ANTIBIOTIC RESISTANCE LANDSCAPES: A QUANTIFICATION OF THEORY-DATA INCOMPATIBILITY FOR FITNESS LANDSCAPES

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ABSTRACT. Fitness landscapes are central in analyzing evolution, in particular for drug resistance mutations for bacteria and virus. We show that the fitness landscapes associated with antibiotic resistance are not compatible with any of the classical models; additive, uncorrelated and block fitness landscapes. The NK model is also discussed.

It is frequently stated that virtually nothing is known about fitness landscapes in nature. We demonstrate that available records of antimicrobial drug mutations can reveal interesting properties of fitness landscapes in general. We apply the methods to analyze the TEM family of β-lactamases associated with antibiotic resistance. Laboratory results agree with our observations. The qualitative tools we suggest are well suited for comparisons of empirical fitness landscapes. Fitness landscapes are central in the theory of recombination and there is a potential for finding relations between the tools and recombination strategies.

1. BACKGROUND

The fitness landscape was introduced as a metaphor for adaptation. Informally, the surface of the landscape consists of genotypes, where similar genotypes are close to each other, and the fitness of a genotype is represented as a height coordinate. Adaptation can then be pictured as an uphill walk in the fitness landscape. [Wright (1931)] It is frequently claimed that we know virtually nothing about fitness landscapes in nature. Scarcity of fitness measurements along with the difficulty in measuring fitness, are cited as reasons. The purpose of this work is to demonstrate that available records of drug resistance mutations can reveal interesting properties of the underlying fitness landscapes. We suggest qualitative tools that are easy to apply and interpret in order to learn properties of fitness landscapes from data. The setting we have in mind is a record of clinically found antimicrobial drug resistance mutations, where there is a well defined wild-type and several mutant variants with some degree of drug resistance. Other cases of adaptation could work as well.

There are several advantages with mutation records as a source of information. The records are already available. The quantity of data is substantial and growing, and the quality tend to be high since the data is of medical importance. Moreover, the data reflects nature, whereas laboratory data sometimes disagree with clinical observations, see our discussion about the TEM-family below. However, the most important reason is that this data reflects adaptation. In contrast, many times empirical studies where fitness is measured consider combinations of deleterious mutations. The majority of such
combinations are probably exceedingly rare in nature. If one is interested in adaptation, one needs knowledge about beneficial mutations as well. For an overview of recent empirical work where fitness is measured, see e.g. Szendro et al. (2012); Weinreich et al. (2005); Carnerio and Hartl (2010) and references. Most existing studies concern few loci (4 or 5) and a specific selective environment. For studies with many loci, see e.g. Kouyos et al. (2012); Schenk et al. (2012), and for a case where fitness ranks for the same genotypes are determined in several different selective environments, see Goulart et al. (2013).

We apply our results to the TEM family of beta-lactamases associated with antibiotic resistance. TEM stands for Temoneira, the name of the patient from whom the enzyme was first isolated. TEM beta-lactamases have been found in *Escherichia coli*, *Klebsiella pneumoniae* and other gram-negative bacteria. TEM-1 is considered the wild-type. The length of TEM-1 is 287, i.e., TEM-1 can be represented as a sequence of 287 letters in the 20-letter alphabet corresponding to the amino acids. Over 170 TEM variants have been found clinically, where 41 are single mutants, i.e., they have exactly one amino acid substitution, and the majority (90 %) have at most 4 amino acid substitutions. We use the record of the TEM family from the Lahey Clinic [http://www.lahey.org/Studies/temtable.asp](http://www.lahey.org/Studies/temtable.asp).

The quality of the TEM record (from now on we will simply refer to “the TEM record”), is assumed to be high. The TEM record represent a case of multiple environments, but probably not an excessive amount of completely different environments. Several antibiotics have similar effects. We have good reason to believe that the TEM record is fairly complete, and that there is not an abundance of neutral mutations.

As a complement, we use laboratory results (Goulart et al., 2013). Our study determines if TEM data is compatible with classical models of fitness landscapes. More precisely, we compare with additive, uncorrelated and block models of fitness landscapes. The NK model is also dicussed.

Throughout our article, our focus is to what extent beneficial mutations combine well. We start with a brief description of our approach in the context of the TEM family. Using standard notation, TEM-2 is a single mutant with the mutation Q39K, which means that the amino acid denoted “Q” (glutamine) at position 39 of the wild-type is substituted by the amino acid denoted “K” (lysine). TEM-174 is the single mutant A213V. One can ask if the double mutant with substitutions Q39K and A213V confer antibiotic resistance, since the double mutant combines two resistance mutations. However, the double mutant does not occur in the record.

Roughly, we compare the candidates for double mutants, such as the one described, with double mutants that do occur in the record, and consider the patterns for how candidates occur of are absent in the record. This approach is motivated by an evolutionary perspective. Provided that the quality of a record of resistance mutations is good, most single mutants are more fit, i.e., confer more drug resistance than the wild-type in some environment. Likewise, if a double mutant occur in a mutation record, it is plausible that the double mutant is more fit than at least one of the corresponding single mutants in some environment.
Put briefly, we consider if “good+good=better” for mutations. The goal is to capture the relation between fitness landscapes and mutation records. One reason for being interested in mutation records, is that laboratory results do not always reflect clinical facts. A striking example is that the triple mutant of the TEM family with substitutions A42G, E104K, and G238S, confer a high degree of cefotaxime resistance according to laboratory results Weinreich et al. (2006), but this mutant has never been observed clinically. Examples where single mutants not found outside of the laboratory confer a high degree of cefotaxime resistance are given in Schenk et al. (2012).

It may seem surprising qualitative information can be useful for analyzing fitness landscapes. However, we will show that predictions from some classical models relate well to qualitative information. Our approach could be useful for comparisons of empirical landscapes, and there is a potential for relating the information derived directly to recombination strategies.

