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LOW FREQUENCY OF MUTATIONS WITH STRONGLY DELETERIOUS BUT NONLETHAL FITNESS EFFECTS

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Most experimentally detectable effects of mutations in cellular organisms are either lethal or mildly deleterious. A possible explanation for the paucity of strongly detrimental but nonlethal mutations is that the processes constituting cellular metabolism are either essential or largely redundant. Alternatively, the partition between lethal and inconspicuous mutations exists within important biological processes. To test this, we measured maximum growth rates of yeast strains each carrying the deletion of a single gene in one of 38 protein complexes. We also used relevant data from previous high-throughput phenotypic studies of the yeast gene-deletion collection. The complexes typified well-defined sets of genes engaged in a common process. Within virtually all essential complexes there were two clear modes of phenotypic effects, that is the cessation of growth or slowdown of growth by a few percent. This uniformity is striking given that complexes differ extensively in function, size, and proportion of essential proteins. The pattern of bimodality is observed both under optimal and suboptimal environmental conditions. The generic paucity of strong effects and abundance of small ones relates to the feasibility of analyses of quantitative traits and epidemiological surveys, irrespective of the particular element of metabolism under study.

KEY WORDS: **Gene deletion, growth rate, protein complex, *Saccharomyces cerevisiae*.**

The distribution of fitness effects of new mutations has been studied experimentally and inferred from DNA sequence data (Eyre-Walker and Keightley 2007). A few generalizations can be already made. First, fitness effects are ubiquitous because truly neutral or effectively neutral mutations are in minority, at least if coding regions of DNA are considered (Eyre-Walker et al. 2002; Loewe and Charlesworth 2006; Subramanian and Kumar 2006). Next, deleterious mutations are clearly more numerous than beneficial mutations (Elena et al. 1998; Sanjuán et al. 2004; Zhang and Li 2005). Among deleterious mutations, there must be some that are not exempt from selection but their individual fitness effects are too small to be detected experimentally. These mutations are likely abundant although difficult to quantify (Davies et al. 1999; Denver et al. 2004; Haag-Liautard et al. 2007). Mutations with experimentally detectable harmful effects have two frequency peaks. A sizable proportion is lethal (Mukai 1964; Mukai et al. 1972; Sanjuán et al. 2004). The other most abundant class includes mutations with relatively weak negative effects (Elena et al. 1998; García-Dorado et al. 1998; Schoen 2005; Burch et al. 2007). Several studies asked about distribution of the fitness effects of new mutations in yeast. The frequency of beneficial effects was originally estimated at 6% (Thatcher et al. 1998) but subsequent studies suggested a lower value, below 1% (Blanc and Adams 2003; Sliwa and Korona 2005). High prevalence of either lethal or slightly negative effects was observed when whole genes were deleted (Steinmetz et al. 2002; Warringer et al. 2003), base substitutions were induced chemically, or spontaneous mutations were accumulated (Wloch et al. 2001; Szafraniec et al. 2003). Thus, phenotypic reaction of the budding yeast to genetic alterations is consistent with commonly observed patterns. We concentrated on the distribution of mutations deleterious to fitness and asked why it is bimodal.

