

Gene loss and compensatory evolution as a source of metabolic preadaptations

Dorottya Kalapis ^{1,2†}, Károly Kovács ^{1,2*†}, Dávid Balogh ¹, Elvin Maharramov ^{1,3}, Walliyulahi Ajibola ^{1,3}, Olin K. Silander ⁴, Tamás Fehér ¹, Csaba Pál ¹, Balázs Papp ^{1,2,5*}

¹Synthetic and Systems Biology Unit, Institute of Biochemistry, National Laboratory of Biotechnology, HUN-REN Biological Research Centre, Temesvári Krt. 62, Szeged 6726, Hungary

²HCEMM-BRC Metabolic Systems Biology Group, Temesvári Krt. 62, Szeged 6726, Hungary

³Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

⁴The Liggins Institute, University of Auckland, Auckland, New Zealand

⁵HUN-REN-SZBK-ELTE-SZTE-NNGYK Genomic Epidemiology Research Group, Temesvári krt. 62, Szeged 6726, Hungary

*Corresponding authors: E-mails: kkaroly@brc.hu (K.K.); papp.balazs@brc.hu (B.P.).

[†]These authors contributed equally.

Associate editor: Laasya Samhita

Abstract

Gene loss is common, yet whether it is merely an evolutionary dead-end or can promote future adaptation to new environments remains debated. Here, we systematically tested whether harmful loss of metabolic genes, followed by compensatory evolution, generates preadaptations to alternative nutrients in *Escherichia coli*. Our analysis revealed a striking outcome beyond simple growth restoration, where repair of a genetic defect yielded growth exceeding the unperturbed wild type on carbon sources not encountered during evolution. Such latent growth gains occurred across multiple deletion backgrounds and carbon sources, with growth rate increases up to 46% relative to the wild type. In some cases, the corresponding ancestral deletion strain failed to grow on these carbon sources, indicating functional innovations relative to the deletion ancestor. Genetic reconstruction and transcriptomic profiling implicate regulatory reprogramming, particularly in carbon catabolite repression and nutrient uptake, as mechanisms underlying latent growth gains. In conclusion, evolutionary recovery from gene loss can rapidly produce preadaptations to future environments in the absence of direct selection.

Keywords compensatory adaptation, exaptation, adaptive laboratory evolution, evolutionary innovation, latent metabolic capacity, deleterious mutations, pleiotropic fitness effects

Introduction

Mutations that disrupt gene function are common and a major source of genetic variation. Yet, the role of gene loss in adaptive evolution remains contentious. While some loss-of-function mutations can be beneficial and drive phenotypic novelties (Olson 1999; Albalat and Cañestro 2016; Murray 2020), most are deleterious or neutral (Baba et al. 2006; Hillenmeyer et al. 2008). Deleterious loss-of-function is thus often treated as an evolutionary dead end. However, its fitness costs can be mitigated by compensatory mutations elsewhere in the genome. In principle, such compensatory evolution may open new adaptive paths and thereby contribute to adaptive evolution (Covert et al. 2013).

Compensatory evolution appears to be common, from RNA and protein structures to transcriptional networks. Compensatory variants are widespread in natural populations (Parts et al. 2011; Rousset et al. 2021; Rosconi et al. 2022), and

laboratory evolution shows that harmful gene disruptions can be rapidly compensated (Blank et al. 2014; Szamecz et al. 2014). Remarkably, fitness recovery is often accompanied by major cellular changes, including regulatory reprogramming, shifts in metabolic pathway usage, and altered morphology (Szamecz et al. 2014; McCloskey et al. 2018a; Farkas et al. 2022), pointing to collateral phenotypic effects beyond restoring growth. What remains unclear is whether compensatory evolution can, as a by-product, generate preadaptations—traits with no immediate benefit that later become advantageous in new environments.

Here, we address this question in laboratory-evolved *Escherichia coli* lines selected for growth recovery following metabolic gene loss. Importantly, gene deletion eliminates the possibility of simple mutational reversion, requiring fitness recovery through novel mutational routes. We focused on nutrient utilization phenotypes, which represent an important class of evolutionary novelties in bacteria. Crucially, in our system,

Received: February 9, 2026. **Revised:** May 27, 2026. **Accepted:** June 4, 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

selection acts only to restore growth in minimal glucose medium; any effects on other carbon sources are collateral. Because compensation after gene loss could simply restore performance toward the unperturbed wild-type (WT) state, we used WT growth as the baseline outcome of the evolutionary process. We define latent growth gains as cases in which compensated lines exceeded this WT baseline on carbon sources not encountered during laboratory evolution. We refer to these cases as compensation-derived latent growth gains to emphasize that they arose during compensatory evolution but were revealed only in alternative nutrient conditions.

We leveraged a collection of laboratory-evolved, growth-recovered *E. coli* lines from Blank et al. (2014), which provides a tractable system to study compensation after a severe metabolic defect. Each line carries a deletion in one of 22 essential metabolic genes; thus, recovery required compensatory mutations to restore fundamental metabolic processes. Importantly, several lines recovered to near-WT growth, reducing confounding by broad growth impairment and enabling a stringent test for latent growth gain. We focused on 18 evolved lines with high fitness compensation and measured their growth phenotypes across 10 alternative carbon sources that were not part of the selection regime. Across this panel, compensated lines repeatedly exceeded WT growth, with growth gains spanning multiple deletion backgrounds and substrates. Genetic reconstruction and transcriptomic profiling implicate regulatory reprogramming, including carbon catabolite control and nutrient uptake, as a mechanistic basis for these latent growth gains.

Results and discussion

Compensatory evolution generates WT exceeding growth outside the selective regime

We systematically tested whether compensatory evolution after gene loss generates latent growth gains—phenotypes that outperform the unperturbed WT in novel environments outside the selective regime. To this end, we analyzed a collection of 68 fitness-compensated *E. coli* lines generated in a previous study (Blank et al. 2014). All these lines carry a gene deletion in one of 22 metabolic genes that are essential for growth on glucose minimal medium (i.e. display close to zero fitness) but show fitness recovery after laboratory evolution in the same environment (i.e. *selection environment*). First, we quantified fitness in the selection environment (minimal glucose medium) using high-throughput growth phenotyping in liquid culture (Methods). Consistent with prior reports, compensation was often substantial, with growth rates approaching that of the unperturbed WT (Table S1). To minimize potential confounding effects from incomplete fitness recovery, we selected 18 lines that had recovered to at least 80% of WT growth (Methods, Table S1). These lines, originating from 12 deletion backgrounds across multiple metabolic functions, were chosen solely based on their recovery in the selection environment, prior to any screening on alternative carbon sources.

Next, we screened the 18 compensated lines across 10 alternative substrates. We chose carbon sources utilizable by the WT

that represent distinct nutrient classes and metabolic routes, including sugars and glycosides, organic acids, glycerol, and amino-acid-derived substrates (Methods). While the WT ancestor can utilize these substrates, the compensated lines have not been exposed to them during laboratory evolution. Importantly, selection in this setting targeted repair of a severe growth defect on glucose medium. While collateral fitness changes across environments are common in experimental evolution and can include improvements (Cooper and Lenski 2010; Leiby and Marx 2014; Choudhury and Saini 2019; Jerison et al. 2020), repairing a growth defect does not inherently predict WT-exceeding performance on substrates not experienced during evolution. We therefore define “compensation-derived latent growth gain” as cases where compensated growth exceeds the ancestral WT in a new environment (Fig. 1a), thereby identifying preadaptations relative to the lineage's unperturbed starting point. To identify this distinct outcome at scale, we screened all 180 line-environment combinations for WT-exceeding growth, measuring the ancestral knockout only where such gains were detected.

