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Masking and purging mutations following EMS treatment in haploid, diploid and tetraploid yeast (*Saccharomyces cerevisiae*)

BARBARA K. MABLE* AND SARAH P. OTTO
Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

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Summary

The yeast, *Saccharomyces cerevisiae*, was used as a model to investigate theories of ploidy evolution. Mutagenesis experiments using the alkylating agent EMS (ethane methyl sulphonate) were conducted to assess the relative importance that masking of deleterious mutations has on response to and recovery from DNA damage. In particular, we tested whether cells with higher ploidy levels have relatively higher fitnesses after mutagenesis, whether the advantages of masking are more pronounced in tetraploids than in diploids, and whether purging of mutations allows more rapid recovery of haploid cells than cells with higher ploidy levels. Separate experiments were performed on asexually propagating stationary phase cells using (1) prototrophic haploid (MATα) and diploid (MATa/α) strains and (2) isogenic haploid, diploid and tetraploid strains lacking a functional mating type locus. In both sets of experiments, haploids showed a more pronounced decrease in apparent growth rate than diploids, but both haploids and diploids appeared to recover very rapidly. Tetraploids did not show increased benefits of masking compared with diploids but volume measurements and FACScan analyses on the auxotrophic strains indicated that all treated tetraploid strains decreased in ploidy level and that some of the treated haploid lines increased in ploidy level. Results from these experiments confirm that while masking deleterious mutations provides an immediate advantage to higher ploidy levels in the presence of mutagens, selection is extremely efficient at removing induced mutations, leading growth rates to increase rapidly over time at all ploidy levels. Furthermore, ploidy level is itself a mutable trait in the presence of EMS, with both haploids and tetraploids often evolving towards diploidy (the ancestral state of *S. cerevisiae*) during the course of the experiment.

1. Introduction

A necessary consequence of sexual reproduction is that there is an alternation between lower and higher ploidy levels, typically haploid and diploid. Nevertheless, the amount of time spent at each ploidy level is not fixed and varies widely among taxa (Raper & Flexer, 1970; Bell, 1994). Currently, we have a limited understanding of the factors that are most important in determining the balance struck between haploid and diploid phases (reviewed in Mable & Otto, 1998). A potentially important factor concerns the genetic advantages of having one or two copies of every gene when these genes are continually subject to mutation (Crow & Kimura, 1965; Kondrashov & Crow, 1991; Perrot *et al.*, 1991; Otto & Goldstein, 1992; Jenkins & Kirkpatrick, 1995). Genetic data suggest that most mutations that affect fitness are both deleterious and partially recessive (Simmons & Crow, 1977; Drake *et al.*, 1998). This implies that diploid individuals tend to 'mask' deleterious mutations with 'normal' alleles carried on the homologous chromosomes. Masking allows diploid individuals with mutations to survive, providing diploidy with a short-term advantage, but there is an associated long-term disadvantage: mutations persist for longer when they are masked from natural selection within diploids. In contrast, selection is much more efficient at eliminating deleterious mutations from haploid individuals (Haldane, 1924).

* Corresponding author. Present address: Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1, Canada. Tel: +1 (519) 824 4120. Fax: +1 (519) 767 1991. e-mail: bmable@uoguelph.ca

Theoretical models have shown that whether evolution favours an expansion of the diploid phase (masking mutations) or the haploid phase (purging mutations) depends on a number of critical parameters, including the strength of selection, the degree of dominance of deleterious mutations and the mode of reproduction of the organism (Perrot *et al*., 1991; Otto & Goldstein, 1992; Jenkins & Kirkpatrick, 1995; Otto & Marks, 1996). Summarizing these models, weaker selection against deleterious mutations and a greater degree of dominance of wild-type alleles tend to favour the expansion of the diploid phase, but only in organisms that frequently undergo sexual reproduction and recombination. An additional factor not considered in the above models is that cells with higher ploidy levels may be more resistant to DNA damage (Long & Michod, 1995), as a result of recombinational repair mechanisms (see Burtscher *et al*., 1988; e.g. Heude & Fabre, 1993). Therefore, higher ploidy levels may evolve in environments characterized by high rates of DNA damage.

In this experiment, we set out to compare the ability of haploid, diploid and tetraploid strains of the yeast, *Saccharomyces cerevisiae*, to survive mutations induced by the mutagen, ethyl methane sulfonate (EMS). In particular, we tested whether cells with higher ploidy levels have relatively higher fitnesses after mutagenesis (in confirmation of the masking hypothesis), whether the advantages of masking are more pronounced in tetraploids than in diploids, and whether purging of mutations allows more rapid recovery of haploid cells than cells with higher ploidy levels. Experiments were performed on asexual cultures of yeast exposed to EMS at various concentrations. We chose the mutagen EMS for study because it has been extensively studied in yeast (Loprieno, 1966; Nasim & Auerbach, 1967; Prakash & Sherman, 1973; Prakash & Higgins, 1982; see also Kilbey & Hunter, 1983; Schiestl & Wintersberger, 1983; van Zeeland *et al*., 1983; Orthen *et al*., 1984; e.g. reviewed by Sega, 1984; Wintersberger & Karwan, 1987; Klein *et al*., 1989; Klein *et al*., 1990; Lee *et al*., 1992). EMS is an alkylating agent that causes DNA damage; repair of this damage induces a high frequency of G/C to A/T transition mutations in yeast (Prakash & Sherman, 1973; Sega, 1984; Kohalmi & Kunz, 1988; Lee *et al*., 1992). The entire experiment was replicated three times using yeast derived from two different laboratory strains.

Experiments with *S. cerevisiae* provide an ideal model system in which to test hypotheses abut the evolution of ploidy levels. Although *S. cerevisiae* are thought to exist in nature predominantly as asexual diploids, laboratory strains can be maintained at different ploidy levels and can be propagated asexually or sexually under certain nutrient limitation conditions. Furthermore, genetic markers are readily available, cultures in liquid or on solid medium are easy to maintain and generation times are short (roughly 90 min when nutrients are plentiful; Sherman, 1991). In our experiments, cultures were maintained under conditions that normally prevent sexual reproduction in order to separate the advantages (or disadvantages) of diploidy from the advantages (or disadvantages) of sexual reproduction. Nevertheless, we observed changes in cell morphology and in population dynamics that suggest that yeast frequently respond to mutagenesis by changes in ploidy level. This occurred even in strains carrying multiple mutations at loci necessary for mating. This phenomenon was further explored by checking nuclear densities in cultures of haploid, diploid and tetraploid yeast in EMS-treated and control lines.

The results from these experiments confirm that while masking deleterious mutations provides an immediate advantage to higher ploidy levels in the presence of mutagens, selection is extremely efficient at removing induced mutations, leading growth rates to increase rapidly over time at all ploidy levels. Furthermore, ploidy level is itself a mutable trait in the presence of EMS, with both haploids and tetraploids often evolving towards diploidy during the course of the experiment.

2. Materials and methods

(i) *Yeast strains*

The experiments were performed on yeast strains derived from two different sources (Table 1). In the first set of experiments, standard laboratory strains of *S. cerevisiae*, a prototrophic diploid (i.e. containing no known mutations at nutritional loci), C276 (MATa/α), and two haploid segregants of this strain C4,1 (MATa) and C4,2 (MATα), were used to establish yeast cultures (obtained from Dr Brian Haarer; strains described in Wilkinson & Pringle, 1974). Throughout the paper, these three strains will be referred to as 'prototrophic' strains. In the second set of experiments, a series of isogenic strains with different ploidy levels from haploidy to tetraploidy were used. These strains were developed by Drs Alison Adams and Sharon Brouwer and will be referred to as 'auxotrophic' strains. The background genotype of the auxotrophic strains was MATa a1-ste6Δ8-694 ura3 leu2 his4 trp1 can1. Not only do these strains share the same genetic background, but they have a mutation at the mating type locus (MATa, mutation a1) and a deletion in the pheromone receptor locus (ste6Δ8-694). This has the double advantage that mating within the strains should be impossible and that pleiotropic effects of the mating type locus on the relative fitnesses of different ploidy levels should be lessened (see Section 4). Initially, the haploid, diploid

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Table 1. *Yeast strains used in the experiments*

Strain designation	Genotype	Source
Prototrophic strains		
C276	MAT a/α	Brian Haarer
C4,1	MAT a	Brian Haarer
C4,2	MAT α	Brian Haarer
Auxotrophic strains derived from SM2185		
BM1N (AA1596)	MAT a a1-ste6Δ8-694 leu trp1 ura3 his4 can1	Alison Adams
BM2N (AA6)	MAT a a1-ste6Δ8-694 leu trp1 ura3 his4 can1	Alison Adams
BM4N (AA12)	MAT a a1-ste6Δ8-694 leu trp1 ura3 his4 can1	Alison Adams

and tetraploid strains carried two plasmids (psm 620 and AAB284) with genotypes CEN6 STE6 URA3 MATα and CEN6 STE6 LEU2 MATa, respectively, which allowed mating when the plasmid with a complementary genotype was present, in order to create higher ploidy levels (A. Adams and S. Brouwer, personal communication). We grew these strains (from frozen stocks) in overnight cultures in 5 ml of rich medium (YPD) and then replica plated onto YPD, ura− and leu− plates to screen for plasmid loss. For each ploidy level, a single colony lacking both plasmids (rendering them unable to mate) was used to establish cultures. These were the cultures used in the second set of experiments, and they will be referred to as BM1N, BM2N and BM4N for haploid, diploid and tetraploid strains, respectively. Except for changes that might have occurred in the rounds of growth following their creation, these strains should have been genetically identical, with the haplotype: MAT a a1-ste6Δ8-694 leu2 trp1 ura3 his4 can1. Cultures were grown overnight in YPD liquid medium at 30 °C and then plated on YPD and stored at 4 °C until required.

(ii) *Media and growth conditions*

Difco YPD solid and liquid media (containing 2% yeast extract, 1% peptone and 2% dextrose), prepared according to the package instructions, were used as rich media. Minimal medium (MM) was prepared using 0·67% 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%. Plates for detecting cells containing URA3 or LEU2 plasmids were made using synthetic complete medium (0·67% yeast nitrogen base without amino acids, 2% dextrose and 2% bacto-agar) with amino acids added in the same concentrations as for minimal media with the exception of uracil (for ura− plates) or leucine (for leu− plates). Liquid cultures and plates were grown at 30 °C. Liquid cultures were rotated continuously during incubation. Under these conditions, yeast typically reproduce asexually about once every 90 min until resources become limiting (Wilkinson & Pringle, 1974).