We define fitness as the expected reproductive success, and use the convention that the wild-type has fitness 1. Fitness is called additive if the fitness effects of mutations sum. Consider a biallelic two-loci system. Suppose that the genotype ab has fitness 1, the genotype Ab has fitness 1.03 and the genotype aB has fitness 1.01. If fitness is additive, then the genotype AB has fitness $1.04 = 1 + 0.01 + 0.03$ (In the literature non-epistatic fitness is sometimes defined as multiplicative, so that the double mutant would have fitness 1.0403. If the fitness effects and the number of beneficial mutations are small, there is not much difference between the definitions.) Values greater than 1.04 implies positive epistasis. Values smaller than 1.04 implies negative epistasis.

Sign epistasis means that a particular mutation is beneficial or deleterious depending on genetic background. For example, if ab, Ab, aB and have fitness values as above (1, 1.03, 1.01), but AB has fitness 1.02, then there is sign epistasis. Indeed, in this case the mutation B is beneficial for the ab-genotype, and deleterious for the Ab-genotype.

The concept of a fitness landscapes has been formalized in different ways. A genotype may be represented as a string in the 20, 4 or 2 letter alphabet, depending on if one considers the amino acids, the base pairs or biallelic systems. Throughout the paper, we will consider amino acids.

Let $\Sigma$ denote the 20 letter alphabet. The genotype space $\Sigma^L$ consists of all $20^L$ strings of length $L$. A fitness landscape $w : \Sigma^L \rightarrow \mathbb{R}$ assigns a fitness value to each genotype. The fitness of a genotype $g$ is denoted $w_g$. If two genotypes differ by a single mutation, they are mutational neighbors.

Remark 1.1. Following the Orr-Gillespie approach we assume that the wild-type has very high fitness also in the new environment as compared to a randomly generated genotype. Consequently, only a small number of mutations of the wild-type are beneficial.

The paper is structured as follows. In Section 1.1 we briefly review classical models of fitness landscapes. Section 2 provides basic observations of the TEM record. Section 3 concerns additive and uncorrelated fitness, and Section 4 block models. For all models,
we compare with the TEM record, and in Section 5 a laborator study of TEM alleles is
used as a complement to the record.

1.1. Classical models of fitness landscapes. Additive fitness landscapes, uncorrelated
fitness landscapes, the block model and Kauffman’s NK model have had a broad influ-
ence in evolutionary biology. We will give a brief overview of the four classical models.

Additive fitness landscapes, or non-epistatic landscape, has been defined. An addi-
tive fitness landscape is single peaked.

In contrast, for an uncorrelated (also called random, rugged or House of Cards [HOC])
fitness landscape, there is no correlation between the fitness of a genotype and the fit-
tess of its mutational neighbors, i.e., alleles that differ by one substitution only.

Consider an uncorrelated landscapes where say 2% of the single mutants are more fit
than the wild-type. It follows that for double mutants corresponding to two beneficial
single mutations, approximately 2% are more fit than the wild-type as well. In other
words, beneficial mutations do usually not combine well for an uncorrelated fitness
landscape by Remark 1.1.

Uncorrelated fitness and additivity can be considered as two extremes with regard to
the amount of structure in the fitness landscape, and most fitness landscape fall between
the extremes. Uncorrelated fitness has been studied extensively in the literature (see e.g.
Kingman, 1978; Kauffman and Levin, 1987; Flyvberg and Lautrup, 1992; Rokyta et al.,
2006; Park and Krug, 2008).

For the block model (see (Macken and Perelson, 1989; Orr, 2006, e.g.) the string
representing a genotype can be subdivided into blocks, where each block makes an in-
dependent contribution to the fitness of the string. Each block has uncorrelated fitness,
and the fitness of the string is the sum of contributions from each block. In particular,
a block model consisting of only one block only is an uncorrelated fitness landscape.
The rational behind this model is that if two blocks have completely different functions,
then the effect of two changes in different blocks should be independent.

Kaufmann’s NK model (see e.g. Kauffman and Weinberger (1989)) is defined so that
the epistatic effects are random, whereas the fitness of a genotype is the average of the
"contributions" from each locus.

More precisely, for the NK model the genotypes have length N (in our notation \( L = N \)), and the parameter \( K \), where \( 0 \leq K \leq N - 1 \), reflects interactions between loci. The
fitness contribution \( \phi_i \) from the locus \( i \) is determined by its state \( g_i \) and the states at \( K \nchoose{1} \) other loci \( i_1, \ldots, i_K \). The key assumption is that this contribution is assigned at random
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from some probability distribution. The fitness of a genotype \( g \) is the average of the
"contributions" from each locus.
means that fitness effects of non-interacting mutations sum. Notice that the fitness
landscapes is additive for $K = 0$ and uncorrelated for $K = N - 1$. The popularity of the NK
model rests on the that the model is ”tunably rugged”. This expression means that the
ruggedness is expected to increase by $K$ from the single peaked additive landscape for
$K = 0$ to the uncorrelated landscape with a maximal number of peaks for $K = N - 1$.
Published results on the NK model of (potential) relevance to evolutionary biology con-
cerns the number of peaks, the length of mutational trajectories, fitness distributions of
genotypes and fitness trajectories.
Notice that also the block model includes additive landscapes and uncorrelated land-
scapes as special cases. More importantly, NK models and block models are similar in
that there is a sharp division between effects which are completely random and effects
which are additive. One should keep in mind that the block models and Kaufman’s NK
model are equipped with very special structures. In order to provide some intuition for
how empirical landscapes relate to the models, we will consider examples.

