Journal of Industrial Microbiology, 2 (1987) 167–174
Elsevier
SIM 00077

Selection in yeast
II: The fitness distribution of new mutations in *Saccharomyces cerevisiae* and its use in a computer simulation

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Received 26 November 1986
Revised 1 May 1987
Accepted 4 May 1987

Key words: Selection; Yeast; Fitness distribution; Mutation; *Saccharomyces cerevisiae*; Computer simulation

SUMMARY

The fitness distribution of new mutations in *Saccharomyces cerevisiae* strain Montrachet was determined for cells on agar irradiated for four periods of time with ultraviolet light. The fitness distributions were obtained by converting a large number of colony diameters into relative fitnesses. The distributions were then used to perform a computer simulation with the purpose of predicting the potential of a stock culture to increase in general fitness through selection, given a frequency and magnitude of mutations.

INTRODUCTION

Mathematical models of fermentation yeast populations have dealt primarily with subjects like the kinetics of fermentation [8], ethanol inhibition [1,2], conjugate inhibition by sugar then alcohol [12], fermentation predictors [4] and computer control of fermentations [11]. There are also several mathematical models for hypothetical or qualitative mutation fitness distributions [3,7,9,10,17]. Fitness in yeast populations is defined as the rate at which viable progeny are produced in a given media. To the best of our knowledge, there are no reports on hypothetical or experimental data for mathematical models dealing with mutational fitness distributions of industrial yeasts and their effects on population dynamics over a very large number of generations. Fitness in microbial populations is a measure of survival and rate of reproduction.

It has been reported that yeasts have the potential for genetic change and improvement of relative fitness when grown on various selective media [15,16]. In the present work, the magnitude and frequency of alleles associated with fitness were established using calculations from survivors of UV mutagenesis.

The mutation fitness distribution thus defined was then used in a computer simulation to observe the effects of fitness selection over a very large number of generations (1000).

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MATERIALS AND METHODS

The strain of yeast, *Saccharomyces cerevisiae,* used throughout the study was the diploid [14] wine yeast Montrachet. The cells were of the same stock as those used in the study complementary to this one [16].

The experiment required the determination of the number of cells in each of a large number (10^3) of colonies growing on agar plates. As direct counting by hemacytometer was impractical for such a purpose, it was necessary to find some easily measurable variable related to cell number. Colonies of various sizes growing on WLN (Difco) agar plates were photographed on slide film and the plates immediately frozen at −20°C. The next day, frozen colonies were individually lifted from the plates with a spatula into 0.8% saline. Using this method, we were able to remove entire intact colonies from the plates, with no appreciable number of cells left behind. The number of cells in each colony was then determined immediately, after dilution, via hemacytometer counting (a minimum of 16 counts per colony). The relationship between the number of cells per colony and the diameter of the colony (obtained from the photographs at 6.2 × magnification) was then determined.

The yeast cells taken from a clone were first synchronized using modifications of the Williamson-Scopes method [5,6,13,18–21]. Once the 2-week synchronization procedure was over, the cells were sonicated at 40 W for 40 s to break clumps, then scored for percentage of buds, as synchronization was not complete. Dilutions of the cells were made with 0.8% saline to obtain final concentrations after UV kill effects of roughly 20 cells per plate. The cells were then spread onto approximately 1500 chilled YEPD plates (10 g yeast extracts, 20 g peptone, 20 g D-glucose and 15 g agar per litre), which were then kept in cold storage (7°C) to minimize growth overnight. (Growth was monitored at 7°C by determining the percentage of buds and doubled cells in a liquid culture after a period of time equivalent to the length of time required to complete the experiment.) The plates, in four lots, were irradiated the following day with a 254 nm Mineralite UV lamp, positioned 30 cm from the plates, each lot being irradiated for a different length of time (20, 28, 36 or 44 s). Plates were then allowed to warm up to room temperature for their 5-day incubation in the dark, and were finally photographed on slide film (Kodachrome 64, six plates per frame). Plates with obvious contaminants were not photographed. The diameter of all colonies not physically touching other colonies was measured, at 6.2 × magnification, with the data being entered directly into a Commodore 64 computer system for further processing. A total of 38 614 colonies were thus measured, with the breakdown as follows: 6888 for the UV-20 s sample, 8234 for UV-28 s, 11 035 for UV-36 s, and 12457 for UV-44 s. Overestimation of ultraviolet kill effects in making the dilutions was responsible for the increased number of colonies on the plates irradiated for the longer periods of time.

