Estimates of the Rate and Distribution of Fitness Effects of Spontaneous Mutation in *Saccharomyces cerevisiae*

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ABSTRACT

The per-genome, per-generation rate of spontaneous mutation affecting fitness (U) and the mean fitness cost per mutation (s) are important parameters in evolutionary genetics, but have been estimated for few species. We estimated U and sh (the heterozygous effect of mutations) for two diploid yeast strains differing only in the DNA mismatch-repair deficiency used to elevate the mutation rate in one (mutator) strain. Mutations were allowed to accumulate in 50 replicate lines of each strain, during 36 transfers of randomly chosen single colonies (~600 generations). Among wild-type lines, fitnesses were bimodal, with one mode showing no change in mean fitness. The other mode showed a mean 29.6% fitness decline and the petite phenotype, usually caused by partial deletion of the mitochondrial genome. Excluding petites, maximum-likelihood estimates adjusted for the effect of selection were $U = 9.5 \times 10^{-5}$ and $sh = 0.217$ for the wild type. Among the mutator lines, the best fit was obtained with $0.005 \leq U \leq 0.94$ and $0.049 \geq sh \geq 0.0003$. Like other recently tested model organisms, wild-type yeast have low mutation rates, with high mean fitness costs per mutation. Inactivation of mismatch repair increases the frequency of slightly deleterious mutations by approximately two orders of magnitude.

THE rate at which spontaneous mutations occur and the frequency distribution of their effects on fitness are of interest both as basic parameters of population genetics and as important factors in the evolution of such diverse features as life histories, sex and recombination, mate choice, and senescence (Kondrashov 1998; Lynch *et al.* 1999). However, estimates of these parameters have been reported for only a few organisms. Rates and effects of mutation can be estimated by allowing spontaneous mutations to accumulate over many generations in replicate populations founded by a common ancestor. With minimal population sizes, the effectiveness of selection against deleterious mutations is minimized and mutations are fixed by genetic drift. As mutations accumulate, the mean fitness of the replicate populations is expected to decline while variance among them increases. From the per-generation changes in mean and variance, a lower bound for the mutation rate per diploid genome (U_{min}) and an upper bound for the mean fitness reduction (s_{max}) can be estimated if it is assumed that all mutations have equal and negative fitness effects (Bateman 1959).

Until quite recently, the only available estimates were based on experiments with *Drosophila melanogaster* (Mukai 1964; Mukai *et al.* 1972; Ohnishi 1977) in which mutations were allowed to accumulate on second chromosomes that were sheltered from selection. The results were interpreted as indicating a relatively high U_{min} of ~1 per genome per generation, with an average reduction of viability of 1–2% per mutation (Simmons and Crow 1977). However, this interpretation has been challenged by reanalyses of the *D. melanogaster* data (Keightley 1996; Garcia-Dorado 1997; Caballero and Keightley 1998) and by additional mutation-accumulation (MA) experiments on *D. melanogaster* (Fernández and López-Fanjul 1996; Fry *et al.* 1999; see reviews by García-Dorado *et al.* 1999; Keightley and Eyre-Walker 1999). These more recent studies imply that U is substantially lower, and s substantially greater, than the values inferred by Mukai *et al.* (1972). An unexpectedly low $U = 0.0052$ was also estimated for the nematode *Caenorhabditis elegans* (Keightley and Caballero 1997), with a very high $s = 0.21$. Keightley and Caballero (1997) introduced a maximum-likelihood (ML) algorithm for estimating U and s from the fitness of MA lines. This allows s to vary among mutations, identifies the most likely distribution of s from a wide variety of gamma distributions, and yields estimates of U and s, rather than U_{min} and s_{max}. The results of a second *C. elegans* experiment (Vassilieva and Lynch 1999) yielded estimates of $U_{min} = 0.05$ and $s_{max} = 0.14$. Likewise, in two independent MA experiments on *Arabidopsis thaliana*, per-generation fitness declines were small (Schultz *et al.* 1999) or undetected (R. G. Shaw, D. L. Byers and E. Darmo, unpublished results). For *Escherichia coli*, Kibota and Lynch (1996) estimated $U_{min} = 0.0002$ and $s_{max} = 0.012$, although given its smaller

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genome size and the occurrence of only one cell division per generation, a low per-generation mutation rate is less surprising for *E. coli* than for multicellular eukaryotes.

