

Rates of fitness decline and rebound suggest pervasive epistasis

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ABSTRACT

Unraveling the factors that determine the rate of adaptation is a major question in evolutionary biology. One key parameter is the effect of a new mutation on fitness, which invariably depends on the environment and genetic background. The fate of a mutation also depends on population size, which determines the amount of drift it will experience. Here, we manipulate both population size and genotype composition and follow adaptation of 23 distinct *Escherichia coli* genotypes. These have previously accumulated mutations under intense genetic drift and encompass a substantial fitness variation. A simple rule is uncovered: the net fitness change is negatively correlated with the fitness of the genotype in which new mutations appear - a signature of epistasis. We find that Fisher's geometrical model can account for the observed patterns of fitness change and infer the parameters of this model that best fit the data, using Approximate Bayesian Computation. We estimate a genomic mutation rate of 0.01 per generation for fitness altering mutations, albeit with a large confidence interval, a mean fitness effect of mutations of -0.01 and an effective number of traits 9 in *mutS* *E. coli*. This framework can be extended to confront a broader range of models with data and test different classes of fitness landscape models.

INTRODUCTION

The distribution of effects of new mutations and how their interactions cause changes in fitness are fundamental quantities in our understanding of evolution. It is generally acknowledged that the genetic background has a strong influence on the phenotypic effect of mutations (De Visser et al. 2011). If the phenotype is relevant for fitness, natural selection will act differently on the mutation depending on its genomic background. Throughout this paper, a mutation is said to be epistatic if its fitness effect, and thus ultimately its fate in the population, depends on the genetic background where it appears.

Numerous population genetics models have been built assuming that mutations affect fitness

1 independently, i.e. that epistasis is absent. In the most classical experiment designed to determine
2 the distribution of fitness effects of mutations, epistasis is typically ignored. In this type of
3 experiment, populations are propagated at the lowest possible population size, minimizing the effect
4 of natural selection and allowing deleterious mutations to accumulate close to the rate at which they
5 appear (Bateman 1959; Mukai 1964). Interestingly, the results of these mutation accumulation
6 (MA) experiments, have been continuously re-analyzed using different methods (Bataillon 2000;
7 Halligan and Keightley 2009), but usually under the same model. The rate and mean effect of
8 mutations are assumed constant and independent of the current fitness of the genotype hit by a new
9 mutation. However, empirical work suggests that epistasis for fitness might be pervasive, with
10 positive or negative mean depending on the organism studied (Sanjuán and Elena 2006) . Many
11 studies are focused on deleterious mutations, as these are the most common. However recent
12 experiments, focusing on discrete sets of beneficial mutations underlying fitness changes observed
13 in experimental populations, support the notion that beneficial mutations may generally interact in
14 an antagonistic way in microorganisms (Chou et al. 2011; Khan et al. 2011; Rokyta et al. 2011).
15 Other studies show mean levels of epistasis ranging from antagonistic to nearly neutral in bacteria
16 (Trindade et al. 2009; Ward et al. 2009).

17 Understanding the type of epistasis underlying fitness has important consequences for a
18 number of evolutionary questions. For example, it is expected that sexual reproduction will be
19 favored if most deleterious mutations interact synergistically (Kondrashov 1988). One important
20 type of epistasis is sign epistasis, in which a mutation is deleterious or beneficial depending on the
21 background. If it is common, then the number of paths that can be taken during adaptation might be
22 severely constrained (Weinreich et al. 2005). The pattern of epistasis is often thought to be specific
23 to a given species. However, recurrent observations suggest an interesting empirical rule: genotypes
24 with lower fitness in a given environment adapt faster to that environment. This is observed across
25 very diverse organisms: phage (Silander et al. 2007), *Escherichia coli* (Moore and Woods 2006;
26 Khan et al. 2011), *Saccharomyces cerevisiae* (Murphy and Zeyl 2012), *Aspergillus nidulans*

1 (Gifford et al. 2011) and *Caenorhabditis elegans* (Estes and Lynch 2003). All of these studies are
2 focused on the fitness effects of beneficial mutations in diverse backgrounds. An open question is
3 whether or not the same rule applies to other types of mutations.

4 Many experiments, particularly those aimed at studying adaptation, can only detect the
5 effects of mutations that survive genetic drift. Since the probability of escaping stochastic loss
6 depends itself on the selection coefficient of mutations, estimating how fitness effects depend on
7 genetic background requires taking into account the effect of natural selection. Three main factors
8 affect the efficiency of selection and thus the probability of empirically detecting a beneficial
9 mutation: population size, spatial structure and recombination. In small or fragmented populations,
10 selection will be relatively inefficient both at purging deleterious and at fixing beneficial mutations.
11 In very large populations, deleterious mutations will have little chance of fixing, while beneficial
12 mutations enjoy a fixation probability directly proportional to their effect on fitness (Haldane 1927),
13 assuming weak selection. In the absence of recombination, the efficiency of selection is also
14 reduced because mutations are locked together in the same genotype. For example, if a beneficial
15 mutation appears in a background that already harbors a deleterious mutation, it will only have a
16 good chance of fixing if its effect outweighs the deleterious effect of the first mutation
17 (Charlesworth et al. 1993; Bachtrog and Gordo 2004). In the same manner, if two beneficial
18 mutations appear in a population on different genetic backgrounds, they will compete for fixation
19 and one will be lost (Gerrish and Lenski 1998; Gerrish et al. 2007). This interference also occurs in
20 the presence of small amounts of recombination (Barton 1995).

