ADAPTATION, EPISTASIS, AND THEIR RELATIONSHIP WITH METABOLIC ENVIRONMENT IN *ESCHERICHIA COLI*

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ADAPTATION, EPISTASIS, AND THEIR RELATIONSHIP WITH METABOLIC ENVIRONMENT IN *ESCHERICHIA COLI*

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Thesis

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ABSTRACT

The benefit or detriment of a mutation cannot be determined on its own, but rather must be considered in connection with the expression of other genes. Furthermore, expression as a whole can be altered by the organism's external environment. In this study, we examine the interactions between five coevolved alleles, and how those interactions may be affected by changes to the external environment. We find that despite being selected for in a single environment, these mutations remain beneficial across a broad range of metabolites. We also find that the epistasis underlying the adaptive landscape is highly dependent on the resource environment. These results have implications for the study of the adaptive landscape in an environmental context.

ACKNOWLEDGEMENTS

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CHAPTER I

ADAPTATION, EPISTASIS, AND THEIR RELATIONSHIP WITH METABOLIC ENVIRONMENT IN *ESCHERICHIA COLI*

Introduction

Adaptation, chance, and history have been shown to influence an organism's fitness, and in turn, evolutionary trajectory (Travisano et al. 1995). The order of the introduction of new mutations is important because the fitness effect of any individual allele depends on the genetic as well as ecological environments in which it arises. This is especially true in asexual populations where beneficial allelic combinations cannot be brought together through recombination. In asexual populations, the order of past mutations establishes the history, each new mutation is a chance event and the ecological environment in combination with these two factors determines the course of adaptation. No studies have looked at the mechanistic interplay of all of these factors across a broad range of environments. Using a set of 32 genetic constructs based on five beneficial alleles (Khan et al. 2011) in concert with 8 different resource environments, we investigate the mechanistic basis of history, chance and adaptation. Exploration of these dynamics can inform us of how evolutionary processes like adaptation and speciation occur or may be constrained.

The fitness consequence of a new allele is not inherent to the allele itself. Instead its effect must be determined functionally in context with the genetic background from which it arose and the challenges and opportunities presented by the physical surroundings (Wright 1982). The most common incarnation of the landscape shows one or more "peaks" corresponding to fitness (sub-)optima of a population. Normally a landscape is intended to represent the influence of genetic interactions in a given environment. In this heuristic, selection is expected to drive populations towards the top of a local peak. Multiple peaks are separated by "valleys." It is not possible for one population to travel through a valley to another peak without the influence of a force other than natural selection (e.g. drift, recombination or an increase in phenotypic variation). In effect, adaptive landscapes define the evolutionary boundaries available to a population (Wright 1982).

Travisano et al. defined three conceptually and mathematically distinct processes useful in illustrating how a clonal population arising from a single genotype explores an adaptive landscape during its evolution. "History" refers to the original genotype from which all future genotypes must arise. "Chance" is the process by which stochastic variation is increased in a population through events like the migration, drift, or mutations in the genetic code. "Adaptation" refers to the selective increase in frequency of an allele that provides a benefit to the organism in its current environment. In context of an adaptive landscape, "history" is a genetic starting position on the fitness surface at the outset of evolution (Wright 1982). "Chance" is any process through which the population is allowed to explore the surface due to the introduction of stochastic

variation. "Adaptation" is the process by which the genotype moves closer to its adaptive peak due to chance events (Travisano et al. 1995).

When attempting to measure the effects of individual alleles in context with others, we must take into account that combinations of alleles may not produce the fitness outcome predicted by the behavior of single ones. Several null models have been proposed in certain circumstances (Mani et al. 2008) but a multiplicative model (Khan et al. 2011) best suits the *E. coli*-based system employed in this study. Under the multiplicative model, we expect genotypes with two or more of our focal alleles to have fitness values equal to the product of the fitness values produced by each allele expressed alone in the same background. Deviations between observed and expected values of fitness are defined as epistatic interactions.

Epistasis can be thought of as having both a magnitude and direction of effect on fitness. Effect direction describes the force of selection on mutations as they are added and can be beneficial (positive) or detrimental (negative) to the organism. Effect magnitude describes the deviation between observed fitness and predicted fitness for a genotype with two or more mutations under a directly multiplicative model. Instances where the observed effect is greater than expected are said to exhibit synergistic epistasis while those effects less than expected are experiencing antagonistic epistasis (Desai et al. 2007). In special cases, sign epistasis may occur, in which selection acts negatively on mutations in some combinations but positively in others (Weinreich et al. 2005).

