

Genetic Dissection of Ethanol Tolerance in the Budding Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Uncovering genetic control of variation in ethanol tolerance in natural populations of yeast *Saccharomyces cerevisiae* is essential for understanding the evolution of fermentation, the dominant lifestyle of the species, and for improving efficiency of selection for strains with high ethanol tolerance, a character of great economic value for the brewing and biofuel industries. To date, as many as 251 genes have been predicted to be involved in influencing this character. Candidacy of these genes was determined from a tested phenotypic effect following gene knockout, from an induced change in gene function under an ethanol stress condition, or by mutagenesis. This article represents the first genomics approach for dissecting genetic variation in ethanol tolerance between two yeast strains with a highly divergent trait phenotype. We developed a simple but reliable experimental protocol for scoring the phenotype and a set of STR/SNP markers evenly covering the whole genome. We created a mapping population comprising 319 segregants from crossing the parental strains. On the basis of the data sets, we find that the tolerance trait has a high heritability and that additive genetic variance dominates genetic variation of the trait. Segregation at five QTL detected has explained ~50% of phenotypic variation; in particular, the major QTL mapped on yeast chromosome 9 has accounted for a quarter of the phenotypic variation. We integrated the QTL analysis with the predicted candidacy of ethanol resistance genes and found that only a few of these candidates fall in the QTL regions.

DISSECTING complex quantitative genetic variation into genes at the molecular level has been recognized as the greatest challenge facing geneticists in the 21st century (RISCH 2000). Identifying the genomic regions that cosegregate with a trait of interest provides a basis for a forward genetic approach for targeting genes that affect genetic variation of a trait. To date, only a few dozen cases of successful identification of genes underlying quantitative trait loci (QTL) have been reported, even though many different theoretical and experimental strategies have been proposed for improving the efficiency of QTL gene identification and have been practiced in tens of thousands of QTL analyses in almost all important animal and plant species and in humans (FLINT *et al.* 2005). This raises some fundamental questions: How efficient and reliable are the current QTL mapping techniques in uncovering information on genome locations of QTL for gene targeting? What are the major obstacles in the path from QTL to genes? Proposing to tackle these complicated questions under the simplest experimental system, we

chose ethanol tolerance (ET) of budding yeast (*Saccharomyces cerevisiae*) as a biological model of quantitative traits to explore these questions.

Ethanol is well known as an inhibitor of microorganism growth. It has been reported that the toxic effects of ethanol on yeast cells involve loss of cell viability and inhibition of both yeast growth and different transport systems such as the general amino acid permease and the glucose transport system (ALEXANDRE and CHARPENTIER 1998). The rising ethanol level during batch fermentation on high concentrations of sugar substrates acts initially to reduce growth and fermentation rates and adversely affects cell viability (PIPER 1995). Thus, a high level of ethanol tolerance for a yeast strain is a prerequisite for a high efficiency of fermentation and, in turn, for a high yield of ethanol.

In recent years, much effort has been devoted to exploring biochemical/physiological determinants of ethanol tolerance in yeast (*e.g.*, reviewed in D'AMORE *et al.* 1990; PIPER 1995; JEFFRIES and JIN 2000). It is clear that variation in ethanol tolerance of budding yeasts can be explained in terms of many factors such as lipid composition of the plasma membrane (JIMENEZ and BENITEZ 1987; LLOYD *et al.* 1993; SAJBIDOR *et al.* 1995; CHI and ARNEBORG 2000; YOU *et al.* 2003; TAKAGI *et al.* 2005), accumulation of trehalose (MANSURE *et al.* 1994;

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SHARMA 1997; LUCERO *et al.* 2000) or heat-shock protein Hsp104 (SANCHEZ *et al.* 1992; PIPER 1995), activity of plasma membrane H⁺-ATPase (ROSA and SACORREIA 1991; SUPPLY *et al.* 1995; AGUILERA *et al.* 2006), and mitochondrial stability (AGUILERA and BENITEZ 1985; IBEAS and JIMENEZ 1997).

So far, several studies have been carried out to identify the genes affecting ethanol tolerance in yeast by testing performance of the reference yeast strain that was genetically modified at different candidate genes under an ethanol stress condition (INOUE *et al.* 2000; KAJIWARA *et al.* 2000; TAKAHASHI *et al.* 2001; FUJITA *et al.* 2006; VAN VOORST *et al.* 2006). The candidacy of the genes was determined mainly according to the genes' involvement in the previously mentioned biochemical or physiological pathways. A collection of the yeast genes whose modification may cause a phenotypic effect on the trait is summarized in supplemental Table S1 (at <http://www.genetics.org/supplemental/>). However, the candidate-gene approach is quite limited in its plausibility for explaining the genetic basis of the character and for its potentiality of application. As with any stress resistance trait, the phenotype of ethanol tolerance of a yeast strain shares the common features of quantitative traits, *i.e.*, polygenic control and environmental influence. The genetic basis of these traits must be presented as a complex architecture of the genes that affect the trait phenotype through their direct and interactive effects (LYNCH and WALSH 1998; MACKAY 2001; STEINMETZ *et al.* 2002).

