Copyright © 1999 by the Genetics Society of America

Unpredictable Fitness Transitions Between Haploid and Diploid Strains of the Genetically Loaded Yeast *Saccharomyces cerevisiae*

Ryszard Korona

Institute of Environmental Biology, Jagiellonian University, 30-060 Krakow, Poland

Manuscript received May 6, 1998
Accepted for publication October 12, 1998

ABSTRACT

Mutator strains of yeast were used to accumulate random point mutations. Most of the observed changes in fitness were negative and relatively small, although major decreases and increases were also present. The average fitness of haploid strains was lowered by ~25% due to the accumulated genetic load. The impact of the load remained basically unchanged when a homozygous diploid was compared with the haploid from which it was derived. In other experiments a heterozygous diploid was compared with the two different loaded haploids from which it was obtained. The fitness of such a loaded diploid was much less reduced and did not correlate with the average fitness of the two haploids. There was a fitness correlation, however, when genetically related heterozygous diploids were compared, indicating that the fitness effects of the new alleles were not entirely lost in the heterozygotes. It is argued here that to explain the observed pattern of fitness transitions it is necessary to invoke nonadditive genetic interactions that go beyond the uniform masking effect of wild-type alleles. Thus, the results gathered with haploids and homozygotes should be extrapolated to heterozygotes with caution when multiple loci contribute to the genetic load.

ACCUMULATION of spontaneous mutations cannot be effectively curbed by natural selection even in relatively large populations when the effects of single mutations are neutral or only moderately deleterious. The resulting genetic load may play a crucial role in the evolution of genetic recombination (Kimura and Maruyama 1966; Feldman *et al.* 1980), sexual reproduction (Kondrashov 1982, 1988; Charlesworth 1990), and alternation of haploid and diploid phases in the life cycle (Perrot *et al.* 1991; Otto and Goldstein 1992; Jenkins and Kirkpatrick 1995). Mildly deleterious mutations are difficult to study empirically for the obvious reason that they manifest themselves only by small alterations of phenotypic traits. Such mutations usually must be accumulated to make their effects detectable, which can be very labor and time consuming. They are also mostly recessive, so measurements of mutational load are generally done in haploids or homozygous diploids. Because of these difficulties, empirical research on mutation accumulation is still insufficient, although several studies have been done employing bacteria (Andersson and Hughes 1996; Kibota and Lynch 1996; Elena and Lenski 1997), haploid lower eukaryotes (da Silva and Bell 1996; de Visser *et al.* 1996, 1997a,b), and homozygotes of higher organisms. The latter were obtained for single chromosomes by applying special genetic techniques (Mukai 1964, 1969), and for the whole genome by comparing inbred and outbred strains or by accumulation of mutations (Charlesworth *et al.* 1990; Johnston and Shoen 1995; Fernandez and Lopez-Fanjul 1996; Deng and Lynch 1997; Keightley and Caballero 1997). The results gathered with haploids and homozygotes are generally considered to be relevant for diploids, except that the negative effects of mutational load should be scaled down in heterozygotes. This is an important assumption because the genetic load of heterozygotes is especially interesting both theoretically and practically.

This work compares the fitness effects of mutational load in haploids, homozygotes, and heterozygotes of one experimental organism. Another aim is to indicate how accumulation of spontaneous mutations can be simplified. Yeast is especially suitable for both undertakings. It can be easily maintained in either haplo- or diplophase. Both genetic phases are very similar phenotypically, making comparisons appropriate. Fitness was estimated by measuring maximum growth rate (MGR). This trait is influenced by many genes and therefore represents a broad mutational target. Accumulation of mutations was done with special strains of yeast in which a gene necessary for mismatch repair, *MSH2* or *PMS1*, was missing (Johnson *et al.* 1996; Kolodner 1996). Such damage elevates the frequency of base substitutions by 20–60 times and the frequency of short deletions or insertions, particularly 1-base deletions, by up to 900 times (Williamson *et al.* 1985; Kramer *et al.* 1989; Marsischky *et al.* 1996).

The study was initiated by accumulation of mutations. It consisted of repeated propagation of yeast colonies from single cells. Such serial reductions of population

Address for correspondence: Ryszard Korona, Institute of Environmental Biology, Jagiellonian University, Ingardena 6, 30-060 Krakow, Poland. E-mail: korona@eko.uj.edu.pl

TABLE 1

Genotypes of the strains used

Strain	Genotype
Y55 2438 or α*m*	*msh2::URA3 ho his4 leu2 MAT*α *THR4 lys2 ADE1 MET13 ura3 CAN1 cyh2*
Y55 2374 or **a***p*	*pms1::URA3 ho his4 leu2 MAT***a** *thr4 lys2 ade1 met13 ura3 can1 CYH2*
Y55 2270	*ho HIS4 LEU2 MAT*α *thr4 LYS2 ade1 MET13 ura3 CANs*
/Y55 2281	*/ho his4 leu2 MAT***a** *THR4 lys2 ADE1 met13 ura3 canR*
αU or αL	*ho MAT*α *LYS ade ura3 CANs*
aU or **a**L	*ho MAT***a** *lys ADE ura3 canR*

size facilitated fixation of spontaneously arising mutations. Mutations were accumulated in haploid mutator strains whose fitness was measured periodically. These data were used to assess the distribution of fitness effects of spontaneous mutations. No mating could be done between these strains as they proved to lack the necessary genetic stability and tended to lose mitochondria.

In further experiments, haploids as well as homozygous and heterozygous diploids with restored mismatch repair and functional mitochondria were obtained and compared. Two main questions were studied: What was the average impact of mutational load on fitness in these three groups, and were the fitness transitions between them correlated? Specifically, the fitness of genetically loaded diploids was expected to be proportional to the fitness of the haploids from which they were derived.

