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Effects of resource packaging on the adaptative and pleiotropic consequences of evolution

Neetika Ahlawat^{1,3}, Pavithra Venkataraman^{1,3}✉, Raman Gulab Brajesh^{1,2} & Supreet Saini¹✉

Adaptation to an environment is enabled by the accumulation of beneficial mutations. How do adaptive trajectories and pleiotropic effects of adaptation change in response to “subtle” changes in the environment? Since there exists no molecular framework to quantify “subtle” environmental change, designing experiments to answer this question has been challenging. In this work, we address this question by studying the effects of evolution in environments which differ solely in the way sugars are presented to a bacterial population. Specifically, we focus on glucose and galactose, which can be supplied to an *E. coli* population as a mixture of glucose and galactose, lactose, or melibiose. We evolve six replicate populations of *E. coli* for 300 generations in these three chemically correlated or “synonymous” environments, and show that the adaptive responses of these populations are not similar. When tested for pleiotropic effects of fitness in a range of non-synonymous environments, our results show that despite uncorrelated adaptive changes, the nature of pleiotropic effects is largely predictable based on the fitness of the ancestor in the non-home environments. Overall, our results highlight how subtle changes in the environment can alter adaptation, but despite sequence-level variations, pleiotropy is qualitatively predictable.

Background

An evolving population accumulates mutations, and natural selection increases the frequency of beneficial ones¹. The environment in which evolution occurs can influence both the mutation rate and the types of mutations that become fixed². The effects of mutations depend not only on the environment but also on the genetic background in which they arise^{3,4}. While similar environments often lead to correlated adaptive responses, there are notable exceptions⁵. In fact, several studies suggest that adaptation in similar environments is, to some extent, predictable^{6–8}. However, designing experimental systems where environmental similarity can be precisely quantified to understand adaptive responses remains challenging.

One approach to measuring environmental similarity is by examining differences in the adaptive responses they induce, under the assumption that similar environments exert similar selection pressures⁹. Phenotypic parallelism may reflect such environmental similarity, though it is important to recognize that similar phenotypic outcomes can arise from different genetic changes. This makes it difficult to predict pleiotropic effects in other environments¹⁰.

Another approach is to compare the pleiotropic outcomes of adaptation itself. For example, if populations evolved in environments *A* and *B* show similar performance in a third environment *C*, this may suggest that environments *A* and *B* are similar^{9,11,12}. A number of laboratory studies have explored the dynamics and variability of pleiotropic effects of adaptation^{9,11,13–22}. While generalist evolution is common, the emergence of specialists has also been observed, highlighting that fitness effects in non-home environments can vary widely.

Both methods for inferring environmental similarity—via adaptive response or pleiotropic performance—have limitations. They often rely on fitness data from laboratory experiments that suffer from a form of circularity: similarity in adaptive effects does not always imply similar pleiotropic outcomes, and vice versa.

To address these challenges, we developed an experimental system in which environmental similarity is defined chemically and metabolically²³, rather than inferred from adaptive or pleiotropic outcomes. We focused on three carbon environments that differ only in how glucose and galactose are presented to *E. coli* populations: (a)

¹Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India. ²Present address: Department of Biomedical Engineering and Bioinformatics, Swami Vivekanand Technical University, Durg, India. ³These authors contributed equally: Neetika Ahlawat, Pavithra Venkataraman.

✉ e-mail: pavithrav@iitb.ac.in; saini@che.iitb.ac.in

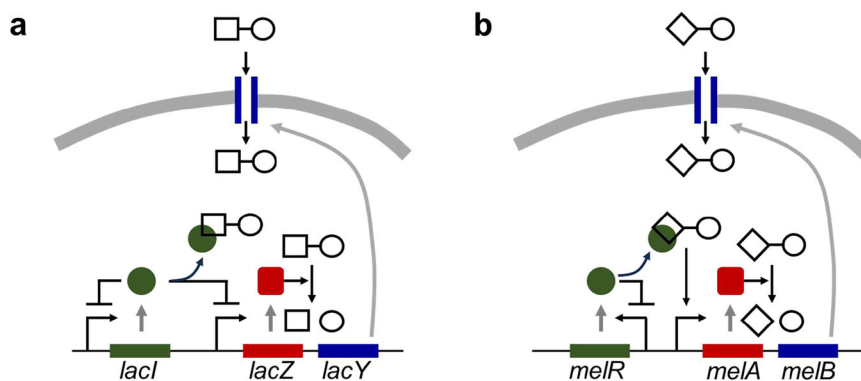


Fig. 1 | Regulatory networks for lactose and melibiose utilization in *Escherichia coli*. **a** The lac operon includes *lacZ* (β -galactosidase) and *lacY* (lactose permease), whose expression is repressed by the LacI repressor protein in the absence of lactose. When lactose (white square linked with white circle) is present, it binds LacI, relieving repression and enabling transcription from the *lacZY* promoter. *lacY*-encoded permease facilitates lactose import, creating a positive feedback loop. Hydrolysis of lactose by LacZ releases glucose (white square) and

galactose (white circle). LacI is also an autorepressor. **b** The mel operon includes *melA* (α -galactosidase) and *melB* (melibiose transporter), regulated by the activator MelR. In the presence of melibiose (rotated white square linked with white circle), MelR activates transcription of *melA* and *melB*, with MelB-mediated import enhancing inducer availability, forming a similar positive feedback loop as in the case of lactose. MelR is an auto-repressor. Hydrolysis of melibiose by MelB releases glucose (white square) and galactose (white circle).

