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Effects of genome duplication on phenotypes and industrial applications of *Saccharomyces cerevisiae* strains

Ke Zhang[1,2] · **Ya-Hong Fang**[1] · **Ke-Hui Gao**[1] · **Yang Sui**[2] · **Dao-Qiong Zheng**[2] · **Xue-Chang Wu**[1]

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Abstract Polyploidy is common in *Saccharomyces cerevisiae* strains, but the physiological and phenotypic effects of ploidy changes have not been fully clarified. Here, isogenic diploid, triploid, and tetraploid *S. cerevisiae* strains were constructed from a haploid strain, CEN.PK2-1C. Stress tolerance and ethanol fermentation performance of the four euploid strains were compared. Each euploid strain had strengths and weaknesses in tolerance to certain stressors, and no single strain was tolerant of all stressors. The diploid had higher ethanol production than the other strains in normal fermentation medium, while the triploid strain showed the fastest fermentation rate in the presence of inhibitors found in lignocellulosic hydrolysate. Physiological determination revealed diverse physiological attributes, such as trehalose, ergosterol, glutathione, and anti-oxidative enzymes among the strains. Our analyses suggest that both ploidy parity and number of chromosome sets contribute to changes in physiological status. Using qRT-PCR, different expression patterns of genes involved in the regulation of cell morphology and the biosynthesis of key physiological attributes among strains were determined. Our data provide novel insights into the multiple effects of genome duplication on yeast cells and are a useful reference for breeding excellent strains used in specific industrial applications.

Keywords *S. cerevisiae* · Ploidy · Stress tolerance · Physiology · Gene expression

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✉ Dao-Qiong Zheng
zhengdaoqiong@zju.edu.cn

✉ Xue-Chang Wu
mblab@163.com

[1] College of Life Science, Zhejiang University, Hangzhou, Zhejiang Province 310058, China

[2] Ocean College, Zhejiang University, Zhoushan, Zhejiang Province 316021, China

Introduction

Saccharomyces cerevisiae strains are widely used in baking, brewing, and bioethanol production. During industrial processes, yeast cells suffer multiple environmental stresses, such as temperature fluctuation, osmotic pressure, and acid stress (Attfield 1997). Additionally, certain inhibitors (weak acids, furans, and phenolic compounds) derived from the pretreatment of cellulosic feedstock would greatly affect the viability of yeast cells and fermentation efficiency (Ravindran and Jaiswal 2016; Sindhu et al. 2016). An effective strategy for breeding of robust yeast strains is a prerequisite to their usefulness in industrial production (Zheng et al. 2011).

Normally, *S. cerevisiae* cells can exist in two forms: haploid and diploid. The haploid cells have a simple lifecycle of mitotic growth, while the diploid cells can undergo sporulation, entering meiosis and producing four spores. Two haploid yeast cells of opposite mating type (*MAT***a** or *MAT*α) as determined by information expressed from the *MAT* locus can mate to form a diploid cell. The gene *HO*, which encodes an endonuclease, is responsible for initiating mating type switching (Hou et al. 2013; Kostriken et al. 1983). Although diploid is the preferential form for *S. cerevisiae*, recent studies revealed extensive ploidy variations in *S. cerevisiae* strains (Zhang et al. 2016; Zhu et al. 2016). Additionally, polyploidization has been used to improve the production of ethanol and the quality of fermentation products in certain yeast stains (Hashimoto et al. 2006; Yamada et al. 2010). These studies

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indicate that ploidy modification may contribute to improvement of specific phenotypes and industrial application of yeast.

In this study, isogenic diploid, triploid, and tetraploid strains were generated from a haploid *S. cerevisiae* strain. Comparative analysis of these euploid strains characterized the effects of ploidy modification on multiple phenotypes, such as tolerance to environmental stresses and ethanol fermentation performance. Additionally, the physiological and transcriptional mechanisms associated with phenotypic changes among the euploid strains were investigated.

Materials and methods

Yeast strains, plasmid, and medium

All *S. cerevisiae* strains used in this study were isogenic to haploid strain CEN.PK2-1C (*MAT***a**; *his3D1 leu2-3_112 ura3-52 trp1-289 MAL2-8c SUC2*; named by CEN-H in this work) (Entian and Kötter 2007). This strain is available from the EUROSCARF collection center (Accession number: 30000A; Frankfurt, Germany). Isogenic diploid (CEN-D), triploid (CEN-TR), and tetraploid (CEN-TE) strains were generated by cycles of mating type switching and mating from the haploid strain CEN-H, as shown in Fig. 1a. Plasmid pSH-HO was constructed by inserting gene *HO* (amplified by PCR using primers HOS: 5′-GCGAATTCATGCTTTCTGAA AACACG-3′ and HOA: 5′-CGACGTCGACGCATATATGA ATATTACTTA-3′) into the *Eco*RI and *Sal*I sites of plasmid pSH47 (Gueldener et al. 2002). Yeast cells were cultured in YPD medium (20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract, pH 5.0) at 30 °C. Yeast transformation was performed using the LiAc/single-stranded carrier DNA/polyethylene glycol method (Gietz and Schiestl 2007). Transformants with plasmid pSH47 were selected on SD-U plates (1.7 g/L yeast nitrogen base, 20 g/L glucose, 5 g/L ammonium sulfate, 0.1 g/L leucine, 0.1 g/L histine, and 0.1 g/L tryptophan, 20 g/L agar, pH 5.0). To induce the expression of the *HO* gene and mating type switching, yeast cells with plasmid pSH-HO were incubated in 5 mL YPG medium (20 g/L galactose, 20 g/L peptone, and 10 g/L yeast extract, pH 5.0) at 30 °C for 24 h with an initial OD_{600} of 0.05. The cells were then plated on YPD agar plates and incubated at 30 °C for 48 h. A diagnostic PCR assay using primers P1: 5′-AGTCACATCAAGATCGTTTATGG-3′, P2: 5′-ACTC CACTTCAAGTAAGAGTTTG-3′, and P3: 5’GCACGGAA TATGGGACTACTTCG-3′ was used to determine the mating type of yeast colonies (Huxley et al. 1990). To remove the intracellular plasmid, yeast cells were incubated in 5 mL YPD at 30 °C for 24 h with an initial OD_{600} of 0.05 and then plated on YPD agar plates. The colonies without plasmid pSH-HO could not grow on SD-U agar plates.