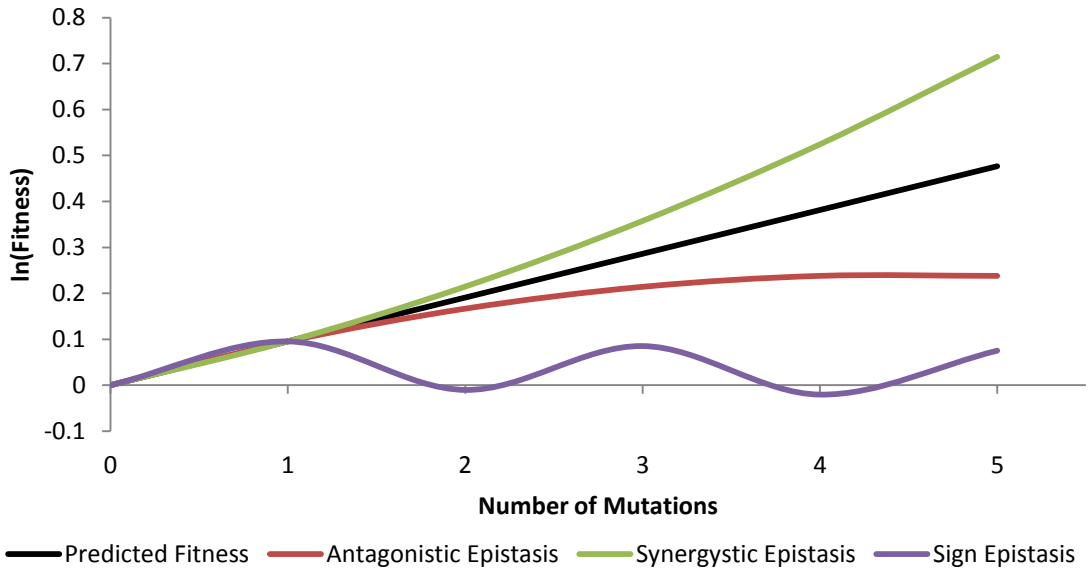


Figure 1 – Potential outcomes of epistasis. Linearized fitness values are plotted against an increasing number of mutations. Predicted fitness (black), antagonistic epistasis (red), synergistic epistasis (green) and sign epistasis (purple) are modeled here for beneficial mutations.

We expand the Desai et al. (2007) epistasis model to include multiplicative fitness effects using the equation:

$$\ln \omega_k = \ln\left(\omega_{k-1}(1+s)^{(1+\epsilon(k-1))}\right)$$

where, ω is relative fitness, *k* is number of mutations, *s* is the cost/benefit of each allele and ∈ is the sign and magnitude of epistasis. This equation produces the green (synergistic), black (expected) and red (antagonistic) lines in Figure 1 above where *s* is 0.1 corresponding to a 10% gain in fitness for each mutation and the value of epistasis is ±0.25. The Desai model does not include sign epistasis. We add one (of many possible) examples of sign epistasis here modeled as:

$$\ln \omega_k = \ln(\omega_{k-1} + (-1)^{k-1} s(\omega_{k-1}))$$

where the direction of effect of a novel mutation is altered by the presence of previous mutation.

In a previous long-term evolution experiment of *E. coli*, Richard Lenski's lab found a clear pattern of decelerating improvement in fitness emerged after 2,000 generations of evolution and persisted through at least 20,000 generations (Lenski et al. 1991; Lenski and Travisano 1994; Barrick et al. 2009). This pattern is found across other strains of *E. coli* (Moore and Woods 2006). Antagonistic epistasis is thought to be the driving force behind this pattern of diminishing returns (Khan et al. 2011). Similar results are found in another study of beneficial mutations in a strain of *Methylobacterium extorquens* (Chou et al. 2011).

Altering genetic history through a single mutational event can have measurable impacts on fitness. Khan et al. (2011) prepared a suite of bacterial constructs consisting of all possible combinations of alleles resulting from five mutations that spread sequentially through Lenski's evolving population. Analysis of these constructs in the context of the evolved environment showed a smooth adaptive landscape with a single peak (Figure 2). Though the evolutionary pathway that was taken followed steps that increased fitness with the addition of each new allele, the steps taken were not necessarily the fittest possible combination. The order in which alleles were added to the ancestral background determined which pathways through the landscape were available without having to cross an adaptive "valley". In this case, where the optimum genotype contained all five of the focal alleles, order may actually seem relatively unimportant. All allowable pathways lead to the same local optimum. However, in a landscape with multiple peaks, order of chance events may determine what optima are possible to reach.

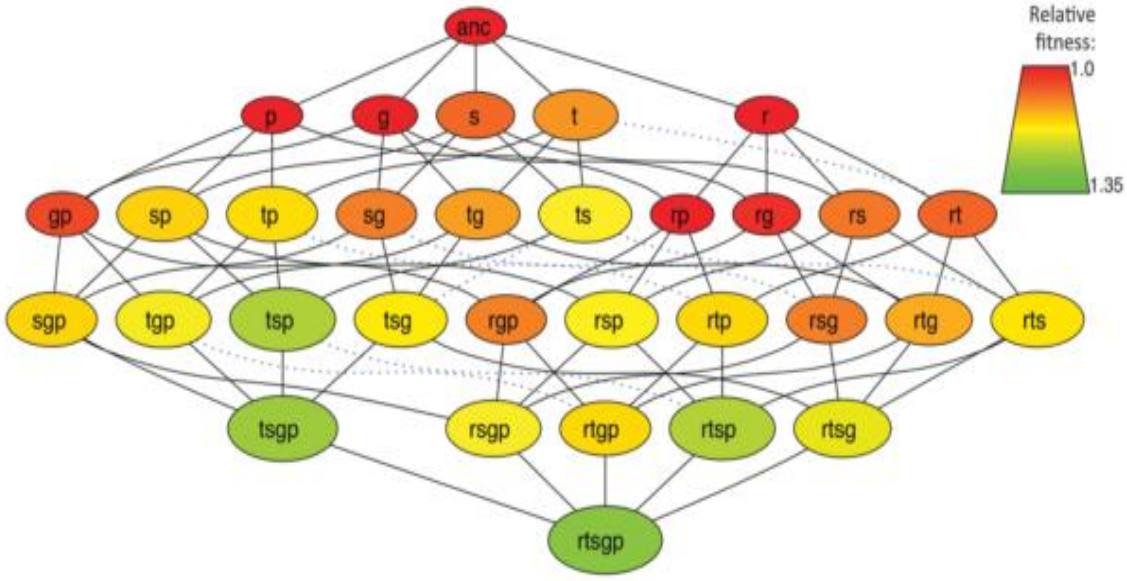


Figure 2 – Mutational network describing the adaptive landscape of 32 genetic constructs in glucose-supplemented media. (From Khan et al. 2011.)

