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Strong dominance of functional alleles over gene deletions in both intensely growing and deeply starved yeast cells

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Abstract

Previous studies with diploid yeast have shown that the deletion of one allele at a single locus typically has little impact on fitness under conditions promoting fast growth. Here, we confirm and quantify this finding. The strong dominance of functional over nonfunctional alleles is predicted by the metabolic control theory which assumes that the cell is a system of metabolic fluxes and that the total metabolic rate is equivalent to fitness. To test whether these requirements are critical, we tested dominance under conditions of long-term starvation when metabolism is low and thus the metabolic activities of proteins are likely inadequate or imbalanced. More fundamentally, the central assumption of the model, that high metabolic rate translates into high fitness, appears implausible. Contrary to these conjectures, we found that the mean rate of survival of starving heterozygotes was affected only slightly more than was the mean rate of growth under good conditions. Under none of the two treatments the central prediction of the model, that fitness of heterozygous strains is higher for the enzymatic proteins than for nonenzymatic ones, was confirmed. Our data add to growing uncertainty whether the metabolic control theory is sufficient to explain the remarkable ubiquity of strong genetic dominance.

Introduction

Research on dominance has played a special role in the study of genetics and evolution. The research led to the realization that the obviously advantageous property of so many genes, the ability to restore required function with just one allele instead of two, does not have to be an evolved adaptation but can be an epiphenomenon of metabolic mechanisms. To see it, it is sufficient to assume that the overall metabolic reward per additional unit of a single enzyme is much higher when the enzyme is scarce than when it is abundant. This argument was originally mostly hypothetical (Wright, 1929; Haldane, 1930). Kacser and Burns (1981) proposed a formal model and, together with corroborative empirical data, presented it as the metabolic control theory (Fig. 1). The metabolic theory was developed in response to suggestions that the dominance of functional alleles over nonfunctional alleles actually evolved and likely involved 'modifier' loci which arose to mask the negative effects of deleterious mutations (Fisher, 1928). The adaptive hypothesis of dominance has been undermined by the theoretical demonstration that the selective pressure required to evolve dominance would be weak (Wright, 1934) and by the empirical discovery that functional alleles also dominate over nonfunctional ones in organisms that are not normally diploid and thus in which dominance could not evolve (Orr, 1991). However, it would be an overstatement to claim that the adaptive hypothesis has been definitively disproven. A genuine, if rather special, example of the evolution of dominance dependent on a modifier locus has been actually found (Tarutani *et al.*, 2010; Billiard & Castric, 2011). Moreover, the adaptive hypothesis is not the only alternative to the metabolic control theory. Another model posits that if selection typically stabilizes intermediate values of traits, then the fitness landscape resembles a rounded hill and, therefore, the fitness of a heterozygote must be greater than expected by averaging the two associated homozygotes. Although the model is restricted to small

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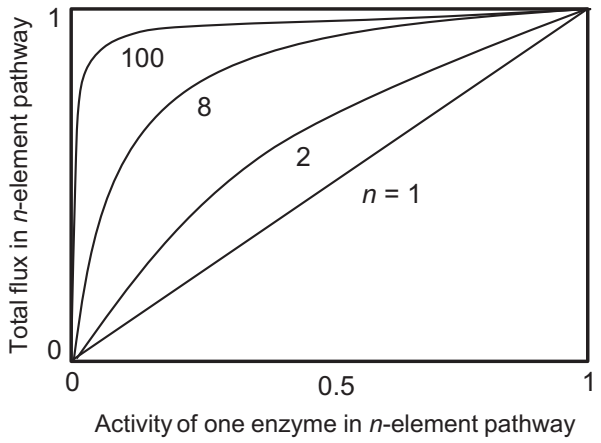

Fig. 1 The metabolic control theory of genetic dominance. The total metabolic flux is considered to be a proxy for fitness. As the number of participating enzymes increases, the relation between the activity or abundance of one enzyme and the total flux is more and more nonlinear (Kacser & Burns, 1981, modified).

mutational effects, it has the advantage of encompassing all traits, not just those dependent on the activity of enzymes (Manna *et al.*, 2011). Still, the explanation of dominance that invokes the nonlinear response of metabolic flux to the amount of gene product is the most popular theory and frequently the only one known. The explanation is appealing because it employs a single and simple mechanism to rationalize a vast array of observations. However, the body of experimental evidence supporting the metabolic control theory is rather small and, as explained below, has weaknesses.

