



The effect of spatiotemporal antibiotic inhomogeneities on the evolution of resistance

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ARTICLE INFO

Article history:

Received 8 May 2019

Revised 31 October 2019

Accepted 9 November 2019

Available online 9 November 2019

Keywords:

Antibiotic resistance

Evolution

Spatio-temporal drug gradients

Evolutionary models

ABSTRACT

Combating the evolution of widespread antibiotic resistance is one of the most pressing challenges facing modern medicine. Recent research has demonstrated that the evolution of pathogens with high levels of resistance can be accelerated by spatial and temporal inhomogeneities in antibiotic concentration, which frequently arise in patients and the environment. Strategies to predict and counteract the effects of such inhomogeneities will be critical in the fight against resistance. In this paper we develop a mechanistic framework for modelling the adaptive evolution of resistance in the presence of spatiotemporal antibiotic concentrations, which treats the adaptive process as an interaction between two mutually orthogonal forces; the first returns cells to their wild-type state in the absence of antibiotic selection, and the second selects for increased *coping ability* in the presence of an antibiotic. We apply our model to investigate laboratory adaptation experiments, and then extend it to consider the case in which multiple strategies for resistance undergo competitive evolution.

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1. Introduction

Combating the evolution of antibiotic resistant pathogens is one of the major clinical challenges faced by modern medicine (Ling et al., 2015; Durão et al., 2018; Baym et al., 2016b). Given sufficient time, resistance to virtually all antibiotic compounds can evolve (Bell and MacLean, 2018), and because resistant strains tend to adapt themselves to their new conditions (MacLean et al., 2010; Andersson, 2006) the return to sensitivity (in the absence of an antibiotic) occurs very slowly (Durão et al., 2018; Schrag et al., 1997; De Gelder et al., 2004). Consequently there is a pressing need for new approaches to predicting and preventing the spread of resistance, and its evolution in the first place (Gifford et al., 2018; Furusawa et al., 2018). This will require novel theoretical descriptions of the development of resistance (Bell and MacLean, 2018; Lukačičinová and Bollenbach, 2017) which take into account a range of environmental and physical factors that regulate the process. If these techniques can be employed to sufficiently slow down the development of resistance, its spread may eventually be stopped entirely (Bell and MacLean, 2018).

Cell-Antibiotic interactions vary greatly depending on the particular species involved: Antibiotics may target a broad range of cellular processes and systems, and can have widely varying ef-

fects on different cell lines or species. These antibiotic effects can be broadly classified as bactericidal (killing of bacteria) or bacteriostatic (preventing of growth) (Kohanski et al., 2010). Antibiotic effects are typically quantified using metrics such as the MBC (minimum bactericidal concentration) or MIC (minimum inhibitory concentration) which represent concentrations required to almost entirely eliminate a bacterial population (Liu et al., 2004; Wang et al., 2016). However, these metrics can be difficult to apply (particularly in clinical settings) due to the wide range of responses cells may have to high antibiotic concentrations. For example, cells can exhibit tolerant (reduced or stalled growth which allows antibiotics that require active growth for killing to be resisted) or persister (non-growing clonal sub-populations) behaviours which allow populations to recover following prolonged antibiotic exposure (Brauner et al., 2016; Harms et al., 2016; Cohen et al., 2013).

At the biochemical level antibiotic resistance can be achieved by a range of mechanisms, depending on the environmental conditions and antibiotic in question (Palmer et al., 2018; Pál et al., 2015). These can be broadly classified into four mechanistic categories: The modification of cellular targets so that antibiotic binding is diminished, physical removal of an antibiotic from the cell via modification of efflux systems, reduction of cellular uptake, and enzymatic inactivation of the antibiotic (Pál et al., 2015). When one of these forms of resistance develops via a series of mutations there are, in many cases, only a limited number of evolutionary pathways that can be taken (Weinreich et al., 2006; Palmer et al., 2015). Evolutionary trajectories have proven to be remarkably

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reproducible (Weinreich et al., 2006; Chevereau et al., 2015; Dideot et al., 2016), and the particular trajectory taken can be influenced by a range of environmental conditions (Gifford et al., 2018; Lukačičinová and Bollenbach, 2017; Zampieri et al., 2017). Similar evolutionary outcomes often have very different effects on other cellular behaviours, such as cross-sensitivity to other antibiotics (Barbosa et al., 2017).

Recent studies have demonstrated that the acquisition of resistance can be significantly influenced by spatial (Zhang et al., 2011; Baym et al., 2016a) or temporal (Toprak et al., 2012; Lindsey et al., 2013; Chevereau et al., 2015; Oz et al., 2014; Zampieri et al., 2017) inhomogeneities in antibiotic concentration. Such inhomogeneities have been shown to arise in both clinical settings (e.g. concentration differences between organs within a patient), as well as in external environments (such as rivers and lakes where antibiotic run-off accumulates) (Andersson and Hughes, 2014). The presence of intermediate antibiotic concentrations allows pathogens to gradually develop greater levels of resistance, making them more able to adapt to large antibiotic concentrations that are subsequently encountered (Bell and MacLean, 2018). During these adaptive processes resistance can increase continuously (e.g. when the expression of many genes is optimised) or in a step-wise manner (e.g. when a small number of mutational changes are responsible for determining overall resistance efficacy) (Toprak et al., 2012; Chevereau et al., 2015; Barrick and Lenski, 2013; Palmer et al., 2018). A particularly extreme case of step-wise resistance improvement is that due to horizontal transfer of genetic information (such as plasmids) that encode resistance genes (Jansen et al., 2013).

A range of mathematical models have been developed to describe the evolution and proliferation of antibiotic resistance (Opatowski et al., 2011; Mozhayskiy and Tagkopoulos, 2013). Competition and transmission models have proven to be valuable for modelling the spread of resistance within cellular populations, patients, or hospital and human populations at large (Spicknall et al., 2013; Jacobs et al., 2016). At the genetic level models of individual base-pair substitutions in DNA can describe adaptive processes over short time-scales (Posada and Crandall, 2001; Hindré et al., 2012), and analyse distributions of mutational effects (MacLean et al., 2010; Gillespie, 1984). Studies of mutational landscapes have demonstrated that these individual genetic mutations may combine to determine a particular trait in highly nontrivial ways (Furusawa et al., 2018; Beerenwinkel et al., 2007; Franke et al., 2011; Palmer et al., 2015). Traditional descriptions of long-term adaptive evolution have generally considered evolutionary pathways at a greater level of abstraction, modelling the variation in quantitative traits as random walk processes (Lande, 1976; Hansen, 1997; Beaulieu et al., 2012; Uyeda and Harmon, 2014). In a few cases stochastic models of evolution have been combined with consideration of spatial inhomogeneities (which models have shown impact genetic diversity (Behrman and Kirkpatrick, 2011) and the evolution of resistance (Kepler and Perelson, 1998; De Jong and Wood, 2018)) for the case of antibiotic resistance: Hermsen et al. proposed a staircase model which discretises bacteria in both space and phenotype, demonstrating that spatial antibiotic variation can encourage development of resistance (Hermsen et al., 2012). This was later developed to provide a continuum treatment of space and phenotype (Hermsen, 2016). Greulich et al. proposed a similar model (they are compared in (Hermsen, 2016)), which demonstrated that environmental inhomogeneities can slow the acquisition of resistance in some cases (Greulich et al., 2012). Finally, Gralka et al. modelled the impact of convection in the presence of spatial inhomogeneities, examining the trade-off between antibiotic efficacy and the potential for resistance to arise (Gralka et al., 2017).