This article presents research on uncovering genetic control of variation in ethanol resistance in a natural population of budding yeast (*S. cerevisiae*) by genome-wide searching and mapping of the QTL. It is a preliminary effort in a series of studies aimed toward the ultimate goal of dissecting the genetic architecture of the quantitative trait at genic and transcriptional levels. In this study, we report five significant QTL, which explain up to 47% of phenotypic variation between two selected parental strains with extreme phenotypes. We compare locations of the mapped QTL to those of the candidate genes reported in the literature.

MATERIALS AND METHODS

Strains: We collected 53 yeast (*S. cerevisiae*) strains that were either purchased (from the China Center of Industrial Culture Collection; Institute of Industrial Microorganisms, Shanghai, China) or donated (by S. H. Tao, Northwestern Agricultural and Forestry University, Yang Lin, China; Y. Y. Li, Fudan University, Shanghai, China; J. H. McCusker, Duke University, Durham, NC; J. Cannon, University of Missouri, Columbia, MO; and H. Shimoi, National Research Institute of Brewing, Tokyo). Of these, 17 are laboratory strains and the remaining are strains used in the brewing industry (for instance, the sake yeast from Japan). The details about these strains' origin, industrial use, morphological characters, and ploidy status are available on request from the corresponding author.

Phenotype scoring of ethanol tolerance: The methodology was modified from the method proposed by OGAWA *et al.* (2000). In detail, cells from a tested strain were first inoculated on a YPD plate and cultured at 30° overnight. A small drop of the cultured cells was then moved to 5 ml YPD liquid medium and cultured at 30° under 280 rpm for 20–22 hr to ensure that the cells reached the stationary phase. The culture was centrifuged at 8000 rpm for 10 sec and supernatant was removed. Cells suspended in 2 ml sterilized water were centrifuged and harvested. The cells harvested were resuspended in sterilized water to 10⁵–10⁶ cell/μl. The cell suspension of 10 μl was added into 5 ml ethanol stress medium with 0.1 M acetate buffer (pH 4.2), 1% glucose, and ethanol at one of the gradient concentrations: 0, 2, 4, . . . , 18% (v/v). The medium was incubated at 30° under 280 rpm shaking for 3 days. A cell suspension of 5 μl from the liquid medium was spotted onto a YPD plate and cultured at 30° for 48 hr. In addition, the control cells cultured in 0% ethanol medium were diluted by 1:100 before spotting onto a YPD plate. The phenotype of ethanol tolerance was scored as the ethanol concentration at which the strain was treated, and formation of colonies was visually the same as that of the diluted control. The phenotype for a strain was the average of two independent records scored in this way.

Selection of parent strains with extreme phenotype: All 53 strains collected were assayed for their ethanol tolerance. Among them, the strain YPQ52, an isogenic haploid strain of the standard reference strain S288c, showed an outstanding viability, as high as 16% (v/v), to the ethanol stress treatment and was chosen as the high-performance parent; it is hereafter referred to as YH1A. To create the low-performance parent, we designed a directional selection for low ET. Two diploid strains, YPQ30 and YPQ51, and one haploid strain, YPQ58, were chosen to initiate the selection scheme for their relatively low ET and good sporulation performance. The HO gene in the two diploid strains was knocked out through homologous recombination to avoid mating-type switching and homothallism (VOTH *et al.* 2001). Genotypes of these strains are illustrated together with their donation sources in supplemental Table S2 at <http://www.genetics.org/supplemental/>.

F₁ zygotes were first generated by crossing YPQ51 to YPQ30 or YPQ58. The zygotes with a truncated value of ET were selected to sporulate and generate segregants. The segregants were assayed for ET performance and those surviving truncation selection were hybridized to form F₂ zygotes. The selection breeding scheme was repeated until the haploid segregant with stable low ET performance was selected. The segregant so selected was assigned as the low-performance parent, labeled as YL1C. A diagram of the selection breeding scheme is detailed in supplemental Figure S1 at <http://www.genetics.org/supplemental/>.

Gene dosage assay: To explore the difference in ethanol performance between haploid and diploid strains, we selected nine haploid strains that showed a wide spectrum of ET phenotypes and created their corresponding homozygous diploids by the diploidization procedure proposed by HERSKOWITZ and JENSEN (1991). Phenotyping each of the nine haploid and diploid strains was repeated three times.

Establishment of a mapping population: The two parental strains, YH1A and YL1C, were crossed to generate F₁ hybrids and the hybrids sporulated to segregants, which are equivalent to gametes, forming an F₂ generation.