The metabolic control theory not only explains why harmful mutations are recessive but also predicts that the most harmful mutations will be the most recessive. This has been shown to be the case in *Drosophila*; lethal alleles are almost completely recessive and slightly deleterious alleles much less so. The effect of this phenomenon is that the average fitness of heterozygous strains is high and indistinguishable for lethal and mild mutations (Mukai *et al.*, 1972; Simmons & Crow, 1977). A very similar result has been obtained for spontaneous and EMS-induced mutations in yeast (Szafraniec *et al.*, 2003). The presence of a strong negative correlation between the selection coefficient and the dominance coefficient (*s* and *h*, respectively) is not predicted by the modifier hypothesis and is considered strong evidence in favour of the metabolic theory (Charlesworth, 1979; Kacser & Burns, 1981). However, in both the fruit fly and yeast, the analysed mutations were random, and neither their molecular nature nor their chromosomal location could be known. Whether the observed degree of dominance related only to the strength of the homozygous effect and not to other factors (e.g. functional features of the mutant protein) is therefore unclear. The construction of a complete collection of yeast gene deletions provided researchers with a qualitatively new tool (Giaever *et al.*, 2002). In each strain, the activity of a single known protein is absent, thus enabling the analysis of gene function. Heterozygous deletion strains have half the gene dose of wild-type strains as well as half the protein dose because compensation through enhanced expression of the remaining allele is rare in yeast (Springer *et al.*, 2010). The heterozygous fitness effects of the engineered deletions were soon found to be very similar regardless of whether the homozygous deletions were lethal or mild (Steinmetz *et al.*, 2002). This was true for all major categories of genes that were analysed, not just for genes encoding enzymes (Phadnis & Fry, 2005). Subsequent reanalysis of the same data set identified several weaknesses: as many as approximately 6000 deletion strains were cultured together making individual estimates weak statistically; a no-deletion strain was not included as a control; deletions generating large fitness effects were often excluded either unintentionally or intentionally; homozygous and heterozygous deletion strains were maintained separately, the former were more affected and therefore could undergo much more effective selection for compensatory mutations (unknown for experimenters) than the latter. Nevertheless, a strong negative correlation between *s* and *h* was still observed after these and other factors were taken into account (Agrawal & Whitlock, 2011). Yet another examination of the same data set suggested that genes with small fitness effects should be excluded from the analysis as the observed effects were likely produced by systematic experimental errors, such as between- and within-treatment interdependences. However, in agreement with previous reports, this reanalysis also found that the heterozygous fitness (1-*hs*) of deletions with large fitness effects remained constant and was unaffected by decreasing homozygous fitness (1–*s*) (Manna *et al.*, 2012).

We reasoned that it is advisable to re-estimate dominance in a way that minimizes both the systematic and random errors present in earlier studies. Furthermore, previous empirical tests were limited to one basic design. The yeast deletion strains were allowed to grow at their maximum rates under laboratory conditions that were either optimal or suboptimal but still allowing relatively steady growth (Steinmetz *et al.*, 2002; Blomberg, 2011). There is, however, another physiological state often experienced by unicellular organisms – starvation. Yeast cells deprived of external resources can survive for weeks or even months by recirculating their biomass and utilizing internal energy reserves. Overall metabolic activity during long-term starvation is much lower than during growth (Werner-Washburne *et al.*, 1993). Therefore, halving the gene dose could be more damaging under long-term starvation (low activity of enzymes) than under growth (high activity of

enzymes) (Fig. 1). We therefore decided that testing survival under prolonged starvation would constitute a valuable extension of the empirical research on dominance.

In this study, we utilized the collection of yeast gene deletions in a way that addressed earlier methodological shortcomings. The deletion strains were maintained individually so that slow growers were not overwhelmed by fast growers. Multiple replicates of a strain with no deletions were included as controls. Finally, we attempted to minimize the chance that spontaneously arising compensatory mutations would play a major role. For nonessential gene deletions, the effect of the complete absence of a single protein was measured in haploid strains and, because the functioning of haploid and diploid cells is very similar in terms of gene expression (Galitski *et al.*, 1999), used as a proxy for homozygous deletions. The haploid deletion strains were then immediately mated with the deletion-free parental strain and the resulting heterozygous diploid strains used to measure the effect of halving the gene dose of single genes. (In this way, new spontaneous mutations are unlikely to be introduced.) For essential genes, an existing collection of heterozygous strains served as the half-dose group; an appropriate full-dose control strain was derived from them by crosses aimed at restoring the genetic background into which single deletions were introduced.

We first tested the experimental and control strains under favourable conditions. Our quantitative estimates indicate that the growth of strains with heterozygous deletions is affected only minimally and that the selection and dominance coefficients are, as expected, negatively correlated. We then examined the same strains for viability by exposing them to long-term starvation. The overall dominance of functional alleles was only slightly lower than that observed under growth conditions and the negative correlation between the selection and dominance coefficients was maintained.