a mixture of glucose and galactose, (b) lactose, and (c) melibiose. The disaccharides lactose and melibiose are formed by linking glucose and galactose via β -1,4-glycosidic and α -1,6-glucosidic bonds, respectively. As shown in Fig. 1, *E. coli* processes both sugars through similar pathways—import via permeases followed by hydrolysis into their monosaccharide components^{24,25}. Due to their shared chemical structure and metabolic processing, we refer to these environments as “synonymous.” This chemically grounded classification allows us to examine how subtle environmental differences impact evolutionary outcomes without relying on circular, outcome-based definitions of similarity.

Using this system, we evolved six replicate populations in each of the three synonymous (or, chemically correlated) environments for 300 generations. We then measured their fitness across both the synonymous environments and a panel of non-synonymous sugar environments using short-term growth assays. Despite the biochemical similarity of the environments, the adaptive responses of the populations diverged significantly, indicating that even minimal environmental differences can lead to distinct evolutionary trajectories. However, when we examined pleiotropic effects in non-home environments, a different pattern emerged: pleiotropic responses were more consistent and, in most cases, predictable. Moreover, we found a correlation between the number of distinct mutational targets and the proportion of unique pleiotropic outcomes, suggesting a mechanistic link between genetic divergence and pleiotropic variability.

While our study, like many others, relies on temporally limited fitness assays, it offers a framework to chemically define environmental similarity independent of evolutionary outcomes. By decoupling the definition of environment from the consequences of adaptation, our work provides a more rigorous foundation for studying how environmental differences shape evolutionary dynamics, and highlights the complex, sometimes decoupled relationship between adaptation and pleiotropy.

Results

Adaptation in synonymous environments yields distinct outcomes

Replicate populations of *E. coli* were evolved in M9 minimal media containing either 0.2% of glucose-galactose (0.1% of each), lactose, or melibiose. After 300 generations, we measured two fitness parameters – growth rate in the exponential phase and yield when bacterial cells enter stationary phase.

As shown in Fig. 2, either the growth rate (r), yield (K), or both, increased when compared to the ancestor ($p < 0.00833$, one-tailed t -test, Bonferroni correction applied for six comparisons) in the eighteen

populations after evolution in one of the three environments for 300 generations (Supplementary Table 1). Upon comparing the average r and K of the three sets of evolved populations with the ancestor, we see that evolution in melibiose results in a significant increase in yield ($p < 0.001$, one-tailed t -test), but not in growth rate ($p > 0.05$, one-tailed t -test). On the other hand, evolution in lactose and glucose-galactose resulted in an increase in both average growth rate ($p < 0.001$, one-tailed t -test), and yield ($p < 0.001$, one-tailed t -test). These findings show that the selection pressure imposed by chemically correlated environments in identical evolution experiments is dissimilar, and as a result, adaptive responses are divergent.

Populations with an increased growth rate (r) are fast growers, while populations with an increased yield (K) are considered better competitors²⁶. While intuition suggests that experimentally evolved populations should exhibit r - K trade-offs, there exists very little empirical evidence in support. Marshall et. al. showed theoretically how and why r and K may covary positively²⁷, and Wei et. al. show that reduction in environment quality leads to such “trade-ups” in r and K ²⁸. In our study, although trade-ups in r and K are common in the evolved populations, the average growth rate of melibiose-evolved populations does not increase compared to the ancestor, indicating that the likelihood of occurrences of trade-ups is dependent on the exact chemical nature of the evolution environment.

Pleiotropic effects of adaptation in synonymous environments

To further understand the pleiotropic fitness effects, we shift these three sets of evolved populations to non-home environments, that contain synonymous sugars as the source of carbon. We calculate the growth r , and K at the end of 16 h, assuming exponential growth. Relative fitness changes [$\frac{f_{\text{evolved}} - f_{\text{ancestor}}}{f_{\text{ancestor}}}$, where f is r , or K] of twelve populations, relative to the ancestor, are calculated in home and away environments, and these values are distributed as shown in Fig. 3a, b.

Next, the fitness gains for each population relative to the ancestor, in home and non-home environments were compared, using one-tailed t -tests (p values are shown in Supplementary Table 2). Each such comparison between relative fitness change in non-home and home environments can be classified into one of the following three categories: (a) identical – the magnitude and sign of fitness effect are statistically identical, (b) magnitude change – the magnitudes of fitness effects are statistically non-identical, and (c) sign change – the magnitude and sign of fitness effects are different in home and non-home environments. Figure 3c, d shows the proportion of these cases (out of 12), for r and K , for the three sets of evolved populations, when grown in non-home synonymous environments.

