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ORIGINAL ARTICLE

Beneficial Mutations from Evolution Experiments Increase Rates of Growth and Fermentation

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Abstract

A major goal of evolutionary biology is to understand how beneficial mutations translate into increased fitness. Here, we study beneficial mutations that arise in experimental populations of yeast evolved in glucose-rich media. We find that fitness increases are caused by enhanced maximum growth rate (R) that come at the cost of reduced yield (K). We show that for some of these mutants, high R coincides with higher rates of ethanol secretion, suggesting that higher growth rates are due to an increased preference to utilize glucose through the fermentation pathway, instead of respiration. We examine the performance of mutants across gradients of glucose and nitrogen concentrations and show that the preference for fermentation over respiration is influenced by the availability of glucose and nitrogen. Overall, our data show that selection for high growth rates can lead to an enhanced Crabtree phenotype by the way of beneficial mutations that permit aerobic fermentation at a greater range of glucose concentrations.

Keywords Beneficial mutations · Fitness effects · Experimental evolution · Yeast · Crabtree · Trade-off

Background

Experimental populations of microbes have facilitated major advances in understanding the molecular mechanisms of adaptation (Lang and Desai 2014). The relative ease of whole genome sequencing and the amenability of microbes to genetic manipulation have colluded to produce a growing number of examples where the genetic causes of adaptation have been unambiguously identified (Hammerschmidt et al. 2014; Frenkel et al. 2015; Meyer et al. 2012; Taylor et al. 2015; Lang et al. 2013; McDonald et al. 2016).

Recently, several experimental studies in *Saccharomyces cerevisiae* have explored in great detail the dynamics and identity of the beneficial mutations that underlie adaptation in glucose-rich laboratory media (Lang et al. 2013; McDonald et al. 2016; Kryazhimskiy et al. 2014; Levy et al. 2015; Venkataram et al. 2016). One of the most consistent results is the recurrent evolution of mutations in the RAS/PKA pathway. The RAS/PKA signaling pathway integrates information on nutrient availability to regulate cell cycle progression, the rate of cell division, and the expression of metabolic genes (Broach 2012). This pathway is well understood and mutations in several of the genes have been demonstrated to confer an increased fitness. However, the mechanism by which mutations in this pathway translate into a growth advantage in glucose-rich conditions remains unknown.

Glucose can be converted into energy via substrate-level phosphorylation (fermentation), or oxidative phosphorylation (respiration). Respiration depends on oxygen and produces a higher yield of ATP per molecule of glucose than fermentation (Verduyn et al. 1991; van Gulik and Heijnen 1995). In oxygen-rich conditions most organisms prefer respiration. However, when glucose is abundant, glycolytic flux increases, resulting in high levels of pyruvate, the product of glycolysis. Instead of utilization via the TCA cycle and respiration, excess pyruvate will "overflow" into the fermentation pathway, resulting in the production of metabolites such as acetate (*E. coli*) or ethanol (yeast) (Otterstedt et al. 2004). Despite the apparent efficiency of respiration, in some conditions, organisms are

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known to preferentially utilize glucose via fermentation rather than respiration.

It has long been established that in glucose-rich, oxidative conditions *S. cerevisiae* prefers fermentation over respiration (Crabtree 1929). This preference for overflow metabolism is referred to as the Crabtree-positive phenotype. *S. cerevisiae* is one of the few Crabtree-positive yeasts (Hagman et al. 2014; Deken 1966) and significant efforts have been expended to explain the preference for this apparently inefficient method of utilizing glucose (Pfeiffer and Morley 2014). A potentially beneficial trait of fermentation is that, despite a lower yield of ATP per unit of glucose, there is a higher rate of ATP production per unit time (Pfeiffer and Morley 2014; Pfeiffer et al. 2001). In other words, fermentation may be less efficient than respiration, but it converts glucose into ATP more rapidly, thereby providing a means to convert the abundant glucose resources into energy more quickly than respiring competitors.

The evolutionary forces that drive the preference for fermentation or respiration can be understood with *R*/*K* selection theory, which predicts the outcomes of evolution in conditions of high or low resource availability (Reznick et al. 2002). An *R*-selected population has evolved with abundant resources and infrequently attains maximum population density. Individuals that most rapidly convert resource into biomass and energy are favored by selection in these conditions, even at the cost of efficiency. This inefficiency is beneficial as long as the population does not encounter resource scarcity. The high levels of resource, and relatively short periods of scarcity that characterize many yeast evolution experiments suggest that they may experience "*R*" selection. Indeed, experimental populations have been used to explore the evolution of trade-offs between *R*- and *K*-selected growth strategies (Luckinbill 1978; Novak et al. 2006; Bennett and Lenski 2007; Jasmin et al. 2012).