Cell densities were determined based on haemacytometer counts. 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 due to the accumulation of waste products and cellular debris from dying cells (unpublished data). 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 $2{\cdot}25 \times 10^5$ cells/ml) were considered below the detectable limit of the haemacytometer and were not included in the results. Buds and cells growing in chains were counted as separate cells.

(iii) *EMS treatments*

EMS (1·06 M) was obtained from Sigma (M0880), and treatment was performed according to standard protocols (Rose *et al.*, 1990; Winston, 1992). Fifty microlitres of the final resuspended volume (on the order of 10^7 cells) was used to innoculate the 0 time cultures. Actual starting density was estimated from haemacytometer counts following the EMS treatment. van Zeeland *et al.* (1983) studied the effects of EMS on the rate of ethylation per nucleotide, estimating rates of $3{\cdot}0 \times 10^{-4}$ and $5{\cdot}8 \times 10^{-4}$ with 26 and 53 nM EMS, respectively (equivalent to our 25 μl and 50 μl treatments). Since *S. cerevisiae* has a genome of approximately $1{\cdot}4 \times 10^4$ kb in size, this would be equivalent to approximately 4 and 8 ethylations per haploid genome. van Zeeland *et al.* (1983) also

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estimated the rates of forward mutations in ade-1 and ade-2 and reversion mutations at his, lys and arg to establish the relationship between ethylations per nucleotide and mutation frequency, but they found large differences among loci and a non-linear correspondence between dosage and mutation induction.

(iv) *Growth curves and fitness measurements*

Previous mutagenesis experiments have estimated changes in viability following treatment based on the relative number of colony forming units (CFUs) when samples from liquid cultures are plated on solid medium. We attempted to do this in all of our trials but found that even for controls the relationship between the number of CFUs and the number of cells counted in the haemacytometer was excessively variable, especially immediately following EMS treatment (data not shown). Since solid medium represents a different selective environment from liquid medium and establishing space to form a colony could be a more complex task than budding in liquid medium, we decided to base our fitness estimates only on growth in liquid medium as estimated by haemacytometer counts. That is, our experiments assess relative fitness changes using population dynamics in liquid batch cultures following EMS treatment and are not intended to estimate colony-forming ability on solid medium.

The classic experiment of yeast in liquid cultures by Carlson (1913), analysed by Renshaw (1991, p. 52), demonstrated that, when a fixed amount of nutrients is supplied, growth rates are density dependent and the number of yeast cells follows a logistic curve over time. The population size at time t (N_t) can then be described using the standard logistic growth model for continuously growing populations:

$$N_t = \frac{KN_0\,e^{rt}}{N_0\,e^{rt}+(K-N_0)}, \qquad (1)$$

where N_0 is the number of cells in the starting culture, r is the intrinsic rate of growth when the number of cells is low and K is the carrying capacity or number of cells maintained during stationary phase. In our experiment, carrying capacity (K) was estimated by averaging across samples taken once a population did not appear to be increasing in size. Intrinsic growth rates (r) were estimated as follows. Equation (1) can be rearranged to give:

$$e^{rt} = \frac{N_t}{N_0}\left(\frac{K-N_0}{K-N_t}\right);$$

taking the log of both sides indicates that

$$\Phi = \log_e\left[\frac{N_t}{N_0}\left(\frac{K-N_0}{K-N_t}\right)\right] \qquad (2)$$

should equal the product of r and times t. Therefore, if one plots Φ as a function of time, the points should fall along a line whose slope is r. For real data, however, the population size will fluctuate around the carrying capacity, which causes (2) to be undefined whenever $N_t > K$. Therefore, we estimated r from the slope of the line that had the least squares fit to plots of Φ over time, using only samples before the population density exceeded half the carrying capacity. For experiments in which only N_0, N_t and K were estimated, (2) was divided by t to provide an approximate estimate of r.

Having estimated growth rates following mutagen treatment in both haploids and diploids, the average dominance coefficient (h) of wild-type alleles over new mutations may be inferred. Let R_{haploid} and R_{diploid} be the growth rates of haploids and diploids following EMS treatments divided by the growth rates of their respective controls. Let n equal the number of mutations induced per haploid genome. Ignoring interactions among these mutations, we can define R_{haploid} as $1-ns$, where s is the average reduction in growth rate per mutation, and R_{diploid} as $1–2nhs$, where the factor of 2 enters because we suppose that diploids receive twice as many mutations as haploids. h is then defined as $(1-R_{\text{diploid}})/(1-R_{\text{haploid}})/2$. If h is zero, mutations are completely masked by wild-type alleles in heterozygous diploids and hence have no effect on their growth rate. If h is 1, mutations in heterozygous diploids have their full effect and reduce growth rate as much as they would in the homozygous or haploid condition. More generally, wild-type alleles mask mutant alleles to some extent if $h < 0{\cdot}5$, whereas mutant alleles are more revealed in heterozygotes than expected based on their average effect in homozygotes if $h > 0{\cdot}5$. Similarly, for tetraploids, we can define the average dominance coefficient for a single mutant allele (a) present in a wild-type (A) background as h_{4N}, where the fitness of AAAa individuals is $1-h_{4N}s$. Following a similar line of reasoning as above, h_{4N} can be estimated as $(1-R_{\text{tetraploid}})/(1-R_{\text{haploid}})/4$. Hence, in tetraploids, if $h_{4N} < 0{\cdot}25$, wild-type alleles mask mutant alleles that are present in a single copy, while if $h_{4N} > 0{\cdot}25$, mutations are more expressed in AAAa heterozygotes than expected based on their average effect in homozygotes.

In experiments with EMS, the assumption that r is constant over time is unlikely to be true. Instead, we expect that natural selection will act on the genetic variation in growth rates created by EMS, causing the average intrinsic growth rate (r) to increase over time in treated yeast cultures. That is, natural selection will cause plots of Φ to curve upwards over time. This hypothesis can be tested by examining whether there is a significantly positive quadratic component in a polynomial regression analysis. Other factors might also alter the shape of plots of Φ over time, but these

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should have different features that can be used to distinguish them from the case of natural selection. If the initial number of cells is incorrectly estimated, plots of Φ should still be linear but will no longer intercept at zero; instead, there will be a negative intercept if N_0 is overestimated, and vice versa. On the other hand, if the number of initial cells is correctly estimated but many of these cells have been killed by EMS, the intercept will still be zero but the slope will initially be flatter due to the lack of growth of the dead cells. This effect is short-lived, however, since the dead cells make up a smaller and smaller fraction of the population over time. Consequently, dead cells present in the initial population have little effect on the slope once $\Phi > 1$, approximately. Errors in estimating the carrying capacity will also cause plots of Φ to be non-linear, but these effects only occur once the population is near carrying capacity and should be minimized by only including data while the population is below half the carrying capacity. Another factor that must be considered is that EMS acts as a poison as well as a mutagen. Therefore, growth rates may increase over time simply because the initial and descendant cells are recovering from treatment rather than evolving. Nevertheless, this should cause only minor changes to plots of Φ over time (akin to the case where a fraction of the initial cells are dead) unless the poisonous effects of EMS are long term and affect cells separated by several generations from the EMS treatment. In summary, the most parsimonious explanation for plots of Φ that curve upwards over the entire period of population expansion is that natural selection is acting to increase the average growth rate within a population.

(v) *Mutagen experiments*

(a) *Varying EMS concentrations using prototrophic strains*

To determine the effects of EMS treatment on the growth characteristics of prototrophic yeast of different ploidy levels, haploid (C4,2) and diploid (C276) strains were exposed to five different levels of EMS: 0 μl (control), 25 μl, 50 μl, 100 μl and 200 μl (one replicate each). In preparation for EMS treatment, a single colony from each strain was picked and grown in 5 ml liquid YPD medium for 18 h at 30 °C with continual rotation at 200 rpm (to allow sufficient time for cultures to reach stationary phase). One millilitre samples were then treated with EMS. Cultures of each ploidy level were started at a density of approximately 2×10^6 cells/ml under both high (50 ml YPD) and low (50 ml MM) nutrient conditions by inoculating 125 ml flasks containing 50 ml YPD (or 50 ml MM) with 50 μl of the treated cells. One hundred μl samples were then drawn after 4, 8, 10, 12, 14, 24, 28, 32 and 48 h of growth to estimate cell densities using haemacytometer counts. To further characterize growth in the YPD cultures, 50 μl of 36 h samples were used to start new cultures in 5 ml fresh YPD and samples taken after 16 h of growth.

(b) *Single dosage of EMS using prototrophic strains*

A more extensive experiment was conducted using only 0 μl (two simultaneous replicates) and 50 μl (three simultaneous replicates) EMS treatments on the prototrophic strains. Again, 50 μl of the treated cells was used to inoculate 125 ml flasks containing 50 ml YPD. Samples of 100 μl were then drawn at 2 h intervals for 36 h and additional samples taken at 48 and 72 h.

(c) *Varying EMS concentrations using auxotrophic strains*

To ensure that mating type switching was not occurring and to extend the experiment to tetraploids, haploid BM1N, diploid BM2N and tetraploid BM4N strains were exposed to EMS. Three replicates were performed per strain, each run contemporaneously and started from a single colony chosen at random from plates. Cultures were diluted to the same density ($1{\cdot}6 \times 10^7$ cells/ml: the density of the least dense strain) and divided into four samples treated with 0, 25, 50 or 100 μl EMS. Cultures were then established in test tubes containing 5 ml of YPD with starting densities recalibrated to 2×10^5 cells/ml. At 24 and 48 h, serial transfers of 100 μl to 5 ml of fresh YPD were performed, so that intrinsic growth rates (r) could be estimated over a 3 day period. Cell densities were based on haemacytometer counts from 100 μl drawn at 6, 12 and 24 h on the first day and at 6 and 24 h on the subsequent two days.