Building upon this past work, in this paper we outline a mathematical framework for describing the evolution of resistance in the

presence of spatiotemporal antibiotic inhomogeneities. We investigate how different aspects of the evolutionary process determine the rate at which resistance emerges, and demonstrate how our framework can be used to analyse the results of past studies and common clinical situations.

2. Methods

2.1. A cell's coping ability

To model the evolution of antibiotic resistance we propose a parameterisation of cell fitness, *coping ability* (γ), which reflects the ability of a particular antibiotic resistance strategy to permit a cell to reproduce when stressed by a *coping challenge* (γ_c): If a given cell has $\gamma < \gamma_c$ (noting that γ_c may be location or time dependent) then it is unable to reproduce. By parameterising resistance in this way we assume that γ is a function of both genetic changes, as well as any other heritable phenotypic differences between cells that impact their interactions with an antibiotic (Deris et al., 2013; Erickson et al., 2016). Many resistance strategies only arise when mutations are present in certain combinations (Weinreich et al., 2006), which motivates a parameterisation of resistance (γ) which is not reducible to a sum of individual effects (i.e. discrete point mutations), since any such reduction would require assumptions be made about all potential combinatorial effects of individual mutations.

The coping challenge (γ_c) is assumed to be a non-decreasing function of local antibiotic concentration, and may be non-linear. The functional dependence of γ_c upon antibiotic concentration is likely to differ between antibiotics depending on their mechanism of action. However, one potential relationship is of the form:

$$\gamma_c = \log([A]/[A]_{50}) \quad (1)$$

where $[A]$ is antibiotic concentration and $[A]_{50}$ is the antibiotic concentration required to prevent growth of 50% of wild-type cells (often referred to as IC_{50} (Soothill et al., 1992)). When no antibiotic is present ($[A] = 0$) we have $\gamma_c = -\infty$, and thus any value of γ is sufficient for all cells to reproduce. Eq. (1) aligns with the results of many experimental studies that have demonstrated that the challenge posed by adapting to an increase in antibiotic concentration is roughly proportional to the fold-change that must be overcome (Baym et al., 2016a; Pennell et al., 2015; Toprak et al., 2012).

A significant obstacle to formulating a general theory for adaptive evolution is the unknown distribution of fitness effects (Rokyta et al., 2005). For antibiotic resistance this metric is difficult to estimate because the beneficial (antibiotic resisting) effects of many mutations are outweighed by their fitness cost (MacLean et al., 2010). To side-step this challenge we decompose the evolutionary process into two orthogonal components. The first is the antibiotic (γ_c) dependent reproduction mentioned above. The second determines the mutational dynamics of γ in the absence of an antibiotic, and is governed by two processes:

Random Mutation (D_γ): Mutational variations in γ are introduced over time as normally distributed increments (as observed in the distribution of fitness effects for non-optimal traits (Schenk et al., 2012; McDonald et al., 2011)) with zero mean, and thus have a 50% probability of increasing γ (but do not affect the cell's typical reproduction rate or longevity). Treatment of γ as a continuous parameter is motivated by the observation that resistance is determined by the cumulative effect of many mutations that may impact diverse processes (Lukačičinová and Bollenbach, 2017; Feng et al., 2016), which often leads to a population's resistance developing continuously in time (Toprak et al., 2012).

Mean Reversion (θ): Mean reversion (drift in γ toward its mean wild-type value μ) is driven by the fitness cost that mutations impart upon a host cell when considered in the absence of

an antibiotic. Long term studies have shown that this rate of reversion can vary significantly depending on the strain, drug, and resistance mechanism in question (Bean et al., 2005). To parameterise this process we assume that a mutational state's fitness cost is independent of the degree to which that state can resist the antibiotic, and as such mean reversion occurs at a constant rate θ . All mutations therefore result in some degree of fitness loss (as they steer the cell away from its optimal wild-type state) when considered independently of the effect of antibiotic-driven selection. A constant (γ -independent) reversion rate represents a minimal null-hypothesis when this dependence is unknown, and is supported by two experimental observations which provide mechanisms for decoupling the potency of resistance mutations from their fitness cost. First, organisms evolve to largely negate the fitness cost of resistance (MacLean et al., 2010; Andersson, 2006), often on a timescale that is faster than the development of resistance itself (Moura De Sousa et al., 2015). Second, a particular γ value may result from a small number of mutations with large effect (or a large number with small effect) on resistance (Bloom and Arnold, 2009), and so in general larger γ values do not necessarily incur a greater fitness cost.

We combine the factors above to model the dynamics of γ in the absence of selection (i.e. zero antibiotic, $\gamma_c = -\infty$) with a stochastic differential equation of the form:

$$d\gamma_t = -\theta \text{sign}(\gamma_t - \mu)dt + \sqrt{2D_\gamma}dB_t \quad (2)$$

Here B_t is a Wiener process (Brownian motion), scaled by diffusion constant D_γ . Eq. (2) is an example of a stochastic differential equation (SDE) with discontinuous drift, for which the stationary solution is a Laplace distribution with $\mathbb{E}[\gamma] = \mu$ and $\text{Var}[\gamma] = 2D_\gamma^2/\theta^2$ (Simonsen et al., 2013). The stationary distribution of coping ability will be (as highlighted by past studies of evolutionary processes (Bloom et al., 2007; Amitai et al., 2007)) an important determinant of a cell's ability to adapt when selection for this trait occurs (i.e. an antibiotic is encountered). We define $\phi = \sqrt{\text{Var}[\gamma]} = \sqrt{2D_\gamma}/\theta$ as a convenient measure of variability for our system. We will also generally set $\mu = 0$, such that $\mathbb{E}[\gamma] = 0$ and a coping challenge $\gamma_c = 0$ (corresponding to $[A]_{50}$) will prevent 50% of cells from replicating. A particle whose motion is described by Eq. (2) has a characteristic speed $\propto \sqrt{D_\gamma}$ and distance $\propto \phi$, meaning that the characteristic timescale over which it traverses its stationary distribution is $t_c \propto \sqrt{D_\gamma}/\theta$. In Fig. 1 we illustrate the interplay between the distribution of γ values for a population of cells and a temporal or spatial step in the value of γ_c .

Past studies of evolutionary processes have similarly employed stochastic differential equations to model the drift in trait values over time (Lande, 1976; Hansen, 1997). In some cases these treatments include pure Brownian motion (Hermesen, 2016), but without mean reversion the variance of a trait value grows linearly in time. Others have described adaptive evolution using an Ornstein-Uhlenbeck process (Hansen, 1997), in which the rate of mean-reversion is proportional to the distance of a trait from an optimal value μ^* . Though this yields a bounded variance, it assumes that the drift process (toward μ^*) is driven by selection for improvement in the trait being studied. The interpretation of the SDE in Eq. (2) differs markedly from past modelling philosophies: Here we have separated out the selection process which drives change in γ in response to environmental pressures (which is instead imposed by γ_c -dependent reproduction) from the underlying mutation process that governs variation in γ in the absence of antibiotic-driven selection (which is entirely described by Eq. (2)). In Note S7 we describe a discretised version of Eq. (2), which constrains γ to only take integer multiples of a discretisation parameter ω . This reflects the outcome of experimental studies which have found that in some cases resistance can develop in discontinuous jumps due