Materials and methods

Strains

We used the BY4741 *MAT***a** YKO collection as our source for haploid strains with deletions of single nonessential genes. As a control, we introduced the *kan*MX4 cassette, which replaces an inactivated *ho* allele and is considered to be neutral for fitness, into the original BY4741 strain (containing only marker gene deletions). The introduced cassette provided resistance for geneticin and was identical to the cassette used to construct the deletion strains (Giaever *et al.*, 2002).

Starting with the haploid strains described above, we derived our own collection of heterozygous nonessential deletion strains by mating the haploid strains (including the control strain) with BY4742 *MAT*α. The BY4742 *MAT*α strain is isogenic to BY4741 *MAT***a**. The resulting diploid strains were considered homozygous except for a single protein-coding gene (experimental strains), the *HO* locus (control) and the marker *LYS2* and *MET15* loci (common to all). To ensure effective selection of diploids, we introduced a centromeric plasmid containing *URA3* and *nat*MX4 (which codes for resistance to nourseothricin) into BY4742. This allowed for the elimination of nonmated haploids on media containing both geneticin and nourseothricin; the plasmid was then removed by growing the resultant diploids on media containing 5-fluoroorotic acid. Only nonessential haploid deletion strains that showed signs of having negative effects on the rate of growth or longevity under starvation in our previous study were mated (see Results).

Our experiments also included all heterozygous deletions of essential genes from the YKO collection. These strains are nearly but not completely identical to the haploid BY4741 and BY4742 (Brachmann *et al.*, 1998). To obtain a relevant control strain, we randomly chose, sporulated and dissected four of the heterozygous deletion strains. Haploid strains with mating types and markers analogous to those of BY4741 and BY4742 were selected from the viable spores and remated to form diploids. A *kan*MX4 cassette was then used to replace one of the *ho* alleles. The resulting diploid strains contained the same combination of auxotrophic markers and the same single resistance cassette as the deletion strains. All four strains grew fast and at equal rates. One of them was selected for use as a control for the heterozygous essential deletions.

Maximum growth rate (MGR)

Strains were cultured individually in standard 96-well titration plates with flat bottoms. Samples of thawed strains were used to inoculate titration plates containing YPD (Yeast Peptone Dextrose), which were then incubated until stationary phase cultures developed. Aliquots of the stationary phase cultures (2 μL) were used to inoculate 200 μL aliquots of fresh YPD to start test cultures. Optical density (OD) of the test 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 as the slope of regression over time. Estimates obtained in this way fit the linear regression model very well; nearly all individual Pearson's coefficients of correlation were higher than 0.99. The strains were arranged in plates in the same order as they were maintained in the YKO collections, although some plates were split in two with the free halves filled with appropriate control strains. This experiment was independently repeated three times. One replicate for the nonessential collection had to be discarded because of an apparent cross-contamination, and therefore only two were used in subsequent analyses.

Maximum lifespan (MLS)

The same set of strains, the same arrangement on plates and the same culture initiation procedure were used to measure MLS as were used to calculate MGR. Following inoculation, experimental cultures were not measured for OD but were instead kept in YPD for a full 7 days to reach a mature stationary phase (Werner-Washburne *et al.*, 1993). They were then diluted 1 : 1000 in 200 μL of water. (Dilution was performed in two steps, 1 : 25 and 1 : 40, in order to avoid imprecise sampling of 0.2 μL aliquots.) The resulting starving cultures were sampled immediately after the transfer to water, 7 days later, and then at 14-day intervals. Every sample contained approximately 1000 cells (initially 5 μL of the starving culture, then incrementally more as recurrent refilling of the starving cultures to approximately 200 μL with water caused gradual dilution). To test for the presence of viable cells, samples were transferred to fresh YPD and incubated for 3 days. The day of starvation at which the last growth in YPD was observed, with no growth at subsequent samplings, was recorded as the MLS. We decided that a log-transformed measure of longevity, ln(MLS), would compare better with MGR, which is a log measure of reproductive ability. Because there were a few cultures with MLS equal zero, the symbol ln(MLS) stands actually for ln(1 + MLS); this was a minor adjustment as a vast majority of MLS scores were much higher than zero.

The procedure applied here to estimate MLS of vegetative cells would not be appropriate for diploid strains if some of starving diploid cells underwent meiosis leaving admixture of (haploid) spores. Fortunately for this experiment, the BY strains require a truly extreme and rapid shift in nutritional conditions to enter sporulation, one that was absent in our protocols (Winzeler *et al.*, 1999). If sporulation happened at least occasionally, some vegetative diploid cultures (with haploid spores in them) would significantly outlive haploid vegetative cultures but this was not observed.

Correction for the plate and position effects

Initial data analyses suggested that both MGR and MLS were affected by the position of a culture on a plate. In the case of MGR, the position effect might have been produced by an OD reader (Tecan Infinity 300); readings within the upper rows of a titration plate appeared slightly different than those taken subsequently. The MLS position effect could have been caused by an increased evaporation, and thus more intense refilling, along the plate's edges.