Mating and hybrid selection

Yeast strains were independently pre-cultured in 5 mL YPD medium for 16 h with an initial OD_{600} of 0.05. The cells

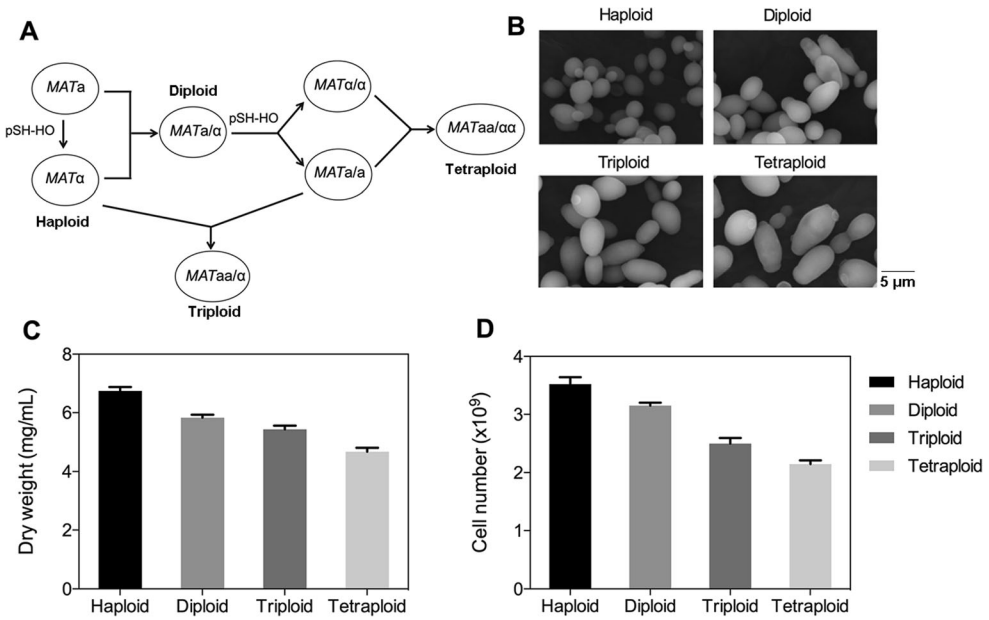

Fig. 1 Cell morphology and biomass formation of four euploid strains. **a** Diagrammatic presentation of mating type switching and crossing used to generate *S. cerevisiae* strains of varying ploidy. Plasmid pSH-HO contains the gene *HO*, which is responsible for initiating mating type switching. **b** Cell morphology of haploid, diploid, triploid, and tetraploid strains detected by scanning electron microscopy. **c** Dry weight and **d** cell numbers of the four euploid strains. Cells were cultured in 25 mL YPD liquid medium at 30 °C for 48 h with an initial OD_{600} of 0.05. *Bars* represent means ± SD of three independent experiments performed in triplicate

(1×10^7) from strains with opposite mating types were collected by centrifugation (6000 rpm for 5 min) and mixed in 5 mL fresh YPD for random mating (static incubation for 24 h at 30 °C). Yeast cells were then plated on YPD plates and the mating types of colonies were determined by diagnostic PCR as described above.

Scanning electron microscopic examination

Yeast cells were incubated in 25 mL YPD (initial OD_{600} of 0.05) at 30 °C for 10 h. Yeast cells (1×10^8) were collected by centrifugation (6000 rpm for 5 min) and washed in 1× PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4) two times. Cells were fixed by 4% glutaraldehyde (2 mL) at 4 °C for 4 h. Cells were washed in 1× PBS three times and postfixed in 1% osmium tetroxide (1 mL) at room temperature for 2 h. Cells were dehydrated through an ethanol series (30% for 15 min, 50% for 15 min, 75% for 15 min, 90% for 15 min, 100% for 15 min, and 100% for 15 min). The dehydrated cells were immersed in 1 mL of hexamethyldisilazane for 10 min and transferred to the specimen holder of a critical point dryer. The dried samples were then mounted on a scanning electron microscopy (SEM) sample stub with double-sided sticky tape, sputtered with gold, and viewed in an S-3000N SEM (Hitachi, Tokyo, Japan).

Stress tolerance test

To determine the tolerance of yeast strains to stressors, overnight-cultured cells were inoculated in 25 mL YPD and YPD with stressors to an initial OD_{600} of 0.05. The pH of the YPD was adjusted to 5 using hydrochloric acid (HCl) unless otherwise specified. The stress conditions were as follows: (A) osmotic pressure, 0.75 M NaCl; (B) heat stress, incubation at 42 °C; (C) oxidative stress, 5 mM H_2O_2; (D) ethanol stress, 8% ethanol (*v*/*v*); (E) low pH stress, culture pH was adjusted to 2.6 using HCl; (F) 3 g/L acetic acid, pH 4.5; (G) 0.9 g/L formic acid, pH 3.5; (H) 3% lactic acid, pH 3.5; (I) 0.8 g/L vanillin; (J) 0.75 g/L furfural; (K) 1.5 g/L 5-hydroxymethyl furfural (HMF); (L) 5 mM $CuSO_4$; (M) 1.5 mM $NaASO_2$; and (N) 2 mg/L fluconazole. Cell growth of the yeast strains was measured by a spectrophotometer. The tolerance of an individual strain to a certain stressor was represented as the ratio of cell growth in the stressful condition to that in normal YPD at the time point of 18 h.