The repeated experimental evolution of mutations in the RAS/PKA pathway suggests that the rate of glucose utilization is under selection. Since glucose utilization also underpins the Crabtree-positive phenotype, understanding the eco-evolutionary forces that drive adaptation in these potentially *R*-selected experimental populations could provide insight into the evolution of this defining trait of *S. cerevisiae*. Here, we re-construct beneficial mutants and measure their growth rates and obtain values for *R* and *K*. We find that beneficial mutations increase growth rate (*R*) at the cost of yield (*K*) and that increases in growth rate result from increased flux through fermentation pathways. We then examine performance of the ancestral strain and mutants across diverse glucose and nitrogen concentrations and show that the balance between fermentation and respiration is driven by both glucose and nitrogen. We find that beneficial mutations in the RAS/PKA pathway can cause an enhanced Crabtree-positive phenotype, whereby some mutants ferment glucose across a wider range of glucose concentrations than the ancestor.

Methods

Host Strain and Construction and Mutants

The host strain used in this study is DBY15084, a haploid derivative of W303 with a genotype of *MAT***a**, *ade2-1, CAN1, his3-11, leu2-3, 112, trp1-1, URA3, bar1Δ::ADE2*, and hmlαΔ::*LEU2*, previously described by Lang et al. (2009). Mutant strains were constructed using lithium acetate (LiOAc) yeast transformation method to swap the gene of interest with a KanMX-promoter cassette. *IRA2, GPB1, GPB2, PDE2, KRE6, and TOH1* genes were separately swapped with a KanMX resistance marker (G418) in DBY15084 using strains from yeast deletion collection (Giaever et al. 2002). The KanMX resistance marker is commonly used in evolution studies and has been found to not confer a cost in competitive fitness assays (Pronk 2002).

Media and Growth Conditions

For growth assays, a single colony was grown overnight in 5 mL of CSM media including 20 g/L glucose and yeast nitrogen base (YNB) without ammonium sulfate. The following day, cell density of cultures was determined using OD_{600} measurements. These measurements were used to standardize the inoculum of host and mutant strains to 0.1 OD_{600}. Cultures were grown on 96-well plates with a final volume of 150 µL media with carbon (glucose) and nitrogen (ammonium sulfate) as limiting nutrients. Glucose concentrations ranged as 6, 12, 14, 18, and 22 g/L while ammonium sulfate concentrations ranged as 0, 0.075, 0.75, 7.5, and 75 g/L of media. Cultures were grown at 28 °C, shaken continuously (fast setting) in the ELx808™ Absorbance Microplate Reader for 15 h. Readings of OD_{600} were taken at every 20-min intervals to generate growth curves of the host and mutant strains.

Statistical Analyses

Maximum growth rate (*R*) and the maximum yield or carrying capacity (*K*) were estimated for each mutant by fitting standard logistic growth models to time-series of optical density with non-linear least squares techniques. All non-linear models were ensured to successfully converge. Linear models followed by Tukey's post hoc tests were then used to assess the effects of each mutant type on *R* and *K*. All analyses were carried out using package nlme with the function nls in statistical software R (Pinheiro et al. 2016; R Development Core Team 2016). Correlations were calculated using

Pearson's correlation coefficient. t was calculated for each correlation coefficient using the following formula:

$$t = \frac{r}{\sqrt{\frac{1-r^2}{n-2}}},$$

and p was calculated using t.dist function in excel, and multiplied by two to give exact, two-tailed p values.

