Exploiting evolutionary non-commutativity to prevent the emergence of bacterial antibiotic resistance

Daniel Nichol[1,5], Peter Jeavons[1], Alexander G. Fletcher[2], Robert A. Bonomo[3], Philip K. Maini[2], Jerome L. Paul[4], Robert A. Gatenby[5], Alexander R.A. Anderson[5] & Jacob G. Scott[2,5]

[1] Department of Computer Science, University of Oxford, Oxford, UK

[2] Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, UK

[3] Department of Medicine, Louis Stokes Department of Veterans Affairs Hospital, Cleveland, OH, USA

[4] School of Computing Sciences and Informatics, University of Cincinnati, Cincinnati, OH, USA

[5] Department of Integrated Mathematical Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Correspondence:

Jacob G Scott, e-mail: jacob.g.scott@gmail.com and

Daniel Nichol, e-mail: dan.nichol@cs.ox.ac.uk

Abstract

The increasing rate of antibiotic resistance and slowing discovery of novel antibiotic treatments presents a growing threat to public health. Here, we develop a Markov Chain model of evolution in asexually reproducing populations which is an encoding of the Strong Selection Weak Mutation model of evolution on fitness landscapes. This model associates the global properties of the fitness landscape with the algebraic properties of the Markov Chain transition matrix and allows us to derive general results on the non-commutativity and irreversibility of natural selection as well as antibiotic cycling strategies. Utilizing this formalism, we analyse 15 empirical fitness landscapes of E. coli under selection by different beta-lactam antibiotics and demonstrate that the emergence of resistance to a given antibiotic can be both hindered and promoted by different sequences of drug application. Further, we derive optimal drug application sequences with which we can probabilistically 'steer' the population through genotype space to avoid the emergence of resistance. This suggests a new strategy in the war against antibiotic–therapy–resistant organisms: drug sequencing to shepherd evolution through genotype space to states from which resistance cannot emerge and by which to maximize the chance of successful therapy.

Background: The increasing rate of antibiotic resistance and slowing discovery of novel antibiotic treatments presents a growing threat to public health. Previous studies of bacterial evolutionary dynamics have shown that populations evolving on fitness landscapes follow predictable paths. In this article, we develop a general mathematical model of evolution and hypothesise that it can be used to understand, and avoid, the emergence of antibiotic resistance.

Methods and Findings: We develop a Markov Chain model of evolution in asexually reproducing populations which is an encoding of the Strong Selection Weak Mutation model of evolution on fitness landscapes. This model associates the global properties of the fitness landscape with the algebraic properties of the Markov Chain transition matrix and allows us to derive general results on the non-commutativity and irreversibility of natural selection as well as antibiotic cycling strategies. Utilizing this formalism, we analyse 15 empirical fitness landscapes of *E. coli* under selection by different β-lactam antibiotics and demonstrate that the emergence of resistance to a given antibiotic can be both hindered and promoted by different sequences of drug application. We show that resistance to a given antibiotic is promoted in 61.4%, 68.6% and 70.3% of possible orderings of single, pair or triple prior drug administrations, respectively. Further, we derive optimal drug application sequences with which we can probabilistically 'steer' the population through genotype space to avoid the emergence of resistance.

Conclusions: Our model provides generalisable results of interest to theorists studying evolution as well as providing specific, testable predictions for experimentalists to validate our methods. Further, these results suggest a new strategy in the war against antibiotic–therapy–resistant organisms: drug sequencing to shepherd an evolving population through genotype space to states from which resistance cannot emerge and from which we can maximize the likelihood of successful therapy using existing drugs. While our results are derived using a specific class of antibiotics, the method is generalisable to other situations, including the emergence of resistance to targeted therapy in cancer and how species could change secondary to changing climate or geographical movement.

1 Introduction

Resistance to antibiotic treatments within bacterial pathogens poses an increasing threat to public health, which coupled with the slowing discovery of novel antibiotics, could soon reach crisis point [Spellberg et al., 2013, French, 2010]. Novel classes of antibiotics discovered since 1987 are few in number [Silver, 2011] . Thus, it is becoming ever clearer that if we are to combat highly resistant bacterial infections, then we must find new ways to prevent resistance and new applications of existing antibiotics to these pathogens. Indeed, public health efforts have attempted to stem the emergence of resistance by reducing unnecessary prescription of antibiotics [Shlaes et al., 1997, Bartlett, 2011, Leuthner and Doern, 2013] and stopping the addition of sub-therapeutic antibiotics in livestock feed [Mathew et al., 2007]. However, such policies require global adoption to be truly effective [Moody et al., 2012], which they have not yet achieved, and is likely infeasible. Recently, there have been efforts to explore how existing antibiotics can be used in new ways to provide effective treatments for resistant pathogens, for example through combination therapy [Chait et al., 2007] or through cycling strategies [Goulart et al., 2013, Pena-Miller and Beardmore, 2010, Bergstrom et al., 2004] – long term hospital–scale treatment protocols that cycle which antibiotics are prescribed over timescales of weeks, months or years. However, our understanding of the mechanisms underlying these strategies remains limited. Indeed, from a mathematical model Pena-Miller and Beardmore [2010] predict that cycling strategies have the potential to perform both better and worse than mixed strategies and hence tools to find optimal strategies are needed.

In order to understand how to minimize the emergence of resistant pathogens, and to decide how best to treat them, we must first understand how their evolution is driven by the selective pressures of different antibiotic drugs — a fundamental problem of biology [de Visser and Krug, 2014]. In particular, if we understand which traits are likely to be selected for by which treatments, then we may be able to avoid selecting for those traits which confer resistance. Recent insights into the evolutionary process have yielded some actionable information. Specifically, Weinreich et al. [2005, 2006] showed that if the genome of a pathogen exhibits sign epistasis, where a given mutation is beneficial on some genetic backgrounds and deleterious on others, then there can exist inaccessible evolutionary trajectories. Further, Tan et al. [2011] studied the evolutionary trajectories of *Escherichia coli* under different antibiotics and found that adaptive mutations gained under one antibiotic are often irreversible when a second is applied. These irreversible trajectories can occur when resistance conferring mutations for one environment carry a cost in another which can be mitigated by other compensatory mutations [zur Wiesch et al., 2010, Tanaka and Valckenborgh, 2011]. These findings lead us to hypothesize that one antibiotic could be used to irreversibly steer the evolution of a population of pathogens to a genotype (or combination of genotypes) which

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35 is sensitive to a second antibiotic but also from which it is unlikely to acquire resistance to that

36 antibiotic. This hypothesis was partly verified by the work of Imamovic and Sommer [2013] who

37 demonstrated that evolving *E. coli* to become resistant to certain antibiotics can increase sensitiv-

38 ity to others. However, this work is limited in two ways. Firstly, in that the experiments do not

39 exhaustively consider all evolutionary trajectories but instead highlight only those that arose in a

40 small number of replicates and secondly, they do not consider how evolution will proceed once the

41 second drug is applied and whether resistance can then emerge.

42 In this paper we present a Markov Chain model of evolution which we use to derive general

43 results on the non-commutavity of evolution and requirements for cycling strategies. Then, using

44 previously measured landscapes for 15 β-lactam anti-biotics, we illustrate that selective pressures

45 are non-commutative, and that the emergence of resistance can be both hindered and promoted

46 by different orderings of these pressures. These findings suggest new treatment strategies which

47 use rational orderings of collections of drugs to shepherd evolution through genotype space to a

48 configuration which is sensitive to treatment, as in the work of Imamovic and Sommer [2013], but

49 also from which resistance cannot emerge.

Evolution on Fitness Landscapes

51 We begin with the concept of a fitness landscape introduced by Wright [1932] and used by Wein-

52 reich et al. [2005] and Tan et al. [2011] to study evolutionary trajectories in asexually reproducing

53 populations. We represent the genotype of an organism by a bit string of length N and model

54 mutation as the process of flipping a single bit within this string. This model of mutation only

55 accounts for point mutations and ignores the possibility of other biologically relevant mutations

56 such as gene insertions, gene deletions and large structural changes to the genotype. This gives a set

57 of 2^N possible genotypes in which individuals of a given genotype, say g, can give rise to mutated

58 offspring which take genotypes given by one of the N mutational neighbors of g — precisely those

59 genotypes g' for which the Hamming distance [Hamming, 1950], $\mathrm{Ham}(g, g')$, from g is 1. As such,

60 our genotype space can be represented by an undirected N-dimensional (hyper–)cube graph with

61 vertices in $\{0, 1\}^N$ representing genotypes and edges connecting mutational neighbors (Figure 1a).