Ethanol fermentation and metabolite analysis

Anaerobic fermentation was performed in 500 mL Erlenmeyer flasks containing 250 mL fermentation medium (pH 4.5). Yeast cells were aerobically pre-cultured in 50 mL YPD for 20 h at 30 °C, and 0.05 g of cells (dry weight) were transferred to the fermentation media. Three fermentation media were used in this study: YPHD, with 184 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract; HA, with 98 g/L glucose, 10 g/L peptone, 5 g/L yeast extract, 3.8 g/L acetic acid, 0.2 g/L formic acid, 1 g/L furfural, and 0.2 g/L 5-HMF; and HB, with 99 g/L glucose, 10 g/L peptone, 5 g/L yeast extract, 3 g/L acetic acid, 0.2 g/L formic acid, 0.3 g/L furfural, 0.2 g/L 5-HMF, and 1.2 g/L vanillin. The different concentrations of inhibitors in HA and HB were used to simulate different types of lignocellulose hydrolysates. Fermentation was then performed at 33 °C and 100 rpm. Samples of fermentation broth for metabolite measurement were immediately centrifuged (12,000 rpm for 10 min at 4 °C), filtered through a 0.45-mm cellulose nitrate filter (Whatman, Maldstone, UK), and stored at −20 °C until analysis. Glucose and ethanol concentrations were measured by high-performance liquid chromatography (HPLC; Agilent, Palo Alto, CA, USA) with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 60 °C and were detected using a refractive index detector (Agilent, Palo Alto, CA, USA). The mobile phase was 4 mM H_2SO_4 at a flow rate of 0.6 mL/min.

Determination of physiological attributes

Overnight-cultured yeast cells were transferred into 25 mL YPD (initial OD_{600} of 0.05) and harvested after incubation for 10 and 30 h. The trehalose of yeast cells (1×10^8 cells) was extracted using 2 mL of 0.5 M trichloroacetic acid (at 4 °C for 45 min) and measured using high-performance liquid chromatography (Guo et al. 2000). Samples for dry weight analysis were washed twice with distilled water and dried at 90 °C for 10 h. Ergosterol from each sample (1×10^9 cells) was extracted using 3 mL of alcohol alkali solution (50 g NaOH was dissolved in 120 mL ddH_2O, and absolute ethanol was added to a volume of 200 mL) at 95 °C for 3 h and then measured using an Agilent 1100 HPLC (Palo Alto, CA, USA) equipped with a Zorbax SB-C18 column (250 × 4.6 mm; Agilent, Palo Alto, CA, USA). Yeast Protein Extraction Kit (CWBIO, Beijing, China) was used to release proteins from yeast samples, and a BCA protein assay reagent (Pierce, Rockford, IL, USA) was used to measure protein concentrations. Catalase (CAT) activity was detected using a CAT assay kit (Beyotime, Beijing, China). One CAT unit was defined as the amount of CAT that decomposed 1 μmol of H_2O_2 per min at 30 °C. Total superoxide dismutase (SOD) activity was measured using an SOD assay kit (Jiancheng, Nanjing, Jiangsu, China) as described previously (Zheng et al. 2011). Total intracellular glutathione (GSH) was extracted in 2 mL of 40% (*v*/*v*) ethanol (cell concentration was adjusted to 1×10^8 cells/mL) and determined by a GSH assay kit (Beyotime, Beijing, China) according to the manufacturer's instructions.

qRT-PCR

A qRT-PCR experiment was used to determine the expression of genes involved in cell morphology and stress tolerance. Yeast cells were cultured in 25 mL YPD medium with an initial OD_{600} of 0.05. At the time points of 10 and 30 h, yeast cells (1×10^8) were collected, and total RNA was extracted as described in our previous study (Zheng et al. 2014). qRT-PCR experiments were performed in an ABI Prism 7500 instrument (AB SCIEX, Foster City, CA, USA). Samples were tested in triplicate in a 96-well plate (reaction volume was 20 μL) using a SYBR® PrimeScript® RT-PCR kit (Takara, Dalian, Shandong, China). The expression was quantified and normalized using *ACT1* as the reference gene (Tao et al. 2012). The primers used in the qRT-PCR experiment are listed in Table S1 (Supplemental file).

Results

Effects of ploidy changes on cell morphology and growth

In this study, isogenic diploid, triploid, and tetraploid yeast strains were generated by cycles of mating type switching and crossing (Fig. 1a). The ploidy of these constructed strains was confirmed by flow cytometry (Fig. S1 in Supplemental file) and aCGH microarray (data not shown). As in other organisms (Frawley and Orr-Weaver 2015), increased sets of chromosomes resulted in an increase in cell size (Fig. 1b). In addition, a greater aspect ratio was observed in polyploid strains than in haploid and diploid strains (Fig. 1b). Additionally, we observed that three connected cells composed of a mother, daughter, and granddaughter accounted for about 15% of triploid and tetraploid cells. This phenomenon might be caused by nuclear and spindle cycles initiated prior to bud emergence (Harrison et al. 2014). When grown in normal YPD medium, biomass formation at stationary phase (at the time point of 48 h) decreased with the increase of ploidy (Fig. 1c, d). The dry weight of the tetraploid cells was only 69% that of the haploid cells (Fig. 1c; $p < 0.01$, t test). The number of haploid cells was 12, 41, and 64% higher than those of the diploids, triploids, and tetraploids, respectively (Fig. 1d; $p < 0.01$, t test).

