RESEARCH ARTICLE

Enhancing Effect of Phenotype Mutational Robustness on Adaptation in *Escherichia coli*

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ABSTRACT

Theoretical and computational studies predict a positive role for widespread phenotype resistance to genetic mutation, or "phenotype mutational robustness," in enhancing adaptation to novel environments through the accumulation of cryptic genetic variation. However, this has not been verified through experimental evolution in biological systems at the level of whole organisms. In a short-term evolution experiment of about 250 generations, we studied the adaptive performances of independently evolving populations of the bacterium *Escherichia coli* in two new nutritional environments, represented by minimal media with either lactate or glycerol as the sole carbon source. At the start of the experiments, all populations expressed identical phenotype, while differing for the amount of cryptic genetic variation, artificially produced by mutagenesis. We found that cryptic genetic variation can promote significantly faster adaptation to a new nutritional environment in *E. coli*. The scale of this effect varies between the two environments, and correlates with an estimation of the phenotype robustness of the ability to grow in a given medium, based on survival rate after mutagenesis in the same medium. *J. Exp. Zool. (Mol. Dev. Evol.) 326B:31–37, 2016.* © 2015 Wiley Periodicals, Inc.

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Widespread phenotype resistance to the impact of genetic mutations, or "phenotype mutational robustness" (Kitano, 2004; Stelling et al., 2004; Wagner, 2005), is a necessary condition for the accumulation of neutral genetic variation (Hayden et al., 2011; Wagner, 2011). This so called "cryptic genetic variation" (CGV) has no effect on the phenotype in a particular genetic or environmental context, but can eventually be expressed in the phenotype as a consequence of genetic mutations or environmental change (Gibson and Reed, 2008).

Since evolutionary adaptation by natural selection requires phenotypic variation, phenotype robustness may seem to be a quality of the organism's genotype–phenotype map that would oppose the process of adaptation. However, somewhat counter-intuitively, robustness can effectively enhance adaptation to novel environments by increasing the number of different phenotypes accessible through mutation (Wagner, 2008; Draghi et al., 2010). This effect has been demonstrated in theoretical studies using computational models (Matias Rodrigues and Wagner, 2009; Barve and Wagner, 2013) and with experimental studies on ribozymes (Hayden et al., 2011), but has not been verified through experimental evolution in more complex evolving systems, such as whole organisms. The possibility of extending this principle of the positive effect of robustness on adaptation to whole organisms by theoretical reasoning is limited by the need for specific assumptions on the features of the organism's genotype–phenotype map. These include the level of epistasis, pleiotropy, and neutrality, for which, despite substantial theoretical modeling (Orr, 2000; Wagner et al., 2008; Pavlicev et al., 2009; Wagner and Zhang, 2011), there are few observational data (Grüneberg, '38; Albert et al., 2008; Rohner et al., 2013).

We designed an experiment to investigate the role of robustness in the adaptation of the bacterium *Escherichia coli* by measuring the effects of CGV in adapting to novel

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environments, represented by minimal media with a single carbon source, different from the original strain's native carbon source which is glucose. The two phenotypic traits under scrutiny were the instantaneous population growth rate in either glycerol or lactate, which can be interpreted as measures of population absolute fitness in these environments (Elena and Lenski, 2003). Our results provide evidence of an enhancing effect of CGV on the process of adaptation to a novel environment, with quantitative differences between the two traits that match an independent measure of trait robustness, based on the survival rate after mutagenesis in the two environments.

MATERIALS AND METHODS

Outline of the Experiment

The experiment consisted in generating *E. coli* populations with different levels of CGV starting from the same genotype and then allowing them to grow in new environments, whereas the level of adaptation (in terms of growth rate) was recorded through time (Fig. 1). We treated different sub-clones of genotype BW30270 with Ethyl-methanesulfonate (EMS), regulating the time of exposure to the mutagen in order to obtain lines with, on average, either 12 or 24 randomly distributed mutations per genome. Control lines (0 mutations), obtained from the same BW30270 clone, were not exposed to the mutagen. Subsequently, all lines underwent stabilizing selection in the native carbon source, to re-establish the original phenotype and to remove phenotypic variation, while preserving the neutral genetic variation produced by the treatment. Combining the level of CGV with the carbon source, five type of lines (treatment groups) were established for the following adaptation experiments. Starting from identical population size ($N = 10^7$), all lines were allowed to grow for 266 generations either in glycerol (lines Gly0, Gly12, and Gly24, with 0 (control), 12 and 24 mutations, respectively) or lactate (lines Lat0 and Lat12, with 0 (control) and 12 mutations, respectively) as the sole carbon sources, with population size varying between 10^7 and 10^{11} during the experiment. The population growth rate was measured at the start of the experiment, after 14 generations and then at intervals of 42 generations. The adaptation experiment was replicated three times for each treatment group, always starting from an independently established line. During adaptation, mutation was the sole source of genetic variation, as BW30270 does not conjugate.

