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RESTRICTED PLEIOTROPY FACILITATES MUTATIONAL EROSION OF MAJOR LIFE-HISTORY TRAITS

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Radical shifts to new natural and human made niches can make some functions unneeded and thus exposed to genetic degeneration. Here we ask not about highly specialized and rarely used functions but those relating to major life-history traits, rate of growth, and resistance to prolonged starvation. We found that in yeast each of the two traits was visibly impaired by at least several hundred individual gene deletions. There were relatively few deletions affecting negatively both traits and likely none harming one but improving the other. Functional profiles of gene deletions affecting either growth or survival were strikingly different: the first related chiefly to synthesis of macromolecules whereas the second to maintenance and recycling of cellular structures. The observed pattern of gene indispensability corresponds to that of gene induction, providing a rather rare example of agreement between the results of deletion and expression studies. We conclude that transitions to new environments in which the ability to grow at possibly fastest rate or survive under very long starvation become practically unnecessary can result in rapid erosion of these vital functions because they are coded by many genes constituting large mutational targets and because restricted pleiotropy is unlikely to constrain this process.

KEY WORDS: **Gene deletion, lifespan, metabolic rate, *Saccharomyces cerevisiae*, starvation.**

Most of nonneutral mutations have negative and rather small effects on fitness (Eyre-Walker and Keightley 2007). If selection against such mutations is inefficient over sufficiently long time, gradual decline in the mean population fitness is unavoidable and may eventually lead to extinction (Lynch et al. 1993). It was also proposed that accumulation of mutations can result in deterioration of not all but only some functions, specifically those that are used too rarely to experience enough selection. This can happen if some functions are specific for specific habitats and these habitats become very rare or "marginal." In effect, populations can survive but in a new ecological niche (Kawecki et al. 1997). In yeasts, partial or complete losses of gene functions have been recorded both for strains that entered new natural or human made niches (Gu et al. 2005; Warringer et al. 2011). The extent and rate of mutational erosion is likely to depend not only on the number of functions that happen to be under relaxed selection. The erosion of functions will not be constrained if some functions can degenerate without affecting negatively those still needed. That is, synergistic pleiotropy should be rare. In recent years it has become possible to test for pleiotropy large collections of mutations that are representative of practically entire genomes of a few model organisms. However, it is still uncertain how to devise a set of test conditions that would constitute an unbiased sample of all relevant phenotypic reactions. The collections have been typically tested for groups of related traits associated with either growth, morphology, or development. It was found that in yeast single gene deletions tend to show phenotypic reactions in zero, one, and only rarely in more than one medium when testing for the rate of growth (Dudley et al. 2005). Similarly, only some yeast deletions produced more than one of many possible morphological

changes (Ohya et al. 2005). An RNAi screen in a nematode worm also suggested that developmental traits depend on a minority of genes and when this happens only few traits depend on a single gene (Sonnichsen et al. 2005; Wang et al. 2010). Based on these findings, the intensity of pleiotropy was judged to be relatively low (Wang et al. 2010). Similar conclusions have been reached in a study in which not thousands of engineered single mutations but hundreds of yeast isolates from different habitats were tested for assimilation of different carbon sources (Opulente et al. 2013). Other researchers, however, advise caution in deriving general conclusions from these specific and still rather few experiments (Paaby and Rockman 2013). Indeed, the engineered yeast deletion strains mentioned earlier have been also used to develop an opposite argument, that pleiotropy can explain much of variation in fitness (Cooper et al. 2007).

In this study, we want to extend the genomic approach on studying pleiotropy in relation to some life-history traits. Namely, we focus on the rate of growth under resource abundance and viability of cells under starvation. We ask whether yeast cells that are unable to grow (metabolize) fast tend also to be short living, and vice versa. We do not necessarily mean here pleiotropy in its strict form when one mutation has direct effects on multiple metabolic pathways or cell structures (Hodgkin 1998; Stearns 2010). We rather ask how often malfunctioning of a gene has any influence, even if indirect and functionally distant, on both the rate at which the cell biomass is synthesized and the rate at which it deteriorates. In a sense, all essential genes could be counted as pleiotropic because absence of them has consequences in all environments (Paaby and Rockman 2013). But, even if this perspective is accepted, essential genes are in minority among those constituting genomes of both unicellular and multicelluar organisms (Korona 2011). In yeast, about one fifth of genes are essential. Nonessential genes tend to be more numerous than essential ones under hundreds of specific physical or chemical conditions tested (Hillenmeyer et al. 2008). It is still inadequately recognized, even for such an intensely studied model organism as the budding yeast, how many nonessential genes are important for fitness regardless of what particular mode of metabolism is induced by external conditions. In particular, how many genes are required for both fast growth and good survival.

