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Resource presentation dictates genetic and phenotypic adaptation in yeast

Neetika Ahlawat[1*], Anjali Mahilkar[1,2] and Supreet Saini[1]

Abstract

Background Environments shape adaptive trajectories of populations, often leading to adaptive parallelism in identical, and divergence in different environments. However, how does the likelihood of these possibilities change with minute changes in the environment remain unclear.

Results In this study, we evolved *Saccharomyces cerevisiae* in environments which differed only in the manner in which the sugar source is presented to the population. In one set of populations, carbon was presented as a mixture of glucose-galactose, and in the other, as melibiose, a glucose-galactose disaccharide. Since the two environments differed in how the two monosaccharides are packaged, we call these environments 'synonymous'. Our results show that even subtle environmental differences can lead to differing phenotypic responses between the two sets of evolved populations. However, despite different adaptive responses, pleiotropic effects of adaptation are largely predictable. We also show that distinct genomic targets of adaptation between the two sets of evolved populations are functionally convergent.

Conclusion This study highlights how subtle environmental differences dictate phenotypic and genetic adaptation of populations. Additionally, these results also suggest the predictive potential of ancestor's fitness in understanding pleiotropic responses. Our work underscores the importance of studying more such environments to understand the generality of adaptive responses in populations.

Keywords Adaptation, 'Synonymous resources', Melibiose, Glucose-galactose, Yeast

Introduction

Environment dictates adaptive trajectories of populations [1–5]. Identical environments tend to drive adaptive parallelism [6–18], while populations evolving in different environments often exhibit adaptive divergence [4, 19–24]. The collateral effects of adaptation, in similar or dissimilar environments, have been reported to be wide-ranging and often predictable [25–27].

However, it remains unclear if adaptive responses of populations evolved in "nearly-identical" environments are predictable or not. For instance, *Escherichia coli* populations evolved in either glycerol or lactate, exhibit phenotypic convergence despite differing in their gene expression profiles [8]. A recent study explicitly asks this question and examines *E. coli*'s adaptation in 'synonymous' environments. In this study, *E. coli* is evolved in environments that differ only in how glucose and galactose are presented– as a mix of the two monomers, lactose, or melibiose [28]. The findings of this study show that while adaptive responses of populations are

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non-identical, pleiotropic effects of adaptation, in a range of non-synonymous carbon environments, are largely predictable.

The above two examples show that adaptation in nearly-identical environments can exhibit qualitatively different signatures of selection. But, how does this variability in adaptation in nearly-identical environments change across the taxa, remains poorly understood. In this study, we address whether the tools of predictability of adaptation reported in the past still hold [28].

To answer this question, we evolve *Saccharomyces cerevisiae* in synonymous environments– a glucose-galactose mix and melibiose [28]. Natural isolates or common laboratory strains of *S. cerevisiae* cannot hydrolyse lactose or melibiose. However, a *S. cerevisiae* strain from several decades ago, carries *MEL1*, an α-galactosidase responsible for extracellular hydrolysis of melibiose into glucose and galactose, and is capable of growth on melibiose as the sole carbon source [29].

After evolution for ~300 generations, we study the adaptive response, genetic basis of adaptation, and pleiotropic effects of evolution in the two 'synonymous' environments. We show that adaptation in nearly-identical environments leads to non-identical phenotypic and genetic changes between the populations. Despite different genomic targets, the two sets of evolved populations are observed to be functionally convergent. Pleiotropic responses of adaptation are largely predictable across a range of non-synonymous (non-identical) carbon environments. Our results provide insights into how even a slight change in the environment can lead to non-predictable adaptive responses, a trend consistent across different taxa.

Materials and methods

Strains used and media composition

We used haploid *S. cerevisiae* (Sc644 *MATa/αMEL1ade1*) [30, 31] to setup selection experiments in the two 'synonymous' environments. Evolution lines were evolved in yeast complete synthetic media (CSM) (composition: yeast nitrogen base, $(NH_4)_2SO_4$, complete amino acid mix) containing either a mixture of glucose and galactose (0.1% each) or melibiose (0.2%).

To perform growth kinetics, we inoculated single colony of evolved populations (300^{th} generation) in YPD (1% yeast extract, 1% peptone and 2% D-glucose) in 25 × 150 mm borosilicate tubes, at 30°C on a rotary shaker at 250 rpm. We transferred 50 µl of the overnight culture to 5 ml fresh glycerol-lactate liquid media (3% glycerol, 2% of 40% lactate, complete amino acid mix and yeast nitrogen base). After 48 h, cells were transferred through two rounds of subculture in 1:100 dilutions in 5 ml glycerol-lactate media, each cycle lasting for about 48 h.

Evolution experiment

For the evolution experiment, Sc644 *MATa* and *MATα* were evolved either in glucose-galactose (0.1% each) or, in melibiose (0.2%) environments. In each sugar environment, there were six independent lines (3 *MATa* and 3 *MATα*) each derived from single ancestor clones of their respective mating types. These lines were diluted 100x in 5 ml yeast CSM and were serially propagated after every 24 h. Evolution experiment was carried out in 25 × 150 mm borosilicate tubes under identical conditions i.e. 30℃ and at 250 rpm. These lines were evolved till ~300 generations and were stored in 50% glycerol solution.