MATERIALS AND METHODS

Strains: In two independent clones, Y55 2438 and Y55 22374, two different genes indispensable for mismatch repair, *MSH2* and *PMS1*, respectively, were disrupted by transplacement, resulting in *msh2* and *pms1* mutants (Chambers *et al.* 1996). These two mutator strains, *msh2* (*m*) and *pms1* (*p*), were used in the mutation accumulation experiment. In subsequent mating experiments, both *m* and *p* strains as well as strains derived from diploid strain Y55 2270/Y55 2281 were used. The latter was never repair deficient and was not subject to mutation accumulation in this experiment. As a result of mating, a collection of genetically unloaded (U) and genetically loaded (L) strains was obtained. The details of mating are described in the following sections. In this article, the mutator strains used only in the accumulation experiment are denoted by *m* or *p*, while U or L refer to the strains that had normal mutation rates and were used only in the comparisons between haploids and diploids. Complete genotypes are provided in Table 1.

All strains used to initiate this study, Y55 2438, Y55 22374, and Y55 2270/Y55 2281, were derived from a single natural isolate, Y55 (McCusker *et al.* 1987). Their subsequent handling under laboratory conditions involved relatively little propagation (R. Borts, personal communication). Thus, they were closely related, although probably not strictly isogenic.

Media: A standard mixture of 1% yeast extract, 2% peptone, and 2% dextrose (YPD) was used as the medium in the accumulation experiment and in the fitness assays. The presence of functional mitochondria was tested by screening for growth on YPG, with 2% dextrose replaced by 3% glycerol, a nonfermentable source of energy. Synthetic minimal medium (SMM), which contained 0.65% yeast-nitrogen base (Difco, Detroit), 2% dextrose, and only the amino acids and nucleobases required by particular strains, was used to screen genetic markers. The media were solidified with 2% Difco-agar when required.

Accumulation of mutations in the mutator strains: Each of the two haploid strains lacking mismatch repair, Y55 2438 and Y55 22374, were used to start eight separate experimental lines designated later as αm_i and $\mathbf{a}p_j$ strains, respectively. α and **a** stand for mating types, *m* and *p* are as described above, and subscripts *i* and *j* range from 1 to 8. These replicate strains were subsequently kept separate and serially transferred between fresh YPD plates. Both the initiation of the experiment and every transfer started with a random choice of a colony. This was done by marking a dot on the bottom of a fresh plate and choosing the closest colony after they appeared. A whole colony was carved out together with some agar and dispersed in 0.5 ml of sterile 0.85% NaCl by vigorous vortexing. A sample of this suspension was streaked to single cells onto a new plate with a sterile toothpick and placed into a 30° incubator. Transfers were done every 54 hr, except that when the colonies were too small they were left to grow for an additional 54 hr. The latter happened more frequently in the late stages of the mutation accumulation experiment.

The strains were monitored for several genetic markers during accumulation of mutations. Every second transfer, 5-μl samples of dispersed colonies were transferred to three different plates: SMM lacking lysine, SMM lacking threonine, and SMM with canavanine. The first plate was tested for external contamination, the other two for cross-contamination between α*m* and **a***p* strains. Cross-contamination among strains of the same genetic type (α*m* or **a***p*) would be undetectable. To minimize such a possibility, transfers of α*m* strains and transfers of **a***p* strains were alternated. Two other traits tested every second transfer were mating ability and mitochondrial function. The test of mating ability was carried out by mixing α*m* and **a***p* strains with **a** and α tester strains, respectively. After overnight incubation on YPD plates, the mixture was transferred to plain SMM plates where only diploid strains could grow, which would indicate successful mating. The presence of mitochondrial function was verified by detecting growth on YPG. The loss of mating ability happened only twice. When it occurred, the strain was restarted from its last positively tested transfer. Inability to grow on YPG occurred much more often. Restarting from previous stages would have been so frequent that the dynamics of population number, and thus mutation accumulation, could differ substantially between strains. Therefore propagation of the populations that had lost mitochondria was continued using the same plates and transfer protocol. These lines were retested for mitochondrial function during the next three consecutive transfers. No revertants were detected. As a result of this procedure, mitochondria were lost in all *m* and *p* lines.

To estimate the number of cell divisions during one transfer,

10 colonies were individually dispersed in 0.85% NaCl. The suspension was serially diluted, transferred on fresh plates, incubated, and the resulting colonies counted. From these counts the number of cells in the original colonies and thus the number of cell divisions were estimated.

Fitness estimation: MGR in liquid YPD was used as the measure of fitness. The medium was supplemented with uracil and lysine or adenine, which enabled the yeast cultures to reach high and similar densities in stationary phase despite their auxotrophic markers.

Each culture was started with inoculation of 1-ml aliquots of fresh medium. A toothpick was used to transfer a sample of cells from a single colony. Cultures were left overnight in a shaking water bath at 30° to allow them to reach stationary phase. The next transfer consisted of 4 ml of fresh medium and a 20-μl sample from the overnight culture. These new cultures were kept in long glass test tubes placed in a water bath on a back-and-forth shaker. They were held at an angle (~25°) to facilitate agitation and aeration of the culture. After 2 hr, a tube was taken out of the bath every 30 min and vortexed and its light absorbance at 600 μm wavelength was measured with a spectrophotometer. Then it was returned to the bath. The data on absorbance were log-normal transformed and regressed. The interval at which the data points fit a straight line well was considered to be the exponential growth phase. Such a procedure is somewhat arbitrary, so the cultures were coded to avoid any bias. Thus the identity of the strain was unknown both during absorbance measurements and when determining the period of exponential growth.

Derivation of repair-proficient strains: *Unloaded haploids:* Unloaded haploids were derived from the diploid strain Y55 2270/Y55 2281, which did not contain mutator genes. The diploid strain was sporulated and the resulting tetrads digested with β-glucuronidase (glusulase). The digest was diluted and spread over selective plates where haploid colonies were screened either for adenine auxotrophy (red color) or canavanine resistance. Sixteen strains were obtained: 8 αU_i and 8 **a**U_j. Full genotype descriptions are provided in Table 1.