In most reported experiments, the distribution of deleterious mutations cannot be functionally interpreted because the location of mutations remains unknown. This is changing as systematic disruptions of large numbers of genes have been carried out for several different organisms. A common result is that only a minority of genes are essential, whereas the inactivation of many others leads to effects that are small or invisible under standard environmental conditions (Oliver 1996; Gerdes et al. 2003; Kobayashi et al. 2003). However, the phenotypic effect of a mutation does not reflect a mere absence of a gene but rather the malfunctioning of a biological process in which it is engaged. The observed paucity of strongly negative but nonlethal mutational effects may suggest that biological processes are either inevitable or largely supplementary. Alternatively, the bimodality of deleterious fitness effects occurs within most biological processes because the mutations typically affect them either very seriously or only marginally. To resolve this question, a reliable separation of genes into processes is necessary. This can be a difficult prerequisite because annotations of a single gene can relate to several functions (Saccharomyces Genome Database). A firm sign of protein cooperation is their physical interaction. Proteomic studies applying high throughput techniques have generated extensive datasets of protein–protein interactions. Their reliability remains limited, however, because they poorly overlap with data acquired in traditional experiments, sharply focused on interactions between few proteins (Reguly et al. 2006). The best defined groups of proteins with the same function are protein complexes. In this study, we used a carefully curated list of yeast protein complexes accessible in the MIPS database (Mewes et al. 2008). One consequence of this choice is that lethality or nonlethality of a particular gene deletion is especially well established. We restricted our analyses to complexes composed of at least four different proteins. We studied 38 complexes containing a total of 1216 proteins. Of these 515 were essential and therefore were not subject to our measurements of growth rate. Essential proteins were present in 27 complexes, the remaining nine complexes did not contain any essential proteins. The proportion of essential genes among those coding protein complexes (0.42) is higher than in the whole genome (0.19) (Giaever et al. 2002; Hillenmeyer et al. 2008). Although not always essential, protein complexes have defined unique roles and therefore are not redundant in any meaningful sense. Furthermore, genes coding for protein complexes are normally not pleiotropic; among the genes studied here, there were 39 contributing to two different complexes and one contributing to three different complexes. In short, we selected a specific sample of groups of cooperating genes. Functional homogeneity within these gene groups is especially high, likewise their importance for metabolism. This was a conservative approach allowing for a test of the hypothesis that fitness effects of mutations within a single biological process generally comply to a binomial distribution. Results of our experiments confirm this expectation, its further corroboration comes from an analysis of data obtained in former high-throughput phenotypic studies of the yeast gene-deletion collection.

Materials and Methods

A collection of gene-deletions was engineered in the laboratory strain BY (Steinmetz et al. 2002). One haploid collection was obtained by deleting genes in BY4741 *MAT*α, the other in BY4742 *MAT***a,** two isogenic strains of different mating types. Both collections were used in this experiment. There is no indication that mating type can influence the functioning of "housekeeping genes," in particular those coding for protein complexes. We used values averaged over two mating types solely to minimize the potential impact of uncontrolled factors (improper designation of a strain, presence of an unknown mutation, etc.)

Growth was monitored in an automated workstation, Bioscreen C. Growth curves were transformed with a polynomial function to convert the optical density (OD) reading into population density and then log-normally transformed. A range of linear relation between the double transformed readings and time was defined based on pilot studies. Its lower and upper limits were the same for all strains. Typically, there were about seven time points for fast-growing strains and proportionally more for slow-growing ones. Regression of optical densities over time matched the linear model with high accuracy, an average squared Pearson's coefficient of correlation between the two variables was 0.99945 with standard deviation 0.00086. The slope of regression line was used as an estimate of the maximum growth rate (Jasnos et al. 2005; Jasnos and Korona 2007).

Several previous studies sought to estimate fitness of the whole collection of viable yeast deletion strains. This was most often done by growing a mixture of strains in a common culture. The deletion cassettes contain unique molecular tags that hybridize to pertinent gene-chips allowing detection and quantification of individual strains. The technique was most intensely used to identify deletions associated with a significant negative growth effect under standard laboratory conditions (Steinmetz et al. 2002; Deutschbauer et al. 2005) or in presence of a variety of drugs (Brown et al. 2006; Parsons et al. 2006; Hillenmeyer et al. 2008). Among studies of that type, the most reliable estimates of individual growth rates were likely obtained in an experiment that involved the highest number of time points (five) of DNA isolation and hybridization (Steinmetz et al. 2002). That study was done with YPD (yeast extract, peptone, and glucose) and YP with different nonfermentable carbon sources of which we selected glycerol as representative of all. The data were presented in the form of individual growth rates. We found a single strong peak at the right side of the growth rate distribution. Assuming that

the peak represents deletions neutral to fitness under the studied conditions, we set the modal value of growth rate to one and normalized accordingly other growth rates. The resulting estimates are referred to as relative growth rates, *R*.