Spectrometric Determination of Residual Ethanol

After 15 h of incubation in the ELx808™ Absorbance Microplate Reader, the cells were pelleted at 1000 rcf for 1 min using Eppendorf 5810 R Centrifuge and 20 µL of the supernatant was used for the subsequent residual ethanol measurements. Ethanol concentrations were assayed using Alcohol Dehydrogenase coupled with (NAD^+) reduction reactions to NADH (Walker 1992). 0.1% (17.1 mM), 0.3% (51.4 mM), 0.5% (85.7 mM), 0.8% (137.1 mM), 1% (171.3 mM), and 1.5% (257 mM) ethanol used as reference concentrations for generating standard curves and estimating residual ethanol concentrations. Reactions were set up on flat-bottom 96-well plates with a total volume of 213.7 µL. Reactions included 87 µL of 50 mM sodium phosphate buffer, 100 µL of 15 mM NAD^+, 20 µL of the samples or ethanol standards, and 6.7 µL of ADH (5 mg/mL). Reactions were left to reach equilibrium at room temperature for at least 4 h, then their absorbance were recorded at a wavelength of 340 nm using ELx808™ Absorbance Microplate Reader. Specific excretion rates of ethanol were calculated by taking measurements of ethanol at 0, 4, 5.5, 6.5, and 7.5 h after inoculation with eight replicates for each time point. Following Basan et al. (2015), the slope of ethanol against cell density (mM/OD_{600}) was multiplied by specific growth rates for each strain that had been calculated for the first 8 h of growth (mOD600/min). This gave a final value with the unit mM/mOD_{600}/min, which we converted to mM/OD_{600}/min. The nitrogen and glucose concentration gradients were carried out in microplates with one replicate for each condition. A single ethanol measurement was taken at 15 h after inoculation and normalized by cell density.

Fitness Assays

Fitness assays were carried out following Lang et al. (2011). Briefly, cultures were grown over night in defined CSM media containing either 20 or 6 g/L glucose. Strains were mixed with a GFP-marked reference strain in a ratio of 1:1 and incubated statically at 28 °C for 24 h. After the period of incubation, cultures were propagated by a $1:2^{10}$ dilution into fresh media. The ratios of the marked reference to the strain of interest were estimated by cell cytometry after 10 and 30 generations, with at least 30,000 cells counted for each assay. A selection coefficient was calculated following Lang et al. (2011).

Results and Discussion

Beneficial Mutants Exhibit a Trade-Off Between Rate and Yield

We constructed six strains, each with a deletion in a different "multi-hit" gene that had evolved mutations in multiple independent populations, across multiple evolution experiments (Table 1). Four of these genes, IRA2, GPB1, GPB2, and PDE2 encode negative regulators within the RAS/PKA growth regulation pathway. Deletion of each of these genes has been previously confirmed to be beneficial (Kryazhimskiy et al. 2014; Venkataram et al. 2016). TOH1 is not part of the RAS/PKA pathway and has an unknown function; however, a TOH1 mutant recovered from an experimental evolution study was shown by genetic reconstruction to confer a benefit in competitive fitness assays (McDonald et al. 2016). We predicted that deletion of TOH1 would also be beneficial, since loss of function mutations are commonly observed in TOH1 in evolution experiments (Lang et al. 2013; Kryazhimskiy et al. 2014). KRE6 is required for beta 1,6 glucan biosynthesis. While certain substitutions in KRE6 are likely to be beneficial, no loss-of-function mutations have been discovered in KRE6 in evolution studies, suggesting that deletion of KRE6 would not be beneficial (Lang et al. 2013; Kryazhimskiy et al. 2014).

Table 1 The number of mutations recovered in previous evolution experiments in the genes deleted in this study

Locus	Systematic	Synonymous	Missense	Nonsense	Indel	Total	Studies
IRA2	YOL081W	0	18	4	2	24	Lang et al. (2013), Kryazhimskiy et al. (2014), Venkataram et al. (2016)
GPB1	YOR371C	0	3	2	2	7	Lang et al. (2013), Kryazhimskiy et al. (2014), Venkataram et al. (2016)
GPB2	YAL056W	0	6	9	6	21	Lang et al. (2013), Kryazhimskiy et al. (2014), Venkataram et al. (2016)
PDE2	YOR360C	1	2	4	6	13	Lang et al. (2013), Kryazhimskiy et al. (2014), Venkataram et al. (2016)
TOH1	YJL171C	0	2	2	0	4	Lang et al. (2013), Kryazhimskiy et al. (2014)
KRE6	YPR159W	0	6	0	0	6	Lang et al. (2013), Kryazhimskiy et al. (2014)

We measured the fitness of the ancestor and deletion strains in conditions matching previous evolution experiments (Lang et al. 2013; McDonald et al. 2016) and confirmed that deletion of IRA2, GPB1, GPB2, PDE2 is beneficial (Fig. 1a). Our fitness assays showed that TOH1 deletion is beneficial and deletion of KRE6 is effectively neutral (Fig. 1a).

