Ploidy evolution in the yeast *Saccharomyces cerevisiae*: a test of the nutrient limitation hypothesis

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Abstract

The nutrient limitation hypothesis provides a nongenetic explanation for the evolution of life cycles that retain both haploid and diploid phases: differences in nutrient requirements and uptake allow haploids to override the potential genetic advantages provided by diploidy under certain nutrient limiting conditions. The relative fitness of an isogenic series of haploid, diploid and tetraploid yeast cells (*Saccharomyces cerevisiae*), which were also equivalent at the mating type locus, was measured. Fitness was measured both by growth rate against a common competitor and by intrinsic growth rate in isolated cultures, under four environmental conditions: (1) rich medium (YPD) at the preferred growth temperature (30 °C); (2) nutrient poor medium (MM) at 30 °C; (3) YPD at a nonpreferred temperature (37 °C); and (4) MM at 37 °C. In contrast to the predictions of the nutrient limitation hypothesis, haploids grew significantly faster than diploids under nutrient rich conditions, but there were no apparent differences between them when fitness was determined by relative competitive ability. In addition, temperature affected the relative growth of haploids and diploids, with haploids growing proportionately faster at higher temperatures. Tetraploids performed very poorly under all conditions compared. Cell geometric parameters were not consistent predictors of fitness under the conditions measured.

Introduction

There have been many hypotheses proposed to explain the trend that most 'higher' plants and animals are characterized by a predominance of the diplophase. However, there are many organisms that maintain both haploid and diploid phases in their life cycles, with time spent in each varying greatly across taxa. Whereas many of the genetically based arguments have concentrated on the increased capacity for genetic buffering and potential for adaptation to novel environmental situations in diploids, other hypotheses have been proposed to explain conditions under which the haplophase might be favoured (see review by Mable & Otto, 1998). The haplophase permits stronger expression and selection of new mutants and thus the potential for early adaptation to changes in the environment, whereas the diplophase provides a stable phenotype because the expression of deleterious recessive genes is masked and the potential exists for heterosis (Fowell, 1969).

One of the most popular nongenetic arguments proposed to explain diversity in the relative length of haploid and diploid phases is the nutrient limitation or nutrient sparing hypothesis (Lewis, 1985). Haploid cells are often smaller than diploid cells (Weiss *et al.*, 1975; Cavalier-Smith, 1978) and thus have a higher surface area to volume ratio. As the ability to transport nutrients across the cell membrane depends on surface area, this increased ratio may lead to improved growth rate or survival, especially under nutrient limited conditions. Furthermore, the energetic costs of DNA replication would be halved in haploid cells. Haploids should thus have an advantage under nutrient limited conditions whereas diploids might be expected to perform the same

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or better than haploids under nutrient rich conditions. In an experiment comparing growth rates of juvenile isomorphic haploid and diploid phases of the red alga, *Gracilaria verrucosa*, Destombe *et al*. (1993) found that haploids had a growth advantage in nonsupplemented seawater (i.e. nutrient poor conditions) whereas diploids had an advantage in enriched seawater (i.e. nutrient rich conditions), precisely as predicted by the nutrient limitation hypothesis.

For single-celled organisms, these arguments rely on a tight correlation between cell size and ploidy level. Weiss *et al*. (1975) found that diploid yeast cells had 1.57 times the volume of haploid cells in minimal medium, whereas under carbon limitation cell sizes were not different. This suggests that cell size can be altered independently of ploidy in response to different environmental conditions, complicating the nutrient limitation hypothesis. They also compared the relative quantity and activity of certain cellular components in haploids and diploids grown under nonlimiting and carbon-limiting nutrient conditions and found that DNA content was the only factor that was closely related to ploidy level under all nutrient conditions. They concluded that the basic biochemical parameters of the cell are determined primarily by cell geometry rather than by ploidy level and predicted that if fitness were determined by cell surface components, diploids would be expected to have lower fitness than haploids because of lower surface area/volume ratios, but if fitness were determined by internal enzyme concentrations, there should not be a fitness difference.

The yeast, *Saccharomyces cerevisiae,* has been used in a number of independent experiments designed to compare fitness of haploids and diploids under varied nutrient conditions, but support for the nutrient limitation hypothesis has been mixed. Adams & Hansche (1974) compared growth rates in asexual haploid and diploid strains maintained in a chemostat. In contrast to the widely held belief among yeast researchers that diploids always tend to outgrow haploids (Roman *et al*., 1955; Fowell, 1969), they concluded that, under nutrient rich conditions, diploids were just 'double haploids' and did not have an intrinsic growth advantage compared with haploids. In agreement with the conclusions of Weiss *et al*. (1975), no differences in fitness were apparent under low nutrient levels but when growth in the chemostat was limited by organic phosphate availability, haploid cells grew more rapidly than diploids. In contrast, using 'quasi-continuous' cultivation of mixed cultures of isogenic haploids and diploids, Glazunov *et al*. (1989) found that diploids outcompeted haploids in rich medium at the preferred growth temperature (30 °C), whereas haploid cells had an advantage in minimal medium and in the presence of a competitor (the yeast, *Pichia pinus*). When the same experiment was repeated using different strains, however, diploids completely displaced haploids under all the conditions compared, except when mitotic recombination was reduced by the addition of potassium chloride to the growth medium (Naidkhardt & Glazunov, 1991). These contrasting results suggest that the particular experimental conditions or the particular yeast strains used influence the outcome of relative competition experiments.

Temina *et al*. (1979) compared the growth rates, biomass accumulation, and electrophoretic spectra of mobile cytoplasmic proteins in nonisogenic haploid and diploid cultures of *S. cerevisiae* with those of isogenic haploid–diploid pairs. On a standard glucose medium (i.e. rich medium), differences in these parameters were found to be because of the genotype of a strain rather than the ploidy level: nonisogenic haploid and diploid cultures displayed considerable variability in these properties whereas no differences were found for isogenic haploid–diploid pairs. Differences in conclusions among researchers could be explained by differences in the types of strains they compared and/or in the way that fitness was evaluated. Adams & Hansche (1974), Glazunov *et al*. (1989) and Naidkhardt & Glazunov (1991) used haploids and diploids that were isogenic (except at the mating type locus), but the former group compared relative fitness of each strain separately against a common competitor, whereas the latter group established the proportion of haploids and diploids growing in mixed cultures via halo assays using mating-type sensitive strains (Sprague, 1991). In addition, heterozygosity at the mating type locus is known to have pleiotropic effects on fitness (Durand *et al*., 1993), which could influence the results of competition experiments comparing haploids and diploids of standard strains.

The goal of this paper was to re-evaluate the nutrient limitation hypothesis in view of the contrasting results of previous researchers and to assess how cell geometric changes associated with combinations of temperature and nutrient levels influence the outcome of relative fitness tests. A set of experiments was performed comparing the relative fitness of an isogenic series of haploid, diploid and tetraploid *S. cerevisiae* that lacked functional mating type loci. These strains not only share the same genetic background but are also functionally equivalent at the MAT locus. Relative fitness was compared by using competition experiments against a common competitor and by estimating intrinsic growth rates of strains grown in isolation under four conditions: (1) in rich medium (YPD) at the preferred growth temperature (30 °C); (2) in minimal medium (MM) at 30 °C; (3) in YPD at a nonpreferred temperature (37 °C) and (4) in MM at 37 °C.