We intended to create experimental conditions that would allow for both good growth and long survival. For a laboratory strain of yeast, both traits reach especially high values during growth or starvation after growth in the nutrient-rich YPD medium (Werner-Washburne et al. 1993; Hampsey 1997). The YKO collection of yeast gene deletions has been already used in many genome-wide growth studies. Different surveys pointed to roughly similar sets of genes required to grow at a high rate, although variation in results was remarkable when different experimental arrangements or particular laboratories were compared (Blomberg 2011). Genome-wide measurements of the chronological lifespan—that is, the time over which nongrowing cells remain viable—were not equally common although a few attempts have been made (Powers et al. 2006; Fabrizio et al. 2010; Matecic et al. 2010; Gresham et al. 2011; Klosinska et al. 2011). None of them, however, was done with cells starving after growth in YPD. In this medium, fermentation and fast growth is rapidly replaced by respiration and slow growth after glucose is depleted. This transition is a strong stimulus to enter deep quiescence enabling long survival (Werner-Washburne et al. 1993). We decided to carry out our own assays of the two traits because only in this way comparisons between them could be made straightforwardly. One goal was to estimate how often single gene inactivation leads to either slow growth, poor survival, or both of them. Another was to determine what cellular functions are especially needed to support each of the two traits. We found that mutational erosion of these two fitness components is largely independent, that is, most mutations that are not neutral tend to affect only one of them and that this independence can be straightforwardly explained on functional grounds.

Methods

SCREEN FOR DELETIONS AFFECTING THE MAXIMUM GROWTH RATE (MGR)

We used the haploid YKO collection, that is, a set of yeast strains which were derived from a single progenitor strain by replacing every single gene from its START to STOP codon by a marker cassette that contained the *kan* gene providing resistance to geneticin, *kan*MX4 (Giaever et al. 2002). To construct a control strain that would be fully comparable to the deletion strains, we inserted the same marker cassette, *kan*MX4, into a neutral locus, *HO*. There were three independent experimental blocks, each consisted of all the deletions strains kept individually in standard titration plates (nonreplicated) and the control strain (replicated $7 \times 96 = 672$ times). The test cultures were initiated by inoculating 200 μL aliquots of fresh YPD with 2 μL aliquots of overnight cultures. Optical density (OD) of the growing cultures was measured after 4, 5, 6, 7, and 8 h of incubation without rotation at 30°C. The densities were log-transformed over time to calculate the MGR. Estimates obtained in this way fit well a linear model, an average Pearson's coefficient of correlation was higher than 0.99 in every of the three experimental replications.

As it turned out, there was an effect of the position on a plate on MGR. In particular, MGR was somewhat higher (about 5%) in the upper part of a plate. The pattern was highly repeatable between and within the three experimental blocks and was apparently produced at the time of OD reading (Tecan Infinity 300). To correct for this effect, we first sought to get the expected values

for each of the 96 positions. An average MGR was calculated for every well across the seven control plates. These averages were used to calculate a smoothened surface of the expected values for every row and column. The difference between the position-specific average (after smoothing) and the plate average was then used as the position-specific correction term. There were also differences between plates in their average MGRs. This was true also for the genetically homogeneous control cultures. We found that the upper quartile of a plate was less varying between plates that the mean or median. We therefore divided all individual MGRs by the upper quartile of a plate and then multiplied by the average upper quartile calculated over all plates within one replication. In effect, the resulting values of MGR were doubly corrected, for the effects of position and plate, and only after that were used in statistical analyses.

SCREEN FOR DELETIONS AFFECTING THE MAXIMUM LIFESPAN (MLS)

This experiment used the same strains and the same initial steps of cultivating as the former one. However, the cultures were allowed to grow for full 7 days to reach deep stationary phase (Werner-Washburne et al. 1993). At that time, cultures were diluted 1:1000 in 200 μL of water. These were sampled first immediately after the transfer to water, then after 7 days, and then after every 14 days. One sample contained about 1000 cells (5 μL of a starving culture, then appropriately more as recurrent refilling with water up to about 200 μL caused gradual dilution). The sampled cells were tested for viability by transferring to fresh YPD for incubation over 3 days. The day at which an uninterrupted array of nonviable samples started was recorded as the MLS of a starving strain. After the data were collected, it become clear that the position of a well on a titration plate can influence MLS. The effects were different and more variable than those observed for MGR. In the first experimental replication the cultures that were positioned more centrally tended to live longer whereas in the two next ones there was an opposite tendency. All estimates were corrected in a way analogous to that developed for MGR.

