to produce and tolerate ethanol is one way in which it may dominate other species in high-sugar environments. Although previous studies showed that *S. cerevisiae* tolerates higher ethanol concentrations than many yeast species, they only included 4/17 of the other yeast species used in this study (Pina et al. 2004; Belloch et al. 2008; Arroyo-López et al. 2010; Salvadó et al. 2011a). To examine the potential impact of ethanol on the growth of each species, we measured the intrinsic growth rate of each species in YPD.
supplemented with ethanol at concentrations ranging from 0-10% and calculated the ethanol concentration that inhibited growth rate by 50% (IC\textsubscript{50}) for each species by fitting dose-response curves to the growth rate (see Materials and Methods).

*S. cerevisiae* had an IC\textsubscript{50} greater than 15/17 yeast species (Figure 2.4 and Table S2.5). The two exceptions to this pattern were *S. cerevisiae*’s closest relative, *S. paradoxus* (FDR = 0.0502) and *C. glabrata* (FDR = 0.0320). *C. glabrata* grew as well as *S. cerevisiae* at moderate ethanol concentrations, and it grew better than *S. cerevisiae* at low ethanol concentrations (Figure S2.2 and Table S2.5). However, most of *S. cerevisiae*’s growth advantage occurred at ethanol concentrations at or above 4% (Figure S2.2 and Table S2.5). Thus, while all yeast species tolerate low concentrations of ethanol (< 4%), *S. cerevisiae* exhibits a growth advantage compared to most yeast species at high-ethanol concentrations.

No evidence for interference competition mediated by other toxic metabolites

In addition to ethanol, previous studies revealed that *S. cerevisiae* produced other toxic metabolites that inhibit the growth of competitor species (van Vuuren and Jacobs 1992; Magliani et al. 1997; Musmanno et al. 1999; Pérez-Nevado et al. 2006; Albergaia et al. 2010; Rodríguez-Cousiño et al. 2011; Branco et al. 2014), although other studies either did not reveal any evidence that *S. cerevisiae* produced an inhibitory compound (Torija et al. 2001; Nissen et al. 2003; Arroyo-López et al. 2011) or revealed that the ability to produce killer toxins varied among *S. cerevisiae* strains (Gutiérrez et al. 2001; Sangorrín et al. 2007; Maqueda et al. 2012).

To determine whether the *S. cerevisiae* strain we used during our assays produces an inhibitor compound, we grew each pre- and post-WGD species in the supernatant obtained from YPD mono-cultures and co-cultures with *S. cerevisiae*. We chose YPD, which contains 2% dextrose,
because ethanol concentrations should not attain inhibitory concentrations during growth. In no instance did the supernatant inhibit the subsequent growth of each species (Figure S2.3 and Table S2.6).

Low-pH and nutrient limitations contribute to *S. cerevisiae’s* intrinsic growth rate advantage in grape juice.

Grape juice differs from high-sugar rich medium in that it has a lower pH (pH = 3.7 vs pH = 6.7) and reduced levels of nutrients, most notably yeast assimilable nitrogen (Henschke and Jiranek 1993). To determine whether *S. cerevisiae’s* higher intrinsic growth rate in grape juice is related to pH or nutrient deficiencies we measured the effects of altered pH of HS and nutrient content of Grape for each species.

To test the effect of pH on the intrinsic growth rate of each species, we grew each species in low-pH HS, which is HS medium adjusted to the same acidity level as our Grape medium. As a control, we compared each yeast species’ growth in low-pH HS to its growth in HS (see Materials and Methods for details). If *S. cerevisiae’s* intrinsic growth rate is greater than other species in Grape due to low-pH, then *S. cerevisiae* should also exhibit a higher intrinsic growth rate than other species in low-pH HS.

*S. cerevisiae* has an intrinsic growth rate advantage in low-pH HS (Figure 2.5). When grown in low-pH HS, 4/18 pre- and post-WGD yeast species exhibited a significantly lower intrinsic growth rate when compared to growth in HS (Figure 2.5A and Table S2.7). Notably, only three species, including *S. cerevisiae*, were not affected by low-pH at a nominal level of significance (alpha = 0.05) compared to a FDR cutoff of 0.01. Additionally, when we compared the intrinsic growth rate of *S. cerevisiae* in low-pH HS to each of the other species in this
environment, *S. cerevisiae*’s intrinsic growth rate was greater than 8/17 pre- and post-WGD yeast species (Figure 2.5B), compared to 0/17 yeast species observed in HS (Figure 2.3B).

To test the effect of nutrient deficiency on the intrinsic growth rate of each species, we grew each species in Grape supplemented with one of several different nutrient sources that varied in complexity: YP, CM, NB, AA, and AS. YP is the rich nutritive base of the HS environment, CM contains vitamins and minerals, amino acids, and a single good nitrogen source, and NB, AA, and AS are the vitamins and minerals, amino acids, and nitrogen source (ammonium sulfate) components of CM, respectively (see Materials and Methods for details). As a control, we compared each species’ growth in Grape with a nutrient supplement to its growth in Grape without the nutrient supplement. If nutrient limitations contribute to intrinsic growth rate differences between species, then nutrient supplements in Grape should increase each species growth rate and reduce or eliminate intrinsic growth rate differences between species.

Most yeast species are nutrient limited in grape juice. Of the 18 species we assayed, 12 exhibited a significant increase in intrinsic growth rate with the addition of one or more nutrient supplements, including *S. cerevisiae* (Figure 2.6, Figure S2.4 A-D, and Table S2.8). However, which nutrients elicited a significant increase in growth varied by species. For example, *S. bayanus* was positively affected by the addition of YP and NB, whereas *C. glabrata* was positively affected by YP, CM and AA. Overall, YP positively affected the intrinsic growth rate of the most species (11), followed by NB (7), CM (6), and AA (3). However, none of the yeast species we assayed grew significantly better with the addition of AS, a good nitrogen source.

Nutrient supplements eliminate the intrinsic growth rate differences between *S. cerevisiae* and nearly all other species. Of the 17 species that grew significantly slower than *S. cerevisiae* in Grape (Figure 2.3A), only 2/18 species, *Naumovozyma castellii* and *V. polyspora*, grow
significantly slower than *S. cerevisiae* in spite of all of the nutrient supplements used in our experiments (Figure 2.6, Figure S2.4 A-D, and Table S2.8). Overall, Grape supplemented with CM had the fewest number of species that still grew significantly slower than *S. cerevisiae* (4), followed by Grape supplemented with AS (7), NB (12), YP (14), and AA (16).

*S. cerevisiae* exhibits a distinct fitness profile

To compare the intrinsic growth rates of all the species while accounting for the strong correlations between environments we used principal component analysis (Figure 2.7). The first two principal components accounted for 59.5% (PCA1) and 19.7% (PCA2) of the variation in the intrinsic growth rate of each species. Growth rate loadings on PCA1 were of similar value and direction for all media (Table S2.9), indicating that PCA1 is a measure of overall growth, independent of the environment. In this regard, *S. cerevisiae* and *C. glabrata* distinguished themselves as the species with the highest growth rates overall. Growth rate loadings on PCA2 were different across media, with the largest positive loadings on Grape and Grape with nutrient supplements and the largest negative loadings on low concentrations of ethanol (< 6%). Taking all axes of variation together, *S. cerevisiae* exhibits a distinct fitness profile and grows particularly well compared to other species in grape juice.