21 Here we analyze the results of two laboratory experiments, where population size is
22 manipulated in order to modulate the efficiency of natural selection. Specifically we re-analyze the
23 results of a MA experiment using a strain of *E. coli* that exhibits a mutator phenotype (*mutS*⁻). In
24 this experiment *E. coli* evolved under drastic periodic bottlenecks consisting of a single cell. We
25 also complement this MA study with a re-adaptation experiment. In this experiment several
26 independent genotypes, stemming from the MA experiment, are allowed to evolve under large

1 effective population size. We explore how fitness changes (decreases in small populations and
2 increases in large populations) depend on the genetic background. We show that the fitness change
3 is strongly correlated with initial fitness in both experiments, suggesting that epistasis is very
4 common.

5 Not accounting for epistasis fails to uncover the salient features of the fitness trajectories observed
6 in each experiment. Therefore we should consider models where epistasis is pervasive. One widely
7 used phenotype-based model of evolution is Fisher's geometrical model, hereafter referred to as
8 FGM (Fisher 1930). This model has been used to interpret patterns of adaptation in large
9 populations and to derive theoretical distributions of fitness effects of mutations and epistasis
10 (Martin et al. 2007). It has also been invoked to help understanding the evolutionary trajectory of
11 small populations (Tenaillon et al. 2007). So far, it has rarely been used to explain the fitness decay
12 typically observed during evolution of very small populations, in particular of MA experiments (but
13 see (Poon and Otto 2000; Martin and Lenormand 2006). Under FGM, one can derive the
14 distribution of fitness effects, s , of a single mutation depending on the current background and
15 pairwise epistasis $\langle \epsilon \rangle$ between random mutations (Martin et al. 2007). The first is expected to
16 follow a shifted gamma distribution with a given mean, $E(s)$, and the latter is predicted to be a
17 normal distribution with mean $\langle \epsilon \rangle = \text{zero}$. Both $E(s)$ and $\langle \epsilon \rangle$, do not depend on the genetic
18 background, although the variances do in the simplest version of FGM. Other versions of the FGM
19 where $\langle \epsilon \rangle$ is non-zero have also been proposed (Gros et al. 2009). The simplest model pictures
20 evolution as a walk in a geometric space of n phenotypic traits. It assumes that, within a given
21 constant environment, a single value for each of the n of traits is optimal, with fitness decreasing
22 smoothly for phenotypes away from that optimum. When a population is located at the optimum,
23 any random mutation, irrespective of its size or direction, will cause a deviation from the optimum
24 and so will necessarily be deleterious. FGM incorporates naturally a dependence of the rate of
25 evolution on the initial phenotype. Importantly this model can account for several empirical patterns
26 (Tenaillon et al. 2007; Gordo and Campos 2012) including the fitness effects of mutations

1 (Schoustra et al. 2009; Bataillon et al. 2011; Sousa et al. 2012; Trindade et al. 2012) and pairwise
2 epistasis (Martin et al. 2007). Here, we use FGM to analyze the results of the measured fitness
3 change, under both small and large population sizes. We introduce a statistical framework that
4 allows combining information from both experiments to estimate the parameters underlying this
5 model. We show that patterns of fitness change in both experiments can be quantitatively captured
6 under FGM.

7

8 **MATERIALS AND METHODS**

9

10 *Bacterial strains and growth conditions*

11 The strains and growth conditions used in this study were as described in (Trindade et al. 2010).
12 Briefly, for the mutation accumulation (MA) experiment and its analysis, the two strains used were
13 *E. coli* K12 MG1655 srl::Tn10 *mutS* and K12 MG1655 srl::Tn10 Δ *ara mutS*. The first strain was
14 the ancestral for the MA experiment, and the second was used as the reference strain for the
15 competition assays in the fitness measurements (see below). The deletion in the arabinose operon is
16 a phenotypic marker that allows distinguishing the two strains in competition, since these mutants
17 give rise to red colonies when plated in Tetrazolium agar (TA) (Lenski et al. 1991).

18 For the recovery experiment, 23 clones derived from the MA lines were studied. These clones cover
19 a range of initial fitnesses from 0.77 to 0.99 and include 16 clones from the 50th bottleneck, 6 clones
20 from the 20th bottleneck and one clone from the 70th bottleneck.

21 To allow the clones to re-adapt, evolution under large population sizes was performed. In the first
22 day, each experimental line was started with 10 ml of LB medium seeded with $\sim 1.5 \times 10^4$ individuals
23 and incubated at 37°C with agitation for 24h. This period allowed for ~ 20 generations after which
24 appropriate dilutions were made from each culture and another $\sim 1.5 \times 10^4$ individuals were used to
25 seed the next passage. This procedure was repeated for ~ 240 generations after which fitness from
26 all the lines was measured. One of the clones was propagated in 3 independent replicates and 5 were

1 propagated in 2 replicates. The remaining clones were propagated once, yielding in total 30 adapted
2 populations.

3

4 *Mutation rate and Fitness estimation*

5 Since it is possible for the mutation rate to evolve, we looked for a phenotypic signature of a change
6 in mutation rate, by a fluctuation assay. We observed that the mutator phenotype present in the
7 ancestral clone was maintained in the 34 lines tested, these comprised the 23 lines used as the
8 ancestors of the recovery experiment and 11 lines after the recovery experiment (Figure S1).