The fitness phenotype is a product of interactions among individual genes, as well as the interaction of the entire genome with its environment. Plasticity refers to variability in phenotype governed by ecological factors, such as temperature and available nutrients. Single mutations are sufficient to produce measureable gene-by-environment interactions. Altering the environment has been shown to change both fitness effect and variance associated with a given set of mutations and external conditions (Remold and Lenski 2001). In that study of random insertion mutations, it appears that plastic effects are dependent on genetic background, meaning phenotypic plasticity consistently depends on underlying epistasis.

Gene-environment interactions tend to follow one of three main patterns. Antagonistic pleiotropy is a system of true trade-offs. Interactions between genes and their surroundings may be beneficial in the environment in which it arose, but deleterious in most others. Examples of antagonistic pleiotropy have been found in Lenski's 10,000-

generation study of clonal populations of *E. coli* in a minimal glucose environment (Cooper and Lenski 2000). Evolved lines in this study had a nearly 25-percent reduction in their ability to utilize multiple sources of carbon for energy. The same mutations that lead to the reduction of the catabolic repertoire were themselves beneficial in the limited glucose environment. This illustrates the importance of the evolutionary environment as a selection pressure that favors reduced costs and increased efficiency of essential functions (Cooper and Lenski 2000). While reduction of diet breadth may prove costly under the wrong environmental conditions, the evolved strains had higher fitness than the ancestor in the evolved environment, as well as in novel environments where resource utilization was possible (Cooper 2002).

A true trade-off system does not account for all the patterns of gene-environment interaction seen in laboratory and wild populations. Allelic changes may have no effect on fitness (i.e. neutral mutations) when they are fixed in a population, but may be deleterious effects in alternate environments (Funchain et al. 2000). This type of interaction may be especially important in asexual populations, where independently-arising beneficial mutation cannot be recombined, but rather compete through clonal interference (Fisher 1930; Muller 1932; Gerrish and Lenski 1998). Moreover, changing environment does not necessitate a decrease in fitness. Some selected mutations may have globally beneficial (or neutral) results, but the degree of benefit may be linked to external conditions (Fry 1993; Fry 1996).

To summarize, epistasis and gene-environment interactions play a critical role in defining the pathways of evolution available to a population. We have a slowly growing body of evidence of this critical role. Travisano et al. (1995) examines how changing the

evolved environment affects evolutionary trajectory in real time, but it lacks mechanistic detail at the genetic level. Khan et al. (2011) provides an excellent breakdown of the epistatic interactions between evolved beneficial mutations, but characterizes these interactions only for the evolved environment (glucose). Flynn et al. (2013) is the first to use a design combining populations of known clonal genotypes with multiple environments. However, the number and types of environments limit the generality of those results. In order to more generally explore the effects of mutational order (genetic history) across environments, we extend the system used in Flynn et al. (2013).

In this study, we investigate the mechanistic basis of adaptation, chance, and history by measuring fitness in 256 combined environments. We examine 32 constructed genotypes representing the adaptive mutations found in one evolving population (Lenski et al. 1991, Khan et al. 2011) across 8 resources. We examine the potential role of epistatic interactions and ecological environment in determining the likely path of adaptation.

Materials and Methods

Mutations and Bacterial Constructs

The first five beneficial mutations to fix in Lenski et al.'s (1991) population of *Escherichia coli* REL606 are utilized in this study. These mutations were fixed in evolving populations in minimal glucose conditions The individual effects of these mutations have been characterized and are presented below (Table 1) in the order in which they appear to have arisen. First, ribose catabolism is lost via the deletion of the *rbs* operon (Cooper et al., 2001). Second, a nonsynonymous point mutation in *topA* leads

to an increase in DNA supercoiling (Crozat et al., 2005). Third, a nonsynonymous point mutation in *spoT* reduces the amount of time spent in lag phase and increases the maximum growth rate (Cooper et al., 2003). Fourth, a 1-base pair insertion reduces the expression of the *glmUS* operon involved in cell wall production (Stanek et al., 2009). Lastly, insertion of *IS150* into the *pykF* gene results in a loss of function of pyruvate kinase I (Schneider et al., 2000). Constructs representing all 31 possible combinations of these five mutations were engineered by the lab of T.F. Cooper (University of Houston). Their assembly has been described previously (Khan et al., 2011).

Table 1 –Five focal mutation events and their consequent effects.

Gene	**Mutation type**	**Effect**
rbs ('r')	deletion (~3kb)	-Loss of ribose catabolism -high mutation rate
topA ('t')	nonsynonymous point mutation	-Increased DNA supercoiling
spoT ('s')	nonsynonymous point mutation	-Increased max growth rate -Stringent response
glmUS ('g')	deletion (1bp)	-Altered promoter -Reduced expression of genes involved in cell wall synthesis
pykF ('p')	insertion (*IS150*)	-Loss of function of *pyruvate kinase I*

Experimental Design

The relative fitness of each combination of mutations ($2^5 = 32$ genotypes) was measured in 8 metabolic environments. The experiment was performed in five blocks for a total $N = 1280$. 42 data points could not be included in the analysis due to non-growth of both strains in competition for a final $N = 1238$. All mutation-by-resource combinations are represented by at least four valid measures.

Competitive Fitness Assays

Each of the 31 constructed strains and the progenitor strain (REL606) used in this study are unable to utilize arabinose (Ara-). This allows for direct competition with an

Ara+ strain that is otherwise identical to the progenitor (REL607). Ara- strains appear red and Ara+ strains appear white on tetrazolium-arabinose (TA) agar. This allows for the proportions of ancestral and recombinant competitors to be counted directly. Competitions took place in Davis minimal salts supplemented 8 different carbon resources as summarized in Table 2. The amount of each supplement was chosen due to its ability to support equivalent densities of the common ancestor REL 606.

Table 2 – Media supplements for fitness assays.

