Genetic background affects epistatic interactions between two beneficial mutations

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The phenotypic effect of mutations can depend on their genetic background, a phenomenon known as epistasis. Many experimental studies have found that epistasis is pervasive, and some indicate that it may follow a general pattern dependent on the fitness effect of the interacting mutations. These studies have, however, typically examined the effect of interactions between a small number of focal mutations in a single genetic background. Here, we extend this approach by considering how the interaction between two beneficial mutations that were isolated from a population of laboratory evolved Escherichia coli changes when they are added to divergent natural isolate strains of E. coli. We find that interactions between the focal mutations and the different genetic backgrounds are common. Moreover, the pairwise interaction between the focal mutations also depended on their genetic background, being more negative in backgrounds with higher absolute fitness. Together, our results indicate the presence of interactions between focal mutations, but also caution that these interactions depend quantitatively on the wider genetic background.

1. Introduction

The phenotypic effect of a mutation can depend on its genetic background. This dependence, known as epistasis, is important to many areas of developmental and evolutionary biology, including speciation [1–4], the maintenance of sex [5,6], adaptation [7–9], the evolution of ploidy [10] and evolutionary contingency [11–14]. As the technology to identify and manipulate specific mutations becomes increasingly available, the influence of epistasis can be examined directly. For example, several studies have examined specific examples of epistasis causing evolutionary contingency [12,13]. Other studies have demonstrated a trend for beneficial mutations to interact antagonistically, causing the rate of adaptation to slow as beneficial mutations accumulate [8,9,15], a relationship predicted by theory [16].

Of note, however, most experimental studies of epistasis focus on interactions between mutations arising within a single population or in replicate populations evolved from a common ancestor. In these cases, relatively few mutational differences separate different genotypes. Consequently, there are relatively few opportunities for differential epistatic interactions to alter the effect of newly occurring mutations. It seems likely that the effects of a single mutation when added to more divergent genotypes might reveal a much wider variety of phenotypes reflecting the many different mutation interactions. Indeed, background-dependent interactions have been proposed as a general explanation for the frequent failure to find genetic determinants that explain a large fraction of common phenotypes in natural populations [17]. Here, we test for higher-order interactions revealed when not just the main effect, but also the interaction between focal mutations, depends on the wider genetic background [18].


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One contribution to a Special Feature on ‘Experimental evolution’ organized by Paul Sniegowski, Thomas Bataillon and Paul Joyce.
We examine the effect of the interaction between two mutations—in the genes topA and pykF—on fitness in a series of distinct genetic backgrounds. These mutations were isolated from a laboratory-evolved population of Escherichia coli and were beneficial in the genetic contexts in which they arose, although the magnitude of this benefit depended on the presence of other evolved mutations. By comparing the effects of these mutations individually and in combination in seven natural isolate strains, we isolate the effects of background dependence on the individual and epistatic interaction between these mutations.

2. Material and methods

(a) Bacterial strains

Strains of E. coli were obtained from the STEC center at Michigan State University (www.shigatox.net) (E267 and R424) and from Francisco Moore (University of Ohio, Akron; ECOR1, VS-126, VS-820, TA135 and TA105). Both collections were isolated with the aim of being representative samples of E. coli. Strain REL606 was used as the ancestor to the evolution experiment in which the mutations we study were isolated. Preliminary sequencing and phylogenetic analysis indicate that the strains we chose represent the major clades of the species. Sequencing of topA in all natural isolate strains indicated no amino acid differences relative to REL606. The effect of transferring the evolved topA allele into these strains is therefore limited to altering intergenic interactions. Because the pykF evolved allele is a large deletion, introduction of this allele can only effect intergenic interactions.

(b) Mutations and genetic manipulations

Mutations in topA [7] and pykF [19] were identified in a population evolved in a minimal glucose environment as part of a long-term evolution experiment [20]. We moved the topA mutation (H33Y) and a deletion allele of pykF that was equivalent to the evolved IS150 insertion mutation [8] individually and in combination into each of our seven natural isolate strains using a suicide vector approach described previously [8]. To address the possibility that secondary mutations occurred during the construction process, for each allele replacement strain we obtained a paired clone that went through the same construction process but that retained the original allele. The allele replacement strain was only kept if the control clone had fitness indistinguishable from the corresponding progenitor strain. We also introduced a mutation into anaA in all natural isolate strains. This mutation facilitated competition experiments by creating a derivative progenitor that could be distinguished from constructed strains on tetrachloroarabinose indicator plates [20].