9 The fitness assays of the MA lines are described in (Trindade et al. 2010). Fitness of the recovery
10 lines (after re-adaptation for 120 and 240 generations) was assayed in the same way as the MA
11 lines. Briefly, competitor and reference strains were mixed in a proportion of 1:1 and used to seed
12 10 ml of LB. The competition tube was then grown for 24 hours at 37°C with agitation. Appropriate
13 dilutions, of both initial and final bacterial populations, were plated in Tetrazolium agar (TA) to
14 access the ratio between competitor and reference strains.

15 Selective coefficients were estimated as the difference in growth between the test strain and the
16 reference strain, normalized by the number of generations elapsed for the reference strain (Chevin
17 2011). For each competition replicate, the difference in growth between the test strain (*a*) and the
18 reference strain (*b*) is given by:

$$19 \Delta r_{ab} = \ln(N_{fa}/N_{ia}) - \ln(N_{fb}/N_{ib}),$$

20 where N_{fa} and N_{fb} are the number of test and reference bacteria, respectively, after the competition,
21 and N_{ia} and N_{ib} are the number of test and reference bacteria, respectively, before the competition.

22 The relative growth rate estimated this way refers to a 24-hour growth cycle and must be
23 normalized by the generation time of the reference in order to be comparable with the population
24 genetics model we use. The number of generations (*G*) elapsed for the reference was estimated for
25 each competition replicate, assuming no death, as follows:

$$26 G = \log_2(N_{fb}/N_{ib}).$$

1 Fitness of strain a relative to the reference b (W_a) is then given by:

2 $W_a = 1 + S_{ab}$, where $S_{ab} = \Delta r_{ab} / G$,

3 The fitness of the ancestral of the MA was measured in the same way and all fitness values were
4 then standardized such that the fitness of this ancestral was 1.

5

6 *Simulations of the evolution process under FGM*

7 In order to simulate datasets under FGM that are directly comparable with our observed empirical
8 data, we performed individual-based Monte Carlo simulations.

9 As the MA experiment involves bottlenecks of one cell every day, in the simulations of MA lines
10 we assumed discrete generations and a demography that reflects this periodic fluctuation in
11 population size. Accordingly, we crashed the populations to a size $N=1$, every 23 generations. After
12 these crashes the population expanded, doubling in size every generation, until the next crash
13 occurred. Therefore the harmonic mean of the population size was 23, which was taken as the
14 effective population size N_e (Wahl et al. 2002)

15 In our MA experiment, the founding clone was highly fit, so we expect the vast majority of new
16 mutations to be deleterious. We started the simulations of the MA experiment with a unique
17 genotype residing at the phenotypic multivariate optimum, i.e. with a maximum fitness of 1. Given
18 these conditions we simulated the mutation-selection-drift process under FGM. FGM assumes that
19 n phenotypic traits underlie fitness differences among individuals and each trait is under stabilizing
20 (optimizing) selection. Without loss of generality we assumed that the optimum phenotypic value
21 was zero for each trait. A special case of FGM was assumed by considering a Gaussian fitness
22 landscape, whereby each individual is represented by a vector of n phenotypic trait values
23 $\mathbf{x}=(x_1, x_2, \dots, x_n)$ and has fitness $W=\exp(-\sum_i x_i^2)$. Mutations are fully pleiotropic and occur each
24 generation. The FGM we considered here is an isotropic version of the ones in Martin and
25 Lenormand (2006) and (Waxman and Welch 2005).

26 We assumed that the number of new mutations in each individual was Poisson distributed with rate

1 U per individual per generation. In addition, each mutation is fully pleiotropic and changes all traits
2 (x_i) and thus ultimately fitness. Each mutation therefore causes a change in each value of x_i , which
3 is normally distributed with mean 0 and variance σ^2 . Individuals are then selected to the next
4 generation according to their fitness. In this model, the mean fitness effect of a mutation “hitting” a
5 genotype initially residing at the multivariate phenotypic optimum is $E(s) = -n\sigma^2$.

6 For the recovery experiment, we assumed a constant effective population size, and three
7 different initial starting fitnesses, covering the range of variation observed in the experiment. N_e was
8 approximated by the harmonic mean of the population size N in the actual recovery experiments.
9 We then let the population evolve for 240 generations and record the values of fitness increase after
10 120 and 240 generations, for each starting fitness. The slope of a linear regression of the fitness
11 change, at each of these time points, as a function of initial fitness was then measured. As these
12 simulations, involving a large N_e , are computationally very intensive, we did not follow all of the
13 possible starting fitnesses considered in the actual experiment.

14 For the simulations in Figures 3, the same model was used, except only one mutation per run
15 was recorded. The probability of each mutation surviving drift was estimated by $(1-e^{-2s})/(1-e^{-2N_e s})$
16 (Haldane 1927). The N_e used was 23.

17 Lastly, under the Gaussian FGM assumed here, the increase in fitness when close to the
18 optimum is expected to be linear with initial fitness (Martin and Lenormand, 2008), and we have
19 checked this assumption with a small subset of simulations performed with many starting fitnesses.
20

21 *Estimation of parameters by Approximate Bayesian Computation (ABC)*

22 Our goal was to estimate jointly the genomic mutation rate for fitness (U) and the parameters
23 underlying FGM (i.e. n the number of independent phenotypic traits under stabilizing selection and
24 σ the scaled mean phenotypic effect of a mutation) from empirical data (collectively termed \mathbf{D}).
25 Hereafter we “collect” the 3 parameters in a vector $\boldsymbol{\theta} = (U, n, \sigma)$.

26 Empirical data available to us were of two distinct kinds: fitness measurement of MA lines at

1 regular time intervals and fitness measurement during the fitness recovery experiment.

