Contribution of individual random mutations to genotype-by-environment interactions in *Escherichia coli*

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Numerous studies have shown genotype-by-environment (G×E) interactions for traits related to organismal fitness. However, the genetic architecture of the interaction is usually unknown because these studies used genotypes that differ from one another by many unknown mutations. These mutations were also present as standing variation in populations and hence had been subject to prior selection. Based on such studies, it is therefore impossible to say what fraction of new, random mutations contributes to G×E interactions. In this study, we measured the fitness in four environments of 26 genotypes of *Escherichia coli*, each containing a single random insertion mutation. Fitness was measured relative to their common progenitor, which had evolved on glucose at 37°C for the preceding 10,000 generations. The four assay environments differed in limiting resource and temperature (glucose, 28°C; maltose, 28°C; glucose, 37°C; and maltose, 37°C). A highly significant interaction between mutation and resource was found. In contrast, there was no interaction involving temperature. The resource interaction reflected much higher among mutation variation for fitness in maltose than in glucose. At least 11 mutations (42%) contributed to this G×E interaction through their differential fitness effects across resources. Beneficial mutations are generally thought to be rare but, surprisingly, at least three mutations (12%) significantly improved fitness in maltose, a resource novel to the progenitor. More generally, our findings demonstrate that G×E interactions can be quite common, even for genotypes that differ by only one mutation and in environments differing by only a single factor.

G×E interaction | GEI | phenotypic plasticity | fitness | evolution

Understanding how genotype and environment interact to determine an organism's phenotype and fitness is a fundamental goal at the interface of ecology, genetics, and evolution (1, 2). However, the extent and underlying form of genotype-by-environment (G×E) interactions are only poorly known, for several reasons. Previous studies that focused explicitly on G×E interactions have typically used genotypes that differ by a large and unknown number of mutations (3, 4). Thus, it is unclear whether a given G×E interaction depends on a single "plasticity" gene or on numerous alleles that together produce the resulting pattern. Moreover, because these genotypes represent standing variation from extant populations, the mutations have been subject to prior selection and cannot be considered a random set with respect to their fitness effects. Based on such studies, it is impossible to say what fraction of new, random mutations contributes to G×E interactions. Other kinds of studies have examined the environment-dependent effects of individual mutations to elucidate the roles of particular genes in biochemical and physiological pathways. However, these mechanistic studies have usually not measured the consequences of single mutations for organismal fitness (but see refs. 5–8). Because of their focus on particular pathways, such studies have not sought to quantify the effects of a random assortment of mutations from throughout the genome. Determining how often the fitness effects of mutations differ across environments, and how this prevalence depends on environmental factors, will contribute to understanding the extent and form of G×E interactions. These are the primary objectives of the present study.

In a statistical context, G×E interaction refers to any nonadditive effect of an organism's genotype and its environment on the expression of a trait of interest. G×E interactions have been documented in many organisms, along many environmental dimensions, and for many different traits (2, 4). There are various forms of G×E interaction (1, 3), two of which are shown in Fig. 1. Fig. 1*A* illustrates a situation in which the genotypic rank order with respect to the trait of interest changes across environments, such that there is no single best genotype. Fig. 1*B* shows a case in which the rank order for the trait is preserved, but the extent of variation is much greater in one environment than in the other. The form of the G×E interaction, its underlying genetic architecture, and the pattern of exposure to the relevant environments exert a major influence on a population's evolution and the fate of its genetic variants (9–18). For example, a change in the rank order of genotypic fitness caused by environment-dependent effects of a single gene may support a balanced polymorphism in a heterogeneous environment. Even without a change in rank order, differences in the extent of variation in fitness among environments will affect the rate at which genetic variants are removed relative to the rate at which new variation arises by mutation and recombination. Investigating the form of G×E interaction caused by single random mutations is an additional goal of this study.