62 We define a selective pressure on our graph that drives evolution, for example through an

63 environmental change or drug application, as a fitness function

$$f : \{0, 1\}^N \to \mathbb{R}^{\geq 0}. \tag{1}$$

64 This fitness function represents a genotype-phenotype map in the simplest sense — assigning to each

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65 genotype a single real-valued fitness. Gillespie [1983, 1984] showed that if the mutation rate u and
66 population size M of a population satisfy $Mu >> 1 >> Mu^2$, and if we assume that each mutation
67 is either beneficial or deleterious, then each beneficial mutation in the population will either reach
68 fixation or become extinct before a new mutation occurs. Further, selection will be sufficiently
69 strong that any deleterious mutation will become extinct with high probability and hence we may
70 assume that this always occurs. In the case that $Mu^2 \approx 1$ stochastic tunneling [Iwasa et al.,
71 2004] through double mutations can occur and we cannot ignore deleterious mutations. Assuming
72 $Mu >> 1 >> Mu^2$, then after each mutation the population will stabilize to consist entirely
73 of individuals with the same genotype and this genotype will be eventually replaced by a fitter
74 neighboring genotype whenever one exists. This observation gives rise to the Strong Selection Weak
75 Mutation (SSWM) model, which models a population as occupying a single vertex on a directed
76 graph on the set of 2^N possible genotypes, $\{0, 1\}^N$, in which there exists an edge from vertex a to a
77 neighboring vertex b if, and only if, $f(b) > f(a)$ (see Figure 1b and 1c). This population undergoes a
78 stochastic walk in which the population moves to an adjacent fitter genotype with some probability.
79 Several 'move rules' have been proposed which can be used to select an adjacent fitter neighbor
80 during this stochastic walk [Orr, 2005] and which of these move rules is most accurate depends on
81 the population size [de Visser and Krug, 2014]. Common move rules include selecting the fittest
82 neighbor [Kauffman and Levin, 1987, Fontana et al., 1993], selecting amongst fitter neighbors at
83 random [Macken and Perelson, 1989, Macken et al., 1991, Flyvbjerg and Lautrup, 1992] or selecting
84 fitter neighbors with probability proportional to the fitness increase conferred [Gillespie, 1983, 1984,
85 1991]. We encapsulate each of these variants of the SSWM model within our model.

A Markov Model of Evolution

87 The SSWM model of evolution reduces the evolutionary process to a random walk on a directed
88 graph and hence can be modeled by a Markov Chain [Grinstead and Snell, 1998]. For a fitness
89 function $f : \{0, 1\}^N \to \mathbb{R}^{\geq 0}$ we can define a transition matrix $P = [\mathbb{P}(i \to j)]_{i,j \in \{0,1\}^N}$ for a
90 time–homogeneous absorbing Markov Chain by setting, for $i \neq j$,

$$\mathbb{P}(i \to j) = \begin{cases} \dfrac{\left(f(j) - f(i)\right)^r}{\displaystyle\sum_{\substack{g \in \{0,1\}^N, \text{ Ham}(i,g)=1 \\ f(g)-f(i)>0}} \left(f(g) - f(i)\right)^r} & \text{if } f(j) > f(i) \text{ and Ham}(i,j) = 1 \\[3em] 0 & \text{otherwise} \end{cases}, \qquad (2)$$
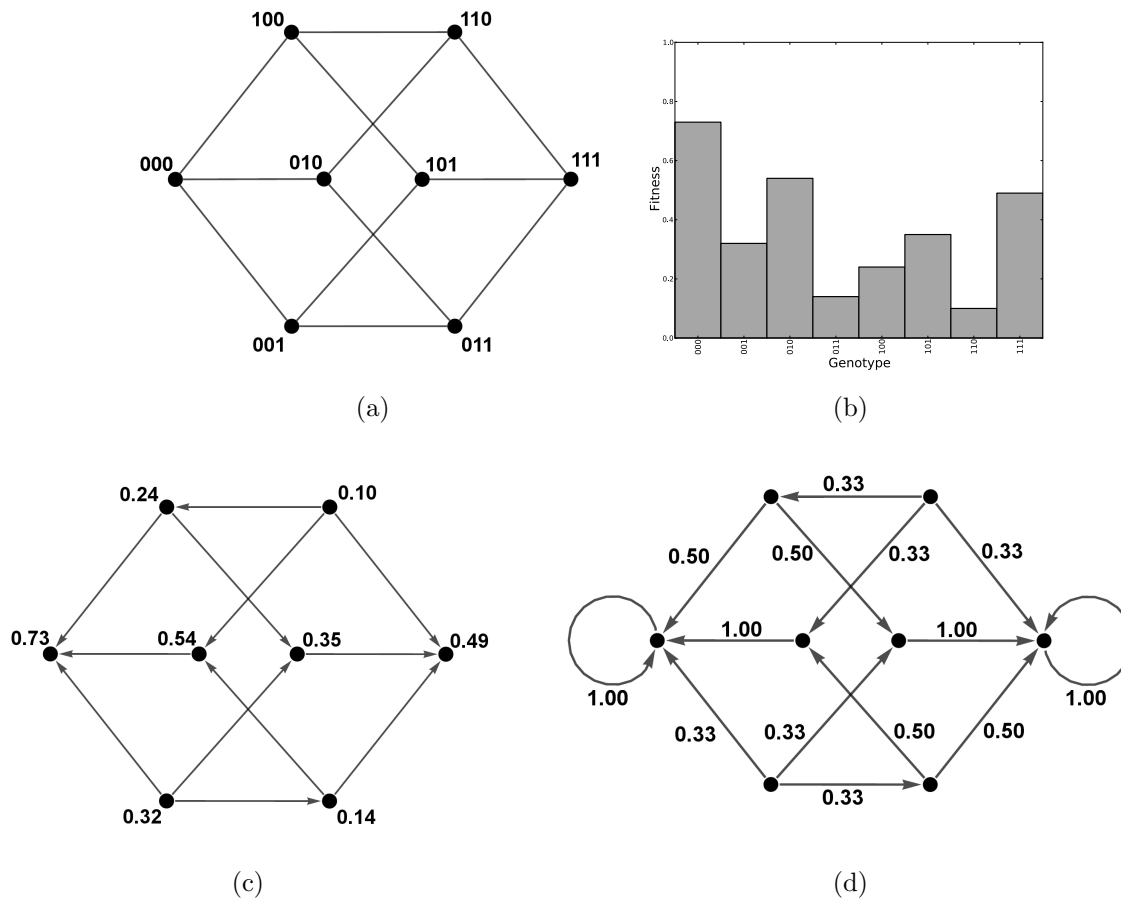
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Figure 1: (a) The space of genotypes comprising bit strings of length $N = 3$. The vertices represent genotypes, and edges connect those genotypes which are mutational neighbors. (b) An example fitness landscape. (c) The directed evolutionary graph according to the landscape in (b) where the vertices still represent genotypes but are labeled by the associated fitness for clarity. The directed graph edges are determined by the fitness function and represent those mutations which can fix in a population (those which confer a fitness increase). (d) The Markov Chain constructed for the same landscape according to equations (2) and (3) with $r = 0$.

and

$$\mathbb{P}(i \to i) = \begin{cases} 1 & \text{if } i \text{ has no fitter one-step mutational neighbors} \\ 0 & \text{otherwise} \end{cases}, \qquad (3)$$

for each i (see Figure 1d). Here the parameter $r \geq 0$ determines the extent to which the fitness increase of a mutation affects its likelihood of determining the next population genotype. In the case $r = 0$, we have the random move SSWM model (as in Macken and Perelson [1989], Macken et al. [1991], Flyvbjerg and Lautrup [1992]), in the limit $r \to \infty$ we have the steepest gradient ascent SSWM model (as in Kauffman and Levin [1987], Fontana et al. [1993]), and for $r = 1$ we have

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97 probability proportional to fitness increase (as in Gillespie [1983, 1984, 1991]). This model differs

98 from the Markov model used by Sella and Hirsh [2005] to study the neutral theory of evolution as

99 we do not allow deleterious mutations to fix in the population.