(d) *Volume and ploidy measurements in EMS-treated auxotrophic strains*

To monitor the effects of EMS treatment on ploidy levels, cell and nuclear volumes were estimated for cells in the 50 μl EMS treatment and control lines from the previous experiment, (c), sampled at 72 h. Cell volumes and eccentricities were calculated by measuring the length (L) and width (W) of five randomly chosen individual yeast cells per ploidy level per treatment, photographed on the haemacytometer grid at a magnification of $\times 400$ (Carter & Sudbery, 1980). Cell volume was calculated using the formula for an oblate spheroid:

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

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and eccentricity using the formula:

$$E = \sqrt{1 - \frac{W^2}{L^2}}. \quad (4)$$

Nuclear densities were determined using a FACScan (Becton Dickinson). Stationary phase cells were fixed and stained with propidium iodide (PI) according to a protocol obtained from the world-wide web (http://pingu.salk.edu/fcm/protocols/ycc.html) based on Sazer and Sherwood (Sazer & Sherwood, 1990). Cells were resuspended in 1 ml of 1 M sorbitol, fixed in 1 ml of 70% ethanol, and stored at 4 °C prior to staining. In preparation for the FACScan analysis, a sample of 300 μl of fixed cells was pelleted, the ethanol removed and samples dried for 5 min. The pellet was then resuspended in 1 ml of 50 mM sodium citrate, repelleted, and resuspended in 500 μl 50 mM sodium citrate containing 0·1 mg/ml DNase-free RNase A. The solution was then incubated at 37 °C for 4 h with slow rotation. After the incubation period, 500 μl sodium citrate containing 4, 12 or 20 μg/ml PI (final concentration of 2, 6, 10 μg/ml) was added to the sample tubes. Control samples for each ploidy level (i.e. not containing PI) were used to adjust for background fluorescence. FACScan settings were chosen to increase the signal-to-noise ratio and were set as follows: FSC = E00; Threshold FSC-H = 0; Amp Gate = 3·84; SSC 372 = linear scale; FL2 556 = log scale. For each ploidy level and for each treatment level (0 and 50 μl EMS), a FACScan analysis was performed on samples drawn at 72 h from experiment (c). The frequency spectrum of the fluorescence distribution was determined and analysed using CellQuest, version 1.0 (Becton Dickinson).

(vi) *Statistical analyses*

For each set of experiments, an initial full model analysis of variance was conducted by fitting a general linear model to the data. In those cases where there was a significant treatment or ploidy effect, pairwise tests were then performed to determine the source of this effect. Independent contrasts were used to compare treated strains with their controls; interpretations of significance were based on corrections for multiple comparisons. When all pairwise comparisons were made (e.g. when comparing measurements among the various ploidy levels), Tukey's honestly significant difference (HSD) test was employed (Tukey, 1953; Kramer, 1956); Tukey's test is designed for such multiple comparisons and ensures that the overall significance level remains at 0·05 when all pairs of means are compared in a one-way ANOVA. For growth rate estimates, regression analyses were performed on Φ over time. The slope of linear regressions was used to obtain estimates of r, whereas the fit of polynomial regressions was used to determine whether a significant quadratic component was apparent in EMS-treated cell lines.

3. Results

(i) *Varying EMS concentrations using prototrophic strains*

In the first experiment, using prototrophic strains, mutagenized lines grew at a slower rate for all dosages of EMS evaluated, but this reduced growth rate was especially pronounced for the haploid lines. Fig. 1 provides a qualitative summary of the effects of varied mutagen concentration on the growth trajectories of haploids and diploids when grown in rich (YPD) or poor medium (MM). Treatment with 100 μl and 200 μl of EMS effectively killed the cell lines (i.e. substantial growth could not be detected in liquid cultures even up to 18 h), although one diploid line treated with 100 μl EMS appeared to recover when grown in YPD. For the remaining EMS levels, treated diploid lines approached the same carrying capacity as the diploid control lines by the end of 48 h. By contrast, treated haploid cells appeared to reach a lower carrying capacity than their controls, especially in YPD. Indeed, new cultures started after 36 h by transferring an aliquot of 50 μl of culture to 5 ml of fresh YPD medium indicated that both haploid and diploid treated lines grew at similar rates and to similar cell densities over the next 48 h as the diploid controls *not* the haploid controls. After 16 h of growth in these serially transferred cultures, cell densities were $2{\cdot}0 \times 10^8$, $1{\cdot}9 \times 10^8$ and $2{\cdot}1 \times 10^8$ cells/ml for diploid cultures treated with 0, 25 and 50 μl EMS, respectively, whereas haploid densities were $3{\cdot}5 \times 10^8$, $2{\cdot}0 \times 10^8$ and $1{\cdot}9 \times 10^8$ cells/ml. Growth in MM did not reveal more sensitive differences, although treated haploids appeared to take longer (more than 30 h) to reach a population size at least half that of the control line.

Because there were few data points, the positive quadratic component was not significantly different from zero. Nevertheless, plots of equation (2) do indeed curve upwards over time in the lines treated with EMS (data not shown), especially for diploids, which is consistent with the hypothesis that natural selection was acting to increase r even over the short time course of this experiment.

(ii) *Single dosage of EMS using prototrophic strains*

Growth curves for the more extensive experiments performed using a single dosage of EMS (50 μl) over 72 h are shown in Fig. 2. Qualitatively, both treated haploids and treated diploids appeared to exhibit slower growth relative to their controls, with this effect again being more pronounced in haploids.

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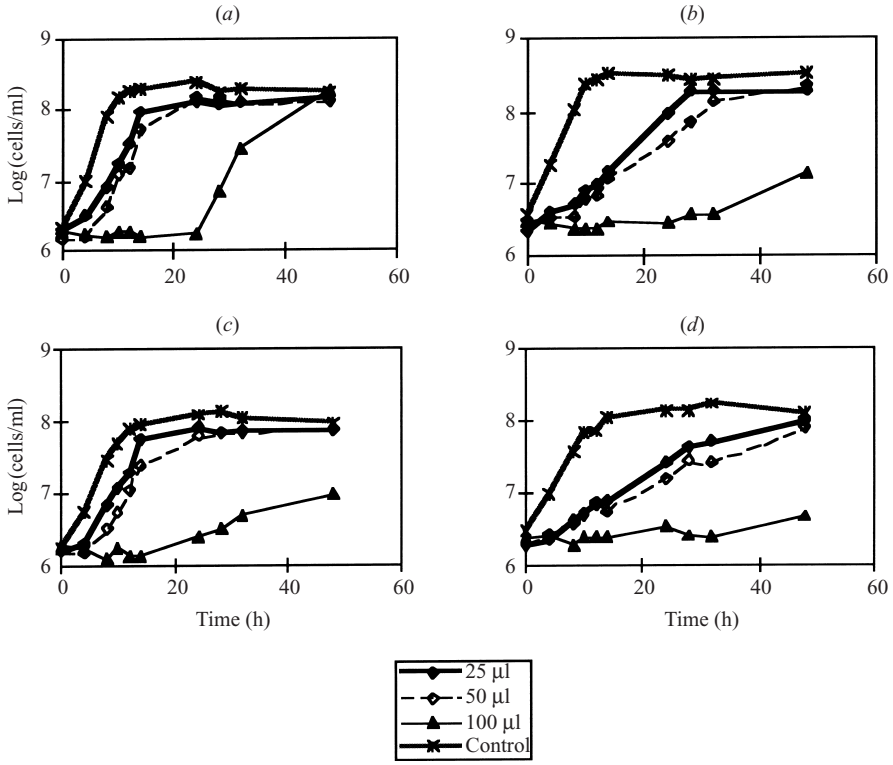

Fig. 1. Growth curves of prototrophic yeast strains following EMS treatment (on a $\log_{10}$ axis). Haploid (C4,2) and diploid (C276) yeast were treated with 0, 25, 50, 100 or 200 μl EMS and grown under high (YPD) and low (MM) nutrient conditions. The 200 μl treatments are not shown, since none of the cultures grew at this level of EMS. (*a*) Diploids grown in YPD; (*b*) haploids grown in YPD; (*c*) diploids grown in MM; (*d*) haploids grown in MM.

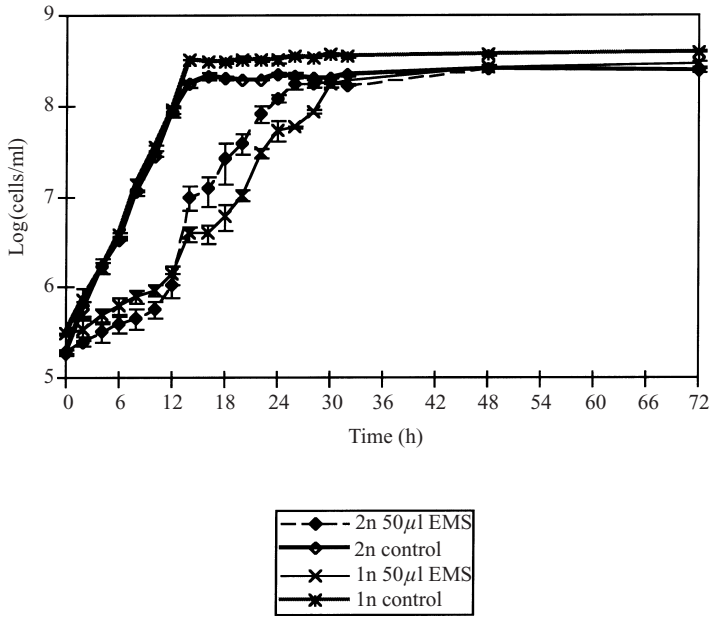

Fig. 2. Growth curves of prototrophic yeast strains following 50 μl EMS treatment. Haploid (C4,2) and diploid (C276) yeast were treated with 50 μl EMS and grown in liquid YPD media. Bars represent ± 1 standard error based on three replicate cultures per treated strain and two replicates for each control strain.

Although treated diploids reached similar densities to their controls by 48 h, treated haploids seemed to approach a carrying capacity closer in size to diploid controls than haploid controls.