2 To estimate θ , we note that the likelihood of our data \mathbf{D} cannot be obtained in a closed form under
3 FGM given the complications introduced jointly by demographics and selection. However, this
4 likelihood can be defined implicitly because we can simulate datasets fully comparable to \mathbf{D} under
5 FGM. We therefore used an approximate Bayesian framework where the likelihood of the full data
6 \mathbf{D} was approximated by replacing the likelihood of \mathbf{D} by the likelihood of a vector of summary
7 statistics, \mathbf{S}_D , that summarizes our data \mathbf{D} . Note that \mathbf{S}_D comprises 39 statistics summarizing both
8 the MA experiment and the fitness recovery experiment (see Table 1).

9 Rejection sampling was then used to approximate the posterior distribution of θ given \mathbf{S}_D . In brief,
10 to conduct rejection sampling, we simulated M “composite” datasets comprising data from both MA
11 and fitness recovery lines under FGM. Each simulation generating an MA + recovery dataset was
12 “seeded” by first drawing parameters from a joint prior distribution $\pi(\theta)$.

13 We chose the following priors for our parameters: $\log_{10}(U)$ is uniform in $[-3.5, -0.5]$ and
14 encompasses very broadly the range of genome wide mutation rates previously reported in *E. coli*;
15 we chose a very flat exponential prior for σ (see Fig. 4); and a uniform discrete distribution on $[1,$
16 $30]$ that covers broadly the range of previously estimated values for this parameter (Martin and
17 Lenormand 2006; Bataillon et al. 2011; Sousa et al. 2012; Trindade et al. 2012)

18 We implemented rejection sampling by retaining the fraction tol of parameters that seeded
19 simulations that yielded the set of summary statistics closest to the vector of observed summary
20 statistics \mathbf{S}_D . In practice, we used $M=10^6$ simulations and conducted rejection sampling by keeping
21 the closest 1000 simulations ($tol=0.001$). The set of parameters yielding these 1000 accepted
22 datasets was used to approximate $\Pi^*(\theta)$ the joint posterior distribution of the parameters.

23 We used a Monte Carlo approach to choose a tolerance level tol and study the statistical
24 performance of our ABC estimators by rejection sampling. One dataset was drawn at random from
25 the pool of 10^6 simulated datasets, rejection sampling was then performed using three tolerance
26 levels on the remaining datasets to obtain ABC parameter estimates. Prediction error was calculated

1 by adding mean (squared) bias and variance as $MSE = \sum_i (\theta'_i - \theta^P_i)^2 / \text{Var}(\theta^P)$, where θ'_i ($i=1..50$)
2 are the ABC estimates and θ^P_i are the true values of the parameters use for simulating data.
3 Preliminary results based on 50 simulated datasets exploring three different tolerance levels
4 ($tol=0.1\%$, 1% , 10%) suggested that the lowest tolerance ($tol = 0.1\%$) yielded the best prediction
5 errors for the ABC estimates (not shown). We cannot exclude that ABC estimators with even lower
6 MSE can be devised by using alternative weighting and/or alternative sets of summary statistics but
7 this is beyond the scope of the present study. Moreover we showed via simulation that at $tol=0.1\%$
8 and with the set of summary statistics we used, ABC estimates do not err systematically (Fig. S3).
9 All rejection sampling, posterior approximations and Monte Carlo simulations for evaluating the
10 performance of the ABC estimator were conducted with the statistical software *R* and using the
11 package *abc* (Csilléry et al. 2012).

13 RESULTS

15 Initial fitness as a predictor of rates of fitness change through time

17 (a) Initial fitness predicts the decrease in fitness in populations experiencing intense genetic drift

18 We re-analyzed the data of a MA experiment in *E. coli* previously reported (Trindade et al.
19 2010) to test the extent to which initial fitness of genotypes can influence subsequent changes in
20 fitness due to mutation accumulation. In this experiment, 50 lines of a mutator strain were
21 propagated by daily transfer. Each transfer consisted of a bottleneck down to a single cell (see
22 methods in (Trindade et al. 2010) and mean fitness was measured every 10 bottlenecks, i.e.,
23 approximately every 230 generations. During the course of this experiment, mean fitness was
24 reduced by 10% on average, and some of the lines reached a fitness level as low as 0.78 (relative to
25 the ancestor). The decline in mean fitness and increase in variance between MA lines through time
26 allowed the estimation of the deleterious mutation rate (U_d) and the mean effect of deleterious