100 Using this Markov Chain we can explore the possible evolutionary trajectories of a population

101 on a given fitness landscape f. We next define a collection of population row vectors $\mu^{(t)}$ for each

102 $t \in \mathbb{N}$, where $\mu^{(t)}$ has length 2^N and k^{th} component which gives the probability that the population

103 has the kth genotype at time t (where the genotypes are ordered numerically according to their

104 binary value). These time steps t are an abstraction which discretely measure events of beneficial

105 mutations occurring and fixing in the population. As such, the actual time between steps t and $t+1$

106 is not constant but may be considered drawn from a distribution parameterized by the mutation

107 rate, reproductive rate and the number of beneficial mutations that can occur. This distribution

108 could, for example, be determined by a Moran [Moran et al., 1962] or Wright–Fisher [Wright, 1932,

109 Fisher, 1958] process depending on how we choose to interpret the fitness values given by f. If the

110 population has a genotype corresponding to a local optimum of the fitness landscape at time t then

111 there are no beneficial mutations that can occur and this definition of a time step is not well defined.

112 In this case there can be no more changes to the population under the SSWM assumptions and

113 for mathematical convenience we define the probability of a local optimum population genotype

114 remaining unchanged as one in equation 3 to ensure our model is a Markov Chain. In this case the

115 step t to $t + 1$ can be chosen to take some fixed arbitrary time.

116 The distribution of a population at time t is related to its initial distribution, $\mu^{(0)}$, by

$$\mu^{(t)} = \mu^{(0)} P^t. \tag{4}$$

117 Since the Markov Chain is absorbing we know that there exists some k such that $P^k P = P^k$ [Grin-

118 stead and Snell, 1998]. Consequently, we know that the matrix

$$P^* = \lim_{t \to \infty} P^t \tag{5}$$

119 exists and in fact this limit is reached after only finitely many matrix multiplications. Thus a given

120 initial population distribution $\mu^{(0)}$ will converge to a stationary distribution μ^* after a finite number

121 of steps in our model. Furthermore, if P^* is known then we compute the stationary distribution

122 μ^* as

$$\mu^* = \mu^{(0)} P^*. \tag{6}$$

123 In particular, provided we assume a drug is applied for sufficiently long to ensure that the disease

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124 population reaches evolutionary equilibrium, we can explore the effects of applying multiple drugs

125 sequentially by considering the matrices P^* for the associated fitness landscapes. In the following

126 discussion we make this assumption.

127 By encoding the evolutionary dynamics in a Markov Chain we can investigate the evolutionary

128 process from an algebraic perspective. In particular, as the transition matrix P encodes all of the

129 evolutionary dynamics of the associated fitness landscape f, we can explore global properties of f

130 by considering the algebraic properties of P. In the following section we present two simple, yet

131 powerful, consequences of this observation.

132 Non-Commutativity and Cycling of Natural Selection

133 We use the Markov Chain model to formally prove that for a large class of fitness landscape pairs,

134 there is non-commutativity in the evolutionary process as described by the SSWM assumptions.

135 More precisely, consider two drugs, X and Y, with corresponding fitness landscapes x and y. We

136 wish to determine what, if any, difference there is between applying X followed by Y to a population

137 as opposed to applying Y followed by X to that population. If we construct the transition matrices

138 P_x and P_y corresponding to x and y, respectively, and take the limits P_x^* and P_y^*, then our model

139 predicts that the ordering makes no difference to the final population distribution on an initial

140 population taking genotype i if, and only if,

$$\mu_i P_x^* P_y^* = \mu_i P_y^* P_x^*, \tag{7}$$

141 where μ_i is row vector of length 2^N whose i^{th} component is one and all of whose other components

142 are zero.

143 If we do not know the starting population genotype we can only guarantee that the order of

144 application is irrelevant when the outcome is the same regardless of the starting genotype. We

145 require for all possible length 2^N unit vectors μ_i that $\mu_i P_x^* P_y^* = \mu_i P_y^* P_x^*$. Since these unit vectors

146 form a basis of $\mathbb{R}^N$ this occurs precisely when

$$P_x^* P_y^* = P_y^* P_x^*. \tag{8}$$

147 Hence drug application will only commute when the corresponding limit matrices commute. In

148 practice we may be able to narrow down which genotypes are likely to constitute the population

149 through bacterial genotyping or by observing that certain strains are not viable in the wild due to

150 the high fitness cost of certain mutations. To determine how common commutativity is we first

151 tested each pair of fitness landscapes from the landscapes empirically determined by Mira et al.

152 [2014] for *E. coli* in the presence of $N = 4$ possible resistance conferring mutations under 15 different

153 antibiotics (listed in Table 1). Of these 15 antibiotics we found no commuting pairs. We then tested

154 10^6 pairs of random fitness landscapes with varying ruggedness generated according to Kauffman's

155 NK model for generating "tunably rugged" fitness landscapes [Kauffman and Levin, 1987, Kauffman

156 and Weinberger, 1989] using a random neighborhood Boolean function for determining the fitness

157 contributions of each locus. We fixed $N = 5$ and generated each landscape by first drawing K

158 uniformly from $\{0, \ldots, N - 1\}$ and then using Kauffman's model to build a landscape. We found

159 that 0.132% of the landscape pairs generated had limit matrices which commuted, suggesting that

160 commutativity is rare.

161   We now turn out attention to finding antibiotic cycling strategies as in Goulart et al. [2013].

162 Unless x is a flat landscape (taking equal values for all genotypes) there must exist a (not necessarily

163 unique) genotype j whose fitness is a minimum and which has a fitter neighbor. Such a genotype

164 satisfies $\mathbb{P}[(i \to j)] = 0$ for all genotypes i. Hence if x is not flat, the limit matrix P_x^* has at least one

165 column of all zeros and is singular, so there cannot exist a second landscape y for which $P_x^* P_y^* = I$.

166 Hence there exists a unit row vector μ_i for which $\mu_i P_x^* P_y^* \neq \mu_i$. As the μ vectors encode probability

167 distributions this means that natural selection in our model is irreversible in the sense that for a

168 given (non-flat) landscape we cannot find another which is guaranteed to reverse its effects. This

169 result precludes the existence of a general cycling strategy through which we can utilize a sequence

170 of drugs to drive evolution through a cycle in genotype space such that the disease population

171 returns to its original genotype, regardless of that starting genotype. If we do in fact know the

172 starting genotype, as we might if the disease is contracted in the wild where resistance conferring

173 mutations often carry a cost [Andersson and Hughes, 2010] making the wild–type genotype most

174 likely, then cycling strategies can be found efficiently by our model. In particular, for a given

175 starting genotype i the initial population distribution will be μ_i and a sequence of drugs $X_1, \ldots X_k$

176 with fitness landscapes $x_1, \ldots x_k$ will constitute a cycling strategy precisely when $\mu_i P_{x_1}^* \ldots P_{x_k}^* = \mu_i$.

177 This criterion will be satisfied when μ_i is a left 1–eigenvector of $P_{x_1}^* \ldots P_{x_k}^*$. As such, we can find

178 cycling strategies using matrix algebra and avoid the graph–search technique used by Goulart et al.

179 [2013].