Fitness measurement in home and non-home synonymous environments

We quantified fitness of evolved strains in their home and non-home synonymous environments. We performed growth kinetics for all six evolved lines across the two synonymous environments: (a) glucose-galactose, and (b) melibiose.

To perform growth kinetics, evolved clones were subcultured in glycerol-lactate medium (mentioned above) in order to remove the cellular memory of their evolution environments. After two rounds of subculture, an appropriate volume of glycerol-lactate was inoculated in 10 ml of complete synthetic media containing either glucose-galactose or melibiose such that the starting optical density at 600 nm (OD) of each culture was 0.01. OD was recoded manually using Thermoscientific Multiscan Go plate reader. Growth kinetics was performed at 30℃ and OD was recorded at a time interval of 8 h till 48 h.

Growth rate was measured as described previously [32]. Maximum OD attained in each replicate population was used as a measure of yield. For every single treatment, ODs were measured in triplicates in 96-well plate and the experiment was repeated three independent times.

Fitness measurement in non-synonymous environments

To study pleiotropic effects of adaptation, fitness of evolved clones was measured in a range of carbon resources, which we termed as non-synonymous environments. Growth kinetics was performed following the same protocol and under identical conditions as mentioned above, except that after the glycerol-lactate propagation, cells were inoculated to 10 ml CSM containing different carbon sources (mannose, fructose, glycerol-lactate, sucrose, raffinose) at a concentration of 0.2%.

Whole-genome sequencing

The evolved and ancestor populations were revived from freezer stock on a YPD plate. A single colony was inoculated and grown in 10 mL of liquid YPD for

about 15–17 h. The cell culture was harvested to isolate genomic DNA following the *S. cerevisiae* genomic DNA isolation protocol [33]. DNA concentration was measured immediately after DNA isolation using Nanodrop Spectrophotometer from Eppendorf (basic), and quality was checked by gel electrophoresis.

Genomic samples were sent for paired-end sequencing using Illumina NovaSeq 6000, with an average read-depth of 150 bp.

Based on the quality report of fastq files, sequences were trimmed to retain only high-quality sequences for analysis and low-quality sequences were removed. The adapter trimmed reads were aligned to the respective reference genome, S288C, using Burrows Wheeler Aligner (BWA). Each sample had a minimum coverage of more than 30x. Variant calling was done for the samples using GATK and further annotated using SnpEff. Variants that were present in the ancestor strain were filtered out manually. After that, remaining SNPs were used for further analysis. We used '*UniprotKB*' to determine the biological function of genes with mutations in the two sets of evolved populations.

Raw sequencing data is available at https://www.ncbi.nlm.nih.gov/sra/PRJNA1150400.

Statistical analysis

Pairwise comparisons between the mean values of the evolved populations were done using one-tailed/two-tailed t-test. For all the analysis, significance level was set to 0.05.

Relative changes in the growth rate and yield of the two sets of evolved populations were compared across home versus non-home environments, using pearson correlation.

Area under the curve was estimated using 'Growthcurver' package in R [68].

Results

Adaptation in nearly-identical environments is non-identical

We evolved six replicate populations of yeast in each of the two synonymous environments - a mixture of glucose-galactose, and melibiose. After ~300 generations of evolution, we quantify growth rate (r) and yield (K) as measures of fitness in the evolved populations in their home environments.

We observe an increase in the fitness parameters (r and K) in all evolved lines relative to the ancestor (Fig. 1A). Populations evolved in glucose-galactose mix, on average, exhibit higher relative increase in yield, compared to the populations evolved in melibiose (*one-tailed t-test*, $pvalue = 0.0094$) (Fig. 1A). On the other hand, populations evolved in melibiose exhibit a higher relative increase in growth rate, compared to the lines evolved in glucose-galactose (*one-tailed t-test*, $pvalue = 0.0015$). These results demonstrate that a subtle change in the environment can change targets of selection. This was further confirmed by estimating the relative change in the area under the logistic growth curve, which was significantly higher for melibiose evolved populations (Figure S1), suggesting different targets of phenotypic selection in the two sets of populations.

Next, we determine variability within replicate lines, by calculating the range of relative fitness gains as the difference between the maximum and minimum increases in the two parameters, r and K, in home environments. The six melibiose-evolved populations exhibit a wider range of these two fitness parameters as compared to glucose-galactose evolved populations (Fig. 1B). Greater phenotypic variability within melibiose-evolved populations suggests that genetic targets of evolution are more diverse in melibiose, relative to that in glucose-galactose.

