

# Predictable Properties of Fitness Landscapes Induced by Adaptational Tradeoffs

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**Abstract** Fitness effects of mutations depend on environmental parameters. For example, mutations that increase fitness of bacteria at high antibiotic concentration often decrease fitness in the absence of antibiotic, exemplifying a tradeoff between adaptation to environmental extremes. We develop a mathematical model for fitness landscapes generated by such tradeoffs, based on experiments that determine the antibiotic dose-response curves of *Escherichia coli* strains, and previous observations on antibiotic resistance mutations. Our model generates a succession of landscapes with predictable properties as antibiotic concentration is varied. The landscape is nearly smooth at low and high concentrations, but the tradeoff induces a high ruggedness at intermediate antibiotic concentrations. Despite this high ruggedness, however, all the fitness maxima in the landscapes are evolutionarily accessible from the wild type. This implies that selection for antibiotic resistance in multiple mutational steps is relatively facile despite the complexity of the underlying landscape.

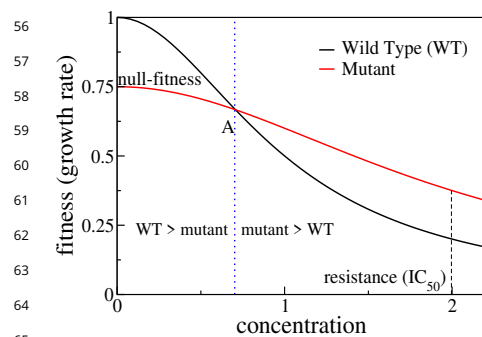
## Introduction

Sewall Wright introduced the concept of fitness landscapes in 1932 (*Wright, 1932*), and for decades afterwards it persisted chiefly as a metaphor, due to lack of sufficient data. This has changed considerably in recent decades (*de Visser and Krug, 2014*). There are now a large number of experimental studies that have constructed fitness landscapes for combinatorial sets of mutations relevant to particular phenotypes, such as the resistance of bacteria to antibiotics (*Weinreich et al., 2006; Marcusson et al., 2009; Schenk et al., 2013; Mira et al., 2015; Knopp and Andersson, 2018*). Mathematical modeling of fitness landscapes has also seen a revival, motivated partly by the need to quantify and interpret the ruggedness of empirical fitness landscapes (*Szendro et al., 2013; Weinreich et al., 2013; Neidhart et al., 2014; Ferretti et al., 2016; Crona et al., 2017; Hwang et al., 2018*). Conceptual breakthroughs, such as the notion of sign epistasis (where a mutation is beneficial in some genetic backgrounds but deleterious in others), have shed light on how ruggedness can constrain evolutionary trajectories (*Weinreich et al., 2005; Poelwijk et al., 2007, 2011; Franke et al., 2011*).

Despite this progress, a limitation of current studies of fitness landscapes is that they focus mostly on  $G \times G$  (gene-gene) interactions, and little on  $G \times G \times E$  (where  $E$  stands for environment) interactions, i.e on how changes in environment modify gene-gene interactions. A few recent studies have begun to address this question (*Flynn et al., 2013; Taute et al., 2014; Gorter et al., 2018; de Vos et al., 2018*). In the context of antibiotic resistance, it has been realized that the

41 fitness landscape of resistance genes depends quite strongly on antibiotic concentration (*Mira*  
42 *et al.*, 2015; *Ogbunugafor et al.*, 2016). This is highly relevant to the clinical problem of resistance  
43 evolution, since concentration of antibiotics can vary widely in a patient's body as well as in various  
44 non-clinical settings (*Kolpin et al.*, 2004; *Andersson and Hughes*, 2014). Controlling the evolution of  
45 resistance mutants thus requires an understanding of fitness landscapes as a function of antibiotic  
46 concentration. Empirical investigations of such scenarios are still limited, and systematic theoretical  
47 work on this question is also lacking.

48 In the present work, we aim to develop a theory of  $G \times G \times E$  interactions for a specific class of  
49 landscapes, with particular focus on applications to antibiotic resistance. The key feature of the  
50 landscapes we study is that every mutation comes with a tradeoff between adaptation to the two  
51 extremes of an environmental parameter. For example, it has been known for some time that  
52 antibiotic resistance often comes with a fitness cost, such that a bacterium that can tolerate high  
53 drug concentrations grows slowly in drug-free conditions. While such tradeoffs are not universal,  
54 they certainly occur for a large number of mutations (*Melnyk et al.*, 2015).



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66 **Figure 1.** Schematic showing dose response  
67 curves of a wild type and a mutant. To the left  
68 of the intersection point A the wild type is  
69 selected over the mutant, whereas to the right  
70 of A the mutant is selected.

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73 swapping the rank order between the two fitness values. The intersection point is known as the  
74 minimum selective concentration (MSC), and it defines the lower boundary of the mutant selection  
75 window (MSW) within which the resistance mutant has a selective advantage relative to the wild  
76 type (*Khan et al.*, 2017; *Alexander and MacLean*, 2018).

77 When there are several possible mutations and multiple combinatorial mutants, a large number  
78 of such intersections occur as the concentration of the antibiotic increases. This leads to a succes-  
79 sion of different fitness landscapes. Whenever the curves of two mutational neighbors (genotypes  
80 that differ by one mutation) intersect, there can be an alteration in the evolutionary trajectory  
81 towards resistance, whereby a forward (reverse) mutation now becomes more likely to fix in the  
82 population than the corresponding reverse (forward) mutation. These intersections change the  
83 ruggedness of landscapes and the accessibility of fitness maxima. In this way a rich and complex  
84 structure of selective constraints emerges in the MSW. To explore the evolutionary consequences  
85 of these constraints, we construct a theoretical model based on existing empirical studies as well  
86 as our own work on ciprofloxacin resistance in *E. coli*. Specifically, we address two fundamental  
87 questions: (i) How does the ruggedness of the fitness landscape vary as a function of antibiotic  
88 concentration? (ii) How accessible are the fitness optima as a function of antibiotic concentration?

89 We find that even when the null-fitness and resistance values of the mutations combine in  
90 a simple, multiplicative manner, the intersections of the curves produce a highly epistatic land-  
91 scape at intermediate concentrations of the antibiotic. This is an example of a strong  $G \times G \times E$

92 interaction, where changes in the environmental variable drastically alter the interactions between  
93 genes. Despite the high ruggedness at intermediate concentrations, however, the topology of  
94 the landscapes is systematically different from the oft-studied random landscape models, such as  
95 the House-of-Cards model (*Kauffman and Levin, 1987; Kingman, 1978*), the Kauffman NK model  
96 (*Kauffman and Weinberger, 1989; Hwang et al., 2018*) or the Rough Mt. Fuji model (*Neidhart et al.,*  
97 *2014*). For example, most fitness maxima have similar numbers of mutations that depend logarithmically  
98 on the antibiotic concentration. Importantly, all the fitness maxima remain highly accessible  
99 through adaptive paths with sequentially fixing mutations. In particular, any fitness maximum  
100 (including the global maximum) is accessible from the wild type as long as the wild type is viable. As  
101 a consequence, the evolution of high levels of antibiotic resistance by multiple mutations (*Hughes*  
102 *and Andersson, 2017; Wistrand-Yuen et al., 2018; Rehman et al., 2019*) is much less constrained by  
103 the tradeoff-induced epistatic interactions than might have been expected on the basis of existing  
104 models.

## 105 Results

### 106 Mathematical model of tradeoff-induced fitness landscapes

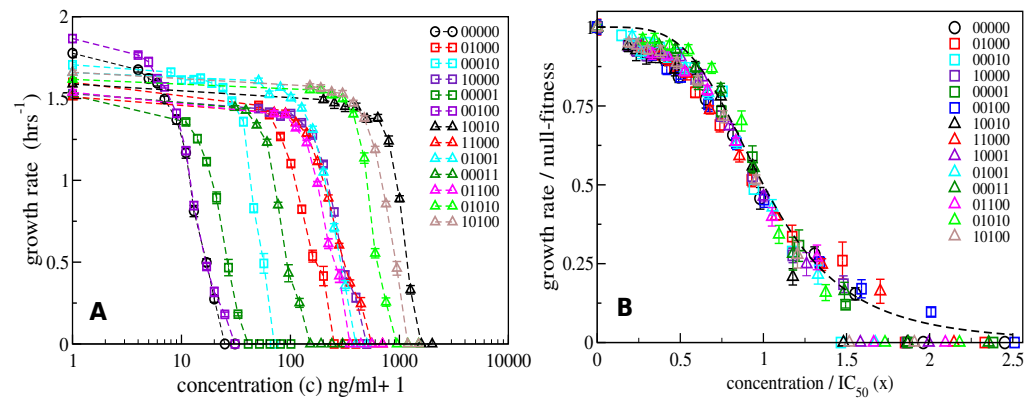
107 The chief goal of this paper is to develop and explore a mathematical framework to study tradeoff-  
108 induced fitness landscapes. We consider a total of  $L$  mutations, each of which increases antibiotic  
109 resistance. A fitness landscape is a real-valued function defined on the set of  $2^L$  genotypes made  
110 up of all combinations of these mutations. A genotype can be represented by a binary string of  
111 length  $L$ , where a 1 (0) at each position represents the presence (absence) of a specific mutation.  
112 Alternatively, any genotype is uniquely identified as a subset of the  $L$  mutations (the wild type is the  
113 null subset, i.e the subset with no mutations).