Strain

Evolving cultures were propagated from fresh cultures of the wild-type *E. coli* K12 BW30270 provided by CGSC (Yale University, New Haven, CT, USA).

EMS Mutagenesis

We treated different sub-clones of the genotype BW30270 with the mutagen Ethyl-methanesulfonate (EMS), performing one or two cycles of mutagenesis in order to establish lines with, on average, either 12 or 24 randomly distributed mutations, respectively. The mean number of 12 mutations per genome induced by each cycle of mutagenesis was calibrated on the basis of previously reported mutation rates, based on the counting of revertants (Cupples and Miller, '89).

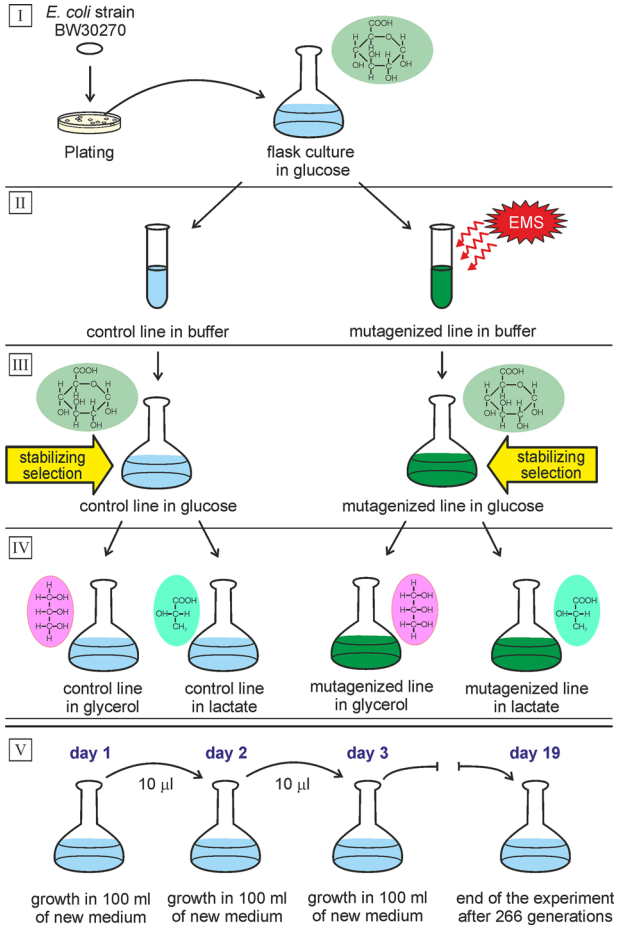

Figure 1. Sketch of the evolution experiments. (I) After plating the original strain, a single colony was randomly selected and sub-cultured in glucose for the following evolution experiments. (II) From this culture, a sub-clone (mutagenized line) was exposed to Ethyl-methanesulfonate (EMS), whereas the control line sub-clone was not exposed to the mutagen. (III) Subsequently, both mutgenized and control lines underwent stabilizing selection for 56 generations in the native carbon source (glucose), to re-establish the original phenotype and to remove phenotypic variation. (IV) Then, sub-samples of each line were transferred to a new nutritional environments (either, glycerol, or lactate) and allowed to growth for 266 generations by daily serial cultures (V), whereas the growth rate was periodically recorded. From step (II), the experiment was replicated three times.