**Example 1.2.**

$$w_{000} = 1, w_{001} = w_{010} = w_{001} = 1.01, w_{011} = 1.02, w_{101} = w_{11} = 1.02, w_{111} = 1.03$$

For every loci, replacing 0 by 1 increases fitness by 0.01. Fitness is additive and the
 genotype 111 is at a peak.

**Example 1.3.**

$$w_{0000} = 1, w_{0001} = w_{0010} = w_{0100} = w_{0001} = 1.02,$$
$$w_{0011} = w_{0101} = w_{0110} = w_{0111} = 1.035,$$
$$w_{1000} = 1.02, w_{1001} = w_{1010} = w_{1100} = 1.04,$$
$$w_{1011} = w_{1101} = w_{1110} = 1.05, w_{1111} = 1.55.$$  

For every loci, replacing 0 by 1 increases fitness. For the first locus the increase is 0.02,
regardless of background. For the other loci, the magnitude of the difference depends
on the background. For instance, the magnitude is 0.02 for the change from 0000 to 0001,
0.01 for the change from 0001 to 0011, and 0.005 for the change from 0011 to 0111.

Fitness is obviously not additive in the second example, since the fitness of the double
and triple mutants are below linear expectations based on the wild-type and the single
mutants. The landscape deviates from expectations for an uncorrelated landscape as
well, since replacing 0 by 1 always gives higher fitness. It remains to consider the more
general models. As for the block model, the first locus is independent, The remaining
three loci interact with each other in a symmetric way. Consequently, the natural can-
didates for blocks would be one block consisting of the first locus, and another block
consisting of the remaining three loci. However, the second block deviates considerably
from random expectations. Consequently, the block model is not a good fit.

For the NK model, the independent fitness contribution for the first loci suggest that
$K = 0$. However, the second locus depends on the third and the fourth loci, suggesting
that $K = 2$. (Similar arguments for the third and the fourth loci, suggests that $K = 2$ as
Since the observations suggest different $K$-values, the NK model does not seem ideal.

**Remark 1.4.** The NK model allows different interactive patterns. However, it is not expected that some loci are more or less independent, whereas other loci have considerable interactions. Some degree of symmetry is expected, reflecting the $K$ value.

Several other models for fitness landscapes have been suggested, including neutral models, see Szendro et al. (2012) for an empirical perspective. For some approaches to fitness landscapes not related to the models mentioned, see the geometric theory of gene interactions Beerenwinkel et al. (2007b), Crona (2013), and the Orr-Gillespie theory Orr (2002). Notice also that fitness landscapes have been used in chemistry, physics and computer science, in addition to evolutionary biology. In combinatorial optimization the fitness function is referred to as the cost function. For a survey on combinatorial landscapes in general see Reidys and Stadler (2002).

2. **The Qualitative Measure of Additivity and the TEM Record**

Throughout the paper, we focus on single and double mutants in a record. The motivation is trivial. If a single mutant has high fitness, it is likely to be found in nature. However, if a $k$-tuple mutant is very fit for some large $k$, the mutant may never be found because of the time span necessary before the substitutions have accumulated. Single and double mutants are likely to appear relatively early in the process of adaptation. Roughly, we are interested in the proportion of beneficial mutations among all possible single mutations, as well as to what extent beneficial mutations combine well. The information we consider is coarse, and a record of mutation will rarely be perfect.

As indicated, we will work with words in the 20 letter alphabet where there is a well defined wild-type. A single mutant is a genotype resulting from one amino acid substitution. However, the amino acid substitutions are not comparable to substitutions of letters in a string. Not every amino acid substitution can occur as the result of a single point mutation. For instance, suppose that the amino acid is Valine at a particular locus, and that the codon is GTT. Then one can obtain exactly 6 single mutants at the locus corresponding to A, D, F, G, I, L (or Alanin, Aspartic acid, Phenylalanine, Glycine Isoleucine, Leucine). On the other hand, one can obtain exactly 8 single mutants (A, D, E, F, G, I, L, M) starting from Valine (the codons for Valine are GTT, GTC, GTA, GTG).

In general, the number of single mutants one can obtain varies depending on amino acid. Moreover, in some cases, such as for Valine, the number depends on if one consider a particular codon for the amino acid or all possible codons. To make matters more complicated, the wild-type allele under consideration may be unique in terms of amino acids, but not in terms of codons. For a precise analysis, one may want to consider codon variations in the wild-type allele. However, for our purposes it is sufficient to consider amino acids.

We assume that there are approximately 7 possible single point mutations for a given locus, so that if the wild-type has length $N$, there are $6N$ mutational neighbors. For the reader’s convenience, we included a table of possible single mutants (see Section 7).
Remark 2.1. Throughout the paper, we assume a genotype of length $N$ has $6N$ mutational neighbors.

We are interested in the proportion of beneficial mutations among all possible mutations. By Remark 1.1, the proportion of beneficial mutations is expected to be small. For the TEM record $N = 287$ and there are 47 single mutants in the record. Consequently,

$$\frac{S_R}{6N} = \frac{6}{6 \cdot 287} = 2.67\%$$

Another interesting property of a fitness landscape, is how beneficial mutations combine. More precisely, consider a double mutant which combines two beneficial single mutations. If the double mutant is less fit than both single mutants, then the double mutant would most likely not appear in the record. The qualitative measure of additivity is motivated by this observation. More precisely, we will use the following definition.

Definition 2.2. Let $B_p$ be the set consisting of all double mutants such that both corresponding single mutations are beneficial. The set $B \subset B_p$ consists of all double mutants in $B_p$ which are more fit than at least one of the corresponding single mutants. The qualitative measure of additivity for a fitness landscape is the ratio $\frac{|B|}{|B_p|}$.