The budding yeast *Saccharomyces cerevisiae* is both a model organism of major importance in molecular genetics and ideally suited to the experimental study of evolutionary and population genetics (Zeyl 2000). A genome-wide mutation rate for yeast of 0.0031 has been extrapolated from fluctuation tests at two loci (Drake 1991). However, it is uncertain whether this extrapolation provides an accurate estimate of the rate of deleterious mutation. Different loci vary greatly in mutation rates, as illustrated by the fact that a third locus (a plasmid-borne nonsense suppressor allele with a mutation rate approximately two orders of magnitude higher than the other two) was excluded as an outlier. Two of ~6100 loci may not represent a sufficient sample to allow an accurate estimate of the genome-wide mutation rate. Also, it is unclear how rates of mutation detected by fluctuation test are related to rates of mutations that reduce fitness. There may be many mutations that reduce fitness but do not entirely abolish the activity of an enzyme and thus are not counted in a fluctuation test; conversely, mutations may eliminate enzyme activity but have no detectable effect on fitness in a given environment.

Yeast can be frozen and revived, allowing samples from each population to be cryopreserved at intervals, and then competed against their ancestors to provide precise and reproducible estimates of fitness. *S. cerevisiae* is also highly amenable to MA, since multiple populations are easily propagated by the transfer of colonies established from single cells (Korona 1999). Although such extreme bottlenecking maximizes genetic drift, selection against mutations still occurs during colony growth. The fraction of cells in a colony carrying a neutral mutation is approximately the product of the mutation rate and the number of generations. The amount by which this fraction is reduced by selection against a deleterious mutation can be estimated from a simple model in which the relative growth rates of all mutant genotypes are reduced below that for wild-type cells by the same fitness cost *s* (Kibota and Lynch 1996). An estimate of *s* can thus be used to correct the inferred mutation rate for the effect of selection.

We ran MA experiments with two founding genotypes: a wild-type (WT) and a congenic mutator (M) strain. Spontaneous mutation rates can be increased by inactivating any of several genes whose products are involved in mismatch repair. Our M strain carried a deletion of the *MSH2* gene, greatly increasing the rates of base substitutions and deletions of one to two nucleotides (Marsischky *et al.* 1996). We estimate the rates and fitness effects of spontaneous mutation in the heterozygous state in congenic *MSH2* (WT) and *msh2* (M) yeast genotypes.

MATERIALS AND METHODS

Founding genotypes and mutation accumulation: The founding WT was a diploid *leu2*Δ genotype derived from strain Y55. The M was an otherwise identical *ura3 leu2*Δ *msh2::URA3* genotype (Chambers *et al.* 1996). From single colonies of each founder, 50 replicate populations were founded. These 100 populations were thus initially identical, except for the *msh2* disruption in the 50 mutator populations. Each population was propagated by streaking a single colony onto a new plate of YPD agar (2% yeast extract, 2% peptone, 1% dextrose, 2% agar) every 2 days. Colony selection was randomized by picking the colony closest to the trademark on the petri plate. At the 18th and 36th transfers, samples from an overnight liquid culture of each transferred colony were frozen at −80° in 15% glycerol.

The number of generations (cell divisions) during each 48-hr growth period was estimated by counting the cells in 10 48-hr colonies using a Coulter particle counter. This gave an estimate of ~16 generations between transfers. The populations frozen after 18 and 36 transfers would thus have undergone ~300 and 600 generations, respectively. Because cell division rates were expected to decrease as fitness declined during MA, this estimate was obtained at transfer 16, near the midpoint of the MA process. The fitness analyses described below were performed only on the samples frozen after 36 transfers.

Fluctuation tests: We wished to estimate mutation rates in both WT and M strains at specific loci, to quantify the effect of mismatch repair deficiency on the rate of mutations affecting fitness, and also to compare genome-wide mutation rates extrapolated from fluctuation tests with those inferred from MA. Luria-Delbruck fluctuation tests were performed as described for *E. coli* (Sniegowski *et al.* 1997), with culture media and plating volumes adjusted for yeast. Mutation rates were estimated at the *CAN1*, *URA3*, and *CYH2* loci, by spreading replicate overnight cultures on canavanine, 5-fluoroorotic acid, and cycloheximide plates, respectively. All three media are toxic to wild-type cells and select for mutant colonies lacking enzyme activity (Brown and Szostak 1983). Because such mutations are presumably recessive, the fluctuation tests were performed on haploid versions of the two strains. The results were analyzed with a maximum-likelihood algorithm (Sniegowski *et al.* 1997).

Fitness assays: The relative fitness of each line after 36 transfers was estimated in replicate competitions with a genotype congenic to the M ancestor that was genetically marked with resistance to the antibiotic G418 (geneticin). Competitions between this marked M genotype and each ancestor have been performed at various times for a variety of experiments and have repeatedly shown the three genotypes to have equal fitness (relative to the marked M genotype, fitness estimates ± 95% confidence intervals pooled across several independent assays are 1.000 ± 0.012 for WT and 1.002 ± 0.007 for M). Since each experimental line was descended from a single colony during the final transfer, genetic variation within lines was assumed to be negligible.

