On repeatability and directionality of collateral effects of drug resistance evolution

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Abstract

Evolution of drug resistance to one drug can alter the minimum inhibitory concentration to another drug. This phenomenon, known as a collateral effect, can manifest as either cross-resistance or collateral sensitivity. Various patterns of collateral effects have been observed experimentally. Repeated adaptation from the same parental strain may result in variable collateral effects; this is non-repeatability. Additionally, adaptation of a pathogen to one drug may produce a specific collateral effect to a second drug, while altering the order of drug exposure can result in a different, or even absent, collateral effect. This phenomenon is termed unidirectionality. The genetic and evolutionary mechanisms underlying these patterns remain incompletely characterised. Here, we propose a framework that integrates pharmacodynamics and population genetics and provide minimal examples to explain these patterns and their combinations. Furthermore, we demonstrate that drug concentration and selection regime strongly influence patterns of collateral effects, including repeatability, directionality, and their temporal dynamics.

1 Introduction

The evolution of drug resistance is a widespread problem that affects a wide range of pathogens and hosts. Despite substantial biological differences among both hosts and pathogens, the evolutionary processes driving the emergence and spread of resistance are fundamentally similar.

In this article, we focus on the evolution of collateral effects, which result when adaptation to one drug alters susceptibility to another. Collateral effects can be either beneficial to the pathogen, termed **cross-resistance**, or detrimental to it, referred to as **collateral-sensitivity** (Casier et al., 2025). Cross-resistance has been documented in multiple experimental studies, and is often, but not always, associated with chemical similarity between drugs (Barbosa et al., 2017; Lázár et al., 2014). Cross-resistance can facilitate multidrug resistance, and it is essential to consider it in order to optimise drug treatment.

On the other hand, collateral sensitivity may be exploited by using drugs cyclically or sequentially to improve treatment success (Nichol et al., 2015; Aulin et al., 2021; Kim et al., 2014). When resistance to one drug evolves, the pathogen becomes more susceptible to another drug, which can then be administered. The pathogen is then more likely to be eradicated by the second drug due to its increased susceptibility to it. Cross-resistance can occur when drug pathways overlap between the two drugs, or when a mutation influences multiple pathways (pleiotropy), which confer resistance to different drugs (Alekshun and Levy, 2007). A specific

example of the former includes mutating a multidrug efflux pump regulator in *Pseudomonas aeruginosa* (Cao et al., 2004). Conversely, collateral sensitivity may result from several mechanisms, including increased drug toxicity or uptake (Roemhild et al., 2020).

Collateral effects of drug resistance evolution receive most attention in studies of bacteria and antibiotic resistance, even though these effects are general and have been documented in multiple classes of pathogens as well as in cancer (Hellinga et al., 2024; Carolus et al., 2024; Ogbunugafor et al., 2016; Zhao et al., 2016). Primarily, studies of collateral effects use large-scale experimental adaptation to different drugs. A parental bacterial strain is evolved in one drug environment, and then the response of the adapted population is measured against a different drug. These studies show diverse collateral effect patterns, particularly in their repeatability, directionality, and temporal dynamics (Sakenova et al., 2025; Maltas and Wood, 2019; Lázár et al., 2014; Nichol et al., 2019; Imamovic and Sommer, 2013; Barbosa et al., 2017; Lázár et al., 2014). Experimental studies often identify mutations conferring resistance and quantify collateral effects as the shift in the minimum inhibitory concentration (MIC).

Defining the patterns of collateral effects of drug resistance

Here, we define a collateral effect as a shift in the minimum inhibitory concentration (MIC) value for a drug other than the one to which the pathogen has adapted. For clarity and simplicity, we will refer to the two drugs as drug A and drug B in the following text, without further specification of their exact nature. In this article, we mainly focus on the patterns of repeatability and directionality of collateral effects.

Repeatability of a collateral effect means that if evolution is repeated from the same parental strain and under the same conditions in drug A, and this strain adapts (evolves resistance), we will *consistently* observe *the same* collateral effect when exposing the adapted population to drug B.

Non-repeatability of a collateral effect means that if we repeat evolution using the same parental strain and under the same conditions in drug A, the resulting adapted populations may display *different* collateral effects when exposed to drug B, or some may show *no collateral effect* at all (Barbosa et al., 2017; Nichol et al., 2019).