Consider the single mutations in a record and the corresponding double mutants. Whenever two single mutants at different sites occur in the record, the corresponding double mutant is considered a candidate for a double mutant of high fitness. Let $\hat{B}_p$ be the set of candidates for double mutants. Let $\hat{B} \subset \hat{B}_p$ be the set of double mutants in the record among the candidates. Loosely speaking, one can consider $\frac{|\hat{B}|}{|\hat{B}_p|}$ the observed qualitative measure of additivity. Under ideal circumstances, the ratios $\frac{|\hat{B}|}{|\hat{B}_p|}$ and $\frac{|B|}{|B_p|}$ are approximately the same, at least in for antimicrobial resistance mutations in the context we consider. We assume that the adaptation, or the resistance development, will take place repeatedly at different geographic locations. If a double mutant is more fit than at least one of the single mutants, the double mutant should occur sooner or later.

If fitness is additive, then $\frac{|B|}{|B_p|} = 1$, and for uncorrelated fitness one expects the value be close to 0 by Remark 1.1. Of course the measure is coarse. However, it is valuable to have a simple method for comparing fitness landscapes in different contexts. Whenever fitness is measured, one can determine $\frac{|\hat{B}|}{|\hat{B}_p|}$, and for any record one can determine $\frac{|B|}{|B_p|}$.

Notice that one expects the qualitative measure to decrease by increasing block size for the block model, as well as by increasing $K$ for the NK model.

For some background, a measure of additivity which reflects quantitative fitness differences is called "roughness" (Carnerio and Hartl, 2010; Aita et al., 2001). Roughness 0 implies that the landscape is additive. A problem with roughness is a possible size bias, i.e., all else equal, the roughness may be greater for a large number of loci. The qualitative measure of additivity does not have any size bias.
Analyzing epistasis is closely related to analyzing additivity. For a thorough discussion about different measures of epistasis and empirical fitness landscapes, see Szen-dro et al. (2012). The most fine-scaled approach to epistasis is the geometric theory of gene interactions, which uses triangulations of polytopes (Beerenwinkel et al. 2007b,c; Crona 2013).

We will consider the \[ \frac{|\hat{B}|}{|B_p|} \] value for the TEM record. The record has 46 single mutants. The substitutions are at position position 69 for 3 single mutants, at position 164 for 3 single mutants, at position 244 for 5 single mutants and at position 275 for 2 single mutants. Each remaining single mutant has its mutation at a unique position.

It follows that the number of candidates are
\[
\binom{46}{2} - \binom{3}{2} - \binom{3}{2} - \binom{5}{2} - \binom{2}{2} = 1018.
\]

The record has 35 double mutants in the set \( \hat{B} \) (see Table 1 for a list of the double mutants in the set \( \hat{B} \)). Consequently,
\[
\frac{|\hat{B}|}{|B_p|} = \frac{35}{1018} = 3.43\%.
\]

We summarize the results for the TEM record in the following observation.

**Observation 1.** For the TEM record,
1. the proportion of beneficial single mutations is 2.67%,
2. the ratio \[ \frac{|\hat{B}|}{|B_p|} = \frac{35}{1018} = 3.43\% \]. Consequently, 3.43% is an estimate of the qualitative measure of additivity \[ \frac{|B|}{|B_p|} \].

3. RECORDS OF MUTATIONS, ADDITIVE FITNESS AND UNCORRELATED FITNESS

Consider a record of drug resistance mutations. We first consider conditions ideal for our purposes. Then we discuss the consequences of relaxing some of the conditions.

3.1. **The perfect record conditions.** Assume that we have a well defined wild-type and several mutant variants associated with drug resistance. Assume that the records of drug resistance mutations satisfy the following conditions.

1. The organism adapts to a single environment. [single environment condition]
2. The record is complete with respect to single and double mutants in the sense that
   a. All single mutants which are more fit than the wild-type occur in the record,
   b. All double mutants which are more fit than both corresponding single mutants occur in the record. [completeness condition]
3. Every single and double mutant in the record is a result of adaption. In particular, the single mutants are the result of beneficial mutations. [Absence of neutral mutations condition]

**Remark 3.1.** Assume that a record satisfies the perfect record conditions, as described.
(i) If $|\hat{B}_{B_p}| < 1$, then the fitness landscape is not additive.

(ii) Suppose that there are $s_R$ single mutants in the record. If the fitness landscape is uncorrelated, then one expects $|\hat{B}_{B_p}|$ to equal $\frac{s_R}{6L}$ under the (simplified) assumption that a genotype has $6N$ mutational neighbors.

The first claim is obvious. Fitness being uncorrelated, approximately $\frac{s_R}{6L}$ of double mutants are more fit than the wild-type. If one restricts to the category of double mutants where both corresponding single mutants are more fit than the wild-type, the proportion is $\frac{s_R}{6L}$ as well. Fitness being uncorrelated, one third of the double mutants in this category are expected to be less fit than both single mutants. Indeed, there are three possible fitness ranks, and the double mutant is as likely to have the lowest fitness as any other rank. The resulting proportion is

$$\frac{2}{3} \cdot \frac{s_R}{6L} = \frac{s_R}{9L},$$

which explains the second claim in the remark.

3.2. Relaxing the perfect record assumptions. The TEM family has adapted to different selective environments, since antibiotics have different effects, so that the single environment condition is not satisfied. First we relax the single environment condition for a record.

Multiple environments and additive landscapes. In contrast to the single environment case, even if the fitness landscape associated with each drug is additive the $|\hat{B}_{B_p}|$-value may be lower than 1. The reason is that if two different single mutants are adapted to different environment, then a combination of the two corresponding mutations may not be fit in any environment. As an illustration, consider the following examples with additive landscapes.