**Discussion**

Fermentation of sugar to ethanol in the presence of oxygen provides post-WGD species the opportunity to exploit novel environments and ecological strategies. One of the post-WGD species, *S. cerevisiae*, consistently dominates wine fermentations and has become widely used to ferment beer, bread and wine. In this study, we investigated when *S. cerevisiae's* ability to
dominate high-sugar environments evolved and whether its dominance is a simple consequence of the fermentative lifestyle. We find that dominance evolved recently along the lineage leading to *S. cerevisiae* and its sibling species *S. paradoxus*, much later than the evolution of the fermentative lifestyle. Although we find coincidental changes in ethanol tolerance, we show that multiple traits besides ethanol tolerance contribute to *S. cerevisiae*’s high fitness in grape juice. Our results suggest that dominance of grape juice is mediated by the evolution of multiple traits that build on an ancient change in metabolism.

**Evolution of ecological dominance in relation to the fermentative lifestyle**

Our study indicates that dominance in high-sugar environments evolved recently along the lineage leading to *S. cerevisiae* and *S. paradoxus*. While previous studies showed that *S. cerevisiae* dominates multiple other pre-WGD species (Holm Hansen et al. 2001; Fleet 2003, 2008; Pérez-Nevado et al. 2006; Goddard 2008), our findings demonstrate that *S. cerevisiae* will also dominate most other post-WGD yeast species. However, a number of alternative but more complex possibilities for the evolution of dominance exist. One possibility is that the ability to dominate high-sugar environments evolved progressively, initiating around the time of the WGD. Since we only measured pairwise competitions with *S. cerevisiae*, the fermentative lifestyle could enable post-WGD species to outcompete pre-WGD species even though they lose to *S. cerevisiae*. We find this scenario unlikely given the absence of a clear separation between the pre- and post-WGD species in regards to ethanol tolerance and intrinsic growth rate in grape juice. Another possibility is that dominance evolved on the lineage leading to the *Saccharomyces* species followed by differentiation of these species based on their thermal preference. While we performed all of our assays at 30°C, *S. bayanus* and *S. kudriavzevii* are considered cryophilic
(Belloch et al. 2008; Gonçalves et al. 2011; Salvadó et al. 2011b) and *S. bayanus* dominates some wines at low temperatures (Torriani et al. 1999; Naumov et al. 2000; Sipiczk et al. 2001; Rementeria et al. 2003; Demuyter et al. 2004). In co-culture competitions, *S. kudriavzevii* does not dominate but competes better with *S. cerevisiae* at low temperatures (Arroyo-López et al. 2011). While certain temperatures may influence dominance, we observed no difference between the growth rate of *S. cerevisiae* and *S. bayanus* in high-sugar rich medium and *S. bayanus*'s growth rate in grape juice increased with supplementation of rich medium (YP) to a rate equivalent to that of *S. cerevisiae*.

Multiple mechanisms of ecological dominance

*S. cerevisiae*'s dominance of high-sugar environments cannot be explained by a single mechanism. While most species are dominated by *S. cerevisiae* in both grape juice and high-sugar rich medium, *C. glabrata* is dominated by *S. cerevisiae* in grape juice but not high-sugar rich medium. These results imply *S. cerevisiae*'s dominance of *C. glabrata* in grape juice is mediated by a different mechanism than the other yeast species. One explanation for *S. cerevisiae*'s dominance of *C. glabrata* in grape juice is that *C. glabrata* is limited by poor nutrients. While *C. glabrata* is as resistant to ethanol and low-pH as *S. cerevisiae*, its growth rate in grape juice is increased by the addition of rich medium (YP) to a level similar to that of *S. cerevisiae*.

Interference competition through the production of ethanol provides one explanation for *S. cerevisiae*'s dominance of high-sugar rich medium. Consistent with previous studies (Pina et al. 2004; Belloch et al. 2008; Arroyo López et al. 2010; Salvadó et al. 2011a), we found that *S. cerevisiae* exhibits greater ethanol tolerance than most yeast species. In support of the role of
ethanol tolerance in dominance, *S. paradoxus* and *C. glabrata* exhibited ethanol tolerance similar to *S. cerevisiae* and were the only two species that were not dominated by *S. cerevisiae* in high-sugar rich medium.

However, previous studies showed that oxygen, cell density and an inhibitory peptide affect *S. cerevisiae*'s dominance of various pre-WGD species (Holm Hansen et al. 2001; Nissen et al. 2003, 2004; Pérez-Nevado et al. 2006; Albergaria et al. 2010; Branco et al. 2014). These studies excluded the effects of ethanol because pre-WGD species initiated cell death before ethanol reached inhibitory concentrations. While we only measured competitions with two of the species used in earlier studies, *T. delbrueckii* and *L. thermotolerans*, we cannot exclude the possibility that these species were dominated for reasons other than ethanol inhibition. One difference between our experiments and those of prior studies is that they were performed with low or no agitation, whereas we performed our competitions under high agitation (400 rpm). Agitation is expected to increase dissolved oxygen and might eliminate cell density and confinement effects (Nissen et al. 2003, 2004; Arneborg et al. 2005).

Multiple lines of evidence suggest that *S. cerevisiae*’s dominance in grape juice is influenced by its high intrinsic growth rate in this environment. *S. cerevisiae* exhibited the highest rate of growth in grape juice, significantly higher than all species except *S. paradoxus*. The absence of any difference in growth rate in high-sugar rich medium implies that *S. cerevisiae*’s high fitness in grape juice is specific to grape juice or similar environments. Furthermore, lowering the pH of high-sugar rich medium did not affect *S. cerevisiae* but affected the growth of other species, and supplementation of nutrients to grape juice increased the growth of many species but had little to no effect on *S. cerevisiae*. Notably, *S. bayanus*, *C. glabrata* and *H. vineae* grew as well as *S. cerevisiae* in low-pH medium and in grape juice supplemented with
rich nutrients (YP), indicating that low nutrients alone may explain their slow growth in grape
juice.

The relative importance of intrinsic growth rate and ethanol inhibition to *S. cerevisiae*’s
dominance of grape juice is uncertain. While both factors are of sufficient magnitude to explain
*S. cerevisiae*’s dominance, their effects are difficult to disentangle from one another. However,
we favor intrinsic growth rate as a driver of dominance as it acts earlier and throughout the
competition. Because most species were not significantly inhibited by ethanol concentrations
below 5%, ethanol inhibition is not likely to be important until the later stages of fermentation, as
findings from other studies also indicate (Goddard 2008; Salvadó et al. 2011a). In comparison, a
fitness advantage of 15% will steadily increase the frequency of *S. cerevisiae* from 50% to 90%
in 15 generations (Hartl and Clark 1989). Excluding *S. paradoxus* and *S. bayanus*, *S. cerevisiae*’s
fitness advantage is between 15-150% based on our intrinsic growth rate measurements in grape
juice. Thus, while it is not possible to know the relative contributions of intrinsic growth rate and
ethanol inhibition, both are expected to influence the outcome of a competition.

Interactions between factors may also contribute to *S. cerevisiae*’s dominance. Ethanol
and high-temperature act synergistically to decrease growth due to their overlapping effects on
lipid membrane integrity (Piper 1995). Lipid membrane integrity importantly affects proton (H+)
transport across the cell membrane, and the combined effects of ethanol and high-temperature
increase the lipid membrane’s H+ permeability (Madeira et al. 2010). Increased H+ permeability
can also result in reduced intracellular pH, particularly in acidic environments such as grape
juice. While we did not measure any interaction effects, Goddard (2008) found interactions
between the effects of temperature, ethanol and media including grape juice on the growth rate of
*Saccharomyces* versus non-*Saccharomyces* species.
Ecology of high-sugar environments

The fermentative lifestyle is hypothesized to coincide with the evolution of flowering plants due to the abundant supply of diverse high-sugar environments (Wolfe and Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Conant and Wolfe 2007). While the ecology of fermentative species is not well known, many have been isolated from insects and may be transported to high-sugar environments (Kurtzman et al. 2011). However, the recent evolution of traits that contribute to S. cerevisiae's dominance of high-sugar environments is perplexing.