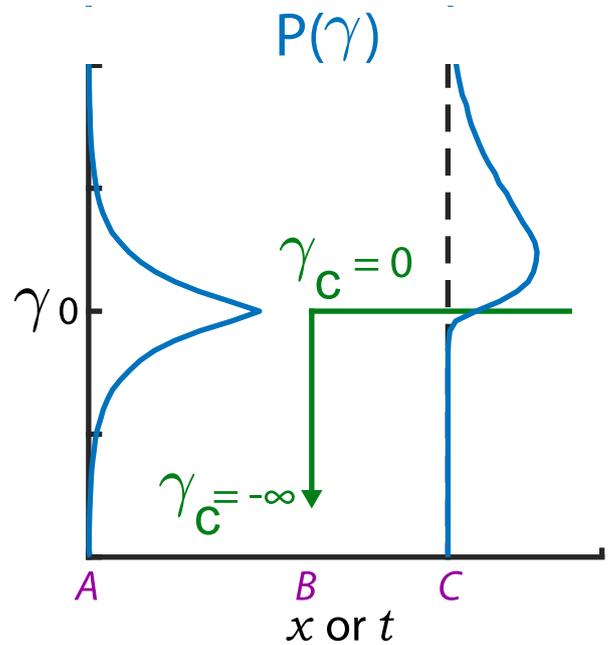


Fig. 1. Probability distributions of coping ability. At point A no antibiotic is present ($\gamma_c = -\infty$) and the stationary distribution of coping ability ($P(\gamma)$) within a population of cells is Laplacian as predicted by Eq. (2). At point B antibiotic is added such that $\gamma_c = 0$, immediately following which cells with $\gamma < 0$ are unable to replicate. At a later point in time/distance C a new stationary distribution has been established, no longer Laplacian since selection for resistance is occurring, and hence selective reproduction interferes with the stationary distribution predicted by Eq. (2). Note that at point C some (small number of) cells have $\gamma < \gamma_c$ due to ongoing mutation, and the population's mean γ is substantially greater than γ_c .

to distinct high-impact mutations (Toprak et al., 2012; Chevereau et al., 2015; Barrick and Lenski, 2013), rather than as a continuous process.

2.2. Spatio-temporal dynamics and selection

Each cell's (unconstrained) spatial diffusion is described by the stochastic differential equation:

$$dx_t = \sqrt{2D_x}dB_t \quad (3)$$

where B_t is a Wiener process (Brownian motion) and D_x is the spatial diffusion rate. The probability density function of the position of a particle whose motion is described by Eq. (3) is a normal distribution with $\mathbb{E}[x_t] = 0$ and $\text{Var}[x_t] = 2D_x t$ meaning that (given sufficient time) unconstrained cells will reach any given spatial location.

Each cell is assumed to die at rate δ , and as such when $\gamma < \gamma_c$ the number of surviving cells in a population will decrease exponentially over time (as is observed experimentally (Brauner et al., 2017)). The reproduction rate of a cell i is given by:

$$R_i = (1 + \delta) \left(1 - \sum_j \frac{\Gamma(x_i - x_j|\sigma)}{k} \right) H(\gamma_i - \gamma_c(x_i, t)) \quad (4)$$

where the $1 + \delta$ pre-factor is a scaling factor of the reproduction rate that arises in non-dimensionalisation (see Note S1), and H is the Heaviside step function. $\Gamma(\Delta x|\sigma)$ is a Gaussian interaction kernel that is convolved across all cell locations x_j to calculate the local cell density (and hence resource availability) at x_i , which has interaction range (standard deviation) σ . Finally, k is the spatial carrying capacity (resource availability) per unit distance, and $\gamma_c(x_i, t)$ is the coping challenge as a function of position and time. When a cell replicates a daughter cell with the same γ is placed at

the same location. For spatial simulations we will generally choose a γ_c that is independent of time. However, if we assume every cell has the same location (equivalent to setting $\sigma \rightarrow \infty$) then our modelling approach can be used to analyse situations in which adaptation is driven by temporal (rather than spatial) variations in antibiotic concentration (i.e. $\gamma_c(t)$).

Considering Eq. (2) alongside the selective reproduction enforced by Eq. (4) allows our modelling framework to be compared to traditional mathematical descriptions of trait evolution. Models that use the Ornstein-Uhlenbeck process and its derivatives employ a single SDE that combines the impact of random mutational changes and selective pressure on a particular trait's value (Hansen, 1997; Beaulieu et al., 2012; Blomberg, 2012). However, in the framework proposed herein these two factors enter as two independent selective forces: First, selection due to an antibiotic challenge γ_c is imposed by selective reproduction following from Eq. (4). Second, a restoring force arising from the fitness cost of resistance mutations (in the absence of antibiotic) is imposed by the drift term (with rate θ) in Eq. (2).

2.3. Model implementation

For many situations that we wish to analyse our model will be analytically intractable, necessitating analysis via numerical simulation. To achieve this the model was implemented in MATLAB (template code is provided with the supplementary material) and numerically integrated as described in Note S2. The parameter values described in Note S3 are used unless otherwise specified in the Supplementary Notes that accompany each result. Though our model considers the impact of local resource competition between cells, it does not explicitly model the consumption of a finite resource pool. To account for this simulations are run for a finite amount of time, and the maximum time permitted is either interpreted as a literal time period, or a limitation imposed by an eventual exhaustion of resources. In simulations and subsequent figures we often express γ values in multiples of ϕ (the standard deviation of the steady-state γ distribution in the absence of antibiotic) to enable direct comparison between different simulated scenarios.

2.4. Modelling multiple resistance strategies

We consider a situation in which our cell's total coping ability $\bar{\gamma}$ is determined by the combination of multiple mutually orthogonal adaptation strategies, γ_i , each governed by Eq. (2). The mutual orthogonality of these strategies implies that the correlation between their Brownian motion terms (as in Eq. (2)) is zero (or at least very small), and that there is no functional dependence between their mutation processes (i.e. D_{γ_i} is not a function of γ_j for $i \neq j$). Examples of orthogonal strategies are those for which a change in the effectiveness of one strategy does not directly affect the potential for improvement in the other. For example, if γ_1 represents adaptation primarily driven by development of efflux systems, and γ_2 represents adaptation primarily driven by active-site mutation, then we anticipate that mutations which cause a substantial increase in γ_1 will not necessarily impact γ_2 (though they may impact $\bar{\gamma}$). An example of two non-orthogonal strategies would be if we chose $\gamma_{1,2}$ to both represent different paths of active site mutation, and thus improvement in one could force improvement in the other (e.g. correlated Brownian motion), or the state of one path could influence the rate of change of the other (e.g. if they can be inter-converted via a small number of mutations (Palmer et al., 2015)). For $\bar{\gamma}$ we propose a function of the form:

$$\bar{\gamma} = \sum \gamma_i \quad (5)$$

which is motivated by the following assumptions: When strategies combine to determine $\bar{\gamma}$ they should do so multiplicatively in terms of antibiotic concentration, and hence additively in terms of γ (following from Eq. (1)). This means that if (for example) γ_i represents an efflux pump strategy which removes 90% of an antibiotic from the cell's interior, then each γ_j ($j \neq i$) evolves as if it is subjected to 10% of the total antibiotic concentration. When a new strategy is considered (i.e. adding an additional γ_i to the calculation in Eq. (5)) the value of D_{γ_i} will depend on how readily this strategy can evolve to impact $\bar{\gamma}$ (if γ_i has minimal impact on a cell's coping ability then $D_{\gamma_i} \approx 0$). Consequently we set each $\mu_i = 0$ such that considering a new strategy in Eq. (5) will not impact $\bar{\gamma}$ (unless it has evolved to contribute some form of resistance). Because each strategy is governed by an independent Wiener process in Eq. (2) we have (when at steady state with $\gamma_c = -\infty$) that $\bar{\phi}^2 = \text{Var}[\bar{\gamma}] = \sum \phi_i^2$.

3. Results

3.1. Adaptation driven by spatial variation in antibiotic concentration

A range of experimental and theoretical studies have demonstrated that spatial gradients (Zhang et al., 2011) or steps (Baym et al., 2016a) in antibiotic concentration can accelerate the acquisition of resistance conferring mutations in bacterial populations. In Fig. 2 we examine the adaptive behaviour predicted by our model to explain these results.