Because there may be many mutations with no detectable effect in a permissive environment but deleterious effects in a more demanding environment (Kondrashov and Houle 1994; Elena *et al.* 1998), during MA the lines were grown on YPD agar (a rich, complex permissive medium) but fitness was assayed in a liquid defined minimal medium (SMM: 0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, and 2% dextrose), supplemented with 60 mg/liter leucine. Liquid medium was used so that the numbers of cell divisions of each genotype could be estimated from the dilution factors used in the competitions.

Before competitions were begun, all lines were acclimated

to the assay environment as follows. Samples were taken from the ultracold freezer and partially thawed, and 20-μl aliquots were used to inoculate 10-ml YPD cultures in 18 × 150-mm borosilicate tubes. After growth for 24 hr, 0.1 ml were transferred to 9.9 ml SMM + leucine and grown for 24 hr again. Competitions were then begun by mixing 50 μl of each line and 50 μl of the G418-resistant ancestor in 9.9 ml of fresh SMM + leucine. A 120-μl sample of each mixture, diluted through a 10-ml tube of sterile deionized water, was spread on a YPD plate, and after 2 days' growth the resulting 100–200 colonies were replica-plated to YPD plates containing G418. Colony counts from each plate provided estimates of the initial frequencies of each competitor. After 24 hr, 0.1 ml of each competition was transferred to a fresh tube of 9.9 ml SMM + leucine. After another 24 hr growth, 120-μl samples of each competition were diluted through two 10-ml water tubes, spread on YPD plates, and replica-plated as above, providing estimates of the final frequencies of each genotype. Fitness was quantified as the ratio of the number of cell divisions of the MA line to that of the marked ancestor during the ~13 generations of the competition (Lenski *et al.* 1991). Fitness assays were performed in five blocks, each block containing one replicate of each of the 50 WT and 50 M lines. Each block included control competitions between the two ancestors and the common competitor to allow correction for any block effect, but no block effect was detected ($P = 0.252$). Between- and within-line components of variance were estimated by ANOVA.

Estimates of *U* and the distribution of *sh*: A maximum-likelihood algorithm, written and kindly provided by P. Keightley (Keightley 1994), was compiled by J. Muday and used to estimate the mutation rate and the distribution of fitness effects for the WT and M genotypes. The program assumes a Poisson distribution of number of mutations per generation and a gamma distribution of fitness effects. The gamma distribution is determined by the values of α and β, the scale and shape parameters, respectively. The mean fitness cost of a mutation *s* (*sh* in our study), is β/α. The program evaluates the natural logarithm of the likelihood of the data as a function of particular parameter combinations, using numerical integration (Keightley 1994, 1998). Parameter combinations were searched over a wide range of combinations of initial values to minimize the risk of missing a global maximum likelihood, and 95% confidence limits for *U* and *sh* were defined by a twofold drop in the log likelihood relative to its maximum values.

RESULTS

Fluctuation tests: Luria-Delbruck fluctuation tests confirmed that mutation rates at three loci were significantly higher in the M strain than in the isogenic WT strain (Table 1) by a geometric mean factor of ~30. Our estimates for the *URA3* and *CAN1* loci agree well with published figures (2.77×10^{-8} and 1.13×10^{-7}, respectively; Drake 1991) and our estimate of the increase in mutation rate caused by *MSH2* deletion is in good agreement with the effect of deleting its homologue *mutS* in *E. coli* (deVisser *et al.* 1999).

Fitness changes: Mean fitness declined significantly for both WT (mean ± 95% confidence limits: 0.890 ± 0.044) and M lines (0.791 ± 0.024). Fitness distributions were clearly bimodal, especially for the WT lines (Figure 1), implying that there were two classes of mutation with different fitness effects. The most likely candidate for the more severe class of mutations is deletion of part or all of the mitochondrial genome. This abolishes the ability to respire and is known as the petite phenotype due to the smaller colonies formed by such mutants (Hampsey 1997). Respiration ability was tested for all lines by plating samples from liquid YPD cultures onto glycerol plates (2% yeast extract, 2% peptone, 3% glycerol), on which respiration is required for growth. Among WT lines there was a clear correspondence between fitness and respiration ability: the upper and lower fitness modes correspond to 31 grande (respiration-competent) and 19 petite phenotypes, respectively (Figure 1). There was no overall fitness decline in WT grande lines (1.003 ± 0.016), while WT petite lines were significantly less fit than their ancestor (0.706 ± 0.034).

Among M lines, the fitness difference between grandes and petites was smaller, but average fitness was still significantly higher for the 34 grandes (0.838 ± 0.027) than for the 16 petites (0.741 ± 0.034; $P = 0.000169$ in a two-tailed *t*-test). Among all petites there was no difference between M and WT lines ($P = 0.166$), but M grandes were significantly less fit than WT grandes ($P = 3.72 \times 10^{-14}$).