Although both S. cerevisiae and S. paradoxus can be found in vineyards (Redzepović et al. 2002; Hyma and Fay 2013), these species are commonly associated with tree bark, soil and decaying leaves (Naumov et al. 1998; Sniegowski et al. 2002; Zhang et al. 2010; Wang et al. 2012; Hyma and Fay 2013). Given their abundance in arboreal habitats, it seems unlikely that their exceptional fitness in grape juice and high-ethanol environments is due to adaptation to grape juice fermentations. One way in which these species may have become adapted to high-sugar but low-nutrient environments is through associations with insect-honeydew, which is high in sugar (>10 g/l) but low in amino acids (Douglas 1993; Fischer and Shingleton 2001; Fischer et al. 2002). While a variety of insects and other animals exploit honeydew for its sugar resources (Beggs and Wardle 2006) and a recent investigation revealed that many taxonomically diverse fungi compete for honeydew (Dhami et al. 2013), the presence and utilization of this carbon source by yeasts has yet to be studied.
Figure 2.1 – Phylogenetic relationships of yeast species used in this study. The phylogeny is based on two previous studies (Kurtzman and Robnett 2003; Rokas et al. 2003) only differ in the placement of *C. glabrata*. The whole genome duplication (WGD) event is shown and pre- (red) and post- (blue) WGD species are colored.
Figure 2.2 – Abundance of *S. cerevisiae* relative to pre- and post-WGD yeast species after co-culture or mono-culture in two high-sugar environments. The abundance of *S. cerevisiae* relative to other species in Grape (A) and HS (B) co-cultures and Grape (C) and HS (D) mono-
cultures. Bars and whiskers represent the mean and 95% confidence interval of the percentage of *S. cerevisiae* present after 48 hours and black diamonds indicate the percentage of *S. cerevisiae* at the start of the experiment (zero hours). Names of pre- (red) and post- (blue) WGD species are colored. Significant changes in the percentage of *S. cerevisiae* from zero hours to 48 hours are shown for FDR < 0.01 (*) and FDR < 0.001 (**).
Figure 2.3 – Intrinsic growth rate differences in high-sugar environments. The intrinsic growth rate of each yeast species in Grape (A) and HS (B). Names of pre- (red) and post- (blue) WGD species are colored. Bars and whiskers represent the mean and standard deviation of the
growth rate. Species that did not differ significantly from *S. cerevisiae* at an FDR cutoff of 0.01 are indicated (NS).
Figure 2.4 – Species differences in ethanol tolerance. Estimate (bars) and standard error (whiskers) of the concentration of ethanol (%) that inhibits growth by 50% (IC$_{50}$) of each yeast species. Names of pre- (red) and post- (blue) WGD species are colored. Species with an IC$_{50}$ that did not differ significantly from S. cerevisiae at an FDR cutoff of 0.01 are indicated (NS).
Figure 2.5 – Intrinsic growth differences in response to low-pH. (A) The effect of low-pH treatment on the intrinsic growth rate \((r)\) of each species in HS \((\Delta r_{\text{Treatment}} = r_{\text{Treatment}} - r_{\text{HS}})\), and (B) the difference in the intrinsic growth rate between \(S. \text{cerevisiae}\) and each species in low-pH HS \((\Delta r_{\text{Species}} = r_{\text{non-S. cerevisiae}} - r_{S. cerevisiae})\). Names of pre- (red) and post- (blue) WGD species are colored.
Whiskers for each bar show 95% confidence intervals. Significant differences in the growth rate of each species with or without low-pH and differences between *S. cerevisiae* and each species in low-pH are shown for FDR < 0.01 (*) and FDR < 0.001 (**).
Figure 2.6 – Intrinsic growth rate in Grape supplemented with nutrients. The mean intrinsic growth rate of each species in Grape supplemented with nutrients (YP). Names of pre- (red) and post- (blue) WGD species are colored. Bars and whiskers represent the mean and standard deviation of the growth rate. Significant differences in the growth rate of each species with or without YP (a) and differences between *S. cerevisiae* and each species in YP (b) are labeled above each bar for FDR < 0.01.
Figure 2.7 – *S. cerevisiae* exhibits a distinct fitness profile. First (PCA1) and second (PCA2) principal components are shown for each species based on 18 measurements of intrinsic growth rate. Names of pre- (red) and post- (blue) WGD species are colored. The percent variation shown by each coordinate is shown in parentheses.
Dissertation Discussion
In order to learn more about how evolutionary innovations evolve and how they contribute to the ecological and evolutionary success of lineages, I explored the relationship of evolutionary innovation, WGD, and the ecological success of diverse yeast species in the *Saccharomyces* complex of yeasts. In particular, I identified a duplicated gene required for a trait that evolved during the transition to the fermentative lifestyle, determined that ecological dominance in high-sugar environments evolved much more recently than the transition to the fermentative lifestyle, and identified multiple traits that contribute to the dominance of *Saccharomyces cerevisiae* in high-sugar environments. In the sections that follow, I summarize the key findings from each of my chapters and discuss their direct implications. I then go on to discuss my findings in the context of other research efforts using the *Saccharomyces* complex of yeast to understand the relationship of WGD, evolutionary innovation and the ecological success of species. Finally, I make recommendations for future evolutionary studies using yeast.

**WGD and evolutionary innovation in the yeast lineage**

In Chapter 1, I synthesized the findings from multiple previous studies (Ragnini et al. 1994; Kerscher et al. 2000; Stribinskis et al. 2001; Dunn and Jensen 2003; Senapin et al. 2003; Byrne and Wolfe 2005; Dunn et al. 2006, 2008; Hwang et al. 2007; Gebert et al. 2011) to identify duplicated genes retained in *Saccharomyces cerevisiae* since a WGD event that are also either known or candidate genes for growth without mtDNA, one of the phenotypes that evolved in yeast during the evolutionary transition to a fermentative lifestyle (Fekete et al. 2007; Merico et al. 2007). Using this approach, I identified 18 duplicated genes that are also known or candidate genes for growth without mtDNA in *S. cerevisiae*. Notably, only one gene in each duplicate-pair had been identified as a candidate for growth without mtDNA in the previous
studies, which suggests these duplicated genes have diverged in function. I then constructed yeast knock-out strains for a subset of candidate genes and evaluated the ability of these knock-out strains to grow following the loss of their mtDNA. Only the knock-out strains for TOM70 and TOM71 did not grow following the loss of their mtDNA, which shows that both of these genes are required for this trait in *S. cerevisiae*. Although previous studies showed that TOM70 is required for growth without mtDNA (Dunn and Jensen 2003), my finding that TOM71 is required for growth without mtDNA is novel.