Remarkably, we found 18 (out of 180) line-environment combinations where the compensated line's growth rate was significantly higher than WT on a specific carbon source (1-sided Wilcoxon test, 5% false discovery rate; Fig. 1b, Table S1). These latent growth gains were distributed across both genotypes and environments: 8/18 compensated lines exhibited WT-exceeding growth on at least one carbon source, and 6/10 carbon sources supported such latent growth gains in at least one line. The magnitude of growth gain was substantial in the affected cases: compensated lines grew, on average, 23% faster than WT in the corresponding alternative environments, with a maximum increase of 46% (Fig. 1b, Table S1). Crucially, these gains were specific to environments outside the selective regime: the same lines did not outperform WT in the original selection environment (Fig. 1b). Thus, compensatory evolution following gene loss can generate large, environment-specific fitness gains above WT in the absence of direct selection. Because compensated lines were previously reported to show longer-than-WT lag times in glucose medium (Blank et al. 2014), we also estimated lag time and OD increment as a carrying-capacity proxy for lines with WT-exceeding growth rates (Table S1). Among these cases, only 2 showed significantly longer lag times than the WT on the same carbon source, metR-E and ptsI-E on pyruvate, and only one case, metR-E on pyruvate, showed significantly reduced OD increment (1-sided Wilcoxon test, 5% false discovery rate). Thus, most WT-exceeding growth rate gains were not accompanied by detectable lag or OD increment costs, supporting an overall growth advantage under the assayed conditions.

Could latent growth gains simply reflect phenotypes inherent to the gene deletion itself? To test this, we assayed the corresponding ancestral deletion strain on each carbon source, where a latent growth gain was observed. In no case did the ancestral deletion strain exceed WT growth (Fig. 1c), ruling out a simple “deletion-only” explanation for latent growth gain. Strikingly, in 5 instances, the ancestral deletion strain showed negligible growth, whereas the compensated line exceeded the WT (Fig. 1c). These instances represent qualitative metabolic gains and hence functional innovations relative to the deletion ancestor: acquiring the ability to grow on a substrate where the

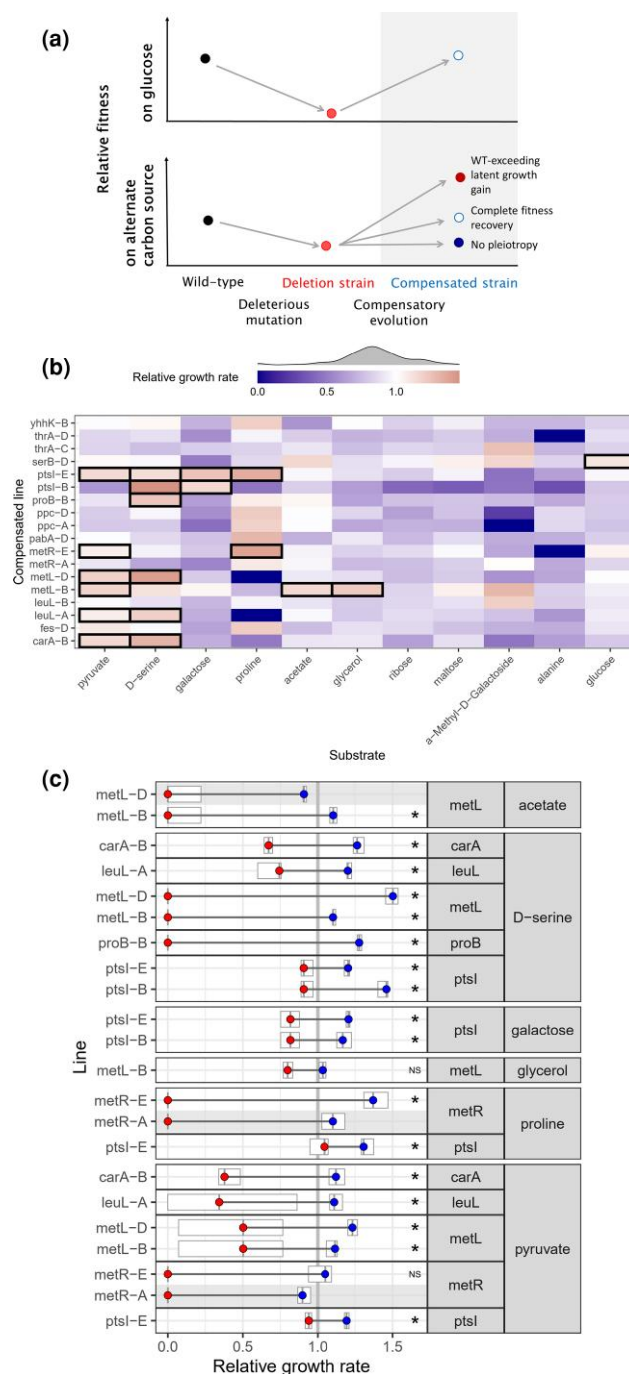


Figure 1 Compensation-derived latent growth gain: WT-exceeding growth as a by-product of defect repair. a) Conceptual illustration of latent growth gains arising from compensatory evolution. Colors of filled circles match the colors of heatmap in panel b. White circle indicates full recovery of WT fitness without WT-exceeding growth gain (fitness = WT). Dark red circle indicates WT-exceeding growth gains in a novel environment (fitness > WT), representing a more extreme gain than expected from simple defect repair. Blue circle represents cases where fitness remains below WT, including incomplete recoveries and potential trade-offs across environments. (b) Heatmap showing relative growth rates of 18 compensated lines across 10 carbon sources (mean of 5 to 6 replicate measurements). Colors denote growth rates relative to the WT (relative growth rate = 1), with red indicating faster-than-WT growth (see above). Black outlines indicate statistically significant increases relative to the WT (latent growth gains; 1-sided Wilcoxon tests; 5% FDR). Columns are clustered using Jaccard distances computed on the binarised growth gain matrix (latent growth gain present/absent). The density plot above the color legend shows the distribution of relative growth rates across all compensated line–carbon source combinations. (c) Dumbbell plots comparing relative growth rates of selected compensated lines (blue) and their ancestral gene deletion strains (red) across carbon sources. Circles mark median growth rates, boxes the interquartile ranges ($n = 6$ to 8); connecting lines highlight the change from ancestor to compensated line. Only lines with significantly higher growth than WT (latent growth gain, Fig. 1a) were included and re-measured alongside corresponding deletion strains (see Methods). Asterisks mark growth significantly higher than WT in the repeated measurement (1-sided Wilcoxon tests, 5% FDR); NS: nonsignificant. Grey-shaded panels display additional parallel lines derived from the same zero-growth ancestors to illustrate further qualitative growth gains (phenotypic innovations) where the strict WT-exceeding growth rate criterion was not met.