114 In this paper, unless mentioned otherwise, we define the fitness  $f$  as the exponential growth  
115 rate of a microbial population. The fitness is a function of antibiotic concentration. This function has  
116 two parameters – the growth rate at zero concentration, which we refer to as the null-fitness and  
117 denote by  $r$ , and a measure of resistance such as  $IC_{50}$  which we denote by  $m$ . Each single mutation is  
118 described by the pair  $(r_i, m_i)$ , where  $r_i$  and  $m_i$  are the null-fitness and resistance values respectively  
119 of the  $i$ th single mutant. We further rescale our units such that for the wild type,  $r = 1$  and  $m = 1$ . We  
120 consider mutations that come with a fitness-resistance tradeoff, i.e a single mutant has an increased  
121 resistance ( $m_i > 1$ ) and a reduced null-fitness ( $r_i < 1$ ) compared to the wild type. To proceed we  
122 need to specify two things: (i) how the  $r$  and  $m$  values of the combinatorial mutants depend on  
123 those of the individual mutations, (ii) how the fitness of the wild type and the mutants depend on  
124 antibiotic concentration, and in particular if this dependence exhibits a pattern common to various  
125 mutant strains. To address these issues we take guidance from two empirical observations.

### 126 Scaling of dose-response curves

127 *Marcusson et al. (2009)* have constructed a series of *E. coli* strains with single, double and triple  
128 mutations conferring resistance to the fluoroquinolone antibiotic ciprofloxacin (CIP), which inhibits  
129 DNA replication (*Drlica et al., 2009*). In their study they measured MIC (minimum inhibitory con-  
130 centration) values and null-fitness but did not report dose-response curves. Some of the present  
131 authors have recently shown that the dose-response curve of the wild-type *E. coli* (strain K-12  
132 MG1655) in the presence of ciprofloxacin can be fitted reasonably well by a Hill function (*Ojtic et al.,*  
133 *2019*).

134 Here we expand on this work and determine dose-response curves for a range of single- and  
135 double-mutants with mutations restricted to five specific loci known to confer resistance to CIP  
136 (*Marcusson et al., 2009*) (see Materials and Methods). Figure 2A shows the measured curves for  
137 the wild type, the five single mutants, and eight double-mutant combinations. The genotypes are  
138 represented as binary strings, where a 1 or 0 at each position denotes respectively the presence or  
139 absence of a particular mutation. If we rescale the concentration  $c$  of CIP by  $IC_{50}$  of the corresponding  
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**Figure 2.** Dose-response curves for *E. coli* in the presence of ciprofloxacin. Each binary string corresponds to a strain, where the presence (absence) of a specific mutation in the strain is indicated by a 1(0). The five mutations in order from left to right are S83L (*gyrA*), D87N (*gyrA*), S80I (*parC*),  $\Delta marR$ , and  $\Delta acrR$ . The names of the strains are given in Table 1 in Materials and Methods. **(A)** Dose-response curves of the wild type, the five single mutants and eight double mutants. Unlike the experiments reported in *Marcusson et al. (2009)*, the mutants were grown in isolation rather than in competition with the wild type. **(B)** The same curves, but scaled with the null-fitness and  $IC_{50}$  of each individual genotype. The dashed black line is the Hill function  $(1 + x^4)^{-1}$ .

141 strain,  $x = c/IC_{50}$ , and the growth rate by the null-fitness  $f(0)$ , the curves collapse to a single curve  
 142 that can be approximated by the Hill function  $(1 + x^4)^{-1}$  (Figure 2B). The precise shape of the curve  
 143 is not important for further analysis. However, the data collapse suggests that we can assume that  
 144 the dose-response curve of a mutant with (relative) null-fitness  $r$  and (relative) resistance  $m$  is

$$f(c) = rw(c/m), \quad (1)$$

145 i.e. it has the same shape as the wild-type curve  $w$  except for a rescaling of the fitness and con-  
 146 centration axes. Similar scaling relations have been reported previously by *Wood et al. (2014)* and  
 147 *Chevreau et al. (2015)*. A good biological understanding of the conditions underlying this feature is  
 148 presently lacking, but it seems intuitively plausible that the shape  $w(x)$  would be robust to changes  
 149 that do not qualitatively alter the basic physiology of growth and resistance.

#### 150 Limited epistasis in $r$ and $m$

151 An interesting recent finding reported by *Knopp and Andersson (2018)* is that chromosomal re-  
 152 sistance mutations in *Salmonella typhimurium* mostly alter the null-fitness as well as the MIC of  
 153 various antibiotics in a non-epistatic, multiplicative manner, i.e. if a particular mutation increases  
 154 (decreases) the resistance (null-fitness) by a factor  $k_1$ , and another mutation does the same with  
 155 a factor  $k_2$ , then the mutations jointly alter these phenotypes roughly by a factor of  $k_1k_2$  (with a  
 156 few exceptions). We have done a similar comparison for the data on the null-fitness and MIC for  
 157 *E. coli* strains in *Marcusson et al. (2009)*. We have analyzed a subset of 4 mutations for which the  
 158 complete data set for all combinatorial mutants is available from *Marcusson et al. (2009)*. The data  
 159 are shown in Table 1. Out of 11 multiple-mutants, only 2 show epistasis in  $r$  and 4 show epistasis  
 160 in  $m$ . Moreover, in all cases where significant epistasis occurs it is negative, i.e. the effect of the  
 161 multiple mutants is weaker than expected from the single mutation effects.

#### 162 Formulation of the model

163 The above observations suggest a model where one assumes, as an approximation, that all the  
 164  $r$  and  $m$  values of individual mutations combine multiplicatively. A genotype with  $n$  mutations  
 165  $(r_1, m_1), (r_2, m_2), \dots, (r_n, m_n)$  has a null-fitness  $r$  and a resistance value  $m$  given by

$$r = \prod_{i=1}^n r_i \quad \text{and} \quad m = \prod_{i=1}^n m_i. \quad (2)$$

Moreover, the dose-response curves of the genotypes are taken to be of the scaling form (1), where the function  $w(x)$  does not depend on the genotype. As indicated before, and without any loss of generality, we choose units such that, for the wild type,  $r = 1$  and  $m = 1$ . Therefore the dose-response curve of the wild type is  $w(x)$  with  $w(0) = 1$ , and choosing  $IC_{50}$  as a measure of resistance we have  $w(1) = \frac{1}{2}$ . Henceforth, we refer to  $x$  simply as the concentration. We also recall that the condition of adaptational tradeoff means that  $r_i < 1$  and  $m_i > 1$  for all mutations.

If the  $r_i$  and  $m_i$  values combine non-epistatically, and if the shape of the dose-response curve is known, it is thus possible to construct the entire concentration-dependent landscape of size  $2^L$  from just  $2L$  measurements (of the  $r_i$  and  $m_i$  values of the single mutants) instead of the measurement of  $2^L$  fitness values at every concentration. In practice we do not expect a complete lack of epistasis among all mutations of interest, and the dose-response curve is also an approximation obtained by fitting a curve through a finite set of fitness values known only with limited accuracy. However, the fitness rank order of genotypes, and related topographic features such as fitness peaks, are robust to a certain amount of error in fitness values (Crona *et al.*, 2017), and our model may be used to construct these to a good approximation.

Lastly, we require that the dose-response curves of the wild type and a mutant intersect at most once, which implies that the equation  $w(x) = rw(\frac{x}{m})$  with  $r > 1$  and  $m < 1$  has at most one solution. This then also implies that the curves of any genotype  $\sigma$  and a proper superset of it (i.e. a genotype which contains all the mutations in  $\sigma$  and some more) intersect at most once. This property holds for all functions that have been used to represent dose-response curves in the literature, such as the Hill function, the half-Gaussian or the exponential function, as well as for all concave function with negative second derivate (see Materials and Methods for details).

### Properties of tradeoff-induced fitness landscapes

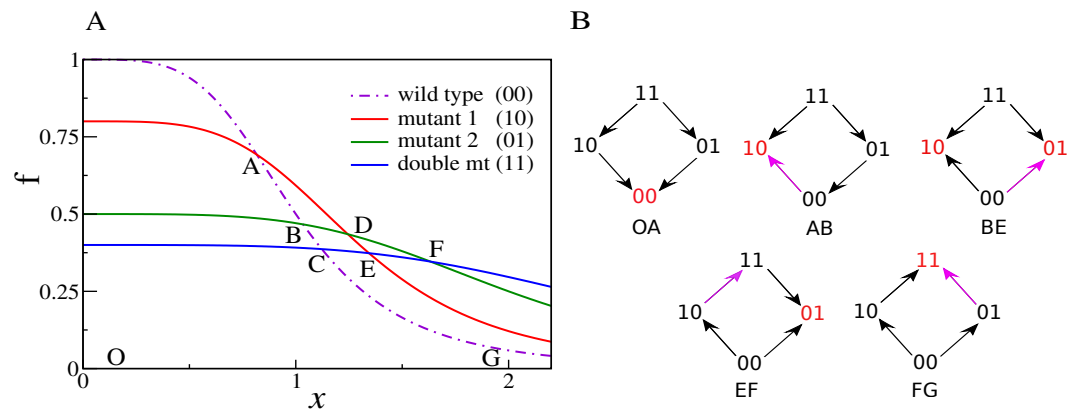
To understand the evolutionary implications of our model, we first describe how the fitness landscape topography changes with the environmental parameter represented by the antibiotic concentration. Next we analyze the properties of mutational pathways leading to highly fit genotypes.