RESULTS AND DISCUSSION

The strongest linear relationship ($r = 0.99624$) between colony diameter and colony cell number was obtained when using the logarithm of both variables. The relation was thus found to be $\log (D) = 0.40178 \log (N) - 2.65511$, where D = true colony diameter (i.e., measured/magnification factor) in mm, and N = number of cells in the colony (Fig. 1). The $\log_2$ of N was defined as F, the number of generations or the fitness equivalent of the colony. Since each colony was grown for the same amount of time, fitnesses were thus directly comparable. Therefore, $F = \log_2 (N) = 8.26799 (\log (D) + 2.65511)$.

Although cell sizes varied greatly within each culture of the yeast strain, they did not apparently vary between cultures. No actual measurements were made; however, the equations presented above would take any such variation into account if it existed, and therefore cell size was of little consequence.

The first stage of processing each of the four sets of raw data was the correction for environmental (plate to plate) variation. This was accomplished by

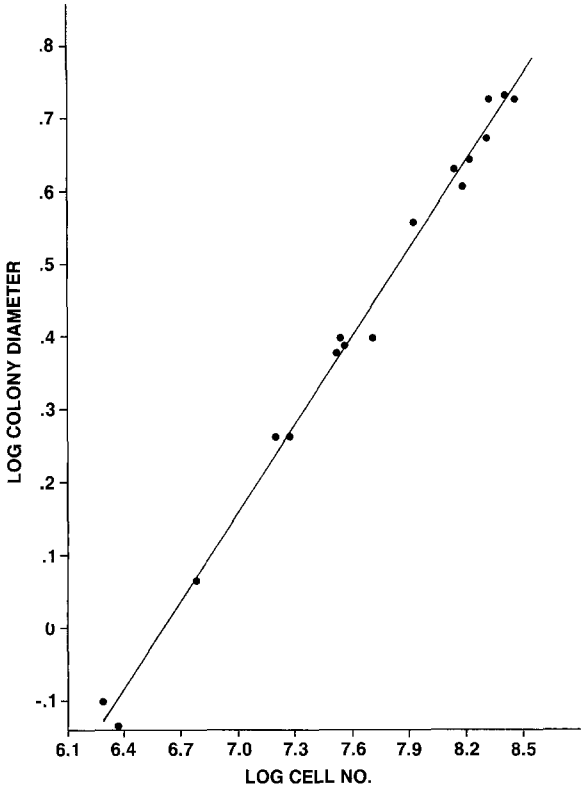


Fig. 1. Relationship between true colony diameter and the number of cells in the colony.

adjusting each plate's modal colony diameter to the overall mean mode for all plates. Thus each colony's diameter was multiplied by the factor required to bring its plate's mode to the mean mode. Modes rather than means were used because means are more prone to vary due to mutants, in addition to the variance due to the environment. Modal diameters therefore were a better estimate of wild-type diameters. The adjustments for environmental variation resulted in a considerably narrower peak in the distribution, corresponding to the wild-type colonies. The second stage of processing involved converting diameters to relative fitnesses, using the formula presented above and setting the distribution's mode arbitrarily to fitness $F = 1$. The final distributions are presented in Fig. 2 and Table 1.