Example 3.2. Consider two different environments and 50 single resistance mutations, where 25 mutants are adapted to each one of two environments. Assume that the fitness landscapes associated with both environments are additive. Moreover, assume that two mutations that constitute adaptations to different environments never combine well, so that the corresponding double mutants do not occur in the record. Then

$$|\hat{B}_{B_p}| = \frac{25}{2} + \frac{25}{60} = \frac{10}{2} = 0.49.$$

Consider exactly the same situation with 50 single mutants but instead 10 different environments, where 5 single mutants are adapted to each different environment. Then

$$|\hat{B}_{B_p}| = \frac{10 \cdot \frac{5}{2}}{\binom{50}{2}} = 0.082.$$ We conclude that in the case of multiple environments the $|\hat{B}_{B_p}|$-value may be low even if each fitness landscape is additive.
The case described, where mutations which are beneficial in different environments never combine well is probably not realistic. However, it is clear that multiple environments may lead to a lower $\frac{|\hat{B}|}{|\hat{B}_p|}$-value.

Multiple environments and uncorrelated landscapes. Consider a situation with multiple environments where the fitness landscape associated with each environment is uncorrelated. For simplicity, we assume that there are not an excessive amount of different environments. By assumption, very few single and double mutants should be more fit than the wild-type in any particular single environment. Multiple environments imply more chance for a mutant to be fit in at least one environment. However, fitness being uncorrelated, that effect is exactly the same for single and double mutants.

Double mutants will be more fit than the wild-type in any of the different environments, so that the $\frac{\hat{B}}{\hat{B}_p}$-value will be very low also in the case of multiple environments. In other words, unless the $\frac{\hat{B}}{\hat{B}_p}$-value is very small, we can rule out that all landscapes are uncorrelated fitness landscapes. (Multiple environments may lead to more beneficial mutations. However, there is no difference between single and double mutants in that respect, so that the $\frac{\hat{B}}{\hat{B}_p}$-value should not be influenced.)

Incomplete records. Missing single mutants in the record will normally have little effect, since $\frac{|\hat{B}|}{|\hat{B}_p|}$ concerns only single mutants in the record and associated double mutants, by definition. However, missing double mutants will make the $\frac{\hat{B}}{\hat{B}_p}$-value smaller as compared to the result for a more Consequently, incompleteness may lead to and underestimate of $\frac{|\hat{B}|}{|\hat{B}_p|}$.

Neutral mutations. An abundance of neutral mutations will make the $\frac{|\hat{B}|}{|\hat{B}_p|}$-value difficult to interpret.

Remark 3.3. An abundance of neutral mutations make the record difficult to interpret. In the case of multiple environments or incomplete records, Observation 2 (i) holds, but not 2 (ii).

The TEM record represents a case of multiple environments, but probably not an excessive amount of completely different environments. We have good reason to believe that the record is fairly complete, and that there is not an abundance of neutral mutations in the record.

For the TEM-record $\frac{\hat{B}}{|\hat{B}_p|} = \frac{35}{1018} = 3.43\%$, from Observation 1, and

$$\frac{s_R}{9L} = \frac{46}{9 \cdot 287} = 1.78\%.$$ 

Observation 2.

(i) Under the perfect record assumptions, the TEM landscape is not compatible with additive or uncorrelated fitness landscapes.

(ii) The TEM record is not compatible with uncorrelated fitness landscapes under realistic assumptions for the TEM record.
(iii) The TEM record combined with knowledge of the context, suggest that fitness is not additive for the TEM family.

Part (iii) rests on the fact that there does not exist an excessive amount of completely different environments for the TEM family. It would be remarkable with \( \frac{B}{|B_p|} = 3\% \) if all the fitness landscapes associated with individual drugs were additive.

The TEM record has in total 46 double mutants, where 35 are included in the set \( \bar{B} \). We conclude the section with some remarks about the remaining double mutants (see Section 7 for a list of them, and Table 2 of the same section for a list of the double mutants in \( \bar{B} \)). For the double mutant TEM-164, none of the single substitutions correspond to single mutants in the record. For the other 9 double mutants, exactly one of the substitutions corresponds to a single mutant. The most likely reason for a double mutant in \( B_p \) not to be included in \( B \) is sign epistasis. Specifically, the single mutation not in the record is selected for only if the other single mutation has already occurred. In such a case, the sign of the effect (positive or negative) of the second mutation depends on background (the effect is negative for the wild-type and positive if the first mutation has occurred). Constraints for orders in which mutations accumulate are known from different contexts (see e.g. [Desper et al., 1999]; [Beerenwinkel et al., 2007a]), including HIV drug resistance.

4. RECORDS OF MUTATIONS, BLOCK MODELS AND POSITION GRAPHS

If fitness is neither additive nor uncorrelated, then one may consider more general models. We will discuss block model with focus on how single beneficial mutations combine. However, in this context one has to consider the structure for how beneficial mutations combine, not only the proportion of good combinations. For simplicity, we will discuss loci rather than amino acid substitutions. The position graph is intended to display the structure of the combinations.

**Definition 4.1.** For a record of mutations, each node of the position graph corresponds to a locus associated with a single mutant in the record. An edge between two nodes indicates that a double mutant occurs in the record, such that the two mutations correspond to the nodes.

Notice that the position graph reflects the sites but ignores the actual amino acid substitutions (such as if the substitution is glutamine or lysine, or if both of them occurs at the site). Single mutants with substitutions at the same site may of course differ in how well they combine with other mutation. One may want to look at more-fine scaled information and distinguish between different substitutions at the same site. However, for simplicity, we ignore this complication.

Figure 1 shows the position graph for the TEM family, except that nodes of degree zero are omitted.

**Remark 4.2.** The position graphs considers loci, but not the amino acid substitution. One may want to look at more-fine scaled information and distinguish between different substitutions at the same site.
Recall that the degree of a node is the number of edges to other nodes. The complement \( \overline{G} \) of a graph \( G \) is a graph on the same nodes, where a pair of nodes are connected by an edge exactly if the pair is not connected by an edge for \( G \). For a \textit{complete bipartite graph}, the nodes can be partitioned into two subsets, such that every pair of nodes from different subsets are connected by an edge, and there are no other edges.