#### Intersection of curves and changing landscapes

We start with a simple example of  $L = 2$  mutations and a Hill-shaped dose-response curve  $w(x) = \frac{1}{1+x^2}$  (Figure 3). At  $x = 0$ , the rank ordering is determined by the null-fitness. The wild type has maximal fitness, and the double mutant is less fit than the single mutants. As  $x$  increases, the fitness curves start to intersect, and each intersection switches the rank of two genotypes. In the present example we find a total of six intersections and therefore seven different rank orders across the full range of  $x$ . This is actually the maximum number of rank orders that can be found by scanning through  $x$  for  $L = 2$ , see Materials and Methods. The final fitness rank order (to the right of the point F in Figure 3A) is the reverse of the original rank order at  $x = 0$ .

Figure 3B depicts the concentration-dependent fitness landscape of the 2-locus system in the form of fitness graphs. A fitness graph represents a fitness landscape as a directed graph, where neighboring nodes are genotypes that differ by one mutation, and arrows point toward the genotypes with higher fitness (de Visser *et al.*, 2009; Crona *et al.*, 2013). A fitness graph does not uniquely specify the rank order in the landscape (Crona *et al.*, 2017). For example, the region BE has a single fitness graph, but three different rank orders in the segments BC, CD and DE.

Because selection drives an evolving population towards higher fitness, a fitness graph can be viewed as a roadmap of possible evolutionary trajectories. In particular, a fitness peak (marked in red in Figure 3B) is identified from the fitness graph as a node with only incoming arrows. Fitness graphs also contain the complete information about the occurrences of sign epistasis. Sign epistasis with respect to a certain mutation occurs when the mutation is beneficial in some backgrounds but deleterious in others (Weinreich *et al.*, 2005; Poelwijk *et al.*, 2007). It is easy to read off sign epistasis for a mutation from the fact that parallel arrows (i.e. arrows corresponding to the gain or loss of the same mutation) in a fitness graph point in opposite directions. For example, in the graph



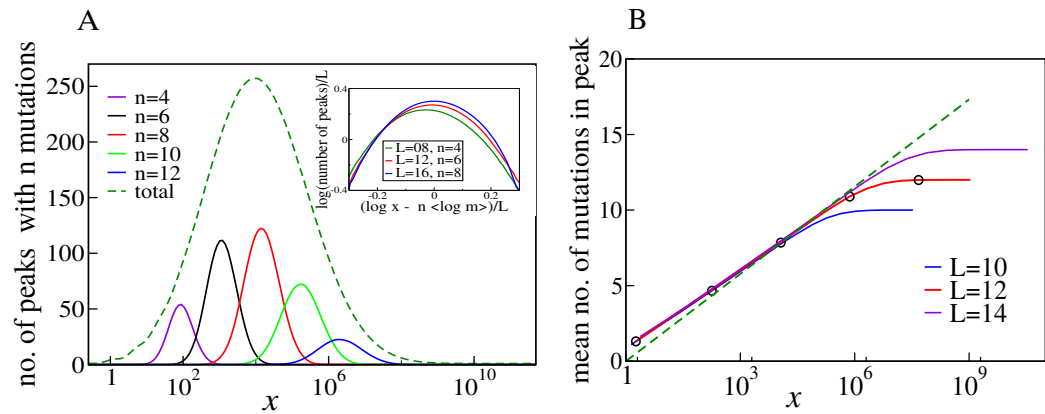
**Figure 3. (A)** An example of dose-response curves of four genotypes - the wild type (00), two single mutants (10 and 01), and the double mutant (11). The parameters of the two single mutants are  $r_1 = 0.8$ ,  $m_1 = 1.3$ ,  $r_2 = 0.4$ ,  $m_2 = 2.5$ . Null-fitness and resistance combine multiplicatively, which implies that the parameters of the double mutant are  $r_{12} = r_1 r_2 = 0.32$  and  $m_{12} = m_1 m_2 = 3.25$ . **(B)** Fitness graphs corresponding to antibiotic concentration ranges from panel A. The genotypes in red are the local fitness peaks. The purple arrows are the ones that have changed direction at the beginning of each segment. All arrows eventually switch from the downward to the upward direction.

215 for the region AB there is sign epistasis in the first position, since the parallel arrows  $00 \rightarrow 10$  and  
 216  $01 \leftarrow 11$  point in opposite directions. Notice that in the current example, we start with a smooth  
 217 landscape at  $x = 0$  (as seen in the fitness graph for OA), and the number of peaks and the degree  
 218 of sign epistasis both reach a maximum in the intermediate region BE. This fitness graph displays  
 219 reciprocal sign epistasis, which is a necessary condition for the existence of multiple fitness peaks  
 220 (Poelwijk *et al.*, 2011). Beyond the point E, the landscape starts to become smooth again, with only  
 221 one fitness maximum and a lower degree of sign epistasis. In the last region FG, the landscape is  
 222 smooth with only one peak (the double mutant 11) and no sign epistasis.

223 These qualitative properties generalize to larger landscapes. To show this, we consider a  
 224 statistical ensemble of landscapes with  $L$  mutations, where the parameters  $r_i, m_i$  of single mutations  
 225 are independently and identically distributed according to a joint probability density  $P(r, m)$ . Figure 4  
 226 shows the result of numerical simulations of these landscapes for  $L = 16$ . The mean number of  
 227 fitness peaks with  $n$  mutations reaches a maximum at  $x_{\max}(n)$  where to leading order  $\log x_{\max}(n) \sim$   
 228  $n(\log m)$ , independent of any further details of the system, as argued in Materials and Methods.  
 229 The asymptotic expression works well already for  $L = 16$  (see inset of Figure 4A). Figure 4B shows  
 230 the mean number of mutations in a fitness peak. This is well approximated by the curve  $n =$   
 231  $\frac{\log x}{(\log m)}$ , showing that the mean number of mutations in a fitness peak grows logarithmically in the  
 232 concentration. This is consistent with what we would expect from the variation in the number of  
 233 peaks with  $n$  mutations as shown in Figure 4A.

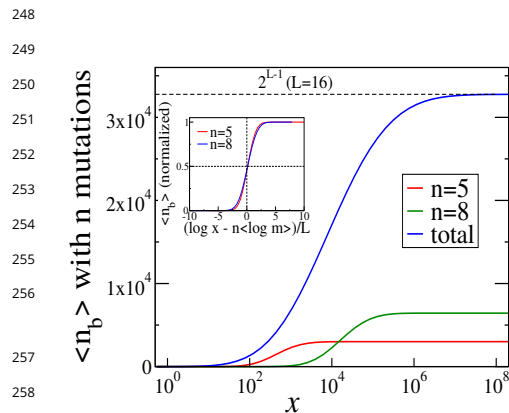
234 As another indicator of ruggedness, we consider the number of backgrounds in which a mutation  
 235 is beneficial as a function of  $x$ . At  $x = 0$ , any mutation is deleterious in all backgrounds, whereas at  
 236 very large  $x$  it is beneficial in all backgrounds. Therefore there is no sign epistasis in either case.  
 237 Sign epistasis is maximized when a mutation is beneficial in exactly 1/2 of all backgrounds. Figure 5  
 238 shows the mean number of backgrounds  $n_b$  (with  $n$  mutations each) in which the occurrence a  
 239 mutation is beneficial, for two different values of  $n$ . The curves have a sigmoidal shape, starting from  
 240 zero and saturating at  $\binom{L}{n}$ , which is the total number of backgrounds with  $n$  mutations. The blue  
 241 curve shows the mean total number of backgrounds (with any  $n$ ) in which a mutation is beneficial,  
 242 which has a similar shape.

243 Since every mutation in every background goes from being initially deleterious to eventually ben-  
 244 efiticial, there must be some  $x$  at which every mutation is beneficial in exactly half the backgrounds.  
 245 The inset of Figure 5 shows that for backgrounds with  $n$  mutations, the average concentration



**Figure 4.** (A) Number of fitness peaks as a function of concentration for different numbers of mutations in the peak,  $n$ , and  $L = 16$ . The dashed green curve is the total number of fitness peaks, summed over  $n$ . The peaks were found by numerically generating an ensemble of landscapes with individual effects distributed according to the joint distribution (8). For this distribution,  $\langle \log m \rangle = 1.19645$ . Inset: The maximal number of peaks for a given value of  $n$  occurs at  $\log x_{\max}(n) = n \langle \log m \rangle$ , and grows exponentially with  $L$ . (B) Mean number of mutations in a fitness peak as a function of concentration  $x$ . The black circles are the mean number of mutations in the fittest genotype. The green dashed line is  $\frac{\log(x)}{\langle \log m \rangle}$ .

246 at which a mutation is beneficial in 1/2 the backgrounds is given by  $\log x \simeq n \langle \log m \rangle$ , which is  
 247 the same concentration at which the largest number of fitness peaks were found in Figure 4.



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**Figure 5.** Numerical averages for the number of backgrounds  $n_b$  for two different values of  $n$  (the number of mutations in the background). The blue curve sums over  $n_b$  for all values of  $n$ . The inset shows the values of  $n_b$  as a fraction of the total number of backgrounds with  $n$  mutations.

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 A derivation of this relation is given in Materials and Methods. Similarly, when summed over all mutation numbers  $n$ , the fraction of beneficial backgrounds reaches 1/2 around the same concentration at which the total number of fitness peaks is maximal. Since the number of backgrounds is largest at  $n = L/2$  for combinatorial reasons, this concentration is approximately given by  $\log x \simeq \frac{L}{2} \langle \log m \rangle$ .