Fig. 2a illustrates a population of cells that encounters a spatial ramp in antibiotic concentration. Near $x \approx 0$, γ_c is well below the wild-type cell's resilience, and so an approximately Laplacian distribution of coping abilities is maintained. As γ_c rises it imparts a selection bias upon the population, favouring cells with larger γ and driving up the mean coping ability. At the leading edge of the adapting front the variability of γ values is substantially decreased because cells that migrate into free spatial regions can rapidly reproduce (due to the lack of resource competition), meaning that colonisation is driven by cells with γ values near to the mean (local) value (and thus the evolutionary process is not driven by only its fittest constituents (Baym et al., 2016a)). There is an optimal antibiotic gradient at which γ grows most quickly (Fig. 2b), originally investigated (using a model that is for this case similar to ours) by Hermsen (2016). In Fig. S1 we provide examples similar to Fig. 2a that illustrate this behaviour for different gradients: When $\alpha < 1$ adaptation is limited by the rate of spatial diffusion (D_x), whereas for $\alpha > 1$ adaptation is limited by rate at which variation in coping ability occurs (D_γ) (Hermsen, 2016). In the large α case variability in γ decreases, with the population's average gathering near its optimum (as found in previous studies of population evolution in changing environments (Droz and Pkalski, 2006)), which impedes adaptation since cells at the adaptive front mix extensively with those with lower γ . This phenomenon has been experimentally observed to slow adaptation at range margins (Bridle and Vines, 2007). In Fig. 2b the observed increase in adaptation rate at greater population densities (k) aligns with the experimental observation that the rate at which resistance develops is increased for larger inoculum sizes (Gould and MacKenzie, 2002).

Fig. 2c illustrates the response of a population of cells to a series of steps in antibiotic concentration: The first step is overcome, but within the given time the population is unable to make the subsequent jump to the region of higher concentration. We find that the probability of overcoming a fixed step in antibiotic is near zero for small population densities, but rises to unity when the cell density increases (Fig. 2d). This mirrors the experimental observations of Hol et al., who found that a critical bacterial density must be overcome in order to overcome a spatial step in antibiotic concentration (Hol et al., 2016). In Fig. 2e we simulate a colony of cells

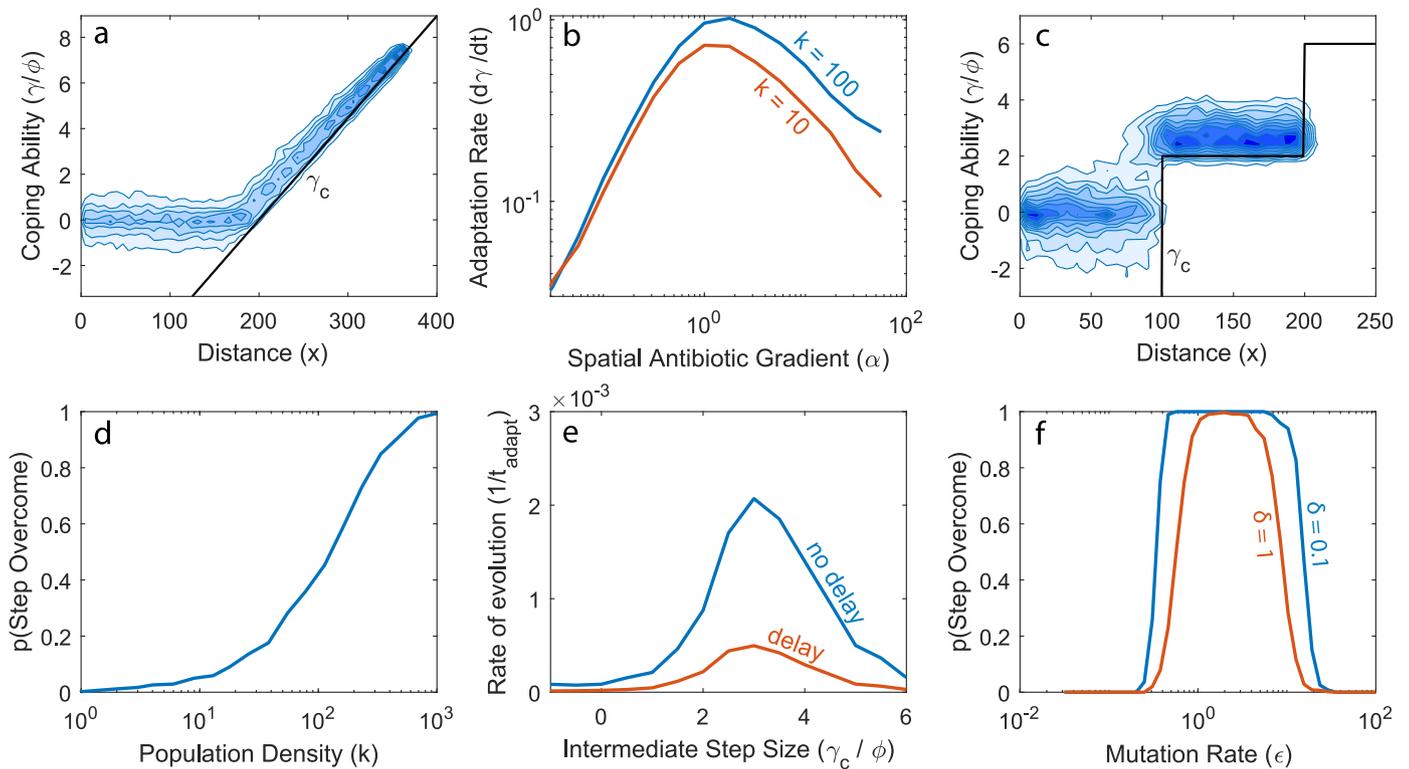


Fig. 2. Spatial antibiotic variation encourages development of resistance. a) Probability density level curves of a population of cells in terms of coping ability γ and position x as they evolve in the presence of a spatial gradient in antibiotic $\gamma_c = x - 200$. b) The adaptation rate as a function of spatial antibiotic gradient, $\gamma_c = \alpha x$, is maximised when $\alpha \approx 1$. Simulation details are presented in Note S8, and examples of adaptation with varying α are presented in Fig. S1a–c. c) Probability density level curves of a population evolving in response to two spatial steps in antibiotic concentration. For $x < 100$ $\gamma_c = -\infty$, for $100 < x < 200$ $\gamma_c = 2\phi$, and for $x > 200$ $\gamma_c = 6\phi$. The population is able to overcome the first step, but not the second. d) Probability of a fixed step in antibiotic concentration being overcome as a function of spatial population density k (Note S9). A similar plot as a function of γ_c is provided in Fig. S1d e) The rate of evolution in a two-step system (similar to c) as a function of the intermediate step size (Note S11). Also plot is the effect of adding a delay to account for extended spatial separation between the steps. f) The probability of a single step in antibiotic concentration being overcome as a function of an organism-wide mutation rate disturbance ϵ , with different values of δ (Note S12). There exists an optimal region of intermediate ϵ where this probability is maximised.

as it attempts to colonise a region of large antibiotic concentration by first colonising a step of intermediate height (the situation illustrated in Fig. 2c). The rate of colonisation is maximal when the intermediate step is approximately half of the final step size, which aligns with the experimental results of Baym et al. (2016a). In Fig. 2f we model the interaction of our system with a mutagen which scales cell-wide mutation rates by a factor ϵ (see Note S12 for full description). This reveals an optimal range of mutation rates within which a population has a high probability of overcoming a step in antibiotic concentration: For small mutation rates variability in γ is too small to overcome the antibiotic step, whilst for high mutation rates accelerated cell death reduces cell density and hence their ability to adapt. This simulation parallels an experimental study by Gerrish and García-Lerma (2003), where it was observed that both low and high mutation rates reduced the ability of a pathogen to adapt to a drug treatment.