We used genotypes of the bacterium *Escherichia coli* to examine the effects of single mutations on the extent and underlying form of G×E interactions. The progenitor genotype was isolated from a population that evolved for 10,000 generations in one of the assay environments (glucose, 37°C). Twenty-six genotypes differ from the progenitor genotype by a single random insertion mutation, and these genotypes have never experienced selection in any of our assay environments. An additional genotype carries a readily scored genetic marker but is otherwise identical to the progenitor. We measured the effect on fitness of each mutation in four environments differing in temperature and limiting resource to address these general questions: Are single, random mutations sufficient to give rise to G×E interactions? Are the fitness effects of mutations equally responsive to temperature and resource, or is one ecological factor more often involved in interactions? Does the form of G×E interaction indicate changing ranks or variances across environments? Are beneficial mutations extremely rare, as is

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Abbreviations: G×E, genotype-by-environment; Cam^R, chloramphenicol resistance/resistant; Kan^R, kanamycin resistance/resistant; Tet^R, tetracycline resistance/resistant.

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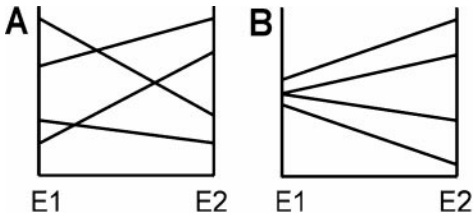

Fig. 1. Schematic of two forms of G×E interaction. In these reaction norms, lines indicate the phenotypic trait values of four hypothetical genotypes in two environments, E1 and E2. (*A*) The rank order of the genotypes changes between environments such that the lines cross. (*B*) The rank order is preserved, but genotypic variance differs between the two environments.

generally accepted, or does their frequency depend on the environment in which a set of mutations is tested?

Materials and Methods

Bacterial Genotypes. The progenitor clone used in this study is a derivative of *E. coli* B, designated REL4548. This clone was isolated from a population that evolved for 10,000 generations in a minimal glucose medium at 37°C as part of a long-term evolution experiment (19, 20). Elena *et al.* (21) constructed 226 mutants derived from REL4548, each of which contains a single insertion of one of three otherwise isogenic mini-Tn*10* transposons (22) that encode resistance to chloramphenicol (Cam^R), kanamycin (Kan^R), or tetracycline (Tet^R). Insertion mutations were constructed by using a phage, λNK, that can neither replicate autonomously nor lysogenize a transformed host (22) and hence serves only as a delivery vehicle for the minitransposon. Because the transposase is expressed from this defective phage, not from the transposon, no secondary transpositions can occur (21, 22). In this study, we randomly chose nine mutations from each of the three resistance classes. One mutant that carried a Cam^R insertion was removed, owing to contamination of its stock culture, and another was determined to be a mislabeled mutant carrying a Kan^R insertion, leaving a total of 7 Cam^R, 10 Kan^R, and 9 Tet^R insertion mutants for our study. The progenitor, REL4548, and all insertion mutants are unable to metabolize arabinose (Ara^-). A spontaneous Ara^+ mutant of REL4548 was isolated for use as a common competitor in the assays of fitness described below.

Fitness Assays. We performed competitions to measure the fitness of each Ara^- genotype (progenitor and 26 mutants) relative to the Ara^+ competitor. The Ara^- genotypes form red colonies when grown on tetrazolium arabinose indicator agar, whereas the Ara^+ competitor produces white colonies (23, 24). This difference in colony color enabled us to quantify the changing densities of the competitors in mixed cultures.

Before mixing the competitors, both genotypes were separately grown for one serial-transfer cycle in the environment in which they would compete, to ensure that they were comparably acclimated to the relevant conditions (19–21, 23). Each pair of competitors was then mixed at a 1:1 volumetric ratio, diluted 100-fold into fresh medium, and allowed to compete for six serial-transfer cycles comprising ≈40 generations (21, 25). Thus, initial frequency and stationary-phase population density were held constant in our study, which sought to examine the effects of other environmental factors on fitness. Initial and final samples of the mixed cultures were spread onto tetrazolium arabinose agar plates, and the initial and final densities of each competitor were determined. From these, we calculated each genotype's Malthusian parameter: $m = \ln (N_6 \times 100^6/N_0)$, where N_0 and N_6 are the initial and final (day 6) population densities. The final population density is adjusted for the six 100-fold dilutions performed during the competition. The relative fitness of each genotype was computed simply as the ratio of its Malthusian parameter to that of its competitor (19). This relative fitness estimate integrates differences in growth and survival during all phases of the serial-transfer regime.