Unidirectionality occurs when a parental strain evolving resistance to drug A leads to a particular collateral effect in drug B, but the reverse is not true: evolving resistance to drug B does not lead to the same effect in drug A, or does not lead to any collateral effect in drug A.

Bidirectionality means that adaptation to drug A or drug B results in the same collateral effect in the other drug, regardless of which drug is used first.

Often, collateral effects are conceptualised through fitness landscapes with distinct peaks in drug A, where different peaks correspond to different or no collateral effects in drug B (Nichol et al., 2019). These fitness landscapes for combinatorially complete sets of genotypes have even been mapped experimentally: sometimes for single drugs (Papkou et al., 2023; Das et al., 2020; Palmer et al., 2015; King et al., 2025) and rarely for multiple drugs (Mira et al., 2015; Ogbunugafor et al., 2016).

Several theoretical and computational approaches have considered collateral effects of drug resistance when comparing different treatment regimes. Some theoretical models studying cross-resistance assume that a single mutation or trait can confer resistance to two different drugs (Hobbs et al., 2023; Caprio and Suckling, 2000). Uni- and bidirectional collateral sensitivity has been modelled for various treatment regimes in bacteria (Aulin et al., 2021). Other models consider arbitrary fitness landscapes in both drug environments (Maltas et al., 2021). Additionally, some rely on experimentally measured fitness landscapes but do not account for different

drug concentrations or different selection regimes (Nichol et al., 2015). Finally, some models are statistical rather than mechanistic, using a data-driven approach to predict collateral effects of drug resistance evolution (Zalounina et al., 2007).

Despite the importance of the collateral effects of drug resistance across clinical and agricultural settings, the genetic architecture combined with evolutionary conditions that lead to collateral effect patterns of repeatability, directionality (sometimes denoted reciprocity), and their temporal dynamics are not yet fully described. In this study, we aim to define a simple theoretical framework to explain multiple experimental observations: uni- and bidirectional, as well as repeatable and non-repeatable collateral effects of drug resistance evolution. Both of these patterns are relevant to define the clinical applicability of collateral sensitivity. Our approach links dose-response curves (pharmacodynamics) to multi-locus population genetics. We demonstrate our reasoning using minimal examples with the fewest loci needed to explain these patterns and discuss the underlying assumptions. Moreover, we show that the temporal dynamics of these collateral effects are a natural consequence of our assumptions.

Importantly, we will show that evolving populations in different drug concentrations can lead to distinct patterns of collateral effects, which may depend on the population and mutation dynamics, here denoted as the selection regime: a) strong selection, weak mutation regime (selective sweeps), and b) clonal interference (competition between mutants). Our approach allows for an intuitive understanding of the assumptions necessary for the phenomena of (non-)repeatable and uni- or bidirectional collateral effects to arise.

2 Model framework

Our model assumes that all changes in drug response are genetic and heritable, and not physiological (Roemhild et al., 2018). We base our model on a combination of pharmacodynamics and population genetics, as reviewed in Witzany et al. (2023). This modelling approach has already been applied to a single-drug environment for models of drug resistance evolution in bacterial biofilms and worms (Trubenová et al., 2022, 2025) as well as to a two-drug environment (Engelstädter, 2014).

Pharmacodynamics is used to describe the relation between drug concentration and some fitness measure of a population, e.g., its growth rate. It is captured by a dose-response curve (Equation 1 and Figure 1) (Regoes et al., 2004):

$$\psi = \psi_{\max} - \frac{(\psi_{\max} - \psi_{\min})(\frac{c}{MIC})^{\kappa}}{(\frac{c}{MIC})^{\kappa} - \frac{\psi_{\min}}{\psi_{\max}}} \tag{1}$$

where ψ is the net growth rate, ψ_{max} is the maximal growth rate in the absence of a drug, ψ_{min} is the minimal growth rate (often negative), c is the drug concentration, MIC is the minimum drug concentration required to inhibit growth (i.e. net growth rate of the population is zero) and κ defines the steepness of the decline in growth rate as drug concentration increases. While often used to describe the growth of bacteria in an environment containing antibiotics, the dose-response curve can be modified for other organisms, such as insects and parasitic worms, for example, to describe the survival probability or inhibition of larval development (Caprio, 1998; Dobson et al., 1996).