My discovery that TOM71 is required for growth without mtDNA makes several important contributions to evolutionary biology. First, this finding highlights the utility of considering gene duplicates as a potential source of additional candidate genes for other traits. TOM71 was not identified as a candidate gene for growth without mtDNA in a previous genetic screen, nor were the duplicates of any of the other known or candidate genes for this trait (Dunn et al. 2006). By considering the duplicates of known and candidate genes for growth without mtDNA, I was able to identify a new gene that the previous screen had missed because it fell below the threshold for consideration. Given the difficulty of identifying the genetic basis of traits, this approach may help to identify more candidate genes for other traits. Second, most evidence to support the role of duplicated genes in evolutionary innovation comes from genome-wide screens of protein evolution and gene expression (e.g., Gu et al. 2005), the discovery that TOM71 is required for growth without mtDNA provides direct evidence that a duplicated gene retained since WGD contributes to an evolutionary innovation in *S. cerevisiae*. Third, both TOM70 and TOM71 are known to transport proteins across the outer mitochondrial membrane (Hines et al. 1990; Söllner et al. 1990; Schlossmann et al. 1996). The requirement of both of these genes for the ability to grow without mtDNA shows that this trait depends on efficient
import of proteins into the mitochondria. Notably, several other duplicated genes that are known or candidate genes for growth without mtDNA are also involved in mitochondrial transport. Direct tests of these genes will help to determine the potential role of these other duplicated genes in the evolution of this trait.

Although WGD is suspected to facilitate evolutionary innovation (Ohno 1970; Otto and Whitton 2000), many duplicate-pairs may be maintained because they evolve complementary loss of function mutations (Force et al. 1999; Lynch 2000). My finding that both TOM70 and TOM71 are required for growth without mtDNA likely indicates that these genes have partitioned the function of the ancestral gene that was also required for this trait. While it is possible that both of these genes have evolved a new function since the WGD, the acquisition of new functions in both of these genes is not required to explain why both of them are required for growth without mtDNA, and it is more parsimonious to assume that the ancestral gene was also required for this trait. While complementation assays in yeast knock-out strains of TOM70 and TOM71 using the corresponding single-copy gene from a lineage that did not experience WGD would help to verify this hypothesis, I believe that characterizing the evolution of other duplicated genes may offer more insight into how WGD contributes to evolutionary innovation.

More direct evidence for duplicated genes involved in evolutionary innovations is needed. While I provide directed evidence that a duplicated gene in yeast is required for an evolutionary innovation, more examples are needed. Candidate genes for future direct tests may come from duplicated genes identified in this study or from genes with known phenotypes that also exhibit accelerated and asymmetric protein evolution since the duplication (Conant and Wagner 2003; Byrne and Wolfe 2007; Scannell and Wolfe 2008). However, it seems that obtaining a precise understanding of how duplicated genes contribute to evolutionary innovation
will depend on the analysis of genes whose functions are already well characterized. While the excitement surrounding the evolutionary implications of WGD lies in its potential to enable the evolution of new functions, the fraction of duplicated genes that actually contribute to the acquisition of new functions remains to be determined through detailed genetic analyses.

**Evolutionary innovation and the ecological success of diverse yeast species**

In Chapter 2, I directly tested the growth of multiple yeast strains representing a taxonomically diverse set of species in co-cultures and mono-cultures in multiple environments to determine when ecological dominance of high-sugar environments evolved in relationship to the evolution of the fermentative lifestyle in yeast and to identify traits that contribute to the ecological success of *S. cerevisiae* in these environments. The results from my co-cultures show that *S. cerevisiae* dominates representative strains for nearly all yeast species in multiple high-sugar environments, including those that are known to exhibit the fermentative lifestyle. The one exception to this pattern is *S. paradoxus*, the closet relative of *S. cerevisiae*. To identify fitness traits in *S. cerevisiae*, I directly tested the fitness of each representative strain in response to different environmental attributes, including pH, nutrients, and ethanol. The results from my mono-cultures show that *S. cerevisiae* exhibits a fitness advantage relative to representatives from other species in response to multiple different environmental attributes, including low-pH, poor nutrients, and high-ethanol, and consistent with co-culture results, *S. paradoxus* is the only species that consistently grows as well as *S. cerevisiae*. Overall, these findings support that ecological dominance in high-sugar environments evolved very recently in the yeast lineage and involved the acquisition of multiple fitness traits, only one of which depends upon the fermentative lifestyle.
Approaches used in this chapter make several novel and important contributions to yeast research. First, while previous studies have assayed the fitness of *S. cerevisiae* and a few representatives of other species (Holm Hansen et al. 2001; Nissen et al. 2003, 2004; Pérez-Nevado et al. 2006; Albergaria et al. 2010; Branco et al. 2014), I used representatives of a taxonomically diverse set of species spanning the evolutionary transition to a fermentative lifestyle. By measuring the fitness of representatives of so many species in the *Saccharomyces* complex, my findings not only provide insight into the evolution of multiple fitness traits within this lineage, but also begin to fill a considerable gap in the current knowledge regarding the growth preferences of many yeast species. Second, directly testing diverse yeast species in direct competition with *S. cerevisiae* presents a considerable technical challenge because the cryptic or aggregate morphology of some species can lead to inaccurate cell counts using standard laboratory approaches. I employed a new approach using pyrosequencing that enabled me to quantify the relative abundance of *S. cerevisiae* and representative strains of many yeast species. Although there is much to be learned about the growth characteristics of different yeast species, the approaches used in this chapter provide useful background for future studies.

My discovery that ecological dominance evolved recently in the yeast lineage challenges current assumptions regarding the ecological success of *S. cerevisiae* in high-sugar environments. The prevailing assumption is the *S. cerevisiae* dominates high-sugar environments because the fermentative lifestyle provides a fitness advantage through the production of toxic ethanol (Wolfe and Shields 1997; Piskur and Langkjaer 2004; Thomson et al. 2005; Piskur et al. 2006). However, other fermenting yeast species are also found within vineyards but are not known for their ecological success in these environments (Fleet 2008; Hyma and Fay 2013). While the fermentative lifestyle may contribute to the fitness advantage of *S. cerevisiae*, my
findings clearly demonstrate that *S. cerevisiae* not only dominates representative strains of multiple yeast species that diverged before the transition to a fermentative lifestyle, but also multiple representative strains of yeast species that diverged after this transition. Furthermore, my results show that *S. cerevisiae* also exhibits an intrinsic fitness advantage in low-pH and poor nutrients environments even when interference via ethanol production is not a factor. These findings indicate that the ecological dominance of *S. cerevisiae* in high-sugar environments was not a direct outcome of the transition to a fermentative lifestyle. Rather, the ecological success of this species depends upon the acquisition of multiple other traits. Notably, previous studies in plants (Wing and Boucher 1998; Edwards et al. 2010; Bouchenak-Khelladi et al. 2014; Spriggs et al. 2014) and animals (Alfaro et al. 2009; Near et al. 2012) also show that dominance may lag behind the emergence of an evolutionary innovation, although the specific traits involved in the dominance of these lineages remain unknown. The findings from this chapter provide insights into the relationship of evolutionary innovation and dominance that can be difficult to discern in other systems because direct fitness tests are either not possible or may be very difficult.

**WGD and evolutionary innovations alter the selective regime of descendant lineages**

Following WGD, the genome of descendant lineages temporarily experiences a new selective regime through the creation of massive genetic redundancy. In yeast, this massive redundancy initially allowed for extensive gene loss and rapid protein evolution (Scannell et al. 2006; Scannell and Wolfe 2008). Over time, as partial or complete functional loss of different genes increased, gene loss and protein evolution decreased due to selection (Scannell et al. 2006; Scannell and Wolfe 2008). In Chapter 1, I show that *TOM70* and *TOM71* are both required for growth without mtDNA in *S. cerevisiae*, and partial loss of the ancestral function in each of these
genes likely explains why both genes persist in the *S. cerevisiae* genome. Although duplicated genes retained in the yeast lineage still appear to experience an altered selective regime relative to single-copy genes, gene loss and protein evolution nonetheless have slowed dramatically (Scannell et al. 2006; Scannell and Wolfe 2008). This decrease in rates of genes loss and protein evolution suggest that the opportunity for extensive functional changes throughout the genome as a result of WGD may be increasingly less, and less likely.