Competitions were performed in four different environments that varied with respect to limiting resource and incubation temperature. In all four environments, 10-ml cultures were held in unshaken tubes. The first environment consisted of Davis minimal medium with 25 μg/ml glucose incubated at 37°C. This regime employs the same medium and temperature as experienced for 10,000 generations by the population from which the progenitor clone was isolated, whereas the other environments are novel in one or both of these respects. The three novel environments consisted of Davis minimal medium with 25 μg/ml maltose incubated at 37°C, the glucose medium incubated at 28°C, and the maltose medium incubated at 28°C.

Fitness assays were conducted in four randomized complete blocks. Each block included one experimental competition of each of the 26 mutants against the Ara^+ competitor, plus five control competitions of the progenitor against the same Ara^+ competitor, in all four environments. Each block therefore comprised 124 competitions. The control competitions were included to adjust for the potential effect of the Ara^+ marker on fitness in any of the environments. Owing to experimental errors, there were two missing values in the full data set.

Statistical Analyses. We first analyzed the fitness of the unmutated progenitor relative to the Ara^+ competitor to test for differences across environments and blocks in the control competitions. We used a mixed general linear model allowing unequal variances across environments (26). Both block and environment had small but statistically significant effects on the fitness of the progenitor relative to the Ara+ competitor (analysis not shown). Therefore, we calculated the average relative fitness of the Ara^+ competitor relative to the progenitor for each environment in each block. Then, each estimate of a mutant's fitness relative to the Ara^+ competitor was standardized to the appropriate control fitness to yield an estimate of the mutant's fitness relative to the progenitor.

The standardized relative fitness data were analyzed with mixed general linear models (26). These models included antibiotic-resistance marker, temperature, resource, and their interactions as fixed effects, and mutation nested within marker and all its interactions with temperature and resource as random effects. The among-mutation variances were allowed to differ across environments, and the temporal block was included in the model as a random effect. The denominator degrees of freedom for *F* tests of fixed effects were estimated by using the Satterthwaite approximation. These estimates may differ among factors at equal levels of the analysis because they depend both on sample sizes and variance structure. Likelihood ratio tests (27) were used to test random factors as well as to test for unequal variances among the effects of mutations across environments. Contrasts within the full model were used to determine which of the sampled mutations contributed to G×E interaction by comparing fitness effects of each mutation across the different environments. After finding that the effects of interactions between temperature and other factors were nonsignificant, we sought to address whether any mutation had a significantly beneficial effect on fitness in either resource. To that end, we ran a total of 52 (two resources, 26 mutations) *t* tests, each of which pooled the eight fitness estimates (four blocks, two temperatures) for a given mutation on each resource. To avoid spurious evidence for beneficial mutations, we used the sequential Bonferroni correction for independent tests (28).