In order to explore the underlying mechanisms of the competitive advantage, we measured maximum growth rates (R) and maximum carrying capacity (K) for each strain. We found that R and K were strongly negatively correlated, suggesting a trade-off between growth rate and yield ($r = -0.90$, $p = 0.0056$). We compared the fitness measurements of mutants to R and K and found that R had a positive correlation with fitness ($r = 0.86$, $p = 0.012$), while K had no correlation with fitness, but showed a negative trend ($r = -0.69$, $p = 0.08$). Previous work has identified a trade-off between growth rate and yield using measurements of experimental yeast populations evolved over 5000 generations (Jasmin et al. 2012), largely explained by a transition from diploid to haploid. Since our beneficial mutants were engineered so that they could not mating-type switch and become diploid ("Methods"), ploidy transitions are unlikely to explain fitness differences.

The High-Yield KRE6 Mutant Has a Superior Fitness Effect in Low Glucose

The KRE6 mutant has significantly higher values of K and lower values of R than the RAS/PKA pathway mutants (Fig. 1c, d). The high K/R ratio suggests that it may perform better in a low glucose environment. We carried out fitness assays in low glucose media to test this prediction. While fitness effects of the RAS/PKA and TOH1 mutants were all reduced relative to fitness measurements made in the high glucose environment, the KRE6 mutant had an elevated fitness in the low glucose environment (Fig. 1b). KRE6 encodes an integral membrane protein essential for cell wall biosynthesis (Roemer and Bussey 1991). The mutations obtained in previous evolution experiments were unlikely to cause a loss of function, but happen frequently enough that they are probably selected for in glucose-rich media (Table 1) (Lang et al. 2013; McDonald et al. 2016; Kryazhimskiy et al. 2014). Combined, these results suggest that selected missense mutations result in changes in function of KRE6 that lead to an R-selected mutant, while complete abrogation of the gene results in a K-selected mutant.

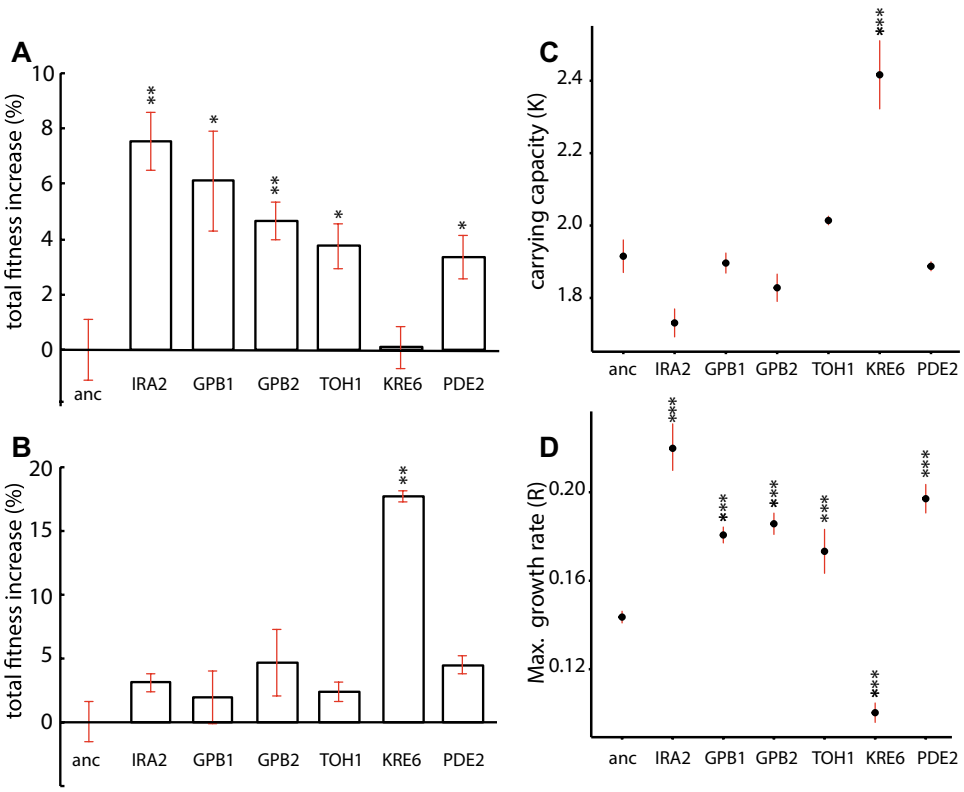

Fig. 1 **a** Competitive fitness assays of deletion mutants measured in 20 g/L glucose. **b** Competitive fitness assays for each mutant measured in 6 g/L glucose. **c** Growth curve data were used to estimate carrying capacity (K) and **d** maximum growth rate (R) for each deletion mutant (Supplementary data). Error bars are ±1 standard error of the mean. Asterisks indicate significant differences from the ancestor (0.05*, 0.01**, and 0.001***)