deletion ancestor failed. Although comparable studies are scarce, prior work on beneficial mutations during the optimization of already high-fitness lineages has mostly reported collateral benefits comparable in magnitude to the directly selected gains. In the methodologically closest study, Ostrowski et al. (2005) found that collateral gains on other carbon sources were generally not larger than the directly selected benefit. Furthermore, the emergence of qualitative growth gains in novel environments is not commonly documented but has been observed after long-term evolution of WT lineages on glucose: Leiby and Marx reported that while largely absent at 20,000 generations, a subset of isolates acquired growth on certain carbon sources after 50,000 generations (Leiby and Marx 2014). Notably, we observe at least 9 such cases across 3 knockout backgrounds and 4 carbon sources in just ~145 generations (including cases where parallel lines from the same ancestor acquired growth without exceeding WT levels; Fig. 1c). This indicates that compensatory evolution can generate qualitative growth gains in novel environments on a timescale orders of magnitude faster than the incremental optimization of high-fitness lineages.

Crucially, the observed latent growth gains are driven by deletion-specific compensatory changes rather than general adaptation to the laboratory environment. To distinguish between these possibilities, we analyzed the genetic parallelism across the diverse backgrounds in our collection (Blank et al. 2014). If adaptation were driven by compensation for a specific gene loss, we would observe high genetic parallelism among replicate lines from the same background, but low parallelism between lines from different genotypes. Our findings support this prediction: replicate lines from the same ancestor shared 32% of their mutated genes, whereas lines from different ancestors shared only 2% [$P < 10^{-3}$, Figure S1a, for the full set of evolved lines from Blank et al. (2014)]. This pattern remained consistent in the high-fitness subset selected for our phenotypic screen (41% vs. 1.7% overlap; $P < 10^{-3}$; Figure S1b). Such strong, background-specific genetic parallelism demonstrates that the selective pressures were largely unique to each gene deletion context and argues against a dominant role for a common culture-medium adaptation in generating latent growth gains. Consistent with this, except for the recurrent gains on D-serine and pyruvate (discussed below), growth gains were idiosyncratic, typically limited to 1 or 2 evolved lines per carbon source. Furthermore, most such lines lacked mutations known to drive general adaptation to the glucose medium (see Table S1; Blank et al. 2014; LaCroix et al. 2015; Knöppel et al. 2018; Wannier et al. 2018; Wytock et al. 2018; Campbell et al. 2023; Venkataraman et al. 2024). Of the 3 lines that did carry glucose-adaptive mutations, 2 harbor mutations only in motility regulators (flhDC and lhrA; Blank et al. 2014). While these genes were found to be mutated and shown to be adaptive in nonshaking liquid medium by increasing motility (Parker et al. 2019), they are not expected to confer the carbon source-specific metabolic preadaptations we observe. Finally, to directly test causality in one instance, we reintroduced specific compensatory mutations into the ancestral knockout strain, as detailed below (Fig. 2 b and c).

In sum, compensatory evolution following metabolic gene loss does not simply restore fitness in the selection environment. It can also generate latent growth gains, where evolved lines surpass the unperturbed WT on carbon sources that were not part of the selection regime. Because our screen covered only 10 carbon sources, the observed frequency is likely conservative; additional latent growth gains may be revealed under other nutrient

conditions. This phenomenon is distinct from prior findings where gene loss potentiated access to higher fitness peaks under direct selection on the focal trait, such as oxidative-stress adaptation (Helsen et al. 2020). Here, the superior phenotypes emerged not from selection in the new environments, but as collateral benefits of recovering from a metabolic defect. These results identify compensatory evolution as a source of metabolic preadaptations.

Regulatory reprogramming can generate latent growth gains

To investigate the mechanisms driving compensation-derived latent growth gains, we focused on a prominent cluster of cases involving D-serine, where multiple lines from diverse backgrounds exceeded WT growth (Fig. 2a). Many of these lines also displayed superior fitness on pyruvate, a metabolically related compound (Fig. 2a). This recurrence across multiple backgrounds pointed to a shared physiological basis.

Genomic analysis identified the CRP–cAMP system, a major regulator of catabolite repression, as the primary candidate. Most lines exhibiting this D-serine/pyruvate growth gain harbor putative loss-of-function mutations in *cyaA* (encoding adenylate cyclase) or *crp* (encoding the cAMP receptor protein). The acquisition of diverse *cyaA* mutations across 5 independent lines (Table S1) suggests convergent evolution at this locus, consistent with an adaptive role for altered CRP–cAMP regulation.

Convergent loss-of-function mutations in the CRP–cAMP system are frequently reported during adaptation to alternative carbon sources (Herring et al. 2006; Conrad et al. 2009; Phaneuf et al. 2020) or long-term starvation (Katz et al. 2021; Behringer et al. 2022, 2024). In contrast, CRP–cAMP inactivation is deleterious under normal glucose batch conditions due to reduced glucose uptake (Perrenoud and Sauer 2005; Pal et al. 2022), consistent with WT CRP–cAMP activity being near-optimal for glucose growth (Towbin et al. 2017). Together, these observations imply that diminished CRP–cAMP activity is favored in stressed genetic backgrounds, during starvation, or on particular nonglucose substrates. Mechanistically, this likely reflects the relief of suboptimal catabolite repression: WT *E. coli* grows slowly on certain carbon sources due to suboptimal regulation, which is relieved by reducing CRP–cAMP activity (Herring et al. 2006; Towbin et al. 2017).

To directly assess the contribution of CRP–cAMP inactivation to WT-exceeding growth gains, we reconstructed the mutations from the *carA*-B compensated line. This line carries a putative loss-of-function mutation in *cyaA*, and a regulatory mutation in *carB*, which has been shown to increase CarB protein expression level (Blank et al. 2014). *carB* upregulation likely compensates for the loss of *carA* (involved in carbamoyl phosphate biosynthesis), as shown by previous studies (Patrick et al. 2007; Blank et al. 2014; Guzmán et al. 2018). We introduced the evolved *cyaA* allele into: the WT (WT + *cyaA**), the *carA* deletion strain ($\Delta carA$ + *cyaA**), and a *carA* deletion strain overexpressing *carB* ($\Delta carA$ + *cyaA** + *carB*_OE) (Fig. 2b and c, see Methods). Consistent with our hypothesis, WT + *cyaA** grew significantly faster than WT on both D-serine and pyruvate. Furthermore, the *cyaA** mutation enhanced growth in the $\Delta carA$ background, but only when *carB* was