The following observation is elementary by Remark 1.1.

**Remark 4.3.** Assume the block model (with at least two blocks). Let \( G \) denote the position graph. Consider \( G \) and the complement \( \overline{G} \). Under the perfect record assumptions, the nodes of \( G \) have degree one or more. Moreover, by Remark 1.1, the following statements hold modulo a few errors:

1. For the case of two blocks, \( G \) is a complete bipartite graph. It follows that \( \overline{G} \) is a disconnected graph with two components, both of which are complete.

2. In general, for \( l \) blocks, \( \overline{G} \) is a disconnected graph with \( l \) components, all of which are complete.

For the TEM record, the single mutants correspond to substitutions at exactly 37 positions. Exactly 21 nodes out of the 37 have degree zero. The position graph has 37 nodes and 25 edges.

Consider the block model (for at least two blocks). The following observations are potentially problematic for a block model.
(1) There is an abundance of nodes of degree zero (21 out of 37),
(2) the total number of edges is (only) 25 and there are 37 nodes.
(3) the maximal degree for a nodes is 8,
(4) $G$ has several triangles, in particular a triangle consisting of the three nodes of
highest degree out of all nodes of $G$.

Under the perfect record assumptions, the block model implies that the degree of each
node is at least one (provided that the nodes are distributed over at least two blocks).
For the case of two blocks, the number of edges is between 36 and $18 \times 19 = 342$,
where the minimum corresponds to the distribution 1 and 36 nodes per block, and the
maximum corresponds to the distribution of 18 and 19 nodes per block. In the first case,
one node should have degree 36. However, the maximal degree of nodes in the position
graph (Fig. 1) is 8. It is clear that the position graph has too few edges for similar block
lengths, and too low maximal degree for unequal block length.

For more than two blocks, even more edges are expected leading to similar problems.
Clearly the block model is not compatible with the data under the perfect record condi-
tions.

Consider the case of exactly two blocks in a more realistic situation. Then the position
graphs should have essentially no triangles by Remark [11] This is because at least two
of the nodes in a triangle are on the same block. (Moreover, for two blocks a reasonable
guess would be that the three nodes of highest degree (39, 69, 164) are on the same block
[the shorter one]. If so, it is unexpected with a triangle consisting of the three nodes.)

It remains to consider the single record condition and consider more than two blocks.
In that case, let us analyze the fact that there are relatively few edges. 21 out of 37 nodes
have degree zero, and in fact one can find a set of 31 nodes (including the 21 nodes) in
the position graph, such that no pairs in the set are neighbors. That implies that among 31
nodes one cannot find a single pair of nodes on different blocks, such that both of them
have high fitness in the same environment.

This is of course possible, especially taken into account that the record may be incom-
plete. However, from knowledge of the context, it does not seem plausible. The number
of completely different environments is limited.

**Observation 3.** From the TEM record and some knowledge of the context, the block
model is probably not a good fit for the TEM family.

5. A laboratory study of TEM alleles

We will compare the results from the TEM record with a laboratory study. The ad-
vantage with the laboratory study is that one can use drug specific information, which
takes care of the difficulties resulting from multiple environments.

The study [Coulart et al.] (2013) considered the antibiotics Ampicillin (AMP), Cef-
tazidime (CAZ) Cefpodoxime (CPD), Cefprozil (CRP), Cefotetan (CTT), Cefotaxime
(CTX), Cefepime (FEP) and Pipercillin/tazobactam penicillin/inhibitor (TZP). Fitness
ranks were determined for the 4 single mutants L21F, R164S, T265M, E240K, as well as
the 6 double mutants than can be obtained from them, for each of the 8 antibiotics.
In particular, for the drug Ceftazidime (CAZ), 4 single mutants were more fit than the wild-type, so that one can obtain 6 double mutants from the single mutants. Out of the 6 double mutants for Ceftazidime, 5 double mutants were more fit than at least one of the single mutants, and one double mutant (the combination of R164S and E240K) was more fit than both corresponding single mutants.

For the 9 antibiotics, we list the number of single mutants with higher fitness than the wild-typ. and below the $\frac{|B|}{|B_p|}$-value.

$$\begin{array}{cccccccc}
4, & 4, & 2, & 2, & 2, & 2, & 2, & 2
\end{array}$$

$$\begin{array}{cccccccc}
5, & 1, & 3, & 1, & 1, & 1, & 0, & 0
\end{array}$$

The mean values is 0.57. Obviously the data deviates considerably from additive fitness as well as uncorrelated fitness.

For a comparison, combinations of 5 beneficial mutations from an experimental *Escherichia coli* population were considered in [Khan (2011)]. Negative epistasis dominated, but sign epistasis was rare. Consider the 10 double mutants combining pairs of the 5 beneficial mutations. Every double mutant had higher fitness than at least one of its corresponding single mutant, so that $\frac{|B|}{|B_p|} = \frac{10}{10} = 1$.

As for the block model, with very few exceptions, the double mutants in $B$ should be more fit than both single mutants, or less fit than both corresponding single mutants, by Remark [1.1]. For the 9 drugs, one can form in total 19 double mutants. 5 of them are more fit than both corresponding single mutants, 6 of them are more fit than exactly one corresponding single mutants, and 8 of them are less fit than both corresponding single mutants. This observation indicates that the block model does not apply. Notice also that the most plausible block distribution of nodes differ from drug to drug (for some drugs node 21 and 265 should be on the same block, and for other drugs not).

**Observation 4** Neither additive fitness, uncorrelated fitness, nor the block model is compatible with data from the laboratory study [Goulart et al. (2013)].