3.2. Adaptation driven by temporal variation in antibiotic concentration

As with spatial gradients, experimental studies have demonstrated that temporal gradients and steps in antibiotic concentration can accelerate the evolution of resistant cell lines (Toprak et al., 2012; Lindsey et al., 2013; Chevereau et al., 2015; Oz et al., 2014; Zampieri et al., 2017). We now adjust our model (for details see Note S3) to consider a single, well mixed population (as might be found in a shaken liquid media experiment) for which spatial organisation of cells is not important.

When presented with a temporal antibiotic ramp ($\gamma_c = \alpha t$) the mean γ value for a cell population approximately coincides with γ_c (Fig. 3a). For large α the cells are no longer able to mutate sufficiently quickly, and we observe a rapid drop in population density and survivability (Fig. 3b). Unlike in Fig. 2b, for temporal gradients we observe that the mean γ value for a population of cells grows at rate α , up to a critical value (in this case $\alpha \approx 1$), after which all cells die out (examples are presented in Figs. S2a–f, and the dependence of adaptation rate on α is plot in Fig. S3). This corresponds to the results of Lindsey et al., who found that cells were unable to adapt when the temporal rate of antibiotic increase was above a threshold value (Lindsey et al., 2013). This result also illustrates that in order to maintain a sub-maximal population density there is a narrow range of feasible rates of antibiotic increase with time. This was observed experimentally by Toprak et al. (2012), who used a temporal antibiotic gradient (which was approximately linear when plot on log-scale, corresponding here to $\gamma_c \alpha t$) to regulate the density of an adapting bacterial population.

When encountering a large step rise in antibiotic concentration the majority of cells (with $\gamma \approx 0$ initially) rapidly die out, however, a few cells with large γ overcome the step and return the population to its maximal density following a short proliferation delay (Fig. 3c). In such a circumstance it is possible for there to be a greater rate of adaptation (change in mean γ value over time) for a short period than for antibiotic ramps (i.e. Fig. 2b), as predicted by Greulich et al. (2012). In Fig. 3d (left axis) we plot the probability of a given cell being able to replicate (i.e. having $\gamma > \gamma_c$) immediately following a step increase in γ_c , which is determined

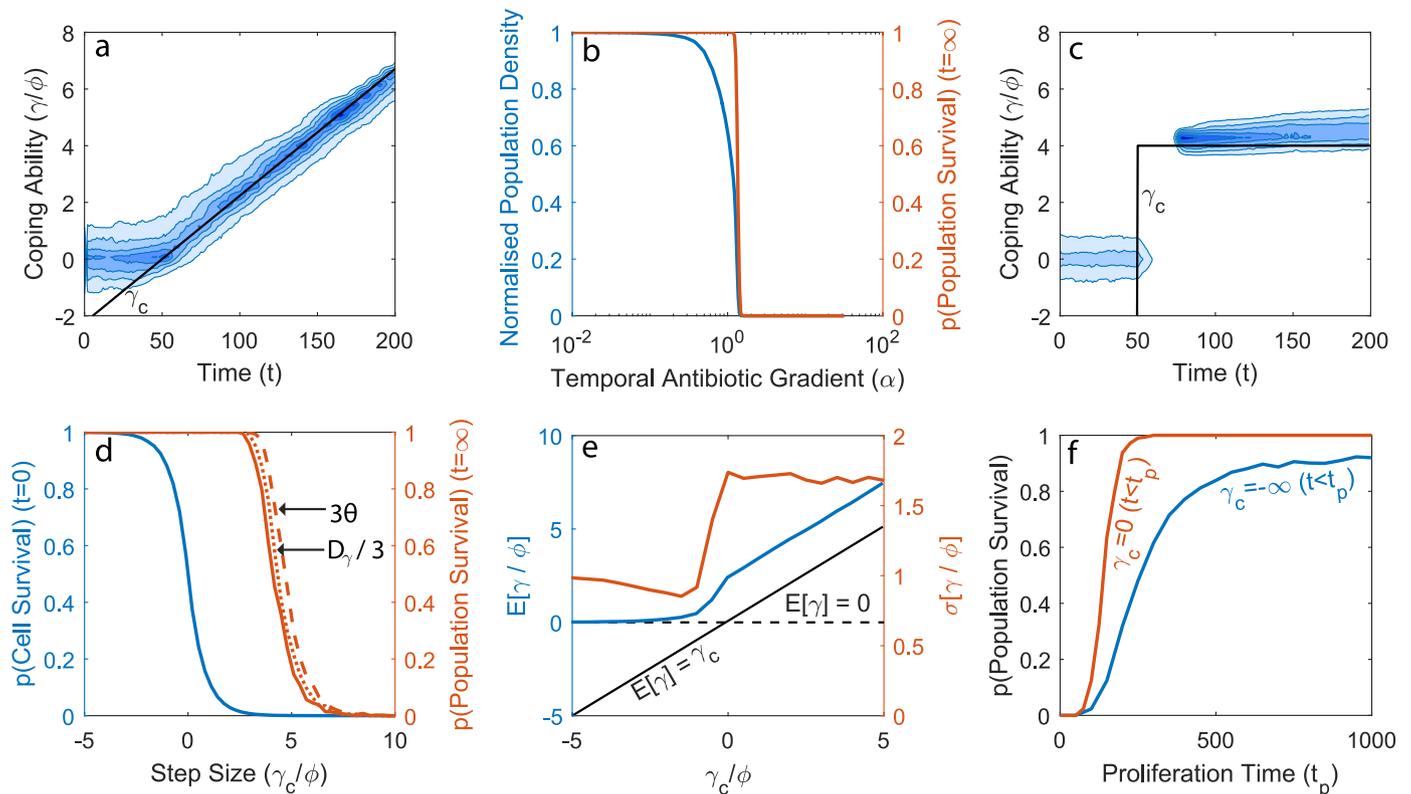


Fig. 3. Temporal antibiotic variation encourages development of resistance. a) Probability density level curves as a function of time for cells evolving in the presence of a temporal gradient in antibiotic concentration $\gamma_c = t - 50$. Here we are plotting a population of cells at a single location at many points in time (rather than a population of cells at many locations at a single point in time as in Fig. 2a). b) The normalised population density (left axis) and the probability of a population of cells being able to survive indefinitely (right axis) as a function of the temporal gradient in antibiotic concentration (with $\gamma_c = \alpha t$). Simulation details are presented in Note S13, and examples of cell populations growing with various α are in Fig. S2a–f. c) Probability density level curves as a function of time for cells that encounter a step rise in antibiotic. For $t < 50$ $\gamma_c = -\infty$, and for $t > 50$ $\gamma_c = 4\phi$. The introduction of antibiotic kills almost all cells at $t = 50$, but survivors have re-established a large population by $t \approx 70$. d) The probability of a single cell surviving (left axis) and the population surviving (right axis) as a function of the size of an antibiotic step rise γ_c (Note S14). We vary the parameters θ and D_γ between trials, observing that the population survival probability changes, though the probability of a given cell surviving remains unchanged (because ϕ varies when θ or D_γ change). e) The mean ($E[\gamma/\phi]$) and standard deviation ($\sigma[\gamma/\phi]$) of γ for a population of cells allowed to evolve for a long period in the presence of a fixed γ_c (Note S15). f) The probability of a clonal population (initially all cells have $\gamma = 0$) surviving a step rise in antibiotic concentration following a period of proliferation (t_p) during which either $\gamma_c = -\infty$ or $\gamma_c = 0$ (Note S17).

