

INVITED REVIEW

Ecological and evolutionary genomics of *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae, the budding yeast, is the most thoroughly studied eukaryote at the cellular, molecular, and genetic levels. Yet, until recently, we knew very little about its ecology or population and evolutionary genetics. In recent years, it has been recognized that *S. cerevisiae* occupies numerous habitats and that populations harbour important genetic variation. There is therefore an increasing interest in understanding the evolutionary forces acting on the yeast genome. Several researchers have used the tools of functional genomics to study natural isolates of this unicellular fungus. Here, we review some of these studies, and show not only that budding yeast is a prime model system to address fundamental molecular and cellular biology questions, but also that it is becoming a powerful model species for ecological and evolutionary genomics studies as well.

Keywords: ecology, genomics, microarray, population genetics, *Saccharomyces cerevisiae*, wine yeast

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Introduction

Model organisms are at the forefront of technical innovation and functional analysis in genetics and genomics as well as in molecular and developmental biology. However, for most laboratory model organisms, we lag far behind in our understanding of the ecological and population genetic features that directed their evolution and shaped their biology. Because genomic tools are becoming increasingly available to all biologists, and because of the increasing interest of developmental biologists and geneticists in naturally occurring genetic variation, non-model organisms whose ecology and evolution are well studied are increasingly studied at the molecular, cellular, and functional genomic levels (see Feder & Mitchell-Olds 2003). Transitions in the other direction, in which a model laboratory organism is studied in an ecological and evolutionary context, remain rare.

Recently, however, the budding yeast *Saccharomyces cerevisiae*, long a model system for functional and comparative genomic studies, has attracted interest as an emerging model in ecological and evolutionary genetics. The potential

of this species to illuminate ecological and evolutionary principles is underscored by a wealth of knowledge of its molecular and cellular biology as well as the diversity of experimental resources available. Here we supply an overview of the current knowledge on *S. cerevisiae* in its natural habitats. We also review recent studies that use genomic tools to gain a better understanding of the evolution and ecology of budding yeast. Finally, we outline opportunities for further research that exploit genomic techniques to help understand the evolutionary and ecological dimensions of this emerging supermodel.

Population biology of *Saccharomyces cerevisiae*

The budding yeast, *Saccharomyces cerevisiae*, was for a long time considered a domesticated unicellular organism distinct from any of its counterparts in natural habitats (Ciani *et al.* 2004). This view emerged in part because *S. cerevisiae* had been used for tens of centuries in baking, brewing, distilling, and wine making, and strains used in these industries were referred to as 'bakers yeast', 'brewers yeast', 'distillers yeast' or 'wine yeast'. Molecular evidence for the historical presence of *S. cerevisiae* in wine fermentation has been obtained from pottery jars concealed in the tomb of King Scorpion I, one of the first kings of Egypt, which dates to 3150 BC (Cavaliere

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Box 1 Yeast life cycle

The life history of yeast is complex. *Saccharomyces cerevisiae* is usually found as a unicellular diploid organism that can reproduce vegetatively through budding. Diploid cells can undergo sporulation (meiosis) to produce an unordered tetrad of four haploid spores called an ascus. These spores can be either of two mating types, *a* or α . Haploid spores can also reproduce vegetatively. Diploid cells are produced by the fusion of two haploid cells of opposite mating type. Some strains, known as heterothallic strains, have a fixed mating type. However, the spores of the majority of natural isolates are able to switch mating type; these are referred to as homothallic strains. The wild-type allele of the gene *HO* for homothallism encodes a site-specific endonuclease that promotes mating type switching from *a* to α or vice versa. *HO* is transcribed only in the mother cell, so that when a homothallic spore divides,

the mother cell switches mating type and immediately fuses with the newly produced daughter cell (Klar 1987). The result is that, in homothallic strains, the haploid phase of the life cycle is transient. The life cycles of heterothallic and homothallic strains are illustrated in Fig. B1. The ecological significance of the polymorphism for homothallism is unclear, but it is known that sporulation can be induced by growth in poor carbon sources or in the absence of a nitrogen source. Asci are also known to have higher resistance to environmental stress, such as heat and dehydration. Although the molecular mechanisms involved in each of these life stages have been studied in great detail, their ecological and evolutionary significance remains unexplored. For instance, how often does mating take place in the wild? How often do cells in the wild propagate as haploids? What are the consequences of this semiclonal life style on yeast population structure?

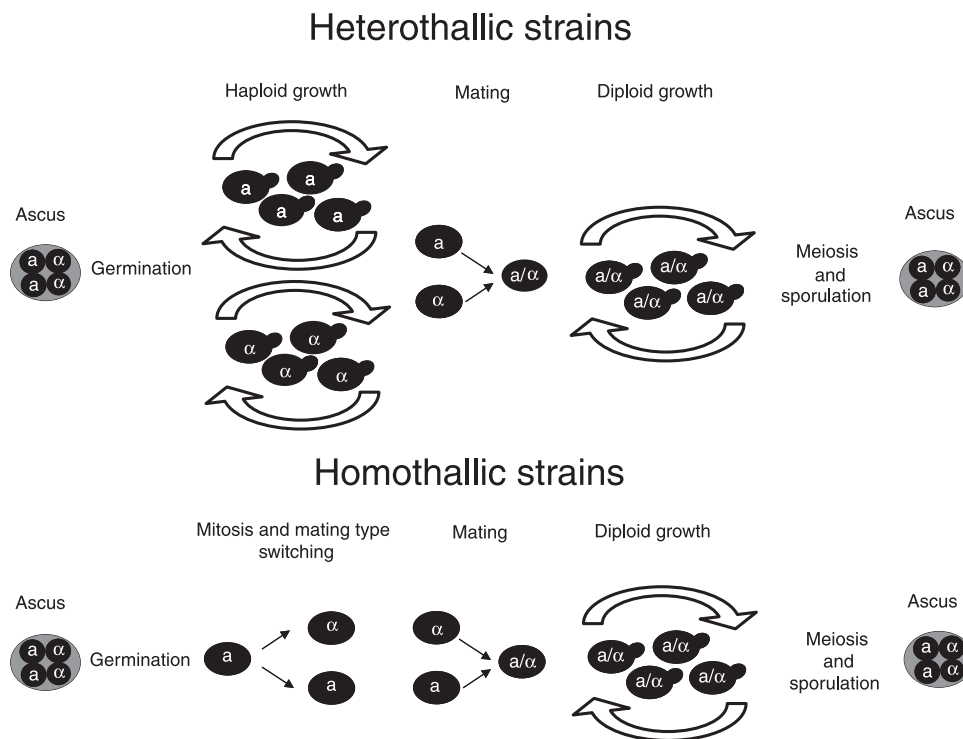


Fig. B1 Life cycle of heterothallic and homothallic strains of *Saccharomyces cerevisiae*.

et al. 2003). Among the first strains isolated for modern scientific purposes was S288c. S288c was derived in the early 1950s from another strain, EM93, a heterothallic strain isolated by Emil Mrak in 1938 from rotting figs in Mercedes, California. EM93 was given to Carl Lindegren for his pioneering studies of yeast genetics (Mortimer &

Johnston 1986). In contrast to EM93, most wild yeast strains (about 70%) are homothallic (Box 1; Mortimer 2000). The isolation of this heterothallic strain facilitated the propagation of haploid cultures and genetic crosses, which led Lindegren to propose this microorganism as an ideal model to study genetics in a unicellular eukaryote. Since

then, *S. cerevisiae* has played a vital role in our understanding of nearly all major eukaryotic cellular processes. Detailed knowledge of the yeast genome, combined with the remarkable experimental tractability of yeast genetics and molecular biology, has positioned *S. cerevisiae* at the centre of research programmes ranging from the study of human disease genes (Botstein & Fink 1988; Foury 1997) to experimental evolution (Ferea *et al.* 1999) to vanguard studies of systems biology (DeRisi *et al.* 1997; Velculescu *et al.* 1997; Ideker *et al.* 2001).