High Growth Rate Is Associated with Increased Ethanol Production

A proposed source of the trade-off between maximum growth rate and yield in a range of organisms is a branch point in glucose metabolism, where the product of glycolysis (pyruvate) is allocated to either respiration or fermentation (Pfeiffer and Morley 2014; Pfeiffer et al. 2001). The relative contributions of fermentation or respiration to growth can be estimated by measurement of the product of fermentation (Basan et al. 2015; Bachmann et al. 2013). In a population of yeast cells, if the preference is for fermentation, this will manifest in detectable levels of ethanol. We measured the specific rate of ethanol production during log growth in media containing 20 g/L glucose, and found that three of the RAS/PKA mutants, PDE2, IRA2, and GPB2 had increased rates of ethanol secretion compared to the ancestor, with a significantly positive effect of R on ethanol secretion rates ($F_{1,4}=13.57$, $p=0.022$) (Fig. 2a). This suggests that, for the PDE2, IRA2, and GPB2 mutants, increased growth rates also result in increased rates of ethanol production.

GPB1 has an elevated fitness, growth rate, and absolute levels of ethanol compared to the ancestor, but does not have an elevated rate of ethanol secretion. One might have predicted that certain RAS/PKA mutants could promote cell division and growth, which would lead to an increase in absolute ethanol production, but not relative ethanol production. This seems to be true for the GPB1 mutant. Alternatively, mutants could lead to increased growth rate, but also increased relative ethanol production, which seems to be true for PDE2, IRA2, and GPB2. These results suggest that the translation of the effect from the RAS/PKA mutants into glycolysis, respiration, and fermentation occurs through a number of pathways, possibly dependent on interactions of the deleted proteins.

IRA2, GPB2, and PDE2 Mutants Prefer Fermentation over Respiration Across a Wider Range of Glucose Concentrations

Previous work has found that ethanol is not produced when glucose concentrations drop below a threshold (Verduyn