Loaded haploids: The α*m* and **a***p* strains from the accumulation experiment could not be used as genetically loaded haploids. They lacked mismatch repair and all lines lost functional mitochondria during the course of the experiment. To restore both functions, every α*m* was mated with a different **a**U and every **a***p* with a different αU. As a result, eight diploid strains α*m*/**a**U and eight diploid strains **a***p*/αU were obtained. These diploids were sporulated, digested, and screened as above. The resulting haploid strains, designated αL or **a**L, contained the same genetic markers (including mating type) as their unloaded parent strains, αU and **a**U, respectively (Table 1). They were also screened to have mismatch repair and mitochondria. L is the genetic load acquired from mutation-accumulating strains α*m* and **a***p*. (Note that the load was only partially transferred to the new strains due to segregation in meiosis.)

In two αL and one **a**L strains functional mitochondria could not be restored. These strains, and their unloaded haploid parent strains, two αU and one **a**U, were not used in later experiments. Comparisons between strains with and without mitochondria would be unfeasible because the loss of mitochondria was usually accompanied by especially pronounced decreases in fitness (see results).

Heterozygous diploids: The haploids described above were mated to get heterozygous diploids with different levels of genetic load. Unloaded diploids are denoted $\alpha U_i/\mathbf{a}U_j$, half loaded as either $\alpha U_i/\mathbf{a}L_j$ or $\alpha L_i/\mathbf{a}U_j$, and fully loaded as $\alpha L_i/\mathbf{a}L_j$. Because *i* ranged from 1 to 6 and *j* from 1 to 7, in each of these four groups a total of 42 diploids was obtained (Figure 1). Diploids were screened on the basis of their markers, but to ascertain that mating was successful each presumptive diploid strain was transferred to sporulation medium and subsequently examined with a microscope for tetrad formation.

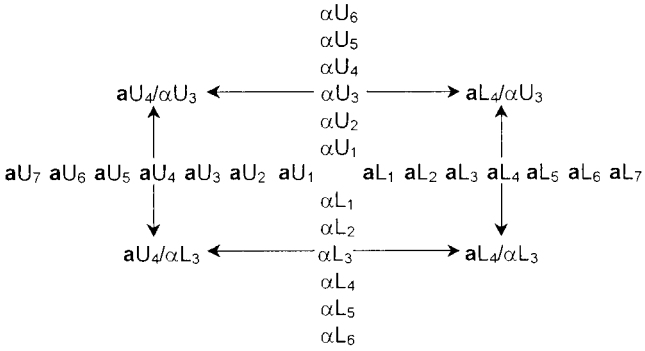

Figure 1.—Matings of haploid strains (mating type **a** or α) from which heterozygous diploid strains (α/**a**) resulted. The arrows show a sample group of four diploid strains related in a particular way: one was unloaded (U/U), two half loaded (U/L or L/U), and one fully loaded (L/L), the last combining loads of the former two. In total, 42 such groups were created.

Homozygous diploids: Both the loaded (αL and **a**L) and unloaded (αU and **a**U) haploids were transformed with a pGAL-*HO* plasmid. The resulting strains were streaked to single cells on YPGal agar plates. During colony growth the galactose present in this medium induced the expression of the *HO* gene that could lead to a switch of the cell's mating type: *MAT*α to *MAT***a** or vice versa. Colonies obtained on YPGal were streaked to single cells on YPD agar plates, stopping the action of the *HO* gene. On average, 83% of these colonies were of the original mating type, 2% of the opposite one, 10% contained cells of both types, and 5% were nonmaters. This generally low efficiency of mating-type switching was advantageous in this case because it reduced the possibility that a mating cell was not a haploid but a diploid, **a**/**a** or α/α (Herskowitz and Jensen 1991). Homozygous diploids were obtained after mating two isogenic strains of the opposite mating type. The diploids were perfectly homozygous except for the *MAT* locus that was heterozygous α/**a**. The *HO* gene could not be expressed in two of the genetically loaded haploids despite repeated efforts, so two homozygous diploids could not be derived.

RESULTS

Fitness decline in the mutator strains

MGR was assayed every 2 transfers, or 46 cell generations. The accumulation of mutations in different strains was terminated after different numbers of transfers (from 15 to 32). Generally, propagation of a strain was terminated when colonies grew very slowly, so that they were small even after incubating for 108 hr. Slow formation of a colony on an agar plate usually but not always coincided with low MGR of the liquid culture initiated from that colony.

Figure 2, a and b, shows average MGRs of seven replicate α*m* (Y55 2438) and six replicate **a***p* (Y55 2374) strains, respectively. (As described in materials and methods, mutation accumulation was carried out for