1 mutations ($E(s_d)$), using a variant of the Bateman-Mukai (BM) method, which accounts for the
2 effects of natural selection between bottlenecks (Gordo and Dionisio 2005; Trindade et al. 2010).
3 The inferred mutation rate was $U=0.005$ deleterious mutations per genome per generation with a
4 mean effect of $E(s_d)=-0.03$. This inference assumes that all mutations are deleterious with identical
5 fitness effects, and that no epistasis occurs. There are two ways in which we can look for evidence
6 of epistasis in an MA experiment. One method that has been widely used is to examine the decrease
7 in fitness, averaged over all MA lines, through time. If there is no directional epistasis ($\langle \epsilon \rangle = 0$), then
8 the decrease in the logarithm of fitness with time is expected to be linear. Deviations from linearity
9 indicate some level of mean epistasis, either positive or negative, depending on the curvature found
10 (De Visser et al. 2011) . For this MA, the regression coefficient of the logarithm of fitness with time
11 was 0.98 (Akaike information Criterion – AIC – of -36.3). A quadratic model applied to this data
12 did not improve the fit (AIC= -36.5). Hence, using this method, we have no evidence that the mean
13 epistatic effect was different from zero. However we note that this approach merely regresses the
14 mean fitness of all lines. Independent lines accumulated different numbers of mutations along time;
15 hence the mean may not be very sensitive. As a complement, we examined the change in fitness
16 after a period of 10 bottlenecks as a function of the initial fitness before these 10 bottlenecks. If
17 epistatic interactions do not exist, no correlation between the fitness of a line and its decrease in
18 fitness in subsequent bottlenecks is to be expected. Contrary to this expectation, we observed that
19 lines with a higher fitness were more likely to show a decrease in fitness than lines with lower
20 initial fitness. Figure 1 shows that the change in fitness and initial fitness are correlated (Spearman
21 correlation coefficient of -0.34, $p < 10^{-7}$). We conclude that the change in fitness depends strongly on
22 the initial fitness. This points to the occurrence of widespread epistasis among spontaneous
23 mutations affecting fitness in *E. coli*. These findings prompted us to re-analyze the data under a
24 model that explicitly accounts for the possibility of widespread epistasis among mutations thus
25 yielding novel estimates of the rate and effects of mutations in this MA experiment (see below).

26

1 ***(b) Initial fitness predicts the increase in fitness in large populations.***

2 Fitness changes observed in the course of MA experiments are generally negative (see
3 (Halligan and Keightley 2009) for a review). We sought to perform a recovery experiment where
4 the change in fitness is expected to be positive. We also wanted to test if the rate of fitness increase
5 depends on the genetic background. We did so by using a sub-set consisting of 23 MA strains
6 covering a wide range of relative fitness (from 0.77 to 0.99) to start our fitness recovery experiment.
7 Among these strains, 16 were obtained from the 50th bottleneck, 6 were from the 20th bottleneck and
8 the 23rd was obtained by propagating one of the MA lines for another 20 bottlenecks.

9 We adapted these clones to the medium where the competitions were performed, during 240
10 generations, under serial dilution thereby imposing an effective population size (N_e) of 3×10^5 . We
11 then measured fitness in the same conditions used for measuring fitness of the MA lines. Figure 2
12 shows the change in mean population fitness as a function of the fitness of the founding clone.
13 Clearly, the rate of adaptation depends on the genetic background (Spearman correlation coefficient
14 of -0.76, $p < 10^{-5}$). In fact, the lower the initial fitness of the clone, the higher its fitness increase.
15 This relationship was observed for the clones sampled from the MA at the 20th and at the 50th
16 bottleneck, as shown in Figure S2. In this experiment, 63% of the variance in fitness could be
17 predicted by the initial fitness, and only two lines exhibited no increase in fitness during the 240
18 generations (Fig. 2).

19 Although not impossible, back-mutation is an unlikely explanation for these results, given
20 the estimates of point mutation rates in *E. coli* (Drake 1991) (Wielgoss et al. 2011). Compensatory
21 mutations happening at other sites throughout the genome are, on the other hand, expected to be far
22 more common (Poon et al. 2005). However, studies addressing their rate and distribution remain
23 rare, with most studies examining compensation in the context of antibiotic resistance (Schrag et al.
24 1997; Levin et al. 2000; Sousa et al. 2012).

25

26

1 Fisher geometrical model predicts the patterns observed in MA and in the recovery 2 experiment

3

4 (a) *The patterns of fitness change expected under FGM*

5 The data of both the MA and the recovery experiment show a clear dependence of fitness
6 change on initial fitness (W_0) as illustrated in Figures 1 and 2. This pattern has been very rarely
7 reported in previous MA experiments (see Bataillon 2000; Halligan and Keightley 2009 for a
8 review). We note however that under FGM, a fitness landscape model that has received a lot of
9 theoretical attention, the distribution of effects of incoming mutations on a genotype, changes
10 drastically as a function of its current fitness relative to the optimum. In particular, under FGM, we
11 expect that in well-adapted populations new mutations will be mostly deleterious, while in poorly
12 adapted populations a sizeable fraction of the mutations will be beneficial (compensatory).

13 We compared the predictions of FGM to the experimental observations, taking into account
14 the demographic parameters used and the parameters that best fit the data (see the next section for
15 the ABC approach we used to estimate them). We modeled the extreme fluctuations in population
16 size in the MA, and allowed selection to occur during the growth of a line before it is bottlenecked
17 (see Methods). When simulating FGM under these conditions, we observed that even with the
18 drastic bottleneck procedure, the distribution of mutations accumulated is skewed by selection for
19 beneficial and small effect deleterious mutations (see Fig. 3A). To evaluate the effect of selection
20 occurring during the MA we simulated MA datasets with and without selection. In these
21 simulations, mutations drawn from FGM accumulated with or without selection and we calculated
22 the slopes of the linear regression of the change in fitness with starting fitness. Without selection the
23 distribution of slopes was centered on zero with a small range of negative or positive values, while
24 for MA data simulated with selection an average negative slope was observed (see Fig 3B). Using
25 the appropriate demography for each experiment, we conclude that FGM can reproduce the patterns
26 seen in the experimental data, both in the MA (Fig. 1) and fitness recovery (Fig. 2).