Tolerance to various stresses among euploid strains

Stress tolerance is key for *S. cerevisiae* strains to perform to necessary commercial standards in industrial processes (Attfield 1997). In this study, the tolerance of the four euploid strains to various stressful conditions, including heat (42 °C), osmotic pressure (0.75 M NaCl), ethanol, weak acids (lactic acid, formic acid, and acetic acid), low pH, oxidative stress, furan derivatives, a phenolic compound (vanillin), metal ions (copper and arsenic), and the antifungal drug fluconazole, was determined. As shown in Fig. 2, we observed that the stress tolerance of these four euploid strains varied widely, especially under lactic acid, H_2O_2, and $NaAsO_2$ stress, and up to 10-fold differences existed between the least and most tolerant strains. Nevertheless, no single euploid strain was tolerant of all stressors. The haploid was the most tolerant strain under lactic acid and low pH, but it was very sensitive to furfural, vanillin, $NaAsO_2$, and H_2O_2 (Fig. 2). The diploid performed best in the presence of 8% ethanol, 0.75 g/L furfural, 1.5 g/L 5-HMF, and 1.5 mM $NaAsO_2$. The triploid showed significant advantages in tolerance to formic acid and osmotic pressure (0.75 NaCl) compared to the other euploid strains. The

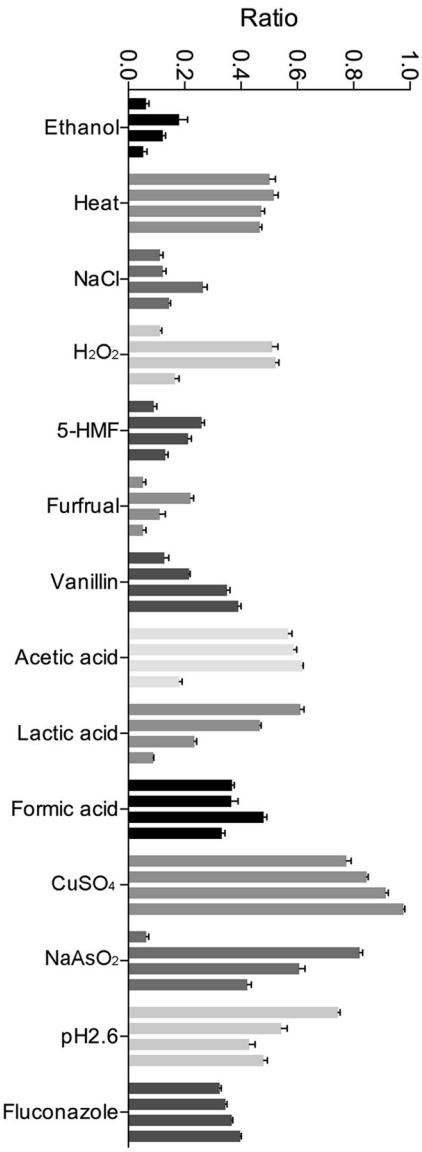

Fig. 2 Relative tolerance (expressed as the ratio of cell growth in a stressful condition to that in the normal condition) of four euploid strains to a range of stresses. For each stressor, the *bars* from top to bottom represent haploid, diploid, triploid, and tetraploid strains. Three independent experiments were performed in triplicate

tetraploid was sensitive to most stressors but was more tolerant of $CuSO_4$ than the other strains. The behavior of the four euploid strains in their tolerance to lactic acid, formic acid, and acetic acid was not consistent (Fig. 2). The triploid was more resistant to acetic acid and formic acid but was less tolerant of lactic acid than the diploid or haploid. This observation indicates that an individual weak organic acid would impose unique toxic effects on yeast cells.

Comparison of ethanol fermentation performance of euploid *S. cerevisiae* strains

Bioethanol produced from wood chips and agricultural residue by *S. cerevisiae* strains is one of the most promising renewable energy sources (Nielsen et al. 2013). During the pretreatment of lignocellulosic biomass (e.g., dilute-acid hydrolysis), numerous by-products, including weak acids, furans, and phenolic compounds, are formed, which would greatly inhibit the viability of yeast cells (Jönsson et al. 2013; Ravindran and Jaiswal 2016; Sindhu et al. 2016). Here, the ethanol fermentation period and ethanol production of the four euploid strains were compared in both normal fermentation medium (YPHD) and inhibitor-containing media (HA and HB). In the YPHD medium, the polyploid strains showed slower ethanol fermentation rates than the diploid and haploid (Fig. 3a). The final ethanol production of the diploid was slightly higher than that of the other euploid strains (Table 1; $p < 0.05$, t test). No significant differences in residual glucose were observed among strains (Table 1). In the presence of inhibitors, a longer lag phase was required for yeast strains to recover from the suppression of inhibitors (Fig. 3b, c). The triploid showed the shortest delay period among the strains in both HA and HB medium (Fig. 3b, c). Fermentation was completed within 42 h by the triploid, while a longer period was required for the other strains (Fig. 3b, c).