A standard measure of the effect of mutation is the per-generation increment of genetic variance, scaled by the environmental variance (V_m/V_e). A nested ANOVA was used to estimate within-line and among-line components of variance in fitness. Within-line variance was used as an estimate of V_e, and V_m was estimated as $V_L/$

TABLE 1

Mutation rates at three loci in WT (*MSH2*) and M (*msh2*) genotypes, estimated by fluctuation tests

Genotype	*CAN1*	*CYH2*	*URA3*
WT	3.2×10^{-7} (2.2×10^{-7}–4.7×10^{-7})	8.0×10^{-10} (2.6×10^{-10}–2.5×10^{-9})	3.3×10^{-8} (1.4×10^{-8}–7.7×10^{-8})
M	1.7×10^{-5} (1.4×10^{-5}–2.1×10^{-5})	4.1×10^{-9} (1.3×10^{-9}–1.3×10^{-8})	4.9×10^{-6} (3.7×10^{-6}–6.6×10^{-6})
Relative mutator strength	35.1	5.1	151.5

The 95% confidence intervals for each genotype at each locus are given in parentheses. Relative mutator strength is the ratio of estimated mutation rate in the mutator genotype to that in the wild type.

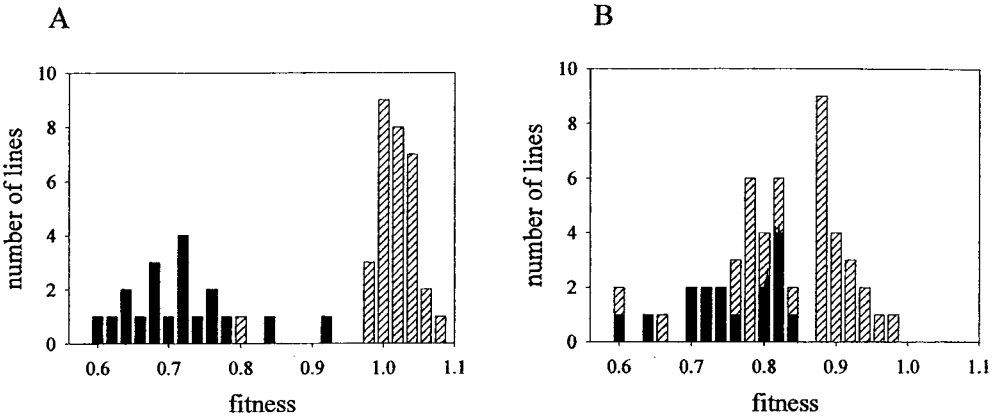


FIGURE 1.—Fitness distribution of 50 mutation-accumulation lines established from (A) wild-type and (B) mismatch-repair-deficient (mutator) ancestors. Hatched bars, respiration-competent (grande) lines; solid bars, petite lines. Ancestral fitness is 1.0 by definition.

$2t$, where V_L is the among-line variance and t is the number of generations (LYNCH and HILL 1986). When all lines are considered, V_m/V_e estimates for both WT and M are well within the usual range (LYNCH and WALSH 1998, p. 338), but for WT grandes alone V_m/V_e is an order of magnitude lower (Table 2).

***U* and the distribution of *sh*:** Separate analyses were performed on three groups of MA lines: WT grande lines alone, all WT lines combined, and M grande lines. We did not expect the fitness effects and frequency of mitochondrial mutations to differ among WT and M lines, a prediction supported by our inability to detect a difference in the mean fitness of WT and M petites. Since we are primarily interested in nuclear mutations due to their relevance for evolutionary models, we did not analyze M petites using ML. MA and fitness estimates were performed on diploid yeast, so all estimates of s are actually estimates of the mean product of s and h, the dominance coefficient. Mutation rate estimates were corrected for the effect of selection during colony growth using the model of KIBOTA and LYNCH (1996). For a given number of cell divisions between single-colony transfers, this model calculates the amount by which the probability of fixation is reduced for a deleterious mutation relative to a neutral one (Figure 2). This selection bias b is then used to correct the estimate for U, where $U_{corrected} = U_{raw}(1 + b)$. Because the model is deterministic, it provides an upwardly biased estimate of the effect of selection, which in reality would be reduced by drift (KIBOTA and LYNCH 1996). Estimates of U and sh are summarized in Table 2.

For the WT grandes, the best ML fit was obtained with $U = 5.5 \times 10^{-5}$, and with a mean $s = 0.217$ and a relatively narrow range of s (Figure 3A). Log likelihoods were rather insensitive to β and α as long as their ratio s was held near 0.22. With β fixed at 60, the 95% confidence intervals are 3.5×10^{-5}–1.5×10^{-4} for U and 0.208–0.236 for sh (Figure 3B). Correction for selection yields a point estimate of $U = 9.46 \times 10^{-5}$.

