Available online at www.sciencedirect.com
SCIENCE DIRECT®
Research in Microbiology 155 (2004) 301–310
Research in Microbiology
www.elsevier.com/locate/resmic

Experimental studies of deleterious mutation in *Saccharomyces cerevisiae*

Ryszard Korona

Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland

Received 18 September 2003; accepted 20 January 2004

Available online 19 March 2004

Abstract

Yeast has proven to be a very useful model organism for studying eukaryotic cell functions. Its applicability for population and quantitative genetics is less well known. Among its advantages is the ease of screening for mutants. The present paper reviews experiments aimed at estimating the parameters of spontaneous mutations deleterious to fitness. The rate of deleterious mutation was found to be moderately high. A large fraction of detectable mutants were lethal; among the non-lethal mutants, the least harmful ones dominated. Deleterious mutations, and especially the lethal ones, were generally very well masked by wild-type alleles when in heterozygous loci. The negative effects of mutations were much stronger under stressful than under benign conditions. Interactions between loci with deleterious mutations did alter their fitness, but no strong overall effect of synergism or antagonisms was observed.

Keywords: Deleterious mutation rate; Selection coefficient; Dominance; Epistasis; *Saccharomyces cerevisiae*

1. Deleterious mutation in population genetics

Systematic collection and analysis of mutants over the past century has been a very successful research program in classical genetics, helping to explain not only how traits are passed over generations but also, and more importantly, how they are produced by organisms. Evolutionary biologists have been familiar with the notion of mutation for even longer, a century and a half, even though the initial concept was imprecise. The essence of Darwin's idea has remained unchanged, with mutations understood as the raw material of evolution. For evolutionary biologists and especially population geneticists, the basic question is not how mutation alters the functioning of an individual organism but whether mutation affects its chances in competition with its conspecifics. The variety of phenotypic changes is converted into a single currency of selection coefficients, that is, proportional changes in fitness relative to the "wild" or typical phenotypes. This methodology has remained unchanged for decades but is still valid, and even "natural", because natural selection has shaped all the marvelous functions of organisms solely by passing judgment on the overall selective value of their phenotypes.

Budding yeast has become one of the top experimental models in modern biology. Its use is based on highly successful genetic and biochemical analyses of cell functioning. It is surprising that, unlike *Drosophila* or *Escherichia*, yeast has not been extensively studied by population geneticists or ecologists. Recent years have witnessed some interest in experimental evolution of yeast [82], but there is still a relative paucity of population studies. This paper is intended to show how the advantages of yeast can be employed to address some longstanding questions of evolutionary biology.

Natural selection may lead to new adaptations, but its everyday job is to maintain the existing ones. If spontaneous mutations have fitness effects, these are much more likely to be negative than positive [6,18]. Deleterious mutations are not only more frequent but also more predictable, and therefore more tractable. Beneficial mutations are likely to be "conditional" in the sense that their selective value can be high in some environments but low in others. Among the deleterious ones, a fair share should be "unconditionally" deleterious because damage to the housekeeping genes would be harmful in all environments. Deleterious mutations are also unavoidable in the sense that errors in replication or maintenance of the genetic material must occur if a population is sufficiently large or long-lasting. Therefore genetic variation of mutational origin is likely to be ubiquitous, even in populations well adapted to their environment.

E-mail address: korona@eko.uj.edu.pl (R. Korona).

0923-2508/$ – see front matter
doi:10.1016/j.resmic.2004.01.015

Haldane [33] noted that even if mutations are removed by selection they remain in a population for some time and therefore decrease its fitness or, in other words, build its mutational load. The purifying effect of selection is reduced in small populations in which chance events may dominate over differences in fitness. This may result in a gradual ratchet-like decline in fitness, especially when organisms reproduce asexually [58], but also sexually [50]. When the load becomes excessive, a population may not even be able to reproduce itself and will "melt down" [26]. Fitness may also collapse in populations of heterozygous diploids in which mutations are masked by wild-type alleles. They become endangered by inbreeding depression if out-crossing becomes less frequent [8]. Another intuitively obvious consequence of spontaneous mutation is degeneration of chromosomes such as mammalian Y, which cannot be repaired by recombination with homologues [7].

Although the most obvious consequence of deleterious mutations is contamination of genomes, their unavoidable presence constitutes a strong selection factor and thus induces adaptive evolution. Several important biological phenomena might have developed in response to spontaneous mutagenesis. Senescence might result from accumulation of those mutations whose negative effects are manifested late in life and therefore were rarely exposed to selection in natural populations where old organisms were rare [55]. The need to remove deleterious mutations from a population has been invoked to explain the evolution of genetic recombination [3,23,31,45] including its most elaborate variant, sexual reproduction. Outcrossing coupled with recombination would result in lower mortality because a fraction of progeny would be freed from an excessive burden of mutations [47]. Another consequence would be uncoupling of the few advantageous mutations from the majority of deleterious ones [66]. The evolution of different genetic life cycles is thought to reflect the fact that neither haploidy nor diploidy is universally advantageous in reducing the mutational load: mutations are twice rarer in haploids but usually less harmful in diploids [36,60,64]. These questions cannot be addressed properly without an estimation of some major parameters of spontaneous mutation, such as the rate of origination, the magnitude of fitness effects, the degree of dominance within a locus, and the intensity of epistasis between loci.

Experimental study of mutations affecting fitness has proved challenging [18,41,51]. Except for lethal and very strong effects, single mutations usually affect fitness too little to be discernible from environmental variation. Therefore mutations are often amassed in experimental populations before their phenotypic effects are measured. When breeding extends over many generations, however, it is difficult to avoid purging selection or adaptation to experimental conditions [39]. In an alternative approach, individuals from extant populations are sampled and their mutational load estimated. In this case it is usually uncertain whether populations have reached a mutation–selection balance and whether the test environments relate well to the natural one [9]. However the mutations accumulated, analyses based on multiple mutations are not straightforward. A general principle has been to compare changes in mean fitness versus changes in variance and to deduce how many and how strong were the effects to produce the observed shifts [4,38,57]. This poses a serious methodological difficulty because the same data have to be used to infer both the number and the selection coefficients of mutations. Deleterious mutation rate estimates obtained in this way can vary by more than two orders of magnitude (selected examples of invertebrates [29,40,52,56]), and it is unclear whether such a discrepancy reflects differences in the genetics of the studied species and populations or results from that methodological difficulty.