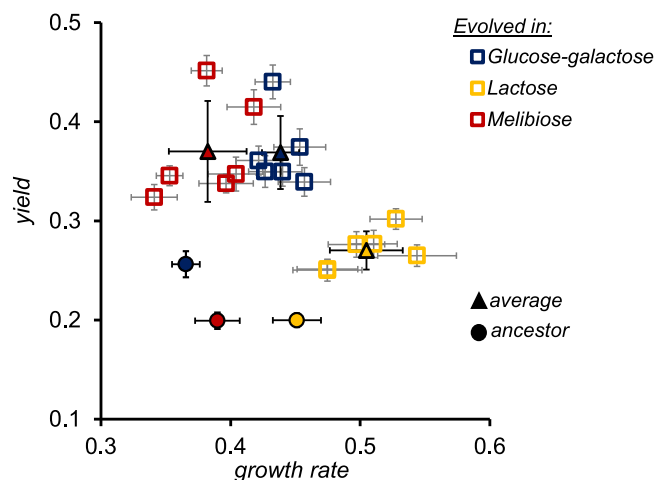


Fig. 2 | Selection is divergent in synonymous environments. Six replicate populations of *E. coli*, founded from a single clone, were evolved in three different, yet synonymous sugar environments – glucose-galactose mixture, lactose, and melibiose. After 300 generations of evolution, growth assays were performed in triplicate for each of the evolved populations and the ancestor to measure growth rate (r) and yield (K). Triangular data points show r and K for each of the replicate populations, and the error bars correspond to the standard deviations obtained from technical replicates. The square blocks show the average r and K for the evolved populations in each of the environments (obtained as the average of the mean of the six replicates), and the error bars correspond to the standard deviation (obtained as the standard deviation of the mean of the six replicates). The circular blocks show the ancestor's average r and K in each of the evolution environments, and the error bars correspond to the standard deviation obtained from technical replicated. For all the eighteen evolved populations, changes in r and K depended on the exact composition on the evolution environment. Clearly, selection acting on a population changes even with a minor change in the environment.

In the lactose-evolved populations, fitness changes in the away environments were identical to the changes in the home environment. Correlated fitness gains are the most common in the lactose-evolved populations, followed by the glucose-galactose-evolved populations. The melibiose-evolved populations, showed the most diverse range of pleiotropic responses.

By and large, our results agree with the observations made by Ostrowski et al. that adaptation in a sugar environment confers positive pleiotropic effects in other sugars¹⁸. However, despite being shifted to synonymous environments, our evolved populations show considerable variability in their pleiotropic responses. What happens to variability in pleiotropic responses in non-synonymous sugars?

Pleiotropic effects of adaptation in non-synonymous environments

The three sets of populations which evolved independently in melibiose, lactose, and glucose-galactose were shifted to non-synonymous sugar environments and tested for fitness. These non-synonymous environments consisted of pentoses (arabinose, xylose), a methyl pentose (rhamnose), sugar alcohols (glycerol, sorbitol), or hexoses (fructose, mannose) as the source of carbon. Again, we measured r in exponential phase and K as cells enter stationary phase. Fitness changes of the eighteen evolved populations, relative to that of the ancestor, were calculated in home and non-home environments, and are as shown in Fig. 4a, b (See Supplementary Fig. 1 for exact fitness changes in each environment). A one-tailed t-test was used to compare the fitness changes between home and non-home environments (p -values are shown in Supplementary Table 2).

We found no environment in which the fitness of all the evolved populations dropped compared to the ancestor. Arabinose and sorbitol were two environments in which both r and K of all the evolved populations increased relative to the ancestor. Therefore, all the populations that evolved in one of the synonymous environments were fit in arabinose and sorbitol. However, we did not find any set of evolved populations in which all the

replicates performed identically in home and any non-home environments. As a result, given a non-home environment, we could only say if an evolved population is fitter than the ancestor or not; we were unable to comment on the exact difference in fitness based on the fitness of the evolved population in the home environment.

In the seven non-synonymous sources of carbon, lactose evolved populations showed the maximum number of similar fitness changes, followed by the glucose-galactose evolved populations. The melibiose-evolved populations continued to show diverse responses in non-synonymous environments as well, as shown in Fig. 4c, d.

Figure 5 shows the overall proportion of cases (considering both r and K , and synonymous and non-synonymous environments) of pleiotropy for the three sets of evolved populations. It is evident that the variability in the type of pleiotropic responses depends on their evolution environment.

Fitness of the ancestor as a predictor of pleiotropic effects

Global epistasis has enabled the prediction of the fitness effect of a beneficial mutation as a function of the fitness of the background on which it occurs²⁹. We tried to identify if ancestral fitness can help predict pleiotropic effects, as reported in the past¹². Specifically, we checked if the fitness of the ancestor can be used as a predictor of the pleiotropic fitness of an evolved population. Therefore, we compared the fitness changes in the evolved populations with that of the ancestor, in both synonymous and non-synonymous environments.

In synonymous environments, there existed no consistent correlation between ancestral fitness and the nature of pleiotropic responses, as shown in Fig. 6a, b. The three sets of evolved populations showed qualitatively similar pleiotropic responses in the non-synonymous environments (refer to Supplementary Fig. 2 and Supplementary Fig. 3). As shown in Fig. 6c, d, in non-synonymous environments, average gains in r and K correlated negatively with ancestral fitness.