An alternative approach is to estimate U by extrapolation from fluctuation tests at specific loci and use that as a fixed value to obtain maximum-likelihood estimates of s. DRAKE (1991) estimated U from fluctuation tests on the *URA3* and *CAN1* loci, after correcting for the number of base pairs at each locus and multiplying by the number of base pairs in the genome, as 3.1×10^{-3}. From our fluctuation tests we obtain a per-locus geometric mean mutation rate in the WT ancestor of 2.0×10^{-8}. Multiplying by the 6×10^3 open reading frames in the yeast nuclear genome and doubling the resulting

TABLE 2

Summary of mutation parameter estimates for wild-type grande lines (WT grande), all 50 grande and petite WT lines, mutator grande lines (M grande), and all 50 M lines

MA lines	Fluctuation tests	V_m/V_e	Bateman-Mukai	Maximum likelihood
WT grande	—	4.80×10^{-4}	—	$U = 9.46 \times 10^{-5}$ $sh = 0.217$
All WT	$U \approx 2.4 \times 10^{-4}$	5.87×10^{-3}	$U_{min} = 2.92 \times 10^{-3}$ $s_{max}h = 0.102$	$U = 1.19 \times 10^{-3}$ $sh = 0.303$
M grande	—	1.54×10^{-3}	$U_{min} = 2.28 \times 10^{-2}$ $s_{max}h = 0.015$	$0.005 \leq U \leq 0.938$ $0.049 \geq sh \geq 0.0003$
All M	$U \approx 8.4 \times 10^{-3}$	1.47×10^{-3}	$U_{min} = 1.97 \times 10^{-2}$ $s_{max}h = 0.018$	—

Bateman-Mukai and ML estimates of U have been corrected for selection against deleterious mutations during colony growth using the KIBOTA and LYNCH (1996) function shown in Figure 2, except for the ML estimate for M grandes, where uncertainty in the estimate of s prohibits this correction.

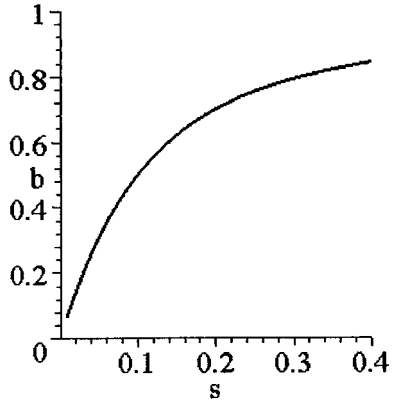
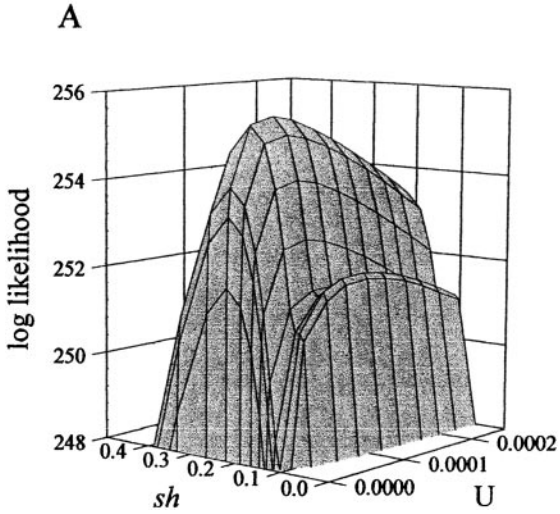


FIGURE 2.—Selection bias b against deleterious mutations during colony growth, as a function of fitness cost of mutations s. This function was obtained by modifying the model of KIBOTA and LYNCH (1996) to account for our 16 generations between each transfer in place of their 25.

haploid estimate gives $U \approx 2.4 \times 10^{-4}$. The higher estimate obtained by DRAKE (1991) gives a significantly poorer fit to our data (log likelihood = 251.87, a reduction of 3.65 from the best-fit model). The log likelihood obtained using $U \approx 2.4 \times 10^{-4}$ is lower but not quite significantly so, even if β is fixed at 60.0 (a reduction of 1.98 from the best-fit model). Because there was no decline in the mean fitness of WT grande lines, the Bateman-Mukai method of estimating a lower bound for the mutation rate per diploid genome (U_{min}) and an upper bound for the mean fitness reduction (s_{max}) from the per-generation changes in mean and variance was not applied