My results show that haploids, if anything, tended to grow faster than diploids under nutrient rich conditions, but there were no apparent differences between them when fitness was determined by relative competitive ability, in contrast to the predictions of the nutrient-limitation hypothesis. In addition, temperature affected the relative growth of haploids and diploids, with haploids having bigger cells and growing proportionately faster at higher temperatures. Tetraploids performed very

poorly compared with both haploids and diploids under all conditions examined.

Materials and methods

Yeast strains

A series of isogenic strains with different ploidy levels from haploidy to tetraploidy (developed by Drs A. Adams and S. Brouwer, personal communication) were used in the experiment. Not only do these strains share the same genetic background, but they have a mutation at the mating type locus (MAT) and a deletion in the pheromone receptor locus (STE6). This has the double advantage – mating within the strains should be impossible and fitness differences in different strains because pleiotropic effects of the mating type should be eliminated. The original haploid strains (AA1526 and AA1569), derived from the strain SM2185, carried one of two plasmids (psm 620 and AAB284) with genotypes CEN6 STE6 URA3 MATα and CEN6 STE6 LEU2 MATa, respectively. By successive rounds of mating and selecting for plasmid loss, a ploidy series from haploid through tetraploid was constructed (A. Adams and S. Brouwer, personal communication). The ploidy of each of the starting strains was confirmed by FACScan analysis of DNA content (Sazer & Sherwood, 1990). I grew these strains (from frozen stocks) in overnight cultures in 5 mL YPD and then replica plated them onto YPD, -ura, and -leu plates to screen for plasmid loss. For each ploidy level, a single colony lacking both plasmids was used to establish the cultures used in the experiments. These will be referred to as BM1N, BM2N and BM4N for isogenic haploid, diploid and tetraploid auxotrophic strains, respectively. Except for changes that might have occurred in the rounds of growth following their creation (approximately 12 doublings in liquid medium followed by growth on plates for 48 h prior to storage at 4 °C), these strains should have been genetically identical. Their shared haplotype, MATa a1-ste6Δ8–694 leu2 trp1 ura3 his4 can1, indicates that they carry (1) a mutation that makes the a1 locus (which is involved in expression of the MATa phenotype) nonfunctional and a deletion between bp's 8 and 694 that makes the ste6 gene (which is involved in pheromone production essential for mating) nonfunctional – together, these mutations make mating impossible in these strains; (2)–(5) mutations that make the strains unable to synthesize their own leucine, tryptophan, uracil, or histidine (-his) and (6) a mutation that made the strains susceptible to canamycin (can1). Cultures were grown overnight in YPD liquid medium at 30 °C (approximately 12 replications prior to stationary phase) and then plated on solid YPD and stored at 4 °C until required.

The experiment was replicated using two different competitors: (1) the prototrophic strain C276 (diploid competitor, MATa/α) and (2) a haploid segregant of this strain, C41 (haploid competitor, MATa, obtained from Brian Haarer; strains described in Wilkinson & Pringle, 1974). Twelve single colonies from the strains BM1N, BM2N and BM4N were picked with a toothpick and diluted in 1 mL ddH_2O. A single colony from each of the competitor strains (haploid, diploid) was also picked and diluted in 1 mL ddH_2O. All samples were then standardized to a density of 2.25×10^6 cells mL^{-1}, which was the density of the least dense strain (based on haemacytometer counts, as described below). This set of 'strains' was used in all of the experiments described below. Initial starting density was thus estimated only once. The strains were stored at 4 °C for a maximum of 2 months; all were stored for the same period of time under the same conditions.

Media and growth conditions

Difco YPD solid and liquid media, prepared according to the package instructions, were used for high nutrient conditions. These media consist of 1% bacto-yeast extract with a complete complement of amino acids, 2% bacto-tryptone, 2% dextrose and 2% bacto-agar (for solid medium). MM was prepared using 0.17% yeast nitrogen base without amino acids and 2% dextrose, with 2% bacto-agar added for solid medium. Essential amino acids were added in the following concentrations: adenine sulphate, uracil, L-histidine and L-tryptophan at 0.0004%, L-leucine at 0.0024%, and L-lysine at 0.0012%. Selective omission plates were made using synthetic complete medium (SC: 0.67% yeast nitrogen base without amino acids, 2% dextrose and 2% bacto-agar) with amino acids added in the same concentrations as for MM except for the omitted amino acid for -his plates. YPD and MM differ mainly in the concentration of nutrients and nonessential amino acids and they both have all essential nutrients for growth. YPD may have additional nutrients provided by tryptone. Comparing growth rates in these media tests the most basic assumption of the nutrient limitation hypothesis: when nutrients are present in low quantities, haploids should have a fitness advantage. We compared the effects of growth under four different sets of conditions: YPD at 30 °C (rich medium, preferred temperature), YPD at 37 °C (rich medium, nonpreferred temperature); MM at 30 °C (nutrient poor medium, preferred temperature) and MM at 37 °C (nutrient poor medium, nonpreferred temperature).

Cell size and shape

Cell volumes, eccentricities and surface areas for the first five 'strains' from each ploidy level were calculated. The length (L) and width (W) of five randomly chosen individual (nonbudding) yeast cells were measured on photographs(Carter & Sudbery, 1990; magnification of 400×) from 48 h samples grown in isolation under each of the nutrient and temperature combinations. Surface area to volume ratios (SA/V) were also computed. As the

cells were roughly prolate spheroids, volume was calculated using the formula

$$V = \frac{4}{3}\pi\left(\frac{L}{2}\right)\left(\frac{W}{2}\right)^2 \quad (1)$$

eccentricity

$$E = \sqrt{1 - \frac{W^2}{L^2}} \quad (2)$$

and surface area

$$SA = 2\pi W(L + (\text{Warcsin}[e]/e)) \quad (3)$$

where $e = [\sqrt{(L^2 - W^2)}/L]$.

Growth rate and stationary phase density

Single strain cultures were grown under each of the treatment conditions to provide estimates of intrinsic growth rate and stationary phase density. Three 'strains' from each ploidy level were grown contemporaneously in 5 mL cultures using 50 μL of the standardized dilutions (i.e. starting at a density of 2.25×10^4 cells mL^{-1}). Samples were taken at 12, 24 and 48 h because previous growth curve experiments had indicated that estimating growth between 12 and 0 h provided a consistent fitness measure whereas sampling at 2 h intervals and fitting a logistic growth curve did not change the relative fitness conclusions (data not shown). These growth rates were estimated at the end of the competition experiments (i.e. after the cells had been stored for 2 months). For YPD and MM cultures at 30 °C, these values were compared with growth rates estimated on the original cultures (i.e. that had not been stored in the fridge) to determine if storage had an influence on fitness (Table 1). The 24 and 48 h samples were taken to estimate stationary phase density of the strains under each set of conditions.