Evolutionary Steering can both Prevent and Promote Resistance

181 Prescriptions of sequences of drugs occur frequently in the clinic, and often without any guidelines

182 as to which orderings are preferable. Common examples of this include, but are not limited to,
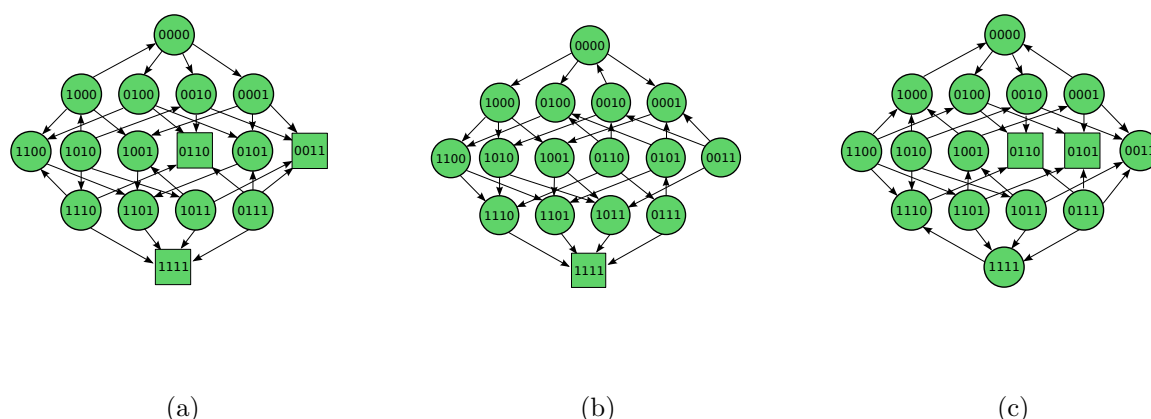
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Figure 2: The evolutionary graphs for the fitness landscapes of *E. coli* with the antibiotics (a) Ampicillin (Amp), (b) Ampicillin + Sulbactam (Sam) and (c) Cefprozil (Cpr) for 4 possible substitutions found in $bla_{\text{TEM-50}}$. Arrows represent fitness conferring mutations which can fix under the SSWM assumptions, the absence of an arrow in either direction corresponds to a neutral mutation which cannot fix under our assumptions. Squares denote local optima within the fitness landscape.

treatment of *H. pylori* [Gisbert et al., 2010], Hepatitis B [Hanazaki, 2004] and the ubiquitous change from broad to narrow spectrum antibiotics [Heenen et al., 2012]. The ordering of the sequence is therefore often determined arbitrarily, by the individual clinician's personal, or historical experience or from laboratory data. Ideally, we would like to be able to identify drug orderings that lower the probability of a highly resistant disease population emerging during the treatment. To consider optimal drug orderings in the context of our model we first need to know the fitness landscapes (or proxies of the fitness landscapes) of a number of antibiotics used to treat a given bacterial infection. Experimentally determining these landscapes requires one to consider all possible 2^N combinations of genotypes in a set of N genes, a task which is prohibitively difficult for all but small values of N. De Visser and Krug [2014] found that there have been less than 20 systematic empirical studies of fitness landscapes and that landscapes have been calculated for a number of model organisms including *E. coli* [Khan et al., 2011, Tan et al., 2011, Schenk et al., 2013, Goulart et al., 2013], *Saccharomyces cerevisiae* [Hall et al., 2010], *Plasmodium falciparum* [Lozovsky et al., 2009] and type 1 Human Immunodeficiency Virus [da Silva et al., 2010]. Recent work by Hinkley et al. [2011] which utilizes regression methods to approximate large fitness landscapes from samples of the space could help ameliorate the complexity of experimentally determining fitness landscapes.

Mira et al. [2014] investigated the fitness landscapes of *E. coli* under 15 different β-lactam antibiotics using the mean minimum inhibitory concentration (MIC) of drug as a proxy for fitness for a total of $N = 4$ resistance conferring mutations. Figure 2 shows the evolutionary graphs
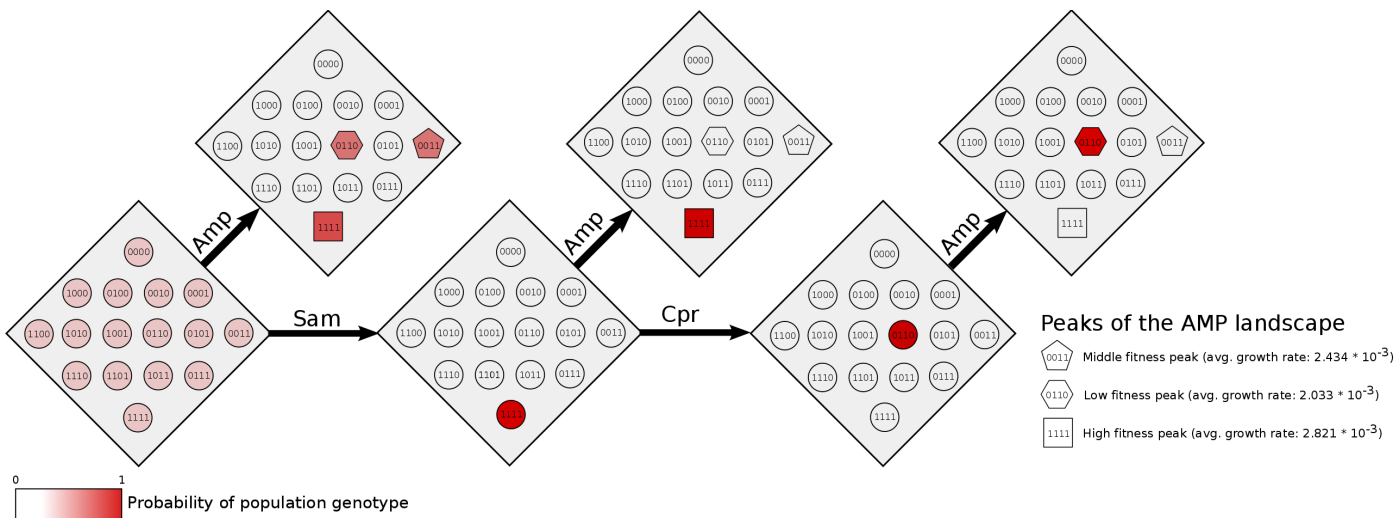
Figure 3: The probability distributions for accessibility of the peaks of the Amp landscape for different steering regimes. The starting distribution is $\mu = [1/2^N, \ldots, 1/2^N]$. When Amp is given first any of the three peaks of the landscape are accessible with most resistant genotype 1111 being most likely. If Sam is given first to steer the population to its sole peak 1111, then resistance to Amp will be guaranteed when it is applied. Alternatively, if Sam is given followed by Cpr then the population evolves to the local optima genotype 0110 of the Cpr landscape. If Amp is applied to this primed population the global optimum is inaccessible.

202 of the fitness landscapes for three of these antibiotics, Ampicillin (Amp), Ampicillin+Sulbactam
203 (Sam) and Cefprozil (Cpr). We will use these three fitness landscapes to demonstrate the steering
204 hypothesis explicitly. In the case of a single peaked landscape, such as that for Ampicillin +
205 Sulbactam, we cannot reduce the likelihood of resistance as all evolutionary trajectories lead to the
206 global fitness optimum. It is only when a drug has a multi–peaked landscape that we may be able
207 to avoid resistance through careful choice of preceding drugs. Of the 15 landscapes determined
208 empirically by Mira et al. [2014] only the landscape for Ampicillin+Sulbactam is single peaked. In
209 their review of empirical fitness landscapes de Visser and Krug [2014] find that biological landscapes
210 show a variable but substantial level of ruggedness suggesting that multi–peaked landscapes could be
211 quite common. Poelwijk et al. [2007, 2011] showed that reciprocal sign epistasis within a landscape
212 is necessary for the landscape to be multi–peaked.

213 In the following we take $r = 0$ in equation (2) and note that changing the value of r will not
214 change the accessibility of an evolutionary trajectory, hence by taking a different value of $r \geq 0$
215 we will only change the result quantitatively (the probabilities may change) but not qualitatively.
216 We begin by supposing that we do not know the initial population genotype. We can model this
217 situation by taking as our prior population distribution $\mu = [1/2^N, \ldots, 1/2^N]$ which specifies that

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218 each genotype is equally likely to constitute the starting population.