**Accessibility of fitness peaks**

Having shown that tradeoff-induced fitness landscapes display a large number of fitness peaks at intermediate concentrations, we now ask how these peaks affect the evolutionary dynamics. We base the discussion on the concept of evolutionary accessibility, which effectively assumes a regime of weak mutation and strong selection (Gillespie, 1984). In this regime the evolutionary trajectory consists of a series of fixation events of beneficial single-step mutations represented by a directed path in the fitness graph of the landscape (Weinreich et al., 2005, 2006; Franke et al., 2011). We say that a genotype is *accessible* from another genotype if a directed path exists from the initial to the final genotype.

The accessibility of peaks in a fitness landscape is determined by the rank ordering of the genotypes. We now show that the rank orders of tradeoff-induced fitness landscapes are constrained in a way that gives rise to unusually high accessibility. Consider two distinct sets of mutations  $A_i$  and  $A_j$  that can occur on the genetic background  $W$ , and the four genotypes  $W$ ,  $WA_i$ ,  $WA_j$

275 and  $WA_iA_j$ , where a concatenation of symbols represents the genotype which contains all the  
276 mutations referred to by the symbols. The **ordering condition** (derived in Materials and Methods)  
277 says that whenever  $W$  is the fittest among these four genotypes,  $WA_iA_j$  must be the least fit, and  
278 whenever  $WA_iA_j$  is the fittest,  $W$  must be the least fit. For the case of two single mutations this  
279 situation is illustrated by the fitness graphs in Figure 3B, where the background genotype  $W = 00$  is  
280 the fittest in the first segment 0A and the genotype  $WA_iA_j = 11$  is the fittest in the last segment  
281 FG. The ordering condition has the immediate consequence that the fittest genotype is *always*  
282 accessible from the background genotype  $W$ . If the fittest genotype is one of the single mutants  
283 (segments AB, BE and EF), then it is of course accessible. If it is the double mutant  $WA_iA_j$  (segment  
284 FG), then the background genotype must be the least fit genotype (from the ordering condition),  
285 and therefore  $WA_i$  and  $WA_j$  should be fitter than  $W$ . Then  $WA_iA_j$  is accessible from the wild type  
286 through the path  $W \rightarrow WA_i \rightarrow WA_iA_j$  and the path  $W \rightarrow WA_j \rightarrow WA_iA_j$ .

287 To fully exploit the consequences of the ordering property we need to introduce some notation.  
288 Let  $\sigma$  be a genotype with  $n$  mutations. We define a *subset* of  $\sigma$  as a genotype with  $l$  mutations,  $l \leq n$ ,  
289 which are all contained in  $\sigma$  as well. Likewise, a *superset* of  $\sigma$  is a genotype with  $l$  mutations,  $l \geq n$ ,  
290 that contains all the mutations in  $\sigma$ . With this, the ordering condition can be seen to imply that  
291 the superset of a fitness peak is accessible from its own supersets. For example, if  $W$  is the fittest  
292 genotype, then  $WA_i$  is a superset of it, and because of the ordering condition,  $WA_i$  must be fitter  
293 than its superset  $WA_iA_j$ , and therefore accessible from it. Similarly, it is easy to show that the  
294 subset of a fitness peak is accessible from its own subsets. This property can be generalized and  
295 constitutes our main result on accessibility of fitness peaks.

296 **Accessibility property:** *Any genotype  $\Sigma$  that is a superset of a local fitness peak  $\sigma$  is accessible from*  
297 *all the superset genotypes of  $\Sigma$ . Similarly, any genotype  $\Sigma'$  that is a subset of a local fitness peak  $\sigma$  is*  
298 *accessible from all the subset genotypes of  $\Sigma'$ .*

299 The proof is given in Materials and Methods. Three particularly important consequences are

- 300 • Any fitness peak is accessible from all its subset and superset genotypes.
- 301 • **Any fitness peak is accessible from the wild type.** This is because the wild type is a subset  
302 of every genotype.
- 303 • For the same reason, when the wild type is a fitness peak, it is accessible from every genotype,  
304 and is therefore also the only fitness peak in the landscape. The same holds for the all-mutant,  
305 which is a superset of every genotype.

306 These properties are illustrated by the fitness graph in Figure 6. We assume that the landscape has  
307 (at least) two peaks at the genotypes 1001 (marked in red) and 0111 (marked in blue). The colored  
308 arrows point towards mutational neighbors with higher fitness and are enforced by the accessibility  
309 property. The edges without arrowheads are not constrained by the accessibility property and the  
310 corresponding arrows (which are not shown in the figure) could point in either direction.

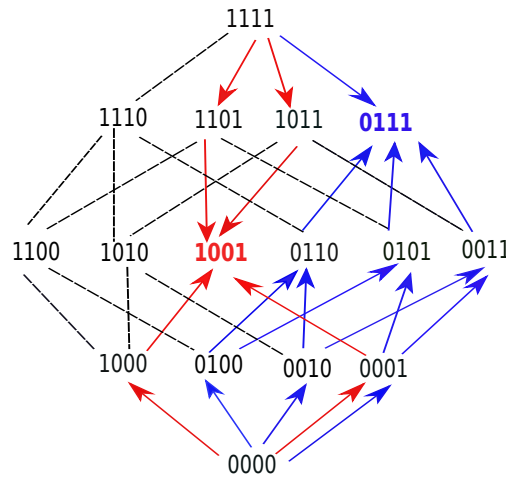
311 Consider the genotype 0111 (marked in blue). It is accessible from all its subsets, namely 0000,  
312 0010, 0010, 0001, 0110, 0101 and 0011, following the upward pointing blue arrows. These subsets  
313 are in turn accessible from their subsets. For example, 0011 is accessible from all its subsets –  
314 0000, 0010, and 0001. The fitness peak is also accessible from its superset 1111. The same property  
315 holds for the other fitness peak. The subsets or supersets may access the fitness peaks using other  
316 (unmarked) paths as well, which would include one or more of the undirected lines in conjunction  
317 with some of the arrows. Moreover, other genotypes, which are neither supersets nor subsets, may  
318 also access these fitness peaks through paths that incorporate some of the undirected edges.

319 A fitness peak together with its subset and superset genotypes defines a sub-landscape with  
320 remarkable properties. It is a smooth landscape with only one peak which is accessible from any  
321 genotype via all direct paths, i.e paths where the number of mutations monotonically increases  
322 or decreases. For example, the fitness peak 1001 is accessible from the all-mutant 1111 by the two  
323 direct paths – 1111  $\rightarrow$  1101  $\rightarrow$  1001 and 1111  $\rightarrow$  1011  $\rightarrow$  1001. Likewise, the peak 0111 is accessible  
324 from its subset 0001 via the paths 0001  $\rightarrow$  0101  $\rightarrow$  0111 and 0001  $\rightarrow$  0011  $\rightarrow$  0111. In general, a peak



325 with  $n$  mutations is accessible from a subset genotype with  $m$  mutations by  $(n - m)!$  direct paths, and  
 326 from a superset genotype with  $m$  mutations by  $(m - n)!$  direct paths. This gives a lower bound on  
 327 the total number of paths by which a fitness peak is accessible from a subset or superset genotype.

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**Figure 6.** A fitness graph of a landscape with  $L = 4$  mutations, illustrating the accessibility property. There are two fitness peaks, 1001 (red) and 0111 (blue). The fitness peaks are accessible from all their subset and superset genotypes following the paths marked by the arrows.

Importantly, the accessibility property formulated above holds under more general conditions than stipulated in the model. We show in Materials and Methods that it holds whenever the null fitness and resistance values of the mutations,  $r$  and  $m$ , do not show *positive* epistasis. This is a weaker requirement than our original assumption of a strict lack of epistasis in these two phenotypes. In this context it should be noted that the rank orderings forbidden by the ordering condition all show positive epistasis for the fitness values, whereas all the allowed orderings can be constructed without positive epistasis. Therefore, any landscape where positive epistasis in the fitness is absent will also display the accessibility property. However, whereas the lack of positive epistasis is a sufficient condition, it is not necessary. In particular, our model does allow for cases of positive epistasis in the fitness values.

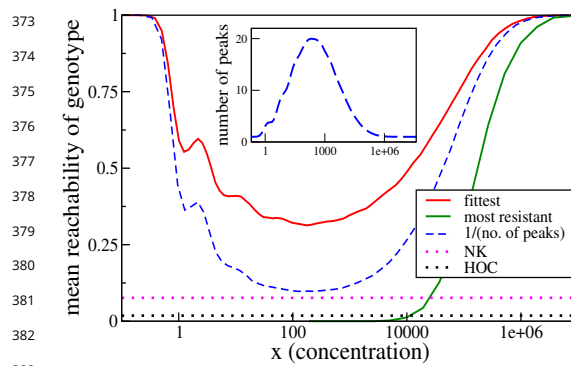
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### Reachability of the fittest and the most resistant genotype

The preceding analyses have shown that within the mutant selection window, where mutants with higher fitness than the wild type exist, every fitness peak is accessible from the wild type. This includes in particular the fittest genotype at a given concentration. However, in general there will be many peaks in the fitness landscape, and it is not guaranteed that evolution will reach the fittest genotype. One can ask for the probability that the fittest genotype is actually accessed under the evolutionary dynamics, which we call its reachability. We assume that the dynamics is in the strong selection weak mutation (SSWM) regime, and the population is large enough such that the fixation probability of a mutant with selection coefficient  $s$  is  $1 - e^{-2s}$  for  $s > 0$ , and 0 for  $s \leq 0$  (Gillespie, 1984). In our setting the selection coefficient is  $s = \frac{f_1}{f_0} - 1$ , where  $f_1$  is the growth rate of a mutant appearing in a population of cells with growth rate  $f_0$ .