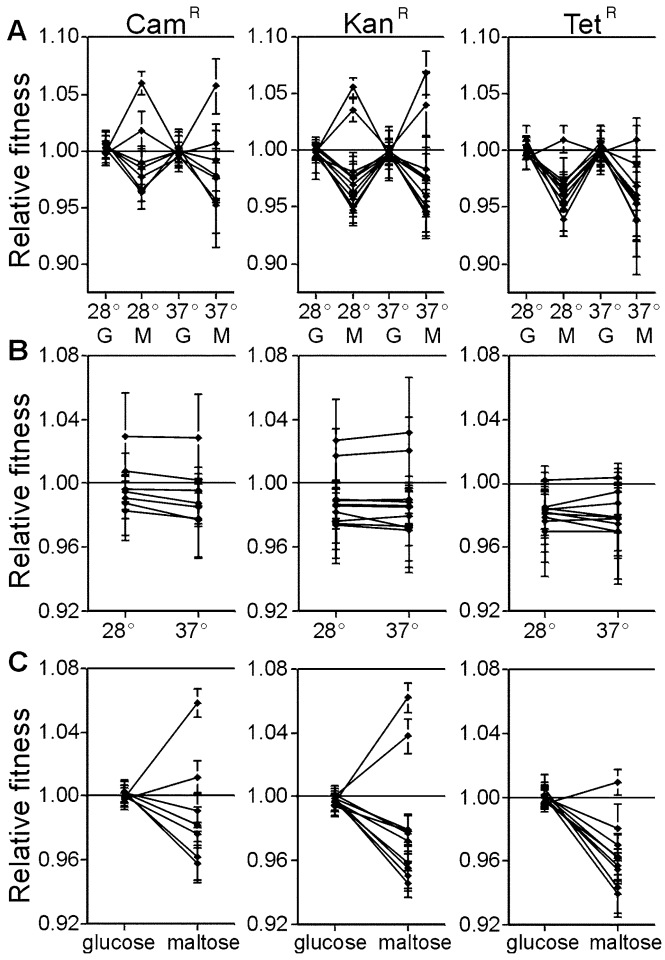

Fig. 2. Reaction norms showing the fitness of each mutant across environments. Cam^R, Kan^R, and Tet^R mutants are depicted separately for ease of viewing. Raw means and 95% confidence intervals of the fitness of a given insertion mutant relative to the progenitor for all four environments (28°C glucose, 28°C maltose, 37°C glucose, 37°C maltose) averaged across blocks (*A*), the two temperatures, averaged across resources and blocks (*B*), and the two resources, averaged across temperatures and blocks (*C*). A relative fitness of 1.0 indicates that the insertion mutation is neutral in that environment.

Table 1. Mixed general linear model of the fitness of all 26 mutants relative to the progenitor

Fixed sources*	ndf	ddf	Type III F
Temperature	1	79.2	3.54^{+}
Resource	1	23.4	10.30**
Temperature × resource	1	79.2	0.16^{ns}
Marker	2	23.4	1.45^{ns}
Marker × temperature	2	79.2	1.71^{ns}
Marker × resource	2	23.4	1.52^{ns}
Marker × temperature × resource	2	79.2	0.54^{ns}

Random sources†	df	Test full model	LR test statistic
Block	1	a	5.75**
Mutation	1	b	124.32***
Mutation × temperature	2	c	0.00^{ns}
Mutation × resource	2	c	458.79***
Mutation × resource variance effect	1	c	82.80***
Mutation × temperature × resource	4	a	0.13^{ns}

ns, $P > 0.1$; +, $0.05 < P < 0.1$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

*Fixed effects tested with approximate *F* tests. ndf, numerator degrees of freedom; ddf, denominator degrees of freedom (estimated using a Satterthwaite approximation).

†Random effects tested with likelihood ratio tests. LR test statistic = −2 × (maximum likelihood from the test's full model − maximum likelihood from a restricted model from which the parameter of interest is removed). The LR test statistic has an approximately Chi-squared distribution; df, difference in the number of parameters fit in the test's full and reduced models. In tests of variance effects, the tested parameter is included in both the full and reduced models, but variances are constraining to be equal in the latter. Test full models: a, full model (all parameters analyzed); b, full model minus all mutation interaction terms; c, full model minus mutation × temperature × resource interaction.

Results

G×E Interactions. Fig. 2 provides a summary of the relative fitness values obtained for the 26 *E. coli* genotypes, each one carrying a single mutation and tested in four environments. The genotypes are grouped by antibiotic-resistance markers, and each group includes an independent set of 7 to 10 insertion mutants. In Fig. 2*A*, the reaction norms show each mutant's fitness in all four environments; Fig. 2*B* shows fitnesses at each temperature (28°C and 37°C) averaged over both resources; and Fig. 2*C* shows the fitnesses in each resource (glucose and maltose) averaged over both temperatures.

Table 1 presents the statistical analysis of the full model including both fixed and random sources of variation. Focusing first on the effects of temperature, we see that neither interaction term involving temperature and mutation is significant (both $P > 0.1$). These negative results can be seen graphically; in Fig. 2*A*, the responses across temperatures for both resources are parallel, and in Fig. 2*B* the among-mutation variances at 28°C and 37°C are similar, and there are few changes in fitness ranks (crossing lines) in the corresponding reaction norms.