219 If we apply the drug Amp to this population distribution we find that in the expected distribu-
220 tion $\mu^* = \mu P^*_{Amp}$ (shown in the first diamond in the top row of Figure 3) each of the fitness peaks
221 could be found. In particular, the most highly resistant genotype 1111 can arise in the population
222 with probability 0.62. Suppose instead we apply Sam first. In this case as the landscape is single
223 peaked the population will converge to the global optimum genotype 1111. This genotype is also
224 the global optimum of the Amp landscape and hence if we apply Amp after Sam we will encounter
225 high resistance. We have steered the population with one drug to a configuration which increases
226 the probability of resistance to a second. Next suppose instead we apply Cpr after Sam, in this
227 case the population is guaranteed to evolve to a local optimum 0110 of the Cpr landscape. 0110
228 is the least fit local optimum of the Amp landscape. Thus if we apply Amp to the population
229 primed by Sam and Cpr then evolution to the global optimum 1111 is not possible. This example
230 demonstrates the steering hypothesis, that evolution can be shepherded through careful orderings
231 of multiple drugs to increase or decrease the likelihood of resistance emerging.

232 To test our steering hypothesis we performed for each of the 15 drugs with landscapes derived
233 by Mira et al. [2014] an *in silico* test of steering using combinations of one, two or three preceding
234 drugs. Table 1 shows for each of the 15 antibiotics the combinations which reduce the probability
235 of evolution to a peak fitness genotype to the lowest possible when applied in order followed by
236 the final drug. We found that for 3 of the 15 drugs there exists another which steers an initial
237 population μ to a configuration which prevents evolution to the global fitness optimum of the
238 landscape entirely. This number rose to 6 when pairs of drugs applied sequentially are used to steer
239 the population and to 7 when triples applied in sequence were considered.

240 We then performed a second *in silico* experiment to find combinations of steering antibiotics
241 that maximize the probability that evolution proceeds to the least fit of the local optima of a final
242 antibiotic. Table 3 show the results of this experiment. We found that, excluding the single peaked
243 landscape for Ampicillin with Sulbactam, there exist 0 drugs for which a single other drug is able
244 to steer the population to a configuration from which evolution to only the least fit optimum is
245 possible. If pairs of drugs are used to steer there are 3 such drugs (including the example presented
246 in the above demonstration) and if triples of steering drugs are considered there remains only 3.
247 These findings suggest that through careful choice of steering drugs we may be able to prevent the
248 emergence of resistance. During these experiments we found that 14/15 of the antibiotics in our
249 experiment (Cefpodoxime (CPD) being excluded) appeared in an optimal steering combination of
250 some length.

251 Whilst careful selection of drugs for steering can prevent the emergence of resistance, arbitrary

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drug orderings can also promote it. We performed an exhaustive *in silico* search of all singles, pairs of, and triples of steering drugs applied sequentially to prime the initial population μ for a final application of each of the 15 antibiotics. We found that steering with a single drug increased the likelihood of the most resistant genotype emerging in 57.3% of cases and decreased the likelihood in 29.8% of cases. Steering with pairs of drugs increased the likelihood in 64.1% of cases and decreased it in 28.4% of cases and steering with triples increased the likelihood in 65.6% of cases and decreased it in 27.5%. For each of the antibiotics except Cefaclor, Cefprozil and Ampicillin+Sulbactam (which is single peaked making steering irrelevant) we found that a random steering combination of length one, two or three is more likely to increase the chances of resistance than to reduce it. Indeed, for Piperacillin+Tazobactam and Ceftizoxime we found that a random steering combination will increase the probability of the most highly resistance genotype occurring in more than 80% of cases, suggesting that sequential multidrug treatments which use these antibiotics should proceed with caution. These findings suggest that the present system of determining sequential drug orderings without quantitative optimisation based guidelines could in fact be promoting drug resistance and that to avoid resistance we must carefully consider the order in which drugs are applied.

Discussion

We have encoded Strong Selection Weak Mutation evolutionary dynamics on fitness landscapes as a Markov Chain. Through this encoding we can explore the dynamics of evolution by considering the algebraic properties of the associated Markov transition matrix. In particular, we have demonstrated that evolution on fitness landscapes is non-commutative through parallels with the non-commutativity of matrix multiplication and that antibiotic cycling strategies can be determined through matrix multiplication. We argue then that the ordering in which a collection of drugs is applied can significantly impact the population that exists after the application is complete.

We have shown that we can find sequences of drugs that can be applied to both avoid and promote the emergence of resistance in the population. In light of the slow pace of novel antibiotic discovery and the rapid emergence of resistance to the presently most utilized antibiotics, this finding suggests a new treatment strategy — one in which we use a sequence of drugs (or even treatment breaks which themselves impose a selective pressure [Andersson and Hughes, 2010]) to steer, in an evolutionary sense, the disease population to avoid resistance from developing. Further, the drugs used to prime the disease population for treatment by an effective antibiotic do not themselves need to be the most effective drugs available. This means that there could be a large pool of potential steering drugs in the form of antibiotics which have gone unused for many years

Final drug	# of Peaks	Fittest peak genotype	Probability of ending at highest fitness genotype (no steering)	Best single steering drug	Probability of ending at highest fitness genotype (with best single steering drug)	Best ordered pair of steering drugs	Probability of ending at highest fitness genotype (with best steering pair)	Best ordered triple of steering drugs	Probability of ending at highest fitness genotype (with best steering triple)
Ampicillin (AMP)	3	1111	0.62	CPR	0.25	AMP→CPR	0.0	-	-
Amoxicillin (AMX)	2	1101	0.75	CTX	0.62	SAM→CPR	0.51	CRO→SAM→CPR	0.51
Cefaclor (CEC)	3	0011	0.19	SAM	0.0	-	-	-	-
Cefotaxime (CTX)	4	1111	0.17	CPR	0.04	AMP→CPR	0.0	-	-
Ceftizoxime (ZOX)	2	0111	0.86	AMX	0.81	SAM→AMX	0.75	CEC→CPR→AMC	0.75
Cefuroxime (CXM)	2	0111	0.56	AMC	0.33	CXM→AMC	0.19	AMC→CXM→AMC	0.11
Ceftriaxone (CRO)	4	1111	0.28	CEC	0.20	TZP→CEC	0.05	AMC→TZP→CEC	0.0
Amoxicillin +Clav (AMC)	2	1101	0.67	CXM	0.39	AMC→CXM	0.23	CXM→AMC→CXM	0.13
Ceftazidime (CAZ)	3	0110	0.39	TZP	0.08	AMC→TZP	0.0	-	-
Cefotetan (CTT)	5	0111	0.18	AMC	0.0	-	-	-	-
Ampicillin +Sulbactam (SAM)	1	1111	1.0	⋆	1.0	⋆	1.0	⋆	1.0
Cefprozil (CPR)	3	0101	0.25	AMP	0.0	-	-	-	-
Cefpodoxime (CPD)	2	1111	0.81	CRO	0.74	CTX→FEP	0.57	SAM→CEC→CRO	0.50
Pipercillin +Tazobactam (TZP)	2	0101	0.80	CTT	0.72	CTT→CTT	0.72	CTX→FEP→CTT	0.68
Cefepime (FEP)	4	1111	0.48	CTX	0.39	CAZ→CEC	0.36	CAZ→CEC→CTX	0.33

Table 1: For each of the 15 antibiotics we have derived the (ordered) sets of one, two and three steering drugs which reduce the probability of evolution to the maximal fitness genotype to the lowest possible. In the case that an ordered set of steering drugs reduced the probability to 0 we have not considered ordered sets of greater length (marked as - in the table). * – as the landscape for SAM is single peaked there can be no combination of steering drugs which reduce the probability of finding the global optimum. In all experiments the initial population distribution is taken as $\mu = [1/2^N, \ldots, 1/2^N]$ and $r = 0$