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Figure 7 shows the numerically obtained reachability for  $L = 10$ , averaged over the distribution  $P(r, m)$  given in Eq. (8). The reachability of the highest peak is 1 at very low and very high concentrations, since there is only peak, the wild type or the all-mutant, at these extremes. The reachability is lower at intermediate concentrations, where there are multiple peaks, all of which are accessible from the wild type. The dashed blue line is the mean of the reciprocal of the total number of fitness peaks, and is therefore the mean reachability of fitness peaks. The reachability of the highest peak follows the qualitative behavior of the mean reachability, but remains higher than the mean reachability everywhere. The green curve is the reachability of the most resistant genotype, i.e the all-mutant. It is extremely low at low and moderate concentrations and grows steeply and saturates quickly at a very large concentration. The all-mutant genotype is less-than-average reachable everywhere except at very high concentration, when it is the only fitness peak and accessible from every other genotype.



**Figure 7.** Reachability of fittest genotype and most resistant genotype. The same model as in the previous subsection has been used, with  $L = 10$ . Inset shows the mean number of fitness peaks as a function of concentration. Dotted horizontal lines show comparisons to the HoC model and an NK model with the same number of mutations. These models were implemented using an exponential distribution of fitness values.

the usual definition of the model, the fitness of a genotype is the sum over the contributions of each of the 10 mutations, and the contribution of each mutation depends only the state of the block to which it belongs. The fitness contribution of each mutation for any state of the block is an independent random number. The mean number of fitness maxima here is  $\approx 28.44$  (Perelson and Macken, 1995; Schmiegelt and Krug, 2014), which is comparable to the maximum mean number in the tradeoff-induced landscapes (see inset of Figure 7). Nonetheless, the reachability of the fittest peak (dotted pink line) is found to be nearly 4 times smaller than the lowest reachability in our landscape. We found that in a fraction of about 0.64 of the landscapes, the fittest maximum is not reached in any of 32000 dynamical runs, indicating the absence of an accessible path in most of these cases (Schmiegelt and Krug, 2014; Hwang et al., 2018). In contrast, an evolutionary path always exists to any fitness peak in the tradeoff-induced landscapes, as we saw in the previous subsection. This endows the tradeoff-induced landscapes with the unusual property of being highly rugged and at the same time having a much higher evolutionary reachability of the global fitness maximum compared to other models with similar ruggedness.

## Discussion

Fitness landscapes depend on the environment, and gene-gene-interactions can be modified by the environment. Systematic studies of such  $G \times G \times E$  interactions are rare, but they are clearly of relevance to scenarios such as the evolution of antibiotic resistance, where the antibiotic concentration can vary substantially in space and time. In this paper we have explored the structure of such landscapes in the presence of tradeoffs between fitness and resistance. We summarize the main findings of our work.

- We have shown experimental evidence that the dose-response curves of various mutant strains of *E. coli* to the antibiotic ciprofloxacin have the same shape, except for a rescaling of the fitness and concentration values. If this shape is known, the fitness of a strain can be estimated at any antibiotic concentration simply by measuring its null-fitness and  $IC_{50}$  (or MIC). This makes it possible to construct empirical fitness landscapes at any antibiotic concentration from a limited set of data.
- Under the assumptions of our model the degree of epistasis, particularly sign epistasis, is

low for zero and high antibiotic concentrations, but it is nevertheless high in the intermediate concentration regime. The number of local fitness peaks scales exponentially in the number of mutations at these concentrations. Epistasis is often discussed as a property intrinsic to mutations and their genetic backgrounds, with limited consideration of environmental parameters. But in the landscapes studied here, the environmental parameter is of paramount importance, since changes in it can dramatically alter gene-gene interactions.

- The expected number of mutations at a fitness peak increases logarithmically with the antibiotic concentration. This implies that, at a given concentration, the highly fit genotypes that make up the fitness peaks carry an *optimal number of mutations* that arises from the tradeoff between fitness cost and resistance.
- Despite the high ruggedness, the landscape displays strong non-random patterns. A rank ordering condition between sets of mutations holds at all concentrations. A remarkable and unexpected consequence of this is that any fitness peak is evolutionarily accessible from the wild type. This is contrary to the common intuition about highly rugged landscapes, where one expects any genotype to have access to only a fraction of the fitness peaks and adaptive walks to terminate after a small number of steps.
- It is well known from experimental studies of antimicrobial resistance evolution that highly resistant genotypes often require multiple mutations which can be acquired along different evolutionary trajectories. Epistatic interactions constrain these trajectories and are generally expected to impede the evolution of high resistance. We find that strong and complex epistatic interactions inevitably arise in the mutant selection window, but at the same time the evolution of the most resistant genotype (the identity of which changes with concentration) remains facile and can occur along many different pathways.

All of these conclusions follow from three basic assumptions that are readily generalizable beyond the context of antimicrobial resistance evolution: the existence of tradeoffs between two *marginal phenotypes* that govern the adaptation at extreme values of an environmental parameter; the scaling property of the shape of the tradeoff function; and the condition of limited epistasis for the marginal phenotypes. How generally these assumptions are valid is a matter of empirical investigation. We have shown that they hold for certain cases, and the interesting evolutionary implications of our results indicate that more empirical research in this direction will be useful.

In the case of antimicrobial resistance, there can be fitness compensatory mutations (*Durão et al., 2018; Levin et al., 2000*) that do not exhibit any adaptational tradeoffs. These mutations are generally found in a population in the later stages of the evolution of antibiotic resistance, which implies that they emerge in a genetic background of mutations with adaptational tradeoffs. An understanding of tradeoff-induced landscapes is therefore a prerequisite for predicting the emergence of compensatory mutations.

In the formulation of our model we have assumed for convenience that the marginal phenotypes combine multiplicatively, but this assumption is in fact not necessary. As shown in Materials and Methods, our key results on accessibility only require the absence of positive epistasis. These results therefore hold without exception for the combinatorially complete data set in Table 1, where epistasis is either absent or negative. More generally, our analysis remains valid in the presence of the commonly observed pattern of diminishing returns epistasis among beneficial mutations (*Chou et al., 2011; Schoustra et al., 2016; Wünsche et al., 2017*). In addition, we expect our results to hold approximately when there is a small degree of epistasis (positive or negative) in  $r$  and  $m$ , but we do not explore that question quantitatively in this paper.

We conclude with some possible directions for future work. Our model provides a principled framework for predicting how microbial fitness landscapes vary across different antibiotic concentrations. This could be exploited to describe situations where the antibiotic concentration varies on a time scale comparable to the evolution of resistance, either due to the degradation of the drug or by an externally imposed treatment protocol (*Marrec and Bitbol, 2018*). From the broader perspec-

472 tive of evolutionary systems with adaptational tradeoffs mediated by an environmental parameter,  
473 our study makes the important conceptual point that it is impossible to have non-epistatic fitness  
474 landscapes for all environments. Using the terminology of *Gorter et al. (2016)*, the tradeoffs enforce  
475 reranking  $G \times E$  interactions which in turn, as we have shown, induce sign-epistatic  $G \times G$  interactions  
476 at intermediate values of the environmental parameter. Notably, this general conclusion does not  
477 depend on the scaling property of the tradeoff function. It would nevertheless be of great interest  
478 to identify instances of scaling for other types of adaptational tradeoffs, in which case the detailed  
479 predictions of our model could be applied as well.

## 480 Acknowledgements

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482 *et al. (2009)*, and Tobias Bollenbach, Michael Brockhurst and Kristina Crona for useful comments.  
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## 487 Materials and Methods

### 488 Experiments

#### 489 Bacterial strains

490 We used strains from Marcusson et al. (2009) (courtesy of Douglas Huseby and Diarmaid Hughes).  
491 The strains are isogenic derivatives of MG1655, a K12 strain of the bacterium *E. coli*, with specific  
492 point mutations or gene deletions in five different loci: *gyrA:S83L*, *gyrA:D87N*, *parC:S80I*,  $\Delta marR$ , and  
493  $\Delta acrR$ . There are 32 possible combinations of these alleles, but we only used the wild type, single  
494 mutants (5 strains) and double mutants (8 strains of 10 possible combinations): LM179 (00000),  
495 LM378 (10000), LM534 (01000), LM792 (00100), LM202 (00010), LM351 (00001), LM625 (11000),  
496 LM862 (10100), LM421 (10010), LM647 (10001), LM1124 (01100), LM538 (01010), LM592 (01001),  
497 LM367 (00011). A binary sequence after the strain's name represents the presence/absence of a  
498 particular mutated allele (order as in the above list of genetic alterations).

#### 499 Growth media and antibiotics

500 LB growth medium was prepared according to Miller's formulation (10g tryptone, 5g yeast extract,  
501 10g NaCl per litre). The pH was adjusted to 7.2 with NaOH, and autoclaved at 121°C for 20 min.  
502 Ciprofloxacin (CIP) solutions were prepared from a frozen stock (10mg/ml ciprofloxacin hydrochloride,  
503 pharmaceutical grade, AppliChem, Darmstadt, in sterile, ultra-pure water) by diluting into LB  
504 to achieve the desired concentrations.