Average carrying capacities were estimated from 34 to 72 h. Untreated diploids showed an average of $2{\cdot}3 \times 10^8$ cells/ml, whereas untreated haploids showed an average of $3{\cdot}7 \times 10^8$ cells/ml. Treated diploids

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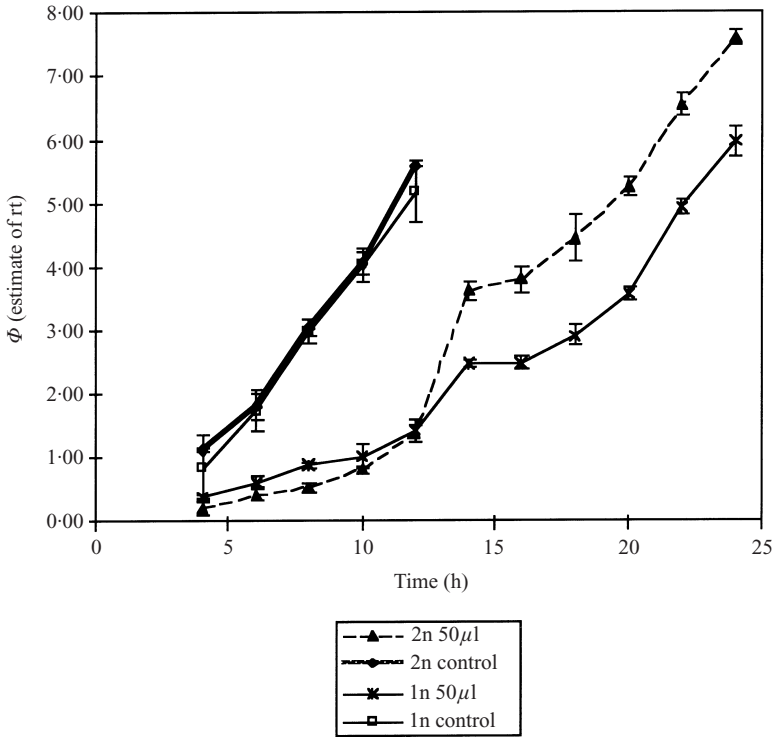

Fig. 3. Plots of Φ (calculated using equation 2) versus time for prototrophic haploid (C4,1) and diploid (C276) cultures treated with 0 and 50 μl EMS. Note that plots for untreated cell lines have a linear slope whereas those for treated cell lines curve upwards.

reached a carrying capacity of $2{\cdot}2 \times 10^8$ cells/ml, whereas treated haploids reached a carrying capacity of $2{\cdot}3 \times 10^8$ cells/ml. Multiple-comparisons tests indicated that the carrying capacities of untreated diploids, treated diploids and treated haploids were not significantly different from one another but were all significantly smaller than the carrying capacity of untreated haploids ($P < 0{\cdot}05$; Tukey's HSD). The population sizes for the three replicates of treated haploid lines were $2{\cdot}1 \times 10^8$, $2{\cdot}2 \times 10^8$ and $2{\cdot}6 \times 10^8$, indicating that all three of the replicates were nearer the diploid carrying capacity than the haploid carrying capacity.

To estimate growth rates (r), the slope of a linear regression of equation (2) against time was determined using these estimates for the carrying capacity (K) and the 0 time densities for N_0 (Fig. 3). Treated diploids showed growth rates of approximately 0·39, 0·37 and 0·35 per hour, whereas control diploids showed growth rates of 0·55 and 0·55 per hour. Growth rates of treated haploids were estimated to be 0·24, 0·23 and 0·26 per hour whereas control haploids showed growth rates of 0·54 and 0·55 per hour. In other words, growth rates of the EMS-treated cells were on average 67% of the control growth rates for diploids and 45% of the control growth rates for haploids. From mutation accumulation experiments in yeast where selection is minimized, the average dominance coefficient of random mutations has been estimated as 0·08 (Korona, 1999*b*). Indeed, in our experiments, haploids were significantly more affected by the mutagen than diploids ($P < 0{\cdot}02$; paired t-test) but not as much as would be predicted if h were 0·08. From the growth rate measurements, the average dominance coefficient of wild-type alleles is estimated as $0{\cdot}30 \pm 0{\cdot}03$ SE, assuming that diploids received twice as many new mutations as haploids. One explanation for why we observed a higher estimated dominance level is that, in haploids, mutations of strong effect may have been rapidly eliminated by selection, which would upwardly bias our estimate of h. Another possibility, however, is that mate switching followed by mating occurred in the haploid lines; this would also have caused h to be nearer 0·5.

Growth rates were not constant over time in the EMS-treated lines but tended to increase after treatment, as seen in plots of equation (2) over time (Fig. 3). A significant quadratic component was found for each of the treated haploid lines ($P = 0{\cdot}005$, 0·017, 0·003) but for only one of the three treated diploid lines ($P = 0{\cdot}30$, 0·12, 0·02). Averaging across lines, the effect was only significant for haploids (haploids: $P = 0{\cdot}002$; diploids: $P = 0{\cdot}08$). However, both ploidy levels showed a similar positive quadratic coefficient (0·0087 for haploids and 0·0084 for diploids). Control cell lines did not show a significant quadratic component. Predicted intercepts were not significantly different from zero for controls (based on a linear fit)

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Table 2. *Intrinsic growth rate (r) estimates over a 6 h period for haploid, diploid and tetraploid cell lines treated with 0, 25, 50 and 100 μl EMS*

EMS dosage	Haploids			Diploids			Tetraploids		
	Day 1[a]	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0 μl	0·53 (0·02)	0·52 (0·01)	0·57 (0·05)	0·54 (0·02)	0·50 (0·04)	0·55 (0·04)	0·47 (0·01)	0·48 (0·01)	0·54 (0·03)
25 μl	0·34* (0·05)	0·60 (0·02)	0·59 (0·05)	0·44 (0·03)	0·59 (0·03)	0·58 (0·02)	0·22* (0·06)	0·64 (0·09)	0·56 (0·08)
50 μl	0·02* (0·01)	0·19* (0·10)	0·89* (0·13)	0·10* (0·03)	0·44 (0·03)	0·59 (0·02)	0·13* (0·03)	0·40 (0·04)	0·61 (0·04)
100 μl	−0·02* (0·01)	0·47 (0·04)	0·66 (0·05)	−0·09* (0·04)	0·44 (0·07)	0·62 (0·02)	−0·07* (0·05)	0·53 (0·15)	0·67 (0·02)

* Significant difference between EMS-treated lines and controls for a given ploidy level on a given day (independent contrasts; $P < 0{\cdot}0001$).
[a] Standard errors based on three replicates are indicated in parentheses below the means.

or for treated cell lines (based on a quadratic fit). Cell densities estimated from the 14 h samples for all the treated lines appeared to result in a change in the projected growth rate (Fig. 3), suggesting that cell density was overestimated at this point. When this sampling point was removed, the quadratic fit improved for the treated haploid lines ($P = 0{\cdot}002$, 0·0007, 0·0009) and two of the three treated diploid lines showed a significant quadratic component ($P =$ 0·14, 0·02, 0·0006). Averaging across cell lines, the effect was significant for both ploidy levels (haploids: $P < 0{\cdot}0001$; diploids: $P = 0{\cdot}009$). The shape of these curves provides evidence that EMS created genetic variation in r within the populations, creating the opportunity for selection to act to increase the average intrinsic growth rate over time.

Differences in cell morphology and replication patterns were also observed in the treated lines. At 8 h, treated diploid cells appeared to be increasing in volume without cell division. Over the next few sampling periods, some treated diploid cells were cigar-shaped, and many formed strings of cells, indicating that some cell division had occurred. Normal buds were not observed until 14 h (compared with 4 h for the diploid controls). Similar observations were made with the treated haploids. Only after about 24 h did most cells in the treated cultures appear normal, although at this point treated haploid cells appeared to be more similar in size to the diploid controls than the haploid controls.

(iii) *Varying EMS concentrations using auxotrophic strains*

The observation that EMS-treated haploid cells appeared to be reaching a carrying capacity more characteristic of diploid than haploid cells called into question the stability of the ploidy levels. Since carrying capacity is a function of cell size in yeast, it is possible that haploids were diploidizing in response to treatment with the mutagen either through mating-type switching and subsequent mating or through endo-diploidization. In this third experiment, haploid (BM1N), diploid (BM2N) and tetraploid (BM4N) strains were used that contain mutations at the mating-type locus and deletions in the pheromone receptor locus, which should ensure that mating does not occur. Meiosis also should not be possible because the presence of gene products from both MATa1 and MATα2 are required to turn off transcription of a repressor gene, RME1 (Kassir & Simchen, 1991). The experiment was extended to include tetraploids, which, if masking increases tolerance to deleterious mutations, would be predicted to be least affected by the mutagen but to recover most slowly. In this experiments, four treatment levels of EMS were used – 0, 25, 50 and 100 μl – each with threefold replication per strain. Cultures were serially propagated every 24 h to provide growth rate estimates over a 3 day period.

Growth rate in this experiment was estimated from formula (2) using counts at 0 and 12 h on the first day and counts at 0 and 6 h on subsequent days (Table 2). For each replicate, cell density after 24 h on the third day of sampling was used as an estimate of K. To be conservative, r was estimated at a later point in time on the first day to compensate, in part, for the possibility that the washes associated with EMS treatment cause a lag in growth for both control and treated lines. The effects of EMS treatment on growth rates were assessed by comparing EMS-treated lines with controls for each ploidy level using independent contrasts. Haploids and tetraploids on the first day

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had significantly lower growth rates than their controls at all dosages of EMS used ($P < 0{\cdot}01$ at 25 μl EMS; $P < 0{\cdot}0001$ at the higher dosages), whereas diploids showed reduced growth rates compared with their controls at 50 and 100 μl EMS ($P < 0{\cdot}0001$) but not at 25 μl EMS. On the second and third days, growth rates of haploids treated with 25 and 100 μl EMS, and those of diploids and tetraploids at all concentrations of EMS, were not significantly different from their controls (i.e. these cell lines had returned to control growth rates). However, growth rates of haploids treated with 50 μl EMS were still significantly lower than controls on the second day ($P < 0{\cdot}0001$), though by the third day, the growth rate of these cell lines had significantly surpassed that of their controls ($P < 0{\cdot}0001$).