Population genetics linked to pharmacodynamics describes the effect of mutations on the dose-response curve. We assume that resistance can be encoded by multiple loci (Nichol

et al., 2019; Ogbunugafor et al., 2016), and that each locus has only one possible mutation, i.e., there are two alleles per locus: wild type, represented by 0, and mutant, represented by 1. Any mutation corresponds to a possible shift in MIC to a given drug, either as an increase relative to wild type, thus corresponding to resistance, or a decrease, corresponding to increased sensitivity. Mutations can shift the MIC in different manners in different drugs. For instance, a mutation in one locus can increase the MIC for one drug, while leaving it unchanged, decreasing, or increasing it for another. We will show this link using mutation-MIC shifts (specific genotype-phenotype maps, represented as tables with pink and green row colours, see an example in Figure 2 and all scenarios in Figure 4). These depict how different loci, when mutated, affect the MIC of different drugs. An empty cell refers to the absence of an effect on the MIC when mutating this locus.

Additionally, mutations may reduce the maximum growth rate, but this is not always the case. This decrease in $\psi_{\max}$ is referred to as a cost. The cost of resistance is often (Andersson and Hughes, 2010), but not always present in adapted strains tested *in vitro* (Dunai et al., 2019; Vogwill and MacLean, 2015).

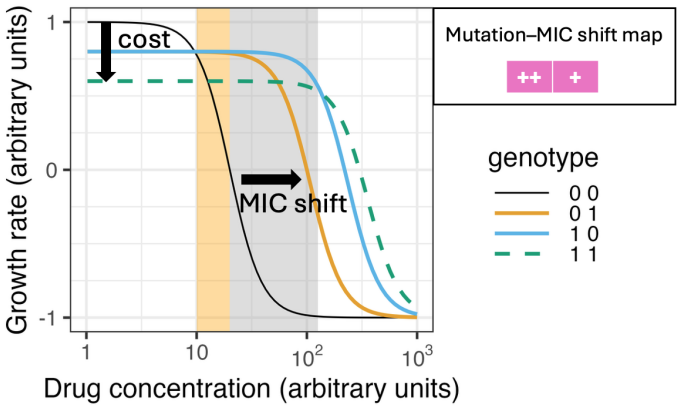

Figure 1: **Dose-response curve example for two-locus drug resistance**. Here, we assume additive MIC shifts and additive costs, lowering ψ_{max}, in the case of two mutations (note the log-scaled x-axis). Note that this figure does not include examples of mutations that induce sensitivity, i.e., lower the MIC compared to wild type, 00. Two mutant selection windows are shown, the orange one where both single mutants (orange, 01, and blue, 10, genotype) are similarly fit and the grey one where the high-resistance single mutant (blue, 10 genotype) is fittest. Mutation-MIC shift (table on the top right): the columns refer to mutations at different loci; each column is a locus. The sign in each cell shows the mutational effect: + means resistance, and ++ refers to a larger increase in MIC than +.

Mutant selection window

A mutant selection window (MSW) defines the drug concentration range where some mutant(s) outcompete the wild type, have a positive growth rate, and are thus selected for. In Figure 1, the orange MSW shows the concentration range where both single mutants are similarly fit, and the double mutant escapes extinction. The grey MSW shows where the high-resistance single mutant (blue) is fittest, and where the double mutant and initially the low-resistance single mutant escape extinction. Concentrations higher than the grey area, but lower than the intercept of the double mutant's dose-response curve with zero net growth rate, define the MSW where the double mutant is fittest, and initially, the high-resistance single mutant escapes extinction.

In all our illustrations, we assume that multiple mutations combine additively to change the two aforementioned parameters, MIC and ψ_{max}, thus modifying the dose-responsecurve. For simplicity, we additionally assume that the steepness of the dose-response, κ, and minimal growth rate, $\psi_{\min}$, are not affected by mutations. It has been shown experimentally that the steepness of the dose-response curve can remain the same across different resistant strains (Das

et al., 2020; Chevereau et al., 2015), though the opposite can also be true (Antunes et al., 2024; Sampah et al., 2011).

