Collateral effects of drug resistance evolution: explaining repeatability and directionality patterns

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Abstract

Drug resistance evolution is hindering the treatment of various diseases. One possible solution could be exploiting trade-offs in resistance to different, existing drugs. Collateral sensitivity means that resistance to one drug increases susceptibility to another. Contrarily, cross-resistance means that resistance to one drug implies resistance to another. These two collateral effects are, however, not always repeatable, nor are they always independent of drug order, i.e. bidirectional. Understanding what drives repeatability and directionality would help define the clinical applicability of collateral sensitivity and avoid cross-resistance. Nevertheless, the genetic and evolutionary mechanisms causing non-repeatability and unidirectionality patterns are not yet fully understood. In this study, we aim to define which drug concentrations, population dynamics, and genetic architectures cause these patterns of collateral effects. We describe the fewest loci and conditions needed for repeatable and non-repeatable, uni- or bidirectional cross-resistance and collateral sensitivity to occur. We show that increasing drug concentration narrows the set of possible adaptive genotypes and thereby increases repeatability. As for selection regimes, clonal interference can explain unidirectional cross-resistance and can increase repeatability, whereas a strong selection, weak mutation regime increases non-repeatability. Overall, we show how non-repeatability and unidirectionality of collateral effects are not properties solely of a drug pair but also of the selection regime: drug dose and population dynamics. Further studies, combining extensive mathematical modelling with measurements of full dose-response curves for drug pairs with known patterns of collateral effects, are needed to shed more light on this problem.

1 Significance statement

Predicting the evolution of multidrug resistance is one of the most pressing challenges in modern evolutionary biology and medicine. On the other hand, exploiting "collateral sensitivity" (where resistance to one drug increases susceptibility to another) is a promising direction for sustainable therapies. Collateral effects are often unpredictable and may depend on the order of drug administration (asymmetry), which hinders their clinical applications. While research traditionally treats collateral effects and their patterns as fixed properties of specific drug pairs, we demonstrate how they are fundamentally shaped by the selection environment.

2 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. Limitations to exploit collateral sensitivity include its predictability and knowing which drug pair in which order to administer (Casier et al., 2025).

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 repeated 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).

Some experimental adaptation studies have investigated selection regimes by changing the gradient at which drug concentration is increased (Brepoels et al., 2022; Chauhan et al., 2025) and additionally, also the maximal drug concentration (Oz et al., 2014; Jahn et al., 2017) or by only changing the maximal drug dose by phenotyping an adapting population at different time points (Maltas et al., 2025). Another study exposed *P. aeruginosa* to two constant sublethal drug concentrations (Sanz-García et al., 2020). Changing only the concentration gradient did not affect collateral effect profiles (Brepoels et al., 2022) or did so inconsistently across population sizes (Chauhan et al., 2025). Additionally varying the maximal drug concentration does not give consistent results across studies: Oz et al. (2014) finds more cross-resistance and collateral sensitivity with stronger selection gradients, whereas Jahn et al. (2017) does not. Sanz-García et al. (2020) found drug-pair dependant changes in collateral effects as the drug concentration to which bacteria were allowed to adapt, increased.

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).

Classical definitions of 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.

Despite the importance of 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 cross-resistance and collateral sensitivity. 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.

3 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 2a and all scenarios in Figure 3). 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 for this drug, 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).

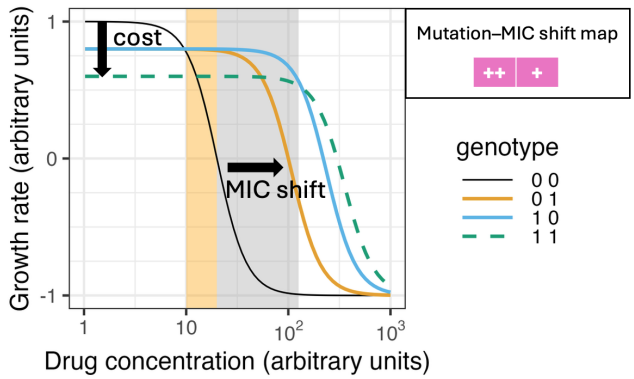


Figure 1: **Dose-response curve example for two-locus drug resistance**. Here, we assume that mutational effects combine additively on MIC shifts and costs, resulting in lower ψ_{max} (log-scaled x-axis). Mutations that decrease MIC are not shown (see Figure 4). Two mutant selection windows are depicted in orange and grey. In the table (top right), each column is a locus that can be mutated; + indicates resistance, ++ a larger MIC increase than +.

Mutant selection window

The 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-response curve (parameter values are given in Table S1). 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). However, note that our reasoning in the rest of this article does not strictly depend on this assumption (see Section S1.1).

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 3). Below, we expand on the necessary reasoning and assumptions, such as drug concentration, the selection regime, and the cost of resistance.

4 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 evaluate 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.

4.1 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 3a, 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 wild type 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.