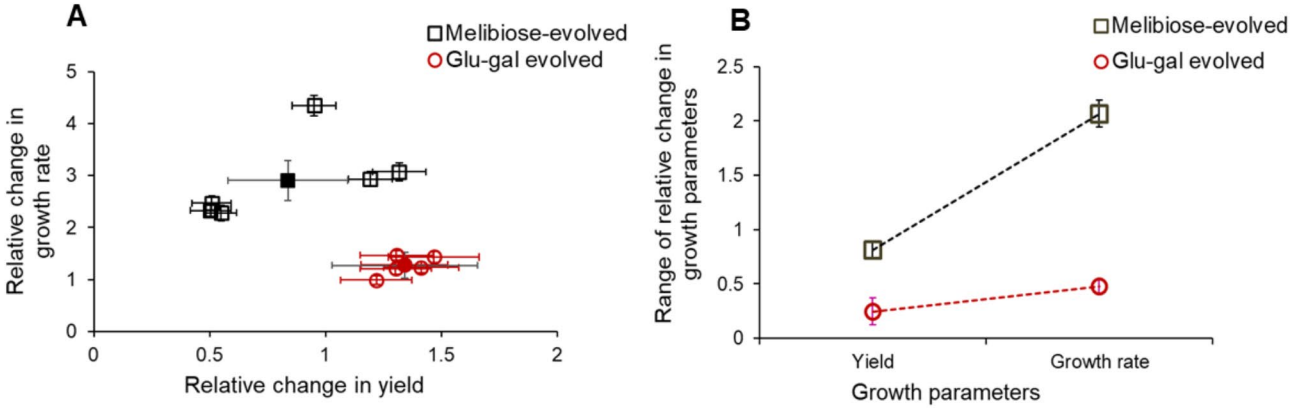

Fig. 1 Populations exhibit different adaptive response in nearly-identical environments. (**A**) Relative change in growth rate (***r***) and yield (***K***) in melibiose and glucose-galactose evolved populations is calculated with respect to the ancestor using, $\frac{Fitness_{evolved} - Fitness_{ancestor}}{Fitness_{ancestor}}$. Filled symbols show the average relative fitness of the two sets of populations. Error bars represent standard deviation (SD) ± 1.96. (**B**) Range of fitness change within two sets of evolved populations is measured as the difference between maximum and minimum relative fitness across the replicate lines

Pleiotropic effects in non-home synonymous environment

We quantify the fitness of populations in non-home synonymous environment by measuring the relative change in r and K of the two sets of evolved populations, with respect to the ancestor. Adaptation in the home environment leads to an increase in fitness in the non-home synonymous environments (Fig. 2A and B).

Even though the melibiose-evolved populations showed a greater increase in growth rate than the glucose-galactose evolved ones (Fig. 1A), the average gains in growth rate in non-home environment was larger for the latter *(two-tailed t-test, melibiose-evolvedp value = 0.0006, glucose-galactose evolvedpvalue = 0.094)* (Fig. 2A). Vice-versa, greater increase in biomass in glucose-galactose evolved populations (Fig. 1A) shows smaller gains in biomass in non-home environment, relative to melibiose-evolved populations *(two-tailed t-test, pvalue for melibiose-evolved populations = 0.121, pvalue for glucose-galactose evolved populations = 0.0006)* (Fig. 2B).

Next, we compare the variability in the fitness parameters (relative growth and relative yield) in home versus non-home environments (Fig. 2C and D). The variability in melibiose-evolved populations decreases in non-home environments, while that of glucose-galactose evolved populations increases, for both the fitness measures under consideration.

Pleiotropic effects in non-synonymous non-home environments

We assess fitness of the two sets of evolved populations in five non-synonymous sugar environments– (a) and (b) hexose sugars (monosaccharide): mannose and fructose, (c) three carbon sugar: glycerol-lactate (non-repressing sugar), (d) complex sugar (trisaccharide): raffinose, and (e) a disaccharide: sucrose. Specifically, we measure relative change in growth rate (r) and yield (K) of the two sets of populations in non-synonymous environments, with respect to the ancestor.

We compare the relative change in fitness of evolved populations in non-synonymous environments with that in home environments. We do not observe any correlated change between the relative change in growth rate in non-synonymous environments with that in their home environments *(melibiose-evolved, correlation coefficient*

