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Mismatch repair and the accumulation of deleterious mutations influence the competitive advantage of *MAT* (mating type) heterozygosity in the yeast *Saccharomyces cerevisiae*

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Summary

Competitions between matched pairs of diploid strains of *Saccharomyces cerevisiae*, one capable of undergoing sexual recombination (*MAT*-heterozygous) and the other not (*MAT*-homozygous), have proved useful for measuring the effects of mitotic and meiotic recombination and DNA repair on competitive ability in this organism. Overall competitive differences between the strains can be enhanced by converting them to petites (aerobic respiration incompetent). Here we report the results of competitions between pairs of strains that also differ in their ability to undergo mismatch repair. In petite strains, the growth rates of mismatch-repair defective strains declined over time regardless of their *MAT* genotype. Mismatch-repair proficient *MAT*-heterozygous strains did not show a decline, while repair-deficient *MAT*-homozygous strains did. The decline appears to be due to the accumulation of deleterious mutations of small effect, which can be corrected by *MAT*-heterozygous strains having intact mismatch repair. The relative competitive abilities of *MAT*-heterozygous and *MAT*-homozygous strains diverged during the course of the competitions, and the variance of this divergence increased significantly when mismatch repair was defective. This large stochastic component indicates that a relatively small number of deleterious mutations may be involved. The accumulation of deleterious mutations and their subsequent repair may have a bearing on the origin of sex in this organism.

1. Introduction

Because of its prevalence, it is obvious that sexual reproduction must be advantageous, but there is as yet little experimental evidence bearing directly on its benefits. There is, however, no shortage of theories (reviewed in Ghiselin, 1974; Williams, 1975; Maynard Smith, 1978; Bell, 1982; Stearns, 1987; and Michod & Levin, 1988). One explanation for the advantage of sex is that it may increase the relative rate of evolutionary change towards higher fitness. Fisher (1930) pointed out that in a genetically heterogeneous population of asexual clones, any new advantageous mutation would be likely to arise in one of the less fit clones. If, however, such a mutation arose in a similarly heterogeneous sexual population, even if it appeared initially in a less fit organism, it might subsequently be recombined into more advantageous genetic backgrounds. Muller (1932) gave a simpler argument that reached the same conclusion, but did not consider the complicating effects of genetic heterogeneity in the population. Later, Muller (1964) examined the consequences of deleterious mutations, and pointed out that asexual populations are locked into a ratchet, unavoidably undergoing decreases in fitness as the least loaded lines are lost. Many variants and elaborations of these and other models have been constructed.

Saccharomyces cerevisiae is an ideal organism for observing the effects of sexual reproduction because, while its asexual phase is normally haploid, asexual diploids can be produced (Herskowitz & Jensen, 1991). This allows for comparisons to be drawn between strains that are capable or incapable of undergoing meiosis, without the confounding effects of differences in ploidy. We have constructed and competed pairs of strains of *S. cerevisiae* that differ

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only in their ability to undergo sexual recombination (Birdsell & Wills, 1996). These strains are heterozygous or homozygous at the *MAT* locus, but no other alterations have been made to the genome. A round of meiotic recombination in a strain that is both capable of meiosis and has a heterozygous genetic background improves its competitive advantage over an otherwise isogenic strain that is incapable of meiosis. In the same paper, we showed that a similar round in a meiosis-capable strain with a homozygous genetic background does not change its competitive ability when measured against an isogenic meiosis-incapable strains.

This experimental system also allowed us to go a step further and ask whether the *ability* to undergo sexual reproduction, which in this organism can be divorced from the process itself, might result in an advantage. Our initial results showed that *even without a round of recombination*, the meiosis-capable strains outcompete the matched meiosis-incapable ones.

The fact that heterozygosity at *MAT* confers advantages even without meiotic recombination may simply mean that heterozygosity for the very different *MATa* and *MAT*α alleles confers an extreme single-gene heterosis. But it also suggests that sexual reproduction in *S. cerevisiae* might not have evolved entirely as a result of the benefits conferred by meiotic recombination. *MAT*-heterozygous strains that were isogenic at all other loci showed enhanced performance in seven of eight properties measured, including mitotic intragenic recombination, DNA repair, and the ability to be transformed with exogenous DNA (Durand *et al*., 1993).

Finally, *MAT*-heterozygous strains that have a heterozygous genetic background will overtake otherwise isogenic *MAT*-homozygous strains more quickly than when the matched pair has a homozygous genetic background. This suggests that at least some of the heterozygous advantage of *MAT* is dependent on the presence of more than one allele at other loci (Birdsell & Wills, 1996). In those experiments, however, we were unable to distinguish between the two possibilities that these heterozygous-background *MAT*-heterozygous strains gained their advantage either from rapid rearrangement of existing genetic variation to produce more favourable genotypes through mitotic gene conversion (Sherman & Roman, 1963) or from rapid removal of deleterious mutations through the same process. We suspected that the latter might be more likely, but had no proof.