Corrections for the inequalities in starting conditions for the different colonies due to imperfect synchronization were found to be unnecessary. Growth rate and kill curve data combined with actual bud frequencies (results not shown) resulted in adjustments due to buds not being large enough to affect the relative fitness distributions at 0.02 fitness intervals. Beginning a colony with one cell or eight affects colony size by much less than would be predicted by the difference of three generations. This is due to the very high initial growth rates (1 gen/h) and the very slow final growth rates (0.04 gen/h). Thus an initial difference of three generations corresponds to a difference of 3 h, which results in an apparent difference of 0.12 generations final or about 0.004 relative fitness units, well below the 0.02 fitness interval width. Also, the killing of one or more nuclei in a group by the ultraviolet light should have resulted in fewer viable buds than were actually counted.

Obvious differences can be seen in the relative fitness distributions of the samples irradiated for different periods of time (see Fig. 2 and Table 1). There were generally more mutants generated by irradiating for longer periods of time, with the greatest differences occurring in the less extreme fitness categories. Although the distributions are less narrow with increasing irradiation time, the overall shapes of these distributions are similar, with the greatest proportion of mutants being near-neutral, with a rapid drop in frequency of high-fitness mutants and a more gradual decline in low-fitness mutants. The proportions of lethal mutations, from kill curve studies is included in Table 1. It must be noted that lethals should not be considered of great importance in a fitness distribution of microorganisms.

One problem encountered during the experiment was the crowding of colonies on plates. An average correlation of -0.4 was found between the number of measurable (i.e., distinct) colonies on each plate and the plate's modal colony diameter. However, since only colonies not physically touching other colonies were measured, it was assumed that the effects observed were average for the plate and equal for each colony. Some errors in the distributions are likely to be attributed to this factor, as no correction for within-plate variation was feasible without tripling the work. If such corrections were