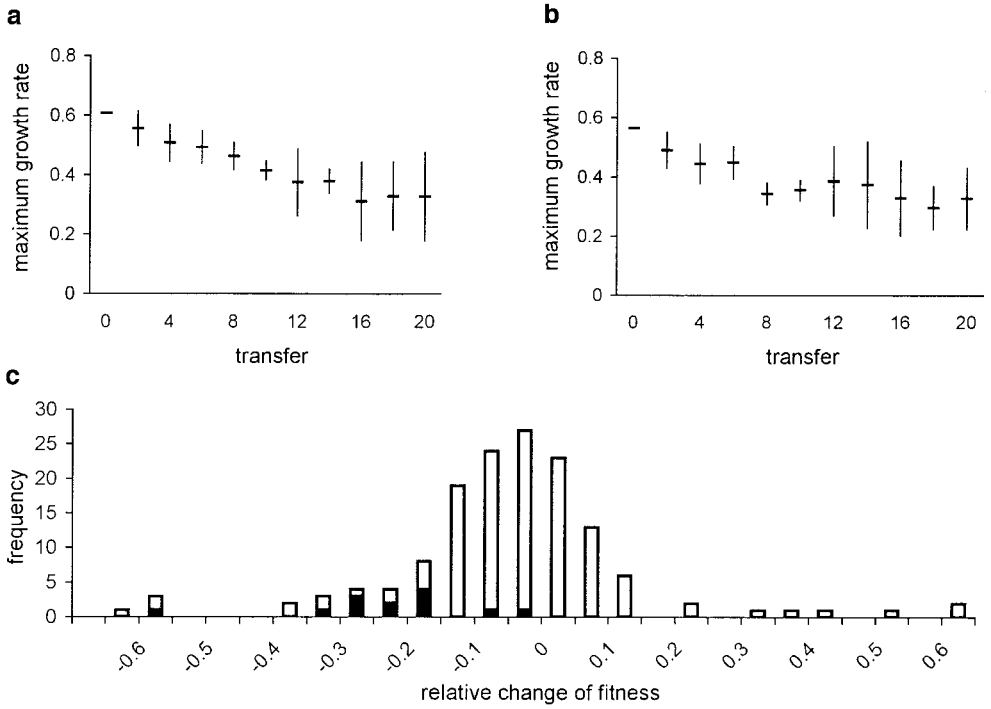

Figure 2.—Average maximum growth rate (MGR) during accumulation of mutations in mismatch repair-deficient strains of haploid yeast. Measurements were made every two plate-to-plate transfers, or 46 generations. (a and b) Averages and 95% confidence limits for the replicate αm (Y55 2438) and **a***p* (Y55 2374) strains, respectively. (c) The distribution of the relative changes in fitness between every two consecutive measurements collected from all strains. The relative change in fitness is defined in a way similar to the selection coefficient *s* (see text for more details). Dark sections of bars indicate the distribution of the fitness changes associated with the loss of mitochondria.

eight αm and eight **a***p* strains, but one αm and two **a***p* strains had to be reinitiated about a month later and their MGRs were not regularly measured.) Propagation of all strains but one was continued up to at least the 20th transfer. The number of strains decreased sharply after the 20th transfer, so further averages are not shown.

There was a significant decrease of MGR over the course of the experiment as evidenced by analysis of means and confidence limits (Figure 2, a and b). To test whether variation between lines increased, the variances among αm and **a***p* lines were pooled and a common estimate of standard deviation was derived. There was a positive correlation between the number of measurements and the standard deviation of MGR ($r = 0.752$, d.f. $= 8$, $t = 3.225$, $P = 0.012$).

To test whether the relative rate of MGR decrease changed over time, the relative change in MGR was calculated as $(\mathrm{MGR}_i/\mathrm{MGR}_{i-1}) - 1$, where i represents the number of fitness measurements and varies from 1 to 10. There was no correlation between the measurement and the average relative change in MGR (Kendall's test for rank correlation; $\tau = 0.333$, $n = 10$, NS). This finding suggests that the relative rate of fitness decrease neither accelerated nor decelerated as the amount of load increased.

Figure 2c presents the distribution of relative changes in MGR, obtained by collecting comparisons between every pair of consecutive measurements in every line. The average relative change in MGR was -0.045. The decrease in MGR was more pronounced during transfers when mitochondria were lost than during the remaining transfers, -0.226 and -0.027, respectively. This difference is statistically significant (Mann-Whitney test; $U = 1510$, $n_1 = 137$, $n_2 = 13$, $t = 4.512$, d.f. $= \infty$, $P < 0.001$). An analysis of the central moments shows that the whole distribution is skewed to the right ($g_1 = 0.648$, $n = 145$, $t = 3.218$, d.f. $= \infty$, $P < 0.001$) and leptokurtic ($g_2 = 6.375$, $n = 145$, $t = 15.932$, d.f. $= \infty$, $P < 0.001$). Deviation from normality is confirmed by the D'Agostino test ($D = 0.2408$, $n = 145$, $P < 0.01$; Zar 1984, pp. 95–96). Thus analysis of the distribution suggests that both the extremely positive and extremely negative relative changes in MGR were overrepresented.

Fitness estimates of repair-proficient strains

Haploids: Figure 3 presents the MGRs of 13 pairs of U and L haploid strains (see materials and methods for a detailed description of their origin). The MGRs of L strains were lower than those of U strains in every comparison. If we define the relative fitness of each U strain as one, and the fitness of the L strain as the ratio of its MGR to the MGR of the corresponding U strain, then the average relative fitness of the 13 L strains was 0.750, which was significantly lower than one ($t = 5.749$, d.f. $= 12$, $P < 0.001$).

Homozygous diploids: Out of 13 unloaded haploids, all were converted to homozygous diploids (U/U). In the case of loaded haploids, only 11 homozygous diploids (L/L) were successfully derived. The average fitness of the 11 L/L strains relative to the U/U strains was 0.705, which was also significantly lower than one ($t = 6.995$, d.f. $= 10$, $P < 0.001$).

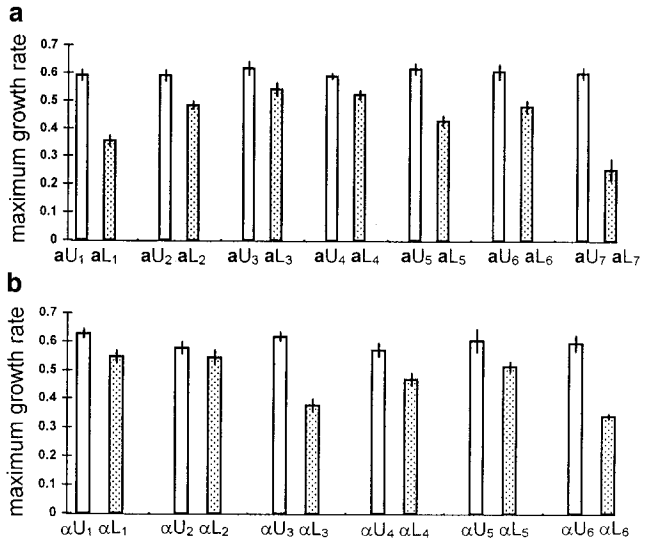

Figure 3.—Maximum growth rates of genetically unloaded (empty bars and letter U) and loaded (dotted bars and letter L) haploid strains in which mismatch repair and mitochondrial function were restored. Vertical lines indicate 95% confidence limits on the slopes of the regression lines. (a) The **a**L strains were derived from α*m* strains (compare Figure 2a). (b) The αL strains were derived from **a***p* strains (compare Figure 2b).