Given the probable large role of the repair of deleterious mutations in both the origin and maintenance of sex, we decided to examine the effects of DNA repair on the competitive advantage of the ability to undergo sexual recombination.

DNA repair may play a fundamental role in the evolution of sex and recombination (Bernstein *et al*., 1981). Further, the diploid, or sexual, stage of a life cycle can offer the organism at least temporary protection against the accumulation of recessive deleterious mutations. The transition from haploidy to diploidy depends on both the mutation rate (Kondrashov & Crow, 1991) and the degree of harmfulness of the mutation (Perrot *et al*., 1991).

Here we examine the effects of a mutational defect in mismatch repair, and the effects of converting the cells from grande to petite. We competed two pairs of *MAT*-homozygous and *MAT*-heterozygous strains of *S. cerevisiae*, isogenic except at the *MAT* locus. Their genetic backgrounds differed from those of the strains employed in the study of Birdsell & Wills. One set of strains was homozygous wild-type at the *PMS1* locus and the other was a homozygous mutant. The *pms1* allele affects mismatch repair (Kramer *et al*., 1989*a*, *b*; Strand *et al*. 1994). Since DNA repair is influenced by the ability to recombine, a *MAT*-homozygous yeast strain with mismatch repair intact should be at a double disadvantage: (*a*) it can no longer recombine meiotically to eliminate harmful mutations, and (*b*) its ability to recombine mitotically is reduced (Durand *et al*., 1993).

Use of these strains allowed us to test three predictions. First, if deleterious mutations are playing a role in the observed difference between the competitive abilities of *MAT*-heterozygous and *MAT*-homozygous strains, then when mismatch repair is intact *MAT*-homozygous strains should decrease in competitive ability with time, relative to the *MAT*-heterozygous strains that are better able to repair them. We found this effect in petite (aerobic respiration incompetent) strains but not grande (respiration competent) strains. In Section 4 we address possible reasons for the differences in outcome between petites and grandes.

Second, if this type of repair is important in changes in the competitive advantage of the *MAT*-heterozygous strain, then defective *MAT*-heterozygous strains should show a smaller relative increase in competitive advantage during competitions. This was not detected in either petite or grande strains, suggesting that this effect, if any, is small.

Third, when mismatch repair is defective, mutations that can be repaired by this system should accumulate more freely in both *MAT*-heterozygous and *MAT*-homozygous strains. This should increase the variance of the change in their relative competitive abilities during the course of the competitions. This effect was detected in competitions involving grande and petite strains.

Cells that are mutant for mismatch repair have higher mutation rates (Kolodner, 1995), and *pms1* cells show enhanced mutation rates even during mitotic division (Strand *et al*., 1994). Without recombination, a finite population can accumulate

mutations in a ratchet-like fashion as each least-loaded line is lost in succession (Muller, 1964). The same process might take place in an infinite (or very large) population, but for it to do so the mutation rate must be so high that all the members of the population begin to accumulate harmful mutations (Kondrashov, 1982). We were therefore interested in exploring the possibility that yeast strains lacking in mismatch repair might, if their mutation rate were sufficiently high, exhibit Kondrashov's variant of the Muller ratchet. Our finding of a slowing of growth rate suggests that the Kondrashov effect is operating even in petite *MAT*-homozygous strains that are able to undergo mismatch repair.

2. Materials and methods

(i) *Media*

Liquid YEPD consisted of 2% dextrose, 1% yeast extract and 2% Bacto Peptone (Difco, all w/v). Solid culture also contained 2% Bacto agar. Galactose medium contained 2% galactose, in place of dextrose.

(ii) *Strains*

We began with *PMS1* (M7S1) and *pms1* (AMY101) strains, kindly provided by T. Petes (Strand *et al.*, 1994), and used them to construct diploid pairs of sexual and asexual yeast, isogenic and homozygous at all loci except at the *MAT* locus. For each strain, the haploid was transformed to the opposite mating type with a non-integrating pGAL-HO plasmid carrying a galactose-inducible *HO* (homothallism) gene. Introduction of this plasmid allowed for mating type interconversion (Herskowitz & Jensen, 1991; Haber, 1992). After this initial conversion, one *a* and one α haploid were mated to create a sexual diploid (a/α) with a homozygous background. The diploid sexual was then transformed to a diploid asexual (α/α) with the pGAL-HO plasmid. We did not examine the equivalent a/a stains, because previous work (Birdsell & Wills, 1996) had shown that the competitive abilities of otherwise matched a/a and α/α strains are indistinguishable. Competitions were begun immediately after these strains had been produced, to minimize any genetic damage that might have accumulated.