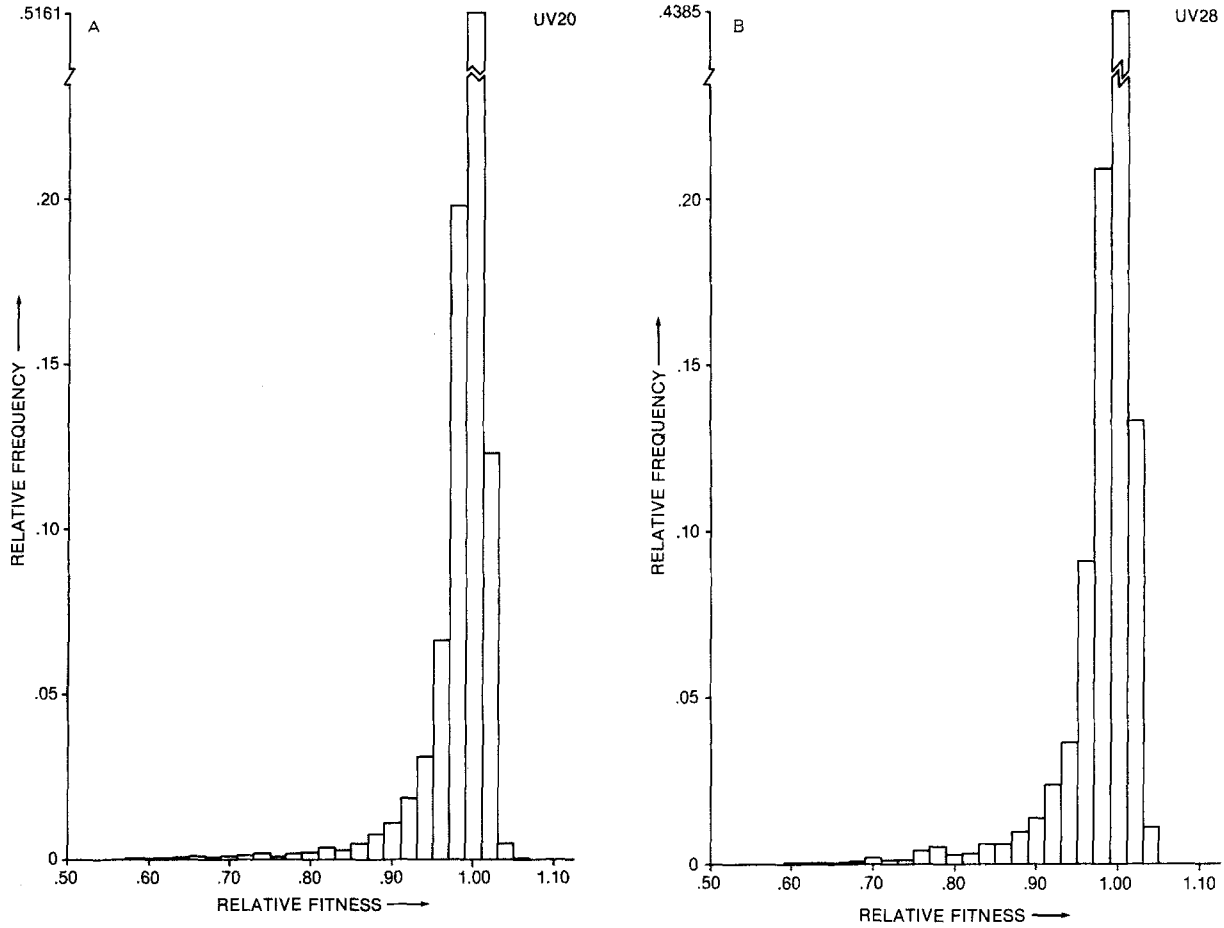
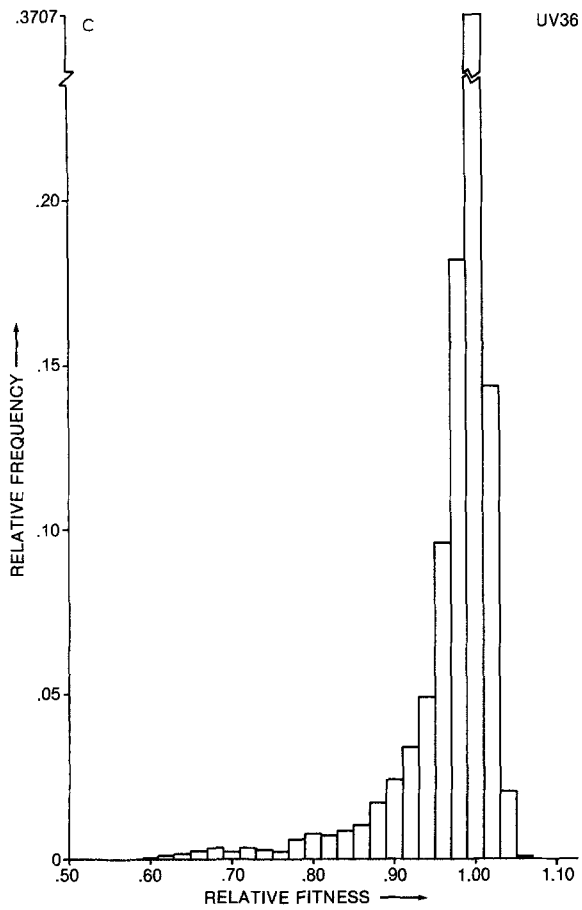