We next check if the variability in pleiotropic responses of these evolved populations is predictable based on ancestor's fitness in the non-home environment. To do so, we consider the variance of fitness gains (in r and K), as a proxy for variability in pleiotropic responses. As shown by the Spearman's rank correlations in Supplementary Table 3, as the fitness of the ancestor increases in the non-home environment, the variability in pleiotropic responses decreases. Therefore, given the fitness of the ancestor in a non-home environment, we show that a qualitative prediction of pleiotropic effects of an evolved population is possible.

Melibiose-evolved populations are the most genetically diverse

We genome sequenced the eighteen evolved populations to identify the genetic changes accumulated in the three synonymous evolution environments. Mutation analyses reveal that mutations in the *rpo* genes were the most common among all sets of evolved populations. *Rpo* genes, that code for proteins that make up RNA polymerase, are known to facilitate adaptation in not only thermal, osmotic, and antibiotic stresses but also to M9 media³⁰. Other mutational targets included *yafW* which is a part of a toxin-antitoxin pair, *nanR* and *acrR*, which are transcription regulators^{31,32}, and the methionine transporter *metN*³³. Overall, the melibiose-evolved populations had the highest number of distinct mutational targets, as shown in Fig. 7a (Supplementary Table 4 lists the mutations in each population). Figure 7b shows the correlation between the proportion of pleiotropic responses and the number of mutational targets.

We counted the number of instances where the three sets of evolved populations showed any type of pleiotropy (change in magnitude and/or sign of fitness effect), across the ten non-home environments (two synonymous, eight non-synonymous) their fitness was measured in. Considering changes in both r and K , we see that pleiotropic effects are the maximum for the melibiose-evolved populations, as shown in Fig. 7b. This proportion of pleiotropic responses, along with that of lactose-evolved and glucose-galactose evolved populations, correlated predictably with the number of distinct genes in which adaptive mutations occurred in replicate populations.

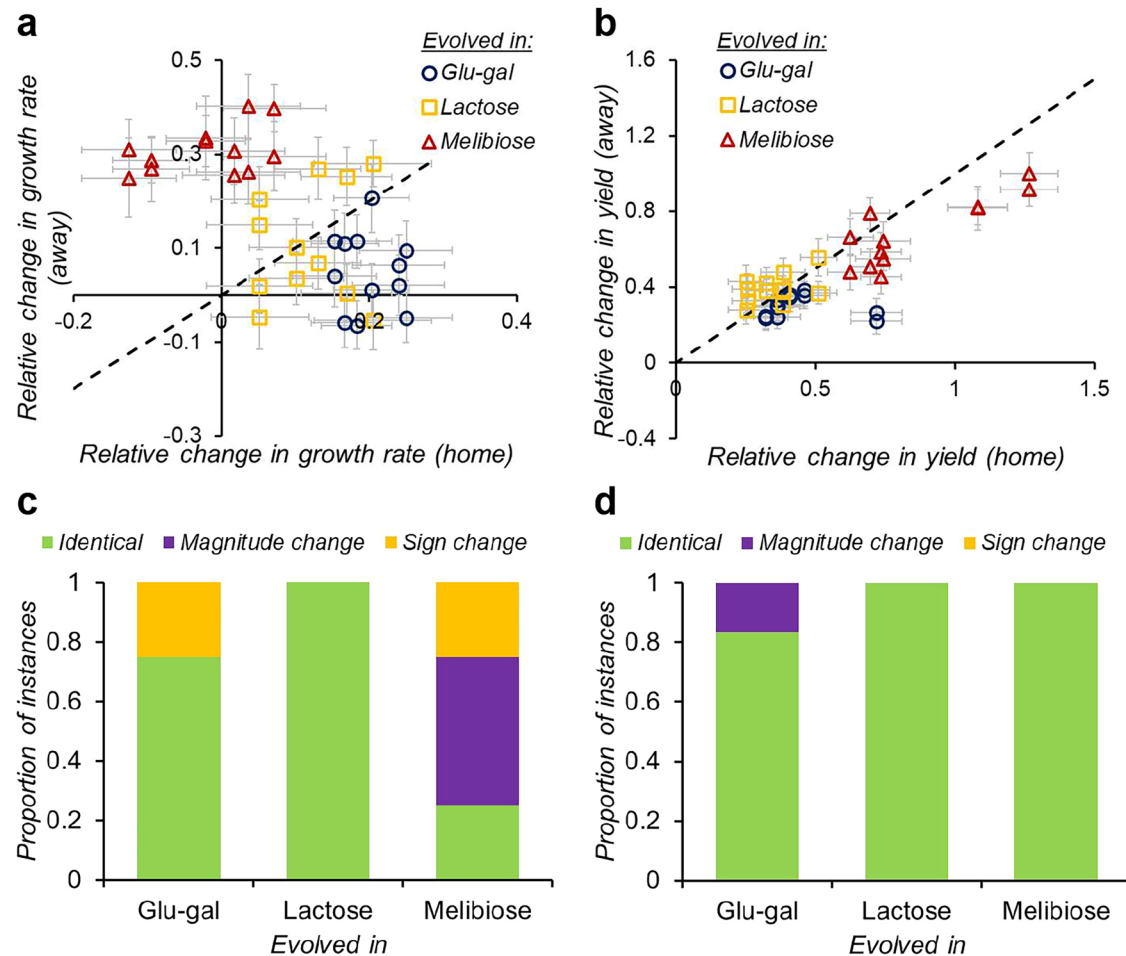


Fig. 3 | Pleiotropic responses of evolved populations in synonymous environments. We tested the fitness (r and K) of the three sets of evolved populations in away environments, in which the source of carbon was a synonymous sugar, and checked if fitness gains in home and away environments were correlated. For each set of evolved population, there were two synonymous away environments in our

experiment. (a, b) show the relative gains in home and away environments, in r and K , respectively. The error bars indicate the propagated uncertainties. We count the number of distinct types of pleiotropic effects. As shown in (c) (growth rate) and (d) (yield), the relative frequencies of the three types of pleiotropic responses are different for different sets of evolved populations.