Supplement	**Type**	**[mg/L]**
Glucose	Monosaccharide (PTS)	25
Acetate	Short chain fatty acid	12.5
Beef Extract	Peptides, amino acids, nucleotides, organic acids, minerals, vitamins	300
Casamino Acids	Amino acids	70
Milk Protein Hydrolysate	Peptides, amino acids, simple and complex carbohydrates	180
N-Acetylglucosamine (NAG)	Monosaccharide (PTS)	125
Rhamnose	Monosaccharide (Non-PTS)	60
Trypsin	Whole protein	20

Prior to the start of competition, strains were allowed to acclimate to the assay conditions for 24 hours. Following this habituation, Ara- strains were mixed in equal volumes and diluted 100-fold into new media. A sample of this culture was taken immediately and plated on TA agar to establish initial proportions. The culture was propagated at 37°C, shaken at 120 rpm for approximately 48 hours with a 100-fold dilution due to transfer to fresh media occurring after 24 hours. Final samples were plated at the close of competition. Malthusian parameters (m) were calculated for both Ara^+ and Ara^- strains in each competition using the formula

$$m = \ln\left(\frac{N_2 * 100^2}{N_0}\right)$$

where N_0 and (N_2*the dilution factor) represent initial and final densities respectively. Relative fitness values were calculated by dividing the Malthusian parameter of the constructed strains by that of their ancestor. Prior to analysis, all relative fitness values were scaled within resource by dividing each measure by the average fitness of the ancestor, REL606 relative to its Ara- competitor REL607, in that resource. Absolute epistasis was calculated as the deviation from predicted fitness determined using a multiplicative model

$$\varepsilon_M = \omega_M - \prod_{i \in M} \omega_i$$

where ω_M is the observed relative fitness of a genotype with two or more mutations from set *M* and predicted fitness is equal to the product of the relative fitnesses of the individual constituent mutations, *i* (da Silva et al., 2010).

Statistical Analyses

All statistical analyses were performed using JMP Pro 10. An ANOVA was used to examine mutation type and resource as potential sources of variance for the fitness of strains containing only a single mutation. ANCOVAs with number of mutations and metabolic environment as main effects were run to assess the contributions of accumulated mutations and external environment both to overall fitness and to epistasis.

Results

Fitness of individual mutations in multiple environments

A full-factorial ANOVA revealed that mutation-type, test environment, and the interaction between these two factors each contribute significantly to the fitness of genotypes containing only one of this study's five focal mutations (Table 3). Figure 3

displays the average fitness of each individual mutation-resource combination. Thirteen of forty (13/40) combinations exhibit greater relative fitness than their ancestor in a given resource, while twenty-two combinations (22/40) were neutral in effect, and five (5/40) yielded relative fitness values lower than that of their ancestor. Significance of these results was determined by 40 separate two-tailed t-tests ($\alpha = 0.05$).

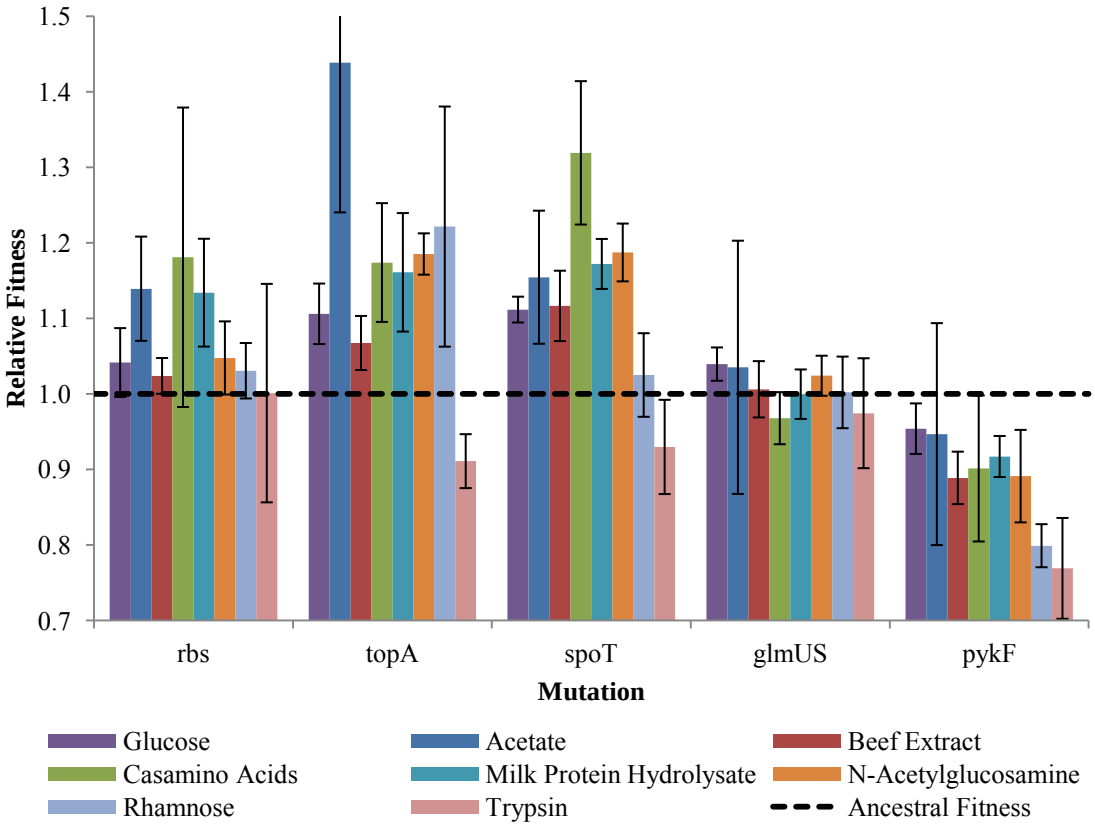


Figure 3 – Average relative fitness values for five focal adaptive mutations (*rbs*, *topA*, *spoT*, *glmUS*, *pykF*) in eight test environments. Dotted line references ancestral fitness. Error bars indicate 95% confidence intervals.