2. Spontaneous mutation rate

2.1. Accumulation of mutations

Yeast, being a microbe, can be maintained in large experimental populations. This is important for studying rare events such as spontaneous mutations. Yeast is also easy and cheap to propagate for many generations. This is not a trivial advantage because studies of spontaneous mutations in multicellular organisms require much time, work—and funds. Samples of experimental yeast populations can be frozen at any time of their history and then used simultaneously in assays of fitness. The experimental environment can be strictly controlled. Most of the experiments described below were carried out in an environment considered optimal for budding yeast, that is, a nutrient broth composed of yeast extract, peptone and glucose (YPD) kept at 30 °C. Any growth defects seen under these favorable conditions are likely to be associated with mutations affecting the basic functions of the cell. Another important feature of yeast cultures is the availability of techniques allowing for relatively efficient shielding of mutations from natural selection. Periodically transferring a population through a single cell will instantly fixate all deleterious mutations that happened to be in the founder of the new population. For all these reasons, study of long-term accumulation of spontaneous mutations seems to be an especially suitable approach in the case of yeast.

Zeyl and deVisser [83] did a study of this kind. They propagated 50 replicate lines of a diploid strain of yeast by transferring them serially on agar plates, beginning each transfer by randomly selecting a colony and streaking it to single cells which were then incubated and developed into colonies of the next generation. Fitness assays were done by competing the mutation-accumulating clones with their founder clone. After 600 generations of such propagation, mitochondria were lost in about half of the replicate lines. Such clones were excluded from further analyses because the loss of mitochondria is not a chromosomal mutation but substantially reduces fitness. Among the remaining lines, the fitness of only one was lower than that of the progenitor clone. The authors based their estimates on this single

mutant phenotype, and found that mutations producing fitness defects arise at the rate of $U = 9.5 \times 10^{-5}$ per division of the whole diploid genome.

Korona [49] performed another study in which mutations were accumulated in yeast by serial transfers through single cells, but he did not estimate the genomic mutation rate, U, because the number of replicate lines, 16, was too low. He used strains that were mutators. They lacked proper post-replication DNA mismatch repair because a gene coding a protein critical for this function was deleted. Earlier, another author found that malfunctioning of mismatch repair led to elevation of the rate of substitution or single base deletion by about two orders of magnitude [46]. The mutator strains in Korona's [49] study were haploid and therefore the emerging mutations were exposed to selection. A substantial decrease in fitness was nevertheless observed. The trajectory of fitness decline strongly suggested that most of the accumulated mutations decreased fitness but that some produced the opposite effect. It was clear that mutations compensating for the accumulating defects appeared and were strongly selected for, at least in populations of mutators. Since the deleterious and beneficial mutations could not be separated, it was impossible to estimate the selection coefficient for any of them.

These examples demonstrate how difficult it is to interpret the results of mutation accumulation experiments. In diploids, many accumulated mutations probably are compensated for efficiently so that only dominant or partially dominant alleles can be detected. In haploids, mutations must be purged by selection when they produce lethal or considerably harmful effects. Positive selection for compensatory mutations is probably even more troubling. Such mutations were found in mutator strains, but they might also occur in strains with a wild-type mutation rate if propagation were sufficiently long. Spontaneous loss or damage to mitochondria may be another source of confusion. The conclusion is that any long-term accumulation of mutations will be unavoidably accompanied by other processes affecting fitness. It may be difficult if not impossible to dissect and quantify those processes.

2.2. Screen for mutations

Experimental accumulation of mutations is an approach devised for organisms in which screening for single mutations is virtually impossible. Imagine that in a fruit fly a mutation decreasing its viability or growth rate by a few percent appears. Initially it would be heterozygous. An individual carrying it would have to be crossed with a wild type. The resulting progeny, generation F_1, could then be used to obtain generation F_2 in which homozygotes would appear. When the expected genetic effects are on the order of 1%, and the environmental effects are at least that large, it is hardly possible to distinguish between the homozygous mutant, heterozygous mutant and wild-type phenotypes even if relatively large numbers of individuals are assayed.

Yeast is a truly exceptional object for classical genetic analysis. It can be maintained stably as a haploid or a diploid organism. The transition from diploidy to haploidy proceeds through meiosis and sporulation. A single diploid cell produces four spores enclosed in an ascus. Each of the four spores contains a single haploid cell which is a direct product of meiosis. The ascus can be digested, and the spores separated and placed on nutrient agar where they can develop into clonal haploid colonies. The spores are equivalent to gametes of multicellular organisms, but the ability to grow and divide mitotically for many generations makes them markedly different from typical gametes. Moreover, both the phenotypes and the patterns of gene expression are very similar for haploids and diploids [27]. Genetic analysis is particularly simple and straightforward because detection of heterozygous loci does not require two rounds of breeding. Instead, tetrads of haploid clones are monitored for segregation of characters. A single heterozygous locus is revealed by the 2:2 segregation pattern: two haploid clones have a wild-type phenotype and the remaining two have a mutant phenotype.

Wloch et al. [80] applied tetrad analysis to an extensive screen for deleterious mutations. A single cell of a homozygous diploid strain was used to initiate replicate mutation-accumulation lines. They grew vegetatively for about two transfers and then went through sporulation and spore separation. This yielded a large number of tetrads, which were strictly isogenic because they had a common ancestor as recently as 60 cell doublings ago. All the resulting haploid clones would be identical to each other if there were no new mutations. The authors tested over 1000 tetrads and scored a few dozen in which two out of four haploid clones were dead or grew more slowly and formed smaller colonies (Fig. 1). The calculated rate of spontaneous mutation affecting fitness was 1.1×10^{-3} per diploid cell division. This figure was obtained by assaying haploid clones; it is about ten times higher than Zeyl and deVisser's [83] estimate from diploids. The difference indicates that mutations probably are well masked in diploids; this question will be analyzed in the following sections.