Discussion

Adaptation in similar environments has often been correlated, offering insights into the predictability of evolution^{34,35}. However, the pleiotropic consequences of adaptation can vary depending on evolutionary timescales^{11,13,15,19,20}. Long-term evolution experiments have shown that prolonged selection in a particular environment can lead to specialization¹¹. Despite this, the role of environment in shaping adaptation and determining whether generalists or specialists evolve remains poorly understood. A central challenge has been the lack of a method to quantify the similarity of environments, making predictions of adaptation and pleiotropy difficult.

In this work, we address that limitation using a novel framework. Instead of relying on phenotypic similarity to infer environmental relatedness—a method confounded by the unpredictability of pleiotropy—we designed environments that are chemically correlated. Specifically, we presented *E. coli* populations with two monosaccharides (glucose and galactose) packaged in three different ways: (a) a mixture of the two, (b) lactose, and (c) melibiose. Lactose and melibiose are disaccharides formed by chemically linking glucose and galactose via β -1,4 and α -1,6 glycosidic bonds, respectively. These “synonymous” sugar environments provide a controlled, chemically quantifiable perturbation.

To probe how such minute chemical differences shape adaptation and pleiotropy, we evolved six replicate *E. coli* populations in each of the three environments for 300 generations—capturing the early onset of adaptation. Notably, all evolution environments supported ancestral growth, avoiding

confounding effects of harsh selection. This setup enabled us to isolate the impact of subtle environmental differences on evolutionary outcomes.

Our results show that evolution in chemically synonymous environments does not lead to correlated adaptive outcomes. Despite similar uptake and hydrolysis mechanisms for lactose and melibiose^{36,37}, the selective pressures they impose differ substantially. In melibiose, selection strongly favored biomass accumulation, sometimes even at a small growth rate cost. In two melibiose-evolved populations, growth rate declined (albeit with low statistical significance)—one of the few empirical demonstrations of an r/K trade-off^{20,28,38–42}. These findings challenge the assumption that cells always prioritize minimizing division time, showing instead that the trait under selection depends on environmental context.

Fluctuating environments are known to drive genetic diversity^{43–45}, and act via global regulators^{46–48}. We show that even stable, chemically similar environments can lead to divergent genotypic and phenotypic outcomes, positioning subtle environmental differences as a driver of microbial diversity.

We hypothesized that evolution in synonymous environments would yield positive pleiotropic effects in each other’s contexts²¹. Fitness assays supported this broadly: positive pleiotropic gains were common across both synonymous and non-synonymous environments. Surprisingly, in some cases, evolved populations performed better in a non-home environment than in their own. For instance, two melibiose-evolved populations had reduced home fitness but increased growth in nearly all other environments.