One of the earlier studies used an approach similar to ours. Growth rates of all viable yeast gene-deletion strains were measured individually in the Bioscreen C workstation (Warringer et al. 2003). There were two important differences. The synthetic defined (SD) medium instead of the rich medium (YPD) was used. The maximum growth rate was calculated not as a slope of multipoint regression line but as an average of selected short-interval slopes (Warringer and Blomberg 2003). The study concentrated on the osmotic stress response and therefore SD with 0.85 M NaCl in parallel to plain SD was used. The data were originally presented in from of doubling times. We used their reciprocals as estimates of the growth rate. Employing the same procedure as described above, we determined the modal growth rate and used it to normalize all individual estimates.

Results

The maximum growth rate was measured four times (two replications for two mating types) for every deletion-strain used in this study. The resulting estimates are listed in Table S1. BY4741 and BY4742 with no gene deletions served as control "wild-type" strains. Their maximum growth rate was measured 36 and 30 times, respectively, and found not to be different at a statistically significant level ($t = 0.580$, df $= 64$, $P = 0.564$). An average maximum growth rate of the wild-type strains was $m_{max} = 0.567$ [1/h]. The maximum growth rates of individual deletion strains were divided by m_{max}. This yielded estimates of the relative growth rate, *R*.

Figure 1 presents the frequency distribution of 1216 *R*'s associated with deletions of single genes coding for proteins constituting complexes in the budding yeast. Among them, there were 701 viable deletion strains assayed in this study and 515 strains that were found lethal in former studies. The figure shows an overall distribution and distributions within the 17 largest complexes. Both the distribution summed over all genes and the individual distributions in particular complexes are bimodal with one peak formed by lethal effects and another by moderately deleterious ones. A roughly similar pattern was also seen in the remaining complexes whose small size makes analysis of individual distributions unfeasible.

We then asked whether distributions of growth effects in individual complexes were homogeneous. Limiting the analysis to complexes with at least four different proteins of which at least three are viable, we found that the average *R* of nonlethal deletions (*ARNLD*) varied between complexes (single classification ANOVA, $F = 2.561$, df $= 34$ and 662, $P < 0.0001$). However, this variation was moderate and did not show any apparent pattern. For example, there was no correlation between *ARNLD* and the number of proteins per complex or the proportion of essential genes in a complex. This is shown in Figure 2. The figure also demonstrates that variation in *ARNLD* was limited to small complexes, whereas large complexes were much more homogenous. Finally, we tested whether *ARNLD* in nine complexes in which no essential genes were present differed from that in complexes in which there were essential genes ($t = 0.224$, df $= 33$, $P = 0.824$). This means that the high peak on the right side of the overall distribution of *R* is built up by strains carrying deletions of some genes coding for essential complexes and a majority of genes coding for nonessential complexes.

Table 1 shows how our results relate to that obtained in earlier studies. *ARNLD*s, with standard deviation and skewness, for 10 largest complexes in four different media (YPD was used twice), are provided. In all media and complexes, growth defects tend to be relatively small, moderately dispersed, and skewed to the left. To test whether the growth rate of particular strains is repeatable across different studies, we correlated our estimates with estimates obtained in two other studies under nonstressful conditions. Figure 3 shows that the estimates do correlate and that the correlation is higher when growth medium is the same as in our experiment (YPD) even though other aspects of our experiment are better reflected in the study in which the SD medium was used.

Discussion

The present experiment tested protein complexes engaged in different cellular functions including anabolic and catabolic processes, intracellular signaling and gene expression, cell cycle progression, structural complexes, and others. Not only metabolic roles but also sizes of complexes differed extensively. It is thus remarkable that in virtually all cases the distribution of fitness effects of single gene deletions showed a striking paucity of strong but nonlethal fitness effects. The same pattern is evident in earlier measurements of growth effects caused by gene-deletions even though they were not concentrated on protein complexes as intensely as in this work. The other studies involved minimal media (extensive anabolism), nonfermentable carbon sources (oxidative energy metabolism), and high osmotic pressure (general stress response). In sum, the bimodality of the distribution of deleterious fitness effects in *Saccharomyces cerevisiae* appears universal across different cellular subsystems and environmental conditions.