#### 505 Dose-response curves

506 We incubated bacteria in 96-well clear flat bottom micro-plates (Corning Costar) inside a plate reader  
507 (BMG LABTECH FLUOstar Optima with a stacker) starting from two different initial cell densities (half  
508 a plate for each), and measured the optical density (OD) of each culture every 2-5 min to obtain  
509 growth curves. Plates were prepared automatically using a BMG LABTECH CLARIOstar plate reader  
510 equipped with two injectors connected to a bottle containing LB and a bottle with a solution of CIP  
511 in LB. The injectors were programmed to create different concentrations of CIP in each column of  
512 the 96 well plate. The injected volumes of the CIP solution were 0, 20, 25, 31, 39, 49, 62, 78, 98,  
513 124, 155, 195  $\mu$ l, and an appropriate volume of LB was added to bring the total volume to 195  $\mu$ l  
514 per well. Since different strains had MICs spanning almost two decades of CIP concentrations, we  
515 used a different maximum concentration of the CIP solution for each strain (approximately 1.5 - 2  
516 times the expected MIC). Bacteria were diluted from a thawed frozen stock  $10^3$  and  $10^4$  times in PBS  
517 (phosphate buffered saline buffer), and 5  $\mu$ l of the suspension was added to each well ( $10^3$  dilution

518 to rows A-D,  $10^4$  dilution to rows E-H). We used one strain per plate and up to 4 plates per strain  
519 (typically 1-2). After adding the suspension of bacteria to each well, the plates were immediately  
520 sealed with a transparent film to prevent evaporation, and put into a stacker (37°C, no shaking),  
521 from which they would be periodically fed into the FLUOstar Optima plate reader (37°C, orbital  
522 shaking at 200rpm for 10s prior to OD measurement). We then used the time shift methods to  
523 obtain exponential growth rates for each strain and different concentrations of CIP, see *Ojkic et al.*  
524 (2019) for further details.

## 525 **Mathematical Methods**

### 526 Rank orders and fitness graphs

527 The total number of possible rank orders with  $L$  mutations is  $2^L!$ , which is 24 for  $L = 2$ . Not all  
528 these rank orders, however, can be realized as one scans through  $x$ . Since any two curves intersect  
529 at most once, the maximum number of distinct rank orders that can be reached is the rank order at  
530  $x = 0$  plus the total number of possible intersections, which is  $\binom{2^L}{2} = 2^{L-1}(2^L - 1)$ . Thus the upper  
531 bound on the number of rank orders found by scanning through  $x$  is  $2^{L-1}(2^L - 1) + 1$ , which is smaller  
532 than  $2^L!$  for  $L \geq 2$ .

533 It is also instructive to determine the number of fitness graphs that can be found by varying  $x$  for  
534 a system with  $L$  mutations. This can be computed as follows: At  $x = 0$  every mutation is deleterious,  
535 and every mutational neighbor with one less mutation is fitter; but due to the tradeoff condition, at  
536 sufficiently large  $x$  every mutation is beneficial and any mutational neighbor with one less mutation  
537 is less fit. In order for this reversal of fitness order to happen, the dose-response curves of any two  
538 mutational neighbors must intersect at some  $x$ . Therefore, the number of fitness graphs generated  
539 is equal to the number of distinct pairs of mutational neighbors, which is  $2^{L-1}L$ , and the number of  
540 distinct fitness graphs encountered is  $2^{L-1}L + 1$ . For  $L = 2$ , this number is 5, as seen in the example  
541 in the main text.

### 542 Condition for two dose-response curves to intersect at most once

543 Consider two DR curves characterized by  $(r, m)$  and  $(r', m')$ , where  $r < r'$  and  $m > m'$ . We need to  
544 show that for the commonly observed cases, the curves  $rw(\frac{x}{m})$  and  $r'w(\frac{x}{m'})$  intersect at most once.  
545 First, notice that it is sufficient to prove this for the case  $r' = 1, m' = 1$ , because any rescaling of the  
546  $x$  and  $w$  axes does not alter the number or ordering of intersection points. Therefore we require  
547  $r < 1$  and  $m > 1$ .

548 Let us consider the case where the dose-response curve is of the form of a Hill function, i.e  
549  $w(x) = \frac{1}{1+x^a}$ , with  $a > 0$ . The intersection of curves happens at the solution of  $w(x) = rw(\frac{x}{m})$ , which  
550 we denote by  $x^*(r, m)$ . In this case the solution is given by

$$x^*(r, m) = \left( \frac{1-r}{r - \frac{1}{m^a}} \right)^{\frac{1}{a}}$$

551 which is positive and unique if  $rm^a > 1$ ; otherwise no solution with  $x^* > 0$  exists. It is similarly easy  
552 to show that at most one intersection point exists for exponentials, stretched exponentials, and  
553 half-Gaussians.

554 The property also holds for any concave dose-response curve with  $w''(x) < 0$ . We prove this as  
555 follows. Any intersection point  $x^*$  is the solution of

$$F(x^*) = r$$

556 where  $F(x) \equiv \frac{w(x)}{w(\frac{x}{m})}$ . We will show that  $F(x)$  is monotonic and therefore the above equation has at  
557 most one solution. We have

$$F'(x) = \frac{w'(x)w(\frac{x}{M}) - \frac{1}{M}w(x)w'(\frac{x}{M})}{w(\frac{x}{M})^2},$$

558 and  $F'(x)$  has the same sign as the numerator  $\mathcal{N}(x) = w'(x)w(\frac{x}{M}) - \frac{1}{M}w(x)w'(\frac{x}{M})$ . Since  $w(x)$  is a  
 559 decreasing function and  $m > 1$ ,  $w(\frac{x}{m}) > w(x) > \frac{1}{m}w(x)$ . When  $w''(x) < 0$ , we also have  $w'(x) < w'(\frac{x}{M})$ .  
 560 Since  $w'(x) < 0$ , this implies  $|w'(x)| > |w'(\frac{x}{m})|$ , and  $\mathcal{N}(x) < 0$ . Therefore  $F(x)$  is monotonically  
 561 decreasing.

### 562 Proof of the accessibility property

563 To derive the ordering condition, let us start with the simplest case of two single mutations  $A_i$ ,  $A_j$   
 564 occurring on the wild type background. There are correspondingly four different genotypes  $W$ ,  
 565  $W_{A_i}$ ,  $W_{A_j}$ ,  $W_{A_iA_j}$ , which are listed in decreasing order of fitness at  $x = 0$ . Let the intersection of  
 566 the DR curves of two genotypes  $\sigma_1$  and  $\sigma_2$  occur at  $x = X_{\sigma_1, \sigma_2}$ . Then  $X_{W, W_{A_j}}$  is given by the solution  
 567  $x^*(r_j, m_j)$  of

$$w(x) = r_j w(\frac{x}{m_j}),$$

568 and  $X_{W_{A_i}, W_{A_iA_j}}$  is given by the solution of

$$r_i w(\frac{x}{m_i}) = r_i r_j w(\frac{x}{m_i m_j}).$$

569 This last equation can be re-written as

$$w(x') = r_j w(\frac{x'}{m_j}),$$

570 where  $x' = \frac{x}{m_i}$ . Comparing this with the first equation above, we have

$$X_{W_{A_i}, W_{A_iA_j}} = m_i X_{W, W_{A_j}} > X_{W, W_{A_j}}. \quad (3)$$

571 This equation tells us that whenever the double mutant is fitter than one of the single mutants, the  
 572 wild type must be less fit than the *other* single mutant. Consequently, when the double mutant is  
 573 fitter than both the single mutants, the WT must be less fit than both the single mutants. In other  
 574 words, the number of single mutants fitter than the wild type cannot be less than the number of  
 575 single mutants less fit than the double mutant. This is the ordering condition given in the main text.  
 576 Any ordering that violates this condition is a *forbidden ordering*. For greater clarity, we list all the  
 577 possible forbidden orderings (up to interchange of indices  $i$  and  $j$ ).

$$\begin{aligned} W &> W_{A_i} > W_{A_iA_j} > W_{A_j} \\ W &> W_{A_iA_j} > W_{A_i} > W_{A_j} \\ W_{A_iA_j} &> W > W_{A_i} > W_{A_j} \\ W_{A_iA_j} &> W_{A_i} > W > W_{A_j} \end{aligned} \quad (4)$$

578 Although we showed this for two single mutations in the wild type background, the same argu-  
 579 ments hold for any two sets of mutations in any background, since the succession of orderings is  
 580 independent of the rescalings of the fitness and concentration axes. To put it more precisely,  $W$ ,  $A_i$   
 581 and  $A_j$  are any three non-overlapping sets of mutations, where  $A_i$  and  $A_j$  are non-empty sets.

582 Next we use this to prove the accessibility property. Let  $\sigma$  have  $n$  mutations. It is sufficient to  
 583 prove that (i) any superset of  $\sigma$  with  $m$  or fewer mutations is accessible from all its own supersets  
 584 with  $m$  or fewer mutations, for all  $m \geq n$  (the statement follows from the case  $m = L$ ); and that (ii)  
 585 any subset of  $\sigma$  with  $m'$  or more mutations is accessible from any of its own subsets with  $m'$  or more  
 586 mutations, for all  $m' \leq n$  (the statement corresponds to  $m' = 0$ ). We prove this by induction.