Screening and genotyping of short tandem repeat markers: The genome sequence of yeast *S. cerevisiae* was downloaded from the Saccharomyces Genome Database (HONG *et al.* 2006) and searched for short tandem repeats (STR) within the genome by making use of the computer software Tandem Repeats Finder 3.21 (BENSON 1999). Among the STR searched, we

selected 561 evenly distributed STR sequences as candidate markers for this study. Primer sequences designed to amplify these candidates are listed in supplemental Table S3 at <http://www.genetics.org/supplemental/>. Polymerase chain reaction (PCR) followed by electrophoresis on 8% polyacrylamide gel (29:1) was performed to test the length difference at each of the candidate STR loci between the two parental strains. The 260 STR loci that exhibited polymorphism between the two parents were determined as basic molecular markers in this study. At each of the STR markers, the 5'-ends of either forward or backward primers were labeled with different fluorescent dyes.

The genotype of an individual was scored by multiplex PCR and electrophoresis running on an ABI 377. In detail, genomic DNA from a tested strain was prepared using the glass-bead method (AUSUBEL and STRUHL 1995). The PCR reaction for multiplex genotyping was prepared in a 10- μ l volume containing 1 \times PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 3 mM MgCl₂, 0.2 mM dNTP, 1.0 unit Taq polymerase (HuaNuo, Shanghai, China), and 6.0 ng yeast genomic DNA. A total of 0.25 μ M of each of the primers was added to the PCR mix for each multiplex panel. PCR amplification was conducted as follows: 94° (5 min) and then 35 cycles at 94° (30 sec)/50° (30 sec)/72° (30 sec) and a final extension at 72° for 7 min. Subsequently, 0.5 μ l of the PCR product was added to 1 μ l loading buffer, which contained 0.68 μ l formamide and 0.15 μ l fragment-size standard labeled with TAMRA (Applied Biosystems, Foster City, CA), and then run on an ABI 377 DNA analyzer (Applied Biosystems). The data were collected automatically by detection of the different fluorescences and analyzed by GeneScan/Genotyper softwares (Applied Biosystems).

In addition to the basic set of STR markers developed above, we added and genotyped four extra SNP markers within the major QTL region to improve the mapping resolution. The primer sequences for these SNP markers are also listed in supplemental Table S3 at <http://www.genetics.org/supplemental/>.

Mapping ethanol-tolerance QTL: We modified and reprogrammed the composite interval mapping (CIM) algorithm developed by ZENG (1994). Modification was made mainly by formulating the regression analysis that accounts for the mechanism of missing marker data (LITTLE 1992). We compared our program with Windows QTL Cartographer 2.5 (WANG *et al.* 2002) by analyzing simulation data and found that the modified program conferred an increased statistical power for detecting the simulated QTL in comparison to QTL Cartographer 2.5, particularly when the proportion of missing marker data is large (our unpublished data). The empirical significance level at 5% was obtained from the distribution of 1000 permutation test statistics as suggested by CHURCHILL and DOERGE (1994). The confidence interval for a mapped QTL was calculated as the interval on either side of the peak value in which the LOD score dropped by 1.0.

RESULTS

Selection for parent strain with low ethanol tolerance:

Figure 1 illustrates the observed response to selection for low ethanol tolerance and phenotypes of the tolerance trait for the two parental lines (YH1A and YL1C). It should be noted that selection was carried out at both gamete and zygote stages in each generation. This prompted a quick accumulation of selection advances, and the strain with a stable ET phenotype as low as <1% was selected after two generations of the two-stage selection (Figure 1A). This rapid and strong selection

response indicates that the trait has a high value of heritability and that additive genetic variation accounts for a major part of the total genetic variation underlying the trait. The realized heritability was estimated to be $h^2 = 0.628$ from the selection experiment (FALCONER and MACKAY 1996).

The phenotype of the two parental strains (haploids) was assayed in six independent ET tests (Figure 1B). Figure 1B shows that the two parental lines have highly divergent ET performance; the low-line parent (YL1C) failed to survive the treatment of ethanol with a concentration as low as 1%, whereas the high-line parent (YH1A) was able to resist treatment of up to 16% ethanol concentration. The phenotype scores were quite uniform across repeated tests, suggesting reliability and repeatability of the method that we developed to score the phenotype.

Performance of haploid and diploid strains: We tested the difference in ET phenotype between haploids and their corresponding homozygous diploids derived from diploidization for nine yeast strains that showed various ET phenotypes. The phenotype of these strains was tested under haploid and double-haploid states for three replicates. Phenotypic means and standard deviations were 12.86 and 2.06 for the haploids and 12.37 and 1.74 for the diploids. The phenotype data were fitted into a linear model with genotype (random) and ploidy level (fixed) as major effects. Table 1 summarized the analysis of variance for testing the significance of these effects. It shows that different genotypes (strains) had a highly significant effect on variation of the trait phenotype, as expected, and that the phenotypic difference was not significant between the haploid and the corresponding double-haploid strains ($P = 0.083$), even though we had observed that diploid strains usually had slightly lower ET values than their haploid counterparts (data not shown but available upon request). This makes it logically plausible that the genetical analysis of this study based on haploids would be largely valid for double haploids. Moreover, the very small value of residual mean square in comparison to that of major effects in the model again reflects the reliability of phenotype assessment.