6. DISCUSSION

We have compared expectations from additive, uncorrelated, and block models of fitness landscapes with empirical data. We argue that the TEM family of $\beta$-lactamases is not compatible with the three models. Under the simplified assumptions of a complete record and a single environment, we found that the TEM data was not compatible with anyone of the three models. Under more realistic assumptions for the TEM family, the data was not compatible with uncorrelated fitness. Similarly, using the record and some knowledge of the biological context, it seems plausible that neither the additive nor the block model is a good fit. Our conclusions for the three models were confirmed by a laboratory study of TEM alleles. We did not compared the TEM family and the NK model. However, the symmetry aspect (see Remark [1.4]) could be problematic.

The literature on additive, uncorrelated block and NK models literature is extensive. Some approaches in the field have been motivated by theoretical considerations, such
as relating epistasis to the number of peaks of a fitness landscape. The purpose of our study was not to debate the value of the classical models. We appreciate that toy models can be used for generating fruitful hypotheses, which can be tested empirically. As for additive and uncorrelated fitness, the extremes will always be of interest as a theoretical starting point.

However, the classical models have been used for interpretations of empirical data as well. A standard assumption for several topics in evolutionary biology and breeding is additive (or multiplicative) fitness, in particular for studies of fitness inheritance and sexual selection (e.g. [Kokko et al. 2003]). Statistical methods which are suitable for uncorrelated fitness landscapes have been used in empirical studies (see e.g. [Crona et al. 2013b] for a discussion), and the NK model is frequently used in empirical contexts. From this perspective, it is reasonable to discuss to what extent the classical models are realistic.

We have suggested elementary tools, including the qualitative measure of additivity and the position graph, for comparing models and data. Our approach demonstrates that one can determine properties of fitness landscapes from a record of mutations. The ideal setting is a single environment. It would be of interest to compare the qualitative measure of additivity with observed behavior for microbes, such as recombination strategies. The position graph can be used as a test of modularity.

We consider it an advantage that our approach does not depend on any structural assumptions of the underlying fitness landscapes. Any case of adaptation where one has a well-defined wild-type and some direct or indirect method for determining fitness ranks of genotypes works.

Qualitative information has its limitations. The ideal information for determining properties of fitness landscapes is of course direct fitness measurements. For obvious reasons such information is sometimes difficult, if even possible, to derive. Moreover, laboratory results do not always agree with clinical findings. Consequently, direct methods for interpretations of nature are of interest as a complement to experimental results.

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TABLE 1. Loci with more than one substitution

| locus | # of substitutions | alleles                      |
|-------|--------------------|------------------------------|
| 69:   | 3                  | TEM–33, TEM-34, TEM-40       |
| 164:  | 3                  | TEM–12, TEM-29, TEM-143      |
| 244:  | 5                  | TEM-30, TEM-31, TEM-51, TEM-54, TEM-79 |
| 275:  | 2                  | TEM-103, TEM-122             |

7. Statistics and tables

We use information from the record of the TEM family from the Lahey Clinic [http://www.lahey.org/Studies/temtable.asp](http://www.lahey.org/Studies/temtable.asp) as of April 2012 for this study. All mutants have been found clinically. The record is continuously updated with new mutants.

The following loci, in total 37, correspond to single mutations:

21, 28, 34, 39, 68, 69, 84, 92, 104, 115, 120, 124, 130, 145, 155, 157, 158, 163, 164, 176, 182, 184, 189, 204, 213, 218, 224, 230, 238, 240, 244, 265, 271, 275, 276, 280, 283

Exactly 4 loci, out of the 37 listed above, correspond to more than one substitutions (see Table 1).

7.1. Double mutants and the position graph. The total number of double mutants in the record is 45, where 35 combine single mutations from the record (see the table). The remaining 10 mutants are as follows: TEM-58, TEM-81, TEM-112, TEM-126, TEM-137, TEM-145, TEM-146, TEM-163, TEM-164, TEM-169.

Information about double mutants in the record are expressed in the position graph (see Fig. 1). Recall that for the position graph a node corresponds to a locus with a single mutation. An edge denotes that there exists at least one double mutant which combines the substitutions at the two loci. The position graph has 37 nodes and 24 edges. The following (21) nodes of the position graph have degree zero.

28, 34, 68, 92, 115, 120, 124, 145, 155, 157, 158, 163, 176, 189, 204, 213, 218, 230, 271, 280, 283

The degree of the remaining (16) nodes are listed by locus (locus:degree).

21 : 3, 39 : 6, 69 : 7, 84 : 1, 104 : 3, 130 : 1, 164 : 8, 182 : 3, 184 : 1, 238 : 4, 224 : 1, 240 : 3, 244 : 2, 265 : 3, 275 : 1, 276 : 1
Table 2. Single mutants of the record