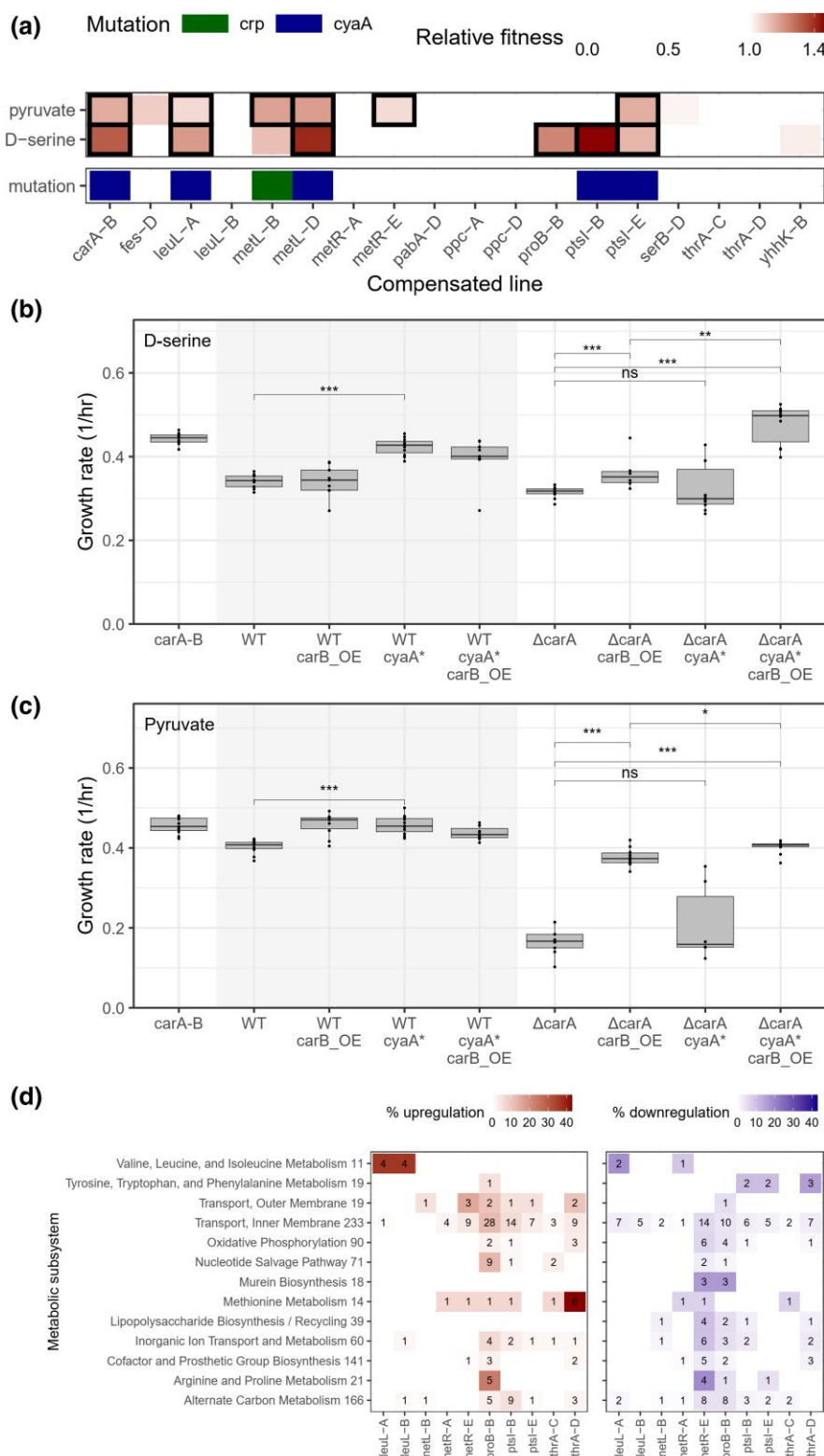


Figure 2 Diminished catabolite repression can generate compensation-derived latent growth gains. a) Heatmap showing, for each compensated line, the presence of mutations in the CRP–cAMP system (either in the genes *crp* or *cyaA*) and relative growth on pyruvate and D-serine. Black outlines mark strain–substrate combinations with significantly higher growth than WT. b, c) Boxplots comparing absolute growth rates on D-serine (b) and pyruvate (c) for the WT, the relevant deletion ancestor, the compensated line and reconstructed strains carrying *carB* overexpression (*carB_OE*) and/or the *cyaA* mutation (*cyaA**), as indicated. Points show individual replicate measurements (at least 6 per strain). *P*-values are from 2-tailed Wilcoxon tests. d) Heatmap showing gene expression changes across metabolic subsystems of compensated lines. Color gradients indicate the percentage of genes in each subsystem that are differentially expressed in each evolved line. Numbers report the counts of up- and downregulated genes (up/down) within the subsystem. The y axis labels give the total number of genes assigned to each subsystem. Only subsystems with differential expression in at least 3 genes across the compensated lines are shown.

also overexpressed, yielding a synergistic effect (Fig. 2b and c). These findings demonstrate that reduced CRP–cAMP activity generates latent growth gains on substrates not encountered during evolution when combined with deletion-specific compensatory changes.

In sum, disrupting a key regulator of carbon catabolism can be beneficial for slow-growing deletion backgrounds, and as a by-product, yield improved growth on specific alternative carbon sources. The recurrence of CRP–cAMP loss-of-function mutations across multiple deletion backgrounds suggests a shared route by which compensatory evolution can generate environment-specific preadaptations. However, reduced catabolite repression can compromise performance in other conditions (Görke and Stülke 2008), underscoring the likely trade-offs associated with this mechanism.

To determine whether regulatory changes drive latent growth gains beyond the CRP–cAMP module, we profiled transcriptomes of 10 compensated lines and the WT in glucose minimal medium. Consistent with prior work (Szamecz et al. 2014; McCloskey et al. 2018a), compensated lines exhibited extensive expression divergence from WT, establishing novel regulatory states even after filtering for potential signatures of general medium adaptation (80 to 322 changes per line; Methods; Figures S2 to S3, Table S4). Mapping these changes onto a genome-scale metabolic network reconstruction (Monk et al. 2017) revealed widespread changes across metabolic subsystems, with altered—often increased—expression of nutrient transporters as a recurrent theme (Fig. 2d). This suggests that compensatory mutations reshape nutrient uptake capacity, incidentally preadapting cells for alternative carbon sources. Two cases illustrate this link, based on integrated evidence from transcriptomes, evolved mutations, and prior literature (Suppl. Notes 1 and 2). First, *ptsI*-compensated lines strongly upregulated *galP*, a promiscuous transporter for both glucose and galactose. Since *galP* upregulation is a known compensatory route for PTS disruption (Henderson et al. 1977; Hernández-Montalvo et al. 2003), enhanced galactose utilization emerges as a byproduct (see Suppl. Note 1). Second, in the *proB*-B line, WT-exceeding growth on D-serine coincides with upregulation of the D-serine transporter gene *cycA*, likely driven by nitrogen-scavenging regulation (Suppl. Note 2). Together, these data point to additional regulatory routes to compensation-derived latent growth gains beyond CRP–cAMP reprogramming.

Collectively, our genetic and transcriptomic analyses indicate that WT-exceeding growth gains arise via multiple regulatory routes, ranging from diminished catabolite repression to substrate-specific shifts in nutrient uptake. The recurrent growth gain on D-serine is particularly notable given its ecological significance: D-serine utilization is a key trait for *E. coli* colonization of the urinary tract, providing both a nutrient source and a competitive edge against rivals susceptible to D-serine toxicity (Moritz and Welch 2006). This demonstrates that repairing a single metabolic defect can inadvertently yield complex, ecologically relevant traits, warranting broader investigation of this process in natural settings.