(iii) *Competitions*

We conducted two types of competition: *PMS1*/*PMS1* sexual (a/α) versus *PMS1*/*PMS1* asexual (α/α), and *pms1*/*pms1* sexual (a/α) versus *pms1*/*pms1* asexual (α/α). Each competition, consisting of eight replicates and one pair of controls in which the strains were simply transferred in separate tubes without competition, was initiated by mixing equal numbers of sexual and asexual cells in 5 ml of YEPD. The strains were allowed to reach plateau, after which 25 μl of the competed culture (approximately 5×10^6 cells) was transferred into 5 ml of fresh liquid medium. Each transfer culture went through approximately seven or eight cell doublings. Samples were taken and plated on 10 plates (to yield approximately 50 colonies per plate) at the start of the competitions and at each transfer. The plates were incubated at 30 °C for 48 h, after which they were sprayed with a fine mist of a *MAT a* assay strain sensitive to the mating pheromone of the opposite mating type. A halo of no growth formed around the α/α colonies, allowing them to be distinguished easily from the a/α colonies. It was therefore not necessary to introduce marker genes in order to determine the proportion of the two types of cell in a culture.

To model the competition we assume that differences in r, the intrinsic rate of growth, K, the carrying capacity, the lag phase, or some combination of all three, cause the differences between the strains. These three factors contribute to the components of competitive ability, C_s and C_a (for strains capable and incapable of undergoing sexual recombination respectively). Further, we assume that no frequency-dependent interaction occurs between the two strains. If the competitive abilities of the strains do not change over time, the proportion of the strain with the smaller C should diminish by a constant factor C_a/C_s each transfer, yielding after t transfers (Birdsell & Wills, 1996):

$$P_{a_t} = P_{a_{t-0}}\left[\frac{C_a}{C_s}\right]^t$$

or

$$P_{s_t} = 1 - \left(P_{a_{t-0}}\left[\frac{C_a}{C_s}\right]^t\right), \quad (1)$$

where P_a is the proportion of *MAT*-homozygous cells, P_s is the proportion of *MAT*-heterozygous cells, and t is the number of transfers. To account for possible changes in relative competitive ability, we used a modified version of this equation. After t transfers:

$$P_{a_t} = P_{a_{t-0}}\left[\frac{C_a}{C_s} - t\Delta\right]^t$$

or

$$P_{s_t} = 1 - \left(P_{a_{t-0}}\left[\frac{C_a}{C_s} - t\Delta\right]^t\right). \quad (2)$$

This second equation can be employed if t does not become too large, and Δ is small. This was true for

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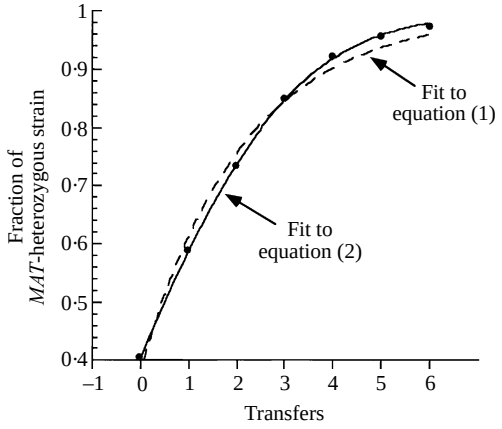

Fig. 1. Comparison of the fit of models, with and without changes in relative fitness, to *PMS1/PMS1* petite competition data. Eqn (1): $P_{s_t} = 1-(P_{a_{t=0}}[C_a/C_s]^t)$; eqn (2): $P_{s_t} = 1-(P_{a_{t=0}}[(C_a/C_s)-t\Delta]^t)$.

each competition. All the data were therefore fitted to both models, allowing comparison between competitions. Values of Δ significantly different from zero indicate that the ratio of C_a to C_s has not remained constant throughout a competition. Changes in the ratio are likely to be small, which would ordinarily make determination of their significance difficult. However, the significance of a non-zero Δ can be determined by examining the standard error of the estimates of Δ that are obtained for each replicate in a competition, and performing a *t*-test to see whether the average Δ is significantly different from zero.

The effect of fitting the two models to a representative set of data is shown in Fig. 1. These data, untransformed, are from the competition in which petite *PMS1/PMS1 MAT*-heterozygous and *MAT*-homozygous strains were competed. It can be seen that the first equation overestimates the rate at which the *MAT*-heterozygous strain displaces the *MAT*-homozygous strain at the outset of the competition, and underestimates the rate by the end of the competition. The addition of the Δ term improves the fit of the model to the data. The improved fit is not, by the usual criteria, significant, but Δ is significantly non-zero when each replicate is considered separately and the standard error of Δ is obtained.