Cell densities were determined based on haemacytometer counts. Samples were vortexed vigorously prior to sampling to reduce problems with clumping. This method was chosen over estimates of density from spectrophotometer readings because it was found that the correspondence between cell density and optical density changed over the growth period, possibly because of the accumulation of waste products and cellular debris from dying cells (data not shown). For the haemacytometer counts, the number of cells in five blocks (each holding 0.004 μL) was determined, and cell densities were estimated based on the average. Cell densities below five cells per five blocks (i.e. below 2.25×10^5 cells mL^{-1}) were considered below the detectable limit of the haemacytometer and were not included in the results.

Intrinsic growth rates were estimated from a rearrangement of the logistic growth equation, (see Renshaw 1991):

$$r = \frac{1}{t}\log_e\left[\frac{N_t}{N_0}\left(\frac{K - N_0}{K - N_t}\right)\right] \quad (4)$$

where N_0 is the number of cells at time 0, N_t is the number of cells at time t, r is the intrinsic rate of growth when the number of cells is low, and K is the number of cells maintained during stationary phase. Stationary phase density (K) was estimated from the 48 h samples. Growth rates were estimated by comparing cell densities between 0 and 12 h because previous growth curves suggested that cells were unlikely to have reached stationary phase density at this point of time.

Competition experiments

An initial pilot study comparing relative competitive ability of haploids and diploids in MM and YPD at 30 °C (based on three replicates per treatment per ploidy level and three plates per replicate, in competition against a haploid prototrophic competitor) was used to design the competition experiments. In the pilot study, diploids appeared to perform better than haploids in YPD but there was no apparent difference in MM. A power analysis indicated that six replicates would be sufficient to detect the difference in YPD at a significance level of 0.05. Based on this information, for each competitor, 12 replicates per ploidy level per treatment were run contemporaneously on two separate days (2 weeks apart). Mixed cultures were started by adding equal volumes (25 μL = 5.6×10^4 cells) of the competitor and test strains to 5 mL of either YPD or MM in test tubes (i.e. starting density of 1.125×10^4 cells mL^{-1} per strain). These cultures were maintained for 48 h at either 30 °C (the preferred temperature) or 37 °C (the nonpreferred temperature) with continuous shaking at 200 r.p.m. This time period was chosen for the competitions to allow cultures to reach their maximum density. After 48 h, 100 μL samples were taken from each of the competition tubes and cell density was roughly estimated using a haemacytometer. Samples were diluted to an appropriate density (i.e. 10^{-5} for YPD treatments and 10^{-4} for MM treatments), to result in approximately 100 colonies per plate. Fifty microlitres of each dilution was plated onto SC medium (on which all strains should grow) and onto SC medium lacking histidine (-his; on which only the competitor strains should grow) and grown at 30 °C. Relative competitive ability of the test strains (i.e. fitness = % test strain) was assessed by counting the number of colonies on SC plates compared with that on -his plates. A single pair of plates was compared for each of the competitions rather than comparing multiple plates per competition to maximize the number of true replicates compared because the pilot study indicated that variation among plates was trivial compared with variation among replicates (data not shown). Final analyses were based on 10 replicates because some cultures were contaminated after the competition cycle. For those treatments that had no contaminants, two contemporaneous replicates were chosen at random to be excluded from the analysis to allow balanced statistical comparisons.

To reflect differences in reproduction and survival during all phases (lag, growth and stationary) of batch culture regimes, Lenski *et al.* (1991) calculated the relative fitness of two genotypes based on the ratio of their growth rates ($m = \ln(N_t/N_0)/t$) during competition for the same pool of nutrients. Based on their formula, in this experiment, per generation fitness of test strains ($m1$) relative to competitor strains ($m2$) can be defined as:

$$\frac{m1}{m2} = \frac{\ln(N_t/N_0)}{\ln(N_t^*/N_0^*)} \quad (5)$$

where N_t is the final density (number of cells mL^{-1}) of the test strain, N_0 is the initial density of the test strain, N_t^* is the final density of the competitor strain, and N_0^* is the initial density of the test strain. Estimates of starting density were based on the standardized dilution estimates. Estimates of final density were approximated by multiplying the number of cells counted on the relevant plates (i.e. for competitors, number of cells on -his plates and for test strains, number of cells on YPD plates minus number of cells on -his plates) by the dilution performed prior to plating. Previous studies have indicated that growth on plates is not always correlated with growth in liquid cultures (G. Bell, personal communication). I was therefore originally concerned that differences among strains or among treatments in the proportion of cells growing on plates, relative to the number counted in liquid cultures, might bias the results. To evaluate this possibility, the relationship between cell density and colony forming units on plates (cfu) was estimated for growth on YPD plates at 30 °C (based both on cultures grown in isolation and on cultures grown in competition). For strains grown in YPD, an average of 38.5% of the plated cells produced colonies (SE = 0.9%). For strains grown in MM, this average was lower (16.4%), but the variance was much higher (SE = 2.4%). Based on this information, I estimated relative growth rates both with and without correcting for reduced growth on plates (dividing N_t by 0.38 for cultures grown in YPD and by 0.16 for cultures grown in MM).

Statistics

For each set of comparisons, an initial full model analysis of variance (ANOVA) was conducted by fitting a general linear model (GLM) to the data using temperature, medium and ploidy as main effects. For the competition experiments, the effects of day were also investigated to determine if subtle differences in media or local temperature variation might influence the results. Significance values in GLMs were adjusted for multiple comparisons using Bonferroni corrections. Independent contrasts were used to test the null hypothesis that there were no differences between haploids and diploids under each combination of temperature and medium. Data were compared with a normal distribution by plotting histograms of the residual errors determined in GLM analyses and by using Shapiro–Wilk *W*-tests. Where necessary, data were transformed to improve the fit to normality, prior to final statistical analyses (see individual results). Correlation analyses were also performed among the various fitness estimates and the cell geometric measurements.

Results

Growth parameters in YPD and MM at 30 °C determined at the end of the experiment for haploids and diploids were not significantly different to those determined in a separate experiment performed when the cultures were first isolated (i.e. prior to storage at 4 °C). Tetraploids, on the other hand, grew significantly more slowly after storage (see Table 1), suggesting that they may have been adversely affected. Values for tetraploids are shown in the figures for general comparison to emphasize that they performed very poorly under all conditions compared with both haploids and diploids, but statistical significance is only reported for comparisons of haploids and diploids. Cell shape characteristics are given in Figs 1 and 2, and Fig. 3 provides a sketch of the average cell size and shape for each of the ploidy levels. Growth parameters estimated from single-strain cultures are given in Fig. 4. Results from the competition experiments are given in Fig. 5 (in terms of percentage test strain) and Table 2 (in terms of generation time). For each of the graphs, untransformed data are presented, even if statistics were calculated based on transformed data. A summary of the results of correlation analyses between the fitness parameters and the cell geometric measures is provided in Table 3. Table 4 provides a qualitative summary of the relative performance of haploids and diploids under each treatment for each of the parameters compared. From this table note that:

1 The volume of haploids and diploids is most similar in YPD at 37 °C.

2 Haploid cells become more similar in shape to diploid cells under each of the stressful conditions compared with the preferred growth conditions (YPD at 30 °C).