by the cumulative density function of the Laplace distribution from Eq. (2). The shape of this curve replicates typical killing curves observed for antibiotics (Liu et al., 2004) when plot as a linear function of antibiotic concentration (see Note S4). The point at which this curve drops to 0.001 ($\gamma_c/\phi \approx 4$) approximates the MBC (minimum bactericidal concentration) of the antibiotic, which is generally defined as the concentration required to reduce 99.9% of bacterial density (Wang et al., 2016). The MIC (minimum inhibitory concentration) takes a similar value (Andersson and Hughes, 2014). The problem with using these metrics for measuring antibiotic efficacy (as discussed by Liu et al. (2004)) is highlighted by the population survival curve (Fig. 3d right axis): Even though the vast majority of cells are killed above the MBC, the survival of even a single cell with high resilience can re-establish the population. The point above which this does not occur (i.e. the point where the population survival probability drops to zero, here at $\gamma_c/\phi \approx 8$) is referred to as the MPC (mutant prevention concentration) (Wang et al., 2016). The distance between these curves represents the mutant selection window (MSW), a range of concentrations which is clinically important as it encourages selection for highly resistant mutants (Bell and MacLean, 2018).

In Fig. 3d we also plot changes anticipated in the Population Survival probability when either D_γ or θ is varied while the step in antibiotic maintained at a constant multiple of ϕ (so that each cell survival probability curve is identical). Adjusting these parameters highlights the relative influence of two means via which a popula-

tion can overcome a temporal step in γ_c : The first is by having at least one cell with $\gamma > \gamma_c$ when antibiotic is introduced (often assumed to be the predominant route to resistance (Andersson and Hughes, 2014)), allowing the step to be overcome (this probability is plot in Fig. S4). The second is having $\gamma < \gamma_c$ immediately following antibiotic introduction, but evolving to a state with $\gamma > \gamma_c$ prior to cell death. If the former was the only mechanism then we would expect changes in D_γ or θ (which determine ϕ) to have no influence on the population survival probability for a step of fixed γ_c/ϕ . This is not the case, demonstrating that some populations in which no cells are able to reproduce post-antibiotic addition can still recover. We can investigate this situation analytically (see Note S5), finding that the probability that a cell with coping ability $\gamma < \gamma_c$ will survive (i.e. it reaches γ_c before dying) thereby sustaining the population is given by:

$$p(\text{Population Survival}) = \exp\left(-\frac{\nu\sqrt{2D_\gamma\delta}}{\theta}\right) \quad (6)$$

where ν is a dimensionless parameter measuring the distance between the cell's initial γ and γ_c . The dependence of Eq. (6) on δ highlights the impact that the development of tolerant cell types (slowly dying in the presence of antibiotic (Brauner et al., 2016)) has upon the emergence of resistance (Cohen et al., 2013; Fridman et al., 2014; Levin-Reisman et al., 2017): Tolerant cell populations (those with a smaller value of δ which die off slowly (Brauner et al., 2017)) often develop as a pre-cursor to actual

resistance (Levin-Reisman et al., 2017). Note S6 derives a similar expression to Eq. (6) for the case in which γ_c depends linearly on time, demonstrating the antibiotic's inefficacy when its (initially large) concentration is reduced rapidly.

In Fig. 3e we plot the steady-state mean coping ability ($\mathbb{E}[\gamma]$) and standard deviation ($\sigma[\gamma]$) of a population of cells subjected to a constant γ_c value. Substantial adaptation occurs well below the MBC ($\gamma_c/\phi \approx 4$ in Fig. 3d), and even below the IC₅₀ ($\gamma_c = 0$). There has been substantial debate regarding whether selection at low antibiotic concentrations is important (Jansen et al., 2013; Andersson and Hughes, 2014), and whether low-concentration treatments should be employed to minimise resistance evolution (Read et al., 2011; Kouyos et al., 2014). According to the classical MSW hypothesis selection is only relevant within the MSW region (Jansen et al., 2013), however, many studies have demonstrated that significant adaptation can occur in response to low antibiotic concentrations (Andersson and Hughes, 2014). Our simulations support the case for adaptation at low concentrations, though they predict that the degree of adaptation in this region will be limited (i.e. cells will not become resistant to very high antibiotic concentrations). This aligns with studies of evolutionary processes which have demonstrated that many traits (such as protein stability (Taverna and Goldstein, 2002)) will typically drift toward their minimum evolutionary requirement, as values much higher do not offer a selective benefit (Bloom et al., 2006). For the variability of γ within a population Fig. 3e suggests the existence of two distinct regimes: When $\gamma_c < \mu$ we have $\sigma[\gamma] \approx \phi$ (following from Eq. (2)), though this decreases as $\gamma_c \rightarrow 0^-$ due to cells with very low γ values being unable to reproduce. When γ_c grows beyond $\gamma_c = 0$ there is a sharp rise in $\sigma[\gamma]$ to a new constant value of $\approx 1.7\phi$, as the population's distribution of γ values is now bounded below by γ_c . This behaviour is observed as a widening of the distribution in Fig. 1, highlighting our model's prediction that the distribution of γ values in a population of cells growing in the presence of $\gamma_c > 0$ will tend to have both an increased mean and standard deviation, making it more likely to adapt to a future rise in γ_c . That said, following any rise in antibiotic concentration there is a period during which $\sigma[\gamma]$ is reduced (Fig. S5) whilst the population re-establishes its genetic diversity (i.e. reaching a new steady state distribution of γ).

In Fig. 3f we initialise a clonal population (in which all cells begin with $\gamma = 0$) and allow cells to proliferate (and mutate) for a period t_p , before subjecting the population to a step rise in antibiotic concentration. We observe that it takes a substantial amount of time for sufficient intra-population variability in γ to develop to enable the population to overcome the antibiotic step when it is encountered. This process is accelerated significantly when cells proliferate in the presence of a low antibiotic concentration ($\gamma_c = 0$), highlighting the clinical role that low concentrations can play in pre-adapting populations, thereby enabling them to overcome large antibiotic doses at later times. Related situations that include the temporary addition or removal (after a period of adaptation) of an antibiotic are presented in Fig. S6. In the latter case we observe that the population survival probability remains at unity for a long period after antibiotic removal, highlighting one of the major clinical challenges posed by antibiotic resistance: Even in the absence of antibiotic a pathogen's return to sensitivity is slow (MacLean et al., 2010; Durão et al., 2018). In Fig. S6e we plot the probability that a population is able to overcome a large temporal step in antibiotic concentration as a function of the time it spends at an intermediate antibiotic concentration. An approximately linear rise is observed, with the probability of survival approaching unity as the time given for adaptation increases, again demonstrating that past environmental conditions can greatly affect a population's survivability following a rapid environmental change (as observed by Gonzalez and Bell (2013)).

3.3. Multiple strategies for resistance

For many antibiotics there is a range of potential mechanisms which can develop in combination to provide resistance (Palmer et al., 2018). For example, efflux pumps (which remove an antibiotic from the cell's interior), or mutation in the protein/s that directly interact with the antibiotic (thereby disrupting its binding) can both contribute to a cell's coping ability. Experimental studies have demonstrated that there are often a number of evolutionary trajectories which a cell can take to acquire resistance (Barbosa et al., 2017; Palmer et al., 2018), and that competition between different trajectories can impact the overall rate of adaptation (Ogbunugafor and Eppstein, 2016). In this section we employ our modelling framework to analyse a system in which there are multiple strategies competing to develop resistance.