In this study, we explain how, by defining the dose-response curves of the same genotypes in two drug environments, we can describe uni- or bidirectional and (non-)repeatable collateral effects (Figure 1 and summarised using mutation-MIC shifts in Figure 4). Below, we expand on the necessary reasoning and assumptions, such as drug concentration, the selection regime, and the cost of resistance.

3 Minimal examples

We present minimal examples that use the fewest loci needed in order to obtain the patterns of (non-)repeatability and uni-/bidirectionality. We will consider adaptation to a single drug and drug concentration, and the evaluation of the collateral effect of the resulting adapted strain. In this section, we consider a drug concentration where the single mutant is the fittest, due to the cost of resistance. We will sometimes comment on a scenario without cost, where the double mutant would be similarly fit to the single mutant at certain drug concentrations. In addition, we assume that mutations are frequent enough to rescue the population from extinction at least sometimes. Finally, we evaluate the collateral effect after a sufficiently long time period, such that the population's genotype composition is stable.

Only bidirectional, repeatable cross resistance can be explained by a single locus

The simplest scenario to explain is **bidirectional, repeatable cross-resistance**, where a single locus confers resistance to both drug A and drug B (Figure 4a, dark blue triangle, Figure S1a). Evolution in an environment containing a drug at a concentration within the mutant selection window will lead to this mutant outcompeting the wildtype and fixing in the population, in any repeated experiment with successful adaptation. Therefore, this scenario is repeatable. The single mutant is resistant to both drugs and will be present regardless of whether the pathogen adapts to drug A or drug B. Therefore, this scenario is bidirectional. This, or a similar structure in which a single locus or trait correlates resistance to one drug with resistance to another, has been used in models of insecticide resistance evolution (Caprio and Suckling, 2000; Hobbs et al., 2023). They compared different treatment strategies for different levels of cross-resistance.

In contrast, a scenario where adaptation to either drug always confers collateral sensitivity to the other drug, denoted **bidirectional, repeatable collateral sensitivity**, requires at least two loci. One locus that gives resistance to drug A and collateral sensitivity to drug B, and another locus that gives resistance to drug B and collateral sensitivity to drug A (Figure 4a, light blue triangle, Figure S1b). Selection by either drug on a wildtype population will select for the locus that gives collateral sensitivity to the other drug as this is the only resistance mutation available. This thus leads to bidirectional, repeatable collateral sensitivity.

Unidirectional cross-resistance requires low and high-resistance mutants

Unidirectional collateral effects occur when adaptation to an environment containing drug A leads to a particular collateral effect in drug B, but adaptation to an environment containing

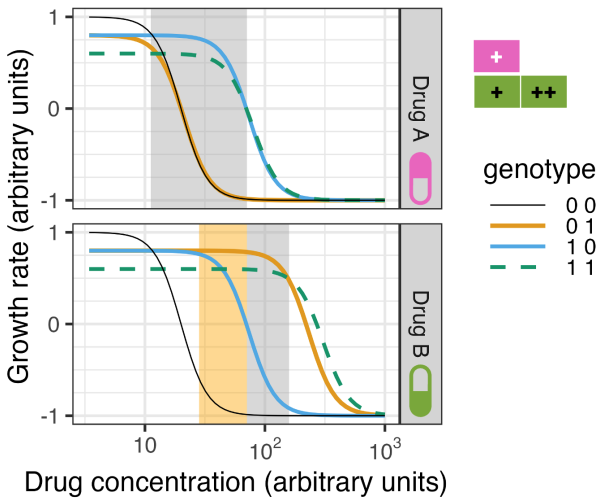

Figure 2: **Unidirectional, repeatable cross-resistance.** Dose-response curve example for two drug environments with corresponding mutation-MIC shift. Mutation-MIC shift: the mutation effect at this locus (column) in drug A (pink top row) and drug B (green bottom row). + means resistance compared to wild type. ++ refers a larger increase in MIC than +. The grey mutant selections windows denote drug concentrations that can lead to cross-resistance only if the population adapted to drug A first (blue, 10 genotype), but not if it adapted to drug B first (orange, 01 genotype). The orange mutant selection window for drug B is the concentration range where competition between mutant strains is necessary to always fix the genotype without a collateral effect in drug A (orange, 01 genotype).

drug B does not lead to the same collateral effect in drug A. Hence, this collateral effect is only present in the direction A to B and not B to A.