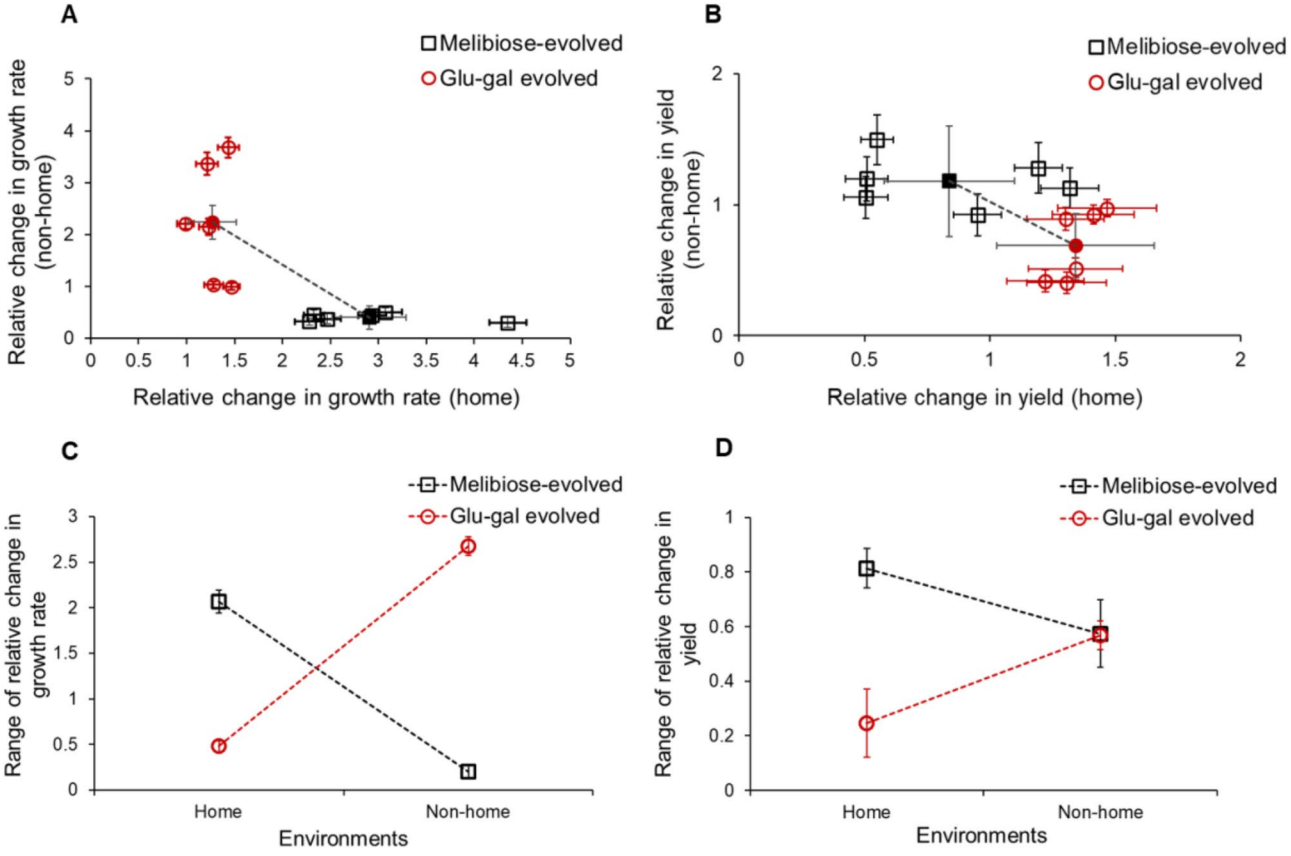

Fig. 2 Pleiotropic responses of the two sets of populations in synonymous non-home environments. (**A**) and (**B**) fitness gains in home versus non-home synonymous environments. Filled symbols represent average gains in the relative fitness. Dotted line connecting average fitness values show the trend in fitness gains between melibiose and glucose-galactose evolved populations. (**C**) and (**D**) range of relative change in growth rate and yield across home versus non-home environments

(r) = 0.16, pvalue = 0.39; glucose-galactose evolved, correlation coefficient (r)=-0.047, pvalue = 0.8) (Fig. 3A). On the other hand, relative change in the yield in non-synonymous environments shows a negative correlation with the relative yield in home environments (melibiose evolved *r*=-0.65, *pvalue = < 0.0001; glu-gal evolvedr*=-0.39, *pvalue = 0.033)* (Fig. 3B).

Next, we compare the gains in the relative change in growth rate and yield between melibiose and glucose-galactose evolved populations across all five non-synonymous environments. Although the two sets of evolved populations show positive pleiotropy, the analysis of variance results show a significant difference between the relative change in fitness of the two sets of populations, across non-synonymous environments (*for relative growth rate, F value = 10.49, pvalue = 0.002; for relative yield, F value = 5.72, pvalue = 0.02*) (Fig. 3C and D). Melibiose-evolved populations exhibit higher gains in the relative growth rate and yield across all five non-synonymous environments, as compared to glucose-galactose evolved populations (Fig. 3C and D). Pairwise comparisons between the two sets of populations show a significant difference between their relative growth rates across all five non-synonymous environments (Table S1). However, the difference between the relative yield of the melibiose and glucose-galactose evolved populations significantly differs only in the fructose and mannose environments (Table S1, Fig. 3D).

Even though the magnitude of relative change in the fitness is different, the two evolved populations exhibit similar pattern of fitness changes across non-synonymous environments (Fig. 3C and D). However, relative change in fitness of the two evolved populations is unpredictable in non-home synonymous environments (Fig. 3C and D).

Our results suggest that while adaptive response of populations in nearly-identical environments is unpredictable, pleiotropic responses can largely be predictable.