Results and Discussion

GENE DELETIONS AFFECTING THE MGR

We worked with haploid strains from the YKO collection of gene deletions, a strain that was isogenic to them served as a control. Experimental cultures were grown in the YPD medium with glucose as a source of carbon. Under such conditions, yeast cells grow at the MGR as long as glucose is no more than half-depleted (Lillie and Pringle 1980). We measured MGR of individual gene deletion cultures and highly replicated control cultures in three independent runs. Complete lists of MGRs are in Tables S1 (deletions) and S2 (control). These tables and all following analyses were restricted to genes that were protein-coding according to the Saccharomyces Genome Database. Subtracting for lethals and strains missing in the version of YKO collection that we worked with, the final number of genes was 3678. Maximum growth rate depended on the position within a titration plate. This effect was accounted for and all subsequent histograms and tests were done with corrected data (see Methods for details). Figure 1 shows frequency distributions of MGR for all three experimental replications separately. Most of the tested nonlethal deletions appeared to have virtually no impact on MGR whereas many potential effects were negative and rather small. Qualitatively similar results were reported earlier on several occasions (Giaever et al. 2002; Steinmetz et al. 2002; Fudala and Korona 2009).

To determine which particular gene deletions slowed down the rate of growth we first asked what was the probability that a strain could be repeatedly recorded as slow growing by chance alone and not because of harboring a gene deletion. Using the distribution of MGR in the control, we calculated the probability of the type I error, p_{ij}, for every deletion strain, i, and every experimental replication, j (see Supporting Information—Methods). We then combined trios of p_{ij} values into single P_i values using the Fisher's formula (Sokal and Rohlf 1995). To determine the false discovery rate (FDR), we first multiplied P_i by the total number of deletions and divided the resulting value by the number of deletion strains that were actually found as growing as slow or slower than the strain i (listed in Table S1). This approach proved effective: as many as 635 deletion strains were identified as slow growing with FDR at 0.01 or lower. In contrast, we could not identify any similarly reliable increases in the rate of growth. The latter finding agrees with that of an earlier extensive screen that was designed specifically to find even small improvements in fitness among the yeast deletion strains competing under conditions similar to those applied here (Sliwa and Korona 2005).

GENE DELETIONS AFFECTING THE MLS

This experiment was initiated in the same way as the former one but the YPD cultures were allowed to grow up to stationary phase and were then left as such for a total of 7 days to make the cells truly quiescent (Werner-Washburne et al. 1993). The cultures were then transferred to water and periodically tested for viability. The MLS was defined as a period of time after which the proportion of viable cells went down to about 0.001 or less. Tables S3 and S4 list all MLSs of the experimental and control strains, both raw and position-corrected (see Methods for details).

Figure 2 presents frequency distributions of MLS recorded in three independent replications. In one experimental replication, the yeast cells lived clearly shorter than in the two others. This could be caused by unidentified differences in media ingredients, water, plates, or incubation conditions. We consider it likely that

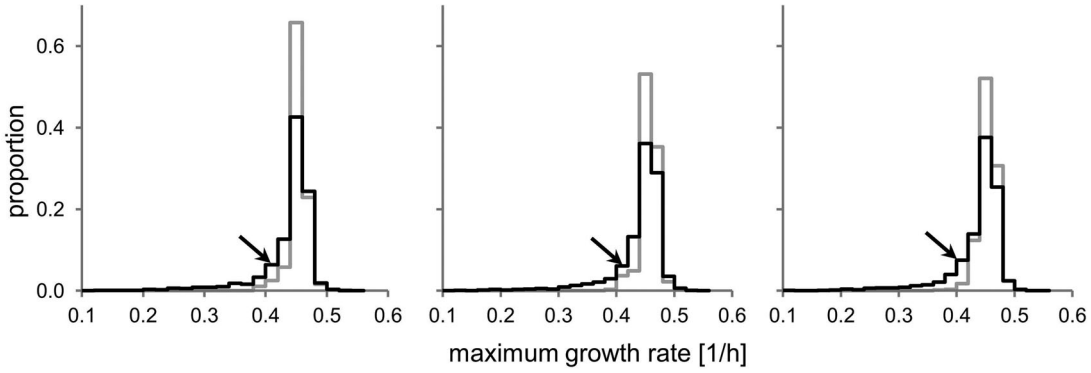

Figure 1. **Frequency distribution of the maximum growth rate (MGR). Maximum growth rate of the 3678 deletion strains and replicated control strain (672 times) was measured three times independently in the phase of exponential growth. Cumulative empirical distributions of the control (gray line) were used to predict the type I error for the deletion strains (see Supporting Information—Methods for more details). Histograms of MGR of the deletion strains are in black. Arrows show three consecutive MGR scores of one particular deletion strain, *rpl17b*Δ. The combined probability that this strain did not differ from the control one was 0.002 (Methods and Results for more details).**