3 SA/V ratio of haploids is higher than diploids under all conditions, except in YPD at 37 °C, where the reverse was found.

Table 1 Comparison of growth rates of haploids, diploids and tetraploids in YPD at 30 °C before and after storage at 4 °C, as estimated between 0 and 12 h (using eqn 5).

	r	
Ploidy	Before	After
1n	0.46 ± 0.025	0.47 ± 0.012
2n	0.41 ± 0.015	0.40 ± 0.023
4n	0.35 ± 0.030	0.14 ± 0.038*

* Significant difference before vs. after storage, based on three replicates per comparison ($P < 0.0001$).

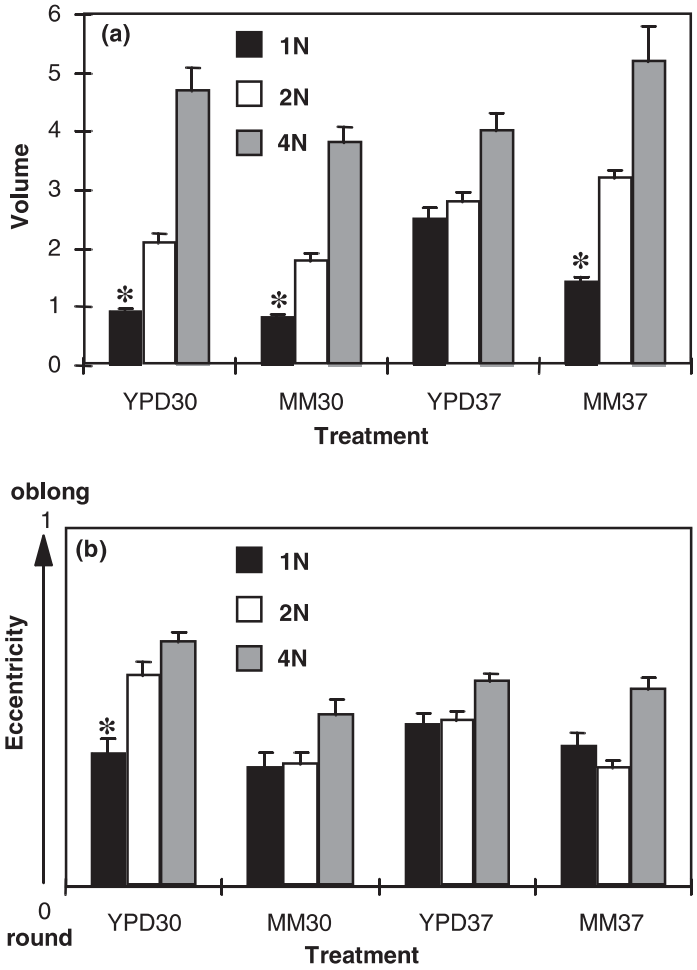

Fig. 1 (a) Volumes and (b) eccentricities of haploid, diploid and tetraploid strains grown under varying temperature and nutrient combinations. Bars indicate means of five replicates (untransformed data) with standard errors. Asterisks indicate significant differences between haploids and diploids within treatments (untransformed data for volumes, arcsin-transformed data for eccentricities; independent contrasts; $P < 0.0001$). (a) Units are in mm^3 as measured on photographs taken at 400× magnification (i.e. 1 mm^3 is equivalent to an actual cell volume of $(1/400)^3$ $mm^3 = 15.63$ μm^3). Note that volume differences among ploidy levels within treatments were significant except for haploid and diploid strains in YPD at 37 °C. (b) Eccentricity. Note that diploids were significantly more oblong than haploids only in YPD at 30 °C; under all other conditions haploids and diploids were more similar in shape.

4 Haploids and diploids have the most similar carrying capacities in MM at 30 °C.

5 Growth rates of haploids tend to be higher than diploids in YPD and growth rates are most similar in MM, whereas the nutrient limitation hypothesis predicts the reverse trend.

6 Haploids and diploids perform roughly equally against both competitors under the preferred growth conditions

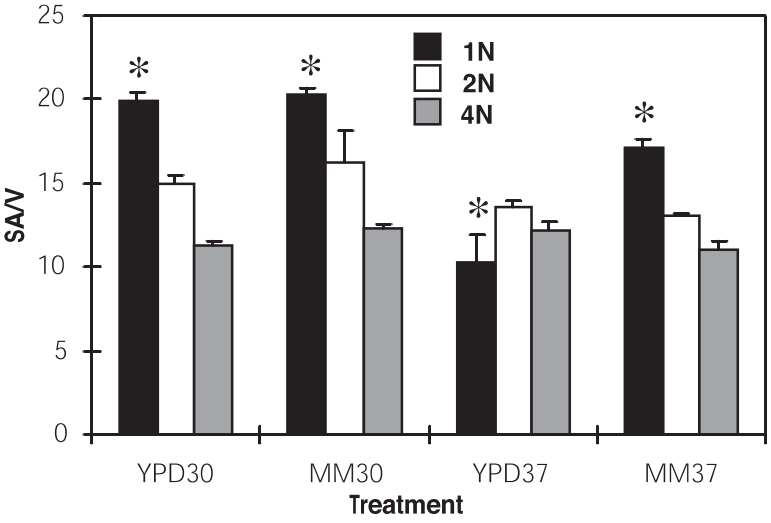

Fig. 2 SA/V ratios of isogenic haploid, diploid and tetraploid strains grown under varying temperature and nutrient combinations. Bars indicate means of five replicates (untransformed data) with standard errors. Asterisks indicate significant differences between haploids and diploids within treatments (untransformed data; independent contrasts; $P < 0.01$). Units are in mm^{-1} as measured on photographs taken at 400× magnification. Note that the SA/V ratio of haploids was larger than that of diploids under all conditions except in YPD at 37 °C, when the reverse was true.

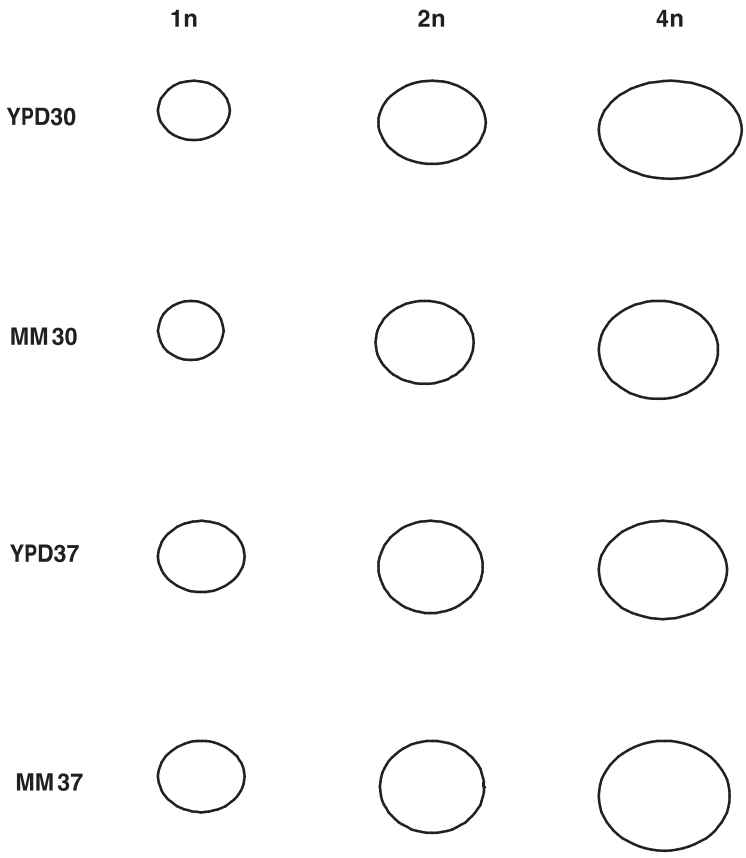

Fig. 3 Sketch of the average size and shape of haploids, diploids and tetraploids under each of the experimental conditions, drawn relative to one another.