Modification of physiological and biochemical attributes among euploid strains

Rapidly adapting cellular activities, such as gene transcription and physiological status, is required for unicellular organisms, including yeast, to survive constant fluctuations in their external surroundings (Gasch and Werner-Washburne 2002). It has been widely recognized that the trehalose, ergosterol, and antioxidative systems are strongly associated with tolerance to multiple stresses in *S. cerevisiae* (Estruch 2000; Ma and Liu 2010; Morano et al. 2012). As shown below, the content or activity of these physiological attributes was measured in cells collected from both exponential and stationary phases of the four euploid strains.

Trehalose is a well-known protectant that preserves the integrity of the plasma membrane and stabilizes proteins in multiple organisms (Mahmud et al. 2010). Many studies have been performed to explore the correlation between trehalose content and tolerance to stresses such as heat (Eleutherio et al. 1993), desiccation (Tapia et al. 2015), saline stress (Mahmud et al. 2009), and ethanol stress (Bandara et al. 2009). In cells in the logarithmic stage, the intracellular trehalose content of the four strains was less than 1 mg/g dry weight (Fig. 4a). Consistent with our previous observation showing that trehalose accumulation was induced when cells entered the stationary phase (Zheng et al. 2011), the trehalose content of the four strains at 30 h increased by at least 10-fold over that at 10 h (Fig. 4a). At the stationary phase, trehalose content in the haploid strain reached 4.1 mg/g dry weight, which is 1.7-fold, 1.2-fold, and 2.7-fold of that in the diploid, triploid, and tetraploid, respectively (Fig. 4a; $p < 0.01$, t test). Interestingly, we observed that odd ploidy strains had higher trehalose content than even ploidy strains. It seems that not only the chromosome sets but also the parity of ploidy affects the regulation of trehalose metabolism.

Ergosterol is a major constituent of cellular membranes in yeast and plays a key role in maintaining membrane integrity (Lees et al. 1995). It has been proven that null mutation of

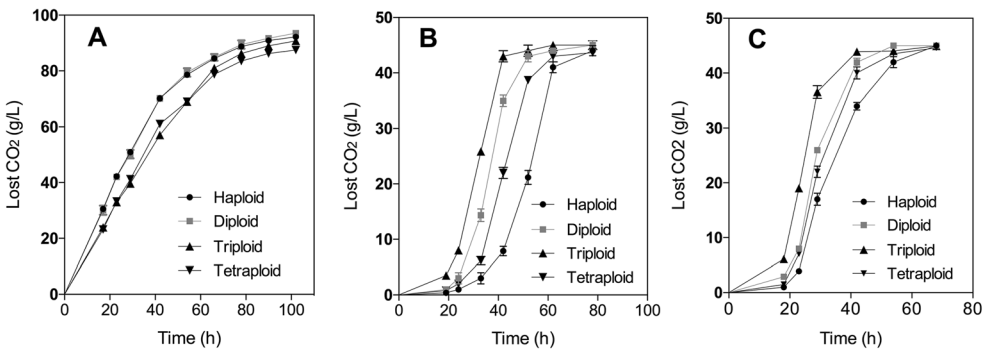

Fig. 3 Lost CO_2 weight of the four *S. cerevisiae* euploid strains during ethanol fermentation. Three fermentation media, **a** YPHD, **b** HA, and **c** HB, were used. The recipes for these media and fermentation parameters are described in the "Materials and methods" section. Three independent experiments were performed in triplicate and the data points represent means ± SD

Table 1 Comparison of ethanol fermentation performances of the four euploid strains in different fermentation mediums

Strains	YPHD		HA	HB
	Ye (g/L)[a]	RS (g/L)[b]	Ye (g/L)	Ye (g/L)
Haploid	81.04 ± 0.55	0.27 ± 0.03	44.10 ± 0.41	44.80 ± 0.56
Diploid	81.90 ± 0.42	0.25 ± 0.01	44.09 ± 0.33	45.01 ± 0.43
Triploid	81.23 ± 0.39	0.26 ± 0.02	44.13 ± 0.28	44.97 ± 0.48
Tetraploid	79.74 ± 0.42	0.28 ± 0.04	43.50 ± 0.42	44.48 ± 0.26

[a] Ye represents the production of ethanol

[b] RS represents the production of residual glucose

genes involved in the ergosterol biosynthesis pathway result in sensitivity to multiple stressors (Endo et al. 2008). Unlike trehalose, in yeast, higher ergosterol content was observed at the logarithmic phase than at the stationary phase (Fig. 4b). At 10 h, the strains with even ploidy showed higher ergosterol content than did the odd ploidy strains. The ergosterol content in the haploid and triploid was 16 and 21% lower, respectively, than that of the diploid (Fig. 4b; $p < 0.05$, t test). No significant difference was observed in ergosterol between the diploid and tetraploid. Ergosterol contents at 30 h among the strains were not significant (Fig. 4b).

Excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) causes oxidative stress (Jamieson 1998). ROS include free radicals such as superoxide ($\bullet O_2^-$) and hydroxyl (•OH), as well as the nonradical species H_2O_2. Under normal conditions, $\bullet O_2^-$ is quickly dismutated to H_2O_2 by superoxide dismutase. H_2O_2 is converted to H_2O and O_2 by GSH peroxidase or catalase, respectively. The activities of SOD and CAT varied substantially among the four euploid strains (Fig. 4c, d). At 10 h, SOD activity decreased with the increase of ploidy, except in the tetraploid (Fig. 4c). The SOD activity in the haploid was 1.9-fold, 3.6-fold, and 1.4-fold of that in the diploid, triploid, and tetraploid, respectively. At 30 h, the ranking of SOD activity observed among the strains had reversed, and the triploid had the highest SOD activity (Fig. 4c). At the logarithmic growth phase, the tetraploid showed the highest CAT activity, which was 15.5-fold, 1.4-fold, and 2.4-fold of that of the haploid, diploid, and triploid, respectively (Fig. 4d). The triploid exceeded the tetraploid at stationary phase and showed the highest CAT activity (Fig. 4d). The extremely low activity of CAT in the haploid agreed well with its lower tolerance than the other strains to H_2O_2 (Fig. 2). In Fig. 4e, no significant differences were observed in GSH among the strains at 10 h. At 30 h, the diploid had the highest intracellular content of GSH (27 μmol/g dry weight), which was 28, 20, and 15% higher than that in the haploid, triploid, and tetraploid, respectively (Fig. 4e; $p < 0.05$, t test).