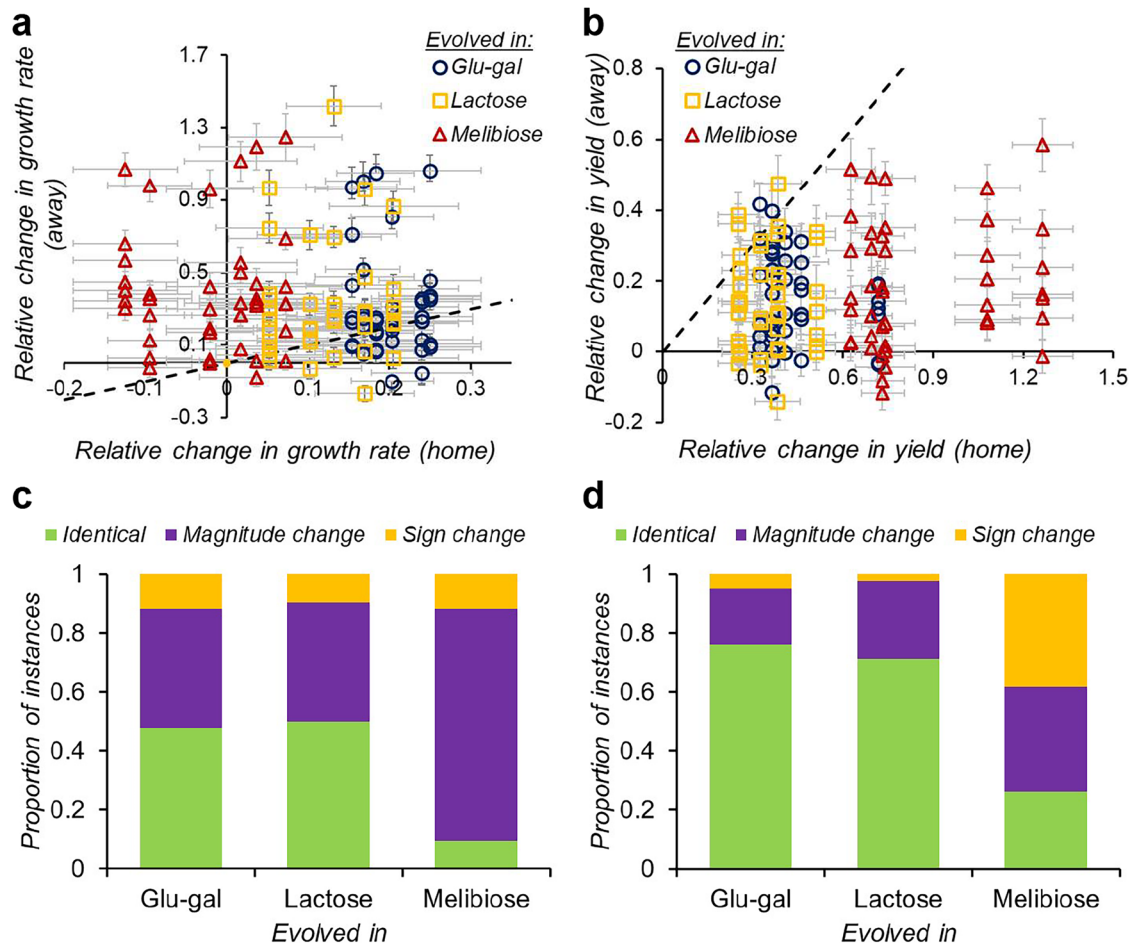


Fig. 4 | Pleiotropic responses of evolved populations in non-synonymous environments. We tested the fitness (r and K) of the three sets of evolved populations in away environments, in which the source of carbon was a non-synonymous sugar, and checked if fitness gains in home and away environments were correlated.

For each set of evolved population, there were eight non-synonymous away environments in our experiment. (a, b) show the relative gains in home and away environments, in r and K , respectively. We count the number of distinct types of pleiotropic effects, and the results are as shown in (c) (growth rate) and (d) (yield).

In fact, populations adapted in environment B sometimes outperformed all A -evolved populations in environment A . This suggests that foreign environments may unlock fitness peaks inaccessible through local adaptation, echoing ideas about epistasis and adaptation roadblocks⁴⁹. While nearly all melibiose-evolved populations gained fitness in non-home environments, the mutations they acquired did not fix in populations evolved in other synonymous environments. This supports the idea that subtle environmental cues can reshape the adaptive landscape, either enabling or blocking specific evolutionary paths.

Interestingly, generalists were more common than specialists even after just 300 generations. To investigate whether ancestral fitness predicts pleiotropic effects, we analyzed correlations. In synonymous environments, no such correlation was found—suggesting early pleiotropic outcomes are not constrained by ancestral fitness. But in non-synonymous environments, a clear pattern emerged: ancestral fitness did predict the qualitative pleiotropic outcome, even amid genetic stochasticity. Moreover, the frequency of pleiotropic responses correlated with the number of distinct mutational targets⁹, reinforcing the need for sequence-level mapping of adaptation using high-throughput experiments¹⁰. For instance, a deep-mutational scan of the *rpo* genes, which are often implicated in nutrient-limiting evolution experiments and shown to have diverse pleiotropic responses¹⁰, may reveal how correlated (or not) the landscape of adaptation and pleiotropy is.

Most prior studies of adaptation and pleiotropy focused on different types of stressful conditions. In contrast, our study shows how related environments with minimal stress can generate both divergent adaptive

responses and predictable pleiotropic effects. While our current analysis is limited to short-term fitness measurements and chemically defined environments, this work sets the stage for exploring more complex environmental differences—including temperature or osmotic stress—in future studies.

We also continue to propagate these populations to investigate long-term outcomes, such as whether early generalists transition to specialists over time. Ultimately, our findings highlight the importance of environmental subtlety in shaping evolutionary trajectories and motivate the development of high-throughput evolution systems to systematically map pleiotropic effects across diverse conditions.

Material and methods

Strains used and media composition

E. coli K12 MG1655 (ATCC 47076) was used as the founder in this study.

Evolution experiments

The evolution experiment was conducted in M9 minimal media (composition: $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (12.8 g/L), KH_2PO_4 (3 g/L), NaCl (0.5 g/L), NH_4Cl (1 g/L), 1 M MgSO_4 and 0.1 M CaCl_2), containing either a mixture of glucose and galactose (0.1% each) or lactose (0.1%) or melibiose (0.1%).

Six independent replicate populations of *E. coli* were evolved at 37 °C and at 250 rpm in three environments - glucose-galactose mixture, lactose, or melibiose. Evolution experiment was performed by serially transferring 50 μL of culture into 4.95 mL of fresh M9 media containing appropriate sugar, every 12 h.