The effects of ploidy level on response to EMS were also assessed using Tukey's HSD test on experimental growth rates measured relative to control growth rates. On the first day, at a dosage of 25 μl EMS, haploids, diploids and tetraploids grew at 64%, 82% and 47% of their control growth rates, respectively; the difference in relative growth rate between diploids and tetraploids was significant ($P < 0{\cdot}01$) but the other differences were not. At a dosage of 50 μl EMS, haploids, diploids and tetraploids grew at 5%, 18% and 27% of their control growth rates, and the haploid growth rate was significantly lower than both the diploid and the tetraploid growth rates ($P < 0{\cdot}01$; Tukey's HSD). At a dosage of 100 μl EMS, however, cell density declined over the first 12 h, generating negative growth rates for all ploidy levels. On the second day, at a dosage of 25 μl EMS, haploids, diploids and tetraploids grew at slightly but not significantly higher rates than their controls (115%, 118%, 133%, respectively), and there were no significant differences between their relative growth rates. At a dosage of 50 μl EMS, haploids, diploids and tetraploids grew at 36%, 87% and 84% of their control growth rates, respectively; only the difference between haploids and diploids was significant ($P < 0{\cdot}05$). At a dosage of 100 μl EMS, haploids, diploids and tetraploids grew at 91%, 86% and 100% of their control growth rates, respectively; the differences between them were not significant. Finally, on the third day, there were no significant differences in relative growth among the three ploidy levels at any concentration of EMS; all had returned to control growth rates.

Again, we can estimate the average dominance coefficients from these data. We concentrate on results from the first day of growth at dosages of 25 and 50 μl EMS, because the density estimates were lower and more error-prone in the 100 μl EMS treatment. For diploids, the dominance coefficient of wild-type alleles over mutant alleles is estimated as $0{\cdot}31 \pm 0{\cdot}11$ SE with 25 μl MS and $0{\cdot}43 \pm 0{\cdot}02$ SE with 50 μl EMS. These estimates are similar to those obtained in the previous experiment and are again higher than expected from experiments where selection has been minimized (Korona, 1999*b*). We can also measure the dominance coefficient of tetraploids by comparing their fitness with haploids, obtaining $0{\cdot}61 \pm 0{\cdot}39$ with 25 μl EMS and $0{\cdot}19 \pm 0{\cdot}01$ with 50 μl EMS. These estimates are both high (recall that $h_{4N} = 0{\cdot}25$ for a mutant allele with no masking), suggesting that mutations are not strongly masked in tetraploids. One must be cautious, however, in interpreting these dominance estimates. It may be that the selective effects of deleterious mutations are more pronounced in tetraploids, because these cells are already highly stressed. Indeed, the selective effects of deleterious mutations have been shown to be more severe in yeast under stressful than under benign environmental conditions (Korona, 1999*a*). Consequently the strength of selection (s) of a mutation in tetraploids might not correspond to that value in haploids. A further caveat is that selection acting to eliminate strongly deleterious mutations from the haploid lines will cause us to overestimate the degree of dominance. A final potential complication is that the ploidy level of tetraploid lines may not have been stable over the first 12 h following EMS treatment (see below).

For each of the 3 days of serial propagation, the densities of cells after 24 h of growth are shown in Fig. 4. With the exception of diploid cells treated at the lowest dose of EMS (25 μl), all treated lines reached a significantly lower density after 24 h of growth on the first day ($P < 0{\cdot}05$). However, by the third day, all lines except the 50 μl treated haploids attained densities after 24 that were not significantly different from the appropriate controls.

As in the previous experiments, there was some indication that by the end of the experiment the haploid lines treated with 50 μl of EMS had lower densities than their controls. After the end of the third day, densities after 24 h of growth for the three haploid replicates treated with 50 μl EMS were $1{\cdot}2 \times 10^8$, $1{\cdot}7 \times 10^8$ and $9{\cdot}0 \times 10^7$ cells/ml compared with the average haploid control density of $2{\cdot}7 \times 10^8$ cells/ml. Conversely, some of the tetraploid lines appeared to approach higher densities than their controls (even though this result was non-significant). At the end of the third day, densities for the tetraploid replicates treated with 50 μl EMS were $2{\cdot}0 \times 10^8$, $1{\cdot}9 \times 10^8$ and $2{\cdot}2 \times 10^8$ cells/ml, which were all more similar to the diploid control densities ($1{\cdot}8 \times 10^8$ cells/ml) than the tetraploid control densities ($9{\cdot}1 \times 10^7$ cells/ml). To determine whether or not these changes may be the result of cell size and/or ploidy changes, cell volumes and nuclear densities were measured on the controls and the 50 μl EMS treatments from the end of the third day (72 h samples).

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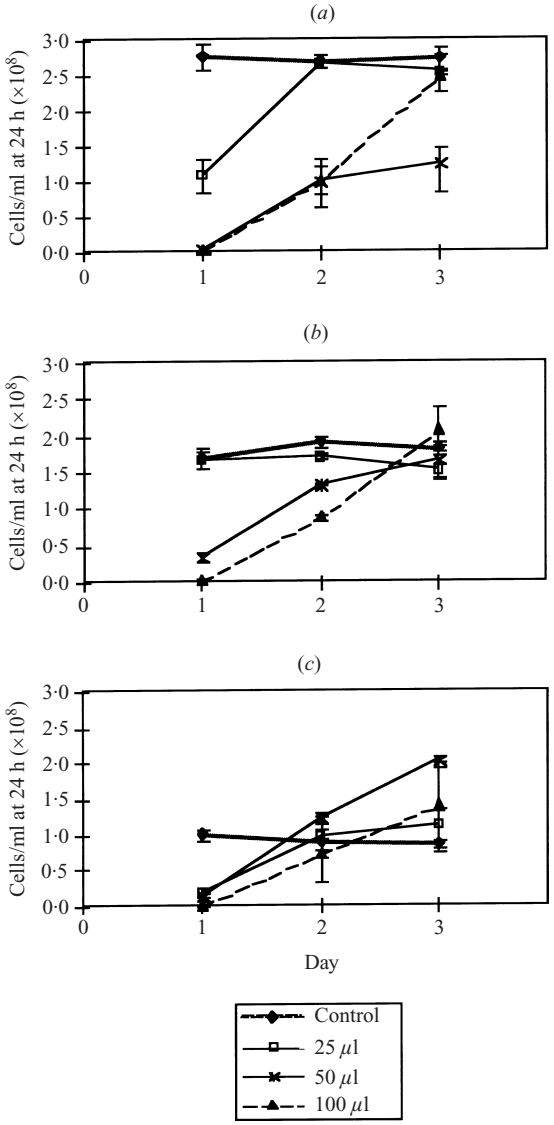

Fig. 4. Estimates of population size at 24 h in EMS-treated auxotrophic strains of yeast. Cell densities for (*a*) haploid (BM1N), (*b*) diploid (BM2N) and (*c*) tetraploid (BM4N) strains were estimated after 24 h of growth following transfer to fresh YPD medium. Measurements were repeated over three consecutive days after treatment with 0, 25, 50 or 100 μl of EMS. Bars represent ± 1 standard error based on three replicates per strain.

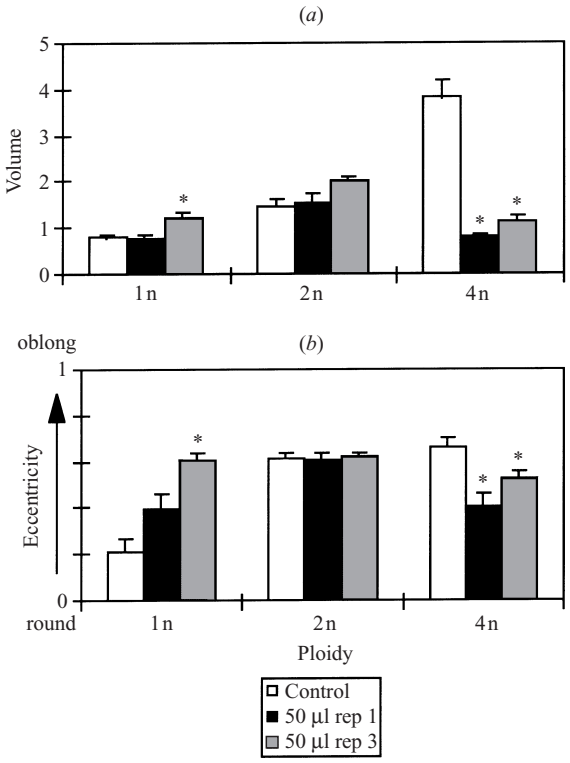

Fig. 5. Volume and eccentricity of haploid, diploid and tetraploid cells 72 h after treatment with 50 μl EMS. Means are shown in rectangles along with bars indicating 1 standard error based on raw measurements of cells. Statistical analyses based on $\log_e$ (volumes) and arcsin (eccentricity) indicated that some cell lines were significantly different from their controls in volume or shape (marked by an asterisk; $P < 0{\cdot}05$, Tukey's HSD). (*a*) Volumes. Units are in cubic millimetres as measured on photographs taken at $\times 400$ magnification (i.e. 1 mm^3 is equivalent to an actual cell volume of $(1/400)^3$ mm^3 = 15·63 μm^3). (*b*) Eccentricities.

(iv) *Volume and ploidy measurements in EMS-treated auxotrophic strains*

Cells from the end of the 72 h period described in the previous experiment were used to assess changes in cell volume and nuclear density. For the cell volume measurements, only controls (first replicate) and 50 μl EMS treatments (first and third replicates) from each of the haploid (BM1N), diploid (BM2N) and tetraploid (BM4N) strains were studied. Using equations (3) and (4), volume and eccentricity of the cells were measured (Fig. 5). Diploid cells did not change significantly in size or shape following EMS treatment. Cells from the third haploid replicate treated with 50 μl EMS were, however, significantly larger and more oblong than their controls and than the first replicate ($P < 0{\cdot}05$; Tukey's HSD). These cells were very similar in shape to diploids but were still significantly smaller than control diploid cells in volume ($P < 0{\cdot}05$; Tukey's HSD). Conversely, cells from both tetraploid replicates examined were significantly smaller and rounder than their controls, appearing to be similar to control haploids in volume but not quite as round in shape.