In contrast, there is a striking and highly significant G×E interaction between mutation and resource ($P < 0.001$, Table 1). This interaction is seen in Fig. 2*C* by the much greater variance in fitness among mutations in maltose than in glucose. A likelihood ratio test indicates that the difference in genetic variance between glucose and maltose is highly significant ($P < 0.001$, Table 1). However, there are no changes in fitness ranks across the two resources because there is no significant fitness variation among the mutations in glucose (Fig. 2*C*, analysis not shown). Thus, the main source of the G×E interaction in this experiment is the difference in mutational variance between environments (as illustrated by Fig. 1*B*), as opposed to changes in genotypic rank (as illustrated by Fig. 1*A*).

There is also a marginally significant main effect of temperature ($P < 0.1$), and a significant main effect of resource ($P < 0.01$, Table 1), as the mutants tended to be slightly less fit at 37°C than at 28°C, and in maltose than in glucose (Fig. 2). The main effect of mutation on fitness is highly significant as well ($P < 0.001$, Table 1), with some mutations having more harmful effects than others. However, one should not place too much emphasis on these significant main effects, owing to the strong interaction between mutation and resource. That is, the significant interaction means that one cannot accurately predict a particular genotype's fitness in a given environment from these main effects. There are two additional "nuisance" variables in our experimental design: the antibiotic-resistance marker and temporal block. The block effect is significant, which implies that uncontrolled temporal factors influenced these fitness data despite our best efforts to standardize all conditions and procedures. Of course, the possibility of uncontrolled temporal factors is precisely the reason for running an experiment in a randomized complete-block design, which ensures that all groups are equally exposed to the unknown factors. The marker

Table 2. Mixed general linear models of the fitness of Cam^R, Kan^R, and Tet^R mutants relative to the progenitor

		Cam^R		Kan^R		Tet^R	
Fixed factors source	ndf	ddf	Type III F	ddf	Type III F	ddf	Type III F
Temperature	1	98.0	6.60**	21.1	0.04^{ns}	129.0	0.15^{ns}
Resource	1	6.1	0.51^{ns}	9.1	1.91^{ns}	8.6	25.31***
Temperature × resource	1	98.0	0.22^{ns}	21.1	0.36^{ns}	129.0	0.52^{ns}

			LR Test Statistic		
Random factors source	df	Test full model	Cam^R	Kan^R	Tet^R
Block	1	a	0.75^{ns}	2.15^{+}	1.75^{+}
Mutation	1	b	973.64***	60.22***	19.95***
Mutation × temperature	2	c	0.00^{ns}	0.00^{ns}	0.00^{ns}
Mutation × resource	2	c	138.38***	236.52***	79.62***
Mutation × resource variance effect	1	c	23.29***	37.30***	19.91***
Mutation × temperature × resource	4	a	0.00^{ns}	1.88^{ns}	0.00^{ns}

See Table 1 for details of test construction and significance categories. *, $0.01 < P < 0.05$.

effect and all its interactions with temperature and resource were not statistically significant ($P > 0.1$).

The three sets of mutations that are associated with the different antibiotic-resistance markers represent independent samples of random insertion mutations. Thus, we can view our overall experiment as comprising three independent subexperiments that allow us to test the same hypotheses repeatedly (Table 2) to judge the robustness of our conclusions. Of course, the marker and its interactions drop out of these analyses. In all three subexperiments, none of the interactions involving temperature is significant. By contrast, in all three cases, the G×E interaction involving resource is highly significant ($P < 0.001$). And in all three subexperiments, the difference in mutational variance for fitness between the glucose and maltose environments contributes significantly to the interaction based on likelihood ratio tests ($P < 0.001$). The main effects of resource and mutation are significant in some or all of the subexperiments; however, as noted above, one cannot attach much importance to main effects involved in strong interactions, as these two factors clearly are. The Cam^R mutants have significantly lower relative fitness at 37°C than at 28°C, but this effect is not seen in either the Tet^R or Kan^R subexperiments (Table 2). In summary, the analyses of the three independent subexperiments support the exact same conclusions regarding G×E interactions as the analysis of the full experiment, thus emphasizing their statistical robustness.