Pleiotropic responses of populations can be predicted depending on ancestor's fitness

We determine whether the relative change in fitness parameters (r and K) of the evolved populations across

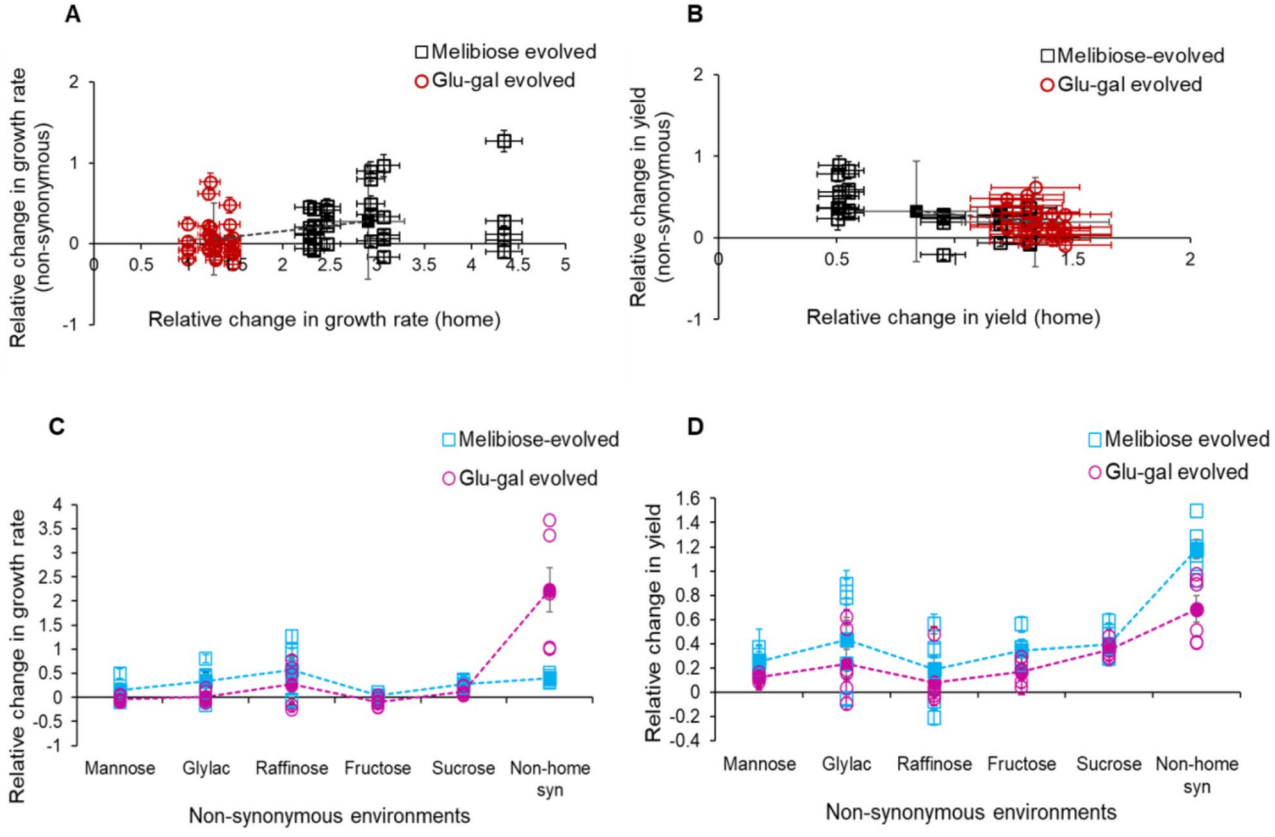

Fig. 3 Pleiotropic responses of the two sets of populations in non-synonymous environments. **(A)** and **(B)** fitness gains in melibiose and glucose-galactose evolved populations in their home versus non-synonymous environments. Filled symbols represent average gains in the relative fitness. Dotted line connecting average fitness values show the trend in fitness gains between melibiose and glucose-galactose evolved populations. **(C)** and **(D)** fitness gains in the relative growth rate and yield in the two sets of evolved populations across all five non-synonymous environments and in non-home synonymous environment. Mean values are represented with error bars indicating standard deviation

two categories of non-home environments (synonymous and non-synonymous), correlate with that of the ancestor's fitness.

In the two synonymous environments, relative change in growth rate and yield of the twelve evolved populations (melibiose evolved and glucose-galactose evolved) correlate negatively with the fitness of the ancestor (*growth rate r=-0.78, p = 0.0026*; yield *r*=-0.75,*p = 0.0048*) (Fig. 4A and B). In case of non-synonymous environments, relative growth rate correlates negatively, whereas the relative yield correlates positively with the fitness of ancestor (growth rate *r=-0.48, p = 0.0001*; yield *r = 0.26, p = 0.041*) (Fig. 4C and D). Evolved populations show smaller increment in the relative change in fitness in environments better suited to the ancestor (Fig. 4A and B 4C). However, we do not observe any such correlation between ancestor's yield and the relative change in yield of evolved populations, across non-synonymous environments (Fig. 4D).

These results suggest that ancestor's fitness can act as a predictor to quantitatively understand pleiotropic responses of adaptation.

Distinct genomic targets of adaptation in glucose-galactose and melibiose-evolved populations

To understand the genetic basis of adaptation in nearly-identical environments, we sequenced the complete genomes of single evolved clones from each population. While sequencing of a single clone may not provide an understanding of population-level genetic diversity, our primary focus is to compare genomic targets of mutations shared between the two sets of evolved populations.