Several lines of evidence suggest that *S. cerevisiae* existed as a distinct species in natural environments long before it was utilized in food production. Although there is no reliable time estimate for the divergence time between *S. cerevisiae* and its closest related species, *Saccharomyces paradoxus*, a recent survey of nucleotide variation in more than 80 isolates collected worldwide supports the hypothesis that domesticated strains derive from natural populations rather than the other way around (Fay & Benavides 2005). It has increasingly become clear that *S. cerevisiae* occupies and flourishes in numerous habitats that are not necessarily associated with human activities. Of course, many natural isolates have been obtained in vineyards in association with grapes. While it is relatively uncommon to find *S. cerevisiae* on the surface of undamaged berries (< 0.1%), the species can easily be found in berries damaged by birds or insects (~24%), which represent about 1 in 1000 grapes. Within any damaged fruit, the population size of *S. cerevisiae* ranges from 10^4 to 10^5 cells (Mortimer & Polsinelli 1999). There is independent evidence that insects and birds are important agents for the dispersal of yeasts in habitats related to, but not necessarily restricted to, wine-making environments (Phaff & Starmer 1987; Mortimer & Polsinelli 1999; Cavalieri D., Calabretta A., & Turillazzi S, unpublished).

Saccharomyces cerevisiae has also been isolated far from vineyards. For instance, Slavikova & Vadkertiova (1997) isolated *S. cerevisiae* from the Danube River, and in the northeastern United States, *S. cerevisiae* can be isolated from soil associated with oak trees (Sniegowski *et al.* 2002). Strains have also been isolated in eastern Asia in association with exudates of trees or various fruits (Naumov *et al.* 2003), and the species is also found in other continents (Fay & Benavides 2005). *S. cerevisiae* is also considered by some to be an opportunistic pathogen, and it can occasionally be isolated from humans that are immunocompromised (e.g. Malgoire *et al.* 2005).

Nevertheless, much of our knowledge of the ecology of *S. cerevisiae* derives primarily from amateur observations and academic studies of fruit fermentation. It is this process that has been vastly expanded by humans into commercial wine fermentation. Early stages of wine fermentation involve many different microorganisms, including bacteria of various kinds as well as molds and yeasts of the genera

Kloeckera, *Metschnikowia*, *Torulopsis*, *Pichia*, *Brettanomyces*, *Dekkera*, *Zygosaccharomyces*, and *Saccharomyces* (Phaff & Starmer 1987; Fleet & Heard 1993). Later stages are ultimately dominated by the wine yeast, *S. cerevisiae*. At the beginning of a natural fermentation of crushed grapes, the population of wine yeast is thought to be small because it derives from only a few berries (Mortimer & Polsinelli 1999). The growth environment is acid: grape juice has a pH of 2.5–3.8 due to organic acids (e.g. tartrate, lactate, and malate). The toxicity of the acid environment is exacerbated by phenolic compounds and high levels of potassium salts (Querol *et al.* 2003). The osmotic pressure is also severe, due to a high concentration of sugar (10–40%) that depends on the grape variety and the time of year. Sources of nitrogen are severely limited, and the oxygen content is very low. Some stressors are fairly homogeneous, but acidity and temperature can differ extensively with geography and with the variety of grapes. Variation in acidity and temperature can significantly affect a strain's ability to survive other stresses (Phaff & Starmer 1987; Fleet & Heard 1993).

Encountering harsh growth conditions and intense competition, *S. cerevisiae* has adapted to survive and dominate its environment by actively modifying the local conditions. Yeasts rapidly begin to ferment sugars to produce ethanol (Querol *et al.* 2003). Although fermentation is widely regarded as a losing strategy owing to the relatively low output of ATP, it is such a rapid process that optimization of glycolysis allows *S. cerevisiae* to produce a similar number of ATP molecules per second as produced in aerobic metabolism (Pfeiffer *et al.* 2001). As long as glucose is present, it is transformed into ethanol. The ability to produce ethanol during fermentation is not unique to *Saccharomyces* species. The uniqueness of *Saccharomyces* rather resides in its ability to produce and to tolerate high levels of ethanol, which may later be utilized as a source of energy once the glucose is depleted (Pretorius 2000; Thomson *et al.* 2005). The high level of ethanol produced, along with the anaerobic conditions, low pH, and the osmotic stress, eliminates other microorganisms. As a result, strains of *S. cerevisiae* are essentially the only organisms remaining alive at the end of a fermentation (Querol *et al.* 2003).

Molecular ecology and population genetics of the budding yeast

Budding yeast provided the first eukaryotic genome to be completely sequenced (Dujon 1996). This early comprehensive knowledge, along with a small genome size, unicellular growth, and rich history of genetic and molecular analysis, has galvanized pioneering work on functional genomics. The yeast genome comprises about 6000 genes located on 16 chromosomes. A large fraction of the genes have been studied and annotated in detail. This wealth of knowledge

is available through numerous databases and resources, which are valuable sources of information for interpreting or formulating hypothesis regarding the genetic and phenotypic variation detected in nature (Table 1).

The community of yeast researchers has developed a keen interest in genetic variation in natural populations and its functional and evolutionary implications, at the same time that other biologists began to consider yeast in an ecological and evolutionary context (Zeyl 2004). This new-found interest blossomed largely because the yeast genome sequence enabled the development of powerful new technologies allowing the investigation of complex natural processes. The first step of this technological innovation was the development of DNA microarrays, which allowed the study of genetic variation and gene expression at a genomic scale in natural populations (see Box 2 for the use of cDNA microarrays for array-based comparative genomic hybridization, aCGH, and gene expression profiling). The technique of aCGH is similar to transcriptional profiling, but genomic DNA rather than cDNA is used in the hybridizations. Approaches based on nucleic acid hybridization offer the potential to facilitate large-scale studies to discover which genes are variable within populations as well as to detect genetic variation in gene copy number among natural isolates or closely related species on a genomic scale (e.g. Fortna *et al.* 2004).

Among the first extensive use of aCGH for the comparison of strains of *Saccharomyces cerevisiae* is the study by Winzeler *et al.* (2003). The technology used in this case was a short oligonucleotide microarray (Affymetrix S98 microarray) consisting of 285 156 different 25-mers from the yeast genomic sequence representing genomic sequence coverage of 16%. Since a single-base change between two sequences 25 bp in length can disrupt their hybridization, a reduction of binding intensity at one probe suggests the presence of a nucleotide polymorphism (Chee *et al.* 1996). Figure 1 shows 14 different strains of yeast, assayed for such variation by Winzeler *et al.* (2003). The dashed arrow shows the location in the dendrogram of S288c, whose sequence was used to design the S98 array, and the closely related X2180-1A. Most of the strains in Fig. 1 are laboratory strains, but four natural isolates are included. W303, a derivative of S288c, has been the strain of choice for studies on cell cycle and mitochondrial metabolism (Myers *et al.* 1985; Folch-Mallol *et al.* 2004). Other strains, such as Σ 1278B and 3962C, are more distantly related to EM93; and still others, such as SK1, are unrelated to EM93. These strains have been used mainly in studies of yeast cellular differentiation programmes (Gimeno *et al.* 1992) or meiosis (Primig *et al.* 2000). YJM789 was derived from a pathogenic yeast strain isolated in San Francisco, M12 is a natural homothallic isolate from Tuscany, and M28F and M28S are homozygous diploid progeny derived from sister spores of another Tuscan isolate M28 (Cavaliere *et al.* 2000).