(c) Fitness and growth rate measurements

The fitness of each constructed strain was measured relative to an anaA derivative of its progenitor using direct competition experiments carried out in Davis minimal (DM) medium supplemented with 25 μg ml⁻¹ glucose [20]. In some strains, the AnA-competition marker was not neutral. In these cases, we normalize relative fitness estimates to account for the marker effect (see the electronic supplementary material, table S1). Maximum growth rates were estimated by growing strains in 96 well plates containing DM medium supplemented with 500 μg ml⁻¹ glucose and measuring changes in OD450 in a VersaMax plate reader. All strains were pre-conditioned in the same media. An R script was used to estimate the maximum growth rate of each strain (table 1).

(d) Statistical analysis and estimation of epistasis

We used a multiplicative model to estimate the absolute epistatic deviation owing to interactions between introduced topA and pykF mutations: εM = wM - ∏iεM wi, where wM is the fitness of a mutant with a set of M mutations and w is the relative fitness of a mutant containing a single mutation from set M [18]. Our results are qualitatively unaffected if we use a relative measure of the magnitude of epistasis.

One-way ANOVAs were used to test for differences in the individual and combined effects of the topA and pykF mutations over different genetic backgrounds. Genetic background was included in these models as a random factor. All analyses were carried out in R v. 2.14.2 (http://cran.r-project.org/).

3. Results and discussion

(a) The fitness effects of topA and pykF mutations depend on their genetic background

The topA and pykF mutations were beneficial in the genetic backgrounds in which they first occurred [8]. Individually, the topA mutation was beneficial in four and neutral in three of the natural isolate strains we consider, and the pykF mutation was beneficial in five and neutral in two (figure 1; significance assessed by 2-tailed t-test at p < 0.05). One-way ANOVA analyses found that the fitness effect of both mutations varied across the different genetic backgrounds, indicating the presence of different gene-by-genotype epistatic interactions that affect fitness (topA: F7,45 = 26.655, p < 0.001; pykF: F7,45 = 38.518, p < 0.001).

(b) Epistasis depends on genetic background

To test for epistasis between the topA and pykF mutations, we added them together into each genetic background. Epistasis was generally negative. In six genetic backgrounds, the fitness of the double mutant was lower than that of the mutant having only the pykF mutation, although this difference was only significant in three cases (VS-126, TA105 and E267; significance assessed by 2-tailed t-test at p < 0.05). Epistasis between the topA and pykF mutations differed significantly between different backgrounds (F7,45 = 23.041, p < 0.001). Of note, in TA105 the double mutant was less fit than either single mutant—an example of reciprocal sign epistasis [11,21], which can constrain adaptation by causing adaptive landscapes to become rugged [12,13].

(c) topA and pykF mutations interact more negatively in strains with faster maximum growth rates

Recent studies have found that epistatic interactions between beneficial mutations tend to become increasingly negative as the fitness of the genetic background increases [8,9,15,22]. This trend can explain the general tendency for the rate of adaptation to decline in populations that are selected in constant environments [16]. However, that work considered genetic backgrounds that differed by only a few mutations and it is not clear if the same pattern will be seen between mutations in divergent backgrounds. To test this, we estimated the correlation between each strain’s maximum growth rate and its deviation in fitness owing to the interaction between topA and pykF. We found that these interactions tended to be...
more negative in strains with higher growth rates (Pearson: $r = -0.810$, $p = 0.015$; Spearman: $\rho = -0.666$, $p = 0.083$; figure 2). Notwithstanding the limited sample size we consider, this result is consistent with the growth rate of a strain determining some portion of the overall direction and magnitude of epistasis. We note, however, that we do not rule out that specific interactions between the genetic background and the focal mutations also play a role in determining epistatic effects.