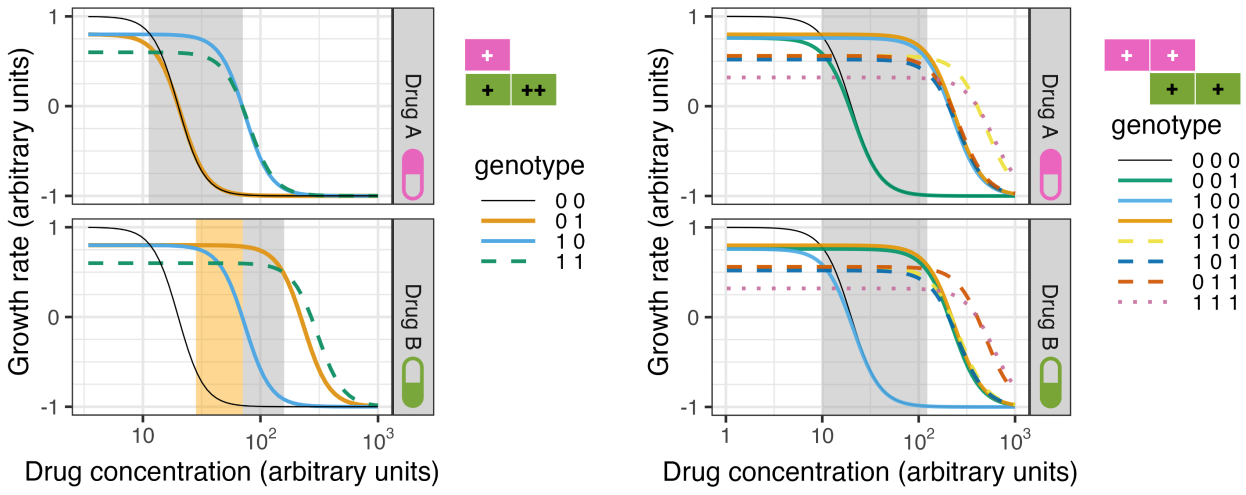
In contrast, a scenario where adaptation to either drug always confers collateral sensitivity to the other drug, denoted **bidirectional, repeatable collateral sensitivity**, requires at least two loci. One locus that gives resistance to drug A and collateral sensitivity to drug B, and another locus that gives resistance to drug B and collateral sensitivity to drug A (Figure 3a, light blue triangle, Figure S1b). Selection by either drug on a wild type 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.

4.2 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 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 2a, summarised in Figure 3b, 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 of drug B.

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 2a; 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 2a), lacking cross-resistance to drug A, will remain. In contrast, adaptation to drug A will always involve a



(a) (b)

Figure 2: We show dose-response curves for two drugs. In the mutation-MIC shift table (top right), columns represent mutable loci; rows indicate drug-specific effects (+ is resistance, ++ is greater resistance). (a) **Unidirectional, repeatable cross-resistance can arise when a low-resistance mutant confers cross-resistance, but a high-resistance mutant does not.** Grey shading marks concentrations where cross-resistance occurs only when adapting to drug A (10 genotype), not drug B (01 genotype). Orange shading for drug B shows where competition fixes the genotype without collateral effect in drug A (01 genotype). (b) **Non-repeatable, bidirectional cross-resistance.** Grey shading highlights concentrations where cross-resistance can occur in both directions, depending on which mutant fixes. Genotypes 100 (blue) and 010 (orange) share the same dose-response curve in drug A; 010 (orange) and 001 (green) share it in drug B. Curves are slightly offset for clarity.

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 3b, light blue triangle). Hence, adaptation to drug A leads to collateral sensitivity to drug B, but not vice versa.

4.3 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.

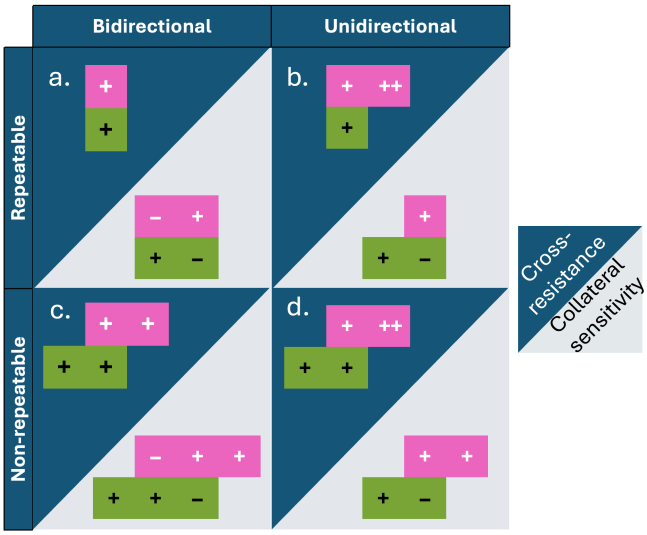