Screening for mutations through tetrad dissection proved to be more sensitive and yielded a higher mutation rate, but

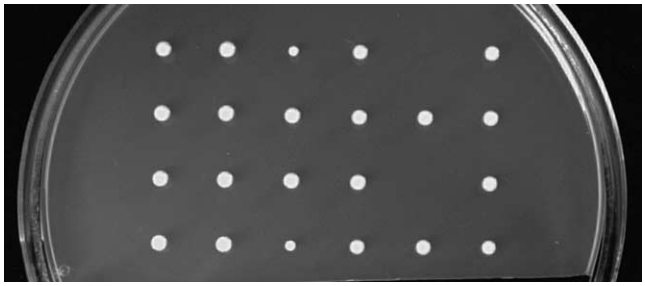

Fig. 1. An agar plate with two-day-old colonies of haploid clones. Each column represents clones grown out of four spores (a tetrad) produced by a single diploid cell. An example of a non-lethal mutation is visible in the third column, and of a lethal one in the fifth column (from [76], modified).

the rate is still low: a deleterious mutation appears every thousand cell divisions. Continuing interest in the deleterious mutation rate was sparked by speculation that the rate might be considerably higher than previously thought, perhaps as frequent as about one mutation per genome per sexual generation [47]. The disproportion with the estimate obtained by Wloch and colleagues seems to be enormous because the two figures differ by three orders of magnitude. However, vertebrates may have several times more genes [75,77] and about a hundred times more cell divisions per sexual generation [12]. These two factors combine to about three orders of magnitude. Thus the data obtained for yeast suggest that the spontaneous mutation rate per gene per cell division is high enough to put higher organisms under strong mutational pressure. Not as strong, however, as was feared after initial studies of *Drosophila* [11,56]. The current estimates of the human spontaneous mutation rate, based on several different approaches, suggest one or a few deleterious mutations per generation [12], and are in very good agreement with the estimates extrapolated from yeast. Sexual reproduction with its associated recombination of genetic material obviously would be advantageous in large multicellular organisms because it would speed up the rate and lower the costs of eliminating the fairly abundant deleterious mutations from populations. In yeast, and probably in other lower eukaryotes, the mutation rate is much lower and the potential link between mutational pressure and the evolution of sex appears to be weaker. These organisms often reproduce vegetatively, and sexual reproduction is "facultative" [5]. However, recombination still can be important for such organisms in dealing with the mutational load. The load will build up during periods of vegetative reproduction, which is analogous to the accumulation of mutations in proliferating cells of multicellular organisms.

3. Selection coefficient

The selection coefficient s is usually defined as $1 - w$ where w stands for relative fitness. The relative fitness of an individual or a clone is calculated as the ratio of its fitness to the fitness of the wild-type individual or clone. The coefficient s is reserved for homozygotes and haploids. The heterozygous fitness effect is denoted hs. The symbol h represents the dominance coefficient. To find it empirically, the heterozygous fitness effect of a considered allele is divided by its homozygous fitness effect. When the homozygous effect of a mutation is not known, however, its heterozygous effect is described by both parameters, which cannot be separated from each other.

The selection coefficient can be estimated in microorganisms by competition experiments in which two strains are mixed in a common culture. The coefficient is calculated from the rate of change in the population sizes of the competitors [19]. Other frequently used tests are based on measurements of maximum growth rate. Such estimates are usually obtained by growing pure clonal cultures. Although no direct competition is involved in such experiments, the obtained estimates are good indicators of fitness in environments in which the ability to grow fast is a major requirement. The maximum growth rate represents a broad mutational target in budding yeast. This was established in studies applying systematic deletions of genes; 17% of all deletions resulted in death, and about 40% in slower vegetative growth [78].

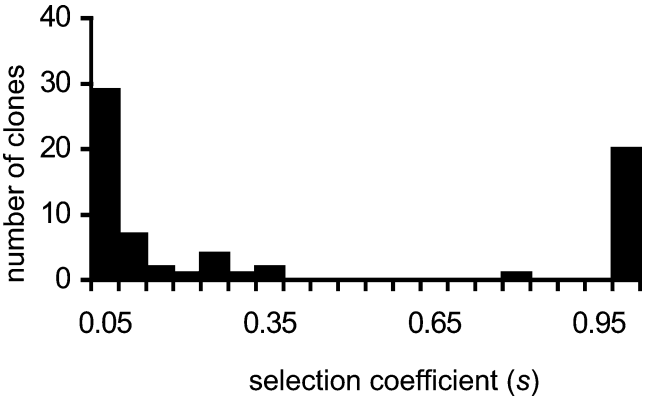

Fig. 2. Frequency distribution of the selection coefficients of spontaneous mutations (from [76], modified).

Using competition experiments, Zeyl and deVisser [83] found that the average heterozygous fitness effect was $hs = 0.217$. This estimate was based on one mutant phenotype. If it were representative, the deleterious mutations in yeast would be very harmful because the selection coefficient would be as high as $s = 0.434$ providing that both alleles were co-dominant. Wloch and colleagues [80] measured the growth rate of thousands of single clones growing as separate colonies on agar surfaces and detected dozens of mutants. The obtained estimates were not true maximum growth rates but average growth rates over the initial 48 h of colony growth. The frequency distribution of the mutants' selection coefficients is presented in Fig. 2. The mean selection coefficient of non-lethal mutations was $s = 0.086$. The modal range was between 0.01 and 0.05; stronger effects were less and less frequent. Even small differences in the rate of colony growth amount to substantial differences in the size of colonies. For example, a mutant growing only 1% slower will produce a colony whose volume is diminished by 22% after 25 generations, according to the calculation $0.99^{25} = 0.778$. The broad range of selection coefficients ranging from 0.01 to one found by Wloch and colleagues is in strong contrast to the findings of Zeyl and deVisser. The latter probably did not overlook small effects because the average fitness of replicate populations remained unchanged, which would not be the case if many individually indiscernible mutations were present.

It is unlikely that methodological differences were responsible for the disparity in selection coefficient estimates between the two described studies. The suitability of competition experiments is not restricted to large fitness effects.

Selection coefficients as low as 0.005 to 0.01 can be reliably estimated in this way [74], although the assays have to last much longer than those reported by Zeyl and colleagues. Short competition experiments produce vague individual estimates but are fairly sensitive when the average fitness of replicate populations is considered [19]. Nor do colony growth rate measurements have to be biased towards small fitness effects; strains growing more than twice as slow as wild-type ones were still able to produce visible colonies [80]. It is proposed here that the two studies gave inconsistent estimates of the selection coefficient because they used strains of different ploidy. Mild fitness effects were most frequent but they were detectable only in haploids. Many mutations, probably the majority, would become effectively neutral in heterozygous diploid loci.