1 The linear relation of change in fitness with starting fitness appears to capture well the
2 pattern of the recovery experiment. Under FGM, and assuming a Gaussian fitness landscape, Martin
3 and Lenormand (2008) predicted a linear relation between the distance to the peak (s_0) and the mean
4 effect of beneficial mutations fixed during the first step of adaptation (i.e. $E(\Delta W|fix) = 4s_0/(4+n)$). This
5 prediction is valid when the starting genotype is close to the optimum and ignoring clonal
6 interference. In order to compare this prediction with our data, we took the clones with highest
7 starting fitness ($W_i > 0.85$). These are the closest to the peak, where clonal interference is least
8 expected to occur. If we assume that the fitness increase observed (ΔW) results from the first step of
9 adaptation, we can calculate the slope of the linear regression of ΔW on $s_0 \sim 1 - W_i$, and get a rough
10 estimate of n . Under these assumptions n is estimated to be around 3.

11

12 ***(a) Estimating parameters of FGM from experimental data***

13 Given the capacity of FGM to reproduce the pattern present in the data (Fig. 1 and 2), we
14 next sought to estimate jointly the parameters of this model (n and σ , see Methods for details) as
15 well as the genomic mutation rate (U) from our experimental data. To do so, we used an ABC
16 framework where we combined empirical data from both experiments using several summary
17 statistics (Table 1). These statistics describe the fitness distribution along bottlenecks of the MA
18 experiment and the fitness change in two time points of the recovery experiment (see Methods). We
19 then confronted these empirically observed summary statistics with those predicted under FGM
20 given a value of U , n and σ . ABC approaches have been very popular when analyzing patterns of
21 nucleotide diversity under various demographic models (Robert et al. 2011). However, to our
22 knowledge, they have never been used systematically to analyze these types of experiments.
23 Previous studies relied on Monte-Carlo simulations of datasets which were used to “fit” data from
24 MA experiments with EMS mutagenesis (Davies 1999) or to estimate fitness effect of two
25 interfering beneficial mutations from patterns of adaptation over time in experimental populations
26 (Hegreness 2006).

1 of epistasis among mutations underlying fitness and lends support to the idea that fitness effects of
2 mutations vary substantially, i.e. the variance in mutational fitness effects is large. Our argument
3 relies first on the observation that patterns of temporal fitness change in both experiments are
4 strongly dependent on initial fitness levels. This qualitative pattern of strong genetic background-
5 dependence in the data can hardly be explained without invoking the existence of epistasis. Second,
6 we note that the fitness effects of new mutations theoretically expected under FGM, exhibit the
7 salient features we empirically observed (Fig. 1 and 2). This motivates the use of FGM as a
8 plausible framework for model-based statistical inference in experimental evolution. FGM has been
9 recently used to derive quantitative predictions for the marginal distribution of fitness effects of
10 single mutations and/or the expected patterns of epistasis among pairs of mutations (Martin et al.
11 2007; Martin and Lenormand 2008; Bataillon et al. 2011). These predictions have been confronted
12 with a few datasets. In particular the distribution of pairwise epistasis between deleterious
13 mutations, in *E. coli*, has been found to follow a Normal distribution with mean zero, following the
14 expectation of this simple model. FGM currently stands as an attractive alternative to statistical
15 heuristics based on extreme value theory (Rokyta et al. 2008; Bataillon et al. 2011). Here we go one
16 step further and demonstrate that parameters underlying FGM can also be estimated jointly with
17 genome wide mutation rate from data on temporal fitness changes in either MA or adaptation
18 experiments.

19 Most of the adaptation detected in the recovery lines is likely due to compensatory
20 mutations. Compensatory mutations are epistatic by definition: they are only beneficial in a specific
21 background as they allow a mutant strain to revert to the ancestral phenotype but do not occur at the
22 same nucleotide (Nagaev et al. 2001). They contribute to adaptation by increasing the number of
23 possible beneficial mutations as fitness decreases. In addition to strictly compensatory mutations, it
24 is possible that the ancestral background was not fully adapted to the medium where the experiment
25 took place. In that case, there can also be new mutations, which are beneficial irrespective of the
26 background. It is possible that both changes in the beneficial mutation rate (U_b) and in the mean

1 effect of beneficial mutations (s_b) are contributing to the pattern observed in Figure 2. As was the
2 case of the MA, our re-adaptation experiment supports the view that there is widespread epistasis
3 between spontaneous mutations in *E. coli*. Empirical evidence that the distribution of fitness effects
4 of compensatory mutations depends on the genetic background has been obtained in several studies
5 examining the cost of antibiotic resistance (MacLean et al. 2010; Gordo et al. 2011). Some of us
6 have recently used FGM in this context and have found that it could provide an accurate
7 quantitative description of experimental data on the effects of these mutations (Sousa et al. 2012;
8 Trindade et al. 2012). However, FGM has not been used to jointly study adaptation of different
9 genotypes, which differ in mutations scattered throughout the whole genome. This is an important
10 test, because it contributes to the establishment of general patterns.

11 Using FGM, one can derive how the rate and effects of both beneficial and deleterious
12 mutations change along the evolutionary process, i.e. as we walk along the single peaked fitness
13 landscape parameterized by the experimental data. Figure 3 illustrates our quantitative predictions,
14 which can be challenged further by performing jointly several MA experiments and many replicate
15 adaptation experiments, starting from distinct initial fitnesses (see for instance Gifford et al. 2011), a
16 task that is beyond the scope of this paper. One salient qualitative feature of the distribution of
17 spontaneous mutations under FGM is that while the mean effect does not depend on the genetic
18 background, the variance does. In particular, as fitness decreases, the variance of the distribution of
19 new mutations increases, with more strongly deleterious and strongly beneficial mutations. Because
20 of that, the mean effect of mutations that survive genetic drift is predicted to depend strongly on
21 initial fitness (Figure 3).