Genomewide scanning for ET QTL and candidate QTL genes:

Among the 561 STR markers screened from the genome of budding yeast (*S. cerevisiae*), 260 were polymorphic between the two parental strains. Figure 2 shows the distribution of the candidate and polymorphic STR markers across 16 yeast chromosomes as gray bars and black bars, respectively. The 260 informative STR markers, together with four extra SNPs, provided an average coverage of 44 kb (equivalent to 14.7 cM)/marker over the whole yeast genome. The genotypes of 319 segregants (or haploid individuals hereafter) derived from crossing the two parental strains with highly divergent ET phenotypes were scored at each of these polymorphic markers. The phenotype for each

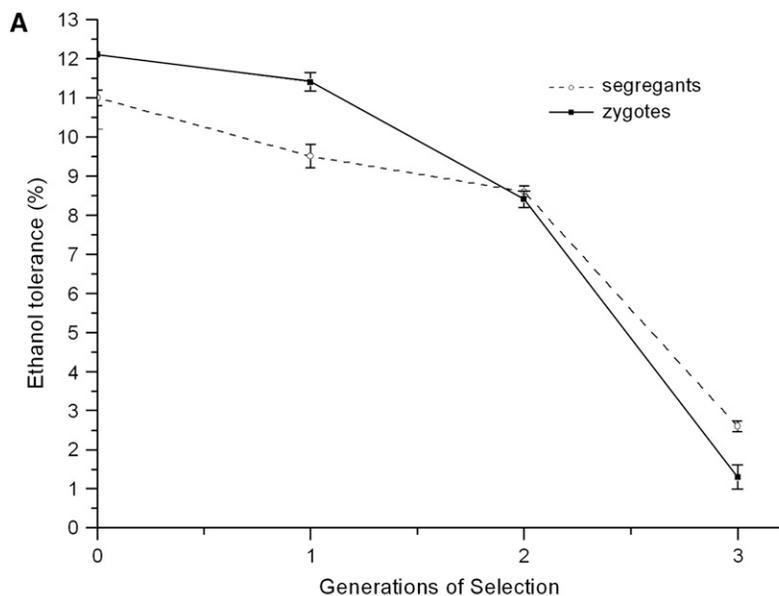
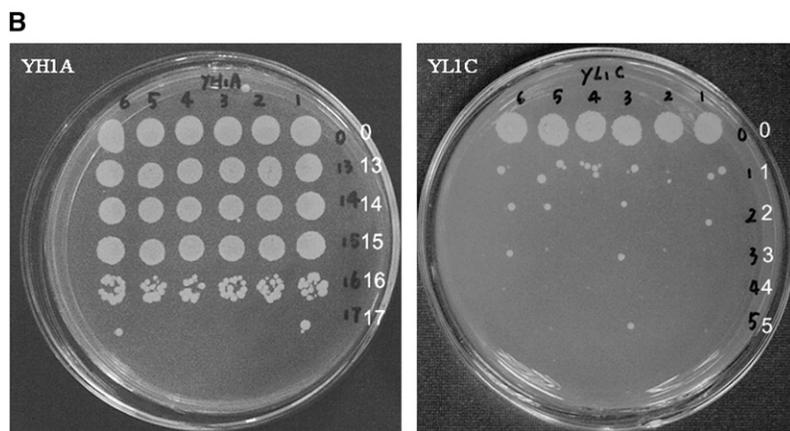


FIGURE 1.—Dynamic change in average ethanol tolerance of yeast segregant (dashed line) and zygote populations undergoing three consecutive generations of phenotypic selection (A) and colonies formed on YPD plates from cells of the two selected parental strains (YH1A and YL1C) under treatment with gradient concentrations of ethanol (B).



of the 319 segregants was determined as the average of two independent ET observations; the phenotypic distribution is shown in Figure 3.

The marker data and ET phenotype data of the segregant population were used to map quantitative trait loci underlying phenotypic variation in ethanol tolerance through the CIM analysis. Figure 4 demonstrates the distribution of LOD scores from the CIM analysis across all 16 yeast chromosomes. The analysis detected five QTL displaying significant effects on the trait phenotype, and they were mapped on chromosomes 6, 7, 9, 12, and 16 accordingly. LOD score profiles in the vicinity of three major QTL (on chromosomes 6, 7, and 9) are highlighted as insets of Figure 4. Estimates of map location and genetic effects of these QTL are summarized in Table 2. It can be seen from Table 2 that the QTL mapped on chromosome 9 has the largest additive effect on the trait and explains up to 25% of phenotypic variation of the trait. It should be noted that we added another four SNP markers in addition to the STR markers within this region and increased the marker coverage to 5 cM/marker. The 1.0-unit LOD score confidence inter-

val for the QTL gene(s) was only ~13 kb. The most likely location of the QTL genes (*i.e.*, the peak value of the LOD score) was between two SNP markers that were separated by a map distance of only 6.5 kb. The five QTL detected in this analysis explained a total of 47% of the variation in the ethanol tolerance trait.