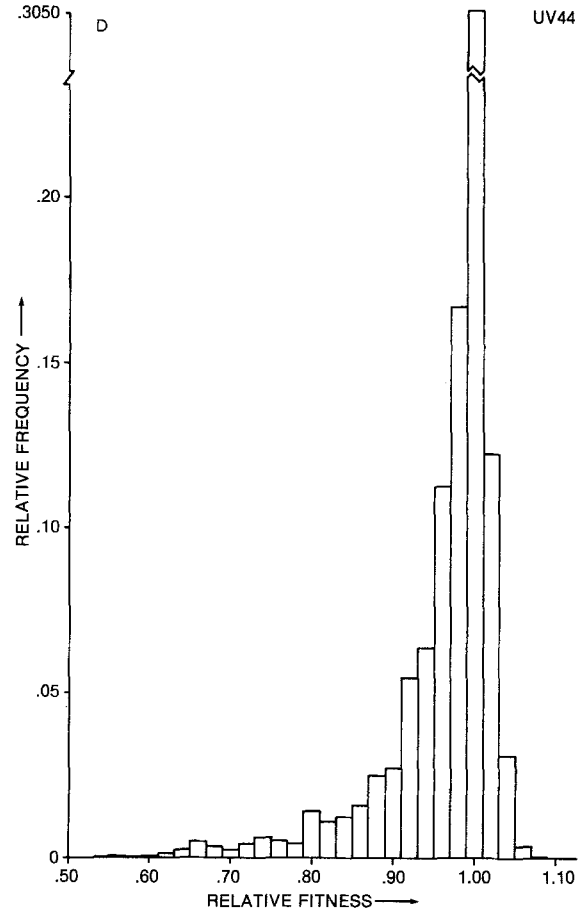
Fig. 2. Final relative fitness distributions of four strains that had received various doses of UV irradiation. Strains A, B, C and D received 20, 28, 36 and 44 s of UV exposure, respectively.

to have been made, they probably would have resulted in somewhat narrower distributions.

Originally, it was hoped that by extrapolation, a distribution for zero-irradiation (spontaneous) mutations would be obtained. However, no simple method for performing this extrapolation was found, with the difficulties compounded by having only four data points per fitness category. The fitness distribution for the 20-s irradiation was therefore subsequently used as the estimate of the spontaneous mutation distribution, since the distributions for the various irradiation times were similar, at least in form. The spontaneous mutation rate was also not available from the data, hence it became a variable in the subsequent computer simulations. It was not possible to directly measure unirradiated cells because the simple growing of cells for spreading on plates would have generated as many mutations as the actual experiment. Therefore, an unirradiated sample would have been overly biased towards the beneficial mutations. By irradiation, this background "noise" should have become negligible compared to the large number of mutants artificially generated.

Fitness distributions published in the literature tend to be somewhat hypothetical and qualitative [10]. The presently determined distribution for yeast tends to agree, at least in general, with the shape of published distributions [3,7,9,17]. Most have a high proportion of near-neutral to neutral mutants, with





tailing off of frequencies rapidly on the beneficial side and more slowly on the detrimental side. The hump of very unfit mutants often depicted [3] was not observed.

The fitness distribution of new mutations in yeast was ultimately used in a computer simulation. The program generated mutants according to a given mutation rate, following the distribution empirically determined for 20-s UV irradiation. Four runs were made, at 2%, 5%, 10% and 20% mutation rates. The initial "population" consisted of "cells" of fitness 1.00. The program passed through 1000 loops (generations), mutating each fitness category according to the mutation distribution, then multiplying each fitness category's frequency by its fitness value, simulating growth at a rate proportional to fitness. Finally, cell numbers were adjusted to maintain a constant population size. Allowable fitness categories were from 0 to 2.06 in 0.02 unit increments. Thus, no "cells" were allowed to be of fitness greater than 2.06 (relative), the arbitrarily chosen maximum adaptation value. Results of the simulations are presented in Fig. 3–5. Three stages were seen to occur in the population. The initial stage consisted of creating a stable fitness distribution from the unrealistic ideal clone of fitness $F = 1$. During this stage, the population initially became less fit due to the greater proportion of harmful mutations, but before long, selection began to take an effect to increase the population fitness with great acceleration. The second stage consisted of the stable distribution increasing its fitness at a rather constant velocity, until stage three, when the population became too well adapted for any further

Table 1

Fitness distributions of new non-lethal mutations in a clone of *Saccharomyces cerevisiae* (Montrachet) cells exposed to 20 s, 28 s, 36 s and 44 s of 254 nm ultraviolet light

After 5 days of incubation in the dark on agar plates, the colonies were photographed and their diameters measured. Diameters were then converted to colony cell numbers and hence generation numbers ($\log_2$ (cell No.)). Relative generation numbers, or relative fitnesses, were obtained by manipulating the data as described in the text. The category consisting of fitness 1.00 contains wild types as well as neutral mutants, and its high proportion in the distributions thus does not necessarily represent a higher frequency of true neutral mutations.