it was an altered dynamics of evaporation refilling. Nevertheless, also in this replication there were large differences in individual MLS values, largely parallel with those in the two remaining replications. Generally, the maximum chronological lifespan was long, both its mean and median reached about 120 days for the control strain. This is a value typical for laboratory conditions similar to those applied here (Werner-Washburne et al. 1993). The estimates of MLS varied considerably even in the genetically homogeneous control. The likely reason was that MLS stands just for the end of a lengthy process in which random events and an unavoidable environmental heterogeneity were likely to occur. Nevertheless, the distributions of MLS in the pool of deletions and in the control were clearly different (see also Supporting Information—Methods). The modal peak was the same as in the control but low values of MLS were visibly enriched. Most importantly, some strains were short-lived repeatedly. Combining results of all three replications we found that for 566 deletions strains the risk of being wrongly classified as short lived was below 0.01 (Table S3).

We searched also for gene deletions that could extend lifespan. Figure 2 shows that the frequency distributions of MLS for the control and experimental strains were similar on their right sides implying that the deletions improving viability was infrequent. Applying an analogous procedure as for the short-lived strains, we identified only two deletions that could be classified as long-lived with FDR set at 0.01. Rising FDR to 0.1 yielded 37 deletions that possibly lived longer than the control strain. We list them in Table S5 but we do not insist that these deletions did extend MLS. They did not appear to have any common role in the cell, they belonged to many (49) and very different Yeast Slim GO Biological Process categories. We therefore conclude that the deletions shortening MLS greatly exceeded those extending

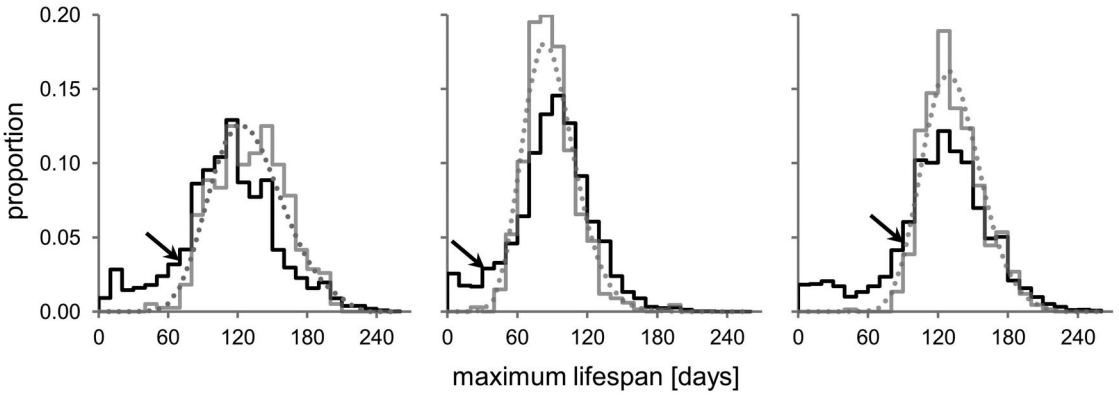

Figure 2. **Frequency distribution of the maximum lifespan (MLS). Gamma distributions (dotted gray line) were fitted to histograms of MLS of the control strain (solid gray line; see Supporting Information—Methods for more details). Histograms of MLS of the deletion strains are in black. Arrows show three consecutive MLS scores of one particular deletion strain, *fmp30*Δ. The probability that this strain did not differ from the control one was 0.0007.**

it. Long-lived variants were generally rare under conditions applied here. Also in previous studies, in which an average lifespan was much shorter, the long-lived mutants were always considerably less frequent than the short-lived ones (Powers et al. 2006; Fabrizio et al. 2010; Matecic et al. 2010).