The mutagenesis protocol followed that by Cupples and Miller ('89). A fresh overnight culture was sub-cultured and grown until it reached a density of 2–3 × 10^8 cells per ml ($A_{600} = 0.3–0.5$). The cells were chilled on ice, spun down at 5,000*g*, at 4 °C, for 10 min, washed twice in M9 salts (ForMedium, UK), and then re-suspended in half the original volume of M9 salts. EMS (Sigma–Aldrich, USA) was added in the cold by pipetting 0.07 mL of EMS into 5 mL of re-suspended cells ([EMS] = 1.4%) and incubated on a roller drum at 30 rpm for 15 min at 37 °C. The reaction was stopped by adding 5 mL of sterile sodium thiosulfate at 20% (w/w) (Sigma–Aldrich, USA). After mutagenesis, the cells were spun down, washed twice in M9 buffer, and then re-suspended in the same volume of M9 buffer and plated for viable cells and contaminations. Samples (1 mL) were added to 100 mL of M9 + glucose broth and the cultures were grown overnight. This process was independently carried out for each mutagenized line, for a total of six (three Gly12 and three Lat12). Samples from the three Gly12 mutagenized populations were mutagenized one more in the same conditions (after stabilizing selection, see below) to produce the three Gly24 lines. Gly0 and Lat0 lines undergone the same treatment, but for the addition of the mutagen.

Control of Phenotypic Variation

The interpretation of the differential grow rates in the different lines as a consequence of CGV is conditional on the absence of genetic variation with phenotypic effect on growth rates at the start of the evolution experiments, possibly introduced by mutagenesis.

In order to eliminate possible mutant phenotypes, the mutagenized populations were subjected to a stabilizing selection regime, cultured in M9 minimal medium + glucose (4 gr/l) and sub-cultured daily (the same procedure adopted for the evolving cultures, see below) for 4 days, for a total of 56 generations. Selection preserved most of the neutral genetic variation produced by the treatment, only slightly reduced by a minimal sampling effect ($N = 10^7$) at the start of each daily culture.

Fifty-six generations of stabilizing selection are sufficient for reducing variation in growth rates well below the phenotypic effect of an average mutation in *E. coli*, estimated between 3% (Trindade et al., 2010) and 10% (Lenski et al., '91). Absence of significant phenotypic variation of growth rates in glucose after stabilizing selection was nonetheless directly assessed through statistical testing on observed growth rates.

Stabilizing selection in glucose was also aimed at eliminating possible epigenetic effects induced by the mutagenesis. It is known, in fact, that *E. coli*, like other bacteria, can epigenetically change mutation rate and expression profile under stressful condition (Rosenberg et al., 2012). However, this effects are transient (Hastings, 2007), and 56 generations of growth in glucose, which is a standard medium for *E. coli* BW30270 strain, are expected to eliminate any possible epigenetic effects produced by mutagenesis (Foster, 2005). Statistical testing on observed growth rates after stabilizing selection attested the absence of significant phenotypic variation of epigenetic origin.

Absence of significant phenotypic variation was further checked through repeated sampling from the starting population of the evolving cultures. For each type of line (Gly0, Gly12, Gly24, Lat0, and Lat12), we extracted 96 independent samples ($N = 50 \times 10^8$) from the starting populations and measured the initial growth rate in their new medium (glycerol or lactate). For the sampling procedure, 100 μL of a fresh overnight culture (in M9 minimal medium + 0.4% glucose) 10^{-6} diluted, were plated to isolate approximately 200 colonies. After 24 hr of incubation at 37°C, a sample of each colony was randomly picked with a sterile stick and put on a well of a 96-well cell culture microplate, each filled with 150 μL of M9 minimal medium + 0.4% glucose. The plates were incubated overnight. After that, 5 μL of each well were transferred in another well of a 96-well microplate, filled with 150 μL of test-medium (M9 minimal medium + 0.4% lactate or glycerol). The five plates were incubated in an incubator shaker at 37°C, 280 rpm and the optical density (*OD*) of each well was periodically recorded (1 hr) at 600 nm with a microplate reader. Growth rates measures underwent statistical testing.

Evolving Cultures

Cultures were conducted in 100 mL of M9 minimal medium supplemented with 4 g/l of lactate (Sigma–Aldrich, USA) or glycerol (Sigma–Aldrich, USA) in covered 250 mL Erlenmeyer flasks in an incubator shaker at 37°C, 180 rpm. Each day, bacteria were grown overnight from an initial population size of 10^7 cells, until reaching the stationary phase at about of 10^{11} cells-($A_{600} \leq 0.9–1.0$), corresponding to about 14 generations. The day after, they were sub-cultured into fresh medium, using a biosafety cabinet and adopting standard sterile technique practices, restabilizing the initial population size of 10^7 cells. Batch growth and serial passage were conducted for 266 generations for all lactate and glycerol cultures. Lines Gly12, Gly24, Gly0 were tested for adaptation in glycerol as sole carbon source, whereas Lat12 and Lat0 lines were tested in lactate. Throughout the course of evolution, samples of each evolving population were frozen in 15% glycerol and stored at −80°C.