We studied engineered gene deletions. Although these are artificial genetic alterations, their net effect is the same as that of any natural changes leading to complete gene inactivation. Spontaneous mutations often lead to only partial inactivation. This

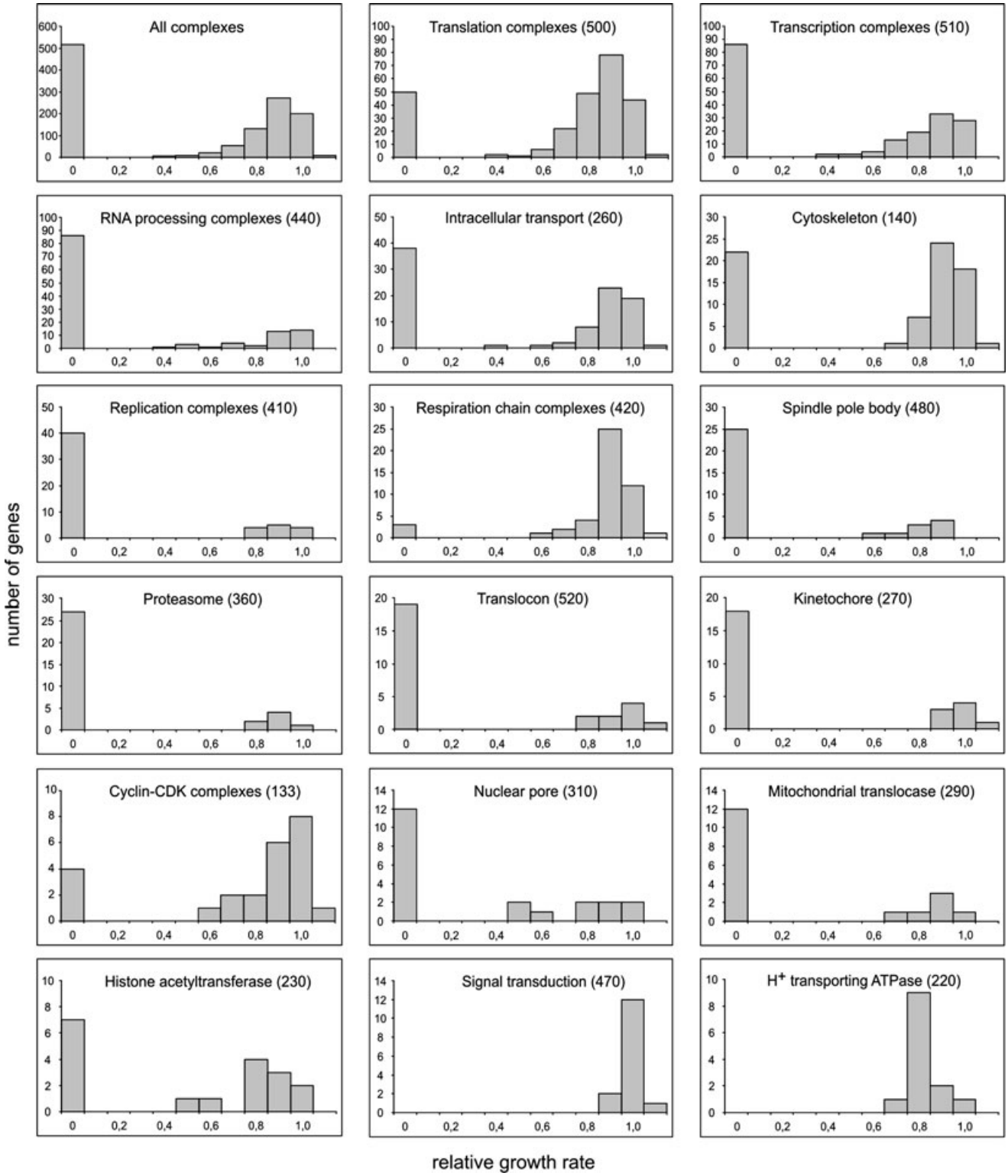

Figure 1. **Distribution of fitness (relative growth rate) effects. Upper-left corner: pooled distribution of fitness effects of 1216 deletions of genes coding for yeast protein complexes; other graphs: individual distributions of fitness effect within 17 largest complexes, numbers in parentheses refer to the MIPS catalogue of yeast protein complexes.**