We then asked specifically about those gene deletions that were found as extending lifespan in earlier studies. A study of starvation for glucose in exhausted minimal media found that the chronological lifespan was substantially extended when one of the 16 genes involved in TOR-dependent signaling and nutrient acquisition was deleted (Powers et al. 2006). To test whether the same deletions lived longer in our screen, we first divided their individual MLSs by an average MLS of the control strains. Averaging these normalized MLS over the 16 genes and 3 replications yielded a value of 1.123. This moderate lifespan extension was statistically significant at $P = 0.0051$ (t-test combined over three experimental replications). We repeated this procedure for two other studies in which deletions of genes other than those related to TOR were identified as long-lived under glucose starvation. One screen found 34 (Fabrizio et al. 2010), the other 57 (Matecic et al. 2010) such genes. In our study, the normalized averages of MLS for the two groups of genes were 1.009 and 0.992, respectively, and did not differ significantly from the control. We conclude that when cells were as deeply quiescent as in our study they tended to live so long that it was difficult to further extend their lifespan through genetic manipulation. Only deletions of genes relating to the TOR-dependent signaling had a positive effect on MLS, although much dampened. These genes are very special, however. Decreased activity of the TOR pathway—either because of environmental signals such as caloric restriction or because of mutations making it ineffective—has been repeatedly shown to extend lifetime in both unicellular and multicellular eukaryotes (De Virgilio and Loewith 2006; Castrillo et al. 2007; Lopez-Maury et al. 2008; Goldberg et al. 2009; Blagosklonny 2010; Fontana et al. 2010).

EFFECTS OF INDIVIDUAL DELETIONS ON GROWTH AND LONGEVITY ARE GENERALLY NOT ANTAGONISTIC

Figure 3 shows the relation between ln(MLS) and MGR (the latter is a logarithmic measure in itself). The Pearson's coefficient of correlation is positive, $r = 0.31$, and statistically significant ($F = 473.692$; df $= 1, 3676$; $P \ll 0.001$), suggesting that slower growth tended to co-occur with shorter lifespan. However, the relation between MLS and MGR was complex and not much suited for an overall test of linear correlation (Fig. 3). Most deletions had none or negligible effects on either growth or longevity. In fact, those with both traits visibly affected appeared underrepresented. An arrow-like shape of the cloud implies that there were two relatively large groups of gene deletions, one in which mostly

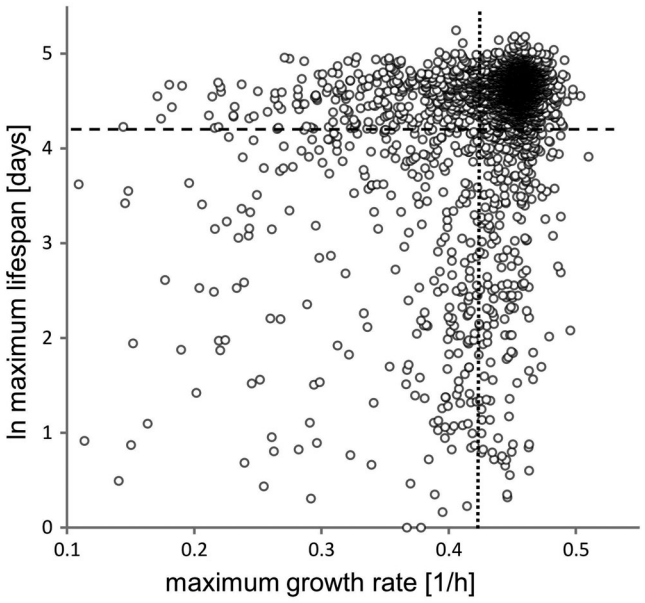

Figure 3. Correlation between lifespan and growth. Log-normal transformed maximum lifespan, ln(1 + MLS), is plotted against the maximum growth rate. The 636 slow-growing ones are to the left of a dotted line. The 566 short-lived deletions are below a dashed line.

MGR but not MLS was affected, and the other with an opposite tendency. This pattern suggests that most functions that were critical for one trait were less important, or entirely unimportant, for the other. This was further tested by comparing the functional annotations of the two groups of genes.

FUNCTIONS OF GENES REQUIRED FOR GROWTH AND LONGEVITY ARE RADICALLY DIFFERENT

Table 1 shows what biological processes were needed to maintain high values of MGR or MLS. The Gene Ontology terms were tested for being enriched (found more frequently than expected) among either the slow growing or short-lived deletions using a standard test based on the hypergeometric distribution (Boyle et al. 2004). The listed categories were ranked beginning with those that were enriched most. It is striking how different the two lists were: among 20 categories that were most important for MGR and 20 most important for MLS, none was shared. Maximum growth rate was most sensitive to slow metabolism of RNAs or proteins, that is, to mutations impairing transcription or translation. Maximum lifespan suffered mostly from the defects in autophagic or mitochondrial functions. We also looked for deletions that affected negatively both MLS and MGR, that is, were affected strongly enough to pass the criterion of FDR lower than 0.01. There were 257 such deletions, and only a few significantly enriched categories among them, and all of them were in some way related to the functioning of mitochondria (Table 2).