Like WGD, evolutionary innovations also alter the selective regime of descendant lineages. Evolutionary innovations involve morphological and physiological changes that can create new selective regimes by reducing the impact of competition. In yeast, evolution of the fermentative lifestyle may have initially altered the selective regime experienced by the yeast lineage through increased glycolytic flux that yielded a faster growth rate for fermenting lineages relative to non-fermenting lineages (Pfeiffer et al. 2001; Conant and Wolfe 2007). Notably, although the fermentative lifestyle also results in the production of toxic ethanol that reduces the growth of diverse yeast species, as shown in Chapter 2 and previous studies (Pina et al. 2004; Belloch et al. 2008; Arroyo-López et al. 2010; Salvadó et al. 2011a), if increased glycolytic flux did yield a faster growth rate, then interference competition through ethanol production was not the primary benefit of this evolutionary transition. However, my findings in Chapter 2 also show that representative strains that span the evolutionary transition to the fermentative lifestyle exhibit similar growth rates in some high-sugar environments. In such a situation, it becomes very tempting to turn again to interference competition via ethanol production to explain the ecological success of fermenting yeast species like *S. cerevisiae*. However, in light of my finding that *S. cerevisiae* exhibits *multiple* fitness traits, I suggest an alternative viewpoint: The evolutionary and ecological implications of evolutionary innovation may occur in two phases,
(1) a “breakthrough” phase, during which the evolutionary innovation becomes established, and
(2) a “refinement” phase in which the selective regime created by the evolutionary innovation
facilitates lineage-specific sophistication. Using this paradigm, the fermentative lifestyle was a
breakthrough innovation, and the phenomenal ecological success of *S. cerevisiae* in high-sugar
environments one potential outcome of the sophistication process.

Unlike WGD that creates a temporary new selective regime that decreases over time,
evolution of the fermentative lifestyle potentially created a selective regime that diverged
increasingly from its former state. Ethanol produced during fermentation may decrease the
growth of competitor yeast species, but it also negatively impacts the growth of fermenting yeast
species. As such, as glycolytic flux increased in fermenting yeast species, so did ethanol
production, which would have necessitated mounting physiological changes in fermenting yeast
lineages in order to cope with the cellular stress imposed by this toxin. From this perspective, the
role of ethanol production in the ecological success of fermenting yeast species would arise not
from its capacity to decrease the growth of competitor species, but rather because it led to
mounting physiological changes necessitated by the selective regime it imposed. If so, then
evolutionary and ecological implications of the fermentative lifestyle may increase over time.

Just as Goddard (2008) recognized the potential of *S. cerevisiae* to function as an
ecosystem engineer (Jones et al. 1994; 1997) through the production of ethanol, I suggest that the
production of ethanol by early fermenting yeast species altered the environment, and thus the
selective regime, for subsequent generations via niche construction. Multiple previous studies
have suggested that niche construction through the production of chemicals will increase the
fitness of constructing organisms (Erwin 2008, 2012; Odling-Smee et al. 2013). This increased
fitness would in turn create a selective feedback that could lead to the “self-propagation” of the
evolutionary innovation (Losos 2010; Erwin 2012). Although no studies have directly tested whether evolutionary innovations create ecological opportunity in this manner, one interesting way to test this idea in yeast is through experimental evolution. If the fermentative lifestyle helped to facilitate the evolution of other traits that contribute to *S. cerevisiae* ecological success, such as the ability to tolerate low-pH, then fermenting species should more readily evolve this ability than non-fermenting species. Given that some of the same physiological responses to ethanol, such as changes in lipid membrane composition, are likely also to increase fitness in low-pH environments, it is not unreasonable to expect that the evolution of the fermentative lifestyle may have precipitated a fitness advantage in low-pH environments as well.

**Recommendations for future evolutionary studies in yeast**

Now more than ever, I find the prospects for evolutionary studies in yeast incredibly exciting. Advancements made by this study raise several interesting questions for future studies: What other traits may contribute to fitness differences between yeast species? How does the interaction of environmental attributes like pH, ethanol, and temperature affect fitness? To what extent does population variation influence some of these findings? Could the fermentative lifestyle facilitate the evolution of other fitness traits? And, of course, what are the genes involved in the evolution of these fitness traits? While few systems come close to yeast in terms of their potential to study the genetic basis of traits, it is imperative for future evolutionary studies using yeast to measure fitness traits for a greater number of yeast species and strains.

To succeed in these research efforts, I recommend that the yeast research community not only collect more isolates along with detailed information about the source of those isolates, but also develop a more efficient way of distributing yeast isolates to other researchers. Current stain
collections are extremely limited for most yeast species, and they are also fragmented across many different labs. Once strains are collected they should be made readily available to other labs through a centralized collection center, akin to plant herbariums. While culture centers do exist, such as the Agricultural Resources Services in Peoria, Illinois, these culture collections are not very accessible, and to my knowledge, there is no current standard of practice for collections to be sent to such resource centers. Depositing isolates into culture collections should be standard practice, and in return, yeast researchers should be granted more access to those collections. It seems to me that better knowledge regarding the natural history of yeast coupled with the incredible power of yeast genetics will lead to much advancement in the field of evolutionary biology.
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Appendix A

Supplementary Information for Chapter 2
Figure S2.1 – Pyrosequencing calibration. Relationship between the known frequency (%) of the reference species based on cell density and the estimated frequency (%) of the reference species based on pyrosequencing relative to *S. cerevisiae* (YPS163). Reference species include *S. cerevisiae* (114) (A), *S. paradoxus* (B), *S. mikatae* (C), *S. bayanus* (D), *C. glabrata* (E), *N. castellii* (F), *V. polyspora* (G), *Z. rouxii* (H), *T. delbrueckii* (I), *L. thermotolerans* (J), *L. waltii* (K), *L. kluyveri* (L), and *H. vineae* (M). Calibration equations based on linear or polynomial regression analysis and $R$-squared values for each model are indicated.
Figure S2.2 – The effect of ethanol on the intrinsic growth rate of each species. Each line shows mean intrinsic growth rate ($r$) of an individual species based on three replicates as a function of ethanol (%) added at the beginning of growth. Significant differences in the growth rate of each species and *S. cerevisiae* are shown for FDR < 0.01 (shaded region). Error bars have been omitted for clarity.
Figure S2.3 – Intrinsic growth rate in YPD made with supernatant. The intrinsic growth rate of each species (A-O) in YPD made with the supernatant from each species’ own supernatant when grown in mono-culture (red), the supernatant from *S. cerevisiae* (YPS163) grown in mono-culture (blue), and the supernatant from co-culture with *S. cerevisiae* (green). Species include *S. cerevisiae* (I14) (A), *S. paradoxus* (B), *S. mikatae* (C), *S. bayanus* (D), *Kaz. lodderae* (E), *Kaz. martiniae* (F), *N. castellii* (G), *C. glabrata* (H), *V. polyspora* (I), *Z. rouxii* (J), *T. delbrueckii* (K), *L. thermotolerans* (L), *L. waltii* (M), *K. lactis* (N), and *H. vineae* (O). Bars and whiskers represent the mean and standard deviation of the growth rate.
Figure S2.4 – Intrinsic growth rate in Grape supplemented with various nutrients. The mean intrinsic growth rate of each species in Grape supplemented with CM (A), NB (B), AA (C) and AS (D). Names of pre- (red) and post- (blue) WGD species are colored. Bars and whiskers represent the mean and standard deviation of the growth rate. Significant differences in the growth rate of each species with or without the added nutrient (a) and differences between S. cerevisiae and each species with the added nutrient (b) are labeled above each bar for FDR < 0.01.
Table S2.1 – Yeast strains used in this study.