First, we tested whether the suspected differences were statistically significant. As mentioned above, replicate cultures of two control strains, one for the collection of nonessential gene deletions and the other for the collection of essential gene deletions, were interspersed with the corresponding deletion stains on 96-well plates. We compared MGRs or MLSs within each control strain according to four factors – time blocs, plate, row within plate and column within plate. Table S1 shows that these factors were indeed often significant. We started by correcting for the time block and individual plate effect. We found that the upper quartile of a plate was less varying between plates that the mean or median. We therefore divided all individual MGR/MLSs by the upper quartile of a respective plate and then multiplied them by the average upper quartile calculated over all plates within one time block. Then, we proceeded to correct for the position of a well on a plate. The (already corrected for the plate effect) measurements were averaged for every particular position (intersection of a row and a column). These averages were then used to calculate a smoothened surface of the expected values across a plate within a replicate, that is time block (as there were small differences between replicates). Examples are shown in Fig. S1. The ratio of the expected position-specific average over the whole-plate average was used as the correction term. Individual estimates of experimental clones were then multiplied by the correction term according to their position. An average MGR correction term was one with standard deviation 0.009 and 0.011 for the nonessential and essential data sets, respectively. In the case of MLS, standard deviations of the correction terms for nonessential and essential deletions were 0.038 and 0.021, respectively. The whole procedure of data correction, applied here for the diploid strains, has been formerly developed and applied in the same way for the haploid strains (Marek & Korona, 2013).

Results

Repeatability of MGR and MLS estimates

The environmental conditions used in this study allowed for either fast growth (doubling time of < 2 h) or survival without growth over long periods of time (up to several months). Two parameters, the MGR or MLS, were used to quantify the performance of the tested deletion strains in the first or second environment, respectively. We started our analyses by asking how repeatable are measurements of MGR and MLS. Table S2 summarizes results of an analysis of variance among strains and replicates (time blocks). One inference is that differences between replicates contribute little to the total variance. Another clear result was that the absolute value of random error was much higher for MLS than for MGR. In relative terms, however, the among-strain component of variance was remarkably high not only for MGR but also for MLS. Thus, genetic

effects were relatively strong when compared with random effects for both studied traits.

MGR and MLS of nonessential haploid deletions

In this study, we sought to compare the effect of losing all or a half of gene dose. The former were represented by haploids tested in our former study. First, we re-examined data on the MGR and the MLS in the haploid collection of nonessential gene deletions reported in our earlier study (Tables S1 and S3 in Marek & Korona, 2013). Figure 2a shows that the right arms and modal values of the distributions for MGR and ln(MLS) were very similar to those of control distributions; the left arms, in contrast, were thickened and extended (Fig. 2a). This pattern implies that the deletion effects in haploids were rarely (perhaps never) positive, most often neutral and sometimes negative. Most of the later were rather small or moderate although large ones were also observed. There were 947 sufficiently large MGR effects and 772 sufficiently large MLS effects (marked in Fig. 2a) that could be judged as caused by gene deletions with a false discovery rate of less than 0.1 (Marek & Korona, 2013).

In the case of MGR, we were able to test quantitatively our assumption that the effects of gene deletions in haploid strains and homozygous diploid strains are similar. We compared our MGR estimates with estimates obtained in a former study (Steinmetz *et al.*, 2002). There were 3574 nonessential gene deletions present in both studies; their fitness estimates correlated with each other (Pearson's $r = 0.591$, $F = 7477.91$, $P \ll 0.0001$). Figure S2 shows that the correlation was best for the most affected strains. Specifically, haploid strains used in the present study as surrogates for homozygotes had an average relative fitness of 0.878, whereas the corresponding homozygotes in the former study had an average fitness of 0.917. Thus, both haploid and homozygous diploid strains were located at the left tail of the fitness distribution indicating that their selection coefficients were not critically affected by the level of ploidy.

MGR and MLS of heterozygous deletions of nonessential genes

The selected haploid strains were mated with a wild-type haploid strain of the opposite mating type to obtain heterozygous strains (see Methods). Individual MGR and MLS estimates of the selected haploid and heterozygous deletion strains are listed in Table S3. Figure 2b presents distributions of MGR and MLS for heterozygous strains. The left arm in these distributions is somewhat extended but by far less than it was for