Growth Rate Measurements

Growth rate was measured at generations 0, 14, and then once every 42 generations until generation 266. At each time point examined, a sub-sample of each culture was used to inoculate 50 mL of fresh preheated medium for a batch culture in the same conditions of the evolution experiment. The growth rate was determined by measuring the *OD* of 2 mL growing cultures over time using a spectrophotometer (A_{600}) by periodic sampling ($\Delta t = 1$ hr) of each batch culture, and interpolating the Log(*OD*)/Log(2) time series of the exponential phase with a linear model in

order to obtain a growth rate estimation in terms of generations per hour (slope of the regression line).

RESULTS

Mutagenesis and Stabilizing Selection

Starting from the same genotype, mutagenesis generated *E. coli* populations with different levels of genetic variation, with on average 0, 12, or 24 randomly distributed mutations per genome. Then, in order to eliminate possible mutant phenotypes, the mutagenized populations were subjected to a stabilizing selection regime in glucose for 56 generations. Growth rates were measured during stabilizing selection at generations 0, 14, 28, 42, and 56, and no significant differences were detected between mutated and non-mutated lines staring from generation 14 (equivalence tests, 95% confidence intervals of the differences within the measurement error of 0.015). This result also supports the effectiveness of the stabilizing selection at eliminating possible epigenetic effects on the mutation rate in the mutagenized lines induced by the mutagenesis stress.

Absence of significant phenotypic variation after stabilizing selection, either of genetic or epigenetic origin, was further assessed through repeated sampling ($n = 96$) from the starting population of each type of line (Fig. 2). We found no statistically significant differences between mutagenized and non-mutagenized lines, either for glycerol or lactate, neither for the means (equivalence tests, 95%CI of the differences within the measurement error of 0.020), nor for the standard deviations (Levene's tests, all comparisons $P > 0.6$).

Absence of effective phenotypic variation after stabilizing selection is also supported by observed adaptive dynamics (see Discussion).

Evolution Experiments

In the process of adaptation to a novel environment, all the lines with some amount of CGV outperformed the corresponding lines with (almost) no CGV in the same environment (Fig. 3). In glycerol, Gly12 lines showed average growth rates significantly higher than Gly0 lines from generation 98 onwards (one-tailed Student's *t*-tests, $P < 0.05$, $n = 3$, significant also after Tukey correction for multiple comparisons). A similar result was obtained for Gly24 lines, with average growth rates significantly higher than Gly0 lines from generation 98 onwards (one-tailed Student's *t*-tests, $n = 3$, $P < 0.05$, also after Tukey correction). In both cases the largest differences were reached at generation 98, when growth rate of Gly12 and Gly24 were 1.36 and 1.49 times that of Gly0, respectively. Likewise, lactate, Lat12 lines showed average growth rates significantly higher than Lat0 lines, although differences were relatively less marked, becoming significant from generation 140 onwards (one-tailed Student's *t*-tests, $n = 3$, $P < 0.05$, from generation 182 after Tukey correction), reaching the largest difference at generation 224, when growth rate of Lat12 was 1.2 times that of Lat0. In all three comparisons, the difference in growth rates between treated and control lines tended to reduce toward the final part of the experiment, mainly due to a deceleration in the growth rate increase of the treated lines.

Considering the effects of different levels of CGV in the same environment (Fig. 3A), Gly24 showed an average growth rate slightly higher than Gly12 from generation 98 to 182 (one-tailed Student's *t*-tests, $n = 3$, $P < 0.05$ at generations 98 and 182, but not significant after Tukey correction). After that, this small gap was rapidly filled in subsequent generations, and the two groups of lines converged to almost identical phenotypes.