The grande and petite WT lines were then combined for ML analysis. For the grande lines there was no decline in mean fitness, and the inferred mutation rate was very low. Therefore, estimates of U and s from the combined WT lines should be dominated by the presumed mitochondrial mutations responsible for the petite phenotype. These estimates are not directly comparable to the estimates for the nuclear genome because of the complications of mitochondrial genetics. Each cell contains several mitochondria, each with dozens of copies of the chromosome, which introduces the potential for selection to act on mitochondrial mutations both among chromosomes within mitochondria and among mitochondria within a cell. We present estimates of U and s with petite lines included simply as a measure of the contribution of presumed mitochondrial mutations to genetic load relative to the contribution of nuclear mutations. As expected, the best fit was obtained with a much higher $U = 6.6 \times 10^{-4}$ (95% confidence limits 4.1×10^{-4}–1.4×10^{-3}) and with mean $sh = 0.303$ (95% confidence limits 0.261–0.344 with β fixed at 18.7). Correction for selection gives $U = 1.19 \times 10^{-3}$. The Bateman-Mukai method yields $U_{min} = 1.94 \times 10^{-3}$ and $s_{max}h = 0.102$ for grande and petite WT lines combined, which when corrected for selection gives $U_{min} = 2.92 \times 10^{-3}$ (Table 2).

For M grande lines, the ML analysis differed in that log likelihoods were highly sensitive to the value of β, so the results cannot be presented as a three-dimensional likelihood surface with β held constant, as for the WT lines. Instead, analyses were run using a series of fixed values for U and searching for optimal α and β values for a given U. An equally good fit was obtained over values of U from 0.005 to 0.938 (Table 2; Figure 4). Lower values of U resulted in significantly lower log likelihoods. With higher mutation rates, no viable models were found. It is possible that higher mutation rates could be accommodated with appropriate parameters, but with increasing U, the range of α and β values that yield best-fit models becomes increasingly narrow, even though the goodness of that fit remains high. Extrapolation from the fluctuation test results as above gives $U \approx 8.4 \times 10^{-3}$ for the M ancestor, and using this value did not significantly reduce the fit of ML models (Table 3). The Bateman-Mukai estimates are $U_{min} = 2.05 \times 10^{-2}$ and $s_{max}h = 0.015$. ML analysis constrained by allowing



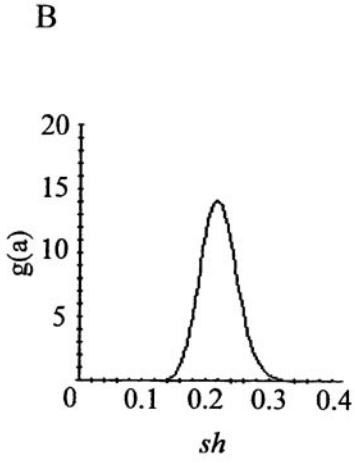


FIGURE 3.—(A) Log-likelihood surface of the fitness distribution of 31 respiration-competent wild-type lines, as a function of the mutation rate U and the mean fitness cost per mutation s. (B) Distribution of mutation effects with $\alpha = 271$ and $\beta = 60$, the parameter values giving the best-fit maximum-likelihood model corresponding to the peak in A. g(a), density function for the gamma distribution.

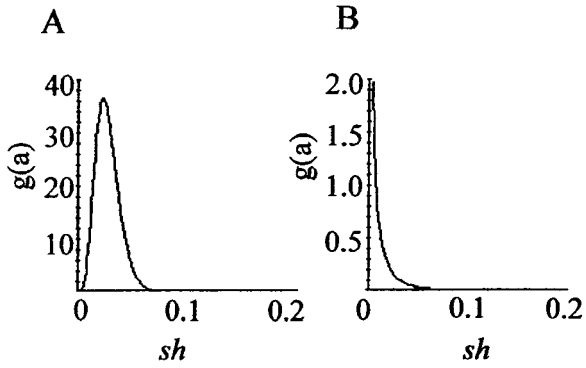


FIGURE 4.—Distributions of mutation effects for two of the best-fit maximum-likelihood models for the fitness distribution of 34 respiration-competent mutator lines (see Table 2). (A) Gamma distribution with $\beta = 6.25$ and $\alpha = 213$, corresponding to the best-fit model with $U = 0.01$. (B) Gamma distribution with $\beta = 0.01$ and $\alpha = 32.214$, corresponding to the best-fit model with $U = 0.94$.

no variation among mutations in fitness effect ($\beta \rightarrow \infty$) did not reduce the likelihood of the best-fit model, which after adjustment for selection gave estimates of $U = 1.19 \times 10^{-2}$ and $sh = 0.031$. By comparison, Bateman-Mukai estimates for all M lines including petites, after correction for selection, are $U_{min} = 1.97 \times 10^{-2}$ and $s_{max}h = 0.018$.

DISCUSSION

As in recent studies of other model organisms, we obtained very low estimates of U and very high estimates of s for wild-type budding yeast. Since the experiment was performed with asexual diploids, mutations were fixed and assayed in the heterozygous state. Thus our estimates of s actually represent hs, the fitness costs of mutations in heterozygotes. The same is true of our adjustments to inferred mutation rates to correct for the bias introduced by selection against deleterious mutations. In similar experiments with mutator yeast strains isogenic to ours, KORONA (1999) assayed fitness using maximum growth rates and estimated $h = 0.08$. This estimate of h is preliminary since the possibility of epistasis between accumulated mutations was not accounted for and is incompatible with our results, since it would imply $s > 1.0$, or negative fitness. However, if many spontaneous mutations have no detectable effects in heterozygotes, then the mutation rates we have inferred are underestimates.