Transcription analysis of genes associated with cell morphology and stress tolerance

In yeast, cell size is associated with the abundance of G_1 cyclins (encoded by *CLN1* and *PCL1*) (Hadwiger et al. 1989). Higher expression of the G_1 cyclins results in faster phase transition and smaller cell size. We found that the expression of *CLN1* was about twofold higher in the haploid than in the other euploid strains (Fig. 5; $p < 0.05$ t test). The diploid strain had the highest expression activity of *PCL1*, which was 1.3-fold, 2.3-fold, and 1.7-fold of that in the

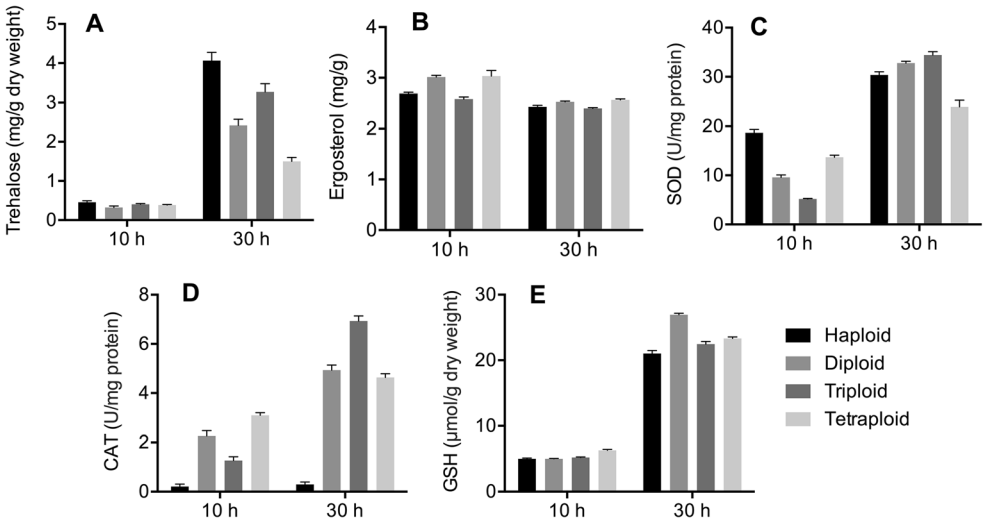

Fig. 4 Effects of ploidy changes on physiological attributes among euploid strains. **a** Trehalose content. **b** Ergosterol. **c** Activity of total superoxide dismutase (SOD). **d** Catalase (CAT) activity. **e** Total glutathione. Yeast cells were cultured in 25 mL YPD medium and collected for physiological analysis at the time points of 10 and 30 h. *Bars* represent means ± SD of three independent experiments were performed in triplicate

haploid, triploid, and tetraploid, respectively. In addition to increased cell volume, polyploid cells showed greater elongation (Fig. 1b). This trend might be caused by the regression of the Gic2 protein, which interacts with the Rho-type guanosine triphosphatase Cdc42 and controls actin organization and polarized cell growth (Galitski et al. 1999). Our results showed that the expression of *GIC2* was repressed gradually with the increase of ploidy (Fig. 5). Previous comparison of gene expression in haploids and tetraploids showed that *CTS1* (encodes a secreted endochitinase involved in the separation of mother cells and daughter buds) was elevated by a factor of 12 and that *FLO11* (encodes a GPI-anchored cell surface glycoprotein that is required for invasive growth and flocculation) was repressed by a factor of 11 (Galitski et al. 1999). Similar trends in the expression changes of these two genes were observed between our haploid and tetraploid, although the magnitude of the variation differed (Fig. 5).

Trehalose is synthesized by a large enzyme complex comprising four subunits (encoded by *TPS1*, *TPS2*, *TPS3*, and *TSL1*) that uses UDP-glucose as substrate (Bell et al. 1998). The conversion of glucose to UDP-glucose is catalyzed by hexokinase isoenzyme (*HXK1*), phosphoglucomutase (*PGM2*), and UDP-glucose pyrophosphorylase (*UGP1*). Trehalose is hydrolyzed into glucose by neutral trehalases encoded by *NTH1* and *NTH2* or by a vacuolar acid trehalase encoded by *ATH1* (Garre and Matallana 2009). The expression activities of these genes in the cells collected at 30 h are shown in Fig. 6a. All the genes involved in trehalose metabolism showed higher expression levels in the haploid and triploid than in the diploid (Fig. 6a). For example, the gene *TPS1* was upregulated by 2.8-fold and 2.2-fold in the haploid and triploid compared to the diploid (Fig. 6a; $p < 0.05$, t test). In contrast, all these genes except *UGP1* showed 10–30% lower expression activity in the tetraploid than the diploid (Fig. 6a; $p < 0.05$, t test).