Table 1 Examples of genomics and bioinformatics resources for *Saccharomyces cerevisiae*

Resources	Description	URL	References
Saccharomyces Genome Database (SGD)	Main resource of the yeast genomics community. Comprehensive genomic resources, sequence, gene ontology, search of orthologues of closely related species, mutant phenotypes and gene expression patterns across a variety of growth conditions	http://www.yeastgenome.org/	Weng <i>et al.</i> 2003
Comprehensive Yeast Genome Database (CYGD)	Information on the molecular structure and functional network	http://mips.gsf.de/genre/proj/yeast/	Guldener <i>et al.</i> 2005
Stanford Microarray Database	General resources for microarray data	http://genome-www5.stanford.edu/	Gollub <i>et al.</i> 2003
Genolevures	<i>Genolevures</i> is a large-scale comparative genomics project between <i>Saccharomyces cerevisiae</i> and 14 other yeast species representative of the various branches of the <i>Hemiascomycetous</i>	http://cbl.labri.fr/Genolevures/index.php	Sherman <i>et al.</i> 2004
Saccharomyces Genome Deletion Project	Data and information on the systematic deletion of all the <i>S. cerevisiae</i> genes and their phenotypic analysis	http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html	Winzeler <i>et al.</i> 1999
GENEMERGE	Tool for the functional analysis of large genomic data set: functional enrichment of biological functions and metabolic network	http://www.oeb.harvard.edu/hartl/lab/publications/GeneMerge/GeneMerge.html	Castillo-Davis & Hartl 2003
Pathway Processor	Analysis and visual mapping of microarray data onto the yeast metabolic network	http://cgr.harvard.edu/cavaliere/pphtml	Grosu <i>et al.</i> 2002
BAGEL	Software for the Bayesian analysis of gene expression level for spotted DNA microarray data sets	http://web.uconn.edu/townsend/software	Townsend & Hartl 2002, Townsend 2004

Box 2 Microarray technologies for the estimation of gene expression variation and array-based comparative genomic hybridization (aCGH)

Gene expression profiling

DNA microarrays usually consist of a set of short DNA fragments immobilized on glass slides similar to slides used in standard light microscopy. These represent DNA fragments that correspond to transcribed regions of the genome. The size and the number of elements present vary according to the array platform. For *Saccharomyces cerevisiae*, several commercially available platforms use short oligonucleotides of 25, 50, or 70 base pairs. First, total or messenger RNA is extracted from two samples of interest, and the reverse-transcribed RNA copies are labelled with fluorochromes (dyes). The two samples are then mixed and hybridized on the glass slide under a cover slip, which acts as a hybridization chamber. The relative abundance of mRNA transcripts can then be estimated using a high precision scanner that quantifies the fluorescence of the two dyes for each probe represented on the microarray. Several statistical methods are routinely used to assess the significance of the difference in fluorescence intensities. Guidelines for experimental design of microarray experiments in ecological and evolutionary genomics are presented in Townsend & Taylor (2005). The genotypes, environments, and developmental states examined should be chosen to answer the questions of interest without undue labour or expense, contrasts of interest should be direct, and chains of comparisons should be closed. Such multifactorial closed-circuit designs may be analysed statistically to yield estimates of gene expression levels by classical ANOVA methods (Kerr *et al.* 2000; Wolfinger *et al.* 2001), Bayesian analysis (Townsend & Hartl 2002; Townsend 2004), and even 'hybrid' empirical Bayes methodologies (Smyth 2004).

Array-based comparative genomic hybridization

The development of comparative genomic DNA hybridization predates DNA microarray technologies and was originally used for the identification of chromosomal abnormalities and gene copy number variation in cancer cells (Kallioniemi *et al.* 1992). This

technique involved the labelling of the test and reference DNA samples and their hybridization to metaphase chromosome spreads (Kallioniemi *et al.* 1992). Lashkari *et al.* (1997) were the first to use a cDNA microarray to detect changes in gene copy number among strains of yeasts. Whereas microarrays developed for gene expression profiling focus on transcribed regions of the genome, array-based comparative genomic hybridization can be used with any DNA template, including complete genomic DNA clones, cDNA, oligonucleotides, or specific targets of interest. Usually, a DNA sample to be tested is compared to a reference sample whose DNA has been used to design the DNA probes immobilized on the glass slide. DNA is extracted from the two samples and fragmented by enzymatic or mechanical means. The two-labelled DNA samples are then hybridized in the same manner as cDNA samples in gene expression profiling. The aCGH techniques may also be used to detect DNA sequence divergence between a test DNA sample and the reference sample (e.g. Edwards-Ingram *et al.* 2004). Estimating sequence divergence rather than quantifying gene duplications and deletions demands careful analysis, because the relationship between the ratios of hybridization and sequence divergence is likely to depend on the platform used. For example, single nucleotide differences between DNA fragments can be detected using short oligonucleotides, such as the 25-bp oligonucleotides in commercial Affymetrix microarrays (Winzeler *et al.* 2003), whereas on other platforms, single nucleotide difference may have a more subtle effect (Brunelle *et al.* 2004). The statistical analysis of aCGH data faces different challenges from gene expression data because one would ultimately like to differentiate gene deletion or duplication from DNA sequence divergence. With the increasing use of aCGH, several statistical methods and tools have been developed (e.g. Hupe *et al.* 2004; Chen *et al.* 2005) to answer these questions. Combined with appropriate experimental confirmation through alternative methods such as PCR, DNA sequencing, FISH, or Southern blot, aCGH can provide a global view of the genomic diversity found in natural populations and among closely related species, and thus help answer fundamental evolutionary questions (e.g. Fortna *et al.* 2004; Giuntini *et al.* 2005).

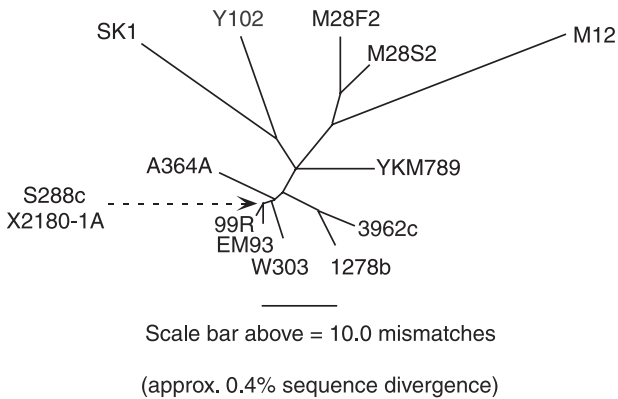


Fig. 1 Phylogenetic relationship among wine and laboratory strains of yeast as established through aCGH. (Modified from Winzeler *et al.* 2003 with permission, © Genetics Society of America.)