(YPD at 30 °C). Under other conditions, whether haploids or diploid perform better depends on the ploidy level of the competitor (see statistical analysis section for details).

7 With either competitor, per generation fitness estimates suggest that haploids and diploids respond similarly to the varied temperature and nutrient conditions.

A brief summary of the statistical results for each analysis is given below.

Cell geometry

Diploids had a significantly larger volume than haploids (*ln*-transformed data; $P < 0.0001$) except in YPD at 37 °C, where the difference was not significant ($P = 0.17$; Figs 1a, 3); all main effects were significant ($P < 0.004$), as were all interactions ($P < 0.003$) except that between medium × temperature ($P = 0.6$).

In contrast, although there were significant effects of ploidy (arcsin-transformed data; $P = 0.05$; difference not significant after correcting for multiple comparisons) and medium ($P < 0.0001$), and significant interactions between ploidy × medium and ploidy × temperature ($P = 0.002$), eccentricity only differed significantly between haploids and diploids in YPD at 30 °C ($P < 0.0001$; Fig. 1b; haploids rounder than diploids).

In terms of surface area: volume ratio (SA/V), all main effects were significant (untransformed data; $P < 0.006$) and all interaction terms were significant except for medium × temperature ($P = 0.10$). The ploidy × medium interaction was not significant after correcting for multiple comparisons ($P = 0.02$). Independent contrasts indicated that haploids had a significantly higher SA/V ratio than diploids (untransformed data; $P < 0.005$; Fig. 3) except for YPD at 37 °C, when diploids had a significantly higher ratio than haploids ($P = 0.02$; difference not significant after correcting for multiple comparisons).

Intrinsic growth rate and stationary phase density

For growth of isolated cultures, there was a significant effect of temperature and of medium (*ln*-transformed data; $P < 0.0001$) but differences due to ploidy ($P = 0.02$) were not significant after correcting for multiple comparisons. All interaction terms were nonsignificant ($P = 0.06$ for ploidy × medium; $P > 0.5$ for the rest). Nevertheless, independent contrasts indicated that haploids grew significantly faster than diploids in YPD at both temperatures (Fig. 1; $P = 0.03$ at 30 °C; $P = 0.04$ at

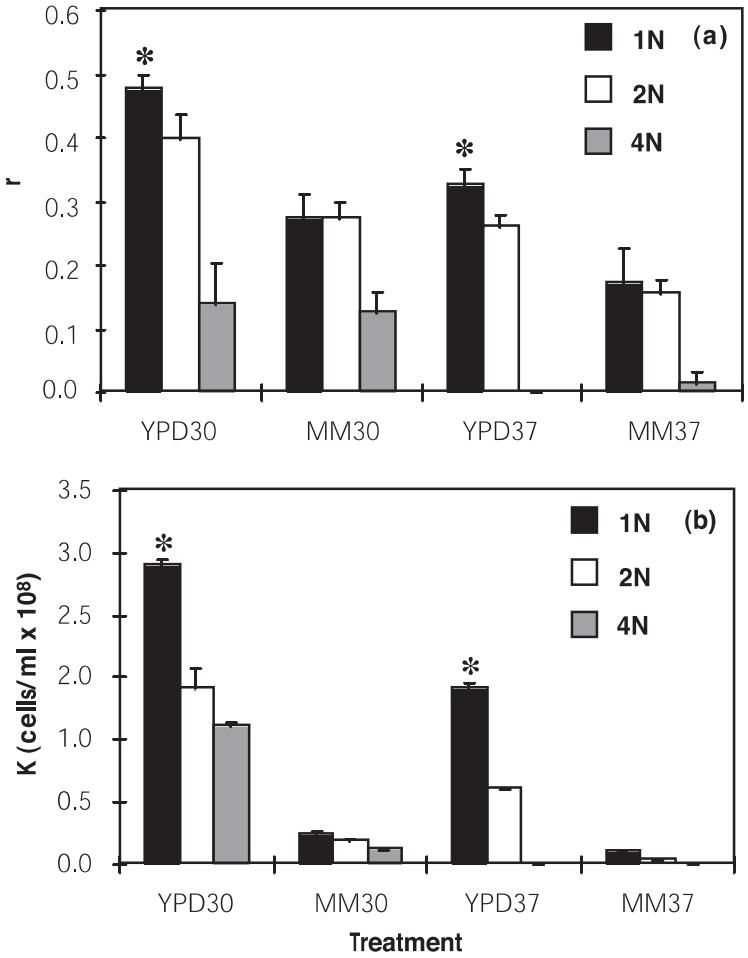

Fig. 4 (a) Growth rate, *r* (growth per hour between 0 and 12 h), and (b) stationary phase density (cell density at 48 h), *K*, for isogenic haploid, diploid and tetraploid auxotrophic strains grown under varying temperature and nutrient conditions. Bars indicate means of three replicates with standard errors (untransformed data). Asterisks indicate significant differences between haploids and diploids within treatments (independent contrasts; $P < 0.03$). Note that haploids showed a higher growth rate than diploids in YPD at both temperatures and that haploids had a significantly higher stationary phase density than the higher ploidy levels in YPD. These differences were much reduced and were not significant in MM.

37 °C), although these values were not significant after correcting for multiple comparisons. Averaged across temperature, haploids grew significantly faster than diploids in YPD ($P = 0.004$), but there were no significant differences in MM ($P = 0.7$).

For stationary phase density estimates, all main effects were significant (untransformed data; $P < 0.0001$) and there were significant interactions between ploidy × medium ($P < 0.0001$) and medium × temperature ($P < 0.0001$). Independent contrasts indicated that the stationary phase density of haploids was significantly greater than that of diploids in YPD at both temperatures ($P < 0.0001$) but not in MM ($P > 0.5$).

Competition experiments

Using the haploid competitor, there were significant effects of treatment (arcsin-transformed data; $P = 0.0008$) and medium ($P < 0.0001$) but not of day or ploidy ($P > 0.3$). Interactions between day × medium ($P = 0.021$) and a three-way interaction between ploidy × day × medium ($P = 0.03$) were significant at an α level of 0.05, although these values were not significant after correcting for multiple comparisons. Comparisons of means using independent contrasts indicated that haploids performed significantly better than diploids in MM at 37 °C when averaged across days ($P = 0.03$), but this was because of a large difference observed on the first day ($P = 0.003$ on day 1; $P = 0.99$ on day 2).

