Metabolic constraints modulate the likelihood and predictability of global epistasis.

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Running Title: *A mechanistic explanation of global epistasis*

Abstract

Epistasis, where a mutation's effect depends on its genetic background, is a key driver of evolution. A key pattern, global epistasis, suggests that mutation fitness effects can be predicted based on background fitness. However, no mechanistic explanation is known for this statistical trend.

Here, we present a first-principles model demonstrating that global epistasis emerges naturally from metabolic constraints on cell growth and division. Using a prokaryotic growth model based on an adder-sizer metabolic principle, we show that: (i) global epistasis becomes less strong as genetic variation increases, and (ii) mutations in rate-limiting terminal reaction module of an enzymatic pathway exhibit the most predictable fitness effects. These findings establish a direct link between metabolism, genetic interactions, and evolutionary predictability.

By uncovering the mechanistic basis of global epistasis, our study advances the understanding of evolutionary constraints and has implications for antibiotic resistance, synthetic biology, and microbial adaptation in dynamic environments.

Keywords: Epistasis; Global epistasis; Mutation Effects; Metabolism.

Introduction

Predicting evolution is a fundamental challenge in biology, with implications on biodiversity, genetics, human health, biogeochemical processes, and agriculture[1–6]. A key obstacle is epistasis, where mutation effects depend on genetic background, shaping evolutionary trajectories in complex ways[7,8]. One striking pattern observed in experimental evolution is global epistasis, where the fitness effects of mutations systematically decline as background fitness increases[9–12]. While this trend has been widely documented, there are also exceptions[13,14]. The mechanistic origins of these patterns, however, remain unclear[15].

Most studies of epistasis rely on statistical modeling approaches[16,17], or empirically characterizing individual mutation effects across backgrounds and analyzing fitness effect distributions (DFEs)[12,18–27]. However, the vast number of possible mutations and genotypes makes full experimental characterization impractical[28,29]. Although metabolic networks and protein interactions have been implicated in epistasis[30–38], no first-principles model explains its statistical patterns or why fitness effects follow predictable trajectories despite the complexity of genetic and metabolic interactions.

Here, we present a mechanistic framework demonstrating that global epistasis arises naturally from metabolic constraints on cell growth and division. Using a quantitative prokaryotic metabolism model[39], we show that mutations in rate-limiting terminal enzymatic steps exhibit strong negative global epistasis, while mutations in interconnected steps display less predictable effects. Furthermore, as background genetic variability increases, epistasis' predictability declines, marking a transition from deterministic to idiosyncratic fitness effects.

Our findings have broad implications for microbial adaptation, antibiotic resistance evolution, and biotechnological strain engineering. Additionally, our model generates testable predictions, making it a valuable theoretical tool for guiding future empirical studies. By moving beyond statistical correlations to mechanistic insights, this study provides a foundational framework for understanding how metabolic constraints shape evolutionary dynamics.

Results

Mutational Fitness Effects Depend on Genetic Background and Exhibit Global Epistasis/

We modelled a prokaryotic cell with three rate-limiting metabolic pathways, each containing two enzymatic reactions (**Figure 1A**). Cell division occurs once predefined threshold amounts of all three metabolites (P_1, P_2, and P_3) are accumulated. The time taken to reach this threshold (t_{div}) determines division timing, with fitness defined as its reciprocal ($1/t_{div}$).

We call reach reaction step a 'module', numbered from one to six in the cell. Each module is defined by four parameters. Two of these parameters, t_{ON} and t_{OFF}, define the promoter activity; while the other two, k_{cat} and K_M, define the enzyme properties.

To study epistasis, we introduced a mutation by altering a parameter (t_{ON1}) in module 1, which dictates the production of intermediate I_1 and directly regulates flux through the other two pathways by drawing up the common substrate (indicated by a triangle in **Figure 1A**). The mutation of interest in this study, unless specified otherwise, is t_{ON1} changing from 0.5 to 1. We assess how this mutation's fitness effect varied across distinct genetic backgrounds (See Methods).