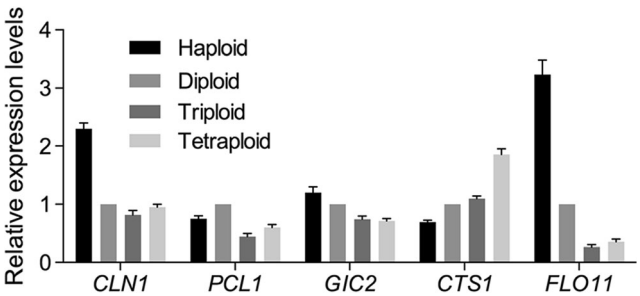

Fig. 5 qRT-PCR reveals different expression patterns of genes controlling the cell cycle and morphology among the four euploid strains. Total RNA was extracted from three independent cultures (at 10 h) and qRT-PCR experiments were performed in triplicate for each culture. The expression levels of genes in the diploid were normalized to 1

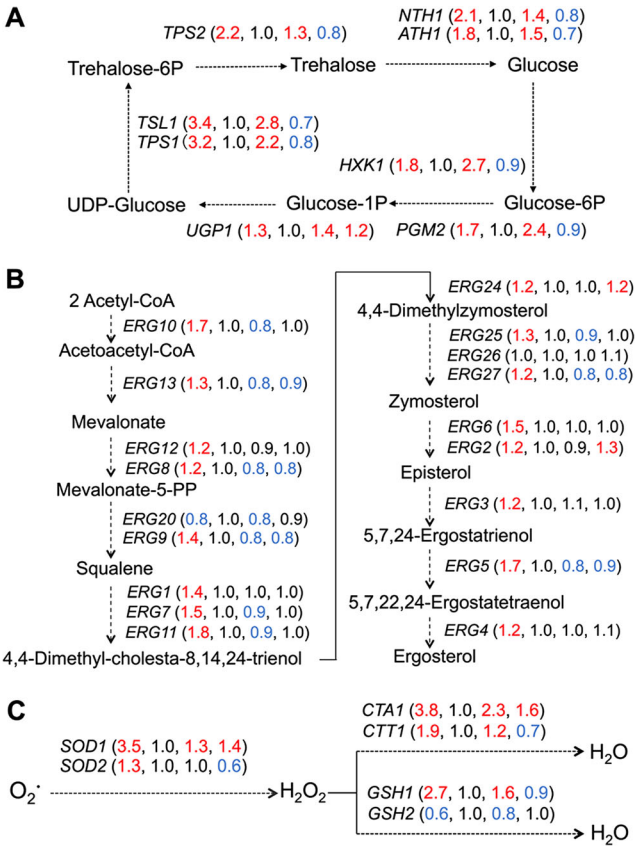

Fig. 6 Various expression levels of genes involved in **a** trehalose metabolism, **b** the ergosterol biosynthesis pathway, and **c** the antioxidative system. Total RNA was extracted from three independent cultures (at 30 h) and qRT-PCR experiments were performed in triplicate for each culture. The expression levels of genes in the diploid were set to 1. The *four values in a set of brackets* show the relative expression of a certain gene in the haploid, diploid, triploid, and tetraploid. The *values marked in red or blue* indicate that the gene was upregulated or downregulated in this strain, respectively, relative to the diploid strain ($p < 0.05$, t test)

The chemical reactions and pathway resulting in the formation of ergosterol in *S. cerevisiae* are shown in Fig. 6b. A total of 18 genes involved in this pathway were determined by qRT-PCR in cells in the logarithmic phase. All these genes except *ERG20* and *ERG26* showed higher expression in the haploid than in the diploid (Fig. 6b; $p < 0.05$, t test), although the ergosterol content in the haploid strain was 11% lower than in the diploid strain (Fig. 4b). No significant or very small differences in the expressions of these 18 genes were observed among the four strains (Fig. 4b).

The gene *SOD1* encodes a Cu-Zn superoxide dismutase, while *SOD2* encodes a manganese-superoxide dismutase that is localized to the mitochondrial matrix in yeast cells. Two yeast catalases, catalase A and catalase T, are encoded by the genes *CTA1* and *CTT1*, respectively. *GSH1* encodes gamma-glutamylcysteine synthetase, which catalyzes the first step in GSH biosynthesis. Glutathione synthetase (encoded by *GSH2*) catalyzes the ATP-dependent synthesis of GSH from

gamma-glutamylcysteine and glycine. The relative expression levels of these genes in the four euploid strains are shown in Fig. 6c. The haploid showed the highest expression levels of *SOD1*, *SOD2*, *CTA1*, and *CTT1*, which were 3.5-fold, 1.3-fold, 3.8-fold, and 1.9-fold that of the diploid strain, respectively (Fig. 6c). This finding was unexpected because the activities of SOD and CAT were lower in the haploid than in the other strains (Fig. 4). The triploid showed higher expression of the genes *SOD1*, *CTA1*, *CTT1*, and *GSH1* but lower expression of *GSH2* than the diploid (Fig. 6c). The expression of *SOD1* and *CTA1* was upregulated by 1.4-fold and 1.6-fold, respectively, in the tetraploid, while *SOD2* and *CTT1* were downregulated by 40 and 30% in this strain compared to the diploid (Fig. 6c; $p < 0.05$, *t* test).

Discussion

In this study, comparative analyses of four euploid *S. cerevisiae* strains were performed to investigate how ploidy increment affects tolerance and ethanol fermentation performance under various conditions. Additionally, certain physiological and transcriptional reprograms associated with ploidy modification were characterized. Below, we discuss the implications of our main findings.