One limitation of this technology is that the functional nature of the polymorphism in each oligonucleotide cannot be defined precisely. However, detailed analysis of the localization of the variable genes revealed that genetic polymorphism is not randomly distributed across the genome (Winzeler *et al.* 2003). Deletion of an entire gene can be detected because all probes to the gene exhibit loss of hybridization. Based on this criterion, 90 genes were scored as missing in at least one strain, of which 29 were within 25 kb of the nearest chromosome end and 61 were in nontelomeric regions. Since the former regions include a total of 265 genes and the latter 5892 genes, the probability of observing such a difference by chance alone is essentially zero. Subtelomeric regions are well known to be rich in redundant sequences such as the X-element or the Y' element, which could serve as initiation points for non-homologous recombination to generate differences in gene copy number (Louis *et al.* 1994). Interestingly, the subtelomeric regions of yeast are enriched for genes with known functions in transport, transport facilitation, fermentation, and C-compound metabolism (Liti & Louis 2004), functions that are all relevant to growth in wine must. Subtelomeric genes include those encoding maltases, alcohol dehydrogenases, and sodium-phosphate transporters. Almost half of the 15 hexose transporter genes known in yeast are located in the subtelomeric regions.

This first study illustrated the power of aCGH for rapid screening of genetic variation, both at the level of nucleotide polymorphism and gene copy number, and has revealed that genetic variation at these levels is abundant among strains. In recent years, several groups have used this approach for answering diverse questions. For instance, a recent study used aCGH to identify gene deletions and duplications that are universal to common commercial strains of yeast (Dunn *et al.* 2005); this study indicated the

absence of extensive chromosomal duplications, a result in contrast to earlier studies (Codon *et al.* 1998). Gene duplication and chromosomal rearrangements have been identified as important mechanisms to produce adaptive mutations in experimental selection experiments (reviewed in Zeyl 2004). The abundance of this type of variation among natural and commercial strains suggests that it might also be important in wine yeast populations. One example of adaptive gene duplication is the tandem gene amplification of *CUP1* that mediates copper resistance (Fogel & Welch 1982). The aCGH technique has also been applied to other questions. For instance, a phylogeny of the *Saccharomyces* complex *sensu stricto* was produced based on the comparative hybridization of genomic DNA on *S. cerevisiae* cDNA microarrays (Edwards-Ingram *et al.* 2004). Also, since single nucleotide polymorphisms can be detected with short oligonucleotide microarrays, this technique can be employed for genotyping potentially hundreds of markers simultaneously and hence be of great use for mapping of quantitative trait loci (QTLs) for ecologically relevant traits. So far, this approach has been used in yeast to map QTLs that affect gene expression between two strains (Brem *et al.* 2002).

The large body of knowledge available on the molecular and cellular biology of yeast (Table 1) and its ease of manipulation in the laboratory offer tremendous advantages. First, candidate genes for phenotypes of interest can be identified from mutant phenotypes, comparative genomics, or gene-expression profiles. Second, several genetic tools facilitate investigations of the functional significance of allelic variants observed in natural populations, including methods such as gene replacement or transformation. On the other hand, very few studies have addressed issues of population structure and molecular population genetics at functional loci in yeast. Only a few studies have used sequencing of multiple loci to address questions about natural populations in species of the *Saccharomyces* group *sensu stricto* (Johnson *et al.* 2004), and only two have examined the population genetics of *S. cerevisiae* (Aa *et al.* submitted; Fay & Benavides 2005). Several studies have, however, employed anonymous molecular markers to estimate the relatedness among strains and their diversity during fermentation. Among the tools employed are DNA fingerprinting, pulsed field gel electrophoresis (PFGE), genomic DNA restriction analysis, mitochondrial DNA restriction analysis (REA), restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), PCR-RFLP on genomic and mitochondrial DNA, amplified fragment length polymorphism (AFLP), and microsatellites (e.g. Barberio *et al.* 1994; Paffetti *et al.* 1995; Baleiras Couto *et al.* 1996; Malgoire *et al.* 2005). These techniques have revealed important aspects of the genetics of wine yeast and its population dynamics during wine fermentation. For example,

analysis of genetic diversity at several time points during natural fermentation revealed a succession of *S. cerevisiae* strains (Schutz & Gafner 1994; Nadal *et al.* 1996).

High levels of genetic diversity are found within at least some wine-producing regions. For example, one study using genetic analysis showed that 28 isolates of *S. cerevisiae* isolated from cellars in the Italian region of Emilia Romagna were all distinct from one another (Mortimer *et al.* 1994), and the estimated level of diversity was even greater when molecular techniques detecting variation in subtelomeric regions were used in combination with genetic analysis (Cavaliere *et al.* 1998). However, Versavaud *et al.* (1995) analysed strains isolated from natural fermentations in different regions of France with PFGE; their results suggested the presence of a limited number of dominant strains that are widespread over the wineries of the region studied. Part of the discrepancy may be due to the use of different techniques to assay genetic diversity. For example, electrophoretic karyotyping of industrial strains (bakers, brewers, distillers, and wine yeast) suggested that most of these strains are aneuploid, having chromosome number that is not a multiple of the haploid number (Codon *et al.* 1998), but this result has been challenged by more recent studies using aCGH (Dunn *et al.* 2005).

Although the use of anonymous molecular markers can be very informative for discriminating among strains, it is difficult to make population genetic or functional inferences from the patterns of variation. With the availability of the complete sequence of the genome, specific genes can easily be studied. Among the first DNA-sequence surveys of *S. cerevisiae* was a study of the molecular evolution of the gene *Sup35* sequenced from a panel of clinical isolates (Jensen *et al.* 2001); *Sup35* codes for the yeast homologue of the translation termination factor eRF3. More recently, a broad survey was performed on natural isolates of *S. cerevisiae* (Aa *et al.* submitted). Four genes in 27 strains collected in Italy, California and Pennsylvania (associated with oak trees) were studied: *SSU1*, encoding a sulfite transporter; *FZF1*, encoding a transcription factor regulating *SSU1*; *CDC19*, encoding a pyruvate kinase implicated in glycolysis and pyruvate metabolism; and *PHD1*, encoding a RNA polymerase transcription factor involved in pseudohyphal growth. A phylogenetic tree based on 78 segregating sites suggests a population structure that includes four clades: (i) a group of strains containing the two laboratory strains (S288c and YPH499) and two strains collected in Elba, Italy; (ii) the oak-associated strains; (iii) a group of four strains from Elba; and (iv) a group of 11 strains from California, Tuscany, Emilia-Romagna, Umbria/California, and Elba. For one of the genes studied, the sulfite transporter *SSU1*, a McDonald–Kreitman test of neutrality suggested that this locus is not evolving neutrally owing to a significant excess of replacement polymorphisms relative to the neutral expectation ($P = 0.037$). Interestingly, *SSU1* is

a sulfite transporter whose expression is genetically variable among strains and is associated with sulfite resistance among vineyard populations (Cavaliere *et al.* 2000; Park & Bakalinsky 2000). The excess of replacement polymorphism at this locus is likely to result from artificial selection, since copper sulfate is used as a microbicide on vineyard grape vines and as a preservative in wine making (Park & Bakalinsky 2000).