Figure 1B illustrates the mutation's fitness effect in four representative cases, where cells differed in one, two, three, or four background parameters. The distributions across all conditions are shown in **Supplementary Figure 1**. Our results demonstrate that mutation effects are not uniform but vary with genetic background, a hallmark of epistasis. This trend persisted even when the number of threshold metabolites was altered (**Supplementary Figure 2**).

A key signature of global epistasis is that the fitness effect of a mutation declines predictably as background fitness increases. To test whether the mutation changing t_{ON1} from 0.5 to 1 in module 1 exhibited this property, we plotted fitness effects of this mutation against background fitness. The strength of this relationship is quantified by R^2 of a linear regression, with higher values indicating stronger global epistasis[40]. Experimental studies have observed epistasis of varying globality in microbial evolution, with R^2 ranging from 0.4 to 0.9[9,10,40–42]. **Figure 1C** presents representative regression analyses, confirming that our model captures global epistasis. A full set of 300 regressions across all background conditions is provided in **Supplementary Figure 3**.

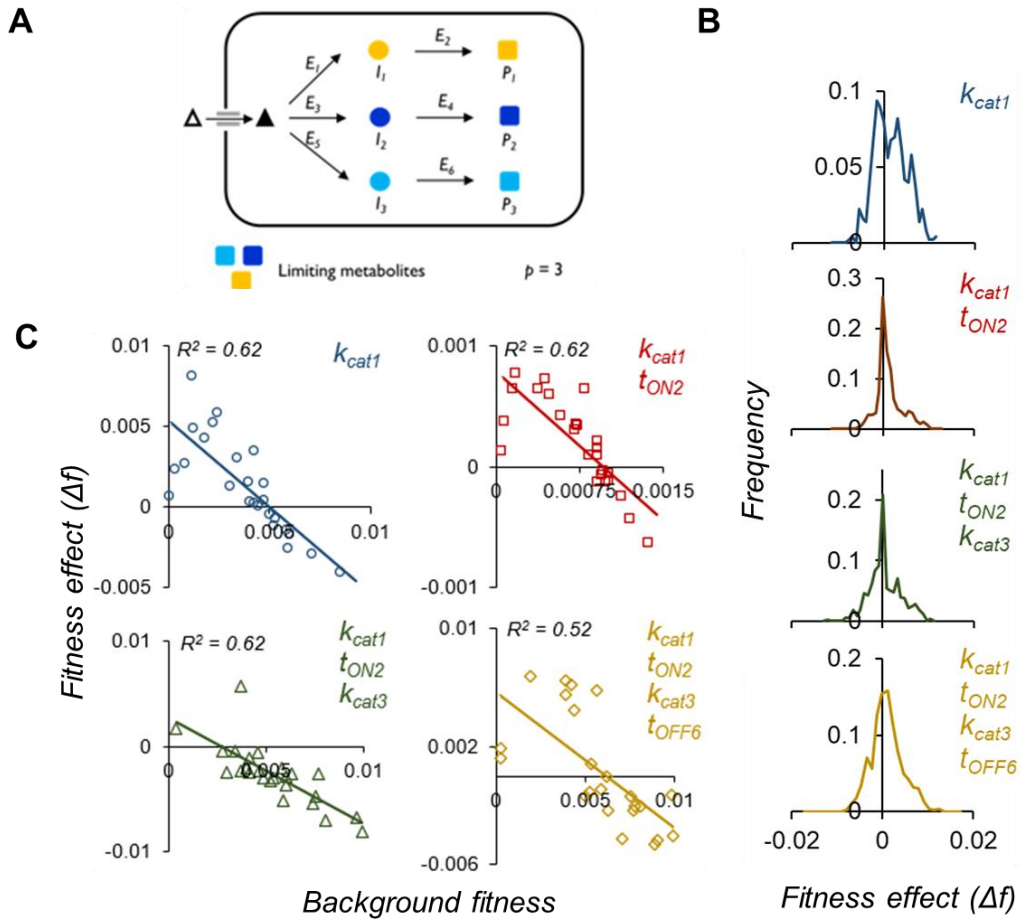

Figure 1. Mutational fitness effects depend on genetic background and vary in predictability. (A) Modeled cells divide upon accumulating threshold amounts of three bottleneck metabolites (P_1, P_2, and P_3), each produced via enzymatic cascades from a common substrate. **(B)** The distribution of fitness effects for a fixed mutation (t_{ON1} changes from 0.5 to 1) is shown across genetic backgrounds differing in one (k_{cat1}), two (k_{cat1}, t_{ON2}), three (k_{cat1}, t_{ON2}, k_{cat3}), or four (k_{cat1}, t_{ON2}, k_{cat3}, t_{OFF6}) parameters. Fitness effects are non-uniform, confirming their dependence on genetic background. **(C)** Regression plots of fitness effects against background fitness for mutations occurring in backgrounds differing by one, two, three, or four parameters. The fitness effect of a mutation is predictable to varying degrees based on background fitness, demonstrating global epistasis.