Unidirectional, repeatable cross-resistance can be explained by assuming that the mutation conferring resistance to drug A is associated with low resistance to drug B, while the mutation that carries high resistance to drug B is not associated with resistance to drug A (Figure 2, summarised in Figure 4b, dark blue triangle). This scenario thus implies that the cross-resistance is low compared to the resistance that could be achieved if the population adapted to a higher concentration drug B environment.

For unidirectional cross-resistance, it is necessary that the mutation conferring resistance to both drugs is unlikely to be present in the adapted population. This outcome can occur for two reasons: either the low-resistance mutation is too minor to provide enough resistance for the mutant to survive (extinction regime), or a high-resistance mutant is likely to emerge and outcompete the weaker mutant before it reaches fixation (competition regime). The extinction regime occurs when the drug concentration lies within the grey mutant selection window for drug B in Figure 2; while in the orange mutant selection window, competition is necessary to prevent cross-resistance. After adaptation to drug B, whether through competition between mutants or extinction, only the high-resistance single mutant (orange, 01 genotype, in Figure 2), lacking cross-resistance to drug A, will remain. In contrast, adaptation to drug A will always involve a genotype that exhibits cross-resistance to drug B. Hence, this defines unidirectional, repeatable cross-resistance.

Note that we assume that there is a cost to resistance that is higher for the double mutant than for the single mutants, so the double mutant should not invade the adapted population. If there were no cost of mutations, the double mutant could sometimes fix by drift, in which case this scenario would become non-repeatable cross-resistance from B to A, but remain repeatable

cross-resistance from A to B.

Unidirectional, repeatable collateral sensitivity is easier to explain. For drug A, a single mutation confers resistance and simultaneously causes collateral sensitivity to drug B. In contrast, resistance to drug B arises from a different mutation, which does not alter the MIC for drug A. (Figure 4b, light blue triangle). Hence, adaptation to drug A leads to collateral sensitivity to drug B, but not vice versa.

Non-repeatability relies on different resistant genotypes having different collateral effects

Several experimental studies have demonstrated that collateral effects may vary when adapting to one drug — even when experiments are conducted under identical conditions and from the same parental strain (Nichol et al., 2019; Barbosa et al., 2017). This phenomenon is referred to as the non-repeatability of collateral effects during the evolution of drug resistance. Its emergence requires that different resistant genotypes can fix, and at least some of these genotypes have distinct collateral effects.

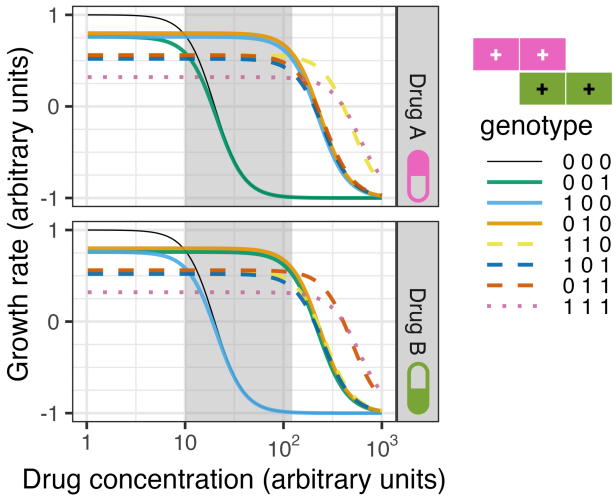

Figure 3: **Non-repeatable, bidirectional cross-resistance.** Dose-response curve example for two drug environments with corresponding mutation-MIC shift. Mutation-MIC shift: the mutation effect at this locus (column) in drug A (pink top row) and drug B (green bottom row). + means resistance compared to wild type. The grey mutant selection window denotes drug concentrations that can *sometimes* lead to cross-resistance in both directions, but not always, depending on which mutant fixes, hence it is non-repeatable. Note that genotypes 100 (blue) and 010 (orange) have the same dose-responsecurve in drug A, and 010 (orange) and 001 (green) the same in drug B. The curves were shifted slightly such that overlapping dose-response curves could be distinguished.