A FACScan analysis on PI-stained cells was performed to determine whether changes in cell volume and shape associated with EMS treatment were caused by changes in nuclear density and hence in ploidy level (e.g. Price *et al.*, 1983). The cells examined were sampled from all three replicates in experiment (c), 72 h after EMS treatment of haploid (BM1N), diploid (BM2N) and tetraploid (BM4N)

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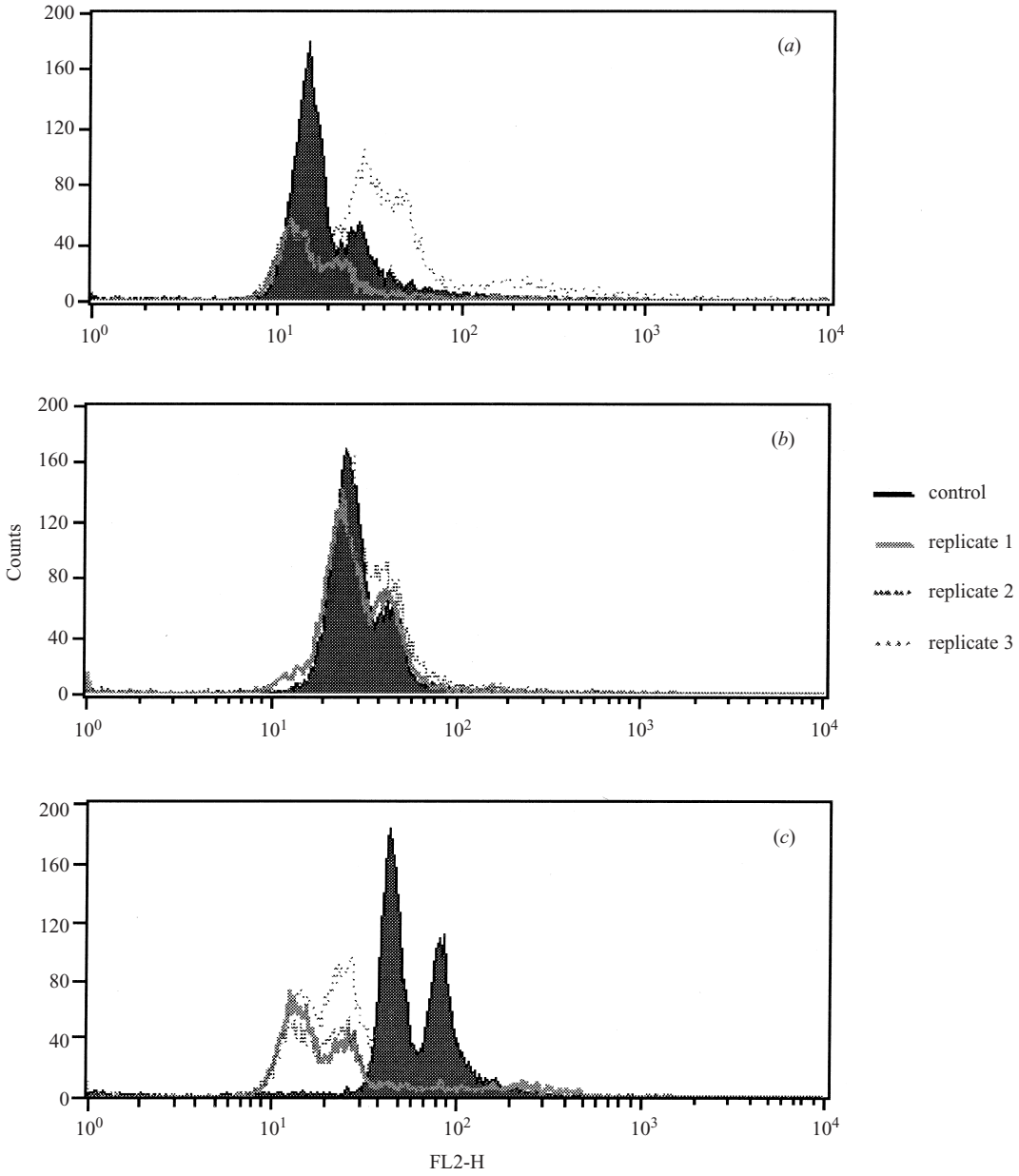

Fig. 6. Histogram showing results from the FACScan analysis of relative nuclear densities of (*a*) haploid, (*b*) diploid and (*c*) tetraploid cells treated with 50 μl EMS compared with their controls. Fluorescence intensity (FL-2H) is plotted on a log scale. For each cell line, the two peaks represent relative DNA content in non-dividing versus dividing cells. Control cell lines are indicated by the shaded histograms. Note that the third replicate of the treated haploid lines increased in DNA content relative to their controls, whereas all three replicates of the treated tetraploid lines decreased in DNA content relative to their controls.

lines. Again, only controls and 50 μl EMS treatments were studied. The results from the FACScan analysis are unambiguous (Fig. 6). Controls for each ploidy level showed two peaks corresponding to the nuclear densities of cells before and after DNA replication (Table 3). Treated diploid cells did not change in ploidy level and showed the same peak positions and relative frequencies as their controls. Treated haploids, however, showed more variable DNA content measurements. From Fig. 6 it is clear that one of the treated haploid replicates (no. 3) increased in nuclear density, probably becoming diploid, whereas the other two appeared to remain haploid. Treated tetraploids all decreased in nuclear density and did not show evidence of an octaploid peak (i.e. corresponding to tetraploid cells in the process of cell division), even though dividing cells were evident from haemacytometer observations.

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Table 3. *Nuclear density distribution based on FACScan analyses performed on haploid, diploid and tetraploid populations of controls and cells treated with 50 μl EMS*

			% ploidy peak[a]			
Ploidy	Treatment	Replicate no.	1n[b] (13·6)[c]	2n (26·0)	4n (47·8)	8n (90·0)
1N	0	1	48·8	32·7	0·0	0·0
	0	2	51·6	33·6	0·0	0·0
	0	3	52·4	38·9	0·0	0·0
	50	1	66·0	27·6	0·0	0·0
	50	2	41·4	47·0	0·0	0·0
	50	3	0·0	49·1	36·6	0·0
2N	0	1	0·0	54·7	42·4	0·0
	0	2	0·0	72·7	26·3	0·0
	0	3	0·0	78·7	19·9	0·0
	50	1	0·0	67·1	34·0	0·0
	50	2	0·0	55·7	39·6	0·0
	50	3	0·0	62·1	43·2	0·0
4N	0	1	0·0	0·0	59·3	27·2
	0	2	0·0	0·0	65·1	24·8
	0	3	0·0	0·0	60·7	31·2
	50	1	55·9	29·6	14·2	0·0
	50	2	48·7	38·9	11·7	0·0
	50	3	35·7	40·6	17·7	0·0

[a] For each FACScan, the percentage of the distribution that fell within the fluorescence peaks corresponding to haploid, diploid, tetraploid and octaploid DNA contents is reported (see also Fig. 6).
[b] The mean of the fluorescence peak for each ploidy level is indicated in parentheses. The range was determined by visual inspection of the minima between peaks in the controls.
[c] Note that one of three of the treated haploid lines appears to have diploidized, but the others have remained haploid.

4. Discussion

(i) *Mutagen experiments*

(a) *Overall results*

In three separate experiments, using both prototrophic and auxotrophic strains of *S. cerevisiae*, diploid strains gained an immediate fitness advantage compared with haploid strains following EMS treatment, presumably through masking of deleterious mutations. At the highest dosages of EMS (100 μl and 200 μl) none of the strains showed evidence of growth in the first 12 h following EMS treatment, so comparisons of relative fitness could not be determined. At the low and intermediate dosages of EMS (25 μl and 50 μl), diploids showed a reduced growth rate following EMS treatment compared with untreated control lines, but this effect was more pronounced in haploids, as predicted by the genetic masking hypothesis. If the increased resistance of diploids to mutations is primarily due to masking and if the strength of masking depends on the ratio of wild-type to mutant alleles, tetraploids should show the least reduction in fitness following EMS treatment. Results from our experiments using auxotrophic strains do not strongly support this prediction. Rather, mutations appeared to be masked to roughly the same extent in diploids and tetraploids (but see caveats below).

The last prediction we examined is that selection should be more efficient at purging deleterious mutations in haploids than in diploids. Because all lines rapidly recovered in fitness, it was difficult to detect differences in the rate of recovery among ploidy levels. Evidence of increasing growth rates through the initial 24 h recovery period (as determined by fitting a polynomial regression to the data) was only significant in treated haploids in the second EMS experiment, but the magnitudes of the quadratic coefficients were similar in the treated diploid lines. Overall, our results suggest that all cultures responded rapidly to selection, and there is no strong evidence that haploids responded more rapidly because mutations were not masked.

(b) *Caveats*

There are several factors that must be taken into consideration in interpreting these results. First and

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foremost, ploidy levels most likely changed over time in many of the cultures (see below), which makes it difficult to determine the extent to which growth and masking properties depend on the original ploidy level. In this regard, estimates of dominance coefficients for the auxotrophic strains may be more accurate, as these are based on growth rates within the first few hours after treatment. Secondly, the observed fitness reductions may be due to toxic effects of EMS rather than DNA damage and mutation (e.g. Sega, 1984), especially at low dosages where DNA damage may not be extensive. Furthermore, cells of different ploidy levels could be more or less sensitive to these toxic effects. For example, EMS may be more toxic for tetraploids because of their increased cell size. Another possibility is that, because gene expression patterns depend on ploidy level in yeast (Galitski *et al*., 1999) the phenotypic effects of mutations may vary among ploidy levels. For example, tetraploid cells express an endochitinase gene product (CTS1) at 12-fold higher rate than haploids, making the polyploid cells less likely to clump together (Galitski *et al*., 1999). Therefore, mutations that are deleterious only when cells are clumped may be more neutral in polyploids as an unexpected side-effect of ploidy-induced changes in gene regulation. Thus, while our data suggest that masking may have contributed to the increased tolerance of the higher ploidy levels to mutagens, other factors may have contributed to the observed effects.