Stronger Global Epistasis is Less Likely with Increased Background Variation

Global epistasis can be characterized by the slope of the regression between fitness effects and background fitness. A negative slope indicates diminishing-returns and increasing-costs epistasis, while a positive slope reflects increasing-returns and diminishing-costs epistasis[40]. To determine whether global epistasis was predominantly negative or positive, we analyzed the

slopes of regression lines (**Supplementary Figure 3**). As shown in **Figure 2A**, negative global epistasis (NGE) remained the dominant pattern across all levels of background variation.

Despite NGE being common, the skewness of the distribution of R^2 values increased as the extent of background variation increased (*1-site*: -0.5864; *2-site*: -0.365; *3-site*: -0.951; *4-site*: 0.2385), though the mean values remained statistically equal (**Figure 2B**, two-tailed *t*-test, $p > 0.05$; see **Supplementary Figure 4** for full distributions across fifteen background variation types).

We defined strong negative global epistasis as cases where $R^2 > 0.5$, distinguishing them from weaker instances. Using this threshold, we quantified how often strong NGE occurred at different levels of background variation. As shown in **Figure 2A**, the likelihood of strong negative global epistasis decreases as background variation increases.

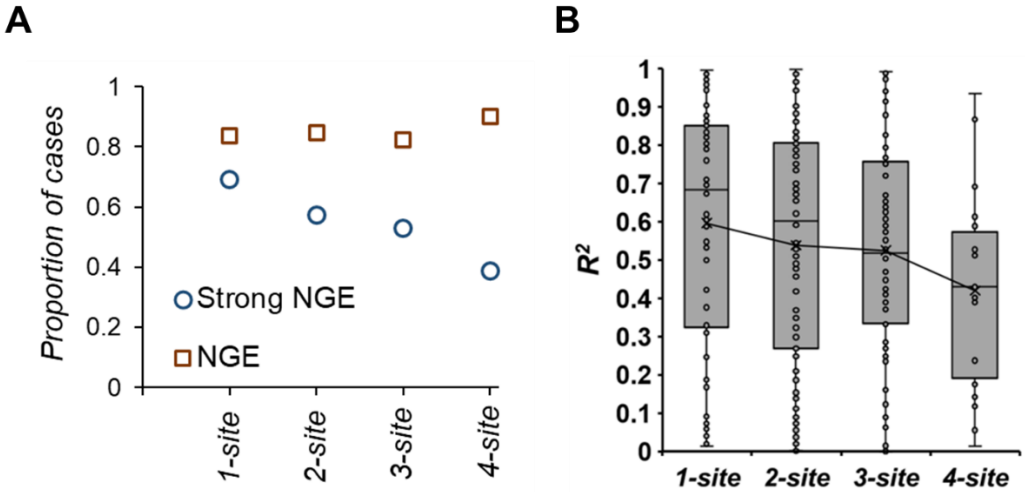

Figure 2. Strong negative global epistasis (NGE) becomes less frequent as background variation increases. (A) The sign of global epistasis was determined from the slope of the regression between mutation fitness effects and background fitness. Negative global epistasis (NGE; squares) remained dominant across all levels of background variation. However, the frequency of strong NGE ($R^2 > 0.5$; circles) decreased as background variation increased. **(B)** Distribution of R^2 values for NGE cases across different background variation levels. While R^2 values varied, their mean remained statistically unchanged ($p > 0.05$, two-tailed *t*-test). See **Supplementary Figure 5** for full distributions across all 15 background variation types.