The frequency distribution of mutational effects must be inferred from the frequency distribution of phenotypes. The latter are often affected both by environmental conditions and by an unknown number of genetic effects. In many previous studies the selection coefficient of deleterious mutations was assumed to be constant only because its distribution could not be determined [29,57,59]. Keightley [38] devised a method in which a broad range of distribution of mutational effects could be tested for best fit with the observed distribution of phenotypes. No single distribution of non-lethal effects was universal. Some results show very small effects to be most frequent, others suggest large effects to be most common, and yet others imply bimodality [14,38,40]. Such discrepancies may reflect real differences between species and traits, but may also indicate that indirect inferences are very sensitive to the type of studied data and the particular assumptions made prior to their analysis.

The experimental results obtained by Wloch and colleagues [80] are in line with the original and probably still dominating presumption that most non-lethal deleterious mutations are of small fitness effect and that therefore the frequency distribution is skewed to the right. This was true not only for spontaneous but also induced mutations (Fig. 2). A similarly skewed distribution was found in a study in which mutations were generated by random transposition across the *E. coli* genome [20]. Another feature of the data collected by Wloch and colleagues [80] was unexpected and even surprising. They were able to compare the frequency of lethal versus non-lethal mutations and found that the former were abundant and accounted for 30–40% of all scored effects. Some previous studies suggested that this fraction should be much lower. For example, only about 5% of spontaneous mutations in the fruit fly were expected to be lethal [57].

4. Dominance coefficient

The dominance coefficient h of deleterious mutations is usually estimated in planned crosses followed by statistical analyses of variance components [53]. Such methods provide mean population estimates of the studied parameter. The classic estimates suggested that in *D. melanogaster* small viability effects caused by new mutations were not markedly recessive, $h = 0.4$ [56,57]. This somewhat unexpectedly high heterozygous effect was confirmed in independent experiments [59], although those results were later questioned [28]. Other studies of *Drosophila* [24,35], *Daphnia* [17], and *Caenorhabditis* [76] showed that alleles with small fitness effects probably retained as much as about a quarter of their impact on fitness in heterozygotes.

Initial studies of dominance of deleterious mutations in yeast provided only average estimates and were not free of drawbacks. Korona [49] obtained haploid strains that contained dozens of mutations accumulated during hundreds of generations of growth with an impaired DNA mismatch repair system. Although the presence of the mutational load was evidenced by a considerable drop in fitness, the strains were also shown to have compensatory mutations that ameliorated some of the harmful effects. Such haploid clones were used in crosses with wild-type clones, and the coefficient of dominance was found to be $h = 0.08$. This value is relatively low, but it may be confounded by the presence of compensatory mutations. Mable and Otto [54] compared large cultures of haploid and diploid yeast treated with EMS. Probably the more harmful mutations were removed by selection before and during the fitness assays. The dominance of the remaining deleterious mutations was relatively high, $h = 0.30$, similar to that reported for multicellular organisms. Studies of adaptive evolution in laboratory yeast populations suggested that beneficial mutations were also moderately recessive [84] although the pattern of dominance and epistasis of such mutations could be very complex [63].

In the experiments described above, the dominance coefficients of individual mutations could not be estimated because the number of mutations involved and their distribution among individuals were not known. Mean estimates of h are often used in both empirical and theoretical studies, but this results from methodological difficulties and not from the researchers' conviction that deleterious mutations are uniform in terms of dominance. Yeast clones with single deleterious mutations can be obtained relatively easily through tetrad analysis and then crossed with each other to obtain homozygotes or with wild-type clones to obtain heterozygotes. In this way Szafraniec et al. [73] were able to estimate the fitness of individual mutations in homozygous and heterozygous loci. Single vegetative cells were placed on nutrient agar plates. The size of the developing colony was used to estimate the average growth rate. The homozygous selection coefficient s was calculated as the relative difference between the growth rates of wild-type and homozygous mutants, and the heterozygous hs as the analogous difference between wild-types and heterozygotes. The mutations used in this experiment were relatively mild because the homozygous coefficient s was in the range of a few percent. The mean heterozygous effect of these

mutations was $\overline{hs} = 0.0042$, and the mean coefficient of dominance was $\bar{h} = 0.20$. Thus the mildly deleterious effects were not strongly recessive because as much as a fifth of their negative effect was still detectable in heterozygous loci. Szafraniec et al. [73] also asked how recessive were the lethal mutations. The heterozygous lethals were viable and their relative fitness could be estimated. The relative fitness w and the selection coefficient s of homozygotes were assumed to be equal to zero and one, respectively, because haploid clones bearing these mutations were not viable and the homozygotes were unavailable. The mean heterozygous fitness effect of lethal mutations was very small, 0.0034. This is not only the value of $\overline{hs}$ but also $\bar{h}$ because the selection coefficient was equal to one. The conclusion is striking: the heterozygous effects of lethal and mildly deleterious mutations are close to each other. The reason is that the dominance of the former is about two orders of magnitude lower than that of the latter.

The estimate of $\bar{h} = 0.20$ for the mildly deleterious effects was not unexpected because the cited studies in other organisms gave similar results. It was also suggested that very harmful and lethal mutations had to be relatively better masked than mild ones [13,57]. However, the yeast system offered an opportunity to directly compare the dominance of relatively large numbers of single lethal and non-lethal mutations. The finding that the heterozygous effect of a mutation is independent of its homozygous fitness has important implications. Natural selection will not purge harmful mutations faster than mild ones if both remain heterozygous. New deleterious mutations arise mostly as heterozygotes, and probably many of them are severe (compare Fig. 2). Accumulation of new severe defects may therefore proceed unnoticed even for a long time, so long as homozygotes are not produced.