|   |      |      |      |       |      |      |      |
|---|------|------|------|-------|------|------|------|
| 1 | TEM-2 | Q39K |      |       |      |      |      |
| 2 | TEM-12 | R164S |      |       |      |      |      |
| 3 | TEM-17 | E104K |      |       |      |      |      |
| 4 | TEM-19 | G238S |      |       |      |      |      |
| 5 | TEM-29 | R164H |      |       |      |      |      |
| 6 | TEM-30 | R244S |      |       |      |      |      |
| 7 | TEM-31 | R244C |      |       |      |      |      |
| 8 | TEM-33 | M69L  |      |       |      |      |      |
| 9 | TEM-34 | M69V  |      |       |      |      |      |
|10 | TEM-40 | M69I  |      |       |      |      |      |
|11 | TEM-51 | R244H |      |       |      |      |      |
|12 | TEM-54 | R244L |      |       |      |      |      |
|13 | TEM-55 | G218E |      |       |      |      |      |
|14 | TEM-57 | G92D  |      |       |      |      |      |
|15 | TEM-70 | R204Q |      |       |      |      |      |
|16 | TEM-76 | S130G |      |       |      |      |      |
|17 | TEM-79 | R244G |      |       |      |      |      |
|18 | TEM-84 | N276D |      |       |      |      |      |
|19 | TEM-90 | D115G |      |       |      |      |      |
|20 | TEM-95 | P145A |      |       |      |      |      |
|21 | TEM-96 | D163G |      |       |      |      |      |
|22 | TEM-103 | R275L |      |       |      |      |      |
|23 | TEM-104 | A280V |      |       |      |      |      |
|24 | TEM-105 | S124N |      |       |      |      |      |
|25 | TEM-117 | L21F  |      |       |      |      |      |
|26 | TEM-122 | R275Q |      |       |      |      |      |
|27 | TEM-127 | H158N |      |       |      |      |      |
|28 | TEM-128 | D157E |      |       |      |      |      |
|29 | TEM-135 | M182T |      |       |      |      |      |
|30 | TEM-141 | K34E  |      |       |      |      |      |
|31 | TEM-143 | R164C |      |       |      |      |      |
|32 | TEM-148 | T189K |      |       |      |      |      |
|33 | TEM-150 | E28D  |      |       |      |      |      |
|34 | TEM-156 | M155I |      |       |      |      |      |
|35 | TEM-166 | R120G |      |       |      |      |      |
|36 | TEM-168 | T265M |      |       |      |      |      |
|37 | TEM-170 | G283C |      |       |      |      |      |
|38 | TEM-171 | V84I  |      |       |      |      |      |
|39 | TEM-174 | A213V |      |       |      |      |      |
|40 | TEM-176 | A224V |      |       |      |      |      |
|41 | TEM-181 | A184V |      |       |      |      |      |
|42 | TEM-183 | F230L |      |       |      |      |      |
|43 | TEM-186 | D176N |      |       |      |      |      |
|44 | TEM-191 | E240K |      |       |      |      |      |
|45 | TEM-192 | M68I  |      |       |      |      |      |
|46 | TEM-198 | T271I |      |       |      |      |      |
**Table 3.** The double mutants of the record which combine single mutations of the record

|   |  TEM-6: |  TEM-7: |  TEM-10: |  TEM-11: |  TEM-13: |  TEM-15: |  TEM-18: |  TEM-20: |  TEM-26: |  TEM-28: |  TEM-32: |  TEM-35: |  TEM-36: |  TEM-37: |  TEM-38: |  TEM-42: |  TEM-45: |  TEM-53: |  TEM-59: |  TEM-65: |  TEM-71: |  TEM-77: |  TEM-82: |  TEM-106: |  TEM-110: |  TEM-115: |  TEM-116: |  TEM-118: |  TEM-120: |  TEM-144: |  TEM-147: |  TEM-154: |  TEM-160: |  TEM-165: |  TEM-189: |
|---|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1 | E104K  | Q39K   | R164S   | Q39K   | Q39K   | E104K  | Q39K   | M182T  | E104K  | R164H  | E104K  | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   | M69L   |
Ceftazidime
\[ w(L21F), w(R164S), w(E240K), w(T265M) > w(TEM-1) \]
\[ w(\{R164S, E240K\}) > w(R164S), w(E240K) \]
\[ w(\{L21F, R164S\}) > w(L21F) \]
\[ w(\{L21F, T265M\}) > w(T265M) \]
\[ w(\{R164S, T265M\}) > w(T265M) \]
\[ w(\{E240K, T265M\}) > w(T265M) \]

Cefotaxime
\[ w(L21F), w(R164S), w(E240K), w(T265M) > w(TEM-1) \]
\[ w(\{R164S, E240K\}) > w(R164S), w(E240K) \]
\[ w(\{L21F, R164S\}) > w(L21F) \]

Pipercillin/tazobactam penicillin/inhibitor:
\[ w(L21F), w(T265L) > w(TEM-1) \]
\[ w(\{L21F, T265M\}) > w(L21F), w(T265M) \]

Cefpodoxime:
\[ w(R164S), w(E240K) > w(TEM-1) \]
\[ w(\{R164S, E240K\}) > w(E240K), w(R164S) \]

Cefotetan:
\[ w(R164S), w(E240) > w(TEM-1) \]
\[ w(\{R164S, E240K\}) > w(E240K), w(E240) \]

Cefprozil:
\[ w(L21F), w(T265M) > w(TEM-1) \]
\[ w(\{L21F, T265M\}) > w(L21F) \]

Ampicillin:
\[ w(L21F), w(T265M) > w(TEM-1) \]
No double mutants to list.

Cefepime:
\[ w(L21F), w(R164S) > w(TEM-1) \]
No double mutants to list.

Amoxillin+Clavulanate:
\[ w(L21F), w(T265M) > w(TEM-1) \]
No double mutants to list.

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| Amino acids | Possible substitutions |
|-------------|------------------------|
| R: C, G, H, I, K, L, M, P, Q, S, T, W |
| S: A, C, F, G, I, L, N, P, T, Y, W |
| L: F, H, I, M, P, Q, R, S, V, W |
| I: F, K, L, M, N, R, S, T, V |
| G: A, C, D, E, R, S, V, W |
| T: A, I, K, M, N, P, R, S |
| Q: D, E, H, K, L, N, P, R |
| V: A, D, E, F, G, I, L, M |
| A: D, E, G, P, S, T, V |
| P: A, H, L, Q, R, S, T |
| D: A, E, G, H, N, V, Y |
| H: D, L, N, P, Q, R, Y |
| K: E, I, M, N, Q, R, T |
| N: D, H, I, K, S, T, Y |
| E: A, D, G, K, Q, V |
| F: C, L, I, S, V, Y |
| M: I, K, L, R, T, V |
| Y: C, D, E, F, H, N, S |
| C: F, G, R, S, W, Y |
| W: C, G, L, R, S |