<table>
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<tr>
<th>Species</th>
<th>Strain name or accession number^4</th>
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<tr>
<td><em>Saccharomyces cerevisiae</em>(^{1,3})</td>
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^1 Strain used in experiments to quantify relative abundance.
^2 Strain used in experiments to measure intrinsic growth rate.
^3 Strain used in experiments to identify unknown inhibtor compounds.
^4 NRRL accession numbers are from the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA.
Table S2.2 – PCR and pyrosequencing primers.

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<td>YGCAGAYGTCGGT</td>
<td>T/T</td>
<td>C/C</td>
<td>1</td>
</tr>
<tr>
<td>ACGATGTTACCCTA</td>
<td>YAAATTCYTTAC</td>
<td>T/C</td>
<td>C/T</td>
<td>1,2</td>
</tr>
<tr>
<td>ATGTTACCCTA</td>
<td>YAATTCYTTWC</td>
<td>T</td>
<td>C</td>
<td>1</td>
</tr>
</tbody>
</table>
Table S2.3 – Significance of changes in the relative abundance of *S. cerevisiae* compared to other yeast species in high-sugar environments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Co-culture</th>
<th>Mono-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grape</td>
<td>HS</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> (114)</td>
<td>0.0927</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>S. paradoxus</em></td>
<td>0.9451</td>
<td>0.0577</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>0.0012</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>S. bayanus</em></td>
<td>0.0061</td>
<td>0.0012</td>
</tr>
<tr>
<td><em>N. castellii</em></td>
<td>0.0021</td>
<td>0.0012</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>0.0072</td>
<td>0.9612</td>
</tr>
<tr>
<td><em>V. polyspora</em></td>
<td>0.0012</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>Z. rouxii</em></td>
<td>0.0013</td>
<td>0.0012</td>
</tr>
<tr>
<td><em>T. delbrueckii</em></td>
<td>0.0007</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>L. thermotolerans</em></td>
<td>0.0011</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>L. waltii</em></td>
<td>0.0005</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>L. kluyveri</em></td>
<td>0.0002</td>
<td>0.0038</td>
</tr>
<tr>
<td><em>H. vineae</em></td>
<td>0.0012</td>
<td>0.0010</td>
</tr>
</tbody>
</table>

FDR values are shown based on the Benjamini–Hochberg procedure applied to one-tailed paired Welch's *t* tests.
Table S2.4 – Significance of intrinsic growth rate differences between *S. cerevisiae* and other yeast species in high-sugar environments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Grape</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. paradoxus</em></td>
<td>0.0284</td>
<td>0.2948</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>0.0008</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>S. bayanus</em></td>
<td>0.001</td>
<td>0.9698</td>
</tr>
<tr>
<td><em>Kaz. Lodderae</em></td>
<td>&lt; 0.001</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>Kaz. Martiniae</em></td>
<td>&lt; 0.001</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>N. castellii</em></td>
<td>&lt; 0.001</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>&lt; 0.001</td>
<td>0.9698</td>
</tr>
<tr>
<td><em>Nak. Bacillsporus</em></td>
<td>&lt; 0.001</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>Tet. Blattae</em></td>
<td>&lt; 0.001</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>V. polyspora</em></td>
<td>&lt; 0.001</td>
<td>0.0852</td>
</tr>
<tr>
<td><em>Z. rouxii</em></td>
<td>0.0017</td>
<td>0.4103</td>
</tr>
<tr>
<td><em>T. delbrueckii</em></td>
<td>&lt; 0.001</td>
<td>0.7347</td>
</tr>
<tr>
<td><em>L. thermotolerans</em></td>
<td>0.0004</td>
<td>0.3901</td>
</tr>
<tr>
<td><em>L. waltii</em></td>
<td>0.0021</td>
<td>0.2948</td>
</tr>
<tr>
<td><em>L. kluyveri</em></td>
<td>&lt; 0.001</td>
<td>0.2948</td>
</tr>
<tr>
<td><em>K. lactis</em></td>
<td>0.0015</td>
<td>0.2948</td>
</tr>
<tr>
<td><em>H. vineae</em></td>
<td>&lt; 0.001</td>
<td>0.7347</td>
</tr>
</tbody>
</table>

FDR values are shown based on the Benjamini-Hochberg procedure applied to one-tailed Welch’s *t* tests.
Table S2.5 – Significance of IC$_{50}$ and intrinsic growth rate differences between *S. cerevisiae* and other yeast species in ethanol (%).

<table>
<thead>
<tr>
<th>Species</th>
<th>IC$_{50}$</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. paradoxus</em></td>
<td>0.0502</td>
<td>0.3403</td>
<td>1</td>
<td>0.6395</td>
<td>0.7946</td>
<td>0.4909</td>
<td>0.1713</td>
<td>0.0409</td>
<td>0.0594</td>
<td>0.4438</td>
<td>0.2953</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>0.0045</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.6685</td>
<td>0.3518</td>
<td>0.0483</td>
<td>0.0800</td>
<td>0.0279</td>
<td>0.3027</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>S. bayanus</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.3518</td>
<td>0.0201</td>
<td>0.0075</td>
<td>0.0032</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>Kaz. Lodderae</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.4871</td>
<td>0.6685</td>
<td>0.3518</td>
<td>0.0104</td>
<td>0.0075</td>
<td>0.0042</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>Kaz. Martiniae</em></td>
<td>&lt; 0.001</td>
<td>0.6404</td>
<td>1</td>
<td>0.4871</td>
<td>0.7114</td>
<td>0.6601</td>
<td>0.0247</td>
<td>0.0094</td>
<td>0.0042</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>N. castellii</em></td>
<td>&lt; 0.001</td>
<td>0.5862</td>
<td>1</td>
<td>0.3267</td>
<td>0.6685</td>
<td>0.2322</td>
<td>0.0107</td>
<td>0.0180</td>
<td>0.0032</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>0.032</td>
<td>0.9740</td>
<td>1</td>
<td>0.8549</td>
<td>0.9403</td>
<td>0.9945</td>
<td>0.9776</td>
<td>0.7828</td>
<td>0.1933</td>
<td>0.4438</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>Nak. Bacilllsporus</em></td>
<td>&lt; 0.001</td>
<td>0.9541</td>
<td>1</td>
<td>0.4871</td>
<td>0.7114</td>
<td>0.4280</td>
<td>0.0046</td>
<td>0.0129</td>
<td>0.0037</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>Tet. Blattae</em></td>
<td>&lt; 0.001</td>
<td>0.5316</td>
<td>1</td>
<td>0.4871</td>
<td>0.6685</td>
<td>0.2419</td>
<td>0.0081</td>
<td>0.0094</td>
<td>0.0032</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>V. polyspora</em></td>
<td>&lt; 0.001</td>
<td>0.0352</td>
<td>1</td>
<td>0.1947</td>
<td>0.4299</td>
<td>0.1665</td>
<td>0.0032</td>
<td>0.0075</td>
<td>0.0042</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>Z. rouxii</em></td>
<td>&lt; 0.001</td>
<td>0.1878</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.2080</td>
<td>0.0180</td>
<td>0.0075</td>
<td>0.0042</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>T. delbrueckii</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.2080</td>
<td>0.0032</td>
<td>0.0075</td>
<td>0.0042</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>L. thermotolerans</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.5278</td>
<td>0.2080</td>
<td>0.0032</td>
<td>0.0075</td>
<td>0.0041</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>L. waltii</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.2080</td>
<td>0.0032</td>
<td>0.0075</td>
<td>0.0041</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>L. kluyveri</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.2080</td>
<td>0.0201</td>
<td>0.0075</td>
<td>0.0041</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>K. lactis</em></td>
<td>&lt; 0.001</td>
<td>0.3403</td>
<td>1</td>
<td>0.3267</td>
<td>0.4380</td>
<td>0.2080</td>
<td>0.0201</td>
<td>0.0075</td>
<td>0.0062</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
<tr>
<td><em>H. vineae</em></td>
<td>&lt; 0.001</td>
<td>0.5316</td>
<td>1</td>
<td>0.5583</td>
<td>0.7946</td>
<td>0.9945</td>
<td>0.1713</td>
<td>0.0282</td>
<td>0.0044</td>
<td>0.1867</td>
<td>0.2381</td>
</tr>
</tbody>
</table>