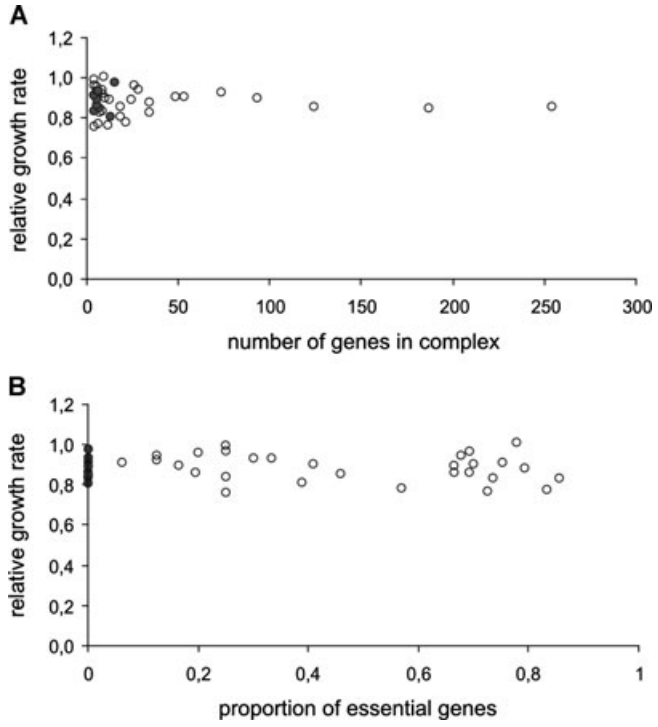

Figure 2. Average fitness (relative growth rate) of nonlethal gene deletions in relation to number of genes in a complex (A) and proportion of essential genes within a complex (B). Complexes containing essential genes and those without essential genes are shown as empty and filled circles, respectively.

should make the fitness costs smaller and thus further augment the peak of slightly deleterious effects. A question arises whether partial inactivation of essential genes might substantially increase the generally low frequency of highly deleterious although nonlethal effects. There is no indication that this is true, at least in the budding yeast. Strong effects are rare also when base substitutions and frame-shifts are considered (Wloch et al. 2001). Another factor that could influence the distribution of fitness effects is the presence of duplicate genes. *Saccharomyces cerevisiae* experienced whole genome duplication in its evolutionary past. It has been shown that deletions of duplicate genes are less frequently lethal than deletions of singletons. Moreover, the weakest effects are more frequent among deletions of duplicates (Gu et al. 2003). Although duplicates do enrich the frequency of small effects, their overall impact is not decisive in the budding yeast. This organism retained only about 12% of the paralogous genes originating from the whole genome duplication (Kellis et al. 2004). The general pattern of bimodality would hold even if only singletons were considered. Extensive gene duplications and high prevalence of nonessential genes are met also in multicellular organisms (Hurst and Smith 1999; Kamath et al. 2003). We therefore believe that generality of our results is not restricted considerably by the choice of yeast as an experimental organism and gene deletion as a model mutation.

Table 1. Fitness (relative growth rate) of viable single gene deletions in 10 largest protein complexes.