Using the diploid competitor, there were significant interactions between day × temperature, temperature × medium and day × medium (arcsin-transformed data; $P < 0.0001$), but there were no significant differences involving ploidy ($P > 0.1$). Nevertheless, independent contrasts indicated that haploids performed significantly better than diploids in MM at 30 °C when averaged across days ($P = 0.02$) but this was again because of a large difference on a single day ($P = 0.007$ on day 2; $P = 0.6$ on day 1).

For per generation fitness estimates ($m1/m2$), whether or not the correction factor for growth on plates was incorporated did not influence the results, hence only calculations without the correction factor are reported. Relative to the haploid competitor (Table 2), the only

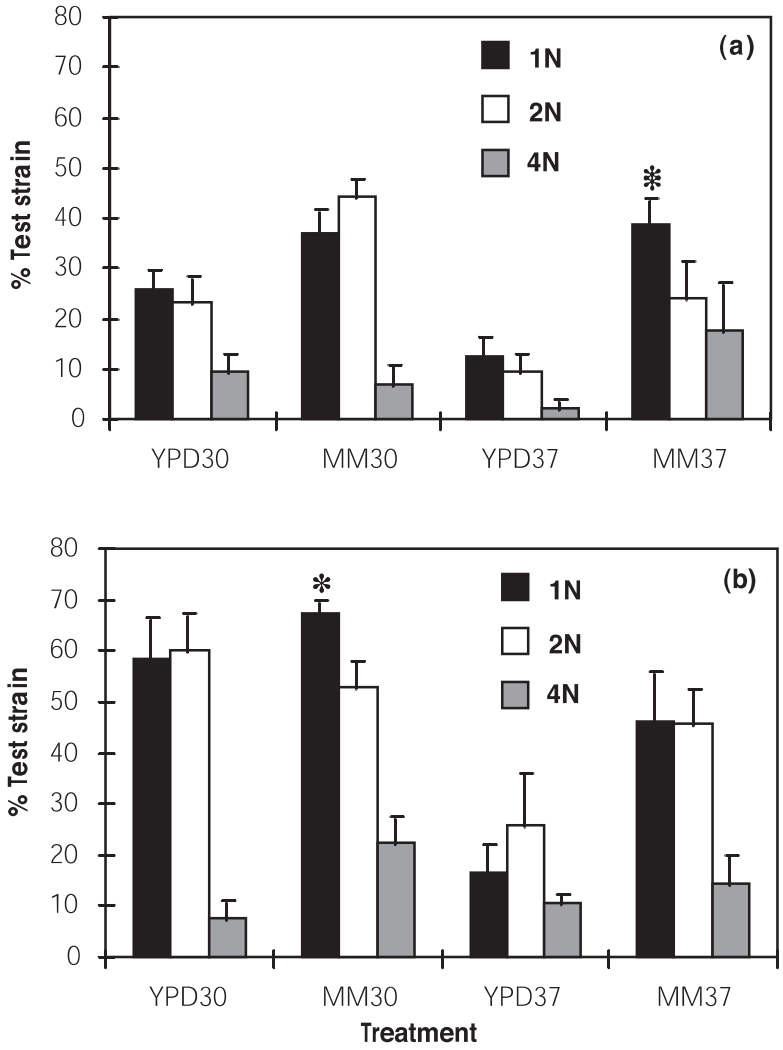

Fig. 5 Relative competitive abilities (% test strain) of haploid, diploid and tetraploid strains against (a) the prototrophic haploid competitor and (b) the prototrophic diploid competitor under varying temperature and nutrient conditions. Bars indicate means of 10 replicates (untransformed data) with standard errors. Asterisks indicate significant differences between haploids and diploids within treatments (arcsin-transformed data; independent contrasts; $P < 0.03$). However, in both cases, significance was the result of comparisons on a single day (see text).

Table 2 Per generation fitness estimates of haploid and diploid test strains under varied temperature and growth conditions, estimated according to eqn 5 (see text for details). Means ± SE are shown relative to the haploid (1n) and diploid (2n) competitors.

	1n Competitor		2n Competitor	
	*m*1/*m*2	*m*1/*m*2	*m*1/*m*2	*m*1/*m*2
Treatment	Haploids	Diploids	Haploids	Diploids
YPD 30 °C	0.91 ± 0.02	0.89 ± 0.02	1.07 ± 0.05	1.08 ± 0.08
MM 30 °C	0.94 ± 0.02	0.98 ± 0.01	1.11 ± 0.04*	1.01 ± 0.03*
YPD 37 °C	0.88 ± 0.01	0.84 ± 0.02	0.88 ± 0.02	0.86 ± 0.04
MM 37 °C	0.95 ± 0.03	0.95 ± 0.02	1.04 ± 0.03	0.97 ± 0.04

*The only significant difference was between haploids and diploids in MM at 30 °C using the diploid competitor ($P = 0.02$), but this difference was not significant after correcting for multiple comparisons.

significant effect was due to medium (untransformed data; $P < 0.0001$) and there were no significant interaction terms except for a weakly significant interaction between temperature × ploidy ($P = 0.05$; not significant after correcting for multiple comparisons). Independent contrasts did not indicate significant differences between haploids and diploids, although haploids appeared to perform better than diploids in YPD at 37 °C ($P = 0.06$). Using the diploid competitor, all main effects except day were significant (day: $P = 0.17$; ploidy: $P = 0.04$; temperature: $P < 0.0001$; medium: $P = 0.01$) and there were significant interactions between medium × temperature ($P = 0.003$), day × temperature ($P = 0.002$), and day × medium ($P < 0.0001$). The interaction between ploidy and medium was not significant ($P = 0.09$). Independent contrasts indicated that haploids had a higher fitness (relative to the diploid competitor) than diploids in MM at 30 °C ($P = 0.03$) but this difference was not significant after correcting for multiple comparisons. As in the

Table 3 Summary of correlations between fitness measurements and cell geometric parameters.

	Volume		Eccentricity		SA/V	
Fitness measurement‡	Probability	Correlation coefficient	Probability	Correlation coefficient	Probability	Correlation coefficient
% test 1n	0.004*,†	−0.45	0.33	−0.16	0.04*	+0.33
% test 2n	0.14	−0.24	0.01*	−0.40	0.02*	+0.36
*m*1/*m*2 1n	0.32	−0.16	0.08	−0.28	0.27	+0.18
*m*1/*m*2 2n	0.02*	−0.38	0.004*,†	−0.44	0.01*	+0.39
r	0.22	−0.26	0.65	+0.09	0.56	+0.12
K	0.0006*,†	−0.65	0.04*	−0.43	0.0003*,†	+0.67

* Significant at a significance level of 0.05.
† Significant after correcting for multiple comparisons.
‡ Comparisons involving *r* and *K* were done using the first three replicates of each of the other parameters; all other comparisons were made using the first five replicates of the competition assays (i.e. day 1 values).