FDR values are shown based on the Benjamini–Hochberg procedure applied to confidence interval overlap (IC$_{50}$) or one-tailed Welch's *t* tests.
Table S2.6 – Significance of intrinsic growth rate differences between each species' own supernatant and supernatant from *S. cerevisiae* mono-culture and co-culture.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mono-culture</th>
<th>Co-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cerevisiae</em> (I14)</td>
<td>0.4568</td>
<td>0.6591</td>
</tr>
<tr>
<td><em>S. paradoxus</em></td>
<td>0.6243</td>
<td>0.5885</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>0.8393</td>
<td>0.7946</td>
</tr>
<tr>
<td><em>S. bayanus</em></td>
<td>0.7623</td>
<td>0.4054</td>
</tr>
<tr>
<td><em>Kaz. lodderae</em></td>
<td>0.6114</td>
<td>0.8351</td>
</tr>
<tr>
<td><em>Kaz. Martiniae</em></td>
<td>0.7356</td>
<td>0.7685</td>
</tr>
<tr>
<td><em>N. castellii</em></td>
<td>0.6075</td>
<td>0.3021</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>0.8433</td>
<td>0.8945</td>
</tr>
<tr>
<td><em>V. polyspora</em></td>
<td>0.0525</td>
<td>0.7437</td>
</tr>
<tr>
<td><em>Z. rouxii</em></td>
<td>0.8528</td>
<td>0.8909</td>
</tr>
<tr>
<td><em>T. delbruecki</em></td>
<td>0.8641</td>
<td>0.5506</td>
</tr>
<tr>
<td><em>L. thermotolerans</em></td>
<td>0.4521</td>
<td>0.4338</td>
</tr>
<tr>
<td><em>L. waltii</em></td>
<td>0.5241</td>
<td>0.4887</td>
</tr>
<tr>
<td><em>L. kluyveri</em></td>
<td>0.2571</td>
<td>0.4796</td>
</tr>
<tr>
<td><em>H. vineae</em></td>
<td>0.1882</td>
<td>0.2304</td>
</tr>
</tbody>
</table>

FDR values are shown based on the Benjamini–Hochberg procedure applied to one-tailed Welch's *t* tests.
Table S2.7 – Significance of low-pH on the intrinsic growth rate of S. cerevisiae and other species in HS.

<table>
<thead>
<tr>
<th>Species</th>
<th>Low-pH</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>0.3419</td>
<td>-</td>
</tr>
<tr>
<td>S. paradoxus</td>
<td>0.7750</td>
<td>0.1191</td>
</tr>
<tr>
<td>S. mikatae</td>
<td>0.0499</td>
<td>0.0102</td>
</tr>
<tr>
<td>S. bayanus</td>
<td>0.0144</td>
<td>0.1600</td>
</tr>
<tr>
<td>Kaz. Lodderae</td>
<td>0.1411</td>
<td>0.0213</td>
</tr>
<tr>
<td>Kaz. Martiniae</td>
<td>0.0499</td>
<td>0.0032</td>
</tr>
<tr>
<td>N. castellii</td>
<td>0.1387</td>
<td>0.0102</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>0.0142</td>
<td>0.3849</td>
</tr>
<tr>
<td>Nak. Bacillisporus</td>
<td>0.0142</td>
<td>0.0033</td>
</tr>
<tr>
<td>Tet. Blattae</td>
<td>0.1987</td>
<td>0.0016</td>
</tr>
<tr>
<td>V. polyspora</td>
<td>0.0062</td>
<td>0.0004</td>
</tr>
<tr>
<td>Z. rouxi</td>
<td>0.3284</td>
<td>0.1422</td>
</tr>
<tr>
<td>T. delbrueckii</td>
<td>0.0010</td>
<td>0.0055</td>
</tr>
<tr>
<td>L. thermotolerans</td>
<td>0.0062</td>
<td>0.0025</td>
</tr>
<tr>
<td>L. waltii</td>
<td>0.0062</td>
<td>0.0032</td>
</tr>
<tr>
<td>L. kluyveri</td>
<td>0.0062</td>
<td>0.0016</td>
</tr>
<tr>
<td>K. lactis</td>
<td>0.0144</td>
<td>0.0102</td>
</tr>
<tr>
<td>H. vineae</td>
<td>0.1065</td>
<td>0.3849</td>
</tr>
</tbody>
</table>

Effect of low-pH = $\Delta r_{\text{Low-pH}} = r_{\text{Low-pH HS}} - r_{\text{HS}}$

Effect of species = $\Delta r_{\text{Species}} = r_{\text{non-S. cerevisiae}} - r_{S. cerevisiae}$

FDR values are shown based on the Benjamini–Hochberg procedure applied to one-tailed paired Welch's $t$ tests.
Table S2.8 – Significance of nutrient supplements on the intrinsic growth rate of *S. cerevisiae* and other species in Grape.

<table>
<thead>
<tr>
<th>Species</th>
<th>Nutrient</th>
<th>Species</th>
<th>Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YP</td>
<td>CM</td>
<td>AA</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>0.0356</td>
<td>0.0029</td>
<td>0.1120</td>
</tr>
<tr>
<td><em>S. paradoxus</em></td>
<td>0.1553</td>
<td>0.0546</td>
<td>0.5710</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>0.2791</td>
<td>0.2626</td>
<td>0.1994</td>
</tr>
<tr>
<td><em>S. bayanus</em></td>
<td>0.0003</td>
<td>0.0479</td>
<td>0.0723</td>
</tr>
<tr>
<td>Kaz. Lodderae</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0069</td>
</tr>
<tr>
<td>Kaz. Martiniae</td>
<td>&lt; 0.0001</td>
<td>0.1217</td>
<td>0.1060</td>
</tr>
<tr>
<td>N. castellii</td>
<td>&lt; 0.0001</td>
<td>0.0003</td>
<td>0.2385</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>&lt; 0.0001</td>
<td>0.0019</td>
<td>0.0069</td>
</tr>
<tr>
<td>Nak. Bacillisporus</td>
<td>&lt; 0.0001</td>
<td>0.0535</td>
<td>0.3618</td>
</tr>
<tr>
<td>Tet. Blattae</td>
<td>&lt; 0.0001</td>
<td>0.0358</td>
<td>0.0069</td>
</tr>
<tr>
<td>V. polyspora</td>
<td>0.0014</td>
<td>0.1019</td>
<td>0.0196</td>
</tr>
<tr>
<td>Z. rouxii</td>
<td>0.0343</td>
<td>0.1761</td>
<td>0.1186</td>
</tr>
<tr>
<td>T. delbrueckii</td>
<td>0.0057</td>
<td>0.1191</td>
<td>0.0108</td>
</tr>
<tr>
<td>L. thermotolerans</td>
<td>0.0854</td>
<td>0.1598</td>
<td>0.1060</td>
</tr>
<tr>
<td>L. waltii</td>
<td>0.1207</td>
<td>0.0458</td>
<td>0.2385</td>
</tr>
<tr>
<td>L. kluyveri</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.0903</td>
</tr>
<tr>
<td>K. lactis</td>
<td>0.8994</td>
<td>0.1191</td>
<td>0.8594</td>
</tr>
<tr>
<td>H. vineae</td>
<td>&lt; 0.0001</td>
<td>0.0004</td>
<td>0.2385</td>
</tr>
</tbody>
</table>