Similar results were obtained when a different way of examining the data, employed in competition experiments by Lenski and co-workers (Lenski, 1991), was used. When the takeover of one strain by another is represented as the ratio of the number of colonies of the more rapidly growing strain divided by the number of colonies of the less rapidly growing strain, then a straight line should result if the logs of these ratios are plotted and the relative competitive abilities of the two strains have not changed. Substantial changes in the competitive abilities would be required to produce a significant departure from a straight line. However, as was done for the formulas above, we modified this relationship to yield:

$$R_t = [R_{t-0}(1+t\Delta)]^t, \quad (3)$$

where R is the ratio of sexual to asexual colonies. In the same sets of competitions as had yielded significant values of Δ in the previous analysis, this approach also yielded significant values.

For presentation of the data, the proportions of sexuals were transformed to the arcsine of the square root (angular transformation) (Sokal & Rohlf, 1981).

We found in both our sets of competitions that the *MAT*-heterozygous strain had a much larger relative competitive ability than the *MAT*-homozygous strain, unlike the strains used by Birdsell and Wills. Our competitions yielded a C_a/C_s of approximately 0·5. The sexual strains therefore displaced the asexual strains very rapidly. In an attempt to slow this rate of displacement, we converted each of the strains to petites by treating cells growing in log phase with 10 μg/ml ethidium bromide for 1 h (Goldring *et al.*, 1970; Wills & Phelps, 1975), then carried out the same competitions using the petite pairs of strains. Although some low levels of genetic heterogeneity may have been induced by the ethidium bromide treatment, the relative rankings of competitive abilities among the strains were not altered by this treatment.

(a) *Measurement of* r *and* K

Change in competitive ability could result from change in rate of growth, *r*, in carrying capacity, *K*, or some combination of both. The parameters C_a and C_s are a function of both these factors, though the contribution of each will depend on the shapes of the logistic growth curves of each strain, and these will change throughout the competitions. We measured *r* and *K* independently, in an attempt to determine which factor has the stronger effect on competitive ability. Measurement of these growth parameters has allowed us to distinguish more than one effect of the *MAT* locus on the various yeast strains.

We measured the plateau levels (*K*, cells/ml) attained by both the mutant and wild type, grande and petite, from samples taken at the beginning and end of each experiment, and from control samples that had been carried through the same number of transfers without competition. Because the sexual strains had effectively displaced the asexual strains by the end of the competitions, we were unable to compare the asexual strains at the beginning and end of the competitions, and we forced to examine any alterations in these strains by comparing them at the beginning and end of an equivalent number of transfers. Eight counts were made per strain.

The growth rates, *r*, of the grande cells were not measured because the carrying capacity, *K*, turned out

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to increase dramatically in the course of the experiment, apparently as a result of enhanced mitochondrial activity. We felt that given this very large effect estimates of r would be unreliable. In the petites, however, no significant effect of transfer on K could be detected. Each yeast strain was grown overnight in liquid culture, and 10^8 cells were inoculated into 100 ml flasks of fresh YEPD at 30 °C, and allowed to enter log phase growth. Samples of 1 ml were removed from the flask over a 2–3 h period, diluted into the same medium, and measured in a spectrophotometer (600 nm). Results were recorded as log (cell density) versus time.

We used slopes with a correlation coefficient of 0·99 or higher, and the data were then normalized among experiments, enabling us to pool the data from three separate experiments. The pooled r values were compared using a two-tailed t-test.

In addition, measurements of cell size were carried out using a Becton-Dickinson cell counter, to determine whether these results were due to changes in cell number or in cell size (see Section 3). Cells were grown to log phase, washed and resuspended in phosphate-buffered saline pH 7·0 (0·1 M KPO_4 with 0·7% (w/v) NaCl), and all the cells in 20 λ samples of this suspension were counted.

3. Results

(i) *Competitions*

In both the mutant and wild-type grande competitions, the sexual strains displaced the asexual strains almost completely within four transfers (Fig. 2). We found that we were able to extend the competitions to six transfers, and to reduce the great advantage of the sexual strains, by converting each pair of competing strains to petites (Fig. 2, Table 2). This manipulation enabled us to detect alterations in competitive ability over time that appeared not to be present in the grande competitions, or alternatively might not have been detectable because of the rapid rate of takeover by the sexual strains. Because Δ was positive in the petite competitions, either the *MAT*-heterozygous strain increased in competitive ability, the *MAT*-homozygous strain decreased, or both, in the course of the competitions. In contrast, the grande mutant competition did not exhibit a significant Δ, and in the grande wild-type competition Δ was slightly negative.