Prevalence of Mutations That Differ in Their Fitness Effects in Glucose and Maltose. The analyses presented above establish that G×E interaction exists within the complete set of 26 mutations, as well as in each of the three subsets of mutations grouped by resistance marker. To quantify more precisely the overall prevalence of resource-dependent fitness effects arising from single insertion mutations, we performed two further analyses. First, for each mutation, we ran a two-tailed paired *t* test comparing the fitness values in glucose with those measured in maltose (pairing measures from the same block and temperature). All 26 tests were significant at $P < 0.05$, and 17 (65%) were significant at $P < 0.001$, indicating that the vast majority of insertion mutations produce different effects on fitness during competition for these two resources. However, because there is a significant resource effect (Table 1), many of these significant contrasts may simply reflect this overall effect of resource. Our second analysis employs contrasts within the full model controlling for all other factors. These contrasts test whether the fitness effect of each mutation differed in glucose and maltose beyond the expectation based on the overall effect of resource. Because it is not possible to separate antibiotic-resistance marker effects from the mean effect of random gene disruption, this approach is conservative with respect to detecting environment-dependent effects of the insertion mutations. Fig. 3 shows the distribution of *P* values for these 26 contrasts. Under the null hypothesis of no mutation-by-resource interaction, the expected distribution is uniform, with 1.3 observations in each of the 20 intervals shown. In fact, we observed a large excess of mutations (11 rather than 1.3) having different fitness effects across the two resources at the level of $P < 0.05$, indicating that some 42% of the sampled genes contributed to the overall significant G×E interaction. Thus, a large fraction of random insertion mutations have distinct effects on fitness in environments that differ only with respect to whether glucose or maltose is the limiting resource.

Some Insertion Mutations Are Beneficial in Maltose. The most surprising result, in our view, is that several random insertions seem to have beneficial effects in maltose. Whereas natural selection applied to large populations demonstrates that beneficial mutations exist (19, 20, 29), it is generally thought by population geneticists that the vast majority of new mutations are neutral or deleterious, and only a tiny fraction are beneficial. To evaluate whether any of the 26 random mutations in our study were beneficial in either resource environment, we ran 52 two-tailed *t* tests to determine whether a given genotype had a fitness different from the null-hypothetical value of 1. (Any

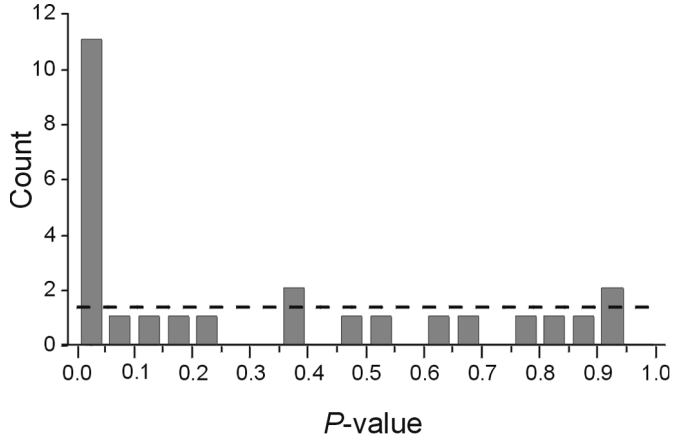

Fig. 3. Histogram of P values from contrasts of fitness effects of the 26 mutants in glucose versus maltose. The expectation under the null hypothesis of equal fitness of all mutants in the two resource environments is indicated by the dashed line.

deviation from the null value includes the effect of the insertion mutation *per se* as well as the effect of carrying a particular resistance marker. Insofar as the possession of an unnecessary resistance gene may be deleterious, this test is conservative with respect to identifying beneficial mutations.) In glucose none of the 26 mutations produced a significant fitness gain relative to the progenitor. In contrast, five (19%) conferred fitness significantly greater than 1 in maltose ($P < 0.05$), and three (12%) remained significantly beneficial when using the sequential Bonferroni criterion for independent contrasts (28). The detection of significant fitness improvements in mutants with at least two different antibiotic-resistance markers (Fig. 2*C*) implies that the benefits cannot be attributed to some unexpected effect of a particular resistance gene. Instead, it indicates that the fitness gains observed in maltose must be attributed to the insertion mutations themselves.

Discussion

In the Introduction, we posed four questions that our experiment would address: Are single, random mutations sufficient to give rise to G×E interactions? Are the fitness effects of mutations equally responsive to temperature and resource, or is one ecological factor more often involved in interactions? Does the form of G×E interaction indicate changing ranks or variances across environments? Are beneficial mutations extremely rare, or does their frequency depend on the environment in which mutations are tested? We will now discuss the answers to each of these questions that are derived from our results.