Another complicating factor is that EMS may continue to induce mutations for several generations following treatment. Klein *et al*. (1989, 1990) performed a pedigree analysis of *S. cerevisiae* cells recovering from EMS damage and found that treated haploid cells continued to show elevated rates of lethal mutation for seven or eight generations following treatment. In fact, descendants were more at risk than cells that were in direct contact with the mutagen, especially at low dosages of EMS. These studies only examined the effects of mutagens on haploids, and it is not known whether similar long-term effects of EMS would be seen with other ploidy levels.

It has been suggested that delay of bud emergence after DNA damage may be due to active inhibition of DNA replication during periods of extensive DNA repair (Wintersberger & Karwan, 1987). In our experiments, prototrophic diploid cells treated with 50 μl EMS first showed evidence of normal budding after 14 h of growth whereas normal budding of treated haploid cells was not apparent until after transfer to fresh YPD medium i.e. after more than 36 h of growth). If 'recovered' diploids carry a larger mutational load at the initiation of DNA synthesis than haploids due to a shorter period of DNA repair, they might actually recover to a lower fitness state than haploids. Orthen *et al*. (1984) found that hidden genetic variability in fitness remained in diploid *S. cerevisiae* after 540 generations post EMS treatment (when fitness was assessed in environments other than those in which selection first took place).

A further complicating factor with the experiments on the prototrophic strains is that the mating-type locus is known to have pleiotropic effects on DNA repair. Durand *et al*. (1993) found increased survival and increased intragenic recombination in a/α heterozygotes compared with a/a homozygotes. Heude & Fabre (1993) found that diploid strains of yeast were more resistant to gamma rays than haploids cells, partly due to heterozygosity at the mating type locus. These problems are not applicable to the experiments on auxotrophic strains, because these were isogenic at the mating type locus.

Finally, reliable estimation of individual cell viability following EMS treatment would be helpful to determine the distribution of mutational effects that occur in the different ploidy levels. The standard methods of plating samples from liquid cultures onto solid medium are not very satisfactory for this purpose because the additional selection imposed by growth on plates could also differ by ploidy level, which would complicate the assessment of EMS effects alone. Alternative methods for estimating viability, such as incorporation of vital dyes or monitoring of individual cell pedigrees (e.g. Klein *et al*., 1990), could provide better solutions to this problem.

(ii) *Ploidy changes following EMS treatment*

(a) *Diploidization of haploid cells*

Although the original purpose of the experiment was to compare the fitness responses of haploids and diploids to treatment with a mutagen, observations in the preliminary experiments suggested that haploids could be diploidizing as a result of EMS treatment. Because the preliminary experiments were conducted on the 'prototrophic' strains with an intact mating type locus, the possibility existed that EMS induced mating type switching (through cassette switching and biased gene conversion: see Herskowitz & Jensen, 1991) that allowed mating within haploid cultures to produce diploids. Schiestl & Winterberger (1983) suggested that mating type interconversion in heterothallic yeast could be part of a general response to DNA damage that might allow increased tolerance to the effects of mutagens. Cells that switched mating type could also take advantage of the pleiotropic benefits of heterozygosity at the mating type locus observed by Durand *et al*. (1993) and Heude & Fabre (1993).

It is thus easy to imagine that haploid cells might benefit from mating to produce heterozygous diploids, both to gain increased protection from deleterious

mutations through masking and for increased DNA repair in cells that are heterozygous at the mating type locus. Indeed, in our experiments using prototrophic yeast, cell size increase in haploids was indirectly evident in all trials, with each replicate population of EMS-treated haploids approaching the carrying capacity typical of diploids. However, in our experiments using the isogenic auxotrophic strains that lacked functional mating type information, it should not have been possible for haploid cells to diploidize through mating type switching. Even if gene conversion between the MAT and the HMR or HML loci occurred in these strains to restore MAT locus information, the deletion at the mating type pheromone locus should have prevented mating. Nevertheless, even in the auxotrophic strains, one out of three haploid replicates treated with 50 μl EMS showed evidence of an increase in nuclear density (Fig. 6). These cells also became more diploid-like in cell shape and volume. In this case, diploidization through endomitosis rather than mating may have been the mechanism underlying the observed increase in nuclear density.

Previous studies have documented similar changes in ploidy level through endomitosis. Karpova *et al.* (1983) found that, in stored haploid samples of the Peterhof strain of *S. cerevisiae*, there were up to 70% autodiploids, which were homozygous for the mating type locus. Another possibility is that EMS may have induced cell cycle division (cdc) mutants (e.g. Hartwell *et al.*, 1973), which could lead to increases in ploidy level without mating. Hartwell *et al.* (1973) described a number of cell cycle mutants, some of which appeared very similar in morphology to the early stages of cell division that we observed in our initial EMS experiment (e.g. cells growing in chains rather than budding). In our study, chromosome gain was only apparent in the haploid strains. However, Whittaker *et al.* (1990) found that EMS also induced chromosome gain in both mitotically and meiotically dividing cells in diploid *S. cerevisiae*. Ploidy increases have also been found following UV treatment in a non-sporulating diploid strain of industrial *S. cerevisiae* (Sasaki, 1992). It may be that EMS is capable of inducing both types of diploidization, via mating type switching and via endomitosis, and that the reason that the prototrophic strains showed more evidence of diploidization is because they could exploit both mechanisms. Our experiments suggest that such ploidy changes may be relatively common in response to genetic damage.

(b) *Chromosome or genome loss in tetraploids*

Although diploid cell lines did not appear to switch ploidy levels, tetraploid cell lines were extremely unstable after EMS treatment. This phenomenon was initially suspected when tetraploid strains appeared to reach a higher carrying capacity than their controls. Volume measurements confirmed that cells were apparently decreasing in size (Fig. 5). The FACScan showed unambiguously that tetraploid cells were decreasing in nuclear content; these cultures showed no evidence of the octaploid peak that results from DNA replication in tetraploids. The fact that FACScans of the EMS-treated tetraploid lines showed peaks typical of haploid and diploid cells suggests that whole chromosome complements were lost rather than single chromosomes, but this was not directly tested. Since we used isogenic cell lines, it is possible that contamination of tetraploid cultures by haploid cell lines would not have been detected. However, this possibility seems unlikely as none of the control strains showed deviations from the expected ploidy peaks in the FACScan analyses. These results are consistent with other studies demonstrating that high ploidy level yeast are genetically unstable. For example, Mayer & Aguilera (1990) monitored the loss of chromosome 7 in strains of various ploidy levels and found that loss was 30-fold higher in triploids and 1000-fold higher in tetraploids than in diploids. They concluded that aneuploidy should be common among yeast with higher ploidy levels, but they did not consider reductions in ploidy level. Aneuploidy and/or polyploidy is widespread among industrial yeast but could be maintained by selection for enhanced dosage of important genes involved in the fermentation process (see Guijo *et al.*, 1997 and references therein).

(iii) *Experimental tests of ploidy evolution*

Our experiments support the hypothesis that diploids gain protection from the effects of deleterious mutations through masking, in that treated diploids generally had a higher growth rate than treated haploids even though they had twice the chance of bearing a deleterious mutation. We had expected haploids to recover more quickly, because each mutation would experience stronger selection than in diploids, where mutations are masked and persist for longer within the populations. A rather surprising result was that selection appeared to be extremely effective in all cultures, with growth rates returning to control levels within a few days. Using growth rate in batch cultures as fitness estimates, small differences remaining between the treated cell lines of the different ploidy levels could be difficult to detect. Nevertheless, the results of our experiment are consistent with purging of mutations in haploids; EMS-treated haploids showed a significant quadratic component in growth rates estimated over a 24 h time period. Furthermore, estimates of dominance coefficients are higher in our experiment than in mutation-accumulation experi-

ments in yeast, which could be explained by a rapid purging of deleterious mutations in haploids (see below). However, EMS-treated diploids also responded rapidly to selection, and there is not sufficient evidence that purging in diploids was significantly less effective than in haploids.

In our experiments, the average dominance level for mutations in diploids was quite high, averaging around 0·35. This is most likely an overestimate for two reasons. First, severe mutations would be rapidly eliminated from the haploid populations where they were not masked, that is, purging may have been faster and more effective in haploids, which would lead to an upwardly biased estimate of h. Second, some of the haploid lines diploidized during the course of the experiment, which would have caused an upwards bias in our estimate of h. Indeed, estimates by Korona (1999*b*) on the dominance level of deleterious alleles from a mutation-accumulation experiment indicated that h is nearer 0·08. In either case, the fact that h is less than 0·5 suggests that, in predominantly outcrossing populations, there would be a selective force due to masking deleterious mutations, which favours longer diploid phases and shorter haploid phases (Crow & Kimura, 1965; Kondrashov & Crow, 1991; Perrot *et al*., 1991; Otto & Goldstein, 1992; Jenkins & Kirkpatrick, 1995). In predominantly asexual or selfing populations, however, h has to be much lower for diploidy to be favoured (Otto & Goldstein, 1992; Jenkins & Kirkpatrick, 1995; Otto & Marks, 1996). Depending on the exact outcrossing rate, dominance values within the range of 0·08–0·5 may be high enough that selection actually favours organisms that spend more time in the haploid phase. Variation in outcrossing rates and average dominance coefficients might help explain why the extent of the haploid and diploid phases of life cycles varies greatly among organisms (Raper & Flexer, 1970; Bell, 1994).

Another unexpected result from our study was the finding that ploidy changes following EMS treatment occurred in both haploids and tetraploids but not, apparently, in diploids. In other words, diploidy appeared to be the more stable ploidy level in *S. cerevisiae*. This may simply be because *S. cerevisiae* is typically diploid and has evolved adaptations to this ploidy level. (Alternatively, it may be that *S. cerevisiae* is typically diploid because this state is, for some reason, more stable.) It would thus be interesting to perform similar EMS experiments with other yeast, such as *Schizosaccharomyces pombe*, which are normally haploid. The influence of the evolutionary history of an organism on life cycle evolution may be important but is easily overlooked. For instance, V. Perrot (personal communication) compared relative fitness of haploid and diploid yeast (as assessed by competition against a common competitor) using *S. cerevisiae* and *S. pombe*. She found that diploid *S. cerevisiae* had an overall advantage over haploid *S. cerevisiae* under both rich and poor media conditions, while haploid *S. pombe* had an overall competitive advantage over diploid *S. pombe*. She concluded that the ploidy history of the species and not nutrient conditions were the most important factor determining the outcome of competition. Consequently, in seeking to understand the forces shaping life cycle evolution, we must remember that 'we are only able to observe the responses of the haploids and diploids of a species which undoubtedly has undergone considerable evolution since the origin of the diploid phase' (Adams & Hansche, 1974).