Significant effects of the ability to repair mismatches were seen. Although the mutant pair of strains did not show any significant difference from the wild-type pair in the mean value of C_a/C_s or the mean value of Δ (t-test, $P > 0{\cdot}05$), both the grande and petite repair-defective competitions exhibited a significantly greater variance in Δ than did the wild-type competitions (F-statistic, Table 1). The variance of C_a/C_s was also

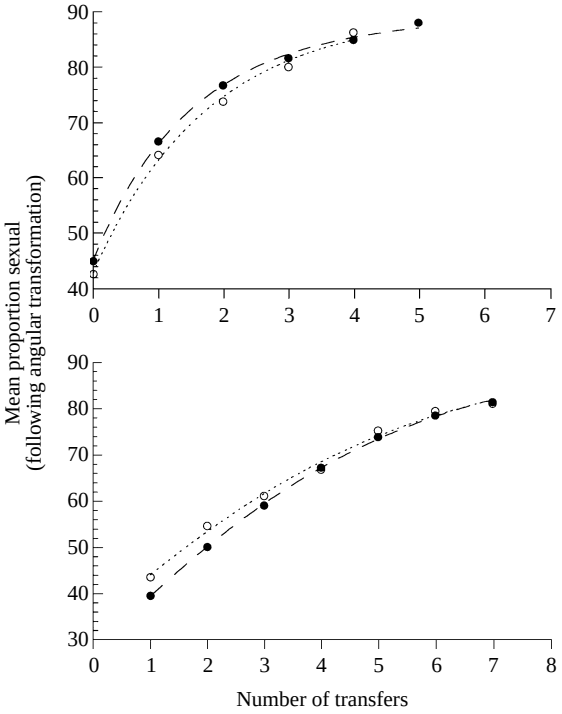

Fig. 2. Results of the competitions between matched pairs of strains capable of undergoing sexual recombination (*MAT* a/α) and strains that are not (*MAT* α/α), one pair damaged in mismatch repair (homozygous for *pms1*) and the other pair wild-type (homozygous for *PMS1*). (—●—, Wild-type sexual *v.* asexual; · · ○ · · ; mutant sexual *v.* asexual). These strains were competed as both grandes and petites. Above: In the grande competitions the steep curve indicates the high competitive ability of the sexual strains. Below: In the petite competitions the competition was extended and the difference in competitive ability was reduced.

larger in both the competitions involving mutant strains, though not significantly so. In the grande competition the change in competitive ability over time was centred around zero, whereas in the petite competition, the values were greater than zero for each sample (Fig. 3). There was one extreme outlier from a mutant competition ($\Delta = 0{\cdot}11$) which we did not use in the statistical analyses, although it would have increased the significance of the differences.

It is unlikely that these changes in relative competitive abilities are due to a differential alteration in cell size between the two strains during the course of the competitions. No significant differences were seen in these strains at the beginning and end of the competitions when cell sizes were measured using the cell-sorter (data not shown). A more extensive experiment, however, shows that repeated serial transfers do eventually have an effect on cell size. Five lines each of four strains of grande cells with genotypes *MAT* a/α, *MAT* α/α, *MAT* a and *MAT* α were transferred for a total of 82 generations. Fig. 4 shows that while there was no significant change in the number of cells per OD_{600} for the first 30 generations

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Table 1. *Comparison of competitive abilities of the strains over the course of both the grande and petite competitions*

Competition	Mean competitive ability C_a/C_s at outset of competition of $MAT\alpha/\alpha$ strains relative to $MATa/\alpha$ strains ± SE	*F*-statistic comparing variances of C_a/C_s between mutant and wild-type	Mean change in advantage of $MATa/\alpha$ strain per transfer, Δ ± SE[a]	*F*-statistic comparing variances of Δ between mutant and wild-type	Mean correlation coefficient, *r*, for the model
Grande					
Mutant	0·561 ± 0·057		−0·014 ± 0·009		0·9979
		2·469, d.f. = 6, 7	NS	7·45, d.f. = 6, 7	
Wild-type	0·520 ± 0·012	NS	−0·008 ± 0·003	$P < 0·02$	0·99925
			$t = -2·644$, d.f. = 7, $P = 0·0332$		
Petite					
Mutant	0·822 ± 0·018		0·01 ± 0·003		0·99665
		3·698, d.f. = 7, 7	$t = 3·325$, d.f. = 7, $P = 0·0127$	6·928, d.f. = 7, 7	
Wild-type	0·819 ± 0·009	NS	0·011 ± 0·001	$P = 0·02$	0·99998
			$t = 10·011$, d.f. = 7, $P = 0·0001$		

C_a/C_s and Δ were calculated for each individual sample and then combined for statistical analysis.
[a] Two-tailed *t*-test for difference from zero.

of transfer, by transfer 53 the variance among lines had increased. By transfer 82 the variance was substantial, although the overall mean cell size had not changed significantly through the course of the experiment.