Protein complex (MIPS number)	This study			SC—individual[1]			SC NaCl – individual[1]			YPD—joint assay[2]			YPG—joint assay[2]		
	Mean	SD	Skew	Mean	SD	Skew	Mean	SD	Skew	Mean	SD	Skew	Mean	SD	Skew
Translation complexes (500)	0.862	0.110	−1.038	0.863	0.102	−0.230	0.900	0.109	−1.067	0.833	0.113	−0.057	0.795	0.195	−0.461
Transcription complexes (510)	0.851	0.145	−1.203	0.869	0.155	−1.006	0.884	0.138	−0.988	0.894	0.108	−0.468	0.870	0.143	−0.896
RNA processing (440)	0.861	0.171	−1.320	0.867	0.127	−0.830	0.910	0.153	−1.927	0.860	0.127	−0.223	0.800	0.210	−0.444
Intracellular transport (260)	0.904	0.119	−2.091	0.926	0.089	−1.632	0.862	0.167	−1.082	0.951	0.080	−1.826	0.906	0.120	−1.239
Cytoskeleton (140)	0.928	0.074	−1.302	0.952	0.074	−1.309	0.902	0.137	−1.030	0.936	0.109	−1.129	0.915	0.151	−0.966
Replication complexes (410)	0.910	0.080	−1.297	0.911	0.135	−1.305	0.866	0.171	−1.025	0.920	0.103	−1.124	0.946	0.141	−0.961
Respiration chain (420)	0.913	0.084	−1.614	0.941	0.080	−1.856	0.952	0.102	−1.608	0.914	0.101	−0.892	0.713	0.197	0.446
Cyclin-dependent kinases (133)	0.895	0.130	−1.144	0.915	0.122	−1.357	0.862	0.153	−0.550	0.941	0.080	−1.268	0.914	0.128	−1.098
Signal transduction (470)	0.974	0.052	1.039	0.966	0.080	−1.804	0.987	0.069	−.0583	0.969	0.073	−2.797	0.874	0.204	−1.826
H^+-transporting ATPase (220)	0.809	0.067	1.369	0.834	0.159	−0.333	0.853	0.126	−0.103	0.769	0.096	0.554	0.618	0.099	0.854

[1]Individual deletion strains assayed in SC or SC with 0.85 M NaCl (Warringer et al. 2003).
[2]Mixture of all viable deletion strains assayed in rich media with glucose (YPD) or glycerol (YPG) as a source of energy (Steinmetz et al. 2002).

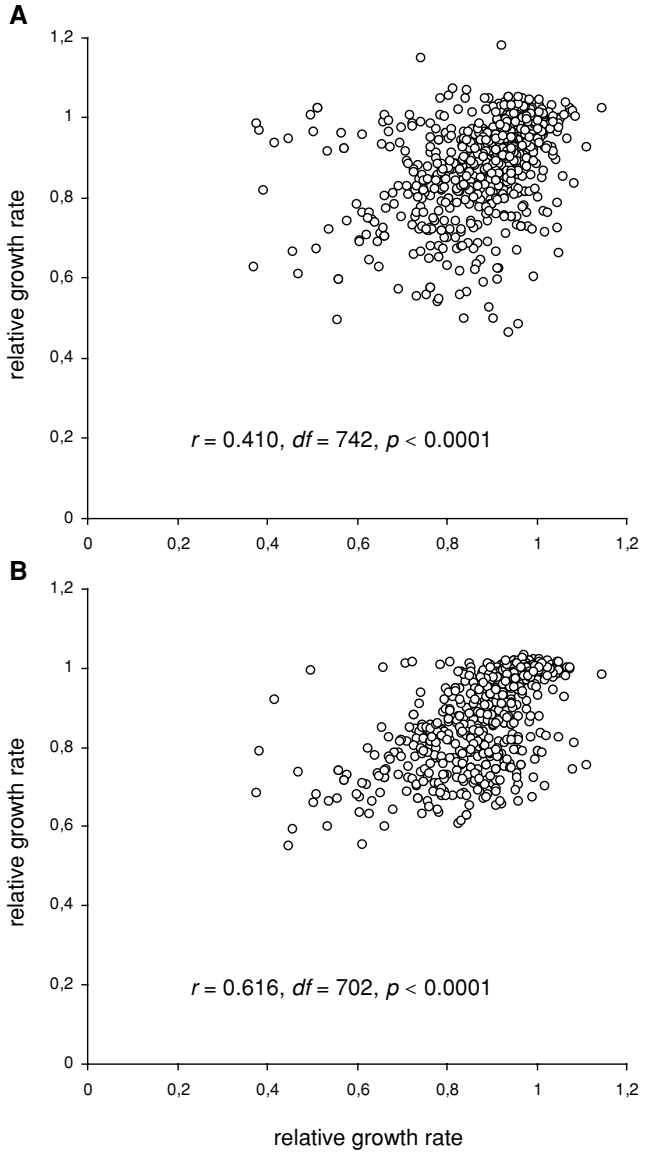

Figure 3. Correlation between estimates of fitness (relative growth rate). In this study, the rate of growth was estimated by growing single-strain cultures in YPD and regressing OD readings over time. The resulting data are used as X-values in the above graphs. (A) Y-values come from a study in which OD readings of single-strain cultures were also used although both medium (SC) and calculations of the growth rate (see Methods) were different (Warringer et al. 2003). (B) Y-values were obtained in a study applying YPD as growth medium but with a mixed culture instead of individual strains and quantitative DNA hybridization instead of OD reading (Steinmetz et al. 2002).