A comprehensive literature survey shows that a total of 251 genes have been identified to date to be ethanol-resistance-related candidates. Among the candidate genes, 65 exhibited significant expression differences before and after treatment of the yeast cells with 7% ethanol (ALEXANDRE *et al.* 2001) and the rest showed varying levels of altered ethanol resistance when the genes were individually knocked out. The survey is summarized in supplemental Table S1 at <http://www.genetics.org/supplemental/>. To compare these candidate genes to our QTL analysis, we found that 37 of the candidates were within the QTL regions with the peak LOD score >2.0 (Table 2).

Five of these candidates fell into the QTL on chromosomes 6, 9, and 16, respectively (Figure 4). Both the yeast gene HXK1, which locates within the QTL on

TABLE 1
Analysis of variance between nine genotypes and two ploidy levels

Source	d.f.	SS	MS	F	P
Genotype	8	167.333	20.917	26.27	0.000
Ploidy level	1	3.130	3.130	3.93	0.083
Genotype × ploidy level	8	3.370	0.796	3.58	0.004
Residual	36	8.000	0.222		
Total	53	184.833			

The genotype effects were modeled as random effects and the ploidy levels as fixed effects.

chromosome 6, and the yeast gene PFK26, which locates within the QTL on chromosome 9, are involved in the glycolytic pathway. HXK1 encodes a cytosolic protein that catalyzes phosphorylation of glucose during glucose metabolism, while PFK26 encodes 6-phosphofructo-2-kinase that has negligible fructose-2, 6-bisphosphatase activity. These two genes were found to be upregulated by 7.3- and 4.2-fold, respectively, after 30 min of ethanol shock (7% v/v) in the experiment by ALEXANDRE *et al.* (2001). RMD8, another candidate falling in the chromosome 6 QTL, encodes a cytosolic protein that is required for meiotic nuclear divisions (ENYENIHI and SAUNDERS 2003). The RMD8 deletion mutant is viable but shows sensitivity to 6% ethanol and reduced growth in the presence of 0.5 mM sorbic acid (VAN VOORST *et al.* 2006). VPS16 and VPS28, members of the VPS gene family, locate within the QTL detected

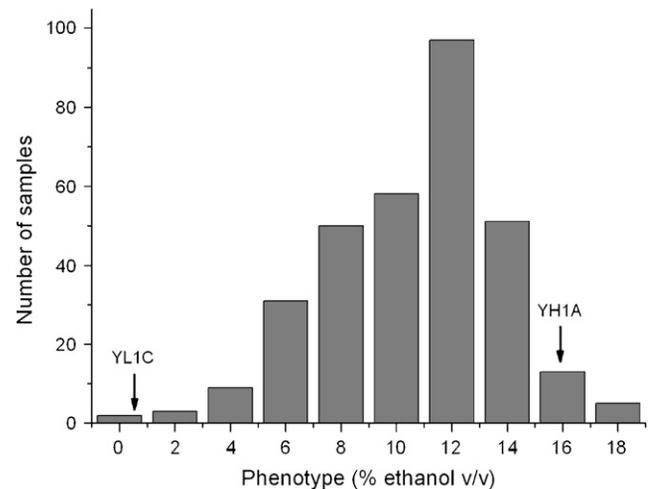


FIGURE 3.—Phenotypic distribution of 319 segregants from crossing yeast parental strains (YH1A and YL1C) with divergent phenotypes of ethanol tolerance.

on chromosome 16. VPS16, which encodes a protein subunit of the homotypic vacuole fusion and vacuole protein sorting complex (SEALS *et al.* 2000), is essential for growth of yeast on plates containing 6% ethanol (VAN VOORST *et al.* 2006). VPS28 encodes a protein that is a component of the ESCRT-I complex involved in ubiquitin-dependent sorting of proteins into the endosome (KATZMANN *et al.* 2001). Cells carrying a VPS28 mutant are sensitive to ethanol and nystatin treatment (FUJITA *et al.* 2006).

Multi-locus association analysis: To explore the joint effect of the five significant QTL on the phenotypic