Table 3 - ANOVA of fitness with respect to type of single mutation and resource.

Source	df	MS	F-ratio	P-value
Mutation	5	0.3893	53.9927	<0.0001
Environment	7	0.0933	12.9341	<0.0001
Mutation*Environment	35	0.0244	3.3841	<0.0001
Error	186	0.0072		

The relationship of fitness to number of mutations across environments

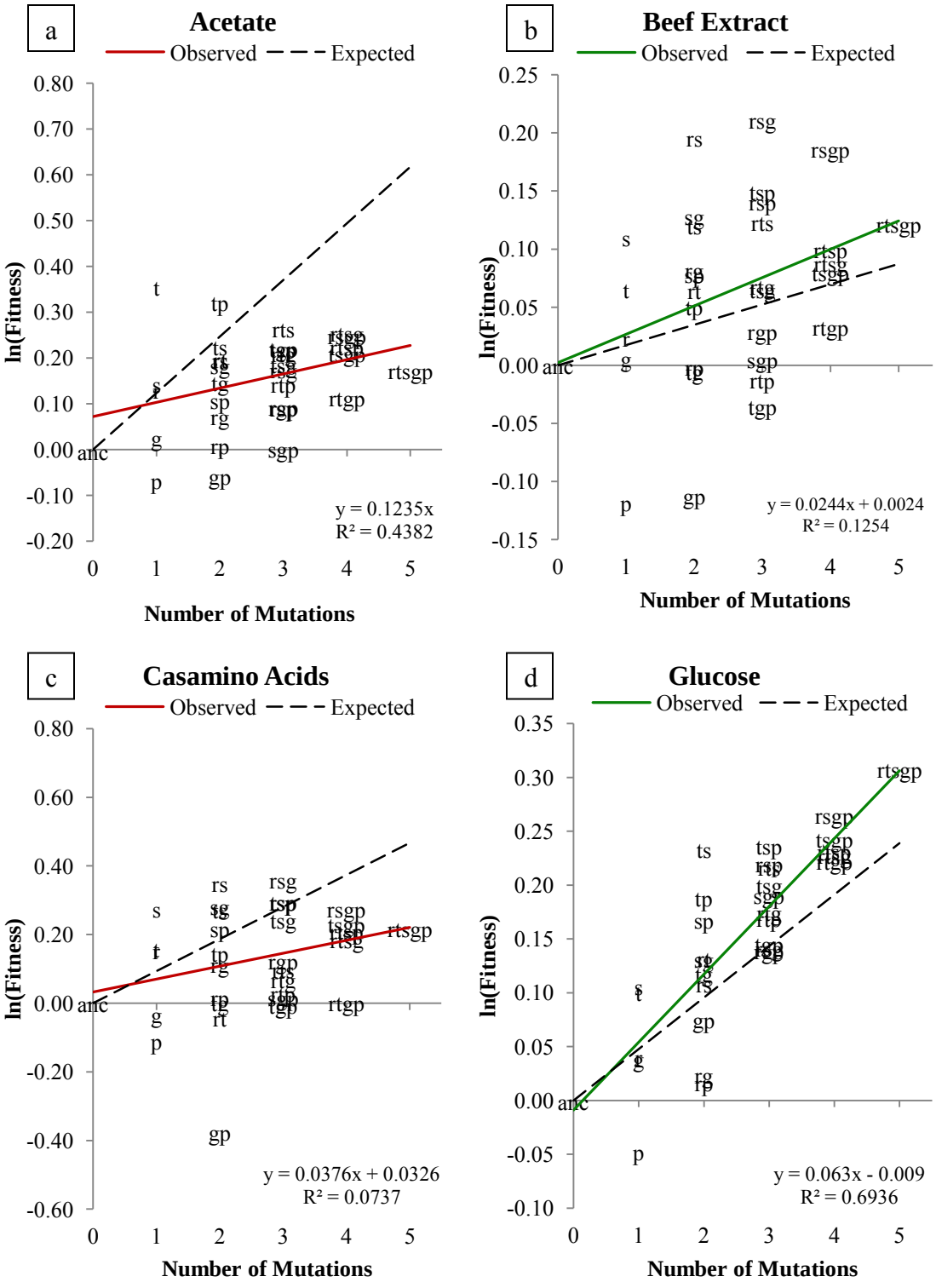
A full-factorial ANCOVA with number of mutations and resource as main effects was calculated based on mean of the natural log transformed fitness for each construct in each resource. The number of mutations present and resource used were significant sources of variation in fitness (Table 4). The effect of number of mutations also varies from resource to resource (Table 4).

Table 4 - ANCOVA of natural logarithm transformed fitness with respect to number of mutations and resource.

Source	df	MS	F-ratio	P-value
Mutation Count	1	2.9375	59.7062	<0.0001
Environment	7	0.8399	17.2872	<0.0001
Mutation Count*Environment	7	0.3020	3.8720	0.0005
Error	240	0.0142		

An additional analysis was performed for each resource comparing expected and observed linearized fitness measures. Values were averaged for each construct and plotted against the number of mutations present. Regression lines based on treatment means were fit to both the observed and expected data. Observed data points are labeled with the corresponding letters of the mutant alleles they contain and, for clarity, expected data points are not shown (Figures 4a-4h). General linear models were used to test for deviation of observed from expected lines for each resource (Figures 4a-4h).