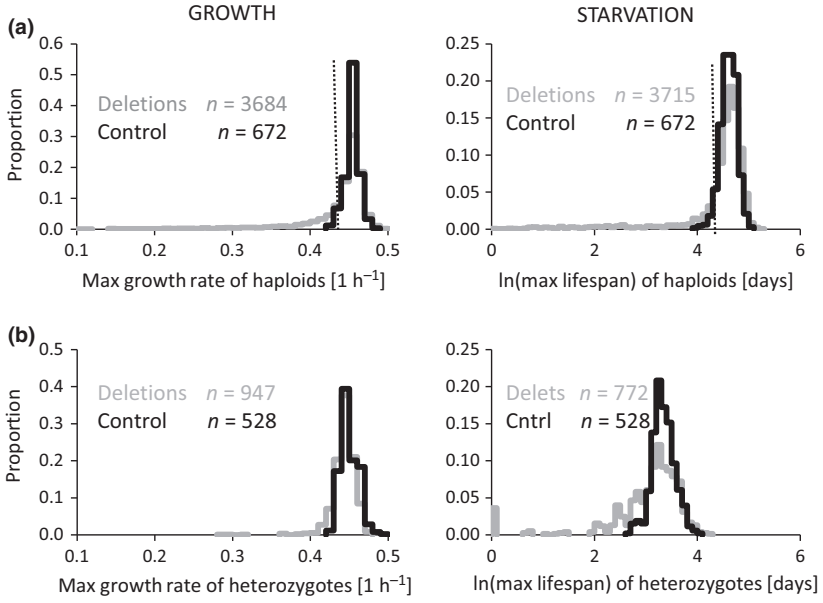

Fig. 2 Dominance of functional alleles in the yeast nonessential gene collection. (a) Plots show frequency distributions of the maximum growth rate and the maximum lifespan of haploid strains in which single nonessential genes were deleted. Only confirmed protein-coding genes were included. The control consists of replicated cultures of an isogenic nondeletion strain. Dashed vertical lines delimit deletion strains classified as slow growing (947 strains) or short living (772 strains) with a false discovery rate of 0.1 or lower. (b) The slow-growing and short-living strains were mated with a nondeletion strain, yielding heterozygous strains. Frequency distributions of the two phenotypic traits of the heterozygous strains are shown. The control haploid strain was also mated with the same nondeletion strain to obtain an appropriate control.

haploids. Still more strikingly, a clear majority of heterozygous strains grew and survived at rates undistinguishable from those of the control. This result indicates that most deletions that are harmful in haploids are effectively neutral in the heterozygous state, although some of the deletions remain deleterious to a varying degree.

A closer examination of graphs in Fig. 2 reveals two more findings. First, a few deletions were inviable on the initial day of starvation in the heterozygous, but not the haploid, state. One interpretation could be that heterozygosity causes about higher sensitivity, a kind of overdominance of negative lifespan effects. This explanation is likely countered by the second of mentioned observations: the lifespan of heterozygous strains was generally much shorter than the lifespan of haploid strains. It is not sure that diploids normally survive for a shorter time than haploids. The two groups were assayed separately and we have noticed that MLS is very sensitive to multiple, and often inscrutable, environmental factors, Thus, 'overdominance' was likely spurious.

To get a common measure of mutational effects, we converted MGR and MLS into relative fitness by dividing every individual estimate by the mean of an appropriate control group. Our chief finding was that, regardless whether fitness of a haploid deletion (1-*s*) was high or low, fitness of a corresponding heterozygote (1-*hs*) was high and nearly unchanging (Fig. 3a). The constantly low average value of *hs* implies that *h* tends to decrease as *s* increases. Figure 3b shows that such a general pattern was present even though individual values of *h* were estimated with relatively high errors. (They were obtained as the *hs* over *s* ratio and therefore combined two errors which led to substantial deviations, especially for MLS.)

MGR and MLS of heterozygous deletions of essential genes

We also tested the collection of essential yeast gene deletions. The fitness estimates for strains with a single heterozygous deletion of an essential gene are listed in Table S3 and shown graphically in Fig. 4. The distributions of both MGR and MLS suggest that most of the lethal effects observed in haploids were fully or nearly fully compensated in heterozygous loci; some deletion effects remained negative but were usually not lethal. In other words, what was true for low fitness mutations in nonessential genes (Fig. 2) was also true for zero fitness mutations in essential genes.

Gene function and dominance

Figure 5 helps to compare the strength of dominance for growth and lifespan in the nonessential collection. The mean fitness of nonessential genes in heterozygous loci was high: it was close to one for the MGR and still over 0.9 for the MLS. Because the metabolic control model relates primarily to enzymes, we then restricted our analyses to genes coding for catalytic proteins (as listed in the Saccharomyces Genome Database\downloads\biochemical_pathways.tab). The means remained nearly unchanged. The dominance for MGR and MLS was even stronger for the essential genes. Restricting the analysis to essential enzyme-coding genes did not change the results substantially (Fig. 5).