587 Firstly, we notice that the case  $m = n$  is trivial, since  $\sigma$  is of accessible from itself. For the case of  
 588 supersets, our base case is  $m = n + 1$ , and the assertion above holds because  $\sigma$  is a local fitness peak,  
 589 and therefore accessible from all its supersets with  $n + 1$  mutations, which are of course accessible  
 590 from themselves.

591 Now we prove the induction step. Assume that all supersets of  $\sigma$  that have  $m$  or fewer mutations  
 592 (where  $m \geq n$ ) are accessible from all their supersets with  $m$  or fewer mutations. Consider a superset

593  $\Sigma$  of  $\sigma$  with  $m$  mutations, and denote it by  $\Sigma = \sigma A$ , where  $A$  is the set of mutations in  $\Sigma$  not present  
 594 in  $\sigma$ . By assumption,  $\sigma$  is accessible from  $\Sigma$ . In the following, we use the notation  $\sigma_1 > \sigma_2$  to indicate  
 595 that a genotype  $\sigma_1$  is fitter than a genotype  $\sigma_2$  (we use the “<” and “=” signs in a similar way).  
 596 Therefore, we have  $\sigma > \Sigma = \sigma A$ .

597 Now consider any superset of  $\Sigma$  with  $m + 1$  mutations, where the additional mutation not  
 598 contained in  $\Sigma$  is denoted  $B$ . Then this superset can be denoted by  $\Sigma B = \sigma AB$ . We must have  
 599  $\sigma > \sigma B$  since  $\sigma$  is a local fitness peak. We now have the relation  $\sigma > \sigma A, \sigma B$ . Therefore we must have  
 600  $\sigma AB < \sigma A, \sigma B$ , for otherwise we violate the ordering condition. Now since  $\Sigma B = \sigma AB < \sigma A = \Sigma$ ,  $\Sigma$   
 601 must be accessible from  $\Sigma B$ , proving that any superset with  $m$  mutations is accessible from any of  
 602 its supersets with  $m + 1$  mutations. This completes the proof of the induction step.

603 The proof for the case of subsets is essentially the same, utilizing the symmetry between the  
 604 wild type and the double mutant in the ordering condition.

605 The accessibility property follows entirely from the ordering condition, and hence any landscape  
 606 that obeys the ordering condition will obey the theorem. The ordering condition follows from  
 607  $X_{W,WA_i} < X_{WA_i,WA_iA_j}$ , as obtained in (3). However, this same inequality obtains under more general  
 608 conditions. To see this, let us define the null-fitness of the double mutant  $WA_iA_j$  as  $r_{ij}$ , and the  
 609 resistance of the double mutant as  $m_{ij}$ . The dose-response curves of  $W$  and  $WA_i$  intersect at  
 610  $X_{W,WA_i} = x^*(r_j, m_j)$ , whereas the curves for  $WA_i$  and  $WA_iA_j$  intersect at

$$X_{WA_i,WA_iA_j} = m_i x^*\left(\frac{r_{ij}}{r_i}, \frac{m_{ij}}{m_i}\right).$$

611 Now it is easy to show that  $x^*(r, m)$  is a decreasing function of both  $r$  and  $m$ . Therefore  $X_{WA_i,WA_iA_j} >$   
 612  $X_{W,WA_i}$  holds if  $r_{ij} \leq r_i r_j$  and  $m_{ij} \leq m_i m_j$ .

### 613 Number of local fitness peaks

614 When dealing with complex fitness landscapes with parameters that can vary across species and  
 615 environments, a useful strategy is to model the fitness effects as random variables that are chosen  
 616 from a probability distribution (*Kauffman and Levin, 1987; Szendro et al., 2013; Hwang et al., 2018*).  
 617 In the limit of large system size  $L$ , many properties emerge that are independent of the details of  
 618 the system. In practice, even relatively small system sizes are often approximated well by results  
 619 obtained in the asymptotic limit.

620 The mean number of peaks with  $n$  mutations in the tradeoff-induced landscapes is

$$K_n(x) = \binom{L}{n} Q_n(x),$$

621 where  $\binom{L}{n}$  is the total number of genotypes with  $n$  mutations, and  $Q_n(x)$  is the probability that  
 622 a genotype with  $n$  mutations is a fitness maximum at antibiotic concentration  $x$ . Then the total  
 623 number of peaks at  $x$  is  $\sum_n K_n(x)$ . Let the resistance of a genotype  $\sigma$  be  $M = \prod_{i=1}^n m_i$ , and likewise its  
 624 null-fitness be  $R = \prod_{i=1}^n r_i$ . The genotype  $\sigma$  is a local fitness maximum if it is fitter than all its subsets  
 625 with  $n - 1$  mutations and all its supersets with  $n + 1$  mutations.

626 To find the concentration at which the curves of  $\sigma$  and its neighboring genotypes intersect, we  
 627 start with the simplest case of the dose-response curves of the wild type and a single mutant  $(r, m)$ .  
 628 These curves intersect at the solution  $x^*(r, m)$  of  $w(x) = rw\left(\frac{x}{m}\right)$ , which is a decreasing function of  
 629  $r$  and  $m$ . The wild type is fitter than the single mutant when  $x > x^*(r, m)$ . Now the intersection of  
 630 the DR curves of a genotype  $\sigma$  with  $n$  mutations and a subset with  $n - 1$  mutations that lacks the  
 631 mutation  $(r_i, m_i)$  occurs at the solution of

$$w\left(\frac{x}{\left(\frac{M}{m_i}\right)}\right) = r_i w\left(\frac{x}{\left(\frac{M}{m_i}\right)m_i}\right)$$

632 which is read off as  $\frac{M}{m_i} x^*(r_i, m_i)$ . Likewise, the intersection of the DR curves of  $\sigma$  and a superset with  
 633  $n + 1$  mutations that contains the additional mutation  $(r_j, m_j)$  occurs at  $M x^*(r_j, m_j)$ . Therefore  $\sigma$  is a

634 fitness maximum if

$$\frac{x^*(r_i, m_i)}{m_i} < \frac{x}{M} < x^*(r_j, m_j) \quad (5)$$

635 for all  $i$  and  $j$  with  $1 \leq i < n$  and  $n < j \leq L$ . Alternatively,

$$\log m_i - \log x^*(r_i, m_i) > \log M - \log x > -\log x^*(r_j, m_j). \quad (6)$$

636 Let us consider the regime where  $L, n \gg 1$ . Then  $\log M \sim n\langle \log m \rangle$ ; if  $\log x$  is smaller than  $O(n)$ ,  
 637 it is clear that the second inequality is almost certainly satisfied whereas the probability of the  
 638 first inequality is vanishingly small. Both the probabilities are finite if  $\log x \sim n\langle \log m \rangle$ . Thus the  
 639 probability of  $\sigma$  being a fitness peak is maximized when  $\log x = \log(M) + \eta$ , where  $\eta \sim O(1)$  and  
 640 depends on the details of the distribution  $P(r, m)$ . Thus the mean number of fitness peaks with  $n$   
 641 mutations is maximal at  $x_{\max}(n)$  where to leading order  $\log x_{\max}(n) \sim n\langle \log m \rangle$ , independent of any  
 642 further details of the system.

643 The total number of genotypes with  $n$  mutations is  $\binom{L}{n}$ , and  $\log \binom{L}{n} \simeq LH(\rho)$ , where  $\rho = \frac{n}{L}$ , and

$$H(\rho) = -[\rho \log \rho + (1 - \rho) \log(1 - \rho)]. \quad (7)$$

644 The mean number of fitness maxima can be found by multiplying this with  $Q_n$ . One may expect  $Q_n$   
 645 to be exponentially small in  $L$ , since a total of  $L$  inequalities (as indicated in (6)) need to be satisfied.  
 646 However, this is complicated by the fact that the probabilities of the inequalities being satisfied are  
 647 not independent. The correlations between the inequalities would depend on the distribution of  
 648  $P(r, m)$  and the dose-response curve. If the correlations are sufficiently weak, one might still expect  
 649 to find an exponential scaling in large  $L$ . To leading order  $\binom{L}{n}$  is itself exponential in  $L$ , and if the  
 650 probability that a genotype is a fitness peak is exponentially small in  $L$ , we expect the mean number  
 651 of peaks  $K_n$  to be exponential in  $L$  as well. This is supported by the scaling shown in the inset of  
 652 Figure 4A.

653 For the simulation results shown in the main text we chose a joint distribution of the form

$$P(r, m) = P(r)P(m|r) = 6r(1 - r)\left(m - \frac{1}{\sqrt{r}}\right)e^{-\left(m - \frac{1}{\sqrt{r}}\right)}. \quad (8)$$

654 The conditional distribution  $P(m|r)$  is a shifted gamma distribution. The shift ensures that the curves  
 655 of a background genotype and a mutant intersect.

656 Sign epistasis in the limit of large  $L$  and  $n$

657 Sign epistasis with respect to a certain mutation occurs when the mutation is beneficial in one  
 658 background but deleterious in another. To understand sign epistasis, we ask for the number of  
 659 backgrounds  $n_b$  in which a mutation is beneficial at concentration  $x$ . If one considers only those  
 660 backgrounds that have  $n$  mutations, then  $n_b$  would depend both on  $n$  and  $x$ .