Another broad-scale survey of nucleotide diversity at 5 loci in 81 strains is also informative (Fay & Benavides 2005). The strains studied were isolated from natural and artificial fermentations, tree exudates, and immunocompromised patients, and represent a diversity of geographical locations: Europe, Africa, Southeast Asia, and North America. A sequence of 7 kb, half coding and half non-coding, identified 184 polymorphic sites. The genealogical relationships among these strains do not offer a clear distinction between domesticated and nondomesticated strains. If natural isolates were all derived from domesticated populations, they should be closely related to them. The phylogenetic tree rather suggests that lineages at the root of the tree were derived from tree exudates in North America and Africa. These studies of DNA-sequence variation, combined with studies that have examined variation at the physiological, morphological, and transcription level, all suggest that populations of *S. cerevisiae* harbour large amount of genetic variation. The comparison of oak-associated and vineyard-associated yeasts suggests that habitats and geographical distance may play a role in shaping the patterns of genetic diversity as they cluster in different phylogenetic groups.

Comparative genomics

Two sequencing centres have recently completed the genome sequences of several species of the *Saccharomyces* complex *sensu stricto* (Cliften *et al.* 2003; Kellis *et al.* 2003). Among the sequenced genomes, four are very close relatives of *Saccharomyces cerevisiae*: *S. paradoxus* [divergence time from *S. cerevisiae* 5–10 million years ago (Ma)], *S. mikatae* (10–15 Ma), *S. kudriavzevii* (15–20 Ma) and *S. bayanus* (20 Ma) (Kellis *et al.* 2003). Comparative genomics of these species and other more distantly related species that have been completely or partially sequenced (e.g. the Génolevures project in Table 1) has provided unprecedented information on the evolutionary dynamics of regulatory elements and the evolutionary fate of duplicated genes (Kellis *et al.* 2003). It also showed that gene order is changing among species by inversion of small segments of DNA (e.g. Seoighe *et al.* 2000). The comparison of closely related species also revealed that more than 100 genes may be specific to *S. cerevisiae*. Confirmation is still required by alternative methods, because the sequencing of the close relatives may be incomplete (Kellis *et al.* 2003). Comparative genomics

of more distantly related species confirmed that the *Saccharomyces* genome originated from a whole genome duplication event followed by massive gene loss and differentiation of gene duplicates (Wolfe & Shields 1997; Kellis *et al.* 2004). The availability of the genome of these closely related species will make possible the combination of comparative genomics, functional genomics, and ecological genetics to address fundamental organismal questions related to the diversification of this group of eukaryotes, a task not possible in any other group of organisms. It will also allow more formal genetic and comparative work among these closely related species.

Speciation and introgression

Model organisms also offer research resources for the study of genetic isolating mechanisms between related species. For instance, the study of *Drosophila* species differences has been facilitated by the *D. melanogaster* genome sequence and functional genomics platforms (e.g. Ranz *et al.* 2003). *Saccharomyces cerevisiae* is no exception to this rule, and a number of closely related species have been examined. Species of the *Saccharomyces complex sensu stricto* (*S. cerevisiae*, *S. paradoxus*, *S. mikatae*, and *S. bayanus*) are not prezygotically isolated: F₁ hybrids can be produced through mating of haploid cells (e.g. Hunter *et al.* 1996; Greig *et al.* 2002; Sebastiani *et al.* 2002). These species are, however, postzygotically isolated because the F₁ diploid cells are mostly sterile, producing 1% or fewer of viable spores. Molecular manipulation of the mismatch repair system showed that it plays a role in hybrid sterility between *S. cerevisiae* and *S. paradoxus* by interfering with proper recombination during meiosis in the F₁ hybrids (Hunter *et al.* 1996). Also, a restoration of colinearity of chromosomes VI and VII in *S. mikatae* with those of *S. cerevisiae* demonstrated elegantly that chromosomal rearrangements also contribute to postzygotic isolation (Delneri *et al.* 2003).

Interspecific hybridization and introgression are known to produce rapid phenotypic variation in natural populations. However, it is still unknown whether these processes play a role in natural populations of wine yeasts. Ecological barriers might exist, but *S. paradoxus*, *S. cerevisiae*, and *S. bayanus* are found in overlapping habitats and are often sampled together either in association with trees or in vineyards (e.g. Sniegowski *et al.* 2002; Johnson *et al.* 2004). One major ecological factor determining boundaries may be temperature regimes under which those species can grow. *S. paradoxus* and *S. bayanus* grow better than *S. cerevisiae* at low temperature (Giudici *et al.* 1998; Greig *et al.* 2002; Sweeney *et al.* 2004). *S. bayanus* is a cryophilic species that can grow at temperature as low as 4 °C. Also, under some conditions, hybrids between species show higher fitness than parental species. For instance, hybrids between *S. cerevisiae* and *S. paradoxus*, or between *S. cerevisiae* and *S. bayanus*, have

the ability to grow over a larger range of temperatures than the parental species (Zambonelli *et al.* 1997; Greig *et al.* 2002).

Molecular markers for specific loci as well as anonymous markers have been used to study the extent of hybridization among these species and possible introgression from other species into the *S. cerevisiae* genome. These studies have focused on commercial strains but nevertheless demonstrate the potential for introgression in the wild. Numerous commercial strains have been shown to be hybrids. For example, the genomes of the wine strain S6U (*S. c. uvarum*), the cider strain CID1, and the beer yeast *Saccharomyces carlsbergensis* have contributions from both *S. cerevisiae* and *S. bayanus* (Hansen & Kielland-Brandt 1994; Masneuf *et al.* 1998; de Barros Lopes *et al.* 2002). There is therefore a potential for interspecific hybridization in the wild, but its contribution to *Saccharomyces* diversity has yet to be systematically explored. With the recent release of the genomic sequence for all of these species, species-specific DNA markers can readily be used to identify hybrids in the wild. High throughput techniques, such as genomic DNA hybridization (Box 2), may also identify which loci are subject to introgression. Indeed, it has recently been shown that genomic DNA sequences from species of the *Saccharomyces complex sensu stricto* are divergent enough to result in differential binding of DNA from different species onto *S. cerevisiae* DNA microarrays (Edwards-Ingram *et al.* 2004; Dunn *et al.* 2005).

Phenotypic diversity

Phenotypic variation among wine yeast isolates was recognized by winemakers before being appreciated by geneticists. Significant heterogeneity is apparent among strains in the production of ethanol, acetic acid, sulfite, and other products of metabolism as well as in colony morphology (Cavaliere *et al.* 1998; Casalone *et al.* 2005). Although most naturally occurring strains ferment to a titre of 11.5–15.5% ethanol, a few strains produce titres as high as 17%. Fermenting yeasts also produce acetic acid that varies in concentration (Polsinelli 1998; Antonelli *et al.* 1999). There is also segregating variation for several other physiological traits in wine yeast populations. For instance, when vineyard isolates are sporulated (Box 1) and their progeny tested for growth on carbon sources such as sucrose, maltose and galactose, approximately 67% of the isolates segregate for the inability to utilize at least one of these sugars (Cavaliere *et al.* 1998). Additionally, there is variation in the ability of strains to grow at extreme temperatures (Steinmetz *et al.* 2002; Mario Polsinelli, personal communication). Variation is also abundant in the ability of strains to switch mating type (Mortimer *et al.* 1994), an aspect that warrants further investigation inasmuch as mating systems are important in affecting the dynamics of genetic variation.