5. Epistatic interactions

In a simple model, selection coefficients of mutations are stable irrespective of the genotypes of other loci. This would result in multiplicative combination of individual effects: the effect of the first mutation would be $w(1 - s_1)$, the second $w(1 - s_1)(1 - s_2)$, and so on. Genetic interactions appear when this simple assumption is violated. The decrease in fitness associated with the increasing number of mutations can be either slower or faster than expected under multiplicity [15,45]. These two tendencies are called antagonism and synergism, respectively. Synergism has attracted much attention because the evolution of genetic recombination and sexual reproduction would be easier to understand if this type of interaction dominated [47]. Initial empirical tests of genetic interactions were based on analyses of the trajectories of fitness decrease in populations accumulating mutations. Mukai [56] found that the trajectory of fitness decrease in *Drosophila* populations accelerated with time, suggesting synergism. Subsequent reanalyses of Mukai's data [25,39,71] and new experiments employing different organisms and experimental designs [16,21,22,65,81] did not confirm that synergism could be ubiquitous and strong.

Wloch et al. [79] accumulated dozens of deleterious mutations in haploid strains of yeast and then mated pairs of strains of opposite mating types. The resulting diploids were sporulated and the tetrads of haploid spores dissected. In this way, two sets of mutations were first pooled and then randomly divided among the tetrads of progeny clones. If genetic interactions were absent, the frequency distribution of the progeny's fitness would be symmetrical and its mean value no different from that of the parents. Only in a few crosses did the fitness of the progeny deviate from that of their parents in a direction indicating synergism. Furthermore, the distributions of progeny fitness were not skewed. The experimental design and the choice of experimental material ensured extensive genetic recombination, because there are as many as sixteen chromosomes in yeast and about a hundred crossovers per meiosis [1,67]. Moreover, all four meiotic products were available for fitness assays, which is not usually the case with multicellular organisms. Despite these generally favorable experimental settings, neither synergism nor antagonism were clearly confirmed. It cannot be ruled out, however, that weak directional interactions, synergism or antagonism, were present but masked by a variety of other more complex interactions, especially since the crosses probably involved not only numerous deleterious mutations but also some compensatory ones.

Szafraniec et al. [73] also crossed yeast haploid clones, but the clones were known to harbor one deleterious mutation at most. There were four types of crosses. Two clones free of mutations gave a wild-type diploid. Two clones each with a different mutation gave a double heterozygote. Each of the two mutated clones crossed with a wild type gave a single heterozygote. The genetic background was the same for all four diploid genotypes used in a single comparison. The effects of the mutations would be multiplicative if the average fitness of two clones each bearing one mutation (disassociation) were equal to the average of the two other clones, one with none and the other with both mutations (association). A result with the association average lower than the disassociation average would mean synergism. Antagonism would be indicated by the opposite relationship. Fig. 3 convincingly shows that most of the genetic variation was attributable to a pair of particular mutations, irrespective of whether they were in association or disassociation. Only about a fifth of the variation was attributable to genetic interaction. This interaction was generally random, with synergism and antagonism equally frequent.

The result further weakens the evidence for synergism as a common and prevailing type of interaction among deleterious mutations. It was obtained within a very narrow range of relative fitness and with a minimum number of interacting loci. Perhaps interaction between many loci or loci with large effects would be different. However, in a large equilibrium population the differences in fitness would also

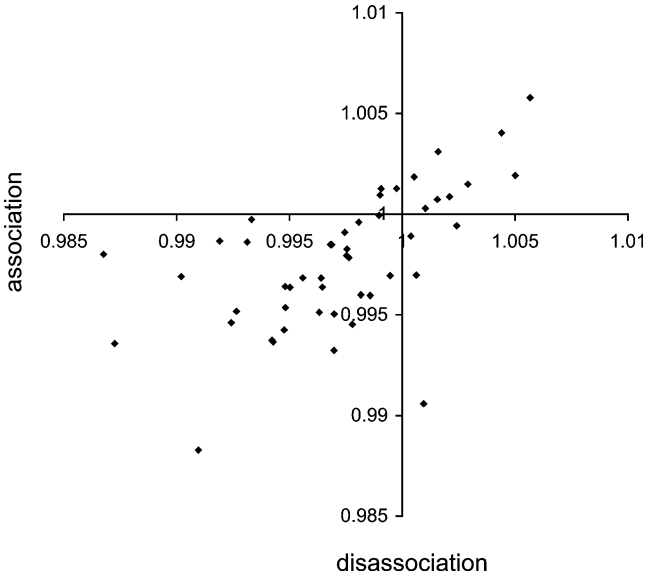

Fig. 3. Interaction between pairs of deleterious mutations. "Disassociation" is the mean fitness of two diploid clones each bearing one deleterious mutation in a heterozygous locus. "Association" is the mean fitness of a clone with both these mutations and a clone free of them (from [69], modified).

be small, because mutations with large fitness effects would be removed readily. It appears that interactions between deleterious mutations, at least those in heterozygous loci, are of minor importance for individuals close to a "wild-type" phenotype. This is an important feature of the experimental system developed by Szafraniec et al. [73]; almost all previous studies concentrated on large fitness effects in haploid or homozygous organisms.

6. Genetic load under stress

One of the basic questions concerning deleterious mutations is whether their negative effects occur only in some environments or in all of them. In other words, the deleterious nature of mutations can be either conditional or unconditional. Korona [48] assayed haploid clones bearing dozens of deleterious mutations in conditions that were either favorable or in different ways challenging: poorly furnished with nutrients, or containing an inferior energetic substrate, or too cold or too warm. The relative fitness of the strains bearing mutations was generally lower in stressful environments than it was in a benign one. The genetic load was largely strain-dependent because some strains tended to be less (or more) fit than others when averaged over all environments. The mutations accumulated in some strains probably were especially severe, and thus the magnitudes of fitness decreases were correlated among different environments. In general, those results suggest that both the average and individual estimates of the genetic load obtained in one environment are indicative of those in other environments.