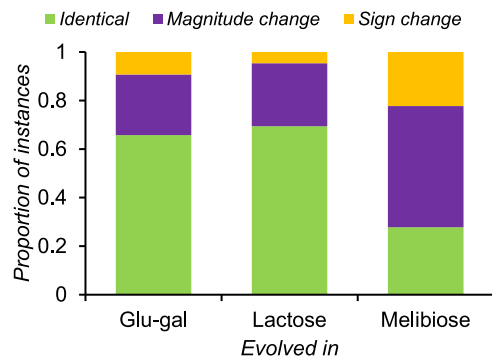


Fig. 5 | Variability in pleiotropic responses. To test whether the pleiotropic fitness changes (both r and K) of glucose-galactose, lactose, and melibiose evolved populations in away environments (both synonymous and non-synonymous) follow any patterns, we compared the relative frequencies of the three types of pleiotropic responses exhibited by the three sets of evolved populations. Melibiose evolved populations exhibited the greatest number of pleiotropic responses, followed by the lactose evolved populations. Overall, we observe that even minute changes in the evolution environment play a significant role in dictating the variability in pleiotropic responses.

Fitness measurements

To measure fitness, cells were grown in triplicate in LB (1% tryptone, 0.5% yeast extract, 0.05% NaCl) and incubated at 37 °C for 12 h with shaking at 250 rpm. Thereafter, cells were sub-cultured 1:100 in fresh M9 media containing 0.2% glycerol for 18 h. To measure fitness, cells were cultured in M9 media containing the appropriate carbon environment (glucose-galactose, lactose, melibiose, L-arabinose, D-xylose, D-mannose, D-fructose, D-sorbitol) to an initial OD600 of 0.01.

Cells were then transferred to a 96-well plate (Costar) and the plate was covered with a breathable membrane (Breathe-Easy) to prevent evaporation. The 96-well plate was incubated at 37 °C with shaking in a microplate reader (Tecan Infinite Pro 200). OD600 was measured every 30 minutes until each culture reached stationary phase (~20 h).

Growth rate was measured as described in the past⁵⁰. Maximum OD attained in each line was used as a measure for biomass accumulation. There was no observable lag phase in the glucose-galactose and lactose-evolved populations, as observed in another study⁵⁰. Therefore, we did not consider it as a measure of fitness change.

Statistical tests

The error bars in the plots shown correspond to standard deviation, unless specified otherwise. A comparison of two means was done using a two-tailed

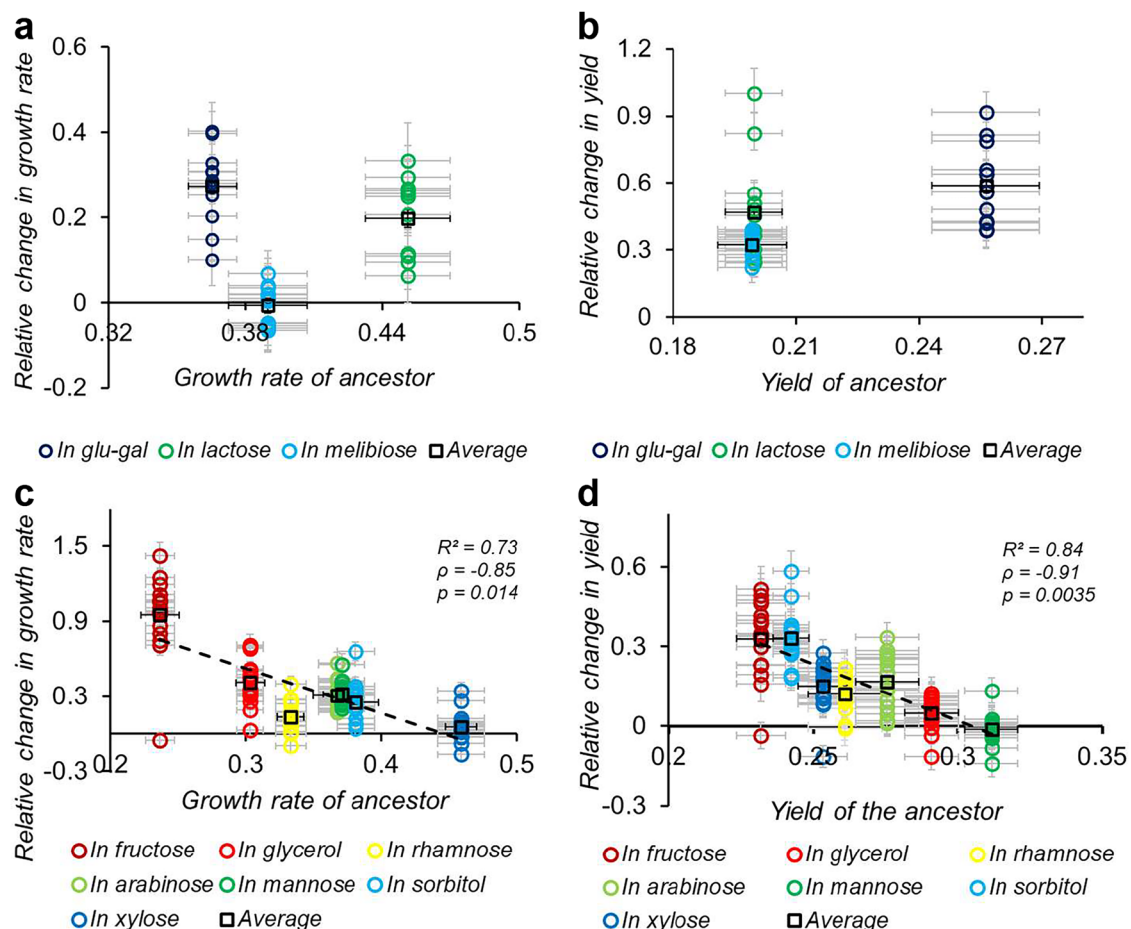


Fig. 6 | Fitness of the ancestor could act as a predictor of pleiotropic fitness gains. We test if the fitness of the ancestral population in an environment can act as a predictor of the fitness of an evolved population. We compare the fitness gain of an evolved population with ancestral fitness in away environments. (a, b) show these comparisons for r and K in synonymous environments, respectively. It must be noted that for each set of evolved populations, there were two synonymous away