Saccharomyces cerevisiae also displays a variety of colony morphologies. While it is mainly known in the laboratory as a unicellular species with ellipsoid cells that divide by budding and form smooth colonies on solid medium, wine yeasts can also form more complex colonies by modifying their budding patterns and forming multicellular pseudohyphae (Gimeno *et al.* 1992). Other phenotypes include the formation of biofilms (Reynolds & Fink 2001) and complex 'fluffy'-structured colonies with cells connected by extracellular glycosylated matrix material (Kuthan *et al.* 2003). Finally, yeast can also form stalk-like structures in response to UV treatments (Engelberg *et al.* 1998). These alternative morphologies are hypothesized to be adaptive responses to environmental signals. For instance, the formation of pseudohyphae is induced by nitrogen deprivation and allows the cell to penetrate in the solid medium and forage into the nutrients (Gimeno *et al.* 1992). The formation of 'fluffy' colonies might also represent an adaptive response for the reason that it provides a protection against desiccation and other environmental factors and can form small channels for nutrient and water flow (Palkova 2004). Filamentous growth is also of medical interest because, in cases of human infection, this mode of propagation is thought to allow the penetration of physiological tissues (Madhani & Fink 1998). A recent screen of 1026 strains of yeast isolated from Italian vineyards showed that roughly half of the strains were able to show invasive filamentous growth when starved for nitrogen. Also, 2.5% of the strains had a filigreed ('rough') or fluffy colony phenotype. Several strains also showed differences in the treatments that induce modification of growth morphology, indicating the presence of genetic variation in the response to stimuli that can produce filamentous growth (Casalone *et al.* 2005). Understanding the evolutionary significance of this variation and identifying its genetic determinants will be important because many of these traits are known to be central for the competition of yeast with other microorganisms and for its tolerance to ecological stress. Also, the proteins involved in the signal transduction and gene regulation of pseudohyphal growth are well characterized (Gancedo 2001), so it would be interesting to know whether genetic variation in those elements contribute to the diversity of phenotypes found in the wild.

Population genetics of gene expression variation

It is still unknown to what extent phenotypic variation within and among species arises from variation in individual gene expression and the rewiring of transcriptional networks, rather than from the evolution of the amino acid sequences of proteins. Assessing the contribution of regulatory polymorphism, as measured by gene-expression variation, and its correlation with various phenotypes, can now be addressed using genomic tools. Studies on gene-expression variation

in yeast are numerous. Significant variation in gene expression is expected among natural isolates in light of their genetic and phenotypic variation and the diversity of environmental conditions in which they live. The first study to address this question, focusing on an isolate with a filigreed colony morphology, uncovered a metabolic phenotype in which hundreds of genes, many of them associated with amino acid biosynthesis or nitrogen or sulfur uptake, which are normally repressed in rich medium, were highly expressed (Cavaliere *et al.* 2000). Another study examined gene-expression variation among natural isolates from Montalcino, Tuscany, grown in rich medium (Townsend *et al.* 2003). Among four isolates, 433 genes were expressed at significantly different levels between at least two isolates. Using the extensive gene annotation of yeast allows one to identify metabolic pathways and biological processes particularly overrepresented among these 433 genes (Table 2). Such an analysis reveals that genes associated with protein synthesis and degradation are particularly variable in expression among these four natural isolates.

Genetic variation in gene expression among natural isolates of *Saccharomyces cerevisiae* was also assessed by Fay *et al.* (2004), who studied eight natural isolates along with S288c in both rich medium and medium containing copper sulfate. Seven of the isolates were obtained from Italian vineyards and one from an oak tree in Pennsylvania. A total of 241 genes showed significant gene expression variation in rich medium. Interestingly, the number of genes differentially expressed between any pair of strains was positively correlated with the number of nucleotide differences between them for three loci examined. As expected from their geographical origin, isolates from Italy tended to show the smallest number of differentially expressed genes and to be more different from the oak strain. On the other hand, some pairs of Italian strains showed about as many differentially expressed genes as observed between Italian and North American isolates. Whether this finding reflects a high level of diversity maintained in Italian populations or recent gene flow between Italian and North American populations will require further investigation. This study also revealed a large number of genes differentially expressed between the laboratory strain S288c and the natural isolates. In fact, 8 of 11 pairwise comparisons showing a significant effect of genotype involved comparisons with the laboratory strain.

Most studies so far have held genotype (mainly a laboratory strain) constant, and exposed the strain to various growth media in order to analyse gene-expression patterns under various physiological conditions. Altogether, these experiments have revealed that the laboratory budding yeast can show extensive regulatory remodelling in response to environment perturbations, i.e. extensive phenotypic plasticity at the transcriptional level. Examples include the use of DNA arrays to study global patterns of gene expression in the uptake and utilization of zinc (Lyons *et al.* 2000),

Table 2 Enrichment of functional categories in the genes that show genetic variation in gene expression level in Townsend *et al.* (2003). GENEMERGE (Castillo-Davis & Hartl 2003) was used to assess the over-representation of Gene Ontology functional categories and metabolic pathways among the set of genes being differentially expressed among the 4 natural isolates. E-scores are Bonferroni corrected *P*-values

Category	Description	Fraction in the genome (total = 5278)	Fraction in genes with genetic variation (total = 1360)	E-score
Molecular function	Structural constituent of ribosome	202	106	2.17E-18
	Endopeptidase activity	35	30	1.08E-12
Metabolic pathway	Ribosome	137	65	1.02E-08
	Oxidative phosphorylation	61	37	1.68E-08
	Lysine biosynthesis	19	13	0.003
	Aminoacyl-tRNA biosynthesis	37	20	0.003
	Glycolysis/gluconeogenesis	45	21	0.030
	Phenylalanine, tyrosine and tryptophan biosynthesis	23	13	0.042
	Fatty acid metabolism	18	11	0.047
Biological process	Protein biosynthesis	244	123	2.14E-19
	Ubiquitin-dependent protein catabolism	72	37	4.34E-05
	Aerobic respiration	51	27	0.001
Cellular component	Mitochondrial large ribosomal subunit	39	25	6.06E-06
	Mitochondrial small ribosomal subunit	28	20	9.48E-06
	Proteasome regulatory particle (<i>sensu</i> Eukarya)	20	16	1.52E-05
	Proteasome core complex (<i>sensu</i> Eukarya)	15	13	4.64E-05
	Cytosolic large ribosomal subunit (<i>sensu</i> Eukarya)	79	38	9.55E-05
	Mitochondrion	145	58	0.0003
	Mitochondrial inner membrane	72	33	0.002
	Cytosol	129	50	0.004

in response to diauxic shift (DeRisi *et al.* 1997), or under aerobic and anaerobic conditions (Ter Linde *et al.* 1999). Growth conditions that have been studied include ethanol (Alexandre *et al.* 2001), salinity (Yale & Bohnert 2001), amino acid starvation (Natarajan *et al.* 2001), and many others. Studies of other phenotypes, such as stages of the life cycle, have also been done; these include the cell cycle (Cho *et al.* 1998), filamentous growth (Madhani *et al.* 1999), sporulation (Chu *et al.* 1998), and ploidy numbers (Galitski *et al.* 1999).