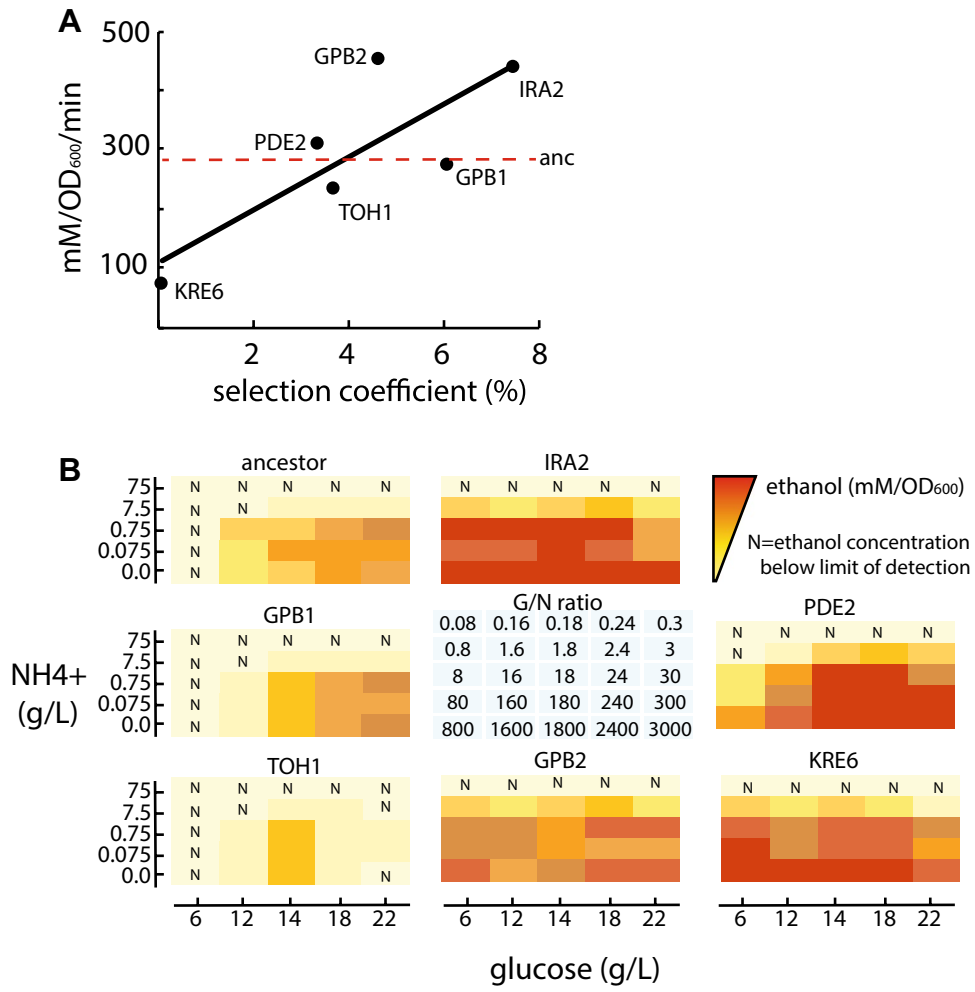

Fig. 2 **a** A plot of ethanol secretion rate and competitive fitness, both measured in 20 g/L glucose. The rate of ethanol secretion by the ancestral strain is given by the red-dashed line. **b** Heat maps show the relative production of ethanol for each strain after growth in media containing 6–22 g/L glucose and 0–75 g/L NH_4^+. Middle panel shows the approximate ratio of glucose: NH_4^+ in each well. (Color figure online)

et al. 1984), suggesting that higher concentrations of glucose should result in more ethanol production. We grew cultures at varying concentrations of glucose, and found a strong positive correlation between glucose concentration and ethanol yield ($r=0.97$, $p=0.005$) (Fig. 2b). At the lowest glucose concentration measured, 6 g/L glucose, the progenitor *S. cerevisiae* strain did not produce any detectable ethanol. Interestingly, the IRA2, GPB2, and PDE2 mutants produced high amounts of ethanol at all glucose concentrations (Fig. 2b) suggesting that the low ethanol production of the ancestral strain at 6 g/L glucose was not due to fundamental constraints on ethanol production caused by the unavailability of glucose.

Increased Nitrogen Supply Reduces Fermentation, but Mutants Do Not Have an Altered Response to Nitrogen Concentration

Recent work has emphasized the high proteomic cost of respiration, and shown that reducing the availability of nitrogen can drive overflow metabolism, even at low carbon source concentrations (Basan et al. 2015). We manipulated glucose and nitrogen concentrations using NH_4^+, a preferred nitrogen source for *S. cerevisiae* (Crepin et al. 2012), and measured ethanol yield (Fig. 2b). The ancestral strain showed a sharp drop in ethanol production when 7.5 and 75 g/L NH_4^+ were added to the media (Fig. 2b), supporting that nitrogen availability, as well as glucose concentrations, plays a role in overflow metabolism in yeast.

Our results show that for most strains, if nitrogen is added up to 0.75 g/L, ethanol production is not perturbed across a range of glucose concentrations (Fig. 2b). However, in low glucose, even when nitrogen is present in a similar ratio to high glucose treatments, fermentation is not evident. For example, in the ancestor strain, ethanol was not produced in 6 g/L glucose and 0 g/L NH_4^+, which has a *G/N* ratio of 800 (Fig. 2b, middle panel). However, ethanol was produced in cultures with a *G/N* ratio of as low as 24 and 30. In agreement with this, we failed to detect any statistically significant interaction of the absolute amounts of NH_4^+ and glucose ($F_{1,220}=0.82$, $p=0.36$). This suggests that it is not the ratio of glucose to nitrogen, but their absolute concentration that influences the fermentation/respiration balance.

These results have implications for the selective forces that underlie the evolution of the Crabtree positive phenotype. Did yeast experience selective pressures driving rapid growth rates or was there selection to circumvent low levels of nitrogen? Our results show that selection for rapid growth can cause the evolution of a more extreme Crabtree-positive phenotype. They also show that high levels of nitrogen can suppress fermentation, implying that nitrogen abundance is a hard constraint on the choice between fermentation and respiration. However, the beneficial mutations studied here arose in experiments that never experienced high nitrogen concentrations. While these mutants may be able to ignore low glucose as a cue to switch from fermentation to respiration, they have not been selected to ignore high nitrogen concentrations. It would be interesting to conduct experiments in media with high nitrogen and high glucose to see if selection for rapid growth could break the repressive effect of high nitrogen concentrations on fermentation.