(ii) *Measurement of carrying capacity* K (*plateau values*)

MAT-heterozygous strains reached much higher plateaus than *MAT*-homozygous strains. Further, in the grande competitions, the plateau values increased dramatically in the course of the competitions. The grande yeast strains of all four types (mismatch-repair mutant and wild-type *MAT*-homozygous, and wild-type *MAT*-heterozygous), which were serially transferred six times, showed increased plateau values. The cause of this rapid change, which was repeatable, remains to be determined, but it is notable that once this change had taken place over the first few transfers no further change was seen. Since no similar change was detected in the petite strains, we are investigating the possibility that this effect is due to a rapid increase in mitochondrial function in the grande strains.

The differences in initial plateau values of the grande strains showed significant effects of both *MAT* heterozygosity and the presence of the *pms1* gene (ANOVA, $F_{pms1} = 45·63$, $P = 0·0001$; $F_{MAT1} = 708·543$, $P = 0·0001$). After six transfers, only the *MAT*-heterozygous yeast strains yielded higher plateau values (ANOVA, $F_{pms1} = 2·215$, $P > 0·05$; $F_{MAT} = 70·34$, $P = 0·0001$).

The petite strains, in contrast, showed the same plateau values before and after transfers. The plateau values of the petite strains were influenced only by *MAT* homozygosity or heterozygosity, both before (ANOVA, $F_{MAT} = 29·37$, $P = 0·0001$) and after (ANOVA, $F_{MAT} = 12·536$, $P = 0·0014$) the six transfers.

(iii) *Growth rates*

Because the grande stains showed such large changes in *K*, we measured growth rate, *r*, for the petite competition only. The wild-type *MAT*-heterozygous strain showed no change in growth rate over time, whereas the wild-type *MAT*-homozygous strain showed a significant decrease in growth rate. In contrast, the *r* values for the mutant strains all decreased significantly over time (Table 2). As with the change in relative competitive values, these reductions in *r* cannot be attributed to changes in cell size.

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Table 2. *Comparison of growth rate*, r, *of the petite strains before and after six transfers, competed and non-competed*

	t-test comparing slopes of growth rates			
	MAT-hom	*MAT*-het		
	Initial vs final	Initial vs final	Initial vs competed	Final vs competed
Mutant				
pms1/pms1	3·668 $P = 0{\cdot}0013$ d.f. = 23	10·968 $P < 0{\cdot}0001$ d.f. = 17	6·2857 $P < 0{\cdot}0001$ d.f. = 23	−0·2542 NS d.f. = 17
Wild-type				
PMS1/PMS1	3·561 $P = 0{\cdot}0017$ d.f. = 22	−0·2397 NS d.f. = 23	1·7747 NS d.f. = 23	0·7575 NS d.f. = 23

MAT-hom, *MAT*-homozygous; *MAT*-het, *MAT*-heterozygous.
For each set of data, the results of three experiments were pooled after normalization. All significant differences from the beginning to the end of the experiment involved decreases in growth rate. There was no difference between the final *r* values of the competed and control *MAT*-homozygous strains (fourth column).