In a large experiment, Gasch *et al.* (2000) studied yeast expression variation in rich laboratory conditions and in the presence of a number of stresses that could mimic fermentation conditions or natural perturbations, such as nitrogen starvation, osmotic shock, and shifts in temperature. These studies have demonstrated that about 900 genes in several laboratory strains show a common environmental stress response (ESR) to several unrelated environmental challenges. It is not known yet whether natural isolates also regulate a congruent set of genes in response to stress, but recent studies suggest that there might be important genetic variation for transcriptional responses to stressful conditions in natural isolates. Phenotypic response to environmental perturbation is known as phenotypic plasticity (in this case, transcriptional plasticity), and genetic variation in those responses is evidenced by significant genotype-by-environment interaction. Among a set of six independent isolates grown in four different environments chosen to mimic

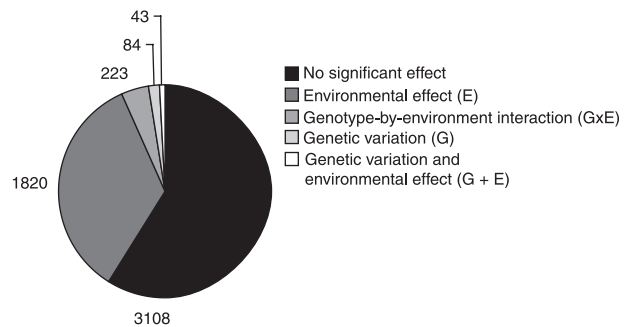


Fig. 2 Fraction of genes whose expression level is affected by the environmental growth condition (E, transcriptionally plastic genes), showing genetic variation independent of the growth condition (G), genetic variation and transcriptional plasticity (G + E) and showing genotype-by-environment interaction (genetic variation for transcriptional plasticity or $G \times E$). Six strains were studied in four conditions representing a gradient of environmental stress, including rich laboratory conditions and nitrogen starvation.

stress that yeast may encounter in the wild, Landry *et al.* (in press) found that natural strains also exhibit important changes in gene expression in response to environmental perturbations. Interestingly, an important fraction of genes show significant genotype-by-environment interaction, meaning that there is genetic variation in transcriptional plasticity for natural selection to act on (Fig. 2) (Landry *et al.* in press).

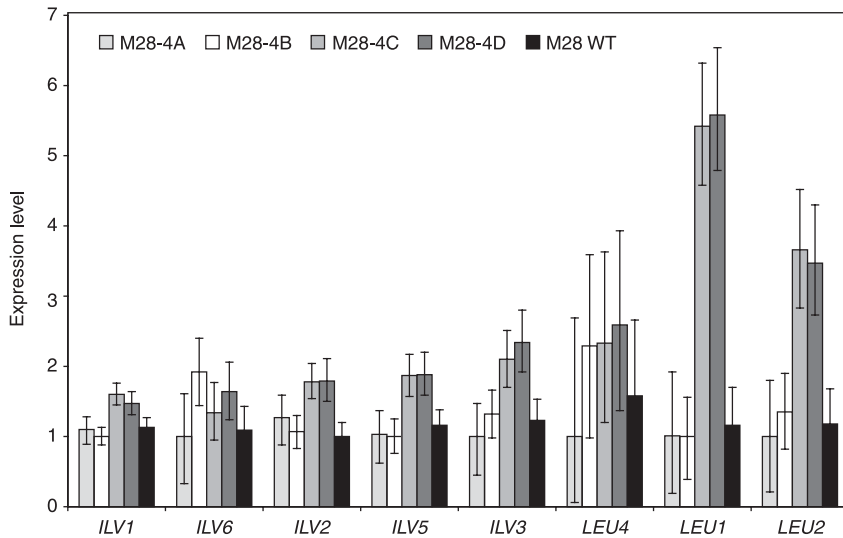


Fig. 4 Expression of the leucine and valine biosynthesis pathway genes, charted in the order of action within the pathway, for the four progeny (A, B, C and D) and the parental M28 strain. Progeny C and D show the filigreed phenotype. Bars indicate 95% Bayesian credible intervals; expression abundance is to scale among strains but not among genes.

yields estimates and credible intervals for the relative gene expression level of each gene. The greatest differences in gene expression distinguishing segregants were genes associated with amino acid synthesis or import. These included genes associated with the pathway from pyruvate to valine and leucine as well as the pathway from phosphoribosyl pyrophosphate to histidine (Fig. 4). Particularly remarkable was the extent of differential expression across the entire biosynthetic pathway from sulfate uptake channels to methionine (Fig. 5). An increased rate of amino acid synthesis demands increased nitrogen intake, and a prediction from these observations would be that the three ammonia permeases of *Saccharomyces cerevisiae*, *MEP1*, *MEP2*, and *MEP3*, would be abundantly expressed as well. *MEP2* has the greatest affinity for ammonia, and it was the most prominently overexpressed in the filigreed segregants compared to the smooth segregants (Fig. 6). *MEP3*, which has the lowest affinity for ammonia, showed the smallest difference between filigreed and smooth segregants.

The genes that were abundantly expressed in the smooth progeny relative to the filigreed progeny present a complementary picture. Amino acid transporters were abundantly expressed in smooth strains. The genes most affected were (i) *BAP2* and *BAP3*, which encode broad-affinity transporters of valine, leucine, isoleucine, alanine, and aromatic and sulfur containing amino acids; (ii) *GNP1*, which encodes a glutamine permease; and (iii) *DIP5* and *VAP1*, which encode amino acid permeases. Taking the overall patterns of abundant and meager expression into account, it is evident that the filigreed progeny of this tetrad meagerly express genes for amino acid transport and instead abundantly express genes for the synthesis of amino acids. An observation supporting this interpretation was that the aminoacyl-tRNA synthetases, which might be differentially expressed if unequal quantities of amino acids were being translated into

proteins, are not differentially expressed. Whether these transcriptional phenotypes cosegregate with the filigreed morphology or not will require the analysis of more tetrads, but the transcriptional profiling provides numerous candidate genes to examine further. For instance, interestingly, *MEP2* is required for pseudohyphal development (Lorenz & Heitman 1998). Another gene involved in fungal development, *PHD1*, which encodes a transcription activator (Gimeno & Fink 1994), is also overexpressed in the filigreed segregants. Note that, notwithstanding the wholesale differences in patterns of gene expression, there was no detectable difference in growth rate between any of the progeny under laboratory conditions. All of the segregants are vigorous. Moreover, none of the stress proteins noted by Wodicka *et al.* (1997) were expressed more abundantly in the filigreed segregants. However, given the different metabolic profiles observed, their fitness may differ in other, non-nutrient-rich conditions. These potential fitness differences require further investigation.

In summary, the segregants from M28 show remarkable differences in the expression of genes involved in central metabolism, particularly amino acid synthesis. This case provides an unusually clear demonstration of how regulatory variation and its pleiotropic effects may be mapped onto metabolic pathways. Comparison of the M28 strain to three other isolates from the same set of vineyards around Montalcino, Italy (Townsend *et al.* 2003) yielded further insight into these segregating metabolic differences by demonstrating that M28 and all its progeny showed extremely meager expression of the sulfur exporter *SSU1*, a key component of the regulation of methionine biosynthesis — and therefore of amino acid biosynthesis in general. *SSU1*, in turn, shows high levels of amino acid replacement polymorphism in naturally isolated vineyard strains (Aa *et al.* submitted). Rejection of a neutral history of molecular