Conclusions

The mutations studied here were obtained from experimental populations subjected to selection for high growth rates in a glucose-rich, and crucially, relatively nitrogen poor environment. Growth curves are relatively easy to obtain for microbial populations, and it may prove fruitful to measure *R* and *K* for evolved populations and putative beneficial mutants from other evolution experiments (Novak et al. 2006; Bennett and Lenski 2007). In this experiment, we found that simple selection for high growth rates, or *R* selection, can drive the evolution of a preference for fermentation at glucose concentrations where respiration is preferred by the ancestral strain. The mutants tend to be in negative regulators of cell growth, suggesting that the yeast has evolved sophisticated regulatory mechanisms to ensure that the Crabtree-positive phenotype is only "engaged" at very specific glucose and nitrogen concentrations. Here, we found that strains adapted to high glucose expanded the range of glucose conditions where fermentation was preferred, but did not evolve a change in response to nitrogen. It may turn out that low nitrogen availability played a key role in the evolution of the Crabtree-positive phenotype. The finding that nitrogen abundance can suppress overflow metabolism is novel for yeast, but is consistent with findings that fermentation requires less protein mass than respiration (Basan et al. 2015). The mounting evidence for the importance of nitrogen abundance in the overflow metabolism suggests that nitrogen as well as glucose should be considered in theories addressing the evolution of the Crabtree-positive phenotype in Yeast.

Author Contributions MJM conceived the study. ALS, MEM, DJM, and MJM designed the study. ALS and MJM conducted experiments. MEM and MJM analyzed the data. ALS, MEM, DJM, and MJM wrote the paper.

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Compliance with Ethical Standards

Conflict of interest We have no competing interests.

References

Bachmann H, Fischlechner M, Rabbers I, Barfa N, Branco dos Santos F, Molenaar D, Teusink B (2013) Availability of public goods shapes the evolution of competing metabolic strategies. Proc Natl Acad Sci USA 110:14302–14307. https://doi.org/10.1073/pnas.1308523110

Basan M, Hui S, Okano H, Zhang Z, Shen Y, Williamson JR, Hwa T (2015) Overflow metabolism in *Escherichia coli* results from efficient proteome allocation. Nature 528, 99–104. https://doi.org/10.1038/nature15765

Bennett AF, Lenski RE (2007) An experimental test of evolutionary trade-offs during temperature adaptation. Proc Natl Acad Sci USA 104(Suppl 1):8649–8654. https://doi.org/10.1073/pnas.0702117104

Broach JR (2012) Nutritional control of growth and development in yeast. Genetics 192:73–105. https://doi.org/10.1534/genetics.111.135731

Crabtree HG (1929) Observations on the carbohydrate metabolism of tumours. Biochem J 23:536–545

Crepin L, Nidelet T, Sanchez I, Dequin S, Camarasa C (2012) Sequential use of nitrogen compounds by *Saccharomyces cerevisiae* during wine fermentation: a model based on kinetic and regulation characteristics of nitrogen permeases. Appl Environ Microbiol 78:8102–8111. https://doi.org/10.1128/AEM.02294-12

De Deken RH (1966) The Crabtree effect: a regulatory system in yeast. J Gen Microbiol 44:149–156. https://doi.org/10.1099/00221287-44-2-149

Frenkel EM, McDonald MJ, Van Dyken JD, Kosheleva K, Lang GI, Desai MM (2015) Crowded growth leads to the spontaneous evolution of semistable coexistence in laboratory yeast populations. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.1506184112

Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B et al (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418, 387–391. https://doi.org/10.1038/Nature00935

Hagman A, Sall T, Piskur J (2014) Analysis of the yeast short-term Crabtree effect and its origin. FEBS J 281:4805–4814. (https://doi.org/10.1111/febs.13019)

Hammerschmidt K, Rose CJ, Kerr B, Rainey PB (2014) Life cycles, fitness decoupling and the evolution of multicellularity. Nature 515:75–79. https://doi.org/10.1038/nature13884

Jasmin JN, Dillon MM, Zeyl C (2012) The yield of experimental yeast populations declines during selection. Proc R Soc Lond B 279:4382–4388. https://doi.org/10.1098/rspb.2012.1659

Kryazhimskiy S, Rice DP, Jerison ER, Desai MM (2014) Microbial evolution. Global epistasis makes adaptation predictable despite sequence-level stochasticity. Science 344:1519–1522. https://doi.org/10.1126/science.1250939

Lang GI, Desai MM (2014) The spectrum of adaptive mutations in experimental evolution. Genomics 104:412–416. https://doi.org/10.1016/j.ygeno.2014.09.011

Lang GI, Murray AW, Botstein D (2009) The cost of gene expression underlies a fitness trade-off in yeast. Proc Natl Acad Sci USA 106:5755–5760. https://doi.org/10.1073/Pnas.0901620106