TABLE 3

Results of ML analysis for M grande lines

U	β	α	Mean sh	Log likelihood
0.005	124.998	2535.826	0.049	218.32
0.0084	285.711	8244.323	0.035	219.93
0.01	6.250	213.171	0.029	220.11
0.05	0.236	39.106	0.006	220.69
0.1	0.106	35.116	0.003	220.74
0.5	0.020	32.426	0.0006	220.76
0.938	0.010	32.241	0.0003	220.76

Each line shows the best-fit model obtained by searching over values of β and α using a fixed value of U.

As in KORONA's (1999) MA experiment, many of our lines lost the ability to respire. Mutations causing this petite phenotype are usually partial deletions of the mitochondrial chromosome, but we cannot rule out the possibility that at least some of our petite mutations were nuclear, since mutations in several nuclear genes can also yield a petite phenotype (HAMPSEY 1997). Meiosis and sporulation in yeast require respiration, so we were unable to sporulate and test-cross our (diploid) petites to confirm cytoplasmic inheritance of the petite phenotype. Similar numbers of petites arose among WT and M lines (19 and 16, respectively; $P > 0.2$ in a G-test), indicating that the frequencies of petites do not significantly differ between WT and M lines. Also, mean fitness did not differ between WT and M petites (see RESULTS). These two observations are consistent with petites arising by a mechanism independent of the accumulation of nuclear mutations of small effect. Mutation rates in mitochondrial genomes are known to be high, probably because of the reactive by-products of oxidative phosphorylation. The somatic accumulation of mitochondrial genome rearrangements is responsible for several degenerative human diseases (WALLACE 1999). The intracellular population dynamics of mitochondrial genomes may lead to conflicts between selection within and between cells. For example, deletion mutants may replicate more quickly than wild-type mitochondrial genomes and therefore go to fixation within initially heteroplasmic cell lineages. By minimizing effective population size to allow mutations to accumulate, we relaxed the among-cell selection that presumably would otherwise oppose this process. The MA transfer protocol may therefore have favored the accumulation of mitochondrial deletions within cells.

Inspection of the fitness data for the WT grande lines reveals that the ML estimates of U and s are determined by the fitness of a single MA line, which declined to 0.802 (see Figure 1A). Multiplying the best-fit estimate $U = 9.5 \times 10^{-5}$ by 31 grande lines and 600 generations gives an expected total of 1.77 mutations, 1.02 of which evade selection under the model of KIBOTA and LYNCH (1996). This is concordant with the observation that the only substantial fitness decline among WT grandes occurred in a single line, by an amount approximately equal to the inferred mean $sh = 0.217$. With this single line removed from the analysis, there is no significant fitness variation among the remaining 30 WT grande lines ($F_{29,120} = 1.08$, $P = 0.371$). Although fitness estimates for some of these lines are slightly above that for the ancestor (Figure 1A), ML analysis in which a variable

fraction of mutations was beneficial did not improve the fit to the data. The detection of only a single mutation limits the confidence that can be placed in a point estimate of U, but the 95% confidence intervals obtained from ML analysis (Table 2) indicate a nonzero mutation rate that is significantly lower than the $U = 0.0031$ extrapolated from fluctuation tests by Drake (1991).

We obtained estimates of U by three methods: (1) extrapolating the results of fluctuation tests at three loci to the entire genome, (2) using the traditional Bateman-Mukai method to infer U from the per-generation rate of fitness decline in MA lines, and (3) ML analysis of the fitness data using Keightley's (1994) program. For the WT, our fluctuation-test estimate of $U \approx 2.4 \times 10^{-4}$ is in reasonable agreement with the $U = 9.5 \times 10^{-5}$ obtained by correcting the ML estimate from WT grandes for selection. The lack of a detectable per-generation fitness decline precluded a Bateman-Mukai estimate. For the M strain, the $U = 8.4 \times 10^{-3}$ from fluctuation tests is simply one of a broad range of mutation rates and effects giving equally good fit to the data (Table 3). This is because U and s are confounded, a common problem with ML analysis of MA results (Keightley 1998). The fit of ML models to the M grande data is significantly reduced by constraining them to the Bateman-Mukai estimates of $U_{\min} = 2.05 \times 10^{-2}$ and $s_{\max}h = 0.015$. However, ML analysis with all mutations having equal fitness effects gave estimates of $U = 1.19 \times 10^{-2}$ and $sh = 0.031$, with no reduction in goodness of fit and in reasonable agreement with the Bateman-Mukai estimates. The latter two values would represent a roughly 100-fold increase in U due to *MSH2* deletion, while the fluctuation tests at three loci indicate a 30-fold geometric mean increase (and a 65-fold arithmetic mean increase).