We first simulate a population of cells growing in the presence of a temporal gradient of antibiotic concentration (e.g. as in Fig. 3a) for which there are two potential strategies for resistance, γ_1 and γ_2 , and the total coping ability is given by $\bar{\gamma} = \gamma_1 + \gamma_2$. Each strategy is assumed to have an equal ability to mutate over time ($D_{\gamma_1} = D_{\gamma_2}$), but their relative drift rate is varied according to $\theta_1 = \theta/\sqrt{1-\zeta}$ and $\theta_2 = \theta/\sqrt{\zeta}$ such that $1/\theta^2 = 1/\theta_1^2 + 1/\theta_2^2$. At steady-state (and in the absence of antibiotic, $\gamma_c = -\infty$) $\text{Var}[\bar{\gamma}]$ is thus independent of ζ , allowing us to compare populations with equivalent initial distributions of coping ability. In Fig. 4a,b we find that when $\zeta = 0.5$ both strategies adapt at half the rate of the temporal gradient (an example is illustrated in Fig. S7a). In the presence of very strong mean reversion for strategy 2 ($\zeta \rightarrow 0$), γ_2 does not develop at all (an example is illustrated in Fig. S7c), as would be expected from a resistance strategy with very high fitness cost when a better alternative is available. Interestingly, for intermediate ζ values ($\zeta \approx 10^{-2}$) at which the mean adaptation rate of γ_2 approaches zero there is a reduced probability (Fig. 4a) that the population survives indefinitely: In this case population die out can occur (an example is illustrated in Fig. S7b) when γ_2 temporarily rises, reducing the pressure on γ_1 to adapt and thus leaving $\bar{\gamma}$ too far below γ_c to keep up when γ_2 's strong mean reversion drives it back toward $\mu_2 = 0$. This phenomenon is primarily observed for intermediate ζ values because when $\zeta \gg 10^{-2}$ selective pressure can overcome the mean reversion to allow γ_2 to permanently adapt, whereas for $\zeta \ll 10^{-2}$ it is very unlikely that γ_2 will deviate from zero at any point. In Fig. S7i,j an alternate situation is investigated, in which $\theta_1 = \theta$ and $\theta_2 = \eta\theta$; in this case when γ_2 has no mean reversion ($\eta \rightarrow 0$) γ_1 still develops, with only a slightly reduced rate.

We now set $\theta_1 = \theta_2$, but vary the mutation rate of each strategy according to $D_{\gamma_1} = D_\gamma\sqrt{1-\xi}$ and $D_{\gamma_2} = D_\gamma\sqrt{\xi}$, such that $D_\gamma^2 = D_{\gamma_1}^2 + D_{\gamma_2}^2$. We observe (Fig. 4c) that as ξ grows our population goes from developing just one strategy to favouring a combination of both. In Fig. 4d we examine the influence of ζ or ξ on the probability of γ_1 developing when the population is confronted with a temporal step in antibiotic concentration (i.e. as in Fig. 3c). We observe that outside of a narrow switching region there is a strong bias in favour of whichever strategy has a greater mutation rate (larger D_{γ_i}) or smaller rate of mean reversion / fitness cost (smaller θ_i). In each case the winning resistance strategy develops largely to the exclusion of the other, such that $\max(\gamma_1, \gamma_2) \approx \bar{\gamma}$ and $\min(\gamma_1, \gamma_2) \approx 0$ (demonstrated in Fig. S7d,e). A similar result is observed when simulations are initialised with one strategy already dominating ($\mathbb{E}[\gamma_1] > \gamma_c > \mathbb{E}[\gamma_2]$) at $t = 0$, Fig. S7f,g: In this case if strategy γ_2 is "better" than γ_1 (large ξ or ζ) then it will (eventually) overcome γ_1 's early lead and take over the population. This result highlights an interesting difference between temporal steps and ramps; for a step the better strategy will eventually dominate in the population (regardless of initial conditions),

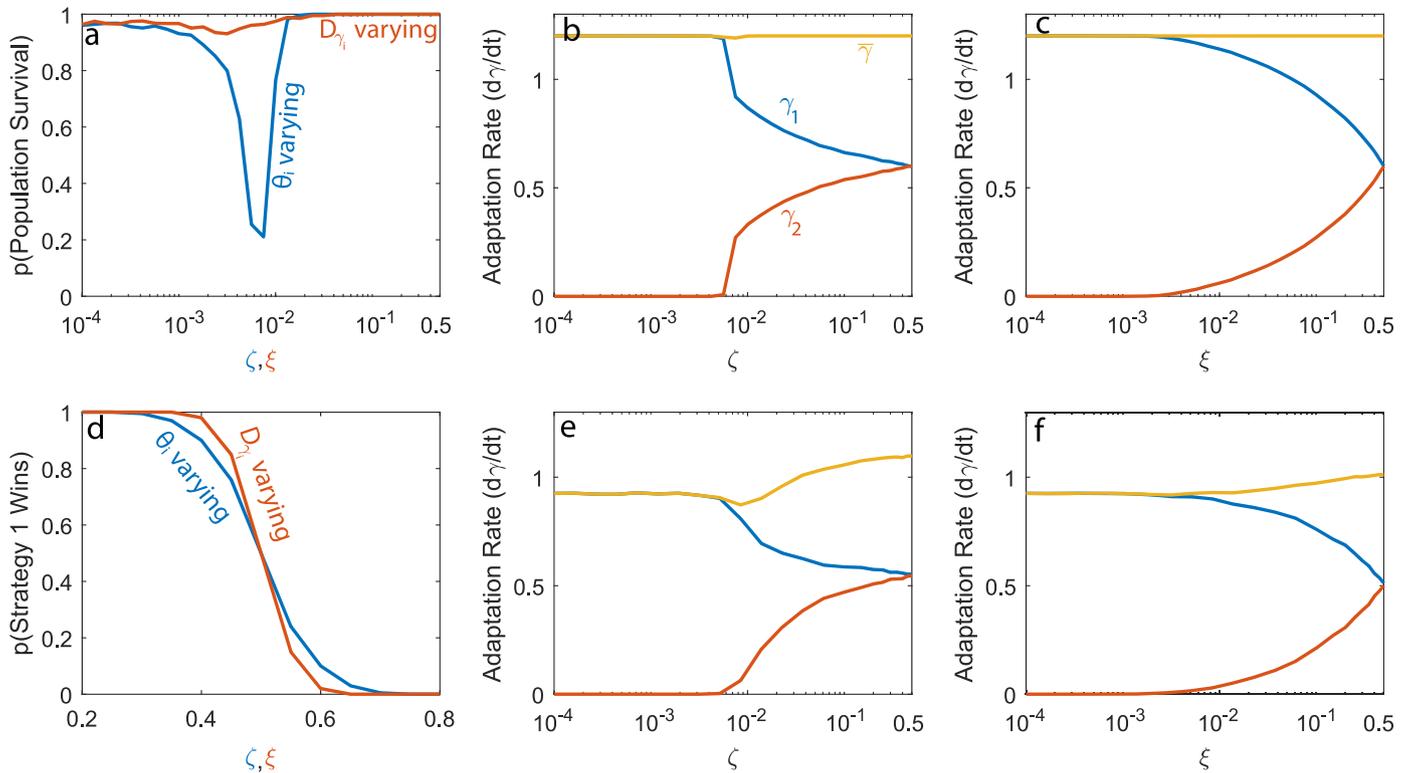


Fig. 4. Competition between multiple paths to antibiotic resistance. We consider two strategies γ_1 and γ_2 which combine as described by Eq. (5), with either $\theta_1 = \theta/\sqrt{1-\zeta}$ and $\theta_2 = \theta/\sqrt{\zeta}$, or $D_{\gamma_1} = D_\gamma\sqrt{1-\xi}$ and $D_{\gamma_2} = D_\gamma\sqrt{\xi}$. a) The probability of the population's survival when subjected to a temporal ramp ($\alpha = 1.2$) in antibiotic concentration as a function of the second strategy's rate of mean reversion. For intermediate values of ζ there is a significant probability that the second strategy causes population die out (Note S21). Examples for various ξ are presented in Fig. S7a–c. b,c) The adaptation rate (defined as in Fig. 2b) of each strategy (labelling is consistent across subplots b,c,e,f) as a function of ζ or ξ (Note S21). The combined rate of change in $\bar{\gamma}$ is constant and equal to the temporal rate of change of antibiotic ($\alpha = 1$). d) The probability that Strategy 1 wins (defined as having a larger mean γ_i value across the population) when the population is subjected to a temporal step in antibiotic concentration (Note S22), as a function of ζ (top axis) and ξ (bottom axis). e,f) Similar to b,c but for a population of cells growing on a spatial antibiotic gradient with $\alpha = 1$ (Note S25).