We then asked whether deletions of genes coding for specific functional groups (as defined by Gene

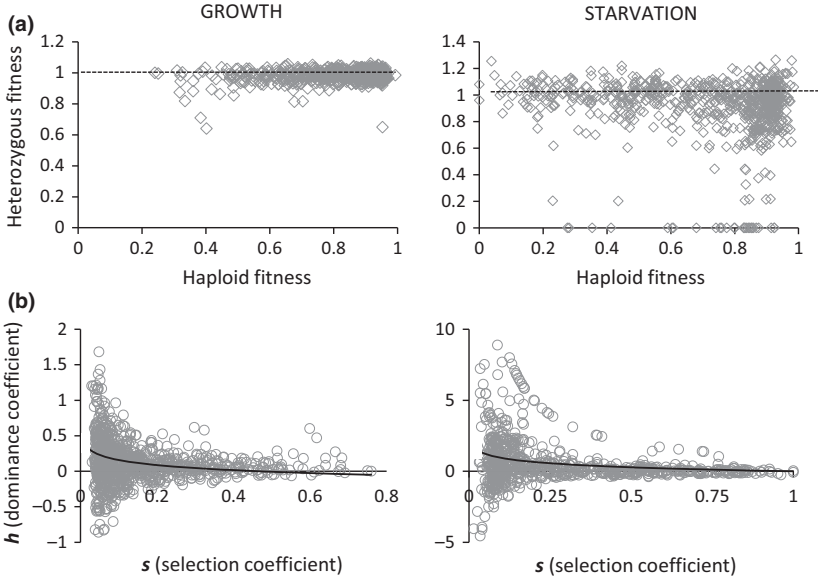

Fig. 3 Relative fitness was calculated by dividing estimates of maximum growth rate (MGR) or maximum lifespan (MLS) by the mean of an appropriate control. (a) Relative fitness of haploids and corresponding heterozygotes plotted against each other. (b) Relation between the dominance coefficient *h* and selection coefficient *s*. MGR: $h = -0.103\ln(s)-0.0787$, $r = 0.199$, $N = 947$, $F = 58.23$, $P < 0.0001$; MLS: $h = -0.411\ln(s)-0.0057$, $r = 0.189$, $N = 772$, $F = 41.75$, $P < 0.0001$.

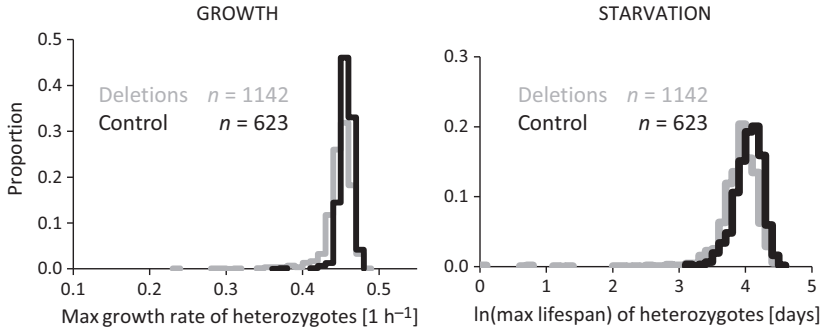

Fig. 4 Dominance of functional alleles in the yeast essential gene collection. Plots show frequency distributions of the maximum growth rate and the maximum lifespan of heterozygous strains with a deletion of a single essential gene. The sample consists of 1142 strains with deletions of genes coding for confirmed proteins that have zero growth rate and zero lifespan when haploid or homozygous. The control consists of replicated cultures of a strain isogenic to the heterozygous essential collection but with no genes deleted (except for common marker genes).

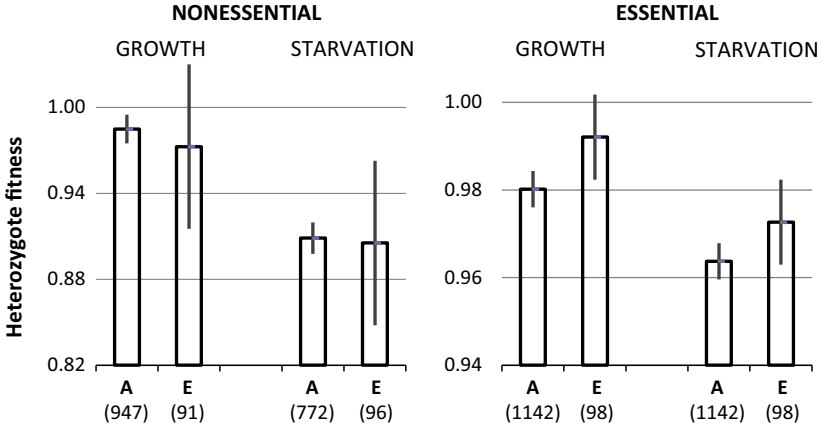

Fig. 5 Dominance of genes encoding enzymes (E) vs. genes encoding all proteins (A). Bars show 95% confidence intervals, and numbers in parentheses indicate sample sizes.