In the experiment described above, the clones were haploid, mutations were numerous, and their joint effect on fitness was substantial. From the perspective of human health and conservation biology, it would be particularly interesting to know the phenotypic effects of relatively few heterozygous mutations in diploids. Szafraniec et al. [72] used an experimental system in which the strain was diploid and the number of mutations was roughly controlled by turning on and off a DNA mismatch repair system. One of the genes essential for the majority of mismatch repair, *PMS1*, was deleted from its chromosomal locus, but a functional copy was present on a plasmid. The mutation rate was normal in the presence of the plasmid, but when it was absent, frameshifts and substitutions increased in frequency by about two orders of magnitude. The accumulation of mutations began when a cell lost its plasmid containing the wild-type *PMS1* gene. Mutagenesis continued for about 35–40 generations of vegetative growth and was terminated when the wild-type gene was reintroduced by transformation. A large number of replicate lines were established in which the losses, and hence mutation accumulation, were independent. The starting strain was strictly homozygous, with two identical alleles in all loci (except for the mating-type locus). The newly generated alleles were expected to be heterozygous, however, because it was highly improbable that both copies of a gene would be mutated. These experimental clones were compared with a control group, that is, clones started from single cells of the same strain as the experimental group but never deprived of the plasmid and therefore not having experienced an elevated mutation rate. The performance of the two groups was identical in a benign environment of 30 °C. At stressful 38 °C, however, the clones contaminated with mutations were about 10–20% less fit. This effect was seen both when the maximum growth rate and when the density of stationary phase cultures were compared. Thus the ability of wild-type alleles to mask deleterious mutations is substantially reduced by environmental stress.

A seemingly straightforward approach based on comparisons of benign and harsh environments is not free of pitfalls. It was suggested that understanding whether and how genetic variation changes under environmental stress is difficult because the history of the studied population is often unsure [34]. For example, one cannot rule out the possibility that alleles deleterious to fitness in unfavorable conditions have been maintained in a population due to their hypothetical advantages in other habitats. Antagonistic pleiotropy [68], purging selection [39] and the ascendance of compensatory mutations [49] can modify the spectrum of deleterious alleles when they are either accumulated in long-term experiments or sampled from extant populations. The experimental system developed by Szafraniec et al. [72] did not suffer from these difficulties. The mutations were few, recent, and virtually unaffected by selection. The influence of history was practically eliminated. Mutations that become deleterious only in stressful environments were not only present but also relatively abundant. An important

conclusion is that studies carried out only under benign environmental conditions may substantially underestimate the incidence and severity of the mutational load.

7. Conclusions and future directions

Budding yeast in a laboratory environment is undeniably a very simplified model of a eukaryote, but the basic metabolic processes are universally shared by eukaryotic cells. Thus, studying the susceptibility of yeast cells to the damaging effects of random mutations is likely to provide information of broad applicability. The genomic rate of spontaneous deleterious mutation was found to be roughly in the middle of estimates obtained with other organisms. It is about 0.001 per diploid yeast cell division; extrapolations accounting for the numbers of genes and cell divisions suggest 0.07 and about one per sexual generation in the fruit fly and human, respectively [70]. The yeast estimate is unique in the sense that it was obtained by direct scoring of single mutational events, an approach virtually impossible in other organisms. The fitness effects of spontaneous mutations in yeast are very different. Small effects dominate among the non-lethal ones, but roughly every third mutation with a detectable fitness effect is lethal. This last finding is rather surprising. Even more striking is the efficiency with which harmful effects of mutations are masked by wild-type alleles. Heterozygous loci cause only a fraction of a percent decrease in fitness, regardless of whether the considered mutation is lethal or only slightly deleterious in a haploid locus. This means that the degree of dominance of slightly deleterious mutations is about two orders of magnitude higher than that of lethal ones. Such a result is not unexpected because theoretical considerations of the mode of metabolic flux imply that mutations severely affecting enzymatic activities will be relatively better masked by the wild-type alleles than mutations causing smaller defects [37]. Recent analysis of yeast gene families provided support for this hypothesis on genomic scale [62]. Not only lethal but also less harmful mutations are usually in heterozygous loci and therefore their elimination from populations by natural selection will be similarly difficult depending mostly on the population size [44]. Finally, the deleterious effects of mutations are generally multiplicative, that is, neither their synergism nor antagonism can be convincingly shown. This conclusion applies to both haploid and heterozygous diploid loci.

The research summarized above concentrated on the phenotypic effects of mutations. The methods of classical yeast genetics have proven very helpful in testing classical hypotheses of population genetics. However, the present development of research technology has already provided tools not even envisaged by the founders of population genetics. The sequence of the whole genome of budding yeast is known [32]. Still better, it can be compared with the sequences of a few other yeast species, making genomic inferences particularly robust [42]. Several planned mutagenesis projects have yielded collections of easily traceable deletions [43] or tagged disruptions [69] in the whole yeast genome. Functions and interactions of proteins are now studied in a systematic manner on a large scale [30]. It is therefore increasingly possible to find the molecular basis of any observable phenotypic variation, both adaptive and maladaptive. The model-based thinking specific to population genetics should not be abandoned, but the terms “selection coefficient”, “dominance” and “epistasis” were invented to describe phenomena; their functional meaning will have to be elucidated progressively. This work should be guided by the recognition that natural selection not only created the structures and functions of organisms in the past but also maintains or modifies them now. The work of the late Michel Blot is an example of successful implementation of this idea. In detailed molecular analyses of transposons and their bacterial hosts, he showed how omnipresent and persistent natural selection can be, even when very simple entities are considered [2,10,61,70]. This is a useful message, especially for those studying more complex systems.

Acknowledgements

This work was supported by grant 0231/P04/2002/22 from the State Committee for Scientific Research of Poland and by grant CRIG 066091 from The Wellcome Trust.

References

[1] M.F.F. Abdullah, R.H. Borts, Meiotic recombination frequencies are affected by nutritional states in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 98 (2001) 14524–14529.
[2] E. Adam, M.R. Volkert, M. Blot, Cytochrome c biogenesis is involved in the transposon Tn*5*-mediated bleomycin resistance and the associated fitness effect in *Escherichia coli*, Mol. Microbiol. 28 (1998) 15–24.
[3] N.H. Barton, A general model for the evolution of recombination, Genet. Res. 65 (1995) 123–144.
[4] A.J. Bateman, The viability of near-normal irradiated chromosomes, Int. J. Radiat. Biol. 1 (1959) 170–180.
[5] G. Bell, The Masterpiece of Nature, Univ. of California Press, Berkeley, 1982.
[6] M. Blot, Transposable elements and adaptation of host bacteria, Genetica 93 (1994) 5–12.
[7] B. Charlesworth, D. Charlesworth, Some evolutionary consequences of deleterious mutations, Genetica 102/103 (1998) 3–19.
[8] B. Charlesworth, D. Charlesworth, M.T. Morgan, Genetic loads and estimates of mutation rates in highly inbred plant populations, Nature 347 (1990) 389–392.
[9] B. Charlesworth, K.A. Hughes, in: S. Singh, C.B. Krimbas (Eds.), Evolutionary Genetics: From Molecules to Morphology, Cambridge Univ. Press, Cambridge, 2000, pp. 369–392.
[10] V.S. Cooper, D. Schneider, M. Blot, R.E. Lenski, Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B, J. Bacteriol. 183 (2001) 2834–2841.
[11] J.F. Crow, The high spontaneous mutation rate: Is it a health risk?, PNAS USA 94 (1997) 8380–8386.
[12] J.F. Crow, The origins, patterns and implications of human spontaneous mutation, Nat. Rev. Genet. 1 (2000) 40–47.