**Table 1.** Estimates of relative fitness and epistatic deviations of different genotypes.

<table>
<thead>
<tr>
<th>progenitor strain</th>
<th>mutations added</th>
<th>fitness relative to progenitor (95% CI)</th>
<th>absolute epistasis (95% CI)</th>
<th>maximum growth rate of progenitor (per hour) (s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOR1</td>
<td>topA</td>
<td>1.010 (0.082)</td>
<td>-0.076 (0.193)</td>
<td>0.654 (0.016)</td>
</tr>
<tr>
<td></td>
<td>pykF</td>
<td>1.120 (0.082)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topA, pykF</td>
<td>1.060 (0.074)</td>
<td>-0.076 (0.193)</td>
<td>0.654 (0.016)</td>
</tr>
<tr>
<td>VS-126</td>
<td>topA</td>
<td>1.160 (0.033)</td>
<td>0.233 (0.085)</td>
<td>0.663 (0.021)</td>
</tr>
<tr>
<td></td>
<td>pykF</td>
<td>1.284 (0.078)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topA, pykF</td>
<td>1.255 (0.109)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS-820</td>
<td>topA</td>
<td>0.988 (0.035)</td>
<td>0.006 (0.035)</td>
<td>0.612 (0.065)</td>
</tr>
<tr>
<td></td>
<td>pykF</td>
<td>1.029 (0.074)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topA, pykF</td>
<td>1.022 (0.028)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA135</td>
<td>topA</td>
<td>1.171 (0.087)</td>
<td>-0.226 (0.111)</td>
<td>0.700 (0.069)</td>
</tr>
<tr>
<td></td>
<td>pykF</td>
<td>1.279 (0.181)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topA, pykF</td>
<td>1.268 (0.058)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R424</td>
<td>topA</td>
<td>0.998 (0.080)</td>
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<td></td>
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<tr>
<td></td>
<td>pykF</td>
<td>1.173 (0.114)</td>
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<tr>
<td></td>
<td>topA, pykF</td>
<td>1.089 (0.048)</td>
<td>-0.081 (0.131)</td>
<td>0.587 (0.005)</td>
</tr>
<tr>
<td>E267</td>
<td>topA</td>
<td>1.383 (0.042)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>pykF</td>
<td>1.054 (0.098)</td>
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<tr>
<td></td>
<td>topA, pykF</td>
<td>1.428 (0.043)</td>
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<tr>
<td>TA105</td>
<td>topA</td>
<td>1.130 (0.065)</td>
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<td></td>
<td>pykF</td>
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<tr>
<td></td>
<td>topA, pykF</td>
<td>1.087 (0.112)</td>
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<tr>
<td>REL606</td>
<td>topA</td>
<td>1.142 (0.023)</td>
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<tr>
<td></td>
<td>pykF</td>
<td>1.000 (0.013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>topA, pykF</td>
<td>1.193 (0.023)</td>
<td>0.051 (0.051)</td>
<td>0.591 (0.014)</td>
</tr>
</tbody>
</table>

**Figure 1.** Relative fitness effect of topA and pykF mutations individually and in combination in each genetic background. Fitness was measured relative to the corresponding natural isolate strains. REL606 is the ancestor used to found the population in which the mutations were originally isolated. Other genotypes are natural isolates. Mean and s.e.m. are shown (natural isolates $n = 4$; REL606 $n = 25$).

**Figure 2.** Relationship between maximum growth rate and the magnitude of epistasis. Points indicate the mean of estimates of maximum growth rate ($n = 9$) and magnitude of epistasis between topA and pykF mutations for the ancestor ($n = 25$) and seven natural isolate strains ($n = 4$). Error bars indicate 95% CI.
Our results demonstrate that both the direct fitness effect of two mutations that were beneficial in a laboratory-evolved population, and the epistatic interaction between these mutations, depend on genetic background. In addition, we found that the effect of epistasis between the topA and pykF mutations was negatively correlated with the growth rate of the strain containing the mutations, which is consistent with previous studies [8,9,23]. This result suggests that our focal mutations may affect a common saturating physiological process, which is related to growth rate. Targeting this process in already fast-growing genotypes would lead to little additional growth rate improvement and, therefore, negative epistasis.

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References