Reaction Speeds Predict the Strength of Global Epistasis

We next examined whether the strength of global epistasis can be predicted based on the relative speeds of the enzymatic modules within the cell. To test this, we constructed four types

of simplified ("toy") cells where six enzymatic modules were predefined as either fast or slow (see Methods). As shown in **Figure 3A**, we created the following variants:

1. 1A – One slow first-step module, with all others fast.
2. 1B – One fast first-step module, with all others slow.
3. 2A – One slow second-step module, with all others fast.
4. 2B – One fast second-step module, with all others slow.

Thus, each cell type contains one outlier reaction step compared to the rest. We simulated twenty-five cells of each type and introduced a hundred random mutations in the outlier step to assess how global epistasis varies.

Our results (**Figure 3B**) show that only mutations in slow second-step modules (2A) exhibit the strongest global epistasis, with fitness effects most strongly correlated with background fitness. In contrast, mutations in other reaction types (1A, 1B, and 2B) show weaker global epistasis. These findings hold even when metabolic network architecture is altered, such that the cell has two threshold metabolites each produced by a cascade of three enzymatic reactions (**Supplementary Figure 5**). Additionally, when we introduced strong metabolic feedback—shut down a reaction arm once a threshold amount of metabolite was produced—we found that mutations in the slowest reaction steps consistently exhibit strong global epistasis (**Supplementary Figure 5**).

Recent studies suggest that as background fitness increases, beneficial mutations become rarer while deleterious mutations increase, reinforcing that global epistasis strongly determines the sign of mutational effects[17,42]. Our findings provide a mechanistic basis for this observation.

Using our dataset of a hundred mutations across twenty-five genotypes in each of the four cell types, we analyzed the mean and variance of these distributions of fitness effects (DFEs). While mean DFE values consistently declined with increasing background fitness (**Supplementary Figure 6**), this decline was most predictable in case 2A, where mutations affected a slow second-step module (**Figure 3C**). This result holds when the metabolic architecture is altered, and when feedback is introduced (**Supplementary Figure 7**). On the other hand, variance of the DFE did not follow a clear pattern, showing no consistent correlation with background fitness (**Figure 3C**, **Supplementary Figure 8**).