Non-repeatable, bidirectional cross-resistance means that both adaptation to drug A as well as to drug B does not always lead to cross-resistance to the other drug. Bidirectional, non-repeatable cross-resistance can be explained by assuming that at least three loci can be mutated, two that confer resistance to drug A and two possible mutations that confer resistance to drug B (Figure 3, summarised in Figure 4c, dark blue triangle). However, only one of these mutations gives resistance to both drugs. Again, we assume a drug dose where the single mutants are fitter than both the wildtype and double mutant (e.g. grey mutant selection window in Figure 3).

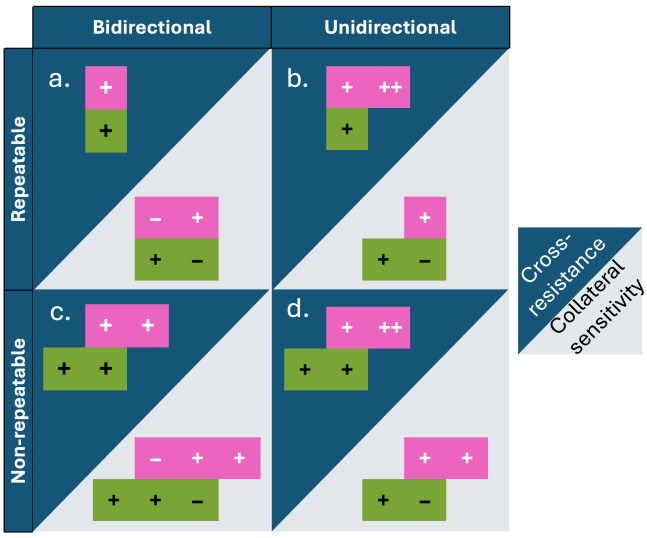

Figure 4: **Overview table of minimal examples of mutation-MIC shifts for two drugs that can lead to different directionality and repeatability for collateral sensitivity and cross-resistance** depending on drug dose, cost, and selection regimes. Note that some scenarios are excluded: repeatable in one direction, not in the other, as well as mixes of collateral sensitivity and cross-resistance. The dark blue upper triangle in each cell denotes cross-resistance, and the light blue lower triangle denotes collateral sensitivity. In each triangle, the columns refer to mutations at different loci, each column is a locus. The rows refer to the effect of the mutation in drug A, pink top row, and drug B, green bottom row. + means resistance and - sensitivity compared to wild type. ++ refers a larger increase in MIC than +.

Non-repeatability can arise when selection is strong enough, and mutations are rare enough for either resistant single mutant to fix before the other one arises. In this case, the fixed genotype may or may not exhibit cross-resistance, leading to non-repeatability. This happens no matter which drug the wildtype population adapted to, leading to bidirectionality.

Alternatively, if mutations are more frequent or selection is not strong enough, for non-repeatability to occur, it is still necessary that either mutation can be fixed. This means that drift, rather than selective sweeps, must ensure fixation, for example, when both single mutants are of similar fitness or the population size is small.

Note that we again assume that single-resistant mutants would outcompete the double-resistant mutant due to the cost of mutations. In the absence of a cost of resistance, the double mutant could drift in frequency. As the double mutant has a collateral effect, for this scenario to remain non-repeatable, the double mutant should not always be present.

Non-repeatable, bidirectional collateral sensitivity is explained in the same way, but requires at least four loci. Two mutations confer resistance to drug A, and only one of them also confers collateral sensitivity to drug B. Another two mutations do the same for drug B. These scenarios, for bi-/unidirectional non-repeatable collateral sensitivity, can be found in Figure 4c and d, light blue triangles.

The degree of repeatability, or the probability of exhibiting a particular collateral effect, can depend on various factors. First, it depends on the number of possible genotypes that confer resistance and have different collateral effects, among which the pathogen cannot transition without undergoing a decrease in growth rate (i.e., fitness peaks). Second, it depends on the probability of clonal interference as opposed to selective sweeps. Third, it is influenced by the probability of a mutation at each locus. Bailey et al. (2017) found that larger population sizes correlate with higher repeatability. Finally, genetic drift also plays a role. We discuss some of

these factors below in greater detail.