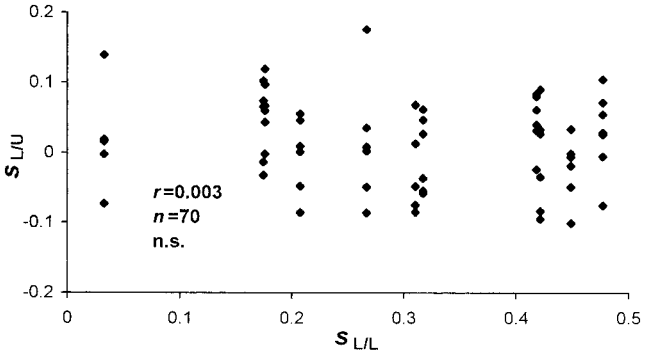

Figure 4.—Selection coefficients of half-loaded heterozygous ($s_{Li/U}$) and loaded homozygous diploids ($s_{Li/Li}$). L_i denotes a loaded haploid genotype that was mated with several unloaded haploids, resulting in half-loaded heterozygotes (L_i/U, compare Figure 1), or used to derive a homozygote (L_i/L_i). In this graph, the $s_{Li/U}$ coefficients form a column over the single $s_{Li/Li}$ coefficient. (The $s_{Li/U}$ coefficients were obtained by relating half-loaded heterozygotes to unloaded heterozygotes. The $s_{Li/Li}$ coefficients were obtained by relating loaded homozygotes to unloaded homozygotes; see the text for details.)

The average relative fitness of the L/L homozygotes was then compared with the average relative fitness of the L haploids. The two L strains for which L/L homozygotes could not be constructed were excluded from the analysis. The average relative fitness of the remaining 11 L strains was 0.752. There was no statistically significant difference when isogenic haploids and homozygous diploids were compared in a paired *t*-test ($t = 1.166$, d.f. = 10, $P = 0.271$). The test of the correlation between haploids and homozygotes is reported in the section on fitness transitions.

Heterozygous diploids: Figure 1 provides details of the matings that resulted in 42 groups of genetically related heterozygous diploids. A group consisted of four strains: one unloaded, two half loaded, and one fully loaded. Within each group the fully loaded diploid combined mutations present in both half-loaded ones. Relative fitness of a loaded diploid was then calculated as its MGR divided by the MGR of an unloaded diploid. This scaling of fitness was done separately within each of the 42 groups.

Selection coefficient (s): The average fitness of fully loaded heterozygotes, 0.954, was significantly lower than one, that is, the fitness of unloaded heterozygotes ($t = 2.705$, d.f. = 41, $P = 0.010$, if individual loaded heterozygotes and the corresponding individual unloaded heterozygotes were compared in pairs; or, $t = 4.175$, d.f. = 12, $P = 0.001$, if a pair of comparison consisted of the average fitness of all heterozygotes sharing a particular L haploid genotype and the average fitness of all heterozygotes sharing the corresponding U haploid genotype). Thus, the average selection coefficient of loaded heterozygotes was $s = 0.046$.

Dominance coefficient (h): The average dominance coefficient of the new mutations can be calculated as the ratio of the selection coefficient of a half-loaded heterozygote, $s_{U/L}$, to the selection coefficient of a loaded homozygote, $s_{L/L}$. Figure 4 shows the distributions of both selection coefficients. The average coefficient of dominance was $h = 0.08$.

Variation among loaded heterozygotes: The *b* coefficients (MGRs) of 42 fully loaded heterozygotes were compared in a single-classification ANOVA. The variance component due to differences among the MGRs was statistically significant [0.0260 ± 0.0122; the 95% confidence limits were found by a jacknife method (Sokal and Rohlf 1995, p. 821)]. The variance component due to error of the MGR measurements was 0.0179 [the 95% confidence limits were 0.0137 and 0.0239; Tate and Klett's method (Sokal and Rohlf 1995, p. 157)].

Fitness transitions between haplo- and diplophase: The fitness of a diploid strain may be determined by the fitness of the haploid strains from which the diploid was derived. This dependence would result in a correlation between both values. There is also a possibility that the fitnesses of diploids sharing a particular L haploid genotype tend to be similar to each other although not correlated with the fitness of this haploid. These two hypotheses were tested and the results are provided in the following two sections.

Correlations: The MGRs of diploid homozygotes were correlated with the MGRs of the haploids from which they were derived (Figure 5a). The results imply that there was a strong correspondence between the MGRs of the haploid and diploid genetic phases as long as the

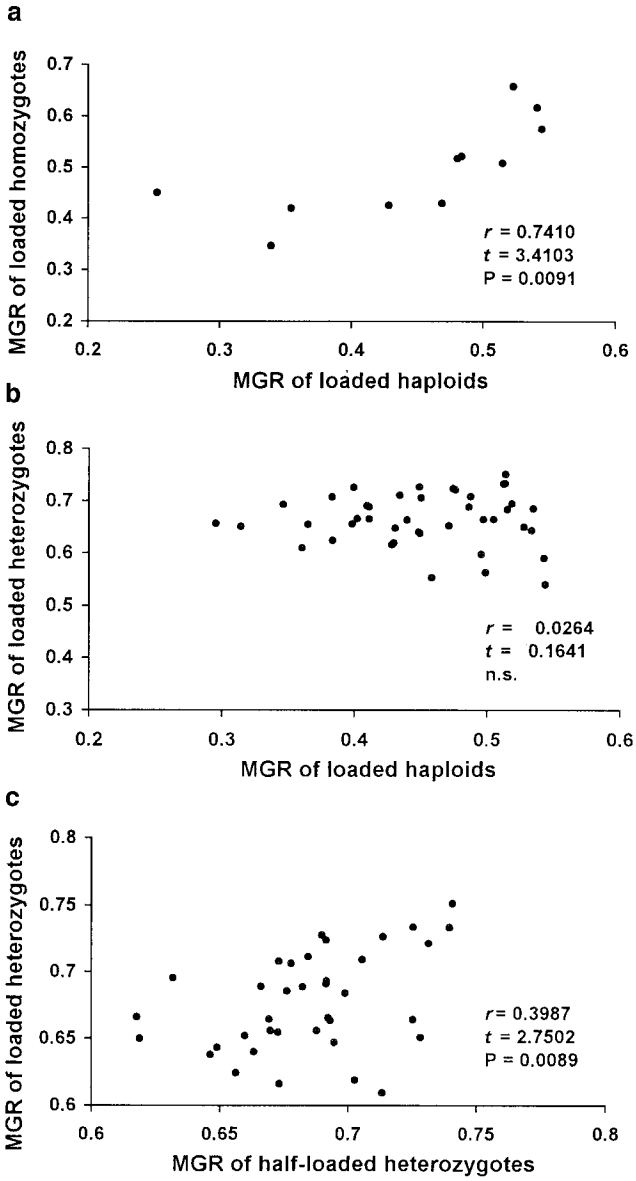

Figure 5.—(a–c) Relationships of maximum growth rates (MGRs) between mutation-loaded strains. Genetic relationships between haploids, half-loaded diploids, and fully loaded diploids are shown in Figure 1.