Lang GI, Botstein D, Desai MM (2011) Genetic variation and the fate of beneficial mutations in asexual populations. Genetics 188:647–661. https://doi.org/10.1534/Genetics.111.128942

Lang GI, Rice DP, Hickman MJ, Sodergren E, Weinstock GM, Botstein D, Desai MM (2013) Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. Nature 500:571–574. https://doi.org/10.1038/nature12344

Levy SF, Blundell JR, Venkataram S, Petrov DA, Fisher DS, Sherlock G (2015) Quantitative evolutionary dynamics using high-resolution lineage tracking. Nature 519, 181–186. https://doi.org/10.1038/nature14279

Luckinbill LS (1978) r and K selection in experimental populations of *Escherichia coli*. Science 202, 1201–1203. https://doi.org/10.1126/science.202.4373.1201

McDonald MJ, Rice DP, Desai MM (2016) Sex speeds adaptation by altering the dynamics of molecular evolution. Nature 531:233–236. https://doi.org/10.1038/nature17143

Meyer JR, Dobias DT, Weitz JS, Barrick JE, Quick RT, Lenski RE (2012) Repeatability and contingency in the evolution of a key innovation in phage lambda. Science 335:428–432. https://doi.org/10.1126/Science.1214449

Novak M, Pfeiffer T, Lenski RE, Sauer U, Bonhoeffer S (2006) Experimental tests for an evolutionary trade-off between growth rate and yield in *E. coli*. Am Nat 168:242–251. https://doi.org/10.1086/506527

Otterstedt K, Larsson C, Bill RM, Stahlberg A, Boles E, Hohmann S, Gustafsson L (2004) Switching the mode of metabolism in the yeast *Saccharomyces cerevisiae*. EMBO Rep 5:532–537. https://doi.org/10.1038/sj.embor.7400132

Pfeiffer T, Morley A (2014) An evolutionary perspective on the Crabtree effect. Front Mol Biosci 1:17. https://doi.org/10.3389/fmolb.2014.00017

Pfeiffer T, Schuster S, Bonhoeffer S (2001) Cooperation and competition in the evolution of ATP-producing pathways. Science 292:504–507. https://doi.org/10.1126/science.1058079

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2016) Linear and nonlinear mixed effects models. (R package version). 3:1–89

Pronk JT (2002) Auxotrophic yeast strains in fundamental and applied research. Appl Environ Microbiol 68:2095–2100

R Development Core Team (2016) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reznick D, Bryant MJ Bashey F (2002) r- and K- selection revisted: the role of population regulation in life history evolution. Ecology 83, 1509–1520. (https://doi.org/10.2307/3071970)

Roemer T, Bussey H (1991) Yeast beta-glucan synthesis: KRE6 encodes a predicted type II membrane protein required for glucan synthesis in vivo and for glucan synthase activity in vitro. Proc Natl Acad Sci USA 88:11295–11299

Taylor TB, Mulley G, Dills AH, Alsohim AS, McGuffin LJ, Studholme DJ, Silby MW, Brockhurst MA, Johnson LJ, Jackson RW (2015) Evolution. Evolutionary resurrection of flagellar motility via rewiring of the nitrogen regulation system. Science 347, 1014–1017. https://doi.org/10.1126/science.1259145

van Gulik WM, Heijnen JJ (1995) A metabolic network stoichiometry analysis of microbial growth and product formation. Biotechnol Bioeng 48:681–698. https://doi.org/10.1002/bit.260480617

Venkataram S, Dunn B, Li Y, Agarwala A, Chang J, Ebel ER, Geiler-Samerotte K, Herissant L, Blundell JR, Levy SF et al (2016) Development of a comprehensive genotype-to-fitness map of adaptation-driving mutations in yeast. Cell 166, 1585–1596. https://doi.org/10.1016/j.cell.2016.08.002

Verduyn C, Zomerdijk TPL, van Dijken JP, Scheffers WA (1984) Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. Appl Microbiol Biotechnol 19:181–185. https://doi.org/10.1007/bf00256451

Verduyn C, Stouthamer AH, Scheffers WA, van Dijken JP (1991) A theoretical evaluation of growth yields of yeasts. Antonie Van Leeuwenhoek 59:49–63

Walker JRL (1992) Spectrophotometric determination of enzyme-activity : alcohol-dehydrogenase (Adh). Biochem Educ 20:42–43. https://doi.org/10.1016/0307-4412(92)90021-D