The results obtained with the yeast genomic collection of deletions help to understand former experiments and make new predictions. For example, even if a sample of mutations (or mutational target) is not truly representative of the whole genome, the general pattern of bimodality should not be altered. Furthermore, because the pattern is observed for very different biological functions, it will likely be seen in assays of different fitness components carried out in different environments. Considering the mutational load of a population, it is interesting to note that the dynamics of selection against deleterious mutations is likely to be similar, irrespective of whether the whole organism or its vital functions are considered. This implies that deleterious mutations accumulating when selection is inefficient will likely compromise many different functions instead of concentrating on some while saving others. Finally, the generic paucity of strong effects and abundance of small ones relates to the discriminatory power of QTL analyses and epidemiological studies, irrespective of the particular element of metabolism under study. Large effects are relatively easy to detect, the challenge will be to estimate the number and strength of minor effects.

The commonly seen partition for lethal and mild effects has several important consequences for population studies, but it is unclear in which functional or molecular mechanisms it is rooted. One possible explanation is that complexes must be arranged in a common way such that their disarrangement leads to a similar spectrum of effects. For example, it has been proposed that complexes are typically comprised of "core" proteins that are functionally homogeneous and necessary for proper activity and "halo" proteins whose roles may be more variable and their association with the complex is less stable. Deletion of the former would lead to strong and uniform effects, for example lethality, deletion of the latter to moderate and more variable defects (Dezso et al. 2003). Although intuitively appealing, this model does not fit the complexes studied here. The "translation complexes" (MIPS no. 500) can be used for illustration. These are cytoplasmic complexes involved in initiation, elongation, and termination of translation, and includes the large and small subunits of the cytoplasmic ribosome, and the large and small subunits of the mitochondrial ribosome. There is no clear distinction between core and halo elements in this ensemble because both essential and nonessential proteins are present in individual complexes. Furthermore, there is no homogeneity of fitness effects associated with the absence of proteins of the same complex. For example, the large subunit of cytoplasmic ribosome is made up of 81 proteins. Their expression is well coordinated and the stoichiometry is stable (Warner 1999). They undoubtedly form a structural and functional "core." However, only 20 deletions of single genes inhibit growth completely, the remaining 61 are rather benign in this respect. As a result, the distribution of fitness effects in this complex is bimodal, with peaks of growth rate at 0 and 0.48 [1/h]. This shows that individual deletions can have very different fitness effects even when proteins are strictly coexpressed and closely connected physically.

The observed bimodality of fitness effects can reflect some basic properties of genetic systems. There is no certainty that the efficiency of a complex translates linearly to the rate of growth.

Perhaps even much reduced activity of a complex is sufficient to uphold relatively good growth. This would be true if the functional capacity of an undisturbed complex is excessive under normal conditions and therefore could remain (nearly) sufficient even after substantial damage. Moreover, the inefficiency of a complex can lead to the accumulation of associated substrates and therefore only slightly affect the overall speed of reaction. Relatively large damage would thus be transformed into a modest reduction of an overall rate of metabolism making strong growth effects rare. The dominance of wild-type alleles over mutated ones is an analogous problem. It has been shown that a pronounced reduction of activity of a single enzyme has usually only a small effect on the rate of flux through the entire metabolic pathway (Kacser and Burns 1981). However, the functioning of protein assemblages in metabolic networks is likely more complex than that of single enzymes in simple pathways. To better understand the reaction of the whole cell to mutational damage further work on codependence of different cellular subsystems and robustness of the global genetic network is clearly needed.

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Supporting Information

The following supporting information is available for this article:

Table S1. Maximum growth rate [1/h] of yeast strains with deleted genes coding for protein complexes. Gene deletions with lethal effects are not listed.

(This link will take you to the article abstract).

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