whereas if the population is driven to continually increase γ (i.e. a temporal ramp) there is a broad parameter regime in which both strategies develop.

In Fig. 4e,f simulations (which are otherwise similar to Fig. 4b,c) are performed for a population of cells that encounters a spatial (rather than temporal) ramp of antibiotic concentration. In this case there is no possibility of the population dying out (as regions of low antibiotic concentration are always accessible), but there is a minimum in the adaptation rate of $\bar{\gamma}$ at $\zeta \approx 10^{-2}$ for similar reasons to Fig. 4a. We also observe that the inclusion of a second strategy (when $\text{Var}[\bar{\gamma}]$ is kept constant) only results in a modest increase in the adaptation rate of $\bar{\gamma}$. Our results thus imply that when resistance is determined by multiple orthogonal strategies adaptation can occur more quickly, even when populations have an equivalent wild-type distribution of resistance (i.e. $\text{Var}[\bar{\gamma}]$ is constant). When a spatial step is simulated (Fig. S8a,b) we again find that it will typically be colonised by γ_2 if it is better than γ_1 (large ξ or ζ). However, in this case both resistance strategies tend to develop to some extent (one does not exclude the other), meaning that $\bar{\gamma}$ is determined by significant contributions from both (Fig. S8c,d).

4. Discussion

In this paper we have developed a theoretical model that describes the evolution of resistance in response to spatially- or temporally-varying antibiotic concentrations. Though both kinds of inhomogeneities can foster the development of high levels of resistance, they are not necessarily directly equivalent. For tempo-

ral steps in antibiotic concentration cells generally require $\gamma > \gamma_c$ at the time of dosing in order for the population to survive (this is often assumed to be the predominant route to resistance (Andersson and Hughes, 2014)), though there is the rare possibility of a “tolerance” path to population resistance, as described by Eq. (6). For spatial steps in antibiotic this path to resistance is less important. Instead, cells can undergo continual variation in γ near a discontinuity in their environment (a point at which γ_c increases), and once a mutant with $\gamma > \gamma_c$ appears they quickly migrate into the now accessible resource-rich region. This corresponds to experimental observations that the evolutionary process is not driven by its fittest constituents, but rather those that are both sufficiently fit and sufficiently close to an environmental inhomogeneity (Baym et al., 2016a). The overcoming of spatial inhomogeneities is aided by the fact that there are continually accessible regions of smaller γ_c in which cells can proliferate, which fundamentally changes the dynamics of competition between multiple resistance strategies in response to each kind of inhomogeneity. For example, we found that the presence of a second adaptive strategy for resistance can lead to a population's extinction in the presence of an otherwise manageable temporal antibiotic gradient (but not for a similar spatial gradient). Furthermore, when cells are presented with temporal steps in antibiotic concentration typically one strategy will be developed to the exclusion of others, whereas a combination of resistance strategies is likely to evolve in response to spatial steps.

The rate at which resistance increases in time ($d\bar{\gamma}/dt$) also differs significantly for the two classes of inhomogeneity: for temporal gradients adaptation rate is determined directly by the

gradient's magnitude ($d\gamma/dt \approx \alpha$) up to a limiting α where population extinction occurs. Meanwhile, for spatial gradients adaptation rate is determined by a trade-off between spatial diffusion (limiting for small α) and the population size near to an accessible (via small γ increase) region of larger γ_c (limiting for large α). These results highlight the importance of avoiding intermediate antibiotic doses which, if they produce intermediate spatial/temporal concentration gradients, maximise the rate at which resistance emerges.

Persister and tolerant cell states have been recognised as playing a prominent part in the development of resistance (Brauner et al., 2016). Our model (see Eq. (6)) supports the experimental observation that tolerance (or greater longevity in the presence of antibiotics) may improve the likelihood of resistance developing (Cohen et al., 2013; Fridman et al., 2014; Levin-Reisman et al., 2017). However, since the death rate (δ) is assumed to be homogenous within each population, we do not account for the wide range of persister behaviours that have been experimentally observed (Brauner et al., 2016): In such cases a small fraction of a clonal cell population may remain dormant in the presence of an antibiotic (via a range of mechanisms (Harms et al., 2016)), allowing them to re-establish the entire population when conditions are more favourable. To capture this behaviour our model would likely need to introduce additional parameters to provide a more complex description of cell states, or separate cells into sub-populations with differing parameter values.

At the level of individual cells, our modelling approach assumes that antibiotic resistance develops in a continuous manner (governed by Eq. (2)) as is frequently observed experimentally (Toprak et al., 2012; Chevereau et al., 2015; Barrick and Lenski, 2013; Palmer et al., 2018). However, in some cases resistance can develop in jumps due to distinct high-impact mutations (Toprak et al., 2012; Chevereau et al., 2015; Barrick and Lenski, 2013) (or by transfer of genes via plasmids). This possibility is investigated in Note S7, where qualitatively similar results to the continuous case are found when γ is constrained to integer multiples of a discretisation parameter ω . Though both treatments of γ allow populations as a whole to make large non-continuous jumps in resistance (e.g. as in Fig. 3c), additional parameters would be required to account for the complex mutational landscapes found in practice for some antibiotics: This might (for example) include defining D_γ as an explicit function of γ to account for features such as fitness valleys in adaptive processes (Greulich et al., 2012) though any such function would be highly antibiotic- and resistance strategy-dependent (Ogbonugafor and Eppstein, 2016; Bollenbach, 2015). Likewise, in some cases an upper bound on the effectiveness of certain resistance strategies may be present (Toprak et al., 2012; Lukačičinová and Bollenbach, 2017), and (for adaptive evolution in general) certain paths may lead to sub-optimal outcomes (Dickinson et al., 2013). Models similar to ours may be useful in describing the trapping of evolution in sub-optimal fitness states (de Vos et al., 2013), which may be realised experimentally using chemical/environmental factors to steer adaptive processes down sub-optimal paths (Furusawa et al., 2018; Gifford et al., 2018).

In recent years it has become apparent that the development of bacterial resistance to antibiotics can be significantly accelerated by spatiotemporal variation in antibiotic concentration. We have developed a mathematical description of these processes, and applied it to analyse a diverse range of experimental results. Our model provides a framework for informing and analysing future studies of the evolution of antibiotic resistance, and may be applicable to many similar adaptive processes in which the rate of fitness cost-driven drift towards a wild type state (here described by θ) can be assumed to be independent of the trait's value (γ).

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

H. Steel is supported by the General Sir John Monash Foundation. A. Papachristodoulou is supported by EPSRC project EP/M002454/1.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtbi.2019.110077.

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