We found two cases of a significant synergistic epistasis trend with increasing number of mutations (milk protein hydrolysate – Fig. 4e and glucose – Fig. 4d) and three cases of significant antagonistic trend in epistasis (acetate – Fig. 4a, casamino acids – Fig. 4c and trypsin – Fig. 4h). We found no significant epistatic trend with increase in loci for three resources (beef extract – Fig. 4b, NAG – Fig. 4f , and rhamnose – Fig. 4g).


a
Acetate
Observed
Expected
ln(Fitness)
Number of Mutations
y = 0.1235x
R² = 0.4382
b
Beef Extract
Observed
Expected
ln(Fitness)
Number of Mutations
y = 0.0244x + 0.0024
R² = 0.1254
c
Casamino Acids
Observed
Expected
ln(Fitness)
Number of Mutations
y = 0.0376x + 0.0326
R² = 0.0737
d
Glucose
Observed
Expected
ln(Fitness)
Number of Mutations
y = 0.063x - 0.009
R² = 0.6936

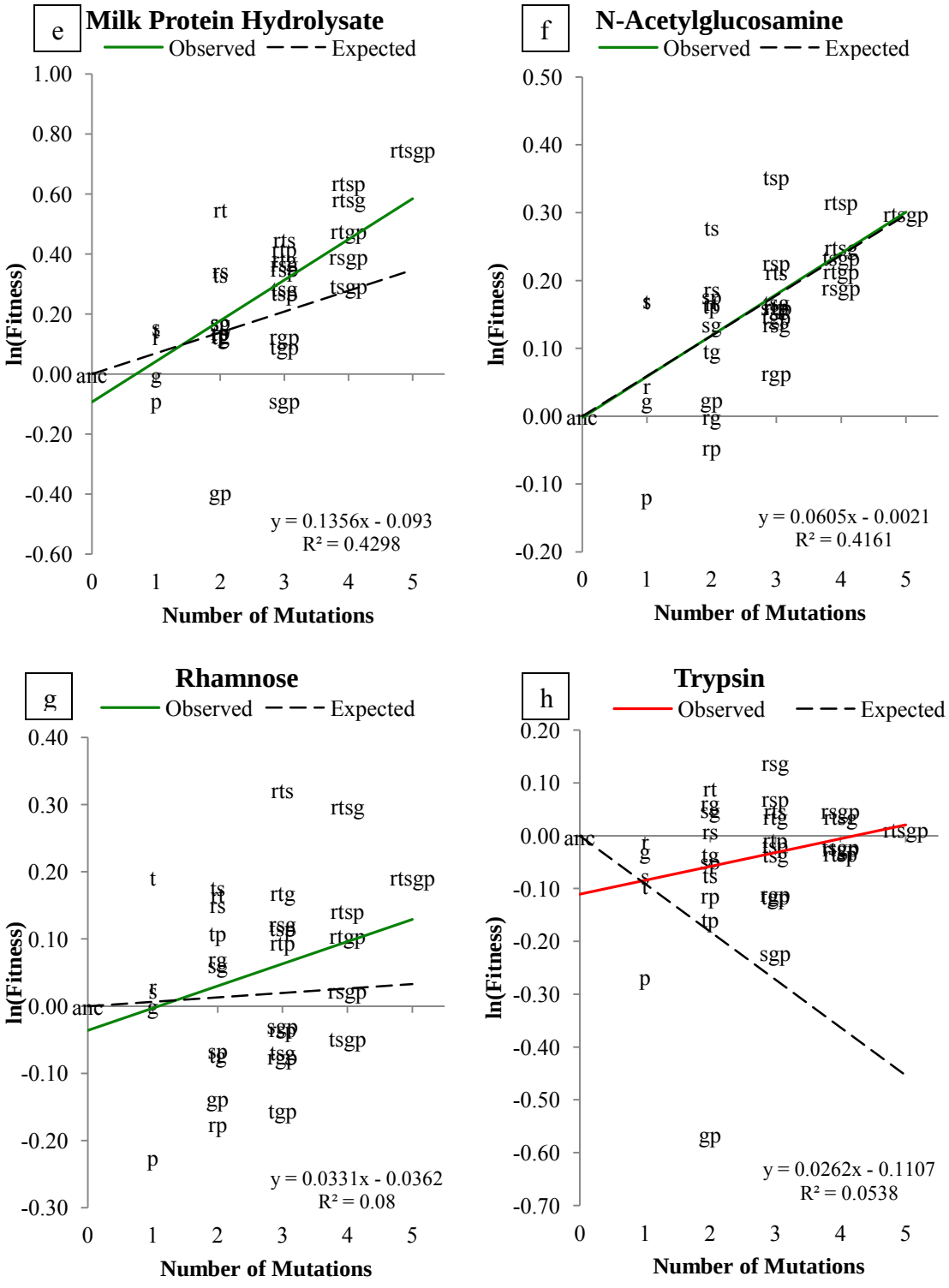


Figure 4 – Expected and observed fitness of constructs grown on (a) acetate ($p < 0.0001$), (b) beef extract ($p = 0.5620$), (c) casamino acids ($p = 0.0289$), (d) glucose ($p = 0.0555$), (e) milk protein hydrolysate ($p = 0.0273$), (f) NAG ($p = 0.9251$), (g) rhamnose ($p = 0.2043$), and (h) trypsin ($p < 0.0001$). P-values associated with testing for differences between slopes of observed and expected lines.

General patterns in adaptive peaks across resource

In all environments except glucose (Figure 4d) and milk protein hydrolysate (Figure 4e), the highest fitness is seen in a clonal population other than the one containing all five mutant alleles ('rtsgp') although these fitness peaks may not differ significantly from all other genotypes. In acetate (Figure 4a), the highest fitness genotype contains only the 't' mutant allele. In beef extract (Figure 4b) and casamino acids (Figure 4c), peak fitness is achieved by the 'rsg' genotype. In NAG (Figure 4f), the maximum fitness value is in the 'tsp' construct.