Table 1. The most enriched GO Biological Process terms among 566 deletions with a decreased maximum lifespan under quiescence (MLS) and 635 deletions with a decreased maximum growth rate (MGR) in rich medium.

Decreased MGR			Decreased MLS		
GO ID	GO Biological Process term	RE	GO ID	GO Biological Process term[1,2]	RE[3]
GO:0006450	Regulation of translational fidelity*	4.74	GO:0015988	Energy coupled proton transport*	4.77
GO:0006407	rRNA export from nucleus**	4.43	GO:0032543	Mitochondrial translation**	4.61
GO:0042273	Ribosomal large subunit biogenesis**	3.99	GO:0000959	Mitochondrial RNA metabolism**	4.33
GO:0030490	Maturation of SSU-rRNA**	3.96	GO:0016236	Macroautophagy**	4.15
GO:0042274	Ribosomal small subunit biogenesis**	3.93	GO:0010821	Regulation of mitochondrion*	4.10
GO:0032786	Positive regulation of transcription**	3.86	GO:0032258	CVT pathway**	3.98
GO:0042255	Ribosome assembly**	3.79	GO:0007035	Vacuolar acidification**	3.96
GO:0006414	Translational elongation**	3.51	GO:0000002	Mitochondrial genome maintenance**	3.82
GO:0006364	rRNA processing**	3.49	GO:0016237	Microautophagy**	3.80
GO:0006360	Polymerase I transciption**	3.48	GO:0000422	Mitochondrion degradation*	3.48
GO:0002181	Cytoplasmic translation**	3.29	GO:0006623	Protein targeting to vacuole**	3.16
GO:0006368	Polymerase II promoter**	2.90	GO:0009267	Cellular response to starvation**	3.14
GO:0022618	Ribonucleoprotein assembly**	2.84	GO:0007005	Mitochondrion organization**	3.13
GO:0031123	RNA 3′-end processing*	2.83	GO:0007033	Vacuole organization**	2.78
GO:0032270	Regulation of protein metabolism*	2.76	GO:0006914	Autophagy**	2.77
GO:0071826	Ribonucleoprotein organization**	2.74	GO:0031667	Response to nutrient levels**	2.51
GO:0034660	ncRNA metabolic process**	2.71	GO:0007034	Vacuolar transport**	2.44
GO:0006412	Translation**	2.52	GO:0045333	Cellular respiration*	2.37
GO:0006403	RNA localization**	2.45	GO:0016044	Cellular membrane organization**	2.21
GO:0051276	Chromosome organization**	1.78	GO:0015980	Energy by organic oxidation*	2.00

[1]Not listed are very broad categories (typically with about 1000 or more genes) or smaller categories with a complete or very large overlap with the shown ones.

[2]Statistical significance in the test of enrichment: *$P < 0.001$, **$P < 0.0001$.

[3]Relative enrichment (RE) is the obtained number of gene deletions divided by the number expected if deletions were distributed randomly.

Table 2. The most enriched GO Biological Process terms among the 257 gene deletions that showed a decrease in both the maximum lifespan under quiescence (MLS) and maximum growth rate (MGR) in rich media.

GO ID	GO Biological Process[1,2]	RE[3]	*N* (*M*)[4]
GO:0006744	Quinone biosynthetic process	12.5***	7 (7)
GO:0043039	tRNA aminoacylation	7.16***	10 (10)
GO:0006397	mRNA processing	3.39***	22 (3)
GO:0007005	Mitochondrion organization	3.14***	56 (54)
GO:0006412	Translation	2.46***	55 (42)

[1]Not listed are very broad categories (typically with about 1000 or more genes) or smaller categories with a complete or very large overlap with the shown ones.

[2]Statistical significance in the test of enrichment: ***$P < 0.0001$.

[3]Relative enrichment (RE) is the obtained number of gene deletions divided by the number expected if deletions were distributed randomly.

[4]*N* is the total number of gene deletions among the 257 with decreased MLS and MGR, *M* is the number of deletions that are also annotated to the GO:0005739 "mitochondrion" cellular component term.

The pronounced differences observed here in the genetic requirements for growth and longevity accord well with those seen in the studies of gene expression. It was repeatedly found that the expression of about one fourth of yeast's genes is dictated by the rate of growth. In particular, genes coding for the synthesis of RNAs and proteins are most active under fast growth whereas those coding for autophagy and oxidative metabolism are turned up when growth slows down (Regenberg et al. 2006; Castrillo et al. 2007; Brauer et al. 2008). This agrees with an intuition that when the cell grows its cellular macromolecules (proteins and RNAs) need to be intensely synthesized whereas starvation examines the cell's ability to recycle them. Although it appears natural that there should be a correlation between the level at which a gene is expressed and the loss of fitness after it is deleted, this was actually rarely seen. The earliest studies in which the growth rate of yeast deletion strains was measured and compared with the already existing data on gene expression found it utterly surprising that there was little to no such dependence (Winzeler et al. 1999; Giaever et al. 2002). In subsequent studies, not only typical media but also specific physical and chemical factors were tested but again the genes that were upregulated under these

Table 3. Probability of the type I error in the tests of enrichment for the GO Biological Process terms among short-lived gene deletion strains.