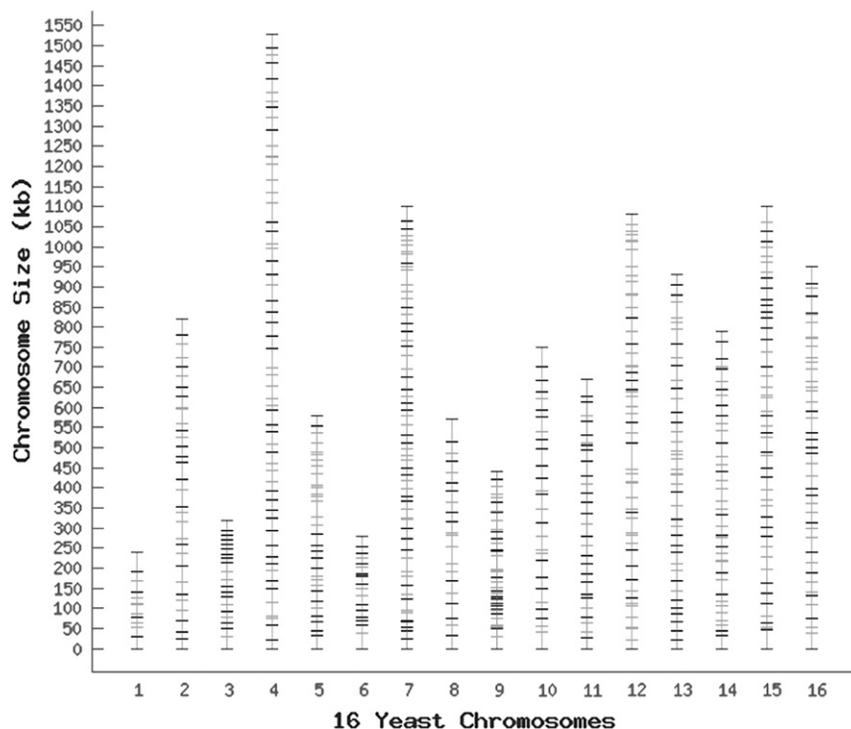


FIGURE 2.—Distribution of 561 STR sites examined (black and gray bars) and 260 polymorphic STR markers (black bars) across 16 yeast chromosomes.

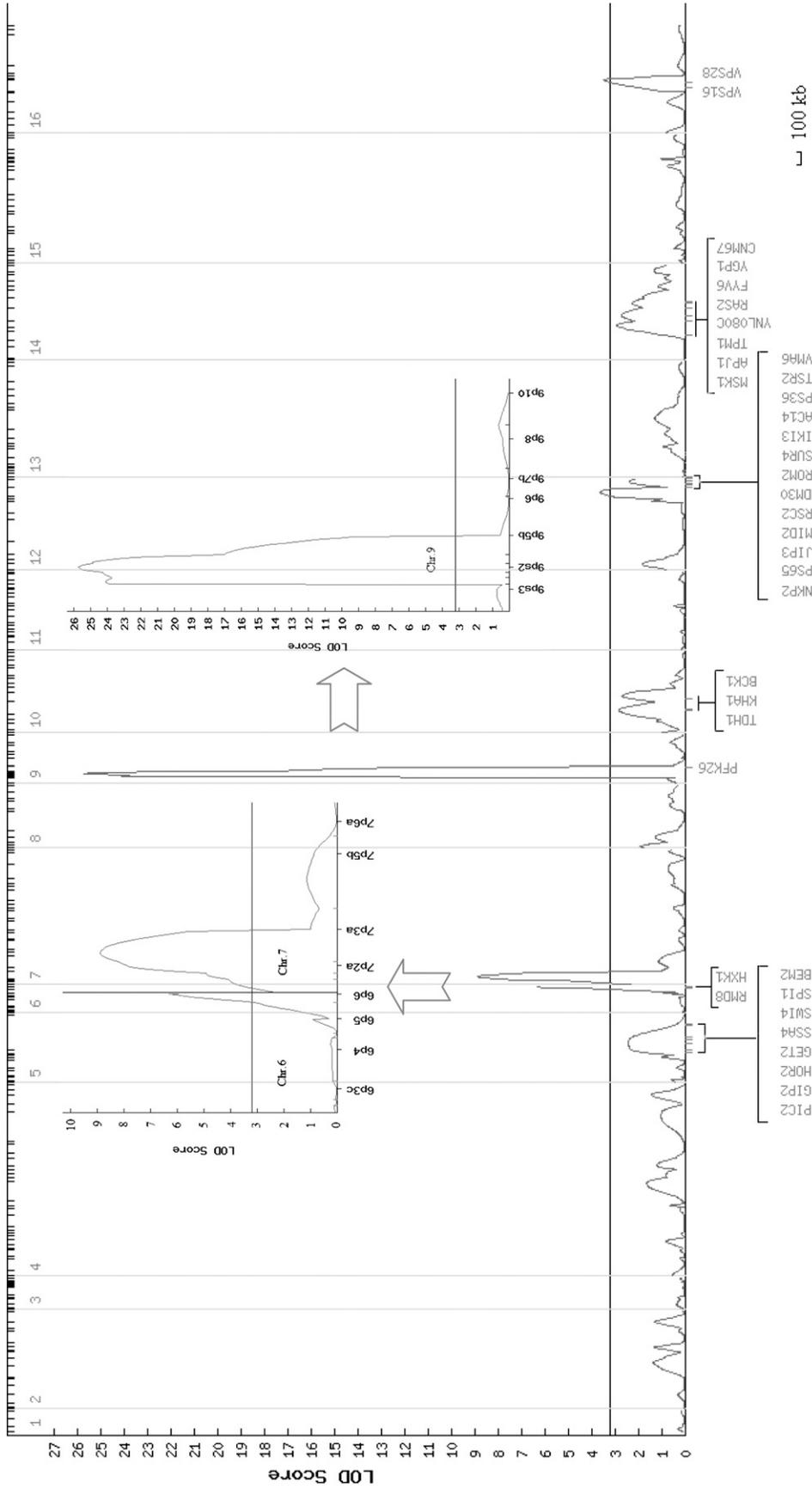


FIGURE 4.—Composite interval QTL maps underlying yeast ethanol tolerance. The horizontal line shows the genome-wide significance threshold calculated by 1000 permutation tests. Marker positions are indicated by short black bars on the top horizontal axis. (Insets) The QTL peaks of chromosomes 6, 7, and 9. Some genes underlying ethanol tolerance documented by previous studies are displayed under the bottom horizontal axis.