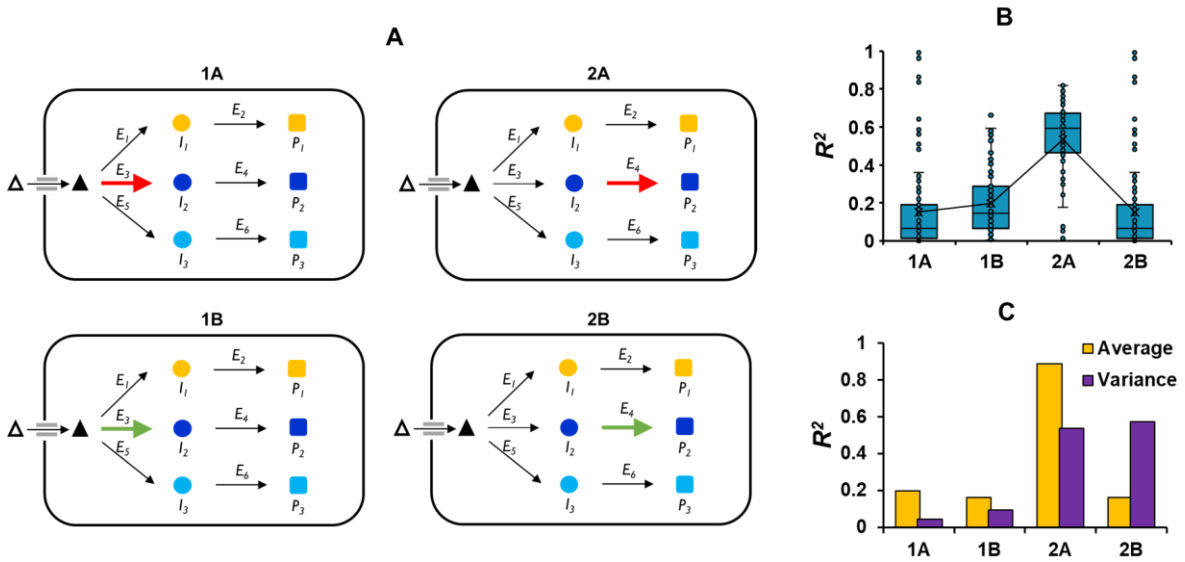

Figure 3. Predictability of mutational fitness effects depends on reaction speed and metabolic control. (A) We constructed four types of simplified ("toy") cells, each with six enzymatic modules pre-defined as either slow (red arrows) or fast (green arrows). The outlier module in each cell type is indicated by bold arrows: 1A: One slow first-step module, with all others fast; 1B: One fast first-step module, with all others slow; 2A: One slow second-step module, with all others fast; and 2B: One fast second-step module, with all others slow. **(B)** To assess global epistasis, we introduced a hundred mutations in the outlier step of twenty-five genetic backgrounds for each cell type and performed a linear regression of mutation fitness effects against background fitness. R^2 values for each mutation are plotted, with mean trends shown by a connecting line. Mutations in slow second-step modules (2A) exhibit the strongest global epistasis, with fitness effects most predictable using background fitness. **(C)** The strength of global epistasis was further quantified by performing linear regressions of DFE mean and variance against background fitness for each cell type. While the mean fitness effect declines predictably in case 2A, variance does not follow a consistent trend.

Discussion

In this study, we developed a mechanistic cell growth and division model that captures epistasis and its globality feature, revealing how metabolic constraints shape mutational effects and drive declining adaptability. Our model defines fitness based on the time required to accumulate threshold levels of key metabolites before division. In living organisms, metabolic networks are complex and reaction arms are mostly interconnected, except terminal steps that lead to the production of metabolites[43]. While the cell that we model does not encode an intricate network, we have two types of reaction modules – ones that control the flux through the other paths, and ones that do not exert control over other paths. This metabolic structure in the cell allows us to comment on the macroscopic effects of coding and non-coding mutations in both types of reaction modules that are part of real cellular networks.

Empirical studies show that negative global epistasis (NGE)—where beneficial mutation's effects diminish as background fitness increases—is common but not universal[13,14,44]. Our model reproduces this trend and predicts that strong NGE becomes less frequent as genetic variability increases. Additionally, we also show that mutations in the slowest, least-controlling reaction modules exhibit strong NGE. As these slow steps improve, beneficial mutations become rarer, contributing to declining adaptability over time[23,26,45].

Conversely, positive global epistasis (increasing-returns epistasis), which implies synergistic mutational effects[46–49], is predicted to occur in highly interconnected modules, where a single mutation can yield a significant fitness gain.

Beyond its theoretical insights, this framework has broad implications for understanding how metabolic complexity influences epistasis. Future work could extend this model by incorporating cellular regulation layers and environmental changes, to explore the role of these factors in dictating epistasis' predictability[40,50]. Additionally, our findings could help in engineering microbial strains with predictable evolutionary trajectories, impacting biotechnology and antimicrobial resistance research[21,51].

Our model provides a quantitative framework for global epistasis. Importantly, it also makes clear testable predictions. In the metabolic map of the cell, several key metabolites feed branches of enzymatic reactions. For instance, when *E. coli* is grown in anaerobic conditions, acetyl-CoA feeds into the Krebs cycle, fatty acid biosynthesis, and mixed acid fermentation[52–54]. Similarly, glutamate is a substrate for the synthesis of glutamine, proline and arginine[55]. Our

model predicts the reaction modules in these pathways in which mutations, under appropriate conditions, would exhibit strong global epistasis. Our model also predicts sign-epistasis between promoter and gene mutations, which is likely to constrain evolutionary trajectories through mechanisms which are not fully understood yet (**Supplementary Figure 9**)[50,56].

Ultimately, this work provides a quantitative foundation for predicting fitness gains in evolving populations. Future studies will be crucial in testing and refining these insights, to further our understanding of the role of cellular processes in dictating evolutionary dynamics.

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