4 Temporal dynamics of collateral effects

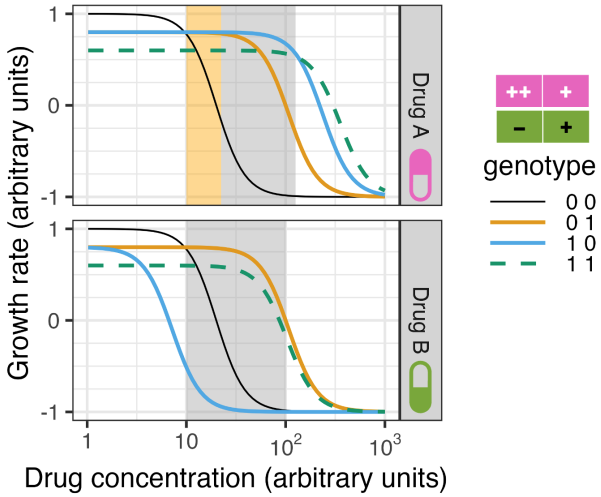

Figure 5: **Mixed cross-resistance and collateral sensitivity.** Dose-response curve example for two drug environments with corresponding mutation-MIC shift. The grey mutant selection window denotes drug concentrations that can lead to collateral sensitivity in one direction (A → B) (blue, 10 genotype) and will lead to cross-resistance in the other direction (B → A) (orange, 01 genotype) (reasoning is found in Section 3). The orange mutant selection window can lead to collateral sensitivity (blue, 10 genotype) or cross-resistance (orange, 01 genotype) in one direction or the co-existence of both phenotypes (A → B).

In our study, we define temporal dynamics as the dependence of the collateral effects in drug B on the time that the population was allowed to adapt to a constant concentration of drug A. If we now relax the assumption that the single mutant is the fittest, the temporal dynamics can become more complex. For instance, we can assume that the double mutant is either the fittest or equally fit as other mutants for certain drug concentrations. For example, in Figure 5 for drug A, when dosing where the double mutant is fittest. Here, the population can go through the single low-resistance mutant, which shows cross-resistance to drug B, towards the double mutant, which still shows cross-resistance to drug B (this depends on how mutational effects combine and how large both single effects are). Alternatively, the population can go through a single high-resistance mutant that shows collateral sensitivity to drug B, and then evolve to a double mutant that shows cross-resistance to drug B.

Another example depends on competition between mutants. Assume a scenario where one resistance mutation confers cross-resistance, and another mutation confers collateral sensitivity to another drug (Figure 5). Within the grey mutant selection window, the fittest mutant in drug A, genotype 10, shows collateral sensitivity to drug B. In a selection regime where competition between mutants is expected, both single mutant strains, 01 and 10, will coexist at some times. Thus, part of the population will show collateral sensitivity, and part will show cross-resistance to drug B. This would be transient until the fitter population, here corresponding to collateral sensitivity, outcompetes the less fit population.

5 Implications of changing selection regime or drug concentration

Mutation rate and selective sweeps can change directionality

Unidirectional cross-resistance can be explained by the presence of both low and high resistance loci, and either a competition or extinction selection regime, see Figure 2 and Section 3. However, if we consider a situation where mutations are rare, this decreases clonal interference and supports selective sweeps. Within the mutant selection window, where both single mutants outcompete both the double mutant and the wildtype, when selective sweeps occur, either of the single mutants may fix in the population.

Because these mutants have different collateral effects, the resulting cross-resistance is non-repeatable in the direction we described and remains repeatable in the other. While the genetic basis is the same as in the scenario of unidirectional, repeatable cross resistance shown in Figure 2, the change in the selection regime altered the repeatability and directionality pattern to become bidirectional: non-repeatable in one direction and repeatable in the other.

Increasing drug concentration can change repeatability patterns

Increasing the drug concentration can imply that the double mutant constitutes the fittest genotype, depending on how mutational effects combine (see Figures 2 and 3). In such a case, if the population avoids extinction, a double mutant will eventually arise and outcompete the rest, fixing in the population.

This has two consequences. First, the evolutionary outcome from Figure 3, which was non-repeatable, becomes repeatable. The double mutant shows a collateral effect to the other drug, hence this is repeatable if enough time is allowed to pass. Second, unidirectional cross-resistance from Figure 2 becomes bidirectional, repeatable cross-resistance.

6 Conclusion

In this study, we build minimal examples that use the least number of loci needed to describe combinations of repeatable and non-repeatable, uni- or bidirectional patterns of cross-resistance and collateral sensitivity, as shown in Figure 4. We show that these patterns can change with drug concentration and selection regime. Furthermore, we demonstrate that dose-response curves for identical genotypes, measured across different single-drug environments, reveal how specific drug concentrations and selection regimes influence the emergence of various collateral effects of drug resistance patterns: repeatability, directionality, and temporal dynamics.