For three of the resource environments, access to peak fitness is potentially blocked by an adaptive valley. In milk protein hydrolysate (Figure 4e), the absolute peak is achieved by 'rtsgp'. If the highest fitness genotypes are fixed in order of benefit a local peak at 'rt' would lead to an adaptive dead end. Similar patterns are seen in rhamnose (with a local peak at 't' and an absolute peak at 'rts' – Figure 4g) and trypsin (with a local peak at 'rt' and an absolute peak at 'rsg' – Figure 4h). While these populations appear to have fitness valleys we did not test for significance of each valley.

General patterns of epistasis across environments

While fitness tends to improve with number of mutations regardless of resource, measures of absolute epistasis vary greatly with environment. An analysis of covariance found no direct effect of the number of mutations on absolute epistasis. However, environment contributes significantly to variation in absolute epistasis. The number of mutations does significantly influence the level of epistasis in a resource environment specific manner with a range of synergistic, neutral and antagonistic epistasis (Table 5,

Figure 5). Significant epistatic trends are seen in five of eight resources (GLM, p<.005), including acetate, casamino acids, glucose, milk protein hydrolysate, and trypsin.

Table 5– ANCOVA of absolute epistasis with respect to number of mutations and resource.

Source	df	MS	F-ratio	P-value
Mutation Count	1	0.0171	0.8591	0.3551
Environment	7	0.6248	31.4280	<0.0001
Mutation Count*Environment	7	0.1759	8.8468	<0.0001
Error	192	0.0199		

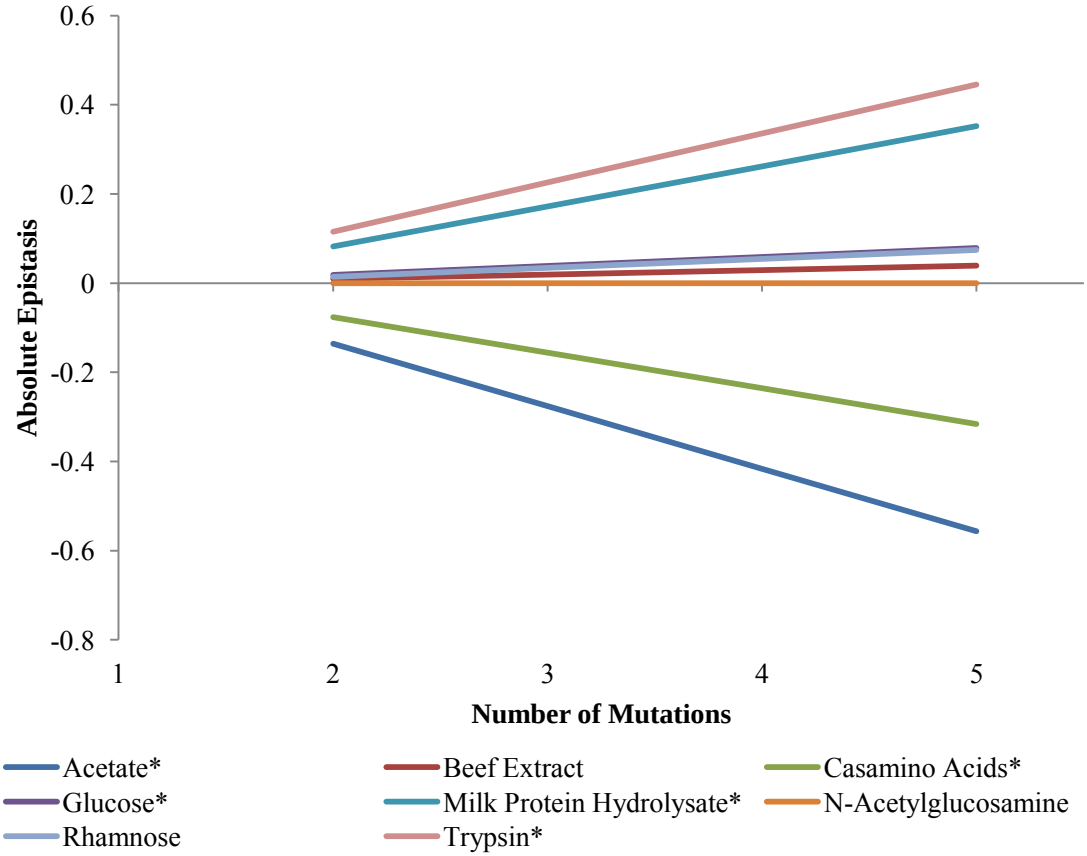


Figure 5 - Plot of absolute epistasis versus number of mutations using only genotypes where two or more of the focal mutations are present. Y-intercept is forced through the point (1,0) to reflect removal of background influence and within-resource scaling.

Discussion

We find that the underlying basis of adaptive landscapes, epistasis, is highly dependent on the resource environment in which those mutations are expressed (Table 5, Figure 4a-h). When looking across a haphazard array of eight resource environments we find that there is no predictable pattern in epistatic effect with increasing number of mutations. Neither synergistic nor antagonistic epistasis dominates in this set of mutations. Current models of adaptation and evolution are flexible but require an understanding of the stability of the architecture of adaptive landscapes. History is recorded in the location of a population in a genetic space. We find that the impact of that history on the expected direction of adaptation is highly contingent on the external environment. Not just because selection on the phenotype changes, but because the genetic interactions creating the phenotype change, altering the way in which a response to selection can occur.

The ruggedness of an organism or population's adaptive landscape has an effect on its ability to repeat evolution given relatively equal genetic and environmental starting conditions. Melnyk and Kassen (2011) found that in *Pseudomonas fluorescens,* as evolution proceeds in two environments adaptation becomes a larger contributor to variance than history or chance. This is consistent with Travisano et al.'s 1995 original study. However Melnyk and Kassen also show that history and chance together contribute more in a novel environment than in the evolved environment, indicating that the adaptive landscape is more "rugged" under unfamiliar conditions. One reason that the landscape may be more rugged in novel environments is because there are more pathways to adaptation (at a metabolic level) than for the evolved environment (Melnyk

and Kassen, 2011). We find evidence of a general basis for this type of observation. Pathways to adaptive optima can be opened or closed by alterations of the genetic and physical environments. In this sense we extend the findings of Flynn et al. (2013) with a broader and more random selection of environments.