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References

Adams, J. & Hansche, P. E. (1974). Population studies in microorganisms. I. Evolution of diploidy in *Saccharomyces cerevisiae*. *Genetics* **76**, 327–338.

Bell, G. (1994). The comparative biology of the alternation of generations. *Lectures in Mathematics for the Life Sciences* **25**, 1–26.

Burtscher, H. J., Cooper, A. J. & Couto, L. B. (1988). Cellular response to DNA damage in the yeast *Saccharomyces cerevisiae*. *Mutation Research* **194**, 1–8.

Carlson, T. (1913). Über Geschwindigkeit und Grösse der Hefevermehrung in Würze. *Biochemische Zeitschrift* **57**, 313–334.

Carter, B. L. A. & Sudbery, P. E. (1980). Small-sized mutants of *Saccharomyces cerevisiae*. *Genetics* **96**, 561–566.

Crow, J. F. & Kimura, M. (1965). Evolution in sexual and asexual populations. *American Naturalist* **99**, 439–450.

Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. (1998). Rates of spontaneous mutation. *Genetics* **148**, 1667–1686.

Dunnett, C. W. (1955). A multiple comparisons procedure for comparing several treatments with a control. *Journal of the American Statistical Association* **50**, 1096–1121.

Durand, J., Birdsell, J. & Wills, C. (1993). Pleiotropic effects of heterozygosity at the mating-type locus of the yeast *Saccharomyces cerevisiae* on repair, recombination and transformation. *Mutation Research* **290**, 239–247.

Galitski, T., Saldanha, A. J., Styles, C. A., Lander, E. S. & Fink, G. R. (1999). Ploidy regulation of gene expression. *Science* **285**, 251–254.

Guijo, S., Mauricio, J. C., Salmon, J. M. & Ortega, J. M. (1997). Determination of the relative ploidy in different

Saccharomyces cerevisiae strains used for fermentation and 'flor' film ageing of dry sherry-type wines. *Yeast* **13**, 101–117.

Haldane, J. B. S. (1924). A mathematical theory of natural and artificial selection: I. *Transactions of the Cambridge Philosophical Society* **23**, 19–41.

Hartwell, L. H., Mortimer, R. K., Culotti, J. & Culotti, M. (1973). Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. *Genetics* **74**, 267–286.

Herskowitz, I. & Jensen, R. E. (1991). Putting the HO gene to work: practical uses for mating-type switching. *Methods in Enzymology* **194**, 132–146.

Heude, M. & Fabre, F. (1993). a/alpha-Control of DNA repair in the yeast *Saccharomyces cerevisiae*: genetic and physiological aspects. *Genetics* **133**, 489–498.

Hughes, J. S. & Otto, S. P. (1999). Ecology and the evolution of biphasic life cycles. *American Naturalist* **54**, 306–320.

Jenkins, C. D. & Kirkpatrick, M. (1995) Deleterious mutations and the evolution of genetic life cycles. *Evolution* **49**, 512–520.

Karpova, T. S., Gordenin, D. A., Andrianova, V. M. & Inge-Vechtomov, S. G. (1983). Genetic rapid test for distinguishing between haploids and autodiploids in *Saccharomyces* yeast. *Genetika* **19**, 1934–1940.

Kassir, Y. & Simchen, G. (1991). Monitoring meiosis and sporulation in *Saccharomyces cerevisiae*. *Methods of Enzymology* **194**, 94–110.

Kilbey, B. & Hunter, F. (1983). Factors affecting mutational yield from EMS exposure of yeast (*S. cerevisiae*). *Mutation Research* **122**, 35–38.

Klein, F., Karwan, A. & Wintersberger, U. (1989). After a single treatment with EMS the number of non-colony-forming cells increases for many generations in yeast populations. *Mutation Research* **210**, 157–164.

Klein, F., Karwan, A. & Wintersberger, U. (1990). Pedigree analysis of yeast cells recovering from DNA damage allow assignment of lethal events to individual post-treatment generations. *Genetics* **124**, 57–65.

Kohalmi, S. E. & Kunz, B. A. (1988). Role of neighboring bases and assessment of strand specificity in ethylmethanesulfonate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis in the SUP4-O gene of *Saccharomyces cerevisiae*. *Journal of Molecular Biology* **204**, 561–568.

Kondrashov, A. S. & Crow, J. F. (1991). Haploidy or diploidy: which is better? *Nature* **351**, 314–315.

Korona, R. (1999*a*). Genetic load of the yeast *Saccharomyces cerevisiae* under diverse environmental conditions. *Evolution* **53**, 1966–1971.

Korona, R. (1999*b*). Unpredictable fitness transitions between haploid and diploid strains of genetically loaded yeast *Saccharomyces cerevisiae*. *Genetics* **151**, 77–85.

Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal number of replication. *Biometry* **12**, 307–310.

Lee, G. S.-F., Blonsky, K. S., Lee van On, D., Savage, E. A., Morgan, A. R. & Borstel, R. C. v. (1992). Base alterations in yeast induced by alkylating agents with differing Swain-substrate constants. *Journal of Molecular Biology* **223**, 617–626.

Long, A. & Michod, R. E. (1995). Origin of sex for error repair. I. Sex, diploidy, and haploidy. *Theoretical Population Biology* **47**, 18–55.

Loprieno, N. (1966). Differential response of *Schizosaccharomyces pombe* to ethyl methanesulfonate and methyl methanesulfonate. *Mutation Research* **3**, 486–493.

Mable, B. K. & Otto, S. P. (1998). The evolution of life cycles with haploid and diploid phases. *BioEssays* **20**, 453–462.

Mayer, V. W. & Aguilera, A. (1990). High levels of chromosome instability in polyploids of *Saccharomyces cerevisiae*. *Mutation Research* **231**, 177–186.

Nasim, A. & Auerbach, C. (1967). The origin of complete and mosaic mutants from mutagenic treatment of single cells. *Mutation Research* **4**, 1–14.

Orthen, E., Lange, P. & Wöhrmann, K. (1984). The effect of newly induced mutations on the fitness of genotypes and populations of yeast (*Saccharomyces cerevisiae*). *Mutation Research* **129**, 237–335.

Otto, S. P. & Goldstein, D. B. (1992), J. C. (1992). Recombination and the evolution of diploidy. *Genetics* **131**, 745–751.

Otto, S. P. & Marks, J. C. (1996). Mating systems and the evolutionary transition between haploidy and diploidy. *Biological Journal of the Linnean Society* **57**, 197–218.

Perrot, V., Richard, S. & Valero, M. (1991). Transition from haploidy to diploidy. *Nature* **351**, 315–317.

Prakash, L. & Higgins, D. (1982). Role of DNA repair in ethyl methanesulfonate-induced mutagenesis in *Saccharomyces cerevisiae*. *Carcinogenesis* **3**, 439–444.

Prakash, L. & Sherman, F. (1973). Mutagenic specificity: reversion of iso-1-cytochrome *c* mutants of yeast. *Journal of Molecular Biology* **79**, 65–82.

Price, H. J., Sparrow, A. H. & Nauman, A. F. (1973). Correlations between nuclear volume, cell volume and DNA content in meristematic cells of herbaceous angiosperms. *Experientia* **29**, 1028–1029.

Raper, J. R. & Flexer, A. S. (1970). The road to diploidy with emphasis on a detour. *Symposia of the Society for General Microbiology* **20**, 401–432.

Renshaw, E. (1991). *Modelling Biological Populations in Space and Time*. Cambridge: Cambridge University Press.

Rose, M. D., Winston, F. & Hieter, P. (1990). *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Sasaki, T. (1992). Induction of ploidy level increments in an asporogenous industrial strain of the yeast *Saccharomyces cerevisiae* by UV irradiation. *Applied Environmental Microbiology* **58**, 948–952.

Sazer, S. & Sherwood, S. W. (1990). Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fission yeast. *Cell Science* **97**, 509–516.

Schiestl, R. & Wintersberger, U. (1983). Induction of mating type interconversion in a heterothallic strain of *Saccharomyces cerevisiae* by DNA damaging agents. *Molecular and General Genetics* **191**, 59–65.

Sega, G. A. (1984). A review of the genetic effects of ethyl methanesulfonate. *Mutation Research* **134**, 113–142.

Sherman, F. (1991). Getting started with yeast. *Methods in Enzymology* **194**, 3–20.

Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.

Tukey, J. W. (1953). *The Problem of Multiple Comparisons*. Princeton, NJ: Princeton University.

van Zeeland, A. A., Mohn, G. R., Aaron, C. S., Glickman, B. W., Brendel, M., de Serres, F. J., Hung, C. Y. & Brockman, H. E. (1983). Molecular dositometry of the chemical mutagen ethyl methanesulfonate: quantitative comparison of the mutagenic potency of *Neurospora crassa* and *Saccharomyces cerevisiae*. *Mutation Research* **119**, 45–54.

Whittaker, S., Moser, S., Maloney, D., Piegorsch, W., Resnick, M. & Fogel, S. (1990). The detection of mitotic and meiotic chromosome gain in the yeast *Saccharomyces cerevisiae*: effects of methyl benzimidazol-2-yl carbamate, methyl methanesulfonate, ethyl methanesulfonate, dim-

ethyl sulfoxide, propionitrile and cyclophosphamide-monohydrate. *Mutation Research* **242**, 231–258.

Wilkinson, L. E. & Pringle, J. R. (1974). Transient G1 arrest of *S. cerevisiae* cells of mating type α by a factor produced by cells of mating type a. *Experimental Cell Research* **89**, 175–187.

Winston, F. (1992). Mutagenesis of yeast cells. *Current Protocols in Molecular Biology* **Suppl. 12**.

Wintersberger, U. & Karwan, A. (1987). Retardation of cell cycle progression in yeast cells recovering from DNA damage: a study at the single cell level. *Molecular and General Genetics* **207**, 320–327.