[13] J.F. Crow, M.J. Simmons, in: M. Ashburner, H.L. Carson, J.N. Thompson (Eds.), The Genetics and Biology of *Drosophila*, vol. 3c, Academic Press, London, 1983, pp. 1–35.
[14] E.K. Davies, A.D. Peters, P.D. Keightley, High frequency of cryptic deleterious mutations in *Caenorhabditis elegans*, Science 285 (1999) 1748–1751.
[15] J.A.G.M. de Visser, R.F. Hoekstra, H. van den Ende, An experimental test for synergistic epistasis and its application in *Chlamydomonas*, Genetics 145 (1997) 815–819.
[16] J.A.G.M. de Visser, R.F. Hoekstra, Synergistic epistasis between loci affecting fitness: Evidence in plants and fungi, Genet. Res. 71 (1998) 39–49.
[17] H.-W. Deng, M. Lynch, Inbreeding depression and inferred deleterious-mutation parameters in daphnia, Genetics 147 (1997) 147–155.
[18] J.W. Drake, B. Charlesworth, D. Charlesworth, J.F. Crow, Rates of spontaneous mutations, Genetics 148 (1998) 1667–1686.
[19] D. Dykhuizen, Experimental studies of natural selection in bacteria, Ann. Rev. Ecol. Syst. 21 (1990) 373–398.
[20] S.F. Elena, L. Ekunwe, N. Hajela, S.A. Oden, R.E. Lenski, Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*, Genetica 103 (1998) 349–358, Spec. Iss.
[21] S.F. Elena, Little evidence for synergism among deleterious mutations in a non-segmentated RNA virus, J. Mol. Evol. 49 (1999) 703–707.
[22] S.F. Elena, R.E. Lenski, Test of synergistic interactions among deleterious mutations in bacteria, Nature 390 (1997) 395–398.
[23] M.W. Feldman, F.B. Christiansen, L.D. Brooks, Evolution of recombination in a constant environment, PNAS USA 77 (1980) 4838–4841.
[24] J. Fernandez, C. Lopez-Fanjul, Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*, Genetics 143 (1996) 829–837.
[25] J.D. Fry, P.D. Keightley, S.L. Heinsohn, S.V. Nuzhdin, New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*, PNAS USA 96 (1997) 574–579.
[26] W. Gabriel, M. Lynch, R. Burger, Muller's ratchet and mutational meltdowns, Evolution 47 (1993) 1744–1757.
[27] T. Galitski, A.J. Saldanha, C.A. Styles, E.S. Lander, G.R. Fink, Ploidy regulation of gene expression, Science 285 (1999) 251–254.
[28] A. Garcia-Dorado, A. Caballero, On the average coefficient of dominance of deleterious spontaneous mutations, Genetics 155 (2000) 1991–2001.
[29] A. Garcia-Dorado, C. Lopez-Fanjul, A. Caballero, Properties of spontaneous mutations affecting quantitative traits, Genet. Res. 74 (1999) 341–350.
[30] A.-C. Gavin, M. Boshe, R. Krause, et al., Functional organization of the yeast proteome by systematic analysis of protein complexes, Nature 415 (2002) 141–147.
[31] D.D.G. Gessler, S.Z. Xu, Meiosis and the evolution of recombination at low mutation rates, Genetics 156 (2000) 449–456.
[32] A. Goffeau, B.G. Barrell, H. Bussey, et al., Life with 6000 genes, Science 274 (1996) 563–567.
[33] J.B.S. Haldane, The effect of variation on fitness, Am. Nat. 72 (1937) 337–349.
[34] A.A. Hoffmann, P.A. Parsons, Evolutionary Genetics and Environmental Stress, Oxford Univ. Press, Oxford, 1991.
[35] D. Houle, K.A. Hughes, S. Assimacopoulus, B. Charlesworth, The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits, Genet. Res. 70 (1997) 27–34.
[36] C.D. Jenkins, M. Kirkpatrick, Deleterious mutation and the evolution of genetic life cycles, Evolution 49 (1995) 512–520.
[37] H. Kacser, J.A. Burns, The molecular basis of dominance, Genetics 97 (1981) 639–666.
[38] P.D. Keightley, The distribution of mutation effects on viability in *Drosophila melanogaster*, Genetics 138 (1994) 1315–1322.
[39] P.D. Keightley, Nature of mutational load in *Drosophila*, Genetics 144 (1996) 1993–1999.
[40] P.D. Keightley, A. Caballero, Genomic mutation rate for lifetime reproductive output and lifespan in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. USA 94 (1997) 3823–3827.
[41] P.D. Keightley, A. Eyre-Walker, Terumi Mukai and the riddle of deleterious mutation rates, Genetics 153 (1999) 515–523.
[42] M. Kellis, N. Patterson, M. Endrizzi, B. Birren, E.S. Lander, Sequencing and comparison of yeast species to identify genes and regulatory elements, Nature 423 (2003) 241–254.
[43] D.E. Kelly, D.C. Lamb, S.L. Kelly, Genome-wide generation of yeast gene deletion strains, Comp. Funct. Genom. 2 (2001) 236–242.
[44] M. Kimura, The Neutral Theory of Molecular Evolution, Cambridge Univ. Press, Cambridge, UK, 1983.
[45] M. Kimura, T. Maruyama, The mutational load with epistatic gene interactions in fitness, Genetics 34 (1966) 1337–1357.
[46] R. Kolodner, Biochemistry and genetics of eukaryotic mismatch repair, Genes Devel. 10 (1996) 1433–1442.
[47] A.S. Kondrashov, Deleterious mutations and the evolution of sexual reproduction, Nature 336 (1988) 435–440.
[48] R. Korona, Genetic load of the yeast *Saccharomyces cerevisiae* under diverse environmental conditions, Evolution 53 (1999) 1966–1971.
[49] R. Korona, Unpredictable fitness transitions between haploid and diploid strains of genetically loaded yeast *Saccharomyces cerevisiae*, Genetics 151 (1999) 77–85.
[50] R. Lande, Risk of population extinction from new deleterious mutations, Evolution 48 (1994) 1460–1469.
[51] M. Lynch, J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, J. Villis, Spontaneous deleterious mutation, Evolution 53 (1999) 645–663.
[52] M. Lynch, L. Latta, J. Hicks, M. Giorgianni, Mutation, selection, and the maintenance of life-history variation in natural population, Evolution 52 (1998) 727–733.
[53] M. Lynch, B. Walsh, Genetics and Analysis of Quantitative Traits, Sinauer Associates Inc., Sunderland, MA, 1998.
[54] B. Mable, S.P. Otto, Masking and purging mutations following EMS treatment in haploid, diploid, and tetraploid yeast (*Saccharomyces cerevisiae*), Genet. Res. 77 (2001) 9–26.
[55] P.B. Medawar, An Unsolved Problem of Biology, Lewis, London, 1952.
[56] T. Mukai, The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability, Genetics 50 (1964) 1–19.
[57] T. Mukai, S.I. Chigusa, L.E. Mettler, J.F. Crow, Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*, Genetics 72 (1972) 335–355.
[58] H.J. Muller, The relation of recombination to mutational advance, Mut. Res. 1 (1964) 2–9.
[59] O. Ohnishi, Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster.* II. Homozygous effect of polygenic mutations, Genetics 87 (1977) 529–545.
[60] S.P. Otto, J.C. Marks, Mating systems and the evolutionary transition between haploidy and diploidy, Biol. J. Linn. Soc. 57 (1996) 197–218.
[61] D. Papadopoulos, D. Schneider, J. Meier-Eiss, W. Arber, R.E. Lenski, M. Blot, Genomic evolution during a 10 000-generation experiment with bacteria, PNAS USA 96 (1999) 3807–3812.
[62] B. Papp, C. Pal, L.D. Hurst, Dosage sensitivity and the evolution of gene families in yeast, Nature 424 (2003) 194–197.
[63] C.E. Paquin, J. Adams, Relative fitness can decrease in evolving asexual populations of *S. cerevisiae*, Nature 306 (1983) 368–371.
[64] V. Perrot, S. Richerd, M. Valero, Transition from haploidy to diploidy, Nature 351 (1991) 315–317.
[65] A.D. Peters, P.D. Keightley, A test for epistasis among induced mutations in *Caenorhabditis elegans*, Genetics 156 (2000) 1635–1647.
[66] W.R. Rice, A.K. Chippindale, Sexual recombination and the power of natural selection, Nature 294 (2001) 555–559.
[67] G.S. Roeder, Meiotic chromosomes: It takes two to tango, Genes Dev. 11 (1997) 2600–2621.
[68] M.R. Rose, Antagonistic pleiotropy, dominance, and genetic variation, Heredity 48 (1982) 63–78.