GO ID	Gene Ontology term[1]	Ours	G_F[2]	G_K	G_M	G_P	P_G	P_K	N_K	Leu_G
GO:0010324	Membrane invagination								1.3E−03	
	-Microautophagy	2.6E−06	1.6E−04		2.8E−06		1.4E−05		5.7E−12	5.9E−05
	-Microautophagy of nucleus	1.1E−08	4.4E−06		9.8E−08		1.8E−06		1.8E−14	1.1E−05
GO:0042594	Response to starvation	1.4E−05			5.6E−05				2.9E−09	6.0E−03
	-Macroautophagy	4.9E−07	5.0E−04		5.0E−07		1.8E−03		2.0E−10	1.7E−04
	-Mitochondrion degradation				3.6E−03				2.4E−04	5.1E−04
GO:0006605	Protein targeting						1.8E−03		2.2E−06	
	-Protein targeting to vacuole	7.0E−08	2.5E−05		5.0E−03		8.4E−06		1.2E−08	
	-CVT pathway	6.7E−05			1.5E−06		8.1E−05		9.8E−12	5.2E−05
G0:0045333	Cellular respiration			3.0E−06						
	-Aerobic respiration	6.6E−03		1.4E−04						
	-Proton transport	7.6E−03								
GO:0007005	Mitochondrion organization	1.9E−34		1.8E−51		6.3E−09	3.0E−19	3.5E−30		
	-Respiratory chain assembly			5.1E−03		7.4E−03				
	-mt genome maintenance	7.3E−05								
GO:0032543	Mitochondrial translation	2.0E−33		6.1E−43		1.1E−04	8.6E−22	1.1E−22		
	-mt tRNA aminoacylation	3.2E−03						2.8E−03		

[1]Terms with an ID number are the Yeast Slim categories.

[2]Starvation for G(lucose), P(hosphorous), N(itrogen), and Leu(cine). The lower index indicates the source of data: F (Fabrizio et al. 2010), G (Gresham et al. 2011), K (Klosinska et al. 2011), M (Matecic et al. 2010), P (Powers et al. 2006).

conditions were different from those that were essential to uphold growth (Birrell et al. 2002; Tai et al. 2007; Jin et al. 2008). The same was found for nongrowing cells in which the genes needed to survive starvation did not overlap the genes that were induced upon starvation (Klosinska et al. 2011). The observed discrepancy has been usually explained as resulting from the low precision and repeatability of estimates, especially those relating to the level of mRNA expression (Ooi et al. 2006; Roemer et al. 2012). In this study, we concentrated on functional classes of genes instead of individual genes. After completing the lists of functions that were most necessary to uphold propagation or survival, we could show that they resembled those most often seen as induced under fast or slow growth (Airoldi et al. 2009). It would be interesting to see whether similar relationships emerge if other fitness components are analyzed in the same way. Some evolutionary biologists have postulated that the intensity of expression correlates with the strength of purifying selection. Consequently, low expression of some genes might in consequence enable their evolution and possibly lead to new adaptations (Lopez-Maury et al. 2008). Our results provide some support for this scenario, at least if the continuum between fast growth and starvation is considered. In this article, however, we concentrate rather on gene erosion than accumulation of gene variants for future evolution.

The remaining three quarters of genes are not dependent in their expression on the rate of growth (Airoldi et al. 2009). This might mean that they are important under both fast growth and starvation. If so, many gene deletions would be scored in both screens and overshadow those which were found as belonging to relatively few and functionally specific classes. This was not the case which indicates that not only those genes (modules) that are downregulated in a particular environment but also many of those that do not react could be inactivated by mutations without causing significant phenotypic changes.