TABLE 2

Genome locations and estimated genetic effects of five QTL for ethanol tolerance of yeast (*S. cerevisiae*)

QTL locations (chromosome/cM) ^a	LOD score ^b	C.I. (kb) ^c	Additive effect	R ^{2d}
6/93.0	6.31	23.3	1.52	0.06
7/22.9	8.88	38.6	1.96	0.09
9/32.8	25.72	12.8	3.24	0.25
12/214.4	3.62	72.1	1.28	0.04
16/118.8	3.50	62.3	-1.10	0.03

^aChromosome/mapping distance in centimorgans from the left end of the chromosome.

^bPositions at which the LOD score reaches the peak value.

^cConfidence interval calculated as the interval on either side of the peak value in which the LOD score dropped by 1.0.

^dProportion of the phenotypic variance explained by genetic segregation at the QTL.

variation, we calculated means and standard deviations for each of the 32 possible haplotypes at the five markers that were in the nearest vicinity of the five detected QTL (the estimates are listed in Table 3). Interestingly, it is found that the largest phenotypic difference was not between the individuals that inherited all five alleles at the marker loci from the ethanol-resistant parental strain (YH1A, or a haplotype genotype “+ + + + +”) and the individuals that inherited all five alleles from the ethanol-sensitive parental strain (YL1C, or a haplotype genotype “- - - - -”). The largest phenotypic variation was observed between the haplotype “+ + + + -” and the haplotype “- - - - +” in the segregant population. This suggests two possible scenarios: a dispersion distribution of the “+” and “-” alleles between the two parental lines or an epistatic effect between the resistant-parental-line transmitted allele and the sensitive-parental-line transmitted allele. However, comparison between the additive effect of the QTL detected on chromosome 16 (Table 2) and the marker-associated effect in the multiple-locus haplotype suggests that epistasis between the QTL and the remaining four seems to be a more likely explanation.

DISCUSSION

Yeast evolution favors fermentation over respiration (WAGNER 2000), and an inevitable product of the fermentation process is ethanol. Viability of a yeast strain in the presence of high ethanol concentration is a prerequisite for a high efficiency of fermentation that is the basis for a high ethanol yield. Thus, selection for yeast strains with a high resistance to ethanol stress is of research importance for understanding the evolution of the organism and of economic value for traditional brewing and rapidly developing biofuel industries. There have been at least 251 yeast genes identified that

TABLE 3

Mean and standard deviations of ethanol tolerance for each of 32 haplotypes at five markers in the nearest vicinity of the five detected QTL

Haplotype	n	Mean ± SD	Haplotype	n	Mean ± SD
- - - - +	14	5.00 ± 3.19	- + + - +	9	10.56 ± 1.88
- - - + +	13	6.92 ± 3.20	+ + - + +	10	10.60 ± 1.84
- - - - -	10	7.30 ± 2.41	- + - + -	8	10.63 ± 1.69
+ - - - +	10	7.50 ± 3.06	+ + - + -	9	11.00 ± 1.87
- - - + -	8	8.00 ± 4.04	- - + + -	10	11.00 ± 2.71
- + - - +	8	8.13 ± 2.47	- - + + +	8	11.63 ± 2.72
- + - + +	10	9.20 ± 2.30	+ + + - +	9	11.89 ± 3.52
+ - - - -	6	9.67 ± 2.34	+ - + + -	8	12.38 ± 1.85
+ + - - +	19	9.74 ± 2.05	- + + + -	17	12.53 ± 1.23
- - + - +	8	9.75 ± 3.92	+ - + + +	10	12.70 ± 1.64
+ + - - -	10	9.80 ± 2.15	+ + + + +	7	12.86 ± 1.68
+ - - + +	6	9.83 ± 2.86	+ - + - -	14	12.93 ± 1.94
- + - - -	11	9.91 ± 2.39	- + + + +	7	13.14 ± 2.34
+ - - + -	13	9.92 ± 2.60	- + + - -	7	13.14 ± 2.48
- - + - -	5	10.00 ± 2.83	+ + + - -	5	13.20 ± 1.10
+ - + - +	23	10.33 ± 2.10	+ + + + -	11	14.91 ± 1.97

“+” represents the marker allele inherited from the ethanol-resistant parental strain YH1A; “-” represents the marker allele inherited from the ethanol-sensitive parental strain YL1C. *n*, the sample size for each of the haplotype groups. The individuals with missing information at any of the five marker loci were excluded from the analysis.

influence this character. However, candidacy of these genes for ethanol resistance was derived from testing their effect on the phenotypic variation by knocking them out (INOUE *et al.* 2000; TAKAHASHI *et al.* 2001; FUJITA *et al.* 2006; VAN VOORST *et al.* 2006) or from exploiting their functional alteration under ethanol stress treatment (ALEXANDRE *et al.* 2001). This indicates that yeast ET is under polygenic control as a typical quantitative trait. This study constitutes the first attempt at dissecting the complex genetic architecture that underlies phenotypic variation of the trait in natural populations of the yeast *S. cerevisiae* through a genomics approach.