We observe a total of 49 mutations, spread over 35 genes, in the two sets of populations (Fig. 5a Table S2). Populations evolved in glucose-galactose had mutations in 18 genes, and those evolved in melibiose had mutations in 19 genes, indicating a nearly identical range of genetic targets of selection in either environment. Of the total number of mutations, we observe 11 single nucleotide variants in the coding regions of glucose-galactose evolved and 12 in melibiose evolved populations. 4 single nucleotide variants are identified in the upstream regions of genes targeted in glucose-galactose evolved and 3 in melibiose evolved populations. We also observe 3 indels in glucose-galactose evolved populations and 5 in melibiose evolved populations.

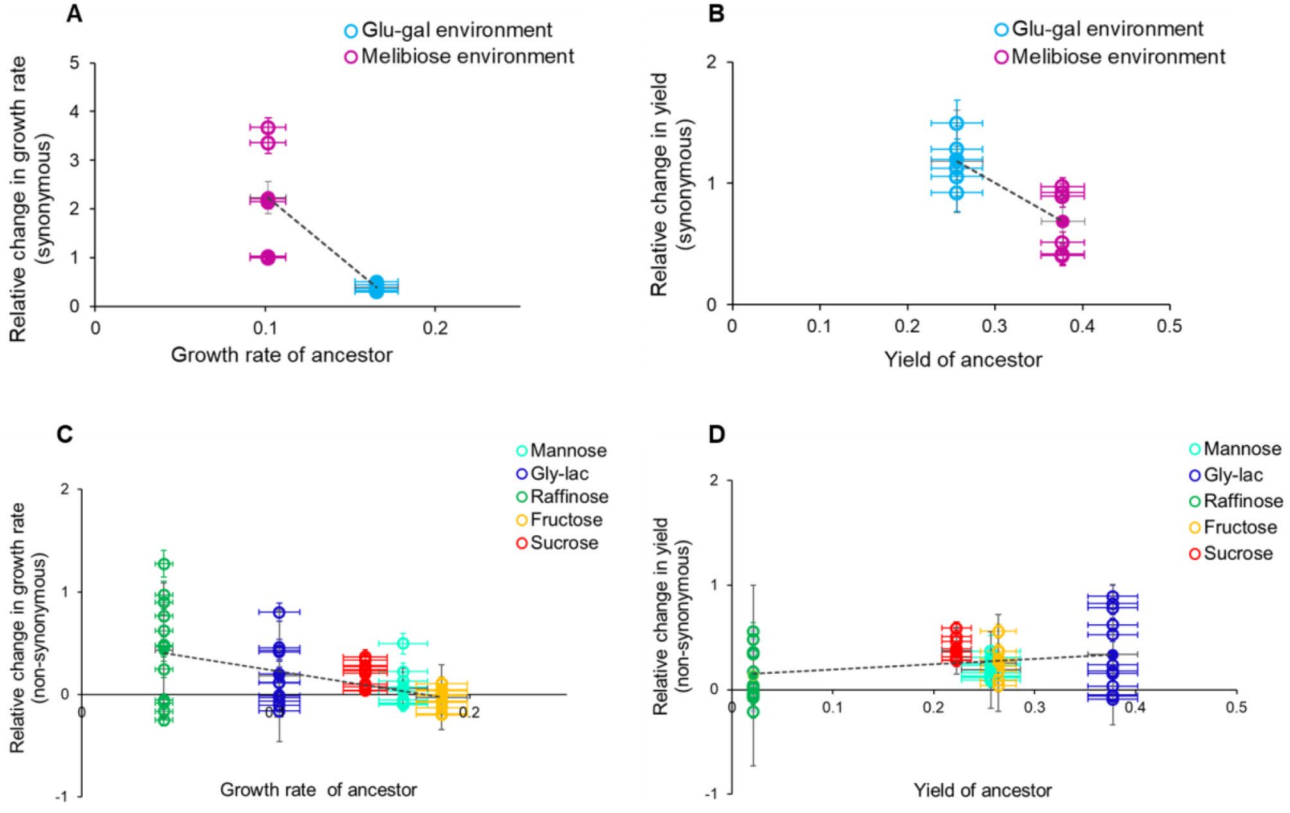

Fig. 4 Predictability of pleiotropic responses in evolved populations based on ancestor's fitness across synonymous and non-synonymous environments. **A**, **B**, **C**, and **D** show the comparison of relative changes in growth rate and yield between evolved populations and ancestor, across both synonymous (non-home) and non-synonymous environments. Pleiotropic responses were predicted by measuring the fitness gains in twelve populations (evolved in melibiose and glucose-galactose) relative to the ancestor's fitness in different environments