22 Our estimate of the genomic mutation rate towards fitness altering mutations in a *mutS*⁻ *E.*
23 *coli* is 0.01 per generation. Considering that this mutator has a 60-fold increase in mutation rate
24 over the wild type, this estimate is similar to previous ones (Kibota and Lynch 1996; Trindade et al.
25 2010) but note that the posterior interval we report U [0.003 - 0.19] is very broad.

26 Our estimate of the mean selection coefficient of a mutation, $E(s) = -0.01$, lies below our

1 previous one (Trindade et al. 2010). This is not surprising because our estimate is based on a model
2 that intrinsically assumes a distribution of fitness effects with a variance larger than zero and
3 explicitly accounts for compensatory mutations. This new estimate is close to that of Kibota and
4 Lynch (1996).

5 Finally we have estimated a relatively small number of traits ($n \sim 9$) under natural selection,
6 which implies a low level of phenotypic complexity in this environment. This estimate is close to
7 comparable measures of phenotypic complexity reported in other bacterial experiments (Bataillon et
8 al. 2011; Trindade et al. 2012) but clearly more work is needed in that area and having a single
9 common model to estimate n would help comparisons.

10 We have emphasized that FGM is an attractive model that naturally incorporates a number
11 of biological important components: epistasis, various degrees of phenotypic complexity underlying
12 fitness, pleiotropic effects of mutations, etc. We have also shown that FGM can account for patterns
13 of fitness decline and recovery observed in empirical data. That being said, a number of alternative
14 fitness models exists. One natural extension of our work is to consider a broader range of FGM
15 based models featuring different forms of pleiotropy (Lourenço et al. 2011), different patterns of
16 epistasis (Gros et al. 2009) , and/or different types of fixed, moving or “shaking” fitness optima
17 (Kopp and Hermisson 2009; Gordo and Campos 2012). Another avenue is to examine radically
18 different fitness landscape models (such as the mutational landscape model (Gillespie JH 1984),
19 seascape model (Mustonen and Lässig 2009) or the recently proposed stickbreaking model (Nagel
20 et al. 2012) and put these in competition with the various versions of FGM for explaining patterns
21 of adaptation. We have shown that our ABC approach, although it is rather brute-force and relies on
22 a large number of simulations, yields fairly accurate parameter estimates of the classical FGM (see
23 Fig. S3) and this framework can be extended to encompass a variety of models. Although model
24 selection under ABC requires caution (see Robert et al. 2011), the methodology is naturally prone
25 towards such an analysis. Our approach can also be a fruitful starting point to devise novel
26 frameworks for the analysis of experimental evolution empirical datasets that borrow strength from

- 1 both temporal patterns of fitness changes and snapshots of the genome at various time points of the
- 2 experiments.
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3 Table 1: Overview of the set of summary statistics used for ABC inference
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Summary Statistic	Observed	Experiment
<0.77 ^a	0	Bot 10
[0.77 - 0.81[0	
[0.81 - 0.85[1	
[0.85 - 0.89[1	
[0.89 - 0.93[3	
[0.93 - 0.97[20	
>0.97	25	
<0.77	0	Bot 20
[0.77 - 0.81[0	
[0.81 - 0.85[4	
[0.85 - 0.89[2	
[0.89 - 0.93[12	
[0.93 - 0.97[24	
>0.97	8	
<0.77	0	Bot 30
[0.77 - 0.81[0	
[0.81 - 0.85[2	
[0.85 - 0.89[10	
[0.89 - 0.93[10	
[0.93 - 0.97[21	
>0.97	7	
<0.77	0	Bot 40
[0.77 - 0.81[2	
[0.81 - 0.85[5	
[0.85 - 0.89[8	
[0.89 - 0.93[16	
[0.93 - 0.97[13	
>0.97	6	
<0.77	0	Bot 50
[0.77 - 0.81[4	
[0.81 - 0.85[10	
[0.85 - 0.89[9	
[0.89 - 0.93[15	
[0.93 - 0.97[11	
>0.97	1	
slope 120 ^b	-0.7986	Fitness Recovery experiment
intercept 120	0.7585	
slope 240	-0.8873	
intercept 240	0.8647	

5
6 a: Observed distribution fitness values in the MA experiment. Distributions at each time point (Bottleneck 10,
7 20,30,40,50) are summarized using counts in 7 classes of fitness values (all counts sum to 50 at each time point).
8 b: The observed slope and intercept of the regression line describing the fitness recovery (after 120 or 240
9 generations) as a function of initial fitness.

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List of Figures

Figure 1. Fitness change as a function of initial fitness during the MA. The fitness change of each line over 10 bottlenecks (or 230 generations) is shown as a function of that line's fitness before that interval: the difference between the 10th and the 20th bottlenecks is shown as a function of fitness at the 10th bottleneck, the difference between the 20th and the 30th is shown as a function of fitness at the 20th, and so on. Each MA line is represented 4 times, once for each interval. The experimental data (shown as dots) is taken from (Trindade et al. 2010). Fifty simulations of datasets under FGM (shown as crosses) were performed as described in the text, using as parameters $n=9$, $U=0.01$ and $\sigma=0.034$. In both cases, a significant correlation between the change in fitness and initial fitness was obtained. For the experimental data, the slope of the linear regression is -0.32 with CI (-0.43, -0.21) and for the data under FGM the slope is -0.15 with the 95% confidence interval (-0.24, -0.05).