A population's genetic history can determine how a particular population evolves. Moore and Woods (2006) founded multiple long-term evolution experiments using different genetic backgrounds of *E. coli* in the same limited glucose environment. Fitness increased after 2000 generations of evolution for all measured lines, though the rate and degree of that improvement differed by strain. This illustrates how the underlying genetic architecture may constrain evolution, even when physical conditions are similar. As in previous studies, rates of fitness improvement decelerated over time, reaching plateaus after approximately 1000 generations (Moore and Woods 2006). This rate was inversely related to the absolute fitness of the ancestor in that new environment (preadaptation). However, even after removing the role of preadaptation to the environment, phylogenetic history played a significant role in determining adaptive rate.

By examining a specifically set of controlled genetic and environmental contexts, our study demonstrates the contingent dynamics of individual beneficial mutations in evolution. Single mutations conferring smaller effect sizes can combine to produce benefits greater than the individual parts under the right conditions. This highlights the importance of mutational neutrality (Kimura 1968) and pleiotropy (Ostrowski et al. 2005) in asexual populations. Mutations may have negligible measurable effect when they arise, but if they are fixed either through drift or by a small selective advantage, they may have larger and more far-reaching effects than originally anticipated when paired with

additional mutations. Individually, these beneficial mutations are generally neutral or beneficial across environments. Notable exceptions include poor performance of all individual mutations in trypsin and poor performance of the *pykF* mutation across all resources (Figure 3). As mutations are added, fitness trends positively in all environments. This suggests that the possible mechanism of gene-environment interaction most heavily influencing this system is selection for globally beneficial mutations (Fry 1993; Fry 1996).

A fundamental question in evolution revolves around the utility of sexual recombination given its inherent cost. The stability of epistatic effects on fitness is central to theories of maintenance of sex. Kondrashov's mutational deterministic hypothesis argues that sexual reproduction is advantageous due to sex's ability to break deleterious epistatic interactions through recombination (Kondrashov 1994). Recombination also allows independent beneficial alleles to come together at a much faster rate than in asexual populations (Muller 1932). In this way, sex can remove the importance of ordering effects.

In the Kondrashov model, deleterious mutations must interact synergistically for sex to be an advantageous way of purging harmful alleles. Elena argues that deleterious alleles do not necessarily act synergistically, and provides evidence that there are no net synergistic interactions between deleterious mutations in *E. coli* (1997). Indeed, Kondrashov and Kondrashov (2001) describe circumstances under which sexual reproduction hampers the fixation of adaptive mutations, and caution that multidimensional epistasis must be examined in an ecological context (Kondrashov and Kondrashov 2001). We find synergistic, antagonistic and multiplicative effects all to

exist in the same genetic combinations, but that the nature of the interactions was environmentally contingent, justifying caution of studies in a single environment (Kondrashov and Kondrashov 2001).

It is not always clear when certain phenotypic changes are due to genetics verses phenotypic plasticity. Inherently, there may be an energetic cost to maintaining a plastic phenotype. When this is the case, and when the environment is stable, plasticity is selected against. Environmental changes, however, can lead to changes in the fitness landscape seen by a population. Plasticity can persist when costs are small enough or environmental perturbations are frequent enough to make maintaining unused genes beneficial on a multi-generational time scale (Price et al. 2003). Cooper and Lenski (2010) evolved replicate populations of the same *E. coli* under either constant or variable resource environments. In all cases, the evolved lines were significantly more fit in the evolved environment than the ancestor, but the degree of fitness increase varied with environment type. Populations that evolved in a fluctuating environment had the most sustained variation, showing that diverse ecological history is left in the genetic record. We further find that each gene combination differs in the extent to which its effects are plastic across environment (Figure 4a-h).

In Lenski's long-term lines, fitness advantages in glucose-evolved replicate populations were correlated with fitness gains when exposed to novel sugar environments whose method of uptake (the PTS system) was the same as glucose and those that did not share this uptake system. However, more variation in fitness was seen with non-PTS sugars. Converging strategies appeared in the pathway under the strongest selection in that environment (Travisano and Lenski, 1996). We find that for these five beneficial

mutations, fixed during evolution in glucose, there are diverse fitness effects that are contingent on genetic environment as well as resource. Interestingly, the highest fitness gains we found were not in glucose but in milk protein hydrolysate.

The dynamics of adaptation depend upon the underlying adaptive landscape of the population under study. Models can be built for predicting mean fitness and number of accumulated mutations based on a landscape. Kryazhimskiy et al. (2009) present a model in which such predictions are governed largely by the expected fixation probability and expected fitness change of each mutation. Under this model, neutral mutations can still arise and propagate even when other mutations are under strong selection. Neutral mutations are important modifiers of adaptive landscapes because in combination with other mutations, they may bring about previously inaccessible benefits. We see many examples of this in our study. For example, the 'g' mutant allele is nearly neutral across all test environments when alone, but can provide significant fitness increases over the ancestor when expressed with other mutant alleles (Figure 4a-4h).

Across a range of resource environments, we found two cases of increasing synergistic epistasis, three cases of increasing antagonistic epistasis and three cases where epistasis did not track with increasing number of mutations. These findings suggest that patterns of absolute epistasis are not inherent to the suite of mutations in question. Rather they are heavily influenced by environment. Because of this influence, adaptive landscapes must be viewed in an environmental context. The effect of genetic history can vary greatly due to present environment.

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