[69] P. Ross-Macdonald, P.R.S. Coelho, T. Roemer, et al., Large scale analysis of the yeast genome by transposon tagging and gene disruption, Nature 402 (1999) 413–418.
[70] D. Schneider, E. Duperchy, E. Coursange, R.E. Lenski, M. Blot, Long-term experimental evolution in *Escherichia coli*. IX. Characterization of insertion sequence-mediated mutations and rearrangements, Genetics 156 (2000) 477–488.
[71] S.A. Shabalina, L.Yu. Yampolsky, A.S. Kondrashov, Rapid decline of fitness in panmictic populations of *Drosophila* under relaxed selection, PNAS USA 94 (1997) 13034–13039.
[72] K. Szafraniec, R.H. Borts, R. Korona, Environmental stress and mutational load in diploid strains of the yeast *Saccharomyces cerevisiae*, PNAS USA 98 (2001) 1107–1112.
[73] K. Szafraniec, D.M. Wloch, P. Sliwa, R.H. Borts, R. Korona, Small fitness effects and weak genetic interactions between deleterious mutations in heterozygous loci of the yeast *Saccharomyces cerevisiae*, Genet. Res. 82 (2003) 19–31.
[74] J.W. Thatcher, J.M. Shaw, W.J. Dickinson, Marginal fitness contributions of non-essential genes in yeast, PNAS USA 95 (1998) 253–257.
[75] The Genome Sequencing Consortium, Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.
[76] L.L. Vassilieva, A.M. Hook, M. Lynch, The fitness effects of spontaneous mutations in *Caenorhabditis elegans*, Evolution 54 (2000) 1234–1246.
[77] J.C. Venter, et al., The sequence of the human genome, Science 291 (2001) 1304–1351.
[78] E.A. Winzeler, D.D. Shoemaker, A. Astromoff, et al., Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis, Science 285 (1999) 901–906.
[79] D.M. Wloch, R.H. Borts, R. Korona, Epistatic interactions of spontaneous mutations in haploid strains of the yeast *Saccharomyces cerevisiae*, J. Evol. Biol. 14 (2001) 310–316.
[80] D.M. Wloch, K. Szafraniec, R.H. Borts, R. Korona, Direct estimate of the mutation rate and the distribution of fitness effects in the yeast *Saccharomyces cerevisiae*, Genetics 159 (2001) 441–452.
[81] H.-P. Yang, A.Y. Tanikawa, W.A. Van Voorhies, J.C. Silva, A.S. Kondrashov, Whole-genome effects of ethyl methanesulfonate-induced mutation on nine quantitative traits in outbred *Drosophila melanogaster*, Genetics 157 (2001) 1257–1265.
[82] C. Zeyl, Budding yeast as a model organism for population genetics, Yeast 16 (2000) 773–784.
[83] C. Zeyl, J.A.G.M. deVisser, Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*, Genetics 157 (2001) 53–61.
[84] C. Zeyl, T. Vanderford, M. Carter, An evolutionary advantage of haploidy in large yeast populations, Science 299 (2003) 555–558.