Comparing adaptation trajectories in glycerol (Fig. 3A) with those in lactate (Fig. 3B), we observed a faster adaptation in the

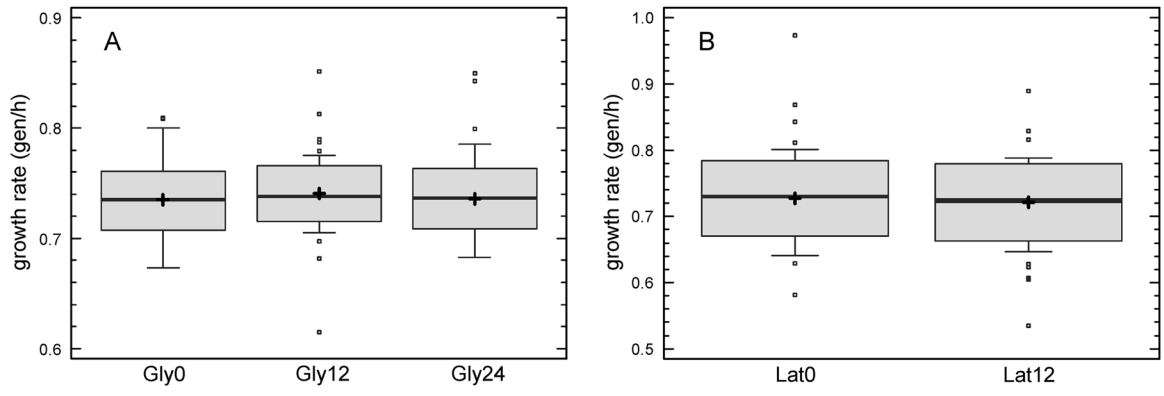

Figure 2. Growth rate variation at the start of culturing experiments in glycerol (A) and in lactate (B). Each line was repeatedly sampled 96 times to estimate growth rate mean (crosses), median (thick horizontal lines), and standard deviation (boxes). There are no statistically significant differences between mutagenized and non-mutagenized lines.

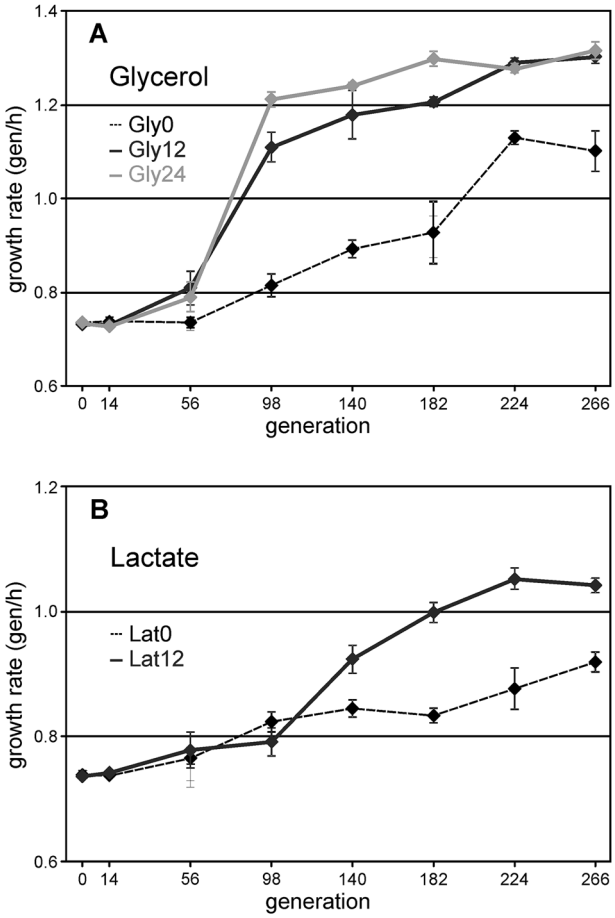

Figure 3. Effect of cryptic genetic variation on adaptation performance in *E. coli*. Average growth rates (diamonds) and standard errors (bars) are based on measurements on three independently evolving lines with the same amount of cryptic genetic variation. (A) Evolution in glycerol. Lines Gly12 and lines Gly24 (12 and 24 neutral mutations per genome on average, respectively) outperformed lines Gly0 (0 neutral mutations per genome). Growth rate differences are significant starting from generation 98. (B) Evolution in lactate. Lines Lat12 (12 neutral mutations per genome on average) outperformed lines Lat0 (0 neutral mutations per genome). Growth rate differences are significant starting from generation 140.

former, for both treated and control lines. Gly12 lines exhibited an average growth rate significantly higher than Lat12 lines from generation 98 onwards (one-tailed Student's *t*-tests, $n = 3$, $P < 0.05$, also after Tukey correction), when the largest difference was reached, with growth rate of Gly12 1.40 times that of Lat12. Similarly, growth rates of Gly0 were significantly higher than those of Lat0 from generation 140 onwards (one-tailed Student's *t*-tests, $n = 3$, $P < 0.05$, from generation 224 after Tukey correction), reaching the largest difference at generation 224, with growth rate of Gly0 1.29 times that of Lat0.