Table 4 Ratios of haploid/diploid values for cell geometric and fitness parameters under varied temperature and growth conditions.

						Fitness in terms of % test strain		Per generation fitness estimate	
Treatment	Volume	Eccen	SA/V	*K*	*r*	1n comp	2n comp	1n comp	2n comp
YPD 30 °C	0.42	1.6	1.3	1.7	1.2	1.1	1.0	1.0	1.0
MM 30 °C	0.45	1.0	1.3	1.3	1.0	0.8	1.3	1.0	1.1
YPD 37 °C	0.91	1.0	0.8	2.3	1.3	1.3	0.6	1.0	1.0
MM 37 °C	0.43	0.9	1.3	3.4	1.1	1.6	1.0	1.0	1.1

unweighted fitness comparisons, this effect was due to a difference only on the second day (day 2: $P = 0.004$; day 1: $P = 0.8$). There were no differences between the ploidy levels under any of the other conditions ($P > 0.1$).

To confirm the results of the above experiments a third set of experiments was conducted using a strain with a mutation in the adenine 2 gene (*ade* competitor, W3031B MATα ura3-1, leu2-3, -112, his3-11, -15, trp1-1, ade2-1 can1-100, obtained from Charlie Boone), which allows comparison of pink vs. white colonies grown on the same plates. Unfortunately, the *ade* strain proved to be a very poor competitor and was almost completely unable to grow in competitions involving YPD, and in MM the results were highly variable. Again, in these experiments, the relative success of haploid strains did not improve under nutrient-limiting conditions (results not shown), supporting the results from the above competition experiments.

Correlation analyses

To investigate whether any of the cell geometric parameters were good predictors of relative fitness, I performed correlation analyses between each of the growth parameters and each of the cell geometric measures. For these analyses, the first five replicates of the competition assays were compared with the five replicates performed for each of the cell geometric properties (as the first five 'strains' had been used to measure these parameters). For comparisons involving growth rate, the first three replicates of each of the parameters were used (as growth rate was estimated from the first three 'strains'). Table 3 provides a summary of the correlations showing the probability values and the correlation coefficients (c.c.). At a significance level of 0.05, there were significantly negative correlations between volume and fitness measured against the haploid competitor (% test 1n: $P = 0.004$) and between eccentricity and fitness measured against the diploid competitor (% test 2n: $P = 0.01$). There were also significantly positive correlations between SA/V and fitness measured against both competitors (% test 1n: $P = 0.04$; % test 2*n*: $P = 0.01$), although neither would be significant after correcting for multiple comparisons. When per generation fitness was compared, there were no significant correlations when fitness was measured relative to the haploid competitor, but there were significantly negative correlations between fitness relative to the diploid competitor and both volume ($P = 0.02$) and eccentricity ($P = 0.004$), and a significantly positive correlation with SA/V ($P = 0.01$). Note, however, that the only values that remain significant after correcting for multiple comparisons are the correlations between volume and fitness measured against the haploid competitor, and between eccentricity and per generation fitness against the diploid competitor. There were no significant correlations between fitness measured by growth rate and the cell geometric parameters. Stationary phase density was negatively correlated

with volume ($P = 0.0006$; as expected based on models of nutrient-limited growth) and eccentricity ($P = 0.04$) and was negatively correlated with SA/V ($P = 0.0003$).

Comparisons were also made among the fitness measurements. Surprisingly, there was not a significant correlation between fitness measured against the haploid vs. the diploid competitor ($P = 0.07$; c.c. = +0.76), and a weakly negative correlation between growth rate and fitness measured against the diploid competitor ($P = 0.03$; c.c. = –0.43) was not significant after correcting for multiple comparisons. There was, however, a significantly positive correlation between fitness measured in terms of generation time using the two competitors ($P = 0.003$; c.c. = +0.78) and a weakly significant negative correlation between growth rate and fitness relative to the diploid ($P = 0.04$; c.c. = –0.43) but not the haploid competitor ($P = 0.64$; c.c. = –0.10). Although the correlations were not significant, growth rate was only positively correlated with fitness measured against the haploid competitor ($P = 0.07$; c.c. = +0.76) and stationary phase density ($P = 0.14$; c.c. = +0.31). Although only the comparisons involving unweighted fitness against the haploid competitor ($P = 0.04$) and per generation fitness against the diploid competitor remotely showed significance ($P = 0.05$; although not after multiple comparisons), stationary phase density was positively correlated with all of the other fitness parameters.

Discussion

Three predictions of the nutrient limitation hypothesis were tested using the results of these experiments.

Prediction 1: Haploids should fare better than diploids under poor nutrient conditions (MM) but not under rich conditions (YPD).

This prediction was not supported by the data. In fact, the single-strain growth rate (r) of haploids was 'higher' than that of diploids in YPD (Fig. 1), but haploids and diploids had nearly equal growth rates in MM. Similarly, haploids and diploids were roughly equal competitors (measured in terms of percentage test strain) regardless of environmental conditions using two different sets of competitors (Fig. 5). Therefore, in none of the experiments did success of haploids relative to diploids improve under nutrient limited conditions. The competition data were relatively noisy because of the necessity of comparing relative growth on different kinds of plates (making the variances quite large), and there were some discrepancies in results using the haploid and diploid competitors (e.g. Fig. 5). However, these discrepant results may have been because of differences in media composition or temperature on different days, as there were some significant interaction terms involving day and in some cases, levels of significance varied dramatically across days. Nevertheless, the interaction of interest (i.e. an interaction between ploidy and medium) was only found for stationary phase density and cell geometric parameters, and not for the fitness measurements. Based on parameter estimates from the GLM analyses, I estimate that a difference in final percentage of the test strain of approximately 5% should have been detectable using the number of replicates used here. Whereas subtle differences might have been obscured by noise, the bottom line is that there was no compelling evidence that haploids performed relatively better than diploids under low nutrient conditions.

Some of the noise in the data could be because the haploid and diploid competitors reach different carrying capacities due to differences in the relative resource space they occupy. Weighting the competition data by stationary phase density and volume (in an attempt to compensate for relative 'biomass') did result in more consistency between results using the two competitors, but, as the data did not meet the necessary statistical criteria of homoscedasticity (equality of variances) and normality (even after transformation), the results are not shown here. It is also possible that different ploidy levels have different generation times under different environmental conditions. Estimations of relative fitness in terms of generation time also did not suggest large differences between haploids and diploids. There was, however, some evidence for an advantage of haploids in nutrient poor medium, but this difference was not significant when corrected for multiple comparisons and was only observed using the diploid competitor at 30 °C on a single day.

Prediction 2: Surface area to volume ratios should be the best predictor of success, especially under low nutrient conditions.

Although there is less data to test this prediction (ideally, multiple strains with different SA/V ratios should be tested), the data do not suggest that the prediction holds under all conditions. As shown in Table 2, the surface area to volume ratio was 30% higher in haploids for all conditions except YPD at 37 °C (where the SA/V ratio was 20% lower in haploids). Nevertheless, the growth rates (r) of haploids and diploids were very similar, especially in minimal medium. In fact, when haploids had a relatively smaller SA/V ratio, they had the highest growth rate relative to diploids (in YPD at 37 °C). This runs counter to the nutrient-limitation hypothesis, which assumes that the main advantage of haploidy comes from better absorption of nutrients, an advantage that should disappear when the SA/V of haploids falls below diploids. The SA/V ratio was a weak predictor of the outcome of competition experiments (i.e. there were weakly significant positive correlations between SA/V and some of the fitness estimates from competition assays) but was not correlated with growth rate (Table 3).