Conclusion

Our study shows that compensatory evolution after metabolic gene loss is not limited to fitness restoration. Instead, it

frequently generates latent growth gains, where repair of a metabolic defect yields growth above the unperturbed WT in nutrient environments not encountered during selection. In our dataset, this outcome arises rapidly (~150 generations) and recurs across multiple deletion backgrounds, indicating that recovery from metabolic defects can readily produce preadaptations to new nutrient conditions. These results indicate that compensation-derived latent growth gain may be an underappreciated outcome of recovery from metabolic gene loss. Our work also identifies latent functional innovations relative to the deletion ancestor: in several cases, compensatory evolution produced qualitative growth gains on carbon sources where the ancestral deletion strain failed to grow. Genetic reconstruction and transcriptomics implicate regulatory reprogramming, including catabolite control and transporter expression, as a plausible mechanism for latent growth gain.

This scenario connects compensatory evolution to broader work on latent traits and nonadaptive origins of innovation. Previous studies have shown that selection in one environment can incidentally generate latent traits that become beneficial in novel environments, including improved use of alternative nutrients or increased resistance to antibiotics (Blount et al. 2008; Meijnen et al. 2008; Barve and Wagner 2013; Lázár et al. 2014; Leiby and Marx 2014). Our study extends this framework from adaptation to external environments to recovery from internal genetic damage. Here, selection primarily favored recovery from metabolic gene loss rather than improved glucose utilization, yet the resulting compensatory changes also produced latent growth gains on carbon sources not encountered during evolution. More broadly, these findings fit with the view that environmental context is central to identifying latent and adaptive innovations in bacteria (Wagner 2017; Karve 2025). How often gene loss and compensatory evolution shape such latent phenotypes in nature remains unknown. However, gene loss is common (Xu and Guo 2020), and gene essentiality varies extensively within bacterial populations (Rousset et al. 2021; Rosconi et al. 2022), suggesting ample opportunity for compensatory evolution to generate hidden phenotypic variation.

Materials and methods

Genotypes

Laboratory-evolved lines carrying specific single-gene deletions were obtained from a previous laboratory adaptation experiment (Blank et al. 2014). Briefly, 22 ancestor single-gene knockout *E. coli* strains were subjected to laboratory evolution in 5 parallel replicates in M9 glucose minimal medium, which allowed growth recovery via the accumulation of compensatory genetic changes. We focused our investigations on 18 evolved lines exhibiting a high degree of fitness recovery (relative growth rate > 0.75). Ancestral deletion strains were taken from the Keio collection (Baba et al. 2006), in which open reading frame coding regions were replaced with a kanamycin resistance cassette. In order to provide a suitable WT control for further experiments, we inserted the same kanamycin resistance cassette into a K-12 BW25113 strain to replace the genomic *IS150* insertion sequence, applying λ -Red mediated recombineering (Nyerges et al. 2016). Briefly, a linear DNA fragment was generated by

PCR using primers mokISFw and mokISRev from the template plasmid pSGT76-K (GenBank: Y098947.1; Pósfai et al. 1997) and was integrated into the *E. coli* BW25113 chromosome after induction of the pORTMAGE-2 plasmid. Recombinants were selected on LB-Km plates, and colonies were PCR-verified using primers IS150up and pSTK-R. The genomic segment was PCR-amplified using primers IS150up and IS150dn, and Sanger-sequenced using primers IS150up and pSTK-F (Table S2).

Growth conditions

To evaluate the impact of compensatory adaptation on fitness in new environments, we measured the fitness of compensated evolved lines, their ancestors, and the control WT strain across 10 different nutrient environments, representing alternative carbon sources (see Table S3). The concentrations of carbon sources in these alternative environments were set to ensure that each contributed an equal amount of carbon to the media. MOPS (3-(N-morpholino)propanesulfonic acid buffered) minimal medium (Neidhardt et al. 1974) was used for all phenotypic profiling experiments.

We grew reconstructed strains containing ASKA (a complete set of *E. coli* K-12 ORF Archive) clones in MOPS minimal medium + 20 µg/ml chloramphenicol. To induce gene expression from the ASKA vector, 25 µM IPTG (isopropyl β-D-1-thiogalactopyranoside) was also added, since cloned ORFs are controlled by IPTG-inducible promoters. In addition to glucose, the selective media was supplemented with D-serine or galactose. For gene expression profiling, cells were grown in M9 minimal medium supplemented with 0.4% glucose. Although this differs from the evolution condition (0.2% glucose), it does not confound our conclusions because all genotypes were profiled under the same 0.4% condition (common-garden assay) and samples were collected at OD 0.3, when glucose remains in excess at both concentrations and no growth rate difference can be detected (Wang et al. 2020).

Fitness measurements

We used established protocols specifically designed to measure fitness, as approximated by growth rate, in microbial populations. The fitness of the ancestor and the evolved lines and the corresponding WT were measured in 8 replicates each. Briefly, bacterial cultures were inoculated from frozen (−80 °C) aliquots onto LB agar plates. After 2 days of incubation at 37 °C, the colonies were suspended in MOPS minimal medium devoid of any carbon source. These cell suspensions were then used to inoculate 96-well plate format precultures (MOPS minimal medium + appropriate carbon source). V&P scientific pin-tool (VP407, V&P Scientific, Inc.) was used to manually inoculate 1 µl of cell suspension into 100 µl of preculture. Depending on the carbon source used, precultures were incubated at 37 °C in a shaking incubator.

Growth curve recordings were carried out using 96-well plates (Greiner) filled with 100 µl rich medium per well, inoculated from preculture plates using a pintool with 1.58 mm floating pins. The pintool was moved by a Microlab Starlet liquid handling workstation (Hamilton Bonaduz AG). Growth curves were monitored over

a 72 h incubation period in a Biotek Powerwave HT automated plate reader in 96 density plates filled with MOPS-defined minimal medium supplemented with the carbon source of interest. During the kinetic run, the optical density of each well was recorded at 600 nm (OD600) every 5 min. Between optical readings, the cultures were incubated at 37 °C, with alternating shaking speed (1,000 to 1,200 rpm). Growth rate was used as a proxy for fitness. Growth rate and lag phase length were estimated as previously described (Warringer and Blomberg 2003; Szamecz et al. 2014). Briefly, raw OD values were background-subtracted, OD-calibrated, locally averaged, and constrained to be nondecreasing before natural-log transformation. Growth rate was estimated from the processed log-transformed OD curve as the mean of the 3rd to 7th highest slopes calculated over 20-min intervals. Lag phase length was estimated by calculating slopes over 70-min intervals, extrapolating each slope back to the mean initial log OD from the first 50 min of measurements, and taking the highest intercept time. OD increment was calculated from the processed nonlog OD curve as the highest OD value minus the mean of the first 5 OD values.

Fitness profiling across environments was performed in 2 distinct rounds. First, we measured the growth rate of 18 compensated lines across 10 alternative carbon sources. Second, to ensure a direct comparison between ancestral and evolved strains, we performed a second round of growth rate measurements for both compensated lines that exhibited higher than WT growth in the initial screen and their respective ancestral strains. Note that this second round of measurements was focused on those 6 alternative carbon sources where WT-exceeding growth rate was observed in the first round. Reassuringly, the compensation-derived latent growth gains of the compensated lines were highly reproducible (~90%, 16/18 significant cases from the first screen were reproduced in the second screen).