Ontology) are overrepresented among those which were least dominant (Boyle *et al.*, 2004). We ranked the tested heterozygous strains from least to most fit for both MGR and MLS and identified any overrepresented functional classes within the first quartile (with all tested heterozygous strains, not the whole genome, serving as a reference group). For nonessential genes, translation as a process and ribosomes as a cell component were enriched significantly in the first quartile of MGR-ranked deletion strains. Ribosome biogenesis as a process and nucleoli and preribosomes as components were enriched in the essential gene deletion strains (full results shown in Table S4). In contrast, there were no exceedingly unfit functional categories of heterozygous deletions observed for either the nonessential or the essential gene sets when MLS data were examined. Together with the reported above absence of difference between enzymatic and nonenzymatic genes, the results of GO analyses indicate that the function of a gene has generally little impact on its heterozygous fitness.

Discussion

Periods of growth interrupted by intervals of starvation are typical for yeast and other free-living microorganisms (Lewis & Gattie, 1991; Gray *et al.*, 2004). Many individual cells in multicellular organisms also experience a high or low rate of metabolism rather than an average and stable one (Coller *et al.*, 2006). As a result, cells need either to increase or preserve biomass, and the applied transcriptional profiles and resulting macromolecular compositions for the two metabolic states differ profoundly. Starvation does not mean full dormancy which in yeast is characteristic for spores. Spores are probably crucial to endure extreme or very long stress. However, the ability to tolerate stress or starvation while retaining vegetative state must be also important for this species. Eukaryotes, from yeast to humans, use remarkably conserved pathways to perceive environmental signals and implement the required adjustments in vegetative metabolism (De Virgilio & Loewith, 2006; Lopez-Maury *et al.*, 2008;

Fontana *et al.*, 2010). We measured the strength of genetic dominance under two major modes of metabolism: either excessive abundance of nutrients and exponential growth or nutrient deprivation and danger of deterioration.

The dominance of the functioning alleles over the nonfunctioning (deleted) alleles was astonishingly strong in our study: most negative effects detected in the haploid phase disappeared in heterozygous diploids under good environmental conditions. The remaining ones caused only a 1–2% loss of fitness averaged over all tested genes. Previous reports on the yeast gene deletions did not provide fitness estimates of comparable accuracy, although the observed dominance was clearly very strong. In agreement with these earlier studies, we also found a negative correlation between the selection and dominance coefficients, such that the single gene deletions with the most harmful effects in haploids generally had the least effect in heterozygotes. There was no difference in the degree of dominance between enzymatic and nonenzymatic proteins, two particularly important functional classes in the testing of the metabolic control theory (Phadnis & Fry, 2005). We did not identify differences in the degree of dominance for any functional class of genes other than the established case of genes coding for the synthesis and functioning of ribosomes (Deutschbauer *et al.*, 2005). The general lack of fitness effects in response to heterozygous gene deletion under normal growth conditions is well known and used to find those relatively rare genes which require a full dose (homozygosity) to function well when confronted with selected chemicals, potential drugs (Baetz *et al.*, 2004). In sum, our improved procedures provided data confirming all major findings about dominance under conditions allowing fast metabolism: dominance is strong, gene function has little effect on the degree of dominance, and the h and s coefficients are negatively correlated.

The degree of dominance under starvation conditions has not been evaluated in previous studies. Starvation enforces a radical reduction of the rate of metabolism, such that growth is impossible and fitness becomes equivalent to the ability to survive as long as possible. We found that the dominance of active alleles for MLS was lower than it was for MGR. Nevertheless, the average lifespan for heterozygous deletion strains was high, exceeding 90% of the lifespan typical for strains with no deletions. In addition, h decreased as s increased and dominance for enzyme-coding genes was comparable to that for other genes. Similar observations were made for the fast-growing cells. The most striking finding of this study is that that the pattern of dominance of functional alleles over deleted ones is nearly the same regardless of whether the MGR of well-nourished cells or the MLS of starved cells is considered.