Prediction 3: When haploids and diploids are similar in volume, diploids should fare better.

An assumption explicit in the argument put forward by Lewis (1985) is that haploids would generally perform

worse than diploids unless smaller cells are ecologically favoured, because of the genetic advantages that diploids gain by having two copies of every gene. If this assumption held, then at a similar volume, the genetic advantages that diploids experience should swing the balance in their favour and they should outcompete the haploids. This prediction is also not supported by the data. The cell volumes of haploids and diploids were most similar in YPD at 37 °C, yet this was the condition under which haploids had the largest advantage over diploids both in terms of growth rate in single cultures (Fig. 4; Table 2). There was a significantly negative correlation between volume and fitness measured against the haploid competitor, and between volume and per generation fitness measured against the diploid competitor ($P < 0.004$). The former was significant only in low medium whereas the latter was significant only in rich medium.

In summary, the nutrient limitation hypothesis is not consistent with the results of the experiments. Haploids did not fare better than diploids under poor nutrient conditions (MM), diploids did not fare better than haploids under rich nutrient conditions (YPD), and cell geometry was not a strong predictor of fitness.

The data from these experiments have several other interesting implications. For one, tetraploids had consistently low fitness, regardless of how fitness was measured (*r*, *K*, competition assays). In fact, they were completely displaced by the competitors in a substantial proportion of replicates under all conditions (Table 5). For tetraploids, the growth rate (*r*), the population size at stationary phase (*K*), and biomass (measured in terms of stationary phase density and cell volume) attained after 48 h of growth with a competitor were all higher at low temperatures than at high temperatures. This suggests that a larger cell volume does not provide tetraploid yeast with much, if any, protection against high temperatures. Indeed, it suggests that yeast that are already stressed by having an atypical ploidy level might be particularly prone to the difficulties of growing at abnormally high temperatures. Tetraploid cultures of *S. cerevisiae* have been found to be highly unstable and chromosome loss is frequent under laboratory conditions (Mayer & Aguilera, 1990), perhaps because spontaneous revertants to lower ploidy levels have a substantial advantage under the growth conditions considered in this study. There might, of course, be conditions where higher ploidy levels might be favoured. For example, aneuploidy and/or polyploidy is widespread among industrial yeast and could be maintained by selection for enhanced dosage of important genes involved in the fermentation process or by increased tolerance to ethanol (see Guijo *et al.*, 1997, and references therein).

The effects of temperature on cell geometric parameters and on relative fitness of haploids and diploids also appeared to be larger than those related to media composition. All the strains (including the competitors) performed more poorly at high temperatures than at the preferred growth temperature, regardless of media composition. At 37 °C, both competitors outcompeted the test strains in a number of replicates (Table 5), but this was most pronounced in YPD. As nutritional effects cannot realistically be separated from other environmental effects in natural populations, perhaps a strictly nutritional advantage might not be sufficient to provide haploids with an advantage over diploids in low-nutrient environments.

Table 5 Number of times per 10 replicates that the competitor completely displaced the test strain in competition experiments (values are shown for 1n competitor/2n competitor).

Treatment	1n	2n	4n
YPD 30 °C	0/0	0/0	4/4
MM 30 °C	0/0	0/0	6/2
YPD* 37 °C	**4/3**	**4/1**	**7/1**
MM 37 °C	0/1	4/1	3/4

* Note that the haploid competitor outcompeted the test strains more often in YPD at 37 °C than the diploid competitor (values indicated in bold).

Results from these experiments are consistent with those of Adams & Hansche (1974) but do not agree with several other studies. Glazunov *et al.* (1989) found support for the temperature nutrient hypothesis (i.e. haploids grew better in minimal medium whereas diploids grew better in rich medium). Using a different set of strains, however, Naidkhardt & Glazunov (1991) found that diploids consistently outcompeted haploids under all conditions. As previously discussed, variation in laboratory conditions and/or strain differences could be quite influential in conclusions related to ploidy differences. In fact, differences in results on different days in my experiment tends to support this view. My strains differed from those of other researchers both in genotype and in being functionally equivalent at the mating type locus (the other studies used isogenic strains that differed at the mating type locus); either of these factors could have influenced competitive outcomes. Discrepancies in conclusions among experiments suggest that haploid strains of yeast in the laboratory do not have a general nutritional advantage over diploids, but there may be conditions under which either ploidy level is favoured.

It is, of course, not really possible to completely separate nutritional factors from genetic factors. For example, Korona (1999), found that genetic load was correlated across environments in repair mismatch-repair deficient strains of yeast but suggested that stressful environments affected genetically loaded and unloaded strains differently. As in my experiments, he found that high temperature posed the most extreme environment, but that strains with accumulated mutations were more affected than mutation-free strains. The effects of het-

erozygosity in diploids could therefore be important in different environments; an effect that could obscure potential nutritional advantages of haploids. As the strains used here should have been effectively isogenic, this should not have influenced the results. However, it is possible that even in isogenic strains, nutritional markers might influence fitness differentially in haploids and diploids.

Historical ploidy level could also be an important factor in determining the outcome of laboratory competition experiments that could override potential nutritional differences. V. Perrot (personal communication) performed competition experiments between haploids and diploids of the yeasts *S. cerevisiae* (which is normally diploid) and *Schizosaccharomyces pombe* (which is normally haploid), and found that diploid *S. cerevisiae* had an overall advantage over haploid *S. cerevisiae* whereas haploid *S. pombe* had an overall competitive advantage over diploid *S. pombe* under both rich and poor media conditions Although *S. cerevisiae* in nature is probably predominantly asexual and diploid, my strains were created from laboratory stocks that had been propagated as asexual haploids for an unknown period of time, perhaps accounting for the apparent growth advantage of haploids in rich medium (i.e. the medium in which they would normally be propagated).

Conclusions

Experiments using an isogenic series of haploid, diploid and tetraploid yeast cells (*S. cerevisiae*) do not support the nutrient limitation hypothesis, which predicts that haploids should outcompete diploids under nutrient-poor conditions whereas diploids should be equivalent or better than haploids under nutrient-rich conditions. In addition, tetraploids appeared to be always at a disadvantage compared with the lower ploidy levels, regardless of how fitness was measured. However, it should be kept in mind that one limitation of all experiments using laboratory strains of microbial species is that a long history of propagation under artificial conditions makes extrapolations to natural populations difficult. For example, results based on laboratory strains used in this experiment (and most other previous experiments) may not necessarily reflect responses in natural populations experiencing natural 'rich' and 'poor' nutrient conditions. Repetition of these types of experiments using field-collected yeast exposed to more natural variation in environmental conditions would be a desirable next step in elucidating the factors that favour the maintenance of haploid–diploid life cycles.

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