Analysis of compensatory mutations

Whole-genome sequences of evolved lines were determined by Blank et al. (2014), identifying mutations using Breseq 0.21 (Barrick et al. 2009).

Visual inspection of the published genome sequence data using Integrative Genomics Viewer (Robinson et al. 2011) revealed that the coverage for the promoter region of *galP* was missing; therefore, we PCR-sequenced this region to look for potential mutations explaining the upregulation of *galP*. Indeed, we found a SNP (T→A, −189) in the *galP* promoter region. Note that the very same SNP (T→A, −189) in the *galP* promoter region was identified in a former lab-evolution experiment, where the simultaneous deletion of 3 genes of the PTS system (*ptsI*, *crr*, and *ptsH*) was compensated on glucose medium (McCloskey et al. 2018b).

Analysis of number of overlapping mutated genes between strains

To determine whether mutations observed in evolved lines primarily reflect general adaptation to the medium or deletion-specific compensatory evolution, we tested whether mutated genes overlap more frequently between lines sharing

the same deletion background than between lines that evolved from different ancestral knockouts. We conducted this analysis on 2 datasets: (i) all sequenced lines reported by Blank et al. (2014), and (ii) the high-fitness lines selected for our phenotypic screen. In both cases, we included only those deletion backgrounds for which at least 2 parallelly evolved lines were available for comparison.

For each pair of lines, we calculated the proportion of overlapping mutated genes using the Jaccard index: the number of shared mutated genes divided by the total number of unique mutated genes across the pair. Genes affected by mutations were defined following (Blank et al. 2014). Because a single mutation can impact multiple genes, we considered such gene sets as a unit and recorded overlap if at least one gene was shared between the sets of 2 lines. Multiple mutations in the same gene were counted only once per pairwise comparison. To avoid inflating overlap estimates due to ancestral mutations (which are necessarily shared among all lines derived from the same deletion background), we excluded any gene that contained an ancestral mutation. Finally, we used a permutation test to assess whether the extent of gene overlap was significantly higher among line pairs with the same deletion background than with different deletion backgrounds. Specifically, we randomized the assignment of mutation sets to strains 10,000 times, each time recalculating the difference between the mean proportions of overlapping genes—computed as the mean for same-background pairs minus that for different-background pairs. *P*-values were computed as:

$$P = \frac{(\text{number of permutations where randomized differences in mean} \geq \text{empirical mean}) + 1}{\text{number of permutations} + 1}$$

Reconstruction of mutations

We inserted the *cyaA* point mutation (GAT300GAA, D->E) found in the compensated line *carA*-B into both WT and Δ *carA* backgrounds using the CRISPR-Cas9 system (Hsu et al. 2014).

We overexpressed *carB* by transforming ASKA overexpression ORF library clones (Kitagawa et al. 2005) into the following 4 backgrounds: WT, WT + *cyaA**, Δ *carA*, and Δ *carA* + *cyaA**.

Transcriptome analysis

Whole transcriptome sequencing was performed for 10 compensated evolved lines and BW25113 WT for control. The 10 lines were selected to provide a diverse and representative sample of successfully compensated lines. This subset includes lines with high fitness recovery, originating from perturbations in various gene functions (e.g. synthesis of various amino acids, carbohydrate uptake), and also features several parallel, independently evolved lineages. Three biological replicates were used for each strain, except metL-B, which had 2 biological replicates. Cells were grown in the same medium as during the evolutionary experiments (M9 minimal medium supplemented with 0.4% glucose). Precultures were inoculated to a standardized optical density of OD 0.1. After overnight growth, sampling cultures were inoculated to an OD 0.05 to a final volume of 2 ml. 24-well polypropylene deep-well plates were used for cultivation.

At an optical density of OD 0.3, cultures were harvested by centrifugation and immediately frozen in liquid nitrogen after removal of supernatant. Total RNA was isolated with Nucleospin RNA Kit (Macherey Nagel) following the manufacturer's instructions. RNA concentration was determined using a Qubit Fluorimeter with Qubit™ RNA Broad Range (BR) Assay Kit. RNA integrity measurement was done by using 1% agarose gel electrophoresis with GelRed staining.

Total RNA was quality control checked using BioAnalyzer 2100 with RNA 6000 Nano Chip (Agilent Technologies, United States, Cat. No. 5067-1511). 500 ng of total RNA input was processed with the NEBNext rRNA Depletion Kit (Bacteria, NEB #E7850X), and NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) following manufacturer's instructions. Sequencing-ready libraries were quality control checked by BioAnalyzer 2100 instrument, using Agilent DNA 1000 Chip (Agilent Technologies, United States, Cat. No. 5067-1504). NGS was carried out on a NextSeq 500 sequencing system with the NextSeq 500/550 High Output Kit v2.5 (75 Cycles) chemistry (Illumina, Inc., United States, Cat. No. 20024906).

Quality control of raw sequences was done by using FastQC (0.11.9) and MultiQC (1.9). Reads from parallel replicates were pooled and then aligned to the *E. coli* BW25113 reference transcriptome (CP009273.1) with Salmon (1.5.2). Transcript abundance was estimated in terms of TPM (transcripts per million) values using Salmon (1.5.2). We further analyzed genes that had at least 5 reads in every replicate of at least one strain.

To identify differentially expressed genes between compensated lines and the WT (BW25113), we performed differential expression analysis using the DESeq2 R package (1.34.0; Love et al. 2014). Fold change (FC) and *P*-values were estimated using default parameters in DESeq2. These *P*-values are corrected for multiple testing using the Benjamini–Hochberg method. Genes were considered differentially expressed when the adjusted *P*-values were below 0.05 and also had greater FC than a chosen threshold ($|\log_2(\text{FC})| > 1$). The $\log_2$ FC and false-discovery rate corrected *P*-values are provided in Table S4.

Information on transcriptional regulatory interactions between genes was downloaded from the EcoCyc database (Keseler et al. 2021) on 07.03.2023.

To filter out potential transcriptomic adaptations to the selection environment (Figure S2b), we excluded genes that exhibited: (i) recurring expression changes in at least 8 out of 10 compensated lines; (ii) involvement in cellular motility, as motility genes are frequently upregulated in deletion strains consistent with a known, general adaptation to the environment (Parker et al. 2019); and (iii) differential expression in strains adapted to glucose medium (LaCroix et al. 2015).

Supplementary material

Supplementary material is available at *Molecular Biology and Evolution* online.

Funding

National Research, Development and Innovation Office grants: “Élvonal” Programme KKP 129814 (B.P.) and KKP 126506 (C.P.), National Laboratory of Biotechnology Grant 2022-2.1.1-NL-

2022-00008 (C.P., B.P., W.A., T.F.), the National Laboratory for Health Security Grant RRF-2.3.1-21-2022-00006 (B.P.), the HUN-REN (TKCS-2024/66; to B.P.), the European Union's Horizon 2020 Research and Innovation Programme no. 739593 (B.P.), National Research, Development and Innovation Office FK 128916 (D.K.), National Research, Development and Innovation Office K146323 (C.P.), and the European Research Council ERC-2023-ADG 101142626 FutureAntibiotics (C.P.)