We developed a simple but reliable experimental protocol to assess performance of ET for a yeast strain and observed a wide spectrum of ET performance among the laboratory and industry strains collected. A strain with ET as low as <1% (YL1C) was achieved by only two repeated generations of two-stage (gamete and zygote) phenotypic selection. In sharp contrast, another strain (YH1A) that may survive ethanol stress at a concentration up to 16% (v/v) was screened from isogenic lines of the well-known standard reference strain S228c. These findings strongly suggest a high heritability of the trait, a dominant part of additive genetic variance within total genetic variation, and the presence of major-effect quantitative trait loci that contribute to the genetic variation (FALCONER and MACKAY 1996).

We tested ET performance of nine strains at haploid and double-haploid states. A preliminary analysis did not reveal a significant difference between the two ploidy levels, although we observed that diploid strains usually had a slightly lower ET than the corresponding haploids. This suggests that gene dosage effect might be trivial for the trait and that the genetical analysis based on haploid data and presented below provides at least a good approximation of the case in double haploids.

We have developed a segregating population that comprises 319 segregants from crossing the two parental strains YH1A and YLIC. A genome scan for QTL underlying the phenotypic variation was carried out on the basis of the phenotype and genotype of the 319 individuals at 264 STR/SNP polymorphic markers. Five QTL were detected to be linked with the genes affecting the quantitative trait. Of the five QTL, the one mapped on chromosome 9 can explain 25% of total phenotypic variation and is between two markers separated by 15 kb. This, together with the other four QTL, explains about half of the total phenotypic variation observed in the mapping population. This illuminates the presence of a major contribution of gene(s) and explains the dominant part of the additive genetic variance component in the genetic control of the trait, as predicted from the selection experiment. It opens an opportunity for identification and molecular cloning of the QTL genes. Within this QTL region, we find the yeast gene PFK26, whose candidacy as an ethanol resistance gene was previously reported, for expression of this gene is markedly upregulated under ethanol treatment (ALEXANDRE *et al.* 2001). However, there are >30 genes across this region. It has been shown that the heat-shock QTL detected in budding yeast masked 3 closely linked genes (STEINMETZ *et al.* 2002). Therefore, finer-scale genetic dissection and functional analysis are needed to decompose the QTL at genic and/or transcriptional levels (GLAZIER *et al.* 2002).

In addition, the QTL analysis is useful for marker-assisted selection for a yeast strain with high tolerance to ethanol stress (LUO *et al.* 1997). However, it should be noted that assembling the chromosomal segments in the vicinity of the QTL from the ethanol-resistant parental strain (YH1A) will not necessarily lead to an expected increase in the resistance performance of the selected line. In fact, a multiple-locus analysis based on representative markers of the QTL reveals the likely epistatic effect of the QTL gene(s) inherited from the sensitive parental strain (YLIC) with that from the resistant strain. This is a reflection of the complexity in genetical control of the polygenic trait.

This study provides a direct assessment of significance for each of the 251 yeast genes that were proposed in previous studies as candidates in influencing phenotypic variation of the ethanol resistance trait. Their candidacy was predicted mainly through knockout or case-control tests on an individual gene basis. Only

~15% (37/251) of these candidate genes are seen to fall into the chromosome regions at which the LOD score is >2.0. This reflects the limitation of the single-gene-based reverse genetics approach, although it may be explained by the different genetic backgrounds on which the inferences were made. Nevertheless, the quantitative genetic analysis presented in this article has succeeded in uncovering the major genetic component for ethanol tolerance of yeast, a typical polygenic trait.

We have demonstrated the theoretical potential of mapping populations created from recurrent selection and backcross (RSB) breeding schemes for mapping quantitative trait loci at the precision and resolution by which molecular cloning of the underlying genes could be directly targeted (LUO *et al.* 2002). The parental strains in this study have been used to create RSB populations for identification of the genes affecting phenotypic variation of ethanol tolerance. Comparison of this QTL analysis with the RSB analysis when it becomes available may provide a direct assessment of the efficiency of the conventional method of QTL mapping and thus answers to the aforementioned questions.

Although the budding yeast (*S. cerevisiae*) is the first eukaryote to have its whole genome sequenced, a matured set of primer sequences for STR markers is not available in public domains or in the literature. We designed and experimentally validated 521 pairs of primers for evenly distributed STR markers in the yeast genome (the information can be found in supplemental Table S3 at <http://www.genetics.org/supplemental/>). This data set will be as useful for a genomic analysis in the species as the well-known Human Linkage Mapping Set commercialized by Applied Biosystems.

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