compared strains were isogenic. However, no correlation was found when the MGR of a fully loaded but heterozygous diploid was related to the average MGR of the two haploids from which it was derived (Figure 5b). The MGR of a fully loaded diploid can also be related to the average MGR of the two relevant half-loaded diploids. This correlation is statistically significant (Figure 5c).

Two-way ANOVA: The fully loaded heterozygotes can be grouped according to the loaded haploids from which they were derived (lower right in Figure 1). A two-way ANOVA without replication is presented in Table 2. The differences between the **a**L genotypes are close to the 0.05 significance level. Closer examination showed

TABLE 2

Fitness of fully loaded heterozygotes grouped according to the haploids from which they were derived

Source of variation	d.f.	MS	*F*	*P*
Among **a**L genotypes	6	0.004852	2.409	0.051
Among αL genotypes	5	0.002923	1.451	0.235
Unexplained	30	0.002014		

Two-way ANOVA without replications was performed.

that this was due to the generally low fitness of the heterozygotes containing genes from the **a**L_3 haploid, which, surprisingly, was the least loaded among the **a**L strains (Figure 3a). The differences among the remaining 12 groups were small. The general conclusion is that there was no significant dependence of loaded heterozygous diploids on loaded haploids.

DISCUSSION

Quantitative traits are usually assumed to be influenced by many genes with small individual effects. A character is shaped by the additive effects of such genes and by their interactions, both within and between loci. Individuals sharing the same alleles will resemble each other only if the interactions between genes are not strong enough to obscure their additive effects. This standard conceptual framework was applied in this study. The maximum growth rate of yeast was chosen as the trait that is quantitative and representative of the organism's fitness. Experimental accumulation of random mutations revealed that the maximum growth rate is indeed influenced mostly by many genes with relatively small effects. However, the genetic relatedness of organisms, such as in the case of a diploid and the two haploids from which it was derived, did not result in similar maximum growth rates. It is suggested that the unpredictability of fitness transitions between haploids and diploids does not merely reflect phenotypic differences between these two genetic phases, but results from the nonadditive fitness effects of the new alleles.

Accumulation of mutations: This study demonstrated that strains deficient in mismatch repair may be used for efficient accumulation of mildly deleterious mutations. The average fitness of mutator strains declined substantially during the course of mutation accumulation, while variation between the replicate lines increased (Figure 2, a and b). Neither acceleration nor deceleration in the rate of fitness decline were observed. Thus, the pattern of fitness decline is in agreement with a simple model assuming that mutations happen at random in different strains over time, and that their average effect does not change during the course of the experiment. This conclusion must be taken cautiously, however, as the accumulation of mutations was interrupted by the

loss of mitochondria that appeared in all lines although at different moments. Loss of mitochondria is a major physiological change; comparing the fitness effects of mutations occurring before and afterward may be problematic.

The average decrease of fitness between two consecutive measurements was negative and rather small, on the order of a few percent. However, there were also mutations with exceptionally large effects, both negative and positive (Figure 2c). Some of the conspicuous drops in fitness were caused by the loss of mitochondria. Restoration of mitochondrial function was not observed. Therefore such simple reverse mutations cannot account for the large fitness increases, although some fitness surges could be caused by mutations that somewhat compensated for the loss of mitochondria. Generally, the nature of most mutations must remain unknown because there are a great number of mutations affecting the maximum growth rate of yeast.

The distribution presented in Figure 2c shows the relative changes in fitness between pairs of consecutive measurements. It is not necessarily a distribution of single mutations, as more than one could happen within each time period (46 generations of cell divisions). Furthermore, the 145 data items are not truly independent as they were collected from 13 separate lines. The results could also be biased by natural selection occurring during the growth of a colony (Kibota and Lynch 1996). A clone with a beneficial mutation increases in frequency during the colony growth while the reverse is true for a clone bearing a deleterious mutation. This affects their chances for being randomly chosen to initiate the next transfer and thus to be fixed. Despite all these reservations, a distribution of the fitness effects of spontaneous mutations was obtained and presented because such data are much needed and still rare.

Fitness of repair-proficient haploids and homozygotes: The strains from the accumulation experiment were used to derive haploids with normal (low) mutation rates and efficient mitochondria. The fitness of the resulting strains was reduced by one quarter on average when compared to strains that were not subject to accumulation of random mutations. These genetically loaded haploids were used to obtain homozygous and heterozygous diploids.

The relationship between the fitnesses of haploids and homozygotes was first determined. Such a comparison is desirable because although the phenotypes of haploids do not differ much from those of diploids they are not identical. For example, the patterns of budding of new cells differ. In this experiment haploids and homozygous diploids also differed genetically at the *MAT* locus, which was α or **a** in the former and α/**a** in the latter. This locus is highly pleiotropic and can influence fitness (Birdsell and Willis 1996). In the present experiment, the transition from haploids to homozygous diploids did not affect fitness: the average fitness of haploids did not differ from that of homozygous diploids (Figure 5a). These results mean that the effects of ploidy and the *MAT* locus cannot account for the observed differences between haploids and heterozygous diploids.

Fitness of repair-proficient heterozygotes: It is unlikely that new mutations occurred in the same genes in two different haploids. Mating of two loaded haploids resulted in a diploid in which the new mutations were most probably paired with wild-type alleles. The average genetic load of heterozygous diploids was found to be substantially lower than the average load of haploids. The average coefficient of dominance for deleterious mutations was 0.08. This estimate falls within the range found in other studies (Deng and Lynch 1997).