Survival Rates After Mutagenesis

Differences between the adaptation processes in the two media also emerge from analyzing the survival rate after mutagenesis to about 12 mutations per genome (R_{12}). Assuming the set of genomes of the viable individuals in one medium belong to the (nearly) neutral network of genotypes mapping on the same phenotype (defined by the viability in that medium) (Matias Rodrigues and Wagner, 2009), the proportion of survivors of the mutagenesis can be taken as a rough measure of the robustness of the ability to growth in that medium (Fig. 4). This must be taken as a crude measure of robustness because factors other than mutation can also contribute to observed mortality, as for instance the direct toxicity of the treatment.

Replicating the measure of survival rate on four independently mutagenized samples for each medium, at an average distance of 12 mutations from the original genotype the ability to grow in glycerol resulted a more robust character ($R_{12} = 0.73$) than the ability to grow in lactate ($R_{12} = 0.56$) (two-tailed Student's *t*-test, $n = 4$, $P < 0.005$).

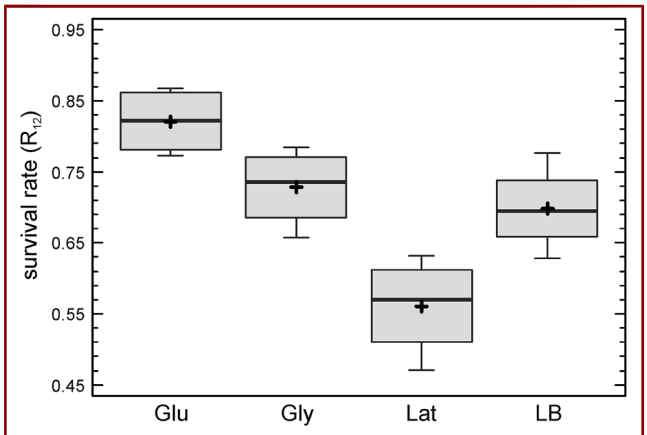

Figure 4. Phenotype robustness estimated as survival rate after mutagenesis in *E. coli*. This is the proportion of viable individuals in a given carbon source after they have undergone a mutagenizing treatment producing on average 12 mutations per genome (R_{12}). The measure assumes that the genomes of the survivors belong to the nearly-neutral genotype network mapping on the same phenotype. Means (crosses), medians (thick horizontal lines), interquartile ranges (boxes), and whole ranges of variation (vertical segments) are based on four independent measures for each substrate. The survival on lactate (Lat) is significantly lower than the survival on glycerol, indicating a lower mutational robustness for the ability to grow on the former substrate. R_{12} in glucose (Glu) and in Luria broth (LB) are shown as reference.

DISCUSSION

In classical models of natural selection, the population rate of change in mean fitness is expected to be proportional to heritable fitness variation (Orr, 2005; Frank, 2012). However, we observed a significant difference in the increase rates of mean fitness between lines that exhibited the same (almost zero) level of fitness variation, but possessed different levels of cryptic genetic variation (CGV). The lines with sizeable CGV exhibited higher *phenotype variability* (Wagner and Altenberg, '96; Willmore et al., 2007) than lines with no CGV, despite starting with the same (almost zero) *phenotypic variation*. This provide evidence in support of more recent views which see an effective role of phenotype robustness in enhancing adaptation, through the accumulation of significant levels of CGV (Wagner, 2012). The two *E. coli*'s metabolic traits under scrutiny appear to show some degree of "innovability" *sensu* Wagner (2011), that is, a propensity to evolve that does not necessary stem from heritable phenotypic variation, or "evolvability" (Pigliucci, 2008).

The roughly sigmoid dynamic of adaptation observed in our study, with the growth rate tending to a plateau, is quite similar to that observed in other experimental evolution studies on *E. coli* (Lenski et al., '91; Fong et al., 2005). Also quantitatively, both growth rate progression and between-lines growth rate variation of Gly0 and Lat0 lines are comparable with those measured in other studies (Fong et al., 2005). However, our specific experimental setting, including direct measurements assessing the absence of effective phenotypic variation between the treatment groups at the start of the evolution experiments, made it possible to expose the counter-intuitive effect of CGV in enhancing adaptation.