Effect of nutrient = \( \Delta r_{\text{Nutrient}} = r_{\text{Grape with nutrient}} - r_{\text{Grape}} \)

Effect of species = \( \Delta r_{\text{Species}} = r_{\text{non-} S. \text{ cerevisiae}} - r_{\text{S. cerevisiae}} \)

FDR values are shown based on the Benjamini–Hochberg procedure applied to one-tailed paired Welch's *t* tests.
Table S2.9 – Growth rate loadings onto principal coordinate analysis.

<table>
<thead>
<tr>
<th>Medium</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape</td>
<td>-0.21</td>
<td>0.34</td>
</tr>
<tr>
<td>HS</td>
<td>-0.25</td>
<td>-0.04</td>
</tr>
<tr>
<td>low-pH HS</td>
<td>-0.27</td>
<td>0.05</td>
</tr>
<tr>
<td>Grape + YP</td>
<td>-0.28</td>
<td>-0.06</td>
</tr>
<tr>
<td>Grape + CM</td>
<td>-0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>Grape + NB</td>
<td>-0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>Grape + AA</td>
<td>-0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>Grape + AS</td>
<td>-0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>YPD</td>
<td>-0.22</td>
<td>-0.33</td>
</tr>
<tr>
<td>YPD + 1% ethanol</td>
<td>-0.14</td>
<td>-0.41</td>
</tr>
<tr>
<td>YPD + 2% ethanol</td>
<td>-0.24</td>
<td>-0.25</td>
</tr>
<tr>
<td>YPD + 3% ethanol</td>
<td>-0.24</td>
<td>-0.25</td>
</tr>
<tr>
<td>YPD + 4% ethanol</td>
<td>-0.25</td>
<td>-0.26</td>
</tr>
<tr>
<td>YPD + 5% ethanol</td>
<td>-0.24</td>
<td>-0.22</td>
</tr>
<tr>
<td>YPD + 6% ethanol</td>
<td>-0.26</td>
<td>-0.04</td>
</tr>
<tr>
<td>YPD + 7% ethanol</td>
<td>-0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>YPD + 8% ethanol</td>
<td>-0.24</td>
<td>0.08</td>
</tr>
<tr>
<td>YPD + 10% ethanol</td>
<td>-0.20</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Curriculum Vitae
EDUCATION

**Ph.D. Evolution, Ecology, and Population Biology**
Washington University in St. Louis, MO
Adviser: Justin Fay

2007-2014

**B.A. Biology and Environmental Studies**
Oberlin College, Oberlin, OH
Advisers: Jane Bennett and David Orr

2002-2006

Intensive course in *Statistical methods in functional genomics*
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

2013

Intensive course in *Tropical plant systematics*
Organization for Tropical Studies and Universidad de Costa Rica, Costa Rica

2007

GRANTS AND SCHOLARSHIPS

- National Institute of Health Genome Analysis Training Grant 2011-2013
- Grand Traverse Band of Ottawa-Chippewa Indians Scholarship 2002-2008
- Bonner Scholar, for social justice, civic engagement, and diversity 2002-2006

PUBLICATIONS

Williams, KM, and Fay, JC. Evolution of ecological dominance of yeast species in high-sugar environments. In revision.
PROFESSIONAL PRESENTATIONS

Oral Presentations
Evolution, Annual Society for the Study of Evolution Meeting, Raleigh, NC 2014
Evolution of ecological dominance in high-sugar environments.

St. Louis Ecology, Evolution, and Conservation Retreat, St. Louis, MO 2013
Evolution of ecological dominance in yeast.

Missouri Grape and Wine Research Alliance Symposium, St. Louis, MO 2013
Evolution of yeast’s ecological dominance in grape juice.

Posters
Society for Molecular Biology and Evolution Annual Conference, Chicago, IL 2013
Evolution of ecological dominance in yeast.

Missouri Grape and Wine Symposium, Columbia, MO 2012
Exploring the traits that make Saccharomyces cerevisiae the dominant wine yeast.

Evolution of strong competitive ability of yeast species in grape juice.

Society for Molecular Biology and Evolution Annual Conference, Iowa City, IA 2009
Divergence in gene regulation is responsible for the inability of two duplicated genes to compensate for one another.

PROFESSIONAL ACTIVITES

• Member – Center for Integrating Research, Teaching and Learning 2012-2013
• Liaison – Lab Sustainability at Washington University 2011
• Coordinator – Evolution, and Ecology Seminar Series 2009-2010
• Senior Intern – Bonner Scholars Program 2005-2006

TEACHING AND EDUCATIONAL OUTREACH

• Guest Instructor – Biology On the Cutting Edge (Biology 4933) 2014
• Mentor – Young Scientist Summer Focus Research Program 2011
• Leader – Evolution Teaching Team 2009-2011
• Tutor – Young Scientist Summer Focus Research Program, 2009-2010
• Teaching Assistant – Evolution (Biology 3501) 2009
• College Essay Tutor – Beaumont High School 2009
• Participant – Evolution Teaching Team 2008-2009
• Education Intern – Ocean Alliance and Cape Anne Whale Watch 2005
• Environmental Education Specialist – Common Ground Earth Camp 2004
• Education Volunteer – Missouri Botanical Garden 2004

PROFESSIONAL DEVELOPMENT AND EXPERIENCE

Graduate rotations and projects, Washington University in St. Louis, MO
Characterized molecular basis of functional divergence of the duplicated genes MLS1 and DAL7 in Saccharomyces cerevisiae. Adviser: Justin Fay 2008


Optimization of PCR protocols for microsatellite genotyping of domesticated populations of the plant Chenopodium berlandieri. Adviser: Ken Olsen 2007

Graduate rotation, Donald Danforth Plant Science Center, St. Louis, MO
Maintained Arabidopsis thaliana and A. lyrata plants in greenhouse and prepared tissues for scanning electron and confocal microscopy. Adviser: Mark Running 2008

Biologist I, Aerotek Scientific at Monsanto, St. Louis, MO

Undergraduate Research, Oberlin College, Oberlin, OH
Quantified pectate lyase expression during lateral root emergence using reverse transcription PCR in Arabidopsis thaliana. Adviser: Marta Laskowski 2006

PROFESSIONAL ASSOCIATIONS

• Society for the Study of Evolution
• Center for the Integration of Research, Teaching and Learning (CIRTL) Network
• Association for Women in Science