Transition between haploids and heterozygous diploids: There was no correlation between the fitness values of loaded haploids and the heterozygous diploids derived from them (Figure 5b). But the fitness of a fully loaded diploid could be predicted from the fitnesses of the two corresponding half-loaded diploids (Figure 5c). Suppose that the larger the negative effect of a mutation, the more it was masked in the heterozygote by the wild-type allele. The coefficient of dominance (h) relates the selection coefficient of heterozygotes to that of homozygotes, $s_{L/U} = hs_{L/L}$. Because in the present study the average value of $s_{Li/U}$ generally did not change with increasing $s_{Li/Li}$ (Figure 4), h must have been negatively correlated with $s_{Li/Li}$ (and s_{Li}), resulting in a lack of correlation between the diploids and haploids (Figure 5b). Figure 4 also shows that the individual selection coefficients of half-loaded heterozygotes varied substantially. The average fitness of a pair of half-loaded heterozygotes is $[(1 - s_{Li/U}) + (1 - s_{U/Lj})]/2 = 1 - (s_{Li/U} + s_{U/Lj})/2$. It must be correlated with the fitness of the corresponding fully loaded heterozygote because the latter is $1 - s_{Li/Lj} = 1 - (s_{Li/U} + s_{U/Lj})$, provided that the loads of the haploid genotypes i and j are additive. This could underline the correlation seen in Figure 5c. Other data also indicate that the coefficient of dominance (h) can be negatively correlated with the selection coefficient (Charlesworth 1979).

Beneficial mutations (Figure 2c) might additionally obscure the relation between the loaded haploids and diploids. These mutations could be partially dominant or at least less recessive than the deleterious ones. The bulk of empirical data and also theory suggest that deleterious mutations are generally recessive (Kacser and Burns 1981). On the other hand, beneficial mutations are often partially dominant, as has been found in the evolution of resistance to herbicides (Jasieniuk *et al.* 1996) and pesticides. Bourguet and Raymond (1998) noted that in the case of pesticide tolerance the partial dominance of beneficial alleles is roughly predicted by Kacser and Burns's theory, which suggests that this phenomenon might be common. Such asymmetry between deleterious and beneficial mutations in their levels of

dominance may have occurred also in this experiment. The haploid strains could have contained mostly deleterious and recessive mutations but also some beneficial and partially dominant ones. Imagine that after the transition to diploidy the prevailing impact of the deleterious mutations was much lessened but the effects of beneficial mutations remained generally strong. The recessivity of deleterious mutations would obscure the correlation between haploids and heterozygous diploids, while the dominance of beneficial mutations would contribute to the correlation between the half-loaded and fully loaded heterozygotes.

There may be no single explanation why the fitness of diploids did not correlate with the fitness of haploids. The masking effect of the wild-type alleles, and especially the negative correlation between the coefficients of selection and dominance, are probably important. However, the partial dominance of the beneficial mutations combined with the recessivity of the deleterious ones would also obscure correlation between the two genetic phases. Other mechanisms are also possible although they remain hypothetical. For example, the fitness of a heterozygote may be higher or lower than the fitness of any single allele, both wild type and mutated. It is also possible that epistatic interactions among haploid or homozygous loci are substantially different from those among heterozygous loci. Discrimination and quantification of these factors is practically impossible when the number of mutations and their individual properties are not known. Therefore the dominance coefficient estimate (0.08) must be taken with caution.

Empirical studies of genetic load: Estimates of the rate at which spontaneous mutations originate (Peck and Eyre-Walker 1997; Drake *et al.* 1998) and descriptions of their interaction (Mukai 1969; da Silva and Bell 1996; de Visser *et al.* 1996, 1997a,b; Elena and Lenski 1997; Charlesworth 1998; West *et al.* 1998) vary widely. It is probably too early to decide whether the measurements are inaccurate or the observed discrepancies reflect the diversity present in nature. Empirical studies usually start by gathering a number of genetically loaded strains; statistical analyses of fitness are then used to obtain estimates of the mutation rate, the distribution of single-mutation effects, dominance coefficients, or assessments of interaction between mutations (Crow and Simmons 1983; Deng and Lynch 1997; Keightley and Caballero 1997). Such estimates are necessarily indirect and susceptible to the assumptions used in modeling, even if fitness variation is largely additive. The task would be even more arduous if diverse effects of dominance and epistasis were abundant.

Unfortunately the implications of this study complicate rather than simplify empirical analyses of the fitness effects of mutational load. In particular they suggest that great care must be taken when the results obtained with haploid or homozygous diploids are extended to heterozygous diploids. This is especially important for organisms that are diploid and usually heterozygous with respect to genetic load (Crow 1997).

I thank R. Borts for providing yeast strains and, most of all, for encouragement and guidance on yeast genetics. A visit to her lab was sponsored by The Wellcome Trust. M. Hanzelka assisted me in the laboratory work. Z. Dabrowski and K. Janeczko provided some instruments. Comments made by A. Lomnicki, J. Kozlowski, J. Radwan, M. Travisano, and an anonymous referee improved the manuscript. M. Jacobs helped edit it. This work was supported by a Polish Committee for Scientific Research grant no. KBN 0284/P04/97/13.

LITERATURE CITED

Andersson, D. I ., and D. Hughes, 1996 Muller's ratchet decreases fitness of a DNA-based microbe. Proc. Natl. Acad. Sci. USA **93:** 906–907.

Birdsell, J. H., and C. Willis, 1996 Significant competitive advantage conferred by meiosis and syngamy in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **93:** 908–912.

Bourguet, D., and M. Raymond, 1998 The molecular basis of dominance relationships: the case of some recent adaptive genes. J. Evol. Biol. **11:** 103–122.

Chambers, S. R., N. Hunter, E. J. Louis and R. H. Borts, 1996 The mismatch repair system reduces meiotic homeologous recombination and stimulates recombination-dependent chromosome loss. Mol. Cell. Biol. **16:** 6110–6120.