Final drug	# of single steering drugs better (*/15)	# of single steering drugs worse (*/15)	# of steering pairs better(*/225)	# of steering pairs worse (*/225)	# of steering triples better (*/3375)	# of steering pairs worse (*/3375)
Ampicillin (AMP)	7 (46.7%)	7 (46.7%)	97 (43.1%)	126 (56.0%)	1373 (40.7%)	1998 (59.2%)
Amoxicillin (AMX)	5 (33.3%)	9 (60.0%)	60 (26.7%)	164 (72.9%)	787 (23.3%)	2578 (76.4%)
Cefaclor (CEC)	9 (60.0%)	5 (33.3%)	132 (58.7%)	88 (39.1%)	2087 (61.8%)	1264 (37.5%)
Cefotaxime (CTX)	4 (26.7%)	10 (66.7%)	67 (29.8%)	155 (68.9%)	989 (29.3%)	2335 (69.2%)
Ceftizoxime (ZOX)	2 (13.3%)	12 (80.0 %)	31 (13.8%)	193 (85.8%)	444 (13.2%)	2930 (86.8%)
Cefuroxime (CXM)	5 (33.3%)	9 (60.0%)	95 (42.2%)	128 (56.9%)	1486 (43.5%)	1885 (55.9%)
Ceftriaxone (CRO)	5 (33.3%)	9 (60.0%)	61 (27.1%)	163 (72.4%)	810 (24.0%)	2564 (76.0%)
Amoxicillin +Clav (AMC)	7 (46.7%)	7 (46.7%)	99 (44.0%)	124 (55.1%)	1428 (42.3%)	1943 (57.6%)
Ceftazidime (CAZ)	4 (26.7%)	10 (66.7%)	76 (33.8%)	147 (65.3%)	1218 (36.1%)	2153 (63.8%)
Cefotetan (CTT)	4 (26.7%)	10 (66.7%)	58 (25.8%)	166 (73.8%)	843 (25.0%)	2531 (75.0%)
Ampicillin +Sulbactam (SAM)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cefprozil (CPR)	7 (46.7%)	7 (46.7%)	113 (50.2%)	110 (48.9%)	1703 (50.5%)	1668 (49.4%)
Cefpodoxime (CPD)	3 (20.0%)	11 (73.3%)	26 (11.6%)	195 (86.7%)	282 (8.4%)	3077(91.2%)
Pipercillin +Tazobactam (TZP)	2 (13.3%)	12 (80.0%)	11 (5.9%)	213 (94.7%)	81 (2.4%)	3293 (97.6%)
Cefepime (FEP)	3 (20.0%)	11 (73.3%)	34 (15.1%)	190 (84.4%)	402 (11.9%)	2972 (88.1%)
Overall	67 (29.8%)	129 (57.3%)	960 (28.4%)	2162 (64.1%)	13933 (27.5%)	33200 (65.6%)
Overall (Without steering for SAM)	67 (31.9%)	129 (61.4%)	960 (30.5%)	2162 (68.6%)	13933 (29.5%)	33200 (70.3%)

Table 2: For each of the 15 antibiotics we determined how many of the possible single, ordered double and ordered triple combinations of steering drugs (allowing drugs to appear multiple times in the combination and also allowing the target drug to appear as a steering drug) improved or worsened the probability of the most resistant genotype being found after the steering drugs are applied in order followed by the final target drug. In each case the initial population was given by $\mu = [1/2^N, \ldots, 1/2^N]$ and $r = 0$. As the SAM landscape is single peaked no combination of steering drugs will improve or worsen the outcome. As such, we have computed the overall numbers both with and without the contribution of the SAM row.

284 due to their inefficacy.

285 However, a major difficulty in using sequential drug treatments to steer disease populations is
286 that in order to predict the outcomes we must know the fitness landscapes of the drugs involved. De
287 Visser and Krug [2014] state that there exist less than 20 systematic studies of fitness landscapes and
288 that these studies consider between 3 and 9 possible mutations. For steering to be fully effective we
289 must account for all likely fitness conferring mutations and their effects on fitness under many drugs.
290 Thus, many of the studies reviewed by de Visser and Krug are insufficient for determining clinically
291 actionable steering strategies for certain diseases. Fortunately, for a number of highly resistant
292 infectious diseases [Woodford and Ellington, 2007, Jensen and Lyon, 2009] and cancers [Lord and
293 Ashworth, 2013, Lito et al., 2013] there are only a small number of mutations which seem to
294 contribute to resistance. Further, recent work by Hinkley et al. [2011] in HIV has introduced a
295 method to approximate large fitness landscapes from relatively fewer data points using a regression
296 method. It follows that determining the landscapes is not an entirely intractable problem. A further
297 complication in determining steering strategies is that fitness landscapes can be dependent on the
298 disease microenvironment and have the potential to change from patient to patient or throughout
299 the course of treatment. The consequences of such effects on fitness landscapes have not yet been
300 experimentally determined.

301 Even in the absence of actual fitness landscapes our findings should be taken as a cautionary
302 warning for multi-drug treatments, particularly those used to treat complex diseases such as mul-
303 tiple infections or cancers. In the same way that the drug ordering can be used to steer away from
304 resistance we have shown it can also be used to make resistance more likely. Our results show that
305 we may be inadvertently selecting for highly resistant disease populations through arbitrary drug
306 ordering in the same way that highly resistant disease can emerge through irresponsible drug dosing.
307 This result corroborates the findings of Pena-Miller and Beardmore [2010] that antibiotic cycling
308 strategies can vary greatly in their efficacy and be both worse and better than mixing strategies.
309 If we are to avoid resistance to our most effective drugs we must carefully consider how they are
310 used together, both in combination and in sequence, with other drugs and take appropriate steps
311 to reduce the risk.

312 Two major assumptions within our modeling are that drugs are administered for sufficiently
313 long that evolution can converge to a local fitness optimum and that this convergence is guaranteed
314 to occur. This assumption poses two potential problems in converting our model predictions to
315 predictions of real–world bacterial evolution. The first is that if selection is strong and mutations
316 are rare then there is a possibility of the population being driven to extinction before an adaptive
317 mutation occurs. We have chosen to ignore this possibility within our modeling as in the context

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318 of treating bacterial infections this would constitute a success. The second is that the time to
319 convergence could be prohibitively long for steering to constitute a realistic treatment strategy.
320 We believe that the assumption of reasonable convergence times could be valid as adaptive walks
321 in rugged landscapes are often short [Orr, 2005]. However, it has been shown that for certain
322 landscapes there can exist adaptive walks of length exponential in the number of loci [Kaznatcheev,
323 2013], but since we get to choose those drugs with which to steer we can avoid landscapes for which
324 the convergence time is prohibitively long. Further, our model is not necessarily restricted to the
325 dynamics within a single patient. Goulart et al. [2013] used fitness landscapes to explore whole
326 hospital scale antibiotic treatment strategies and our model, as an encoding of evolution on fitness
327 landscapes, is capable of making predictions at this scale also. As such, even if evolutionary
328 convergence is experimentally determined to be prohibitively slow for steering to be effective as
329 a treatment for bacterial infection within a single patient, our results will still hold in scenarios
330 which admit longer timescales. Specifically, evolutionary steering could provide an effective means
331 to avoid the emergence of drug resistance at the hospital scale or in life–long diseases such as HIV.

332   The Strong Selection Weak Mutation model we have used here is a highly simplified, yet well
333 studied model of evolution. The model ignores much of the complexity of the evolutionary process,
334 specifically simplifying the genotype–phenotype map, ignoring the disease microenvironment and
335 making the assumption of a monomorphic disease population in which deleterious and neutral mu-
336 tations cannot fix. Under certain regimes of population size and mutation rates these simplifying
337 assumptions break down. For example, if the population is sufficiently large then stochastic tun-
338 neling [Iwasa et al., 2004] – the situation where double mutations can occur allowing the crossing
339 of fitness valleys – can arise causing a breakdown of the Strong Selection assumption. Similarly,
340 if the mutation rate is sufficiently high then the population ceases to be monomorphic and forms
341 a quasispecies [Nowak, 1992, Bull et al., 2005]. Conversely, if the population is sufficiently small
342 then it becomes possible for deleterious mutations to fix [Moran et al., 1962, Wright, 1932, Fisher,
343 1958]. Finally, we have ignored the possibility of neutral spaces in the fitness landscape which have
344 been shown to have significant impact on whether non-optimal genotypes can fix in the population
345 as well as the time taken for evolution to find a locally optimal genotype [Schaper and Louis, 2014,
346 Schaper et al., 2012]. We believe that each of these breakdowns of the SSWM model will have
347 important consequences for the possibility and efficacy of steering and hence a proper treatment
348 of their implications is beyond the scope of this paper. In our future work we aim to undertake
349 a comprehensive study of the implications of population size, mutation rate, neutral drift and
350 evolutionary convergence times on the steering hypothesis.