Conflicts of interest

None declared.

Data availability

The data underlying this article are available in the article and in its online [supplementary material](#).

References

- Albalat R, Cañestro C. Evolution by gene loss. *Nat Rev Genet*. 2016;17:379–391. <https://doi.org/10.1038/nrg.2016.39>.
- Baba T *et al*. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol*. 2006;2:2006.0008. <https://doi.org/10.1038/msb4100050>.
- Barrick JE *et al*. Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. *Nature*. 2009;461:1243–1247. <https://doi.org/10.1038/nature08480>.
- Barve A, Wagner A. A latent capacity for evolutionary innovation through exaptation in metabolic systems. *Nature*. 2013;500:203–206. <https://doi.org/10.1038/nature12301>.
- Behringer MG *et al*. Complex ecotype dynamics evolve in response to fluctuating resources. *mBio*. 2022;13:e03467–e03421. <https://doi.org/10.1128/mbio.03467-21>.
- Behringer MG *et al*. Trade-offs, trade-ups, and high mutational parallelism underlie microbial adaptation during extreme cycles of feast and famine. *Curr Biol*. 2024;34:1403–1413.e5. <https://doi.org/10.1016/j.cub.2024.02.040>.
- Blank D, Wolf L, Ackermann M, Silander OK. The predictability of molecular evolution during functional innovation. *Proc Natl Acad Sci U S A*. 2014;111:3044–3049. <https://doi.org/10.1073/pnas.1318797111>.
- Blount ZD, Borland CZ, Lenski RE. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2008;105:7899–7906. <https://doi.org/10.1073/pnas.0803151105>.
- Campbell RP, Whittington AC, Zorio DAR, Miller BG, Ozkan B. Recruitment of a middling promiscuous enzyme drives adaptive metabolic evolution in *Escherichia coli*. *Mol Biol Evol*. 2023;40:9427. <https://doi.org/10.1093/molbev/msad202>.
- Choudhury D, Saini S. Evolution of *Escherichia coli* in different carbon environments for 2,000 generations. *J Evol Biol*. 2019;32:1331–1341. <https://doi.org/10.1111/jeb.13517>.
- Conrad TM *et al*. Whole-genome resequencing of *Escherichia coli* K-12 MG1655 undergoing short-term laboratory evolution in lactate minimal media reveals flexible selection of adaptive mutations. *Genome Biol*. 2009;10:R118. <https://doi.org/10.1186/gb-2009-10-10-r118>.
- Cooper TF, Lenski RE. Experimental evolution with *E. coli* in diverse resource environments. I. Fluctuating environments promote divergence of replicate populations. *BMC Evol Biol*. 2010;10:11. <https://doi.org/10.1186/1471-2148-10-11>.
- Covert AW, Lenski RE, Wilke CO, Ofria C. Experiments on the role of deleterious mutations as stepping stones in adaptive evolution. *Proc Natl Acad Sci U S A*. 2013;110:E3171–E3178. <https://doi.org/10.1073/pnas.1313424110>.
- Farkas Z *et al*. Gene loss and compensatory evolution promotes the emergence of morphological novelties in budding yeast. *Nat Ecol Evol*. 2022;6:763–773. <https://doi.org/10.1038/s41559-022-01730-1>.
- Görke B, Stülke J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nat Rev Microbiol*. 2008;6:613–624. <https://doi.org/10.1038/nrmicro1932>.
- Guzmán GI *et al*. Reframing gene essentiality in terms of adaptive flexibility. *BMC Syst Biol*. 2018;12:143. <https://doi.org/10.1186/s12918-018-0653-z>.
- Helsen J *et al*. Gene loss predictably drives evolutionary adaptation. *Mol Biol Evol*. 2020;37:2989–3002. <https://doi.org/10.1093/molbev/msaa172>.
- Henderson PJ, Giddens RA, Jones-Mortimer MC. Transport of galactose, glucose and their molecular analogues by *Escherichia coli* K12. *Biochem J*. 1977;162:309–320. <https://doi.org/10.1042/bj1620309>.
- Hernández-Montalvo V *et al*. Expression of galP and glk in a *Escherichia coli* PTS mutant restores glucose transport and increases glycolytic flux to fermentation products. *Biotechnol Bioeng*. 2003;83:687–694. <https://doi.org/10.1002/bit.10702>.
- Herring CD *et al*. Comparative genome sequencing of *Escherichia coli* allows observation of bacterial evolution on a laboratory timescale. *Nat Genet*. 2006;38:1406–1412. <https://doi.org/10.1038/ng1906>.
- Hillmeyer ME *et al*. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science*. 2008;320:362–365. <https://doi.org/10.1126/science.1150021>.
- Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157:1262–1278. <https://doi.org/10.1016/j.cell.2014.05.010>.
- Jerison ER, Nguyen Ba AN, Desai MM, Kryazhimskiy S. Chance and necessity in the pleiotropic consequences of adaptation for budding yeast. *Nat Ecol Evol*. 2020;4:601–611. <https://doi.org/10.1038/s41559-020-1128-3>.
- Karve S. Evolutionary novelties in bacteria and the missing back-drop of the environment. *Environ Microbiol*. 2025;27:e70044. <https://doi.org/10.1111/1462-2920.70044>.
- Katz S *et al*. Dynamics of adaptation during three years of evolution under long-term stationary phase. *Mol Biol Evol*. 2021;38:2778–2790. <https://doi.org/10.1093/molbev/msab067>.
- Keseler IM *et al*. The EcoCyc Database in 2021. *Front Microbiol*. 2021;12:711077. <https://doi.org/10.3389/fmicb.2021.711077>.
- Kitagawa M *et al*. Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): unique resources for biological research. *DNA Res*. 2005;12:291–299. <https://doi.org/10.1093/dnares/dsi012>.
- Knöppel A *et al*. Genetic adaptation to growth under laboratory conditions in *Escherichia coli* and *Salmonella enterica*. *Front Microbiol*. 2018;9:385. <https://doi.org/10.3389/fmicb.2018.00756>.
- LaCroix RA *et al*. Use of adaptive laboratory evolution to discover key mutations enabling rapid growth of *Escherichia coli* K-12