Charlesworth, B., 1979 Evidence against Fisher's theory of dominance. Nature **278:** 848–849.

Charlesworth, B., 1990 Mutation-selection balance and the evolutionary advantage of sex and recombination. Genet. Res. **55:** 199–221.

Charlesworth, B., 1998 The effect of synergistic epistasis on the inbreeding load. Genet. Res. **71:** 85–89.

Charlesworth, B., D. Charlesworth and M. T. Morgan, 1990 Genetic loads and estimates of mutation rates in highly inbred plant populations. Nature **347:** 389–392.

Crow, J. F., 1997 The high spontaneous mutation rate: Is it a health risk? Proc. Natl. Acad. Sci. USA **94:** 8380–8386.

Crow, J. F., and M. J. Simmons, 1983 The mutational load in *Drosophila*, pp. 1–35 in *The Genetics and Biology of Drosophila*, Vol. 3c, edited by M. Ashburner, H. L. Carson and J. N. Thompson. Academic Press, London/New York.

da Silva, J., and G. Bell, 1996 The ecology and genetics of fitness in *Chlamydomonas*: VII. The effects of sex on the variance in fitness and mean fitness. Evolution **50:** 1705–1713.

Deng, H.-W., and M. Lynch, 1997 Inbreeding depression and inferred deleterious-mutation parameters in Daphnia. Genetics **147:** 147–155.

de Visser, J. A. G. M., R. F. Hoekstra and H. Van Den Ende, 1996 The effect of sex and deleterious mutations on fitness in *Chlamydomonas*. Proc. R. Soc. Lond. B Biol. Sci. **263:** 193–200.

de Visser, J. A. G. M., R. F. Hoekstra and H. Van Den Ende, 1997a An experimental test for synergistic epistasis and its application in *Chlamydomonas*. Genetics **145:** 815–819.

de Visser, J. A. G. M., R. F. Hoekstra and H. Van Den Ende, 1997b Test of interaction between genetic markers that affect fitness in *Aspergillus niger*. Evolution **51:** 1499–1505.

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

Elena, S. F., and R. E. Lenski, 1997 Test of synergistic interactions among deleterious mutations in bacteria. Nature **390:** 395–398.

Feldman, M. W., F. B. Christiansen and L. D. Brooks, 1980 Evolution of recombination. Proc. Natl. Acad. Sci. USA **76:** 4838–4841.

Fernandez, J., and C. Lopez-Fanjul, 1996 Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. Genetics **143:** 829–837.

Herskowitz, I., and R. E. Jensen, 1991 Putting the *HO* gene to work: practical uses for mating-type switching, pp. 132–145 in *Guide to Yeast Genetics and Molecular Biology: Methods in Enzymology*, Vol. 194, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego.

Jasieniuk, M., A. L. Brule-Babel and I. N. Morrison, 1996 The

evolution and genetics of herbicide resistance in weeds. Weed Sci. **44:** 176–193.
Jenkins, C. D., and M. Kirkpatrick, 1995 Deleterious mutation and the evolution of genetic life cycles. Evolution **49:** 512–520.
Johnston, M., and D. J. Shoen, 1995 Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. Science **267:** 226–229.
Johnson, R. E., G. K. Koali, L. Prakash and S. Prakash, 1996 Requirement of the yeast *MSH3* and *MSH6* genes for *MSH2*-dependent genomic stability. J. Biol. Chem. **271:** 7285–7288.
Kacser, H., and J. A. Burns, 1981 The molecular basis of dominance. Genetics **97:** 639–666.
Keightley, P. D., and A. Caballero, 1997 Genomic mutation rate for lifetime reproductive output and lifespan in *Caenorhabditis elegans.* Proc. Natl. Acad. Sci. USA **94:** 3823–3827.
Kibota, T. T., and M. Lynch, 1996 Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli.* Nature **381:** 694–696.
Kimura, M., and T. Maruyama, 1966 The mutational load with epistatic gene interactions in fitness. Genetics **34:** 1337–1357.
Kolodner, R., 1996 Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev. **10:** 1433–1442.
Kondrashov, A. S., 1982 Selection against harmful mutations in large sexual and asexual populations. Genet. Res. **40:** 325–332.
Kondrashov, A. S., 1988 Deleterious mutations and the evolution of sexual reproduction. Nature **336:** 435–440.
Kramer, W., B. Kramer, M. S. Williamson and S. Fogel, 1989 Cloning and nucleotide sequence of DNA mismatch repair gene PMS1 from *Saccharomyces cerevisiae*: homology of PMS1 to prokaryotic MutL and HexB. J. Bacteriol. **171:** 5339–5346.
Marsischky, G. T., N. Filosi, M. F. Kane and R. Kolodner, 1996 Redundancy of *Saccharomyces cerevisiae* MSH3 and MSH6 in MSH2 dependent mismatch repair. Genes Dev. **10:** 407–420.
McCusker, J. H., D. S. Perlin and J. E. Haber, 1987 Pleiotropic plasma membrane ATPase mutations of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 4082–4088.
Mukai, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50:** 1–19.
Mukai, T., 1969 The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. Genetics **61:** 749–761.
Otto, S. P., and D. B. Goldstein, 1992 Recombination and the evolution of diploidy. Genetics **131:** 745–751.
Peck, J. R., and A. Eyre-Walker, 1997 A muddle about mutations. Nature **387:** 135–136.
Perrot, V., S. Richerd and M. Valero, 1991 Transition from haploidy to diploidy. Nature **351:** 315–317.
Sokal, R. R., and F. J. Rohlf, 1995 *Biometry*, Ed. 3. Freeman, New York.
West, S. A., A. D. Peters and N. H. Barton, 1998 Testing for epistasis between deleterious mutations. Genetics **149:** 435–444.
Williamson, M. S., J. Game and S. Fogel, 1985 Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I. Isolation and characterization of PMS1-1 and PMS1-2. Genetics **110:** 609–646.
Zar, J. H., 1984 *Biostatical Analysis.* Prentice-Hall International, Englewood Cliffs, NJ.

Communicating editor: P. D. Keightley