		Relative frequency			
		UV20	UV28	UV36	UV44
Relative fitness:	0.54	0.00004	0.00000	0.00011	0.00020
	0.56	0.00007	0.00000	0.00023	0.00040
	0.58	0.00022	0.00009	0.00011	0.00020
	0.60	0.00036	0.00018	0.00045	0.00054
	0.62	0.00040	0.00033	0.00091	0.00108
	0.64	0.00065	0.00049	0.00125	0.00221
	0.66	0.00098	0.00046	0.00238	0.00500
	0.68	0.00065	0.00064	0.00326	0.00343
	0.70	0.00098	0.00182	0.00227	0.00259
	0.72	0.00131	0.00085	0.00335	0.00433
	0.74	0.00160	0.00121	0.00326	0.00614
	0.76	0.00087	0.00401	0.00308	0.00502
	0.78	0.00189	0.00504	0.00634	0.00466
	0.80	0.00203	0.00285	0.00788	0.01421
	0.82	0.00348	0.00316	0.00761	0.01108
	0.84	0.00290	0.00607	0.00888	0.01204
	0.86	0.00494	0.00607	0.01047	0.01694
	0.88	0.00755	0.00959	0.01726	0.02497
	0.90	0.01118	0.01385	0.02474	0.02689
	0.92	0.01873	0.02405	0.03407	0.05435
	0.94	0.03107	0.03643	0.04948	0.06326
	0.96	0.06635	0.09096	0.09574	0.11223
	0.98	0.19759	0.20889	0.18165	0.16641
	1.00	0.51611	0.43855	0.37068	0.30497
	1.02	0.12282	0.13299	0.14314	0.12234
	1.04	0.00501	0.01129	0.02066	0.03075
	1.06	0.00022	0.00012	0.00072	0.00361
	1.08	0.00000	0.00000	0.00000	0.00016
		1.00000	0.99999	0.99998	1.00001
Proportion of survivors		0.519	0.436	0.294	0.130
Relative fitness distribution mean		0.98557	0.98175	0.97257	0.96274
Relative fitness distribution variance		0.00173	0.00208	0.00369	0.00486
Raw data mean (mm)		4.79722	4.37719	3.92936	3.51782
Raw data variance		1.12974	1.14066	1.40677	1.48129
Number of plates		339	347	343	348
Number of colonies		6888	8234	11035	12457
Mean mode (mm)		5.26739	4.90541	4.57326	4.28068
Model variance		0.35488	0.32878	0.24936	0.24027
Corrected mean (mm)		4.87848	4.47138	4.00310	3.57687
Corrected variance		0.98776	1.05608	1.38711	1.52218

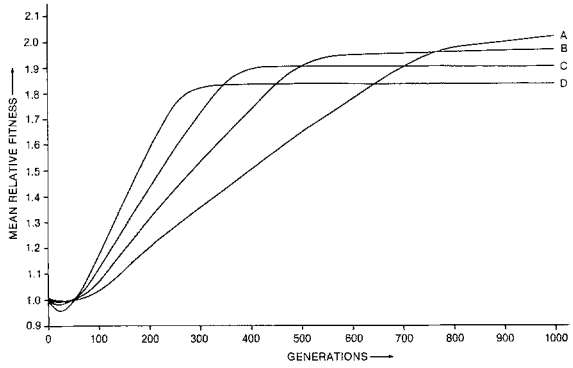


Fig. 3. Computer simulation results based on the mutation fitness distribution of *S. cerevisiae* (strain Montrachet). Distribution based on strain UV 20 data. Curve A represents a 2% overall mutation rate, B = 5%, C = 10% and D = 20%. Cells were not allowed to exceed the fitness value of 2.06, hence plateaus approach this value.