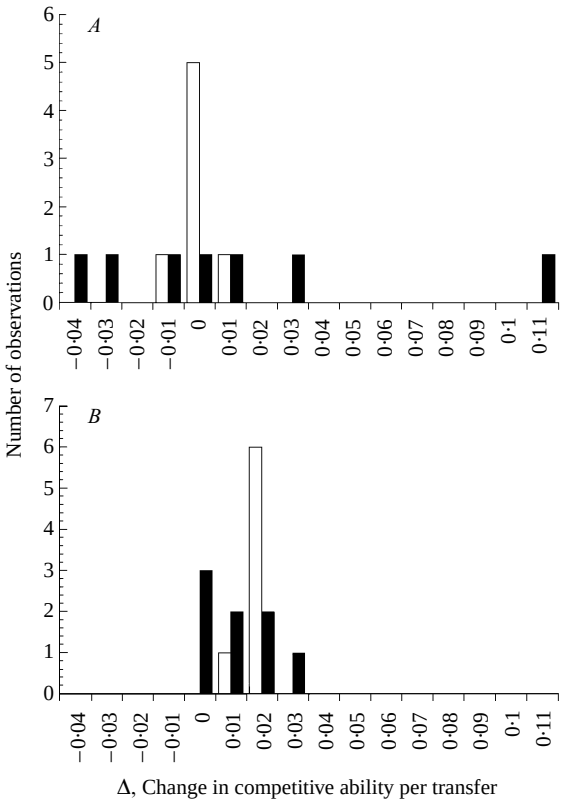

Fig. 3. Variation in the change in competitive abilities per transfer. (*A*) refers to competitions between grande strains (□, wild-type grande; ■, mutant grande), and (*B*) to competitions between petite strains (□, wild-type petite; ■, mutant petite). The grande strains have a mean not significantly different from zero, whereas the petite stains centre about a positive value. In both competitions the mutant has a greater amount of variation. The outlier in the grande competition is not included in the statistical analyses.

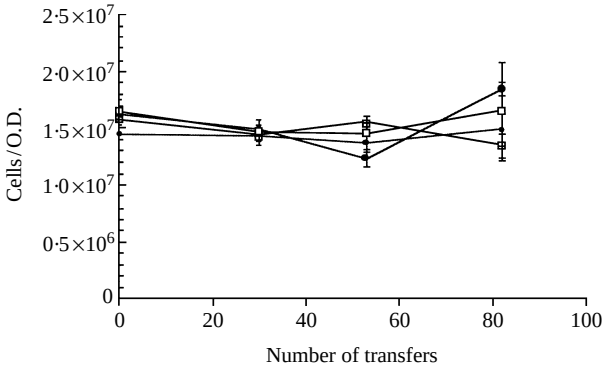

Fig. 4. Long-term transfer experiment in which grande cells of four different strains were transferred for 82 generations. Cell density was determined spectrophotometrically, and cell number by counting in a cell-sorter. —●—, *MAT* α/α diploid; —●—, *MAT* a/α diploid; —□—, *MAT* α haploid; —□—, *MAT* a haploid.

4. Discussion

(i) *The nature of the advantage of* MAT *heterozygotes*

The sexual yeast strains outcompeted the asexuals in each competition. Therefore, even when lacking *PMS1* activity, a sexual strain had an advantage. The absence of mismatch repair did not produce a significant difference in initial competitive abilities of the sexual and asexual strains.

We have attributed the nature of that initial difference to the very large number of pleiotropic effects exhibited by heterozygotes at the *MAT* locus (Durand *et al.*, 1993; Birdsell & Wills, 1996). It remains possible, however, that an intrinsic effect of

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homozygosity for *MAT*, such as the requirement to produce mating pheromone, may be entirely responsible for slowing down growth in these cells. It does not seem to us likely that this could be the entire explanation, in view of the numerous effects of *MAT* heterozygosity, but specific gene-disruption experiments will be required to distinguish these possibilities.

In earlier competitions between different sexual and asexual strains of yeast, the mean competitive ability of the sexuals was only approximately 10% greater than that of the asexuals (Birdsell & Wills, 1996). Our competitions, however, showed an almost 50% advantage to being sexual, indicating a strong influence of this particular genetic background on the pleiotropic effects of *MAT*.

Although the petites show a reduction in plateau numbers, the petite *MAT*-heterozygous strains still reach higher plateau numbers than the petite *MAT*-homozygous strains, indicating that the *MAT* locus affects expression of genes in the nuclear genome. Destruction of mitochondrial function allows the effects of heterozygosity for the *MAT* locus on the nuclear genome to be separated from its effects on the mitochondrial genome. While the petite *MAT*-heterozygous strains still outcompeted the petite *MAT*-homozygous strains, their competitive advantage was substantially reduced compared with that seen in the grande competitions.

(ii) *Reduction in* r *in an effectively infinite population due to deleterious mutations*

(a) *Changes in growth rate*

In the competitions between petite strains able to undergo mismatch repair, *MAT*-homozygous strains showed a significant decline in r in the course of transfers or competitions, while *MAT*-heterozygous strains did not. A *MAT*-homozygous strain will be expected to accumulate more deleterious mutations than the wild-type, since in both cases the level of DNA repair is reduced and in *MAT*-homozygous strains there are also reduced levels of mitotic recombination. To cause a decrease in the growth rate in such a very large population, the mutation rate must be high (Kondrashov, 1982). Since the *MAT*-heterozygous strains have not undergone a round of meiosis in the course of the competitions, the increased rates of mitotic recombination and DNA repair probably account for the retention of a high growth rate by the wild-type *MAT*-heterozygous strains.

In competitions between strains deficient in mismatch repair, in contrast, both *MAT*-heterozygous and *MAT*-homozygous strains show a decline in r with time. This provides strong evidence that the ability to undergo mismatch repair is an important component of the competitive ability of these strains.