Single Mutations Produce G×E Interactions. Previous studies of G×E interaction have used genotypes that differ by an undetermined, and usually very large, number of mutations. Such studies cannot address whether G×E interactions depend on one or many of the underlying mutations, nor whether any particular mutation contributes to the overall interaction. By contrast, our study used 26 genotypes that differ by only a single mutation from their common progenitor. Our findings demonstrate that these single mutations are sufficient to produce a strong G×E interaction (Fig. 2). This interaction is highly significant across all 26 mutants as a whole (Table 1) as well as in analyses of all three subexperiments (Table 2). Moreover, these mutations had not experienced prior selection in any of the assay environments, and they were randomly chosen with respect to their phenotypic effects. The random nature of this variation also contrasts with many previous studies of G×E interactions, in which investigators have studied extant genetic variation that was presumably subject to prior selection. Our results clearly indicate that random individual mutations in the *E. coli* genome can contribute substantially to G×E interactions. Therefore, phenotypic plasticity must often depend on many different genes rather than a handful of "plasticity" genes.

Ecological Factors Differ in the Prevalence of G×E Interaction. Our experimental design included two environmental factors: resource and temperature. *A priori*, we had no reason to expect that one factor or the other would be more extensively involved in G×E interaction with single mutations. Indeed, previous experiments (29–32) using *E. coli* genotypes closely related to the progenitor in this study have demonstrated phenotypic plasticity and G×E interaction involving both temperatures and resources. However, the genotypes from earlier studies differed from one another by mutations at multiple loci and had been subjected to natural selection in one of the test environments.

In contrast to the view that both resource and temperature might contribute more or less equally to G×E interactions, the 26 random mutations studied here showed a strong tendency to interact with resource but not with temperature. The interaction between resource and mutation was highly significant ($P < 0.001$) in analyses of the overall experiment (Table 1) and all three subexperiments, each comprising an independent set of 7–10 mutations (Table 2). By contrast, the interaction between temperature and mutation was nonsignificant ($P > 0.1$) in every case. Eleven of the 26 mutations contributed to the G×E interaction through their significantly different fitness effects in glucose versus maltose (Fig. 3). Within the context of this experiment, the fraction of new mutations whose effects depend strongly on resource is clearly much greater than the fraction whose effects depend strongly on temperature. In other words, phenotypic plasticity across the two assay temperatures, 28°C and 37°C, is affected by many fewer genes than plasticity across the two resources, glucose and maltose. These divergent results indicate that it would be difficult to predict what proportion of genes are involved in plasticity with respect to other environmental factors, such as pH or population density, that might be examined in similar experiments.

Form of G×E Interaction Indicates Changing Variances Across Environments. Fig. 1 contrasts two distinct forms of hypothetical G×E interaction, one in which the rank order of genotypes changes across environments (Fig. 1*A*) and the other in which the genetic variance changes across environments (Fig. 1*B*). Changing ranks and variances are not mutually exclusive, and thus G×E interactions can involve both patterns simultaneously. In our experiment, the form of the interaction between resource and mutation is one of changing variance in mutational effects between glucose and maltose (Fig. 2*C*), whereas there was no interaction of either type between temperature and mutation (Fig. 2*B*). G×E interaction involving changing variance, like that observed across resources, implies that the relative influence of selection and drift on the evolutionary fate of alleles will depend on environmental conditions. Exposure to environments where the variance in fitness effects is low should slow the rate at which alternative alleles are removed from or become fixed in the population.

The maintenance of genetic variability because of G×E interaction requires that selection favor different alleles at a given locus in different component environments. Fry *et al.* (33) found that new mutations (detected in mutation accumulation lines) commonly contribute to this form of G×E interaction in *Drosophila melanogaster*. However, we did not detect individual mutations with fitness ranks that changed across environments in this study. The lack of evidence for changing ranks across resources reflects, at least in part, the absence of variability in fitness in glucose among the 26 mutants (Fig. 2*C*). In the absence of any significant variation in the glucose environment, there was no opportunity to detect significantly changing ranks between glucose and maltose.