The metabolic control theory relates to flux, not growth or survival. With this reservation in mind, it appears correct to say that the metabolic theory is the only model offering a straightforward explanation why the dominance of functional alleles can be so strong. In a network of 3000–5000 enzymatic proteins, the serious malfunction of one enzyme would decrease metabolic flux by a factor of 10^{-3}–10^{-4}. Any difference between diploids bearing one or two functional alleles of a single gene would be virtually undetectable (Kacser & Burns, 1981). However, there are numerous genes encoding proteins that are not linked to any particular flux of metabolites. Kacser and Burns exclude them from their analyses and suggest codominance as a more appropriate model. However, only genes encoding ribosomal proteins appear partly codominant. But, they are a special case because they function within large stoichiometric complexes of dozens of other proteins together with large amounts of RNA. A shortage of one protein would both lower the rate of translation and burden the cell with unnecessary synthesis and disposal of vast amounts of protein and RNA (Warner, 1999). The average heterozygous fitness for deletions of structural proteins participating in small complexes and other nonenzymatic proteins does not differ from the mean fitness (Fig. 5). The standard metabolic model appears implausible here. But, the rate of metabolic flux is only a proxy for fitness. Fitness pay-offs associated with nonenzymatic proteins may depend on factors related to the proteins in question rather than the total flux. Production and maintenance of proteins is costly as suggested by both experimental (Tomala & Korona, 2013) and comparative analyses (Zhang & Yang, 2015). A theory linking fluxes of small metabolites with the economics of macromolecule production is much needed. It could provide support for the model of stabilizing selection in which an intermediate amount of a protein (as in heterozygotes) typically results in higher fitness than expected from the extremes (homozygotes) (Manna *et al.*, 2011).

The almost universally strong dominance of functional alleles over deletions is even more puzzling when starving cells are considered. In principle, the metabolic control theory can also be applied in this case. It assumes that the flux through a pathway has two basic features: (i) all enzymes participate in determining the rate of flux, and (ii) the control of the flux by a single enzyme drops hyperbolically with the concentration of the enzyme (Kacser & Burns, 1981). There are no *a priori* limits on the efficiency of particular enzymes or the output of the whole pathway. Thus, the general features of the system remain similar regardless of whether the rate of metabolism is very high or very low. However, the model requires that the concentrations of all enzymes and metabolites reach equilibrium. It would be very difficult, if not impossible, to determine empirically whether a cell is in the metabolic equilibrium prescribed by the current catalytic potential of all its proteins. The pools of metabolites and

concentrations of proteins have been estimated under conditions of growth. Remarkably, a large percentage of the proteins in a cell are represented by no more than dozens or hundreds of molecules per cell (Ghaemmaghami *et al.*, 2003). There are no comparable estimates for starving cells. The number of active protein molecules is likely to be even lower in such cells. Many metabolic activities are likely too low, too unstable or not connected well enough through shared metabolites to produce a balanced state in which the activity of every (quasi)enzyme counts. Indeed, applicability of the metabolic control theory is more constrained than is usually acknowledged (Cornish-Bowden, 1987; Savageau & Sorribas, 1989; Grossniklaus *et al.*, 1996).

There is another basic difficulty with nongrowing cells. The metabolic control theory equates fitness with flux (Hurst & Randerson, 2000). However, starving cells actually suffer when flux is unnecessarily high because energetic reserves are used up too quickly. Therefore, our data on survival should not be seen as a strict test of the metabolic control theory and definitely not as a confirmation of it. The theory assumes that the metabolic system is self-regulating with no external signals playing a role in it. In fact, the state of a cell under starvation (as well as under growth) is governed by environmental signalling followed by extensive alterations in gene expression (Regenberg *et al.*, 2006; Lopez-Maury *et al.*, 2008; Klosinska *et al.*, 2011). The distortion of fluxes caused by the mutation of a single gene can be overwhelmed by concerted regulation that is stabilized by multiple correctly functioning genes. The relative importance of automatic reactions predicted by the metabolic model vs. cellular programs that evolved to adapt the cell to changing external and internal conditions is largely unstudied (Levy *et al.*, 2007). An ambitious framework would be to measure actual fluxes within the metabolic network of the cell and then evaluate how the fluxes translate into major phenotypic traits, which are the determinants of fitness. The goal of this paper was to test whether strong dominance is restricted to conditions under which fitness is likely correlated with the rate of metabolic flux. The answer is largely negative. Strong dominance is not formally forbidden under conditions of low metabolism, but there are reasons to believe that these conditions make the appearance of high dominance less likely. It is not clear whether (at least partial) applicability of the metabolic control model extends down to the very low metabolism of starving cells or other mechanisms underpin the observed here strong dominance of functional over nonfunctional alleles.

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Conflict of interest

The authors declare that they have no competing interests.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Typical surfaces of expected values calculated for the 8 × 12 titration plates used for the MGR (left) and MLS (right) assays.
Figure S2 Relative fitness (growth rate divided by respective average) for the 3574 gene deletions strains which were assayed both as haploids (X axis) and homozygous diploids (Y axis).
Table S1 Variation* within replicated control strains; ANOVA according to time block, plate number, row within plate and column within plate.
Table S2 Components of variance* in ANOVA.
Table S3 Individual estimates of MGR and MLS.
Table S4 Gene Ontology analysis.

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