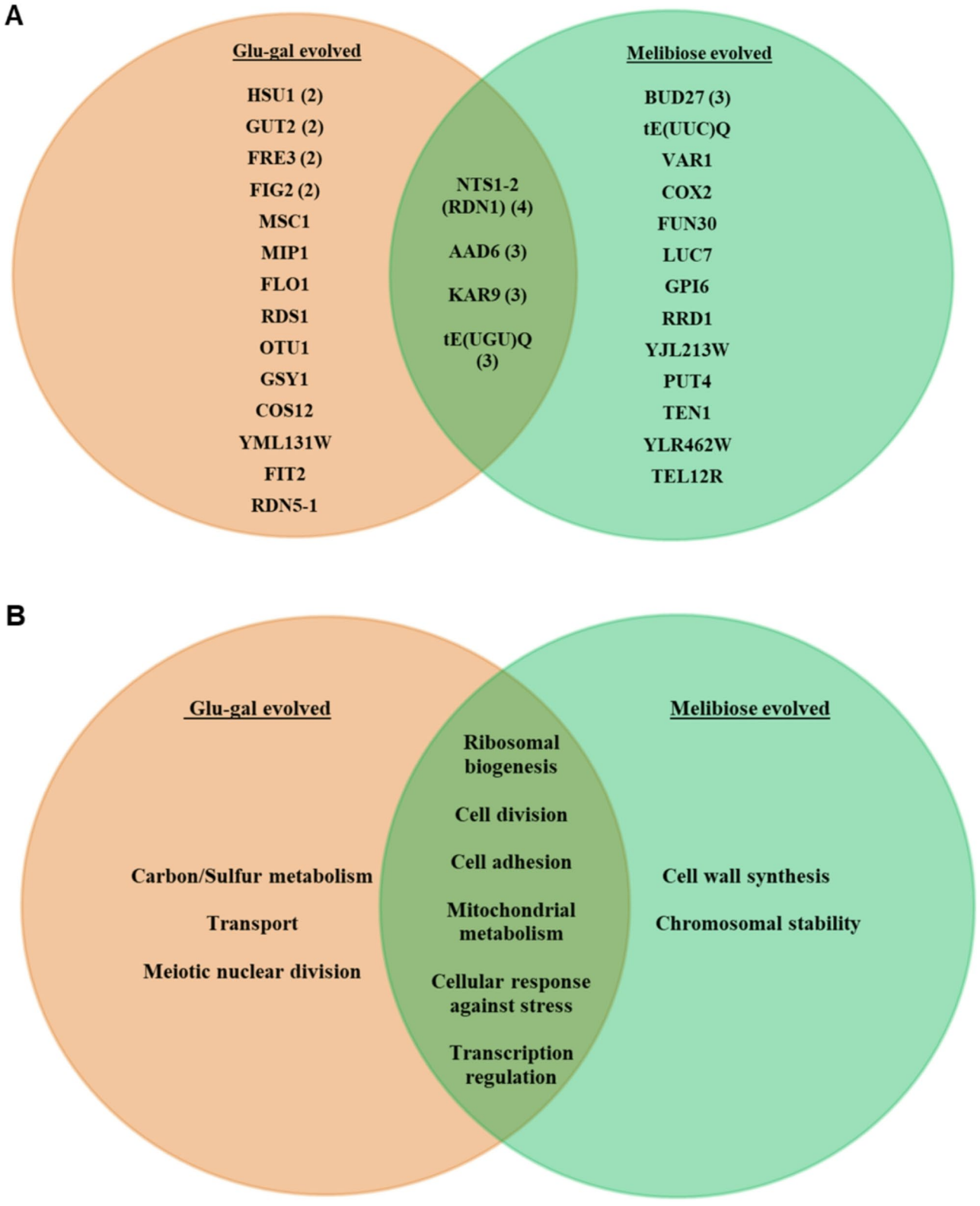

Fig. 5 Genomic targets of adaptation. Venn diagram showing **(a)** distinct targets of adaptation, and **(b)** biological function of targeted genes within and between glucose-galactose and melibiose evolved populations. Overlapping regions show shared **(a)** mutational targets, and **(b)** molecular processes targeted between the two sets of evolved populations. Numbers in parentheses represent number of replicate populations that share mutations in the same gene

Glucose-galactose evolved and melibiose evolved populations share 4 genomic targets each within their replicate populations (Fig. 5a, Table S2). We also observe that only 4 genes (Non-transcribed regions of *RDN1*, *AAD6* and *KAR9*, *tE(UUC)Q1*) were the common targets between the two sets of populations (Fig. 5a).

On comparing the biological implications of different genomic targets, we observe a convergence in the functions of genes targeted between the populations evolved in glucose-galactose or melibiose environments (Fig. 5b, Table S2). This includes ribosomal biogenesis (*RDN1*, *RRB1*, *BUD27*), cellular response against stress (*AAD6*), transcription regulations (*RDS1*, *OTU1*, *RRD1*, *TEN1*), mitochondrial metabolism (*MIP1*, *COX1*, *tE(UUC)Q*, *tT(UGU)Q1*), cell adhesion (Fig. 2, *FLO1*, *GPI6*) and cell division (*KAR9*) (Fig. 5b, Table S2).