There are two, non-mutually exclusive ways in which CGV is thought to be able to promote faster adaptation (Wagner, 2012). Firstly, among the different genotypes with the same phenotype, some variants may be accidentally "pre-adapted" or "exapted" to the new environments (Hayden et al., 2011). Part of the cryptic variation is thus, "unveiled" and immediately converted to effectively advantageous phenotypic variation. Such fortuitous events were not expected to play a significant role in our short-term evolutionary experiment, as genetic variation produced with mutagenesis was relatively modest, less than 1/10 of the genetic variation recorded in wild-type populations of *E. coli* (Zhang et al., 2006). Secondly, the scattering of genotypes through the genotype space allows the population to access a greater number of new phenotypes trough mutation, increasing the probability of finding phenotypes that happen to have higher fitness (Matias Rodrigues and Wagner, 2009). This second mechanism confers a wider-ranging advantage to a population with significant CGV, because it does not depend on the specific mutations accumulated. Genotype scattering, which can be maintained even under a selective regime (Barrick and Lenski, 2013), was expected to affect more consistently the adaptive dynamics of our experiment.

The observed adaptation trajectories in our experiments suggest indeed a dominance of the effects of genotype dispersal. If a phenotype with a growth rate in the order of the value reached toward the end of the experiments (about 1.30 gen/h for glycerol and 1.05 gen/h for lactate) was already present at generation 0, even in one single individual (frequency 10^{-7}), the growth rate would have nearly reached the observed plateau within about 40 generations for glycerol and 70 for lactate (Fig. S1). But, in all lines, after 56 generations, average growth rates are still below 0.85 gen/h and the differences between treated and control lines are all statistically not significant (Fig. 3). The observed pattern is instead completely compatible with the progressive emergence of novel phenotypes with increasingly higher growth rates, in a sort of a stepwise adaptive progression, as described in other studies on bacterial evolution (Lenski et al., '91). This gradual increase in growth rate also makes very unlikely that some residual, undetected standing phenotypic variation might have remained in the treated lines after the stabilizing selection, and thus represents further assessment of the absence of significant phenotypic differences between treated and control lines at the start of the evolution experiments.

From comparing the adaptation dynamics of lines Gly24 with lines Gly12, it appears that the difference in CGV does not affect the final level of adaptation, but only the adaptation dynamics. In fact, both groups of lines tend to level to the same growth rate plateau, although in lines Gly24 the increase in growth rate is faster and the plateau is reached earlier (Fig. 3). This growth rate value seems to correspond to a not uncommon high-fitness phenotype, as not only the average adaptive paths of the two groups reached the same final value, but also those of each of the six individual lines (Fig. S1). In effect, across a relatively small number of generations, as those of our experiment, consistent adaptive patterns can more easily emerge because of some general features of genetic variation, like the dispersal of genotypes through the genotype space, rather than depending on the finding of rare advantageous mutations.

The observed slower adaptation rates in lactate with respect to glycerol can hardly be explained by simply assuming a phenotype optimum in the lactate closer to the starting growth rate value than in the glycerol, such that the slowing down of adaptation rate would depend on the relative proximity of the fitness plateau (*plateau effect*). Actually, longer adaptation experiments on *E. coli*, conducted for up to 600 generations, found a similar growth rate plateau for glycerol and lactate (Fong et al., 2005), although the plateau tends to be reached later in lactate than in glycerol. Conversely, the slower adaptation rate in lactate is in agreement with a lower robustness of the ability to grow in this medium with respect to the ability to grow in glycerol (*robustness effect*), as independently emerged from the different survival rates after mutagenesis in the two environments (Fig. 4).

Overall, this study provides experimental support on the view that phenotype robustness, through the accumulation of cryptic genetic variation, can promote faster adaptation at the level of a whole organismal system, here a bacterium. It also suggests that this can be achieved by allowing genetically more variable

populations to access a greater amount of phenotype variation, and that can be effective even in short-term evolution. Further studies, complementing measures of adaptive performances with a genetic analysis of mutation patterns, will be necessary to clarify the precise dynamics underlying the influence of cryptic genetic variation on adaptation.