351   The SSWM model is a simplification of the evolutionary process and given that non-commutativity

is present in this highly simplified model it is unlikely that commutativity will emerge as more complexity is introduced. It follows that the cautionary message regarding sequential drug application which results from our simplified model merits serious consideration. Whether or not measuring fitness landscapes provides sufficient information to correctly identify optimal drug orderings *in vivo* is a question that cannot be answered through mathematical modeling alone. It is only by verifying the predictions of steering strategies given by our model through biological experiment that we can determine whether they are viable. Supposing our model predictions are indeed viable then knowledge of some approximation to the fitness landscapes of the presently most used antibiotics could, in combination with our model, provide at least a good heuristic for how to proceed with multi-drug treatments, future antibiotic stewardship programs and clinical trial design.

Acknowledgment and Supplementary Information

Final drug	# of Peaks	Least fit peak genotype	Probability of ending at lowest fitness genotype (no steering)	Best single steering drug	Probability of ending at lowest fitness genotype (with best single steering drug)	Best ordered pair of steering drugs	Probability of ending at lowest fitness genotype (with best steering pair)	Best ordered triple of steering drugs	Probability of ending at lowest fitness genotype (with best steering triple)
Ampicillin (AMP)	3	0110	0.19	CPR	0.51	SAM→CPR	1.0	-	-
Amoxicillin (AMX)	2	0010	0.25	CTX	0.38	SAM→CPR	0.49	AMP→SAM→CPR	0.49
Cefaclor (CEC)	3	0100	0.41	TZP	0.70	CXM→AMC	0.78	AMC→CXM→AMC	0.87
Cefotaxime (CTX)	4	1010	0.21	FEP	0.27	CTX→FEP	0.43	SAM→CEC→CRO	0.50
Ceftizoxime (ZOX)	2	1001	0.14	AMX	0.19	SAM→AMX	0.25	AMP→SAM→AMX	0.25
Cefuroxime (CXM)	2	0100	0.44	AMC	0.67	CXM→AMC	0.81	AMC→CXM→AMC	0.89
Ceftriaxone (CRO)	4	0100	0.30	CXM	0.59	AMC→CXM	0.76	CXM→AMC→CXM	0.86
Amoxicillin +Clav (AMC)	2	0100	0.33	CXM	0.61	AMC→CXM	0.77	CXM→AMC→CXM	0.87
Ceftazidime (CAZ)	3	0011	0.26	FEP	0.28	CAZ→CEC	0.40	CAZ→AMX→FEP	0.45
Cefotetan (CTT)	5	1101	0.19	AMX	0.75	SAM→AMX	1.0	-	-
Ampicillin +Sulbactam (SAM)	1	1111	1.0	⋆	1.0	⋆	1.0	⋆	1.0
Cefprozil (CPR)	3	0011	0.25	ZOX	0.26	CAZ→CEC	0.34	CAZ→CEC→FEP	0.43
Cefpodoxime (CPD)	2	1010	0.19	CRO	0.26	CTX→FEP	0.43	SAM→CEC→CRO	0.50
Pipercillin +Tazobactam (TZP)	2	1000	0.20	CTT	0.28	CTT→CTT	0.28	CTX→FEP→CTT	0.32
Cefepime (FEP)	4	0000	0.14	FEP	0.14	TZP→CEC	0.17	CXM→AMC→CTX	0.20

Table 3: For each of the 15 antibiotics we have derived the (ordered) sets of one, two and three steering drugs which increase the probability of evolution to the least fit optimum genotype to the highest possible. In the case that an ordered set of steering drugs increases the probability to 1 we have not considered ordered sets of greater length (marked as - in the table). ⋆ – as the landscape for SAM is single peaked there can be no combination of steering drugs which change the probability of finding the global optimum. In all experiments the initial population distribution is taken as $\mu = [1/2^N, \ldots, 1/2^N]$ and $r = 0$

Bibliography

Brad Spellberg, John G Bartlett, and David N Gilbert. The future of antibiotics and resistance. *New England Journal of Medicine*, 368(4):299–302, 2013.

Gary L French. The continuing crisis in antibiotic resistance. *International Journal of Antimicrobial Agents*, 36:S3–S7, 2010.

Lynn L Silver. Challenges of antibacterial discovery. *Clinical Microbiology Reviews*, 24(1):71–109, 2011.

David M Shlaes, Dale N Gerding, Joseph F John, William A Craig, Donald L Bornstein, Robert A Duncan, Mark R Eckman, William E Farrer, William H Greene, Victor Lorian, et al. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the Prevention of Antimicrobial Resistance in Hospitals. *Clinical Infectious Diseases*, 25(3):584–599, 1997.

John G Bartlett. A call to arms: the imperative for antimicrobial stewardship. *Clinical Infectious Diseases*, 53(suppl 1):S4–S7, 2011.

Kimberly D Leuthner and Gary V Doern. Antimicrobial stewardship programs. *Journal of Clinical Microbiology*, 51(12):3916–3920, 2013.

Alan G Mathew, Robin Cissell, and S Liamthong. Antibiotic resistance in bacteria associated with food animals: a united states perspective of livestock production. *Foodborne Pathogens and Disease*, 4(2):115–133, 2007.

Julia Moody, Sara E Cosgrove, Russell Olmsted, Edward Septimus, Kathy Aureden, Shannon Oriola, Gita Wasan Patel, and Kavita K Trivedi. Antimicrobial stewardship: a collaborative partnership between infection preventionists and healthcare epidemiologists. *Infection Control and Hospital Epidemiology*, 33(4):328–330, 2012.

396 Remy Chait, Allison Craney, and Roy Kishony. Antibiotic interactions that select against resistance.
397   *Nature*, 446(7136):668–671, 2007.

398 Christiane P Goulart, Mentar Mahmudi, Kristina A Crona, Stephen D Jacobs, Marcelo Kallmann,
399   Barry G Hall, Devin C Greene, and Miriam Barlow. Designing antibiotic cycling strategies by
400   determining and understanding local adaptive landscapes. *PloS one*, 8(2):e56040, 2013.

401 R Pena-Miller and Robert Beardmore. Antibiotic cycling versus mixing: the difficulty of using
402   mathematical models to definitively quantify their relative merits. 2010.

403 Carl T Bergstrom, Monique Lo, and Marc Lipsitch. Ecological theory suggests that antimicrobial
404   cycling will not reduce antimicrobial resistance in hospitals. *Proceedings of the National Academy
405   of Sciences of the United States of America*, 101(36):13285–13290, 2004.

406 J Arjan GM de Visser and Joachim Krug. Empirical fitness landscapes and the predictability of
407   evolution. *Nature Reviews Genetics*, 2014.

408 Daniel M Weinreich, Richard A Watson, and Lin Chao. Perspective: sign epistasis and genetic
409   costraint on evolutionary trajectories. *Evolution*, 59(6):1165–1174, 2005.

410 Daniel M Weinreich, Nigel F Delaney, Mark A DePristo, and Daniel L Hartl. Darwinian evolution
411   can follow only very few mutational paths to fitter proteins. *Science*, 312(5770):111–114, 2006.

412 Longzhi Tan, Stephen Serene, Hui Xiao Chao, and Jeff Gore. Hidden randomness between fitness
413   landscapes limits reverse evolution. *Physical Review Letters*, 106(19):198102, 2011.

414 Pia Schulz zur Wiesch, Jan Engelstädter, and Sebastian Bonhoeffer. Compensation of fitness costs
415   and reversibility of antibiotic resistance mutations. *Antimicrobial agents and chemotherapy*, 54
416   (5):2085–2095, 2010.