These results can be contrasted with those obtained by Sniegowski *et al*. (1997), who found no decline in growth rate of *E. coli* serially transferred over 10000 generations, even though mutants that increased the mutation rate arose in the course of the transfers. The strains in which we found declining growth rates were unable to carry out aerobic respiration, while we were unable to make similar measurements on the less metabolically damaged cells. It will be most instructive to determine how much metabolic damage to *E. coli* is necessary to reveal the effects of accumulating deleterious mutations.

(b) *Increases in the variance of* Δ

The largest difference between the grande and petite competitions lies in the magnitude of Δ. In grande competitions Δ was not significantly different from zero, and in petite competitions Δ was substantially larger. This suggests that the ability to respire aerobically can overcome the disabilities imposed by homozygosity for *MAT* and for the *pms1* mutation.

In view of the declines that were seen in r in all petite strains except for the repair-competent *MAT* heterozygotes, we would have predicted that Δ should be larger in the repair-competent competitions than in the repair-incompetent competitions. This is because deleterious mutations should be free to accumulate in the repair-incompetent *MAT* heterozygotes as well as the repair-incompetent *MAT* homozygotes. Such an effect was not seen, however. It remains to be discovered whether the effect was too small to be detected in these experiments, or whether some compensatory mechanism was at work.

There was, however, a detectable effect of the inability to undergo mismatch repair on the variance of Δ. In both the grande and petite competitions, the *pms1* mutants exhibited a greater degree of variation in Δ between replicates than the wild-type, although the mean Δ was approximately the same for mutant and wild type (Table 1, Fig. 2). Since the mean Δ in the grande competitions is close to zero, this raises the possibility that in this genetic background some of the mutations arising from mismatch repair can actually be beneficial. Alternatively, it is possible that in different replicates of the competitions the wild-type strains and those defective in mismatch repair are accumulating different numbers of mutations of small effect, and that the increase in variance is entirely due to the chance accumulation of more negative mutations in one of the lines than the other. Further studies are needed to distinguish these possibilities.

Removing the ability of the cells to metabolize aerobically has the effect of removing large transfer-dependent changes in growth patterns as a confounding variable. Since Δ in the petite competitions is only a measure of changes in r, then either the

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growth rates of the petite *MAT*-heterozygous strains are increasing over time, those of the *MAT*-homozygous strains are decreasing, or both. Increases in *r* were not seen as a result of transfers. Since all the petite strains examined showed a reduction in *r* after transfers except for the *MAT*-heterozygous wild-type, and none shows an increase in *r*, it appears that the significant Δ values are due to the accumulation of predominantly disadvantageous mutations.

In their explanations for the maintenance of sex, Fisher emphasized the effects of positive mutations and Muller emphasized the effects of negative mutations. These experiments provide solid evidence for the accumulation of negative mutations in this organism and hint that positive mutants may be playing a role as well, at least in grande strains. It will be important to use this yeast system and similar systems to investigate whether both positive and negative mutations can actually play a role in the maintenance of the ability to undergo sexual reproduction.

(iii) *The evolution of sex in yeast*

Zeyl & Bell (1997) mixed together strains of haploid yeast cells of both mating types that were otherwise largely genetically identical. They found that, when these strains were allowed to undergo repeated rounds of mating and sporulation, the survivors showed increased rates of increase in colony diameter even when grown on a medium (galactose) to which they had not become adapted. A diploid of these same strains, subject to the same number of generations of growth but without being put through sporulation medium, did not show this effect. They proposed, on the basis of some suggestive but not statistically significant further results, that this increase in growth rate may be because the rounds of recombination are removing deleterious mutations. The mutations had presumably accumulated since the haploids had been established.

Their results are quite consistent with the observations of Birdsell & Wills (1996) that a single round of recombination actually resulted in a temporary lowering of the competitive ability of a homozygous-background strain, presumably because deleterious mutations were ether being made homozygous or were being produced by the process of meiosis itself (Magni & von Borstel, 1962). They are also consistent with the present results, which show that, even in the absence of meiosis, deleterious mutations can arise in the course of competitions. However, it should be cautioned that it is premature to attribute the advantage of sexual recombination entirely to the ability to remove deleterious mutations, since there are so many other reasons for this advantage in this organism. The presence of so many pleiotropic effects provides strong support for the concept of the origin of sex through contagion (Hickey & Rose, 1988). The plasmids or other vectors that originally introduced the mating type idiomorphs into the genome of the ancestor of yeast may have also carried genes involved in DNA repair and uptake, which would have aided in the retention of the plasmids (Birdsell & Wills, in preparation).

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