661 In a statistical ensemble of landscapes, one may compute the probability  $P_b$  that a mutation  
 662 is beneficial in a background with  $n$  mutations, and of course  $\langle n_b \rangle = P_b \binom{L}{n}$ . In the limit of large  $L$   
 663 and  $n$ ,  $P_b$  exhibits some universal properties to leading order. When  $\log x > n\langle \log m \rangle$ , we are in the  
 664 regime of high concentration relative to  $n$ , and we expect a mutation to be beneficial. We find that  
 665 to leading order  $P_b(\rho, x) = 1$ , with corrections that are exponentially small in  $n$ . When  $\log x < n\langle \log m \rangle$ ,  
 666 we are at concentrations that are too low to prefer additional mutations, and  $P_b$  is exponentially  
 667 small in  $n$ . When  $\log x = n\langle \log m \rangle$ , we are at the threshold concentration where a new mutation  
 668 becomes beneficial. Here we find that  $P_b \simeq \frac{1}{2}$ . For large  $L$  we therefore expect a steep transition  
 669 from 0 to 1 as the concentration crosses the threshold value (see inset of Figure ??).

670 Consider a mutation  $(r, m)$  in a background with  $n$  mutations  $(r_1, m_1), (r_2, m_2) \dots (r_n, m_n)$ . The mutation  
 671 is beneficial in this background if

$$m_1 m_2 \dots m_n x^*(r, m) < x \quad (9)$$

672 Taking logarithms, we have

$$-\log x^*(r, m) > \sum_{i=1}^n \log m_i - \log x. \quad (10)$$



673 Define  $\xi = \frac{\log x}{L}$  and  $\rho = \frac{n}{L}$ , and  $z = -\log x^*(r, m)$ . Then the above inequality becomes

$$\frac{z}{n} > \frac{1}{n} \sum_{i=1}^n \log m_i - \frac{\xi}{\rho}. \quad (11)$$

674 Let the distribution of  $z$  be  $P(z)$ , and let  $C_z(z) = \int_z^\infty P_z(x) dx$ . Define the random variable  $\omega =$   
 675  $\frac{1}{n} \sum_{i=1}^n (\log m_i - \frac{\xi}{\rho})$ , and denote its distribution  $P(\omega)$ . Then the probability that a mutation is beneficial  
 676 in a background with  $n$  mutations is

$$P_b(\rho, \xi) = \int_{-\infty}^{\infty} P(\omega) C_z(n\omega) d\omega \quad (12)$$

$$(13)$$

677 The mean number of backgrounds with  $n$  mutations in which a mutation is beneficial is  $n_b(\rho, \xi) =$   
 678  $P_b(\rho, \xi) \binom{L}{n}$ . Note that  $\langle \omega \rangle = \langle \mu \rangle - \frac{\xi}{\rho}$  where  $\mu = \log m$ . When  $n \gg 1$ ,  $C_z(n\omega) \simeq 1$  for  $\omega < 0$  and  $C_z(n\omega) \simeq 0$   
 679 for  $\omega > 0$ , with a sharp transition from 1 to 0 that happens within a region of width  $\sim O(1/n)$  of the  
 680 origin. Also for large  $n$ ,  $P(\omega)$  is sharply peaked around  $\langle \omega \rangle$  over a region of width  $O(1/\sqrt{n})$ .

681 When  $\langle \omega \rangle < 0$ ,  $C_z(n\omega) \simeq 1$  over this entire region, as observed before. Thus to leading order,  
 682  $P_b(\rho, \xi) = 1$ . The mean number of backgrounds in which a mutation is beneficial is  $n_b(\rho, \xi) =$   
 683  $P_b(\rho, \xi) \binom{L}{\rho L}$ .

$$n_b(\rho, \xi) \simeq \sqrt{\frac{2\pi}{L}} \frac{1}{\sqrt{\rho(1-\rho)}} e^{LH(\rho)} \quad (14)$$

684 where  $H(\rho)$  is defined in (7). Therefore

$$\log n_b \simeq LH(\rho) \quad (15)$$

685 to leading order.

686 When  $\langle \omega \rangle > 0$ , the dominant contribution to the integral in (12) comes from  $\omega \leq 0$ , since  $C_z(n\omega)$   
 687 quickly drops from 1 to zero for  $\omega > 0$ . Further, since  $C_z(\omega) \simeq 1$  for  $\omega < 0$  (except for a region of width  
 688  $O(1/n)$  around  $\omega = 0$ , as observed before), we can approximate  $\log P_b(\rho, \xi)$  simply by the probability  
 689 that  $\omega < 0$ . Then

$$\log P_b(\rho, \xi) \simeq -nI\left(-\frac{\xi}{\rho}\right)$$

690 where  $I$  is the large deviation function of  $-\mu$ , and

$$\log n_b(\rho, \xi) \simeq L\left[H(\rho) - \rho I\left(-\frac{\xi}{\rho}\right)\right].$$

691 This implies that  $n_b$  is reduced by a factor that is exponentially small in  $L$  compared to (15), and  
 692 therefore the fraction of backgrounds in which a mutation is beneficial is very small.

693 Finally, when  $\langle \omega \rangle = 0$ , i.e.  $\xi = \frac{n}{L} \langle \mu \rangle$ ,  $P(\omega)$  is centered at the origin and decays over a width  $O(1/\sqrt{n})$ .  
 694 For  $\omega > 0$ ,  $C_z(n\omega)$  is 0 except over a much smaller width  $O(1/n)$  to the right of the origin, whereas  
 695 for  $\omega \leq 0$ , it is 1 except for a small region of width  $O(1/n)$  left of the origin. Thus the dominant  
 696 contribution to the integral in (12) comes from  $\omega \leq 0$ , and as before,  $P_b$  can be approximated by the  
 697 probability  $\omega \leq 0$ . Due to the central limit theorem,  $P(\omega)$  is approximately Gaussian and therefore  
 698 symmetric around  $\omega = 0$ , and therefore  $P_b \simeq \frac{1}{2}$ . Consequently, we should have

$$n_b(\rho, \xi) \simeq \frac{1}{2} \sqrt{\frac{2\pi}{L}} \frac{1}{\sqrt{\rho(1-\rho)}} e^{LH(\rho)},$$

699 which is  $\frac{1}{2}$  times the total number of backgrounds given by (14). This proves that the concentration  
 700 where the mutation is beneficial in half of the backgrounds is given by  $\langle \omega \rangle = 0$  or  $\log x = n \langle \log m \rangle$  for  
 701 large  $L$  and  $n$ .

702 **Epistasis in null-fitness and MIC for *E. coli* in the presence of ciprofloxacin**

703 Primary data shown in Table 1 were obtained from *Marcusson et al. (2009)*. In the third and  
 704 fifth columns, the errors in the  $\log(x)$  are calculated as  $\frac{|\Delta x|}{x}$ , where  $|\Delta x|$  are the standard error as  
 705 calculated from the standard deviations reported in the paper. The errors in columns four and  
 706 six were estimated as  $\sum_i \frac{|\Delta x_i|}{x_i}$  where the sum is over the mutations present in the combinatorial  
 707 mutants. The detectable cases of epistasis are marked in blue. Negative epistasis is found in all  
 708 these cases. Also, all the cases with epistasis correspond to two or more mutations that affect the  
 709 same chemical pathways.

Strain	String	log null-fitness	Non-epistatic	log MIC	Non-epistatic
MG1655	00000	0.00 (± .004)	NA	0.00 (± .35)	NA
LM378	10000	0.01 (± .016)	NA	3.17 (± .70)	NA
LM534	01000	-0.01 (± .018)	NA	2.75 (± .70)	NA
LM202	00010	-0.19 (± .020)	NA	0.69 (± .70)	NA
LM351	00001	-0.094 (± .014)	NA	1.08 (± .70)	NA
LM625	11000	-0.030 (± .011)	0.0 (± .038)	3.17 (± .70)	5.92 (± 1.1)
LM421	10010	-0.15 (± .019)	-0.18 (± .040)	4.13 (± .70)	3.56 (± 1.1)
LM647	10001	-0.051 (± .013)	-0.084 (± .034)	3.44 (± .70)	4.65 (± 1.1)
LM538	01010	-0.19 (± .020)	-0.20 (± .042)	4.13 (± .70)	3.46 (± 1.1)
LM592	01001	-0.083 (± .015)	-0.10 (± .036)	3.16 (± .70)	3.83 (± 1.1)
LM367	00011	-0.20 (± .026)	-0.28 (± .038)	2.06 (± .70)	1.77 (± 1.1)
LM695	11010	-0.24 (± .017)	-0.19 (± .058)	3.85 (± .70)	6.61 (± 1.1)
LM691	11001	-0.073 (± .013)	-0.094 (± .052)	3.85 (± .70)	7.00 (± 1.4)
LM709	10011	-0.24 (± .027)	-0.274 (± .054)	4.54 (± .70)	4.94 (± 1.4)
LM595	01011	-0.51 (± .051)	-0.294 (± .056)	4.54 (± .70)	4.52 (± 1.4)
LM701	11011	-0.42 (± .037)	-0.284 (± .072)	4.83 (± .70)	7.69 (± 1.8)

**Table 1.** The names of the strains and values of null-fitness (in competition assays with the wild type) in the third column and MIC (of ciprofloxacin) in the fifth column are obtained from *Marcusson et al. (2009)*. The binary strings represent the same genotypes as given in the caption of Figure 2. The values in parentheses are error estimates. The fourth and sixth columns are respectively the null-fitness and MIC values expected in the absence of epistasis. NA denotes the cases where this is not applicable.

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