GENETIC REQUIREMENTS FOR LONGEVITY FORM A COMPACT AND UNIVERSAL REPERTOIRE

It is generally agreed that the synthesis of macromolecules is a critical requirement for rapid growth under many environmental conditions not only in yeast but also in bacteria and metazoans (Warner 1999; Wada et al. 2000; Ma and Blenis 2009). The metabolism of nongrowing cells is not known as well and is considered to depend on the limiting agent. Were it indeed strongly dependent on environmental conditions, the validity of our findings would be potentially limited. We therefore reexamined the results of all earlier starvation studies and compared them with our results (see Supporting Information—Methods for details on the compared sets of data). Table S6 shows a list of all GO terms referring to biological processes that were found enriched in either the present or any earlier study. Its simplified version, limited to the Yeast Slim terms, is given in the main text (Table 3). In all nine screens examined, only two broad functions of the cell were found as enriched among the deletions shortening lifespan. One was the recycling of proteins through sorting them out of the cytosol and nucleus into the vacuole through several specific processes that could be collectively described as autophagy. The other was the assembling, functioning, and removal of mitochondria. Notably, the list of required processes is longest for the present screen. The reason could be that the procedure applied here to get deeply quiescent cells resulted in especially strong signaling so that an especially broad set of functions needed to prepare for long

starvation was turned on. If so, however, the lists of requirements under starvation in synthetic media (8 days to 7 weeks depending on study) should be not only shorter, as they were, but also characteristic for specific limiting nutrients. This was not observed, the four screens under glucose deficiency yielded different results as did the two under phosphorous deficiency. An alternative explanation is that the experimental and statistical procedures applied here proved more powerful in discovering the short-lived mutants and thus our results are not different but simply more complete. We applied an especially long exposure to starvation. When lifespan is generally short, because of environmental conditions, only the strongest negative effects can be seen readily whereas those weaker may become undistinguishable from random/environmental variation. This conjecture is supported by an observation that in the two starvations for phosphorous more functions were found when the starvation lasted for 21 as opposed to 8 days. We conclude that there is a relatively small set of functions which are required when the cells stop to grow regardless of what nutrient they are lacking and for how long are they prepared to sustain the starvation. Therefore, our conclusions regarding the differences in genetic requirements for growth (here MGR) and longevity (here MLS) are likely to be broadly applicable.

Conclusions

We measured the rate of growth and longevity under starvation for some 3600 yeast strains with individual genes deleted. There were hundreds of strains in which one of these traits was affected strongly enough that we could qualify them safely as deleterious mutations. These relatively strong effects were weakly correlated suggesting that pleiotropy was generally synergistic. This overall correlation was caused by a limited number of deletions that affected both traits. Most of nonneutral deletions, however, either slowed down growth or shortened lifespan. This statistical dichotomy was clearly supported by analyses of molecular and cellular functions that dominated among genes affecting either of these traits. Their functional profiles were radically different: functions needed for the synthesis of macromolecules dominated among those important for growth, whereas functions required for recycling of macromolecules were overrepresented among genes supporting long survival. We thus propose that mutational erosion of any of these two fitness components is largely independent from each other. The mutational targets were large, about 600 genes were identified individually for every trait. However, only those relatively strong effects were detected in this way. Figures 1 and 2 suggest that these numbers could be perhaps doubled if smaller effects were included. This means that large portions of the yeast genome can start to accumulate mutations after transferring to environments in which either bursts of fast growth or prolonged periods of starvation became very rare. Such scenarios may become possible in case of emerging parasitism, even more endosymbiosis, and potentially in some environments created by humans.

For decades pleiotropy was believed to be universal, or very common, as this was suggested by the geometric model of adaptation, observations of physiological geneticists, and concepts envisaging metabolism as virtually a single network of fluxes (Fisher 1930; Wright 1968; Kacser and Burns 1981). More recently, the geometric model of adaptation has been modified to explicitly assume that each locus can affect only a limited number of traits (Martin and Lenormand 2006; Chevin et al. 2010). It has also been proposed that organisms are not quite entities but rather assemblages of functional modules and therefore pleiotropy is also modular, that is, restricted to smaller networks of gene–trait interactions (Welch and Waxman 2003; Wagner and Zhang 2011). Our question was whether modules that were most important for growth tended also to considerably affect longevity. Only the mitochondrion appeared universally needed and thus genes coding for it were pleiotropic. However, this element of the cell is hardly a typical "module." It is very large, used for multiple purposes, and thus unavoidably harming many processes when damaged. We find it revealing that there are so many other functional modules that can be only partly effective under some conditions but not visibly affected under others. This means they are largely independent from one another at least when mutational erosion is considered.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Maximum growth rate (MGR) of deletion strains.

Table S2. Maximum growth rate (MGR) of the control strain.

Table S3. Maximum lifespan (MLS) of deletion strains.

Table S4. Maximum lifespan (MLS) of the control strain.

Table S5. List of deletions associated with the longest recorded MLSs.

Table S6. The test of GO terms enrichment among short-lived deletions.