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Figure S1

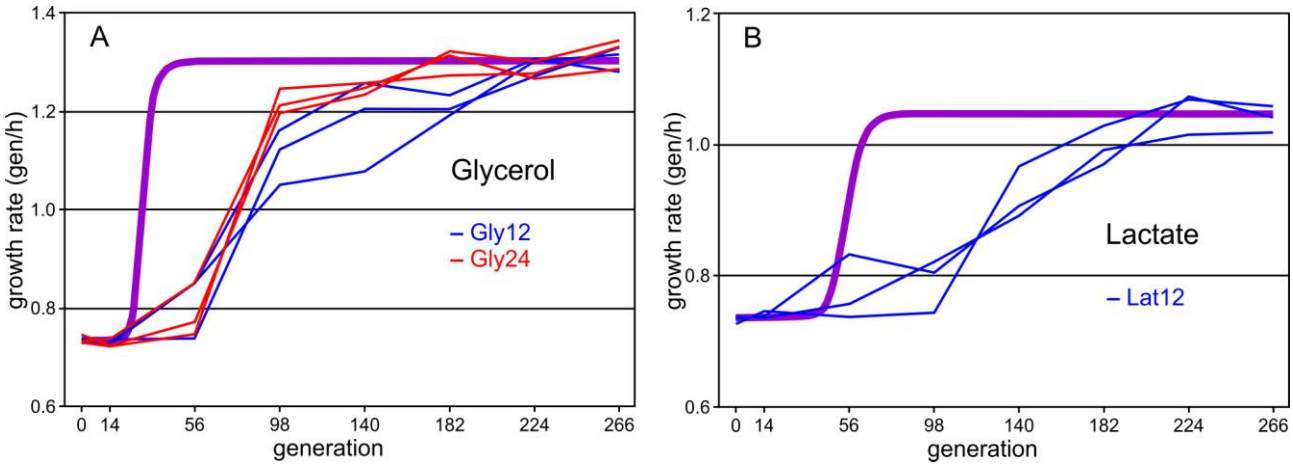

Figure S1. Observed adaptive paths of individual mutagenized lines. Observed adaptive paths in glycerol (**A**) and in lactate (**B**) of lines with 12 (blue) and 24 (red) neutral mutations per genome on average. Purple curves in the two graphs is the expected evolution of the growth rate in the case of one single individual with a phenotype with growth rate equal to value reached toward the end of the experiments (1.30 gen/h for glycerol and 1.05 gen/h for lactate) was already present at generation 0 (frequency 10^{-7}). Growth rate plateau would have been reached at about generation 40 in glycerol and at generation 70 in lactate. This simple evolution model (drawn from [1]), considers the Malthusian growth of two independent populations with constant growth rates (r_1 and r_2) expressed as generations/hour. The instantaneous population average growth rate is $GR(t) = \ln(f(t)2^{r_1} + (1-f(t))2^{r_2})/\ln(2)$, where $f(t)$ is the frequency of the mutant at time t. This is calculated as $f(t) = f_0 2^{(r_1-r_2)t}/(1+f_0(2^{(r_1-r_2)t}-1))$, where f_0 is the frequency of the mutant at time t=0, i.e. 10^{-7}. As the observed evolution trajectories are plotted on the number of generations, $GR(t)$ values are accordingly plotted on the number of generations that have succeeded at time t, calculated as $g(t) = \ln(f_0 2^{r_1 t} + (1-f_0)2^{r_2 t})/\ln(2)$. The use of a pure deterministic model, which does not take explicitly into account the effects of random drift, is justified by the effective population size ($Ne \cong 10^8$, the harmonic mean of the population size along the daily culture). In fact, calculating as $\tau_{0.5} \approx \ln_2(0.5Ne)/s$ [2] the number of generations needed to reach a frequency of 0.5 for the mutant phenotype (with a selective advantage of s=0.76 for glycerol and s=0.42 for lactate), the effect of drift in delaying the establishment of an advantageous phenotype already present at the start of the culturing experiment is in the order of 2 generations for glycerol and 5 generations for lactate.

References

1. Lenski RE, Rose MR, Simpson SC, Tadler SC (1991) Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am Nat 138: 1315–1341.
2. Barrick JE, Yu DS, Yoon SH, Jeong H, Oh TK, Schneider D, Lenski RE, Kim JF (2009). Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. Nature 461: 1243–1247.