Despite the uncertainty in ML results for the M grande lines, the confidence intervals for s indicate a major difference between the M (0.0003–0.049) and WT (0.208–0.236) strains in the mean fitness effect of mutations. Comparing the WT and M strains therefore illustrates not only the obvious point that mismatch repair greatly reduces mutation rates, but also that it is slightly deleterious mutations that are eliminated. This may be simply because in the absence of mismatch repair they are the most abundant type. Gabriel *et al.* (1993) predicted this property of DNA repair systems because it is slightly deleterious mutations that cause the greatest loss of population mean fitness, mutations with larger effects being efficiently removed by selection.

A similar rarity of slightly deleterious mutations in wild-type strains has also been observed in other recent MA experiments (Fernández and López-Fanjul 1996; Keightley and Caballero 1997; Fry *et al.* 1999; Keightley and Eyre-Walker 1999; Vassilieva and Lynch 1999). It may be that mutations with small effects accumulate but are not detected in competitive fitness assays. Most of the mutations induced by ethyl methanesulfonate in *C. elegans* probably have fitness costs of <0.1% (*i.e.*, $s < 0.001$; Davies *et al.* 1999). This was suggested by combining DNA sequence comparison between *C. elegans* and *C. briggsae* with ML analysis of fitness data from mutagenized lines of *C. elegans*. Although our fitness assays would not detect individual mutations with such small fitness effects, our results suggest that mutations with $0.05 > s > 0.01$ are infrequent in wild-type *S. cerevisiae*. We would otherwise expect that during 600 generations of MA, our lines would have accumulated enough such mutations that a decline in mean fitness would have been detected, along with relatively little among-line variance in fitness.

The results reported here illustrate both the motivation and the drawback to MA as a method for studying mutation load: it may prove impossible to derive independent point estimates of U and s, but MA experiments can reveal biologically important differences between the fitness effects of spontaneous mutations in mismatch-repair-proficient and -deficient strains and the effects of experimentally induced insertions or deletions.

For most yeast genes, transposon insertions (Goebl and Petes 1986; Burns *et al.* 1994) and targeted deletions (Oliver *et al.* 1992) had no apparent phenotypic effect, even in a range of stressful environments. Fewer than a third of the genes identified by the yeast genome sequencing project have known functions, which has been interpreted to mean that mutations in many of these genes have fitness effects only in environments that have not yet been identified. However, analyses of mutagenized yeast have often identified only major, qualitative phenotypic effects and probably would not detect effects as subtle as reduction of competitive fitness by a few percent. This was confirmed by Thatcher *et al.* (1998), who found that competitive fitness was significantly reduced in 19 of 27 isogenic yeast strains carrying transposon insertions that had shown no qualitative phenotypic effects. This argues against the hypothesis that many yeast genes function only in specific, as yet unknown, environments. Similar observations have come from a collection of 2026 yeast strains, in each of which a single open reading frame has been precisely deleted (Winzeler *et al.* 1999). Only 17% of these deletions were lethal in haploids, but competitions among a pool of 558 deletion mutants revealed reduced growth rates in almost 40%. Most transposon insertions in *E. coli* (Elena *et al.* 1998) reduce fitness by <5%. For *E. coli*, the fitness costs of mutation inferred from mutagenesis are slightly greater than, but in reasonable agreement with, s as estimated by MA (Kibota and Lynch 1996). Our results suggest that this is not so for wild-type yeast, although greater numbers of spontaneous mutations must be observed before it can be concluded with confidence that spontaneous and experimentally induced mutations have different effects in yeast. Gene

deletions and transposon insertions are major mutations in molecular terms, so it is surprising that they seem to have smaller fitness effects than the spontaneous mutations that accumulated in our WT lines. Spontaneous loss of an entire chromosome (aneuploidy) is possible in asexually propagated diploid yeast and may have occurred in some of our lines.

A major motivation for estimating mutation rates and effects is the deterministic mutation hypothesis for the evolution of sex, one of the few theories featuring both an individual-level fitness advantage for sex and a readily tested requirement. According to this hypothesis, the advantage of sex is the increased variance in mutation load among recombinant offspring. This advantage can outweigh the twofold cost of sex only if $U \geq 1$ and there is synergistic epistasis among deleterious mutations (Kondrashov 1988). At least for the wild-type yeast strain we tested, U is clearly too low to support this hypothesis. However, since *S. cerevisiae* is isogamous, there is also no twofold cost of sex in this species, which greatly relaxes the requirements for any theory of sex. The potential for sex to reduce the mutational load in yeast mitochondria is of potential interest since, like many fungi, yeast have biparental inheritance and recombination of their mitochondrial genomes.

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