environments. We also plot relative fitness gains against ancestral fitness in eight non-synonymous environments. As shown in (c) (for growth rate) and (d) (yield), in most cases, there exists a trend – in non-synonymous environments, average pleiotropic fitness gains decrease with an increase in the fitness of the ancestor. However, there exists no such quantitative trend in the case of synonymous environments.

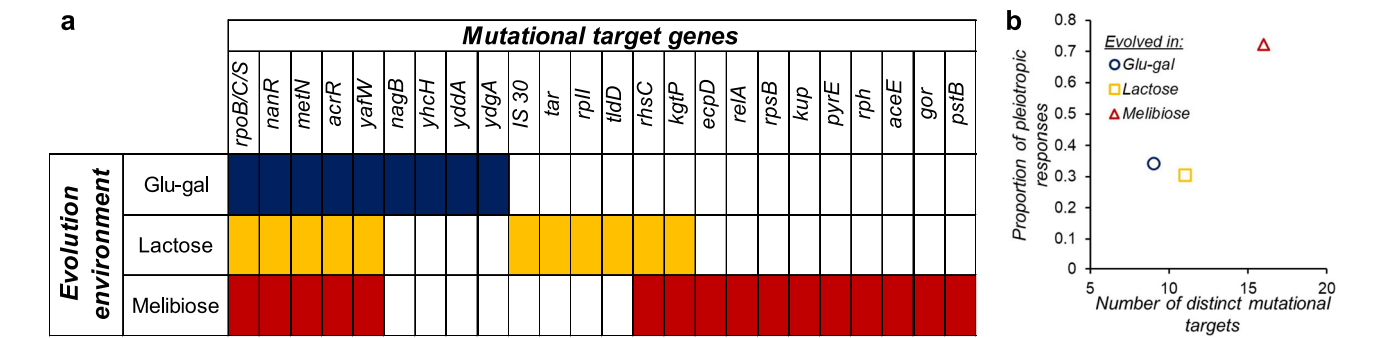


Fig. 7 | Genetic basis of pleiotropic effects. a The mutational targets in the three sets of evolved populations are shown. **b** The proportion of pleiotropic responses is correlated with the number of distinct mutational targets in the evolved populations.

t test. A one-tailed *t* test was used to ascertain if one mean is greater than or lesser than another. In all cases, the significance level was set to 0.05.

Whole-genome sequencing

Single clones of ancestor and evolved *E. coli* populations were used to isolate genomic DNA using the standard phenol/chloroform DNA isolation protocol⁵¹. DNA quality and concentration were measured using Nanodrop Spectrophotometer (Eppendorf), and band size was confirmed through gel electrophoresis. Genome sequencing was carried out using Illumina NovaSeq 6000 platform with paired-end reads of 150 base pairs, and an average read depth of 100×.

Raw sequencing reads were assessed for quality using FastQC (v- 0.11.9)⁵², and fastq mcf (v-1.04.803) was used to trim adapters and remove low-quality bases, retaining reads with a minimum Phred score ≥20. The filtered high-quality reads were aligned to *E. coli* K-12 MG1655 reference genome (ATCC 47076) using Burrows Wheeler Aligner (BWA) tool (v- 0.7.17)⁵³ with default parameters (bwa mem -t 8). SAM files were converted to BAM, sorted, and indexed using SAMtools (v- 1.21)⁵⁴.

Variant calling was performed using GATK HaplotypeCaller (v- 4.6.1.0)⁵⁵, with default settings optimized for haploid bacterial genomes (--ploidy 1). For downstream analysis, only variants with a minimum read depth of 30 were considered to filter out variants. Variants identified in the ancestral population were filtered out manually using custom R scripts. The remaining unique variants were annotated using SnpEff (v- 5.1)⁵⁶ with the *E. coli* K-12 MG1655 genome database. Raw sequencing data is available at ncbi.nlm.nih.gov/sra/PRJNA1022868.

Data availability

Genome sequencing is available at ncbi.nlm.nih.gov/sra/PRJNA1022868.

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Author contributions

N.A.: Performed Characterization Experiments, Wrote manuscript. R.G.B.: Performed Evolution Experiment. P.V.: Performed data analysis, Wrote manuscript, Performed Experiments. S.S.: Conceived & supervised the study, wrote manuscript. All authors reviewed the manuscript.

Competing interests

N.A., P.V., R.G.B. declare no financial or non-financial competing interests. S.S. serves as Guest Editor for the collection on Evolutionary Systems Biology of this journal and had no role in the peer-review or decision to publish this manuscript. S.S. declares no financial competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Pavithra Venkataraman or Supreet Saini.

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