- MG1655 on glucose minimal medium. *Appl Environ Microbiol*. 2015;81:17–30. <https://doi.org/10.1128/AEM.02246-14>.
- Lázár V *et al.* Genome-wide analysis captures the determinants of the antibiotic cross-resistance interaction network. *Nat Commun*. 2014;5:4352. <https://doi.org/10.1038/ncomms5352>.
- Leiby N, Marx CJ. Metabolic erosion primarily through mutation accumulation, and not tradeoffs, drives limited evolution of substrate specificity in *Escherichia coli*. *PLoS Biol*. 2014;12:e1001789. <https://doi.org/10.1371/journal.pbio.1001789>.
- Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol*. 2014;15:550. <https://doi.org/10.1186/s13059-014-0550-8>.
- McCloskey D *et al.* Evolution of gene knockout strains of *E. coli* reveal regulatory architectures governed by metabolism. *Nat Commun*. 2018a;9:3796. <https://doi.org/10.1038/s41467-018-06219-9>.
- McCloskey D *et al.* Adaptive laboratory evolution resolves energy depletion to maintain high aromatic metabolite phenotypes in *Escherichia coli* strains lacking the phosphotransferase system. *Metab Eng*. 2018b;48:233–242. <https://doi.org/10.1016/j.ymben.2018.06.005>.
- Meijnen J-P, de Winde JH, Ruijsenaars HJ. Engineering *Pseudomonas putida* S12 for efficient utilization of D-xylose and L-arabinose. *Appl Environ Microbiol*. 2008;74:5031–5037. <https://doi.org/10.1128/AEM.00924-08>.
- Monk JM *et al.* iML1515, a knowledgebase that computes *Escherichia coli* traits. *Nat Biotechnol*. 2017;35:904–908. <https://doi.org/10.1038/nbt.3956>.
- Moritz RL, Welch RA. The *Escherichia coli* argW-dsdCXA genetic island is highly variable, and *E. coli* K1 strains commonly possess two copies of dsdCXA. *J Clin Microbiol*. 2006;44:4038–4048. <https://doi.org/10.1128/JCM.01172-06>.
- Murray AW. Can gene-inactivating mutations lead to evolutionary novelty? *Curr Biol*. 2020;30:R465–R471. <https://doi.org/10.1016/j.cub.2020.03.072>.
- Neidhardt FC, Bloch PL, Smith DF. Culture medium for enterobacteria. *J Bacteriol*. 1974;119:736–747. <https://doi.org/10.1128/jb.119.3.736-747.1974>.
- Nyerges Á *et al.* A highly precise and portable genome engineering method allows comparison of mutational effects across bacterial species. *Proc Natl Acad Sci U S A*. 2016;113:2502–2507. <https://doi.org/10.1073/pnas.1520040113>.
- Olson MV. When less is more: gene loss as an engine of evolutionary change. *Am J Hum Genet*. 1999;64:18–23. <https://doi.org/10.1086/302219>.
- Ostrowski EA, Rozen DE, Lenski RE. Pleiotropic effects of beneficial mutations in *Escherichia coli*. *Evolution*. 2005;59:2343–2352. <https://doi.org/10.1111/j.0014-3820.2005.tb00944.x>.
- Pal A, Iyer MS, Srinivasan S, Narain Seshasayee AS, Venkatesh KV. Global pleiotropic effects in adaptively evolved *Escherichia coli* lacking CRP reveal molecular mechanisms that define the growth physiology. *Open Biol*. 2022;12:210206. <https://doi.org/10.1098/rsob.210206>.
- Parker DJ, Demetci P, Li G-W. Rapid accumulation of motility-activating mutations in resting liquid culture of *Escherichia coli*. *J Bacteriol*. 2019;201:10.1128/jb.00259-19. <https://doi.org/10.1128/JB.00259-19>.
- Parts L *et al.* Revealing the genetic structure of a trait by sequencing a population under selection. *Genome Res*. 2011;21:1131–1138. <https://doi.org/10.1101/gr.116731.110>.
- Patrick WM, Quandt EM, Swartzlander DB, Matsumura I. Multicopy suppression underpins metabolic evolvability. *Mol Biol Evol*. 2007;24:2716–2722. <https://doi.org/10.1093/molbev/msm204>.
- Perrenoud A, Sauer U. Impact of global transcriptional regulation by ArcA, ArcB, Cra, Crp, Cya, Fnr, and Mlc on glucose catabolism in *Escherichia coli*. *J Bacteriol*. 2005;187:3171–3179. <https://doi.org/10.1128/JB.187.9.3171-3179.2005>.
- Phaneuf PV *et al.* Causal mutations from adaptive laboratory evolution are outlined by multiple scales of genome annotations and condition-specificity. *BMC Genomics*. 2020;21:514. <https://doi.org/10.1186/s12864-020-06920-4>.
- Pósfai G, Koob MD, Kirkpatrick HA, Blattner FR. Versatile insertion plasmids for targeted genome manipulations in bacteria: isolation, deletion, and rescue of the pathogenicity island LEE of the *Escherichia coli* O157:H7 genome. *J Bacteriol*. 1997;179:4426–4428. <https://doi.org/10.1128/jb.179.13.4426-4428.1997>.
- Robinson James T *et al.* Integrative genomics viewer. *Nat Biotechnol*. 2011;29:24–26. <https://doi.org/10.1038/nbt.1754>.
- Rosconi F *et al.* A bacterial pan-genome makes gene essentiality strain-dependent and evolvable. *Nat Microbiol*. 2022;7:1580–1592. <https://doi.org/10.1038/s41564-022-01208-7>.
- Rousset F *et al.* The impact of genetic diversity on gene essentiality within the *Escherichia coli* species. *Nat Microbiol*. 2021;6:301–312. <https://doi.org/10.1038/s41564-020-00839-y>.
- Szamecz B *et al.* The genomic landscape of compensatory evolution. *PLoS Biol*. 2014;12:e1001935. <https://doi.org/10.1371/journal.pbio.1001935>.
- Towbin BD *et al.* Optimality and sub-optimality in a bacterial growth law. *Nat Commun*. 2017;8:14123. <https://doi.org/10.1038/ncomms14123>.
- Venkataraman P, Nagendra P, Ahlawat N, Brajesh Raman G, Saini S. Convergent genetic adaptation of *Escherichia coli* in minimal media leads to pleiotropic divergence. *Front Mol Biosci*. 2024;11:1286824. [about 13 p.]. <https://doi.org/10.3389/fmolb.2024.1286824>.
- Wang M, Liu Q, Kang X, Zhu Z, Yang H *et al.* Glycogen Metabolism Impairment via Single Gene Mutation in the glgBXCAP Operon Alters the Survival Rate of *Escherichia coli* Under Various Environmental Stresses. *Front Microbiol*. 2020;11:588099. <https://doi.org/10.3389/fmicb.2020.588099>.
- Wagner A. The white-knight hypothesis, or does the environment limit innovations? *Trends Ecol Evol*. 2017;32:131–140. <https://doi.org/10.1016/j.tree.2016.10.017>.
- Wannier TM *et al.* Adaptive evolution of genomically recoded *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2018;115:3090–3095. <https://doi.org/10.1073/pnas.1715530115>.
- Warringer J, Blomberg A. Automated screening in environmental arrays allows analysis of quantitative phenotypic profiles in *Saccharomyces cerevisiae*. *Yeast*. 2003;20:53–67. <https://doi.org/10.1002/yea.931>.
- Wytock TP *et al.* Experimental evolution of diverse *Escherichia coli* metabolic mutants identifies genetic loci for convergent adaptation of growth rate. *PLoS Genet*. 2018;14:e1007284. <https://doi.org/10.1371/journal.pgen.1007284>.
- Xu Y-C, Guo Y-L. Less is more, natural loss-of-function mutation is a strategy for adaptation. *Plant Commun*. 2020;1:100103. <https://doi.org/10.1016/j.xplc.2020.100103>.