Figure 2. Fitness change as a function of the initial fitness. Fitness change after 12 bottlenecks (shown as dots) as a function of the initial fitness. Clones with different starting fitness were adapted for 240 generations under a large effective population size (see Methods for details). The results of FGM simulations with parameters values set to $n=9$, $U=0.01$ and $\sigma=0.034$ are shown as crosses. In both cases, a significant correlation between the change in fitness and initial fitness was obtained. For the experimental data, the slope of the linear regression is -0.77 with CI (-0.97, -0.57) and for the data under FGM the slope is -0.84 with the 95% confidence interval (-0.86, -0.82).

Figure 3. A) Distribution of fitness effect of mutations before and after selection. The distribution of spontaneous mutations was generated by at least 100000 Monte-Carlo simulations of

1 FGM as described in the text, using as parameters $n=9$, $U=0.01$ and $\sigma=0.034$. The distribution of
2 mutations that escape genetic drift was estimated as the fraction of spontaneous mutations that
3 escape drift in a population of effective size of 23 individuals, corresponding to the population
4 expansion occurring in the MA (Gordo and Dionisio 2005). **B) Distribution of slopes of the linear
5 regression of change in fitness with initial fitness under FGM** with no selection (black bars,
6 mean = -0.003) and with selection (grey bars, mean = -0.015) in the MA lines. The dashed line
7 shows the location of slope 0. Parameter values used are the same as in panel A.

8

9 **Figure 4. Approximate posterior densities for parameters of FGM.** All posterior densities are
10 approximated using a set 1000 accepted simulations (tolerance rate of 0.001, see methods for
11 details). Prior densities are in orange. Marginal posteriors are in blue and regions of highest
12 posterior densities are in grey. **(A)** Prior and approximate marginal posterior for the genome wide
13 mutation rate for fitness (U). Note the \log_{10} scale. **(B)** Prior and approximate marginal posterior for
14 the scaled phenotypic effect of mutations, σ . **(C)** Prior and approximate posterior probability
15 distributions for the number of dimensions, n . Note that these are discrete probability distributions
16 **(D)** Bivariate approximate posterior densities for U and σ . A blue circle depicts the location of the
17 region with highest posterior density. The regions comprising 0.5, 0.9, 0.95 and 0.99 of the
18 probability mass of the posterior density are depicted using grey shadings. **(E)** Bivariate
19 approximate posterior densities for U and n . Legend as in D. Note that for graphical convenience
20 the joint posterior distribution over n , and U was smoothed by “jittering” the values of n to make
21 them apparently continuous. **(F)** Bivariate approximate posterior densities for σ and n . Legend as in
22 3D. Note that for graphical convenience the joint posterior distribution over n , and σ was smoothed
23 by jittering the values of n to make them apparently continuous.

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1 List of supporting information figures caption

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3 **Supporting Figure S1 – Fitness changes as a function of initial fitness for two points in the MA**

4 **experiment.** The blue dots show the data for the 6 clones taken from the 20th bottleneck (B20), the

5 red dots show the clones taken from the 50th bottleneck (B50) and the green dot corresponds to the

6 clone from the 70th bottleneck (B70).

7

8 **Supporting Figure S2. Change in fitness after 120 (red symbols) and 240 generations of**

9 **adaptation (black symbols).**

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12 **Supporting Figure S3. Distribution of ABC estimates for data simulated under FGM and**

13 **known genomic mutation rates.** We used 500 simulated datasets to estimate the bias and sampling

14 variance of the ABC estimator for the joint parameters $\theta=(U, n, \sigma)$ of the model. Datasets were

15 simulated using a broad range of parameters values (spanning the prior we used for our data

16 analysis) and to match the data we analyze here (i.e. an MA experiment and a fitness recovery

17 experiment). Bias is visualized on the scatter plots. Perfect unbiased estimation would follow the y

18 $= x$ line (orange) and the difference between the ideal line and the actual local robust regression

19 (blue line) reveals the bias. The number of dimensions underlying the fitness landscape, n , is

20 estimated with some imprecision (see scatter above and below the diagonal). Bias in estimation

21 varies with the true number of dimensions: n is systematically overestimated by about 1 dimension

22 for $n < 10$, and underestimated for $n > 15$. Estimation of the mean phenotypic effect of a single

23 mutation, σ is fairly precise with bias and variance being minimum in the area where our data lies

24 ($\sigma < 0.1$). Estimation of U is virtually unbiased in the range of U values that are likely of empirical

25 interest ($U=0.005$ to $U=0.05$).

26 Figure S4. Posterior distributions obtained by fitting separately MA and Fitness Recovery datasets.

1 The ABC inference was carried as described in the methods but using separately the fitness
2 recovery dataset (4 summary statistics) and the fitness of all 50 MA lines at 5 times points ($7*5=35$
3 summary statistics).

4

5 **Figure S4. Marginal Posterior distributions obtained by fitting separately MA and Fitness**
6 **Recovery datasets.**

7 The ABC inference was carried as described in the methods but using separately the fitness
8 recovery dataset (4 summary statistics) and the fitness of all 50 MA lines at 5 times points ($7*5=35$
9 summary statistics). We report in orange the prior used for the each analysis (an identical prior was
10 used for each inference) and the marginal posteriors obtained for the genome-wide mutation rate
11 (**panel A**), the phenotypic effect of a mutation (**panel B**) and the number of dimensions underlying
12 the fitness landscape (**panel C**). For all the parameters there is a large overlap between the posterior
13 distributions for both experiments.