The lack of fitness variation among mutations in glucose that we observed differs somewhat from the results of Elena *et al.* (21), who studied a larger set of 226 mutations from which the genotypes that we used were drawn. In particular, Elena *et al.* (21) reported that most insertion mutations had slightly deleterious effects in glucose at 37°C, whereas about 5% of these mutations had very deleterious effects. By chance, our random sample did not contain any mutants from the severely deleterious tail. This limited our ability to detect variation among mutants in glucose performance and thus also the possibility of changing ranks. Nonetheless, it is clear from both studies that the majority of insertion mutations have little or no fitness effect in glucose. It is unclear, however, why the modal fitness effect in our study was centered on neutrality, whereas the previous study indicated a mode with a slightly negative fitness effect.

Prevalence of Beneficial Mutations. In general, beneficial mutations are thought to be rare (34). However, commonly used approaches to estimating genomic mutational effects do not accu-

rately estimate the rate of beneficial mutations (35). Our results indicate that, at least under some conditions, mutations that improve fitness may be much more common than previously believed. Because all five mutations that showed evidence of being beneficial did so in maltose but not in glucose, they are only conditionally beneficial. Two nonmutually exclusive causes of this resource dependence are similar in that they both invoke a footprint of adaptation to glucose use in the progenitor genotype. They differ in the time frame over which they suggest this adaptation has occurred.

First, the resource dependence could be caused by recent adaptation in response to the 10,000 generations of selection in minimal glucose medium. During this period of adaptation, fitness increased rapidly for the first 2,000 generations but more slowly thereafter (20). This fitness trajectory suggests that the population is nearing a fitness peak in glucose. Theoretically, the closer a genotype is to the peak, the smaller the number of possible mutations that can be beneficial (36). Moreover, in the absence of selection for performance on maltose, the progenitor may have lost fitness in the maltose environment, leaving more opportunities for improvement in maltose. As a result of both of these effects, more mutations should be beneficial in the unselected maltose environment than in the glucose environment. The reduced slope of the fitness trajectory after 2,000 generations also suggests that, during this period, many traits experienced stabilizing selection. Such stabilizing selection has been hypothesized to result in genetic canalization (37–41). Genetic canalization, the insensitivity of the phenotype to mutation, reduces the variance in mutational effects in the selected environment but not in other environments. It could therefore cause the observed pattern of resource-dependent variance in mutational effects. Genetic canalization could also contribute to resource dependence of beneficial mutations by reducing the frequency of beneficial mutations with effects large enough to be detectable in glucose.

Alternatively, the resource dependence may be because of ancient differences between the genetic architectures of uptake and use of glucose and maltose. The central metabolic role of glucose may have caused selection for robustness in metabolic pathways involved in growth on glucose to exceed selection for robustness in maltose pathways. This robustness could include not only genetic canalization over a longer time scale but also biochemical redundancy arising as a consequence of adaptations that decrease sensitivity to environmental fluctuations (42, 43). Both *in vivo* and *in silico* studies of knockout mutations in *E. coli* suggest that growth on glucose involves more redundancies than growth on other resources (44). If ancient evolution of genetically robust growth in glucose contributes to the observed resource dependence in this system, then similar patterns may exist in other taxa as well.

In summary, the pattern of fitness effects of random mutations across resources and temperatures demonstrates that individual mutations commonly contribute to G×E interactions with respect to at least some environmental factors. Therefore, phenotypic plasticity may often depend on many genes rather than only a few "plasticity" genes. We showed that the contribution of new mutations to G×E interactions differed between resource and temperature. This indicates that the resource and temperature G×E interactions observed in previous studies, which used selected genotypes differing at many loci (29–32), may be caused by different underlying genetic architectures. We observed significantly lower variance in mutational effects in glucose than in maltose but no change in the ranks of mutations across resources. The latter outcome was due in part to the absence of genetic variability for fitness in glucose. Most surprising was the high frequency (12%) of mutations conferring fitness advantages in the novel resource, maltose. The resource dependence of both the variance in mutational effects and the number of beneficial mutations may reflect recent adaptation to the glucose environment or ancient differences in the genetic robustness of growth on these two resources.

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