Our study highlights the importance of experimentally measuring the full dose-response curves of resistant genotypes in multiple drug environments. This information can then be used to inform expected patterns of collateral effects when combined with information on population and mutation dynamics. We hope that this framework will be useful for understanding the patterns of repeatability and directionality of collateral effects for both theoretical modellers and experimental biologists studying the evolution of drug resistance, not only in bacteria, but across taxonomic boundaries.

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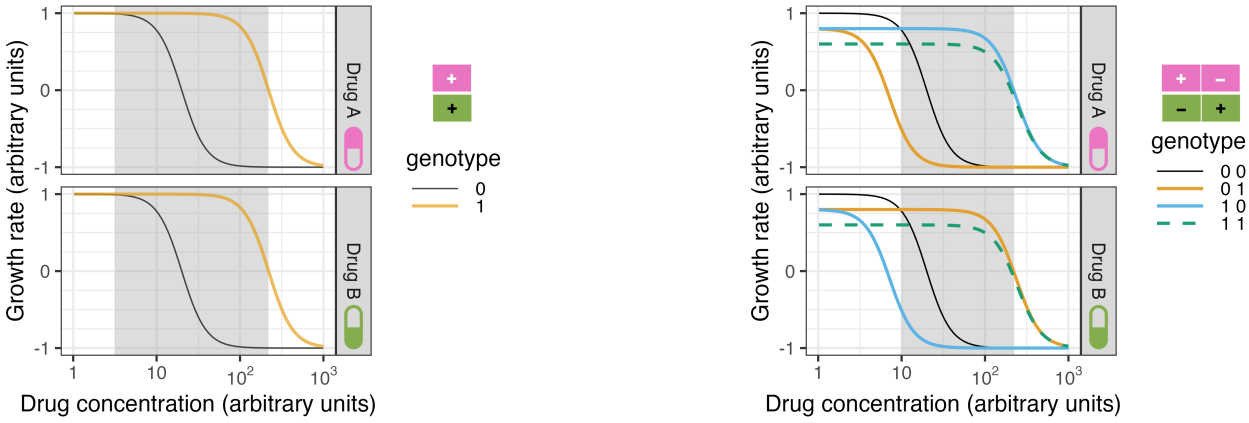
7 Supplementary

Bidirectional, repeatable in one direction, non-repeatable in the other cross-resistance can be explained by assuming that there are two possible mutations. Two of these mutations confer resistance to drug A, but only one confers resistance to drug B. This corresponds to the dose-response curves in Figure 3 without the third locus. Following the same reasoning and assumptions as Section 3, for an intermediate drug concentration range, adaptation to drug A can sometimes but not always lead to cross-resistance to drug B, and adaptation to drug B will always lead to cross-resistance to drug A.

The code used to create the illustrations is found in the following github repo



(a) (b)

Figure S1: Dose-response curve examples for two drug environments with corresponding mutation-MIC shifts that can lead to different collateral effect patterns. Mutation-MIC shift: the mutation effect at this locus (column) in drug A (pink top row) and drug B (green bottom row). + means resistance compared to wild type. - means increased susceptibility compared to wild type. (a) **Bidirectional, repeatable cross-resistance.** The grey mutant selection window denotes drug concentrations that will lead to cross-resistance if the population adapted to either drug (orange, 1 genotype). (b) **Bidirectional, repeatable collateral sensitivity.** The grey mutant selection window denotes drug concentrations that will lead to collateral sensitivity if the wildtype population adapted to either drug A (blue, 10 genotype) or drug B (orange, 01 genotype).

ψ_{max}	1
ψ_{min}	-1
κ in Figure 2	4
κ in all other figures	3
cost per locus	0.2
MIC_{wildtype}	20
MIC shift of low benefit loci in Figure 2	50
MIC shift of low benefit loci in all other figures	80
MIC shift of high benefit loci	200
MIC shift of collateral sensitivity locus	-13.3

Table S1: **Parameters of illustrated dose-response curves.** The parameters of Equation 1, used in all figures, are shown in Table S1.