Among all 12 replicate populations, six show mutations in genes involved in ribosomal biogenesis which might have adaptive benefits in the populations evolved in the two nearly-identical carbon-limiting environments [34] (Table S2). We also observe changes in the cytoplasmic element i.e. mitochondrial genome across four replicate populations which might confer adaptive advantage and has previously been reported in yeast populations evolved in galactose environments [35]. Some processes are unique to a particular environment. For instance, mutations in genes involved in glycerol and carbohydrate metabolism are shared within the replicate populations evolved in glucose-galactose environment, suggesting environment-specific adaptive response (Table S2).

These observations clearly demonstrate that (a) despite non-identical phenotypic responses, biological functions of genomic targets are more or less the same between the populations evolved in nearly-identical environments, and (b) there seems to be no correlation between genetic and phenotypic variability.

Functional convergence in different genes targeted between the two sets of populations suggests that selection acting on populations in nearly-identical environments might favour similar processes for adaptation. This might also suggest predictability of adaptation at the level of molecular processes, despite differing phenotypic and genomic responses.

Discussion

Adaptation in identical environments has been studied in the past [6–18, 36], and both parallelism and divergence have been observed depending on the evolutionary context. But how does the likelihood of these possibilities change with minute changes in the environment? How does this likelihood itself change with increasing complexity in the organism? We address these questions by evolving yeast populations in two nearly identical environments (glucose-galactose mix, and melibiose) that have been previously reported as 'synonymous' sugar environments in an adaptive laboratory study conducted using *E. coli* [28].

Our results, in concordance with the previous study using *E. coli* [28], show non-identical adaptive responses in populations that evolved in 'synonymous' environments. However, we observe that the targets of selection differ with the change in the organism - the biomass yield of melibiose-evolved yeast cells exhibit lesser fitness gains than their growth rate, unlike the melibiose-evolved *E. coli* cells where fitness gains were higher in biomass yield than in growth rate. *r-K* trade-offs have been reported in the past [37–41], but the findings of this work show that evolution in a given environment leads to different trade-off patterns, that differs between the organisms. In addition, we also show that in *S. cerevisiae*, adaptation in an environment can be predicted based on ancestor's fitness.

At the molecular level, we see that the mutational targets are different for the two organisms evolved in the same environments. While *E. coli* internalizes melibiose, *S. cerevisiae* breaks it down into glucose and galactose outside the cell, and subsequently imports the monosaccharides. Hence, in melibiose and glucose-galactose environments, importing monosaccharides is vital for the yeast cells, especially because of the involvement of different hexose transporters (*HXT* family) [42, 43] for glucose uptake in the cell.

Our expectation based on this mechanism of sugar utilization was that the genomic targets of selection would be largely shared between the glucose-galactose and melibiose-evolved populations. To test this, we compared genomic targets in single clones from each evolved populations. While this approach may not capture allele frequencies and genetic diversity in a population, and the potential role of clonal interference in adaptation [44, 45], our focus is to compare mutations that are either fixed or higher in frequency. Through genomic analysis, we observed that only four genes were the common mutational targets between the two sets of evolved populations.

Although the genomic targets of selection are different, they are found to be functionally convergent. Furthermore, rarely any mutation was found to be involved in glucose and galactose utilisation pathways [46, 47]. This is not surprising, as adaptive mutations in yeast populations evolved in either glucose or galactose environments have previously been reported to occur outside the canonical pathways of these genes [35, 48, 49]. This suggests that selection in carbon-limiting environments might act on broader cellular processes rather than solely on the pathways directly involved in carbon metabolism [50].

Correlating the exact genotype with phenotype is challenging. As a result, predicting effects of adaptation based on macroscopic traits like fitness, has been a recent focus [20, 36, 51–54]. In this context, our study shows that the ancestor's fitness serves as a predictor of pleiotropic effects of adaptation in non-home non-synonymous sugar environments. It remains to be tested if this rule of predictability holds in other environments as well.

Overall, this work provides insights into the effects of minute environmental changes on adaptation and pleiotropic effects in eukaryotic asexual populations of yeast. However, the role of ploidy, which is an important factor in dictating evolutionary trajectories [55–58], has not been explored in this study. Also, most eukaryotes are sexually reproducing species, and we do not yet know how the effects of evolution in synonymous environments change if the evolving population reproduced sexually.

In yeast, hydrolysis of some complex sugars such as, melibiose, sucrose, raffinose, involves production of public-goods [30, 59, 60]. Like melibiose, these complex sugars are hydrolysed into monosaccharides in the extracellular environment, which are internalised in the cell. However, it remains unclear whether the 'synonymous' sugar combinations such as, raffinose and a mixture of glucose-galactose-fructose; sucrose and a mixture of glucose-fructose, elicit identical adaptive responses. Studying evolution in these synonymous environments would offer insights into how the presentation of resources influences the processes of diversification [61], niche specialization [30, 62–64], and sympatric speciation [65–67].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-025-02361-3.

Supplementary Material 1

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Author contributions
NA performed experiments, designed experiments, analyzed data, and wrote the manuscript. AM performed and designed experiments. SS conceived the study, designed experiments, and wrote the manuscript.

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Data availability
Genomic sequencing data is available at https://www.ncbi.nlm.nih.gov/sra/PRJNA1150400.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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