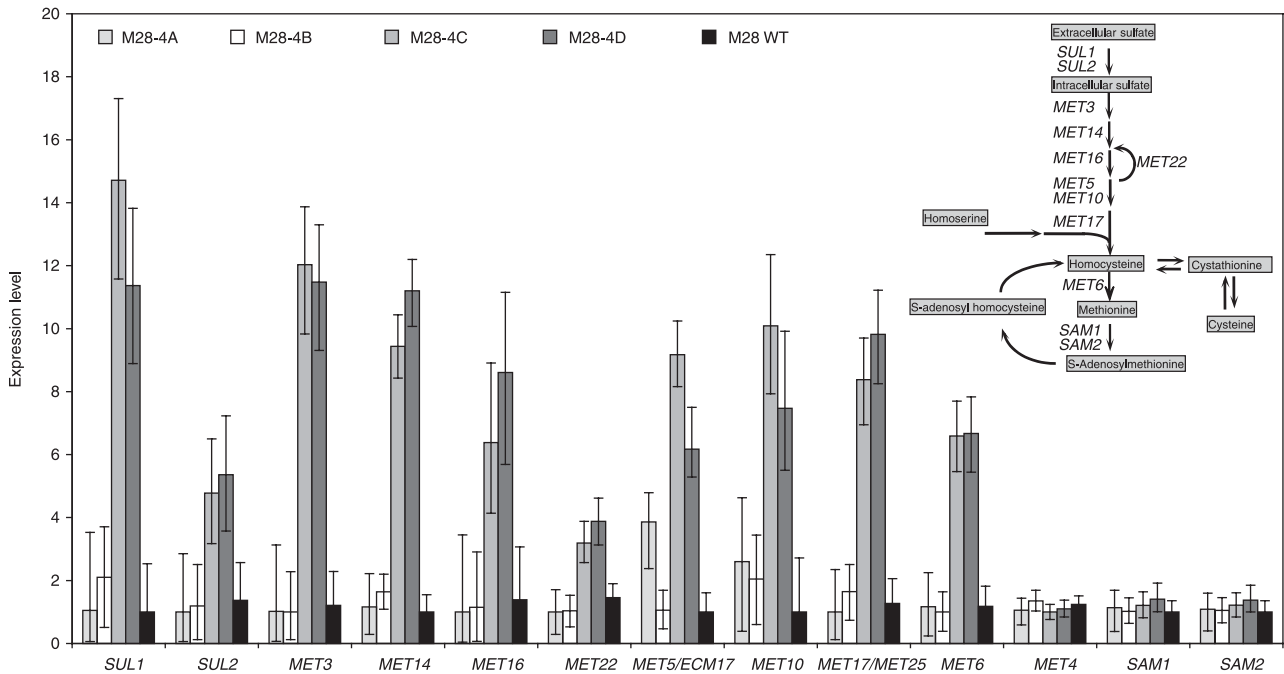


Fig. 5 Expression of sulfur-uptake and methionine biosynthesis pathway genes, charted in the order action within the pathway, for the four progeny and the parental M28 strain. Also represented is the position of those genes along the metabolic pathway. *MET4* is not represented in the metabolic pathway because it encodes a transcription activator. Bars indicate 95% Bayesian credible intervals; expression abundance is to scale among strains but not among genes.

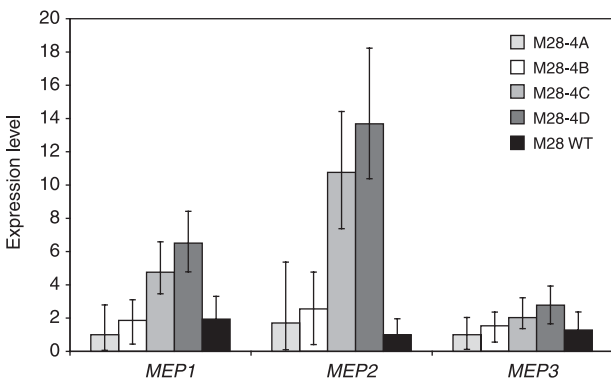


Fig. 6 Expression of genes for nitrogen uptake *MEP1*, *MEP2* and *MEP3* for the four progeny and the parental M28 strain. Bars indicate 95% Bayesian credible intervals; expression abundance is to scale among strains but not among genes.

evolution for *SSUI* suggests that this gene is subject to selection in vineyard populations, perhaps because of its wide-ranging metabolic effects and relevance to sulfur-based fungicide tolerance.

Concluding remarks

Ecology deals with phenotypic variation in relation to the habitat of an organism, whereas functional genomics

has among its objectives to describe and understand the mapping of phenotypes onto genotypes. A merger of these disciplines is the grand enterprise of ecological genomics. *Saccharomyces cerevisiae* has been a subject of intensive research in genetics, molecular biology, cellular biology, genomics, and proteomics, and the accumulated knowledge of its biology and physiology should help illuminate the ecological and evolutionary significance of phenotypic variation in natural populations. The first steps in this direction have been realized by genetic and phenotypic studies revealing extensive variation (physiological, morphological, and regulatory) in natural isolates. The challenge will now be to understand the ecological significance of this variation and whether it has been shaped by natural selection or neutral processes.

With a significant fraction of the genome of *S. cerevisiae* comprehensively studied and annotated, the adaptive significance of amino acid polymorphism in natural populations may be assessed directly. The growth rates of allelic variants may be compared in an isogenic background in conditions relevant to the function of the gene of interest. Population-based approaches, such as the association of phenotypic and genotypic variation with ecological variables, should shed light on the evolutionary significance of this variation. Further characterization of the genotype-phenotype map in yeast will be facilitated by the high throughput genotyping techniques for use in QTL mapping

in *S. cerevisiae* that are available. For example, aCGH can be used for genotyping of segregants (Brem *et al.* 2002; Steinmetz *et al.* 2002). So far, this has been applied to the mapping of gene expression QTL in a cross between a laboratory strain and a natural isolate, but it can easily be applied to other traits of ecological importance that variable in natural populations, such as those involved in stress resistance. QTL mapping is a promising avenue as ecologically important traits are likely to be polygenic.

Ecological studies of *S. cerevisiae* will also help better understand several recent findings in laboratory strains. Large-scale studies of *S. cerevisiae* in the laboratory have revealed intriguing features of its genomic architecture. For example, 80% of *S. cerevisiae* genes are not essential for cell propagation under rich laboratory conditions (Winzeler *et al.* 1999). A large fraction of genes might therefore play an important role in specific ecological settings. Since the deleted genes in the yeast deletion collection have been replaced by specific cassette containing unique tag sequences, strains can be competed in specific growth conditions in order to identify genes involved in susceptibility to specific treatments. This approach has been used for instance to identify genes involved in UV radiation sensitivity (Birrell *et al.* 2001).

Another useful approach will be the comparison of polymorphism and divergence in gene expression between natural isolates of *S. cerevisiae* with those of its closely related species inhabiting similar environments, namely *Saccharomyces paradoxus* and *Saccharomyces bayanus*. This will allow, for instance, examination of whether closely related species tend to harbour genetic variation in similar regulatory pathways. Considering gene expression levels as quantitative traits, comparison of polymorphism and divergence is a powerful approach in trying to estimate which evolutionary forces predominate in shaping interspecific differences in gene expression (Lemos *et al.* 2005). No microarrays have yet been constructed for these closely related species, but *S. cerevisiae* DNA microarrays may well be useful for such purposes, as heterologous hybridizations have been demonstrated for species that are more distantly related (e.g. Renn *et al.* 2004). This kind of analysis would also likely shed light on the regulatory divergence that has accompanied the ecological diversification of these species. With their complete genomic sequences available, and the numerous molecular tools available for yeast genetics and genomics, no other species group offers such potential and such promising possibilities.

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