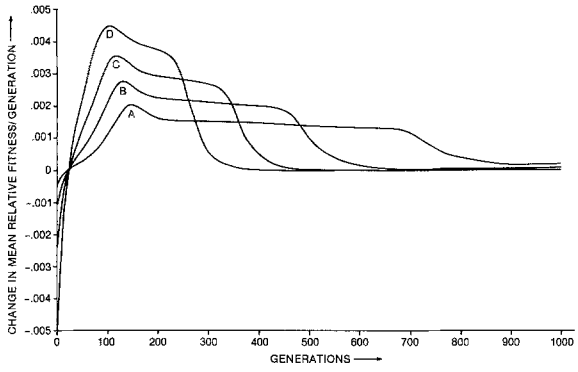


Fig. 4. Rates of change of population mean in the computer simulation. Curve A represents 2% overall mutation rate, B = 5%, C = 10% and D = 20%.

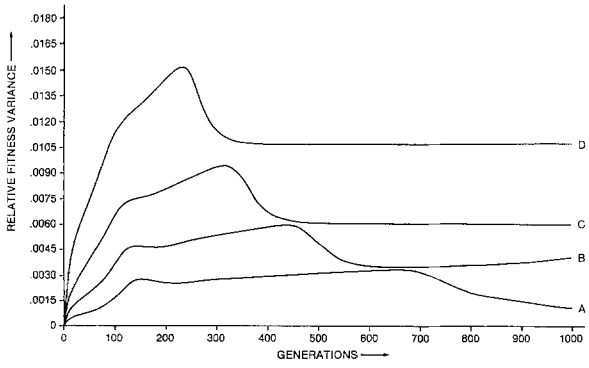


Fig. 5. Variance of the population fitness in the computer simulation. Curve A represents a 2% overall mutation rate, B = 5%, C = 10% and D = 20%.

increase as the maximum 2.06 fitness was approached. It can clearly be seen from the graphs that the higher mutation rates resulted in a much faster attainment of maximum fitnesss. The maximum population fitness was slightly lower with increased mutation rate, however, due to the increased variance.

It must be noted that only one fitness distribution of mutations was available to mutate all of the fitness categories. The distribution is that of wild-type cells, and therefore may be totally invalid for cells of different fitness from that of the wild type. It is plausible that cells of already high relative fitness might have a distribution containing fewer beneficial mutations, and that cells of low relative fitness might have a proportionately higher frequency of good mutations. The simulations performed in the present study cannot be expected to be accurate in toto; however, they likely are reliable for population changes near wild-type fitness values. Leveling off of the rise in population fitness may thus occur much sooner than depicted in Figs. 3–5; the graphs merely should be taken to indicate trends. Thus, from an initial ideal clone of cells containing no mutants, a mutant load on the population is initially formed, lowering the mean population fitness, then the small proportion of better-fit mutants eventually take over the population and improve it. In order to determine actual rates, mutation distributions of already mutant cells would need to be determined first. The most important point is that the population was shown to improve by natural selection with the actual fitness distribution of mutations taken into account, i.e., that the cells used to determine the distribution had potential to improve.

Applying the results of the computer simulation to actual yeast populations, it can be concluded that the natural relative fitness distribution of yeast will tend to increase the population's growth rate, given enough generations of selection. Although the relative fitness of a clone-inoculated culture may initially drop, this drop is only temporary, and real gains in growth rates can be achieved in a reasonable time. The implication for industry is a mathematical support for the argument that large num-

bers of selected generations should precede the expansion of stock cultures.

ACKNOWLEDGMENTS

The authors wish to thank Susan Moore, Jane Pursey and Leonard Seed for assistance during this study. Financial support of an NSERC Canada grant is acknowledged.

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