Using cycles of mating type switching and crossing (Fig. 1a), euploid *S. cerevisiae* strains varying from haploid to tetraploid were constructed. Although two types of haploid (*MAT***a** and *MAT*α) and triploid (*MAT***a**/*MAT***a**/*MAT*α and *MAT*α/*MAT*α/*MAT***a**) strains were available in this study, only one type each of these odd ploidy strains was used for analyses because the opposite mating types imposed little effect on the phenotypes and physiological status determined (data not shown). As previously reported (Galitski et al. 1999), ploidy increase did result in increased cell volume and aspect ratio (Fig. 1b). qRT-PCR confirmed that the cyclin-encoding genes (*CLN1* and *PCL1*) whose expression levels are negatively correlated with the length of the G_1 phase were repressed in polyploids (Fig. 5). Meanwhile, the higher expression activity of the gene *GIC2* in polyploids (Fig. 5) might contribute to cell elongation. In some cases, mature daughter cells were not separated from their polyploid mother cells, resulting in a high ratio of three connected cells composed of a mother, daughter, and granddaughter bud (Fig. 1b). This observation indicates that polyploidy would lead to deviation from normal cell cycle regulation. Such an obstacle to cell separation might reasonably be the higher expression of the endochitinase-encoding gene *CTS1* in polyploid strains (Fig. 5).

Previous studies focused on the mechanisms involved in sensing stress, the signaling pathways, and the resulting compensatory changes in physiology have greatly enriched our understanding of how yeast cells adapt and survive under non-ideal conditions (Attfield 1997; Gibson et al. 2007; Morano et al. 2012). In this work, our results suggest that ploidy changes would greatly affect the patterns of cellular responses and tolerance (Fig. 2). To be sure, no single euploid strain could be tolerant of all stressors, and each strain had its advantages and disadvantages. Tolerance to certain stressors (i.e., vanillin, $CuSO_4$, and fluconazole) was increased in polyploids, while tolerance to lactic acid and low pH was repressed as ploidy increased (Fig. 2). These observations suggest that ploidy modification could allow yeast cells to better adapt rapidly to changing circumstances. As expected, these euploid strains also showed differences in ethanol fermentation performance (Fig. 3). The diploid strain was the best performing, with highest ethanol yield and fermentation rate in YPHD medium (Fig. 3a). In the presence of inhibitors (i.e., acetic acid, formic acid, 5-HMF, furfural, and vanillin), the triploid showed the fastest fermentation rates (Fig. 3b, c), although this strain was not most tolerant of any single stressor (Fig. 2). This result indicates the potential application of triploids for bioethanol production using cellulosic feedstock. It should be noted that chromosome loss occurred at a higher frequency in polyploid strains than in haploid and diploid strains under normal conditions (Storchova 2014). Certain selection pressures indicated by this work may contribute to the stability of polyploidy strains during cell passages.

Exposure of yeast cells to low levels of stress often triggers an adaptive response that would result in improved resistance to higher levels of the same stress or to other types of stress (Berry and Gasch 2008; Carmelo et al. 1998). This phenomenon suggests that the proper physiological status of yeast cells is critical to tolerating impending stress. In Fig. 4, we observed different contents of trehalose, ergosterol, and antioxidative factors among the euploid strains. On the one hand, the physiological attributes that varied among the strains were growth phase-dependent. For example, the triploid strain had the lowest SOD activity at the logarithmic phase, while it showed highest activity of this enzyme at the stationary phase (Fig. 4). This result demonstrated that ploidy changes influence the flexibility of physiological adjustments in response to environmental stimuli. On the other hand, the parity of ploidy would also contribute to the diverse physiological status among the four euploid strains. It was observed that strains of odd ploidy had higher trehalose content and lower ergosterol content than strains of even ploidy (Fig. 4). Compared to other euploid strains, the haploid showed significantly lower activity of SOD and CAT (Fig. 4). This trait might at least partly explain the limited application of haploid yeast strains in industry, given that these two enzymes are necessary for resistance to multiple environmental stimuli, such as heat, ethanol, and osmotic pressure (Morano et al. 2012; Zyrina et al. 2016), but the precise nature of this phenomenon needs further investigation.

Using qRT-PCR, the expression of genes involved in trehalose and ergosterol metabolism and of genes encoding anti-

oxidative factors was determined (Fig. 6). In response to stressors such as heat and ethanol, yeast cells would accumulate trehalose, although both the genes encoding trehalose-6-phosphate synthase/phosphatase and trehalase would be upregulated (Mahmud et al. 2010). Our observations showed that all the genes involved in trehalose metabolism were upregulated in the haploid and triploid strains compared to the diploid strain, but to different degrees (Fig. 6a). The extent of upregulation of the genes *TPS1*, *TPS2*, and *TLS1* was greater than that of *ATH1*, *NTH1*, and *NTH2* (Fig. 6a) and resulted in higher trehalose contents in the haploid and triploid strains. Much to our surprise, unlike the other euploid strains, the relationship between gene expression and certain physiological attributes in the haploid could not be explained in a simple way. In contrast to the lower ergosterol content and activities of SOD and CAT in the haploid than in the diploid (Fig. 4), the genes involved in these pathways were all significantly upregulated in the haploid (Fig. 6b, c). This conflicting result might imply a specific mechanism of posttranscriptional regulation in haploid strains. Despite being involved in the same pathway, the expression patterns of *GSH1* and *GSH2* were not similar (Fig. 6c). We assumed that the lower expression of *GSH2* might be the main cause of the lower GSH in the triploid and haploid than in the diploid.

To the best of our knowledge, this work is the first to characterize the effects of ploidy at the levels of both phenotypes and physiologies that are highly desired for industrial applications. Our results not only provide novel insight into how ploidy changes affect the physiology and phenotypes of yeast cells but also have significance as a reference for strain construction for specific industrial fields.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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