417 Mark M Tanaka and Frank Valckenborgh. Escaping an evolutionary lobster trap: drug resistance
418   and compensatory mutation in a fluctuating environment. *Evolution*, 65(5):1376–1387, 2011.

419 Lejla Imamovic and Morten OA Sommer. Use of collateral sensitivity networks to design drug
420   cycling protocols that avoid resistance development. *Science translational medicine*, 5(204):
421   204ra132–204ra132, 2013.

422 Sewall Wright. The roles of mutation, inbreeding, crossbreeding and selection in evolution. In
423   *Proceedings of the Sixth International Congress on Genetics*, volume 1, pages 356–366, 1932.

424 Richard W Hamming. Error detecting and error correcting codes. *Bell System Technical Journal*,
425   29(2):147–160, 1950.

426 John H Gillespie. A simple stochastic gene substitution model. *Theoretical Population Biology*, 23
427   (2):202–215, 1983.

428 John H Gillespie. Molecular evolution over the mutational landscape. *Evolution*, pages 1116–1129,
429   1984.

430 Yoh Iwasa, Franziska Michor, and Martin A Nowak. Stochastic tunnels in evolutionary dynamics.
431   *Genetics*, 166(3):1571–9, Mar 2004.

432 H Allen Orr. The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*, 6(2):
433   119–127, 2005.

434 Stuart Kauffman and Simon Levin. Towards a general theory of adaptive walks on rugged land-
435   scapes. *Journal of Theoretical Biology*, 128(1):11–45, 1987.

436 Walter Fontana, Peter F Stadler, Erich G Bornberg-Bauer, Thomas Griesmacher, Ivo L Hofacker,
437   Manfred Tacker, Pedro Tarazona, Edward D Weinberger, and Peter Schuster. RNA folding and
438   combinatory landscapes. *Physical Review E*, 47(3):2083, 1993.

439 Catherine A Macken and Alan S Perelson. Protein evolution on rugged landscapes. *Proceedings of
440   the National Academy of Sciences of the United States of America*, 86(16):6191–6195, 1989.

441 Catherine A Macken, Patrick S Hagan, and Alan S Perelson. Evolutionary walks on rugged land-
442   scapes. *SIAM Journal on Applied Mathematics*, 51(3):799–827, 1991.

443 Henrik Flyvbjerg and Benny Lautrup. Evolution in a rugged fitness landscape. *Physical Review A*,
444   46(10):6714, 1992.

445 John H Gillespie. *The causes of molecular evolution.* Oxford University Press, 1991.

446 Charles Miller Grinstead and James Laurie Snell. *Introduction to Probability.* American Mathe-
447   matical Soc., 1998.

448 Guy Sella and Aaron E Hirsh. The application of statistical physics to evolutionary biology. *Pro-
449   ceedings of the National Academy of Sciences of the United States of America*, 102(27):9541–9546,
450   2005.

451 Pat A P Moran et al. The statistical processes of evolutionary theory. 1962.

452 Ronald A Fisher. *The genetical theory of natural selection.* , 1958.

453 Portia M Mira, Kristina Crona, Devin Greene, Juan C Meza, Bernd Sturmfels, and Miriam Barlow.
454 Rational design of antibiotic treatment plans. *arXiv preprint arXiv:1406.1564*, 2014.

455 Stuart A Kauffman and Edward D Weinberger. The NK model of rugged fitness landscapes and
456 its application to maturation of the immune response. *Journal of Theoretical Biology*, 141(2):
457 211–245, 1989.

458 Dan I Andersson and Diarmaid Hughes. Antibiotic resistance and its cost: is it possible to reverse
459 resistance? *Nature Reviews Microbiology*, 8(4):260–271, 2010.

460 Javier P Gisbert, Xavier Calvet, Anthony O'Connor, Francis Mégraud, and Colm A O'Morain.
461 Sequential therapy for helicobacter pylori eradication: a critical review. 44(5):313–325, 2010.

462 Kazuhiro Hanazaki. Antiviral therapy for chronic hepatitis b: a review. *Current Drug Targets-*
463 *Inflammation & Allergy*, 3(1):63–70, 2004.

464 Sarah Heenen, Frédérique Jacobs, and Jean-Louis Vincent. Antibiotic strategies in severe nosoco-
465 mial sepsis: Why do we not de-escalate more often? *Critical Care Medicine*, 40(5):1404–1409,
466 2012.

467 Aisha I Khan, Duy M Dinh, Dominique Schneider, Richard E Lenski, and Tim F Cooper. Negative
468 epistasis between beneficial mutations in an evolving bacterial population. *Science*, 332(6034):
469 1193–1196, 2011.

470 Martijn F Schenk, Ivan G Szendro, Merijn LM Salverda, Joachim Krug, and J Arjan GM de Visser.
471 Patterns of epistasis between beneficial mutations in an antibiotic resistance gene. *Molecular*
472 *biology and evolution*, 30(8):1779–1787, 2013.

473 David W Hall, Matthew Agan, and Sara C Pope. Fitness epistasis among 6 biosynthetic loci in the
474 budding yeast saccharomyces cerevisiae. *Journal of Heredity*, 101(suppl 1):S75–S84, 2010.

475 Elena R Lozovsky, Thanat Chookajorn, Kyle M Brown, Mallika Imwong, Philip J Shaw, Sumalee
476 Kamchonwongpaisan, Daniel E Neafsey, Daniel M Weinreich, and Daniel L Hartl. Stepwise
477 acquisition of pyrimethamine resistance in the malaria parasite. *Proceedings of the National*
478 *Academy of Sciences of the United States of America*, 106(29):12025–12030, 2009.

479 Jack da Silva, Mia Coetzer, Rebecca Nedellec, Cristina Pastore, and Donald E Mosier. Fitness
480 epistasis and constraints on adaptation in a human immunodeficiency virus type 1 protein region.
481 *Genetics*, 185(1):293–303, 2010.

24

Trevor Hinkley, João Martins, Colombe Chappey, Mojgan Haddad, Eric Stawiski, Jeannette M Whitcomb, Christos J Petropoulos, and Sebastian Bonhoeffer. A systems analysis of mutational effects in hiv-1 protease and reverse transcriptase. *Nature genetics*, 43(5):487–489, 2011.

Frank J Poelwijk, Daniel J Kiviet, Daniel M Weinreich, and Sander J Tans. Empirical fitness landscapes reveal accessible evolutionary paths. *Nature*, 445(7126):383–386, 2007.

Frank J Poelwijk, Sorin Tănase-Nicola, Daniel J Kiviet, and Sander J Tans. Reciprocal sign epistasis is a necessary condition for multi-peaked fitness landscapes. *Journal of Theoretical Biology*, 272 (1):141–144, 2011.

Niel Woodford and Matthew J Ellington. The emergence of antibiotic resistance by mutation. *Clinical Microbiology and Infection*, 13(1):5–18, 2007.

Slade O Jensen and Bruce R Lyon. Genetics of antimicrobial resistance in staphylococcus aureus. *Future Microbiology*, 4(5):565–582, 2009.

Christopher J Lord and Alan Ashworth. Mechanisms of resistance to therapies targeting brca-mutant cancers. *Nature Medicine*, 19(11):1381–1388, 2013.

Piro Lito, Neal Rosen, and David B Solit. Tumor adaptation and resistance to raf inhibitors. *Nature Medicine*, 19(11):1401–1409, 2013.

Artem Kaznatcheev. Complexity of evolutionary equilibria in static fitness landscapes. *arXiv preprint arXiv:1308.5094*, 2013.

Martin A Nowak. What is a quasispecies? *Trends in Ecology & Evolution*, 7(4):118–121, 1992.

James J Bull, Lauren Ancel Meyers, and Michael Lachmann. Quasispecies made simple. *PLoS computational biology*, 1(6):e61, 2005.

Steffen Schaper and Ard A Louis. The arrival of the frequent: How bias in genotype-phenotype maps can steer populations to local optima. *PLOS ONE*, 9(2):e86635, 2014.

Steffen Schaper, Iain G Johnston, and Ard A Louis. Epistasis can lead to fragmented neutral spaces and contingency in evolution. *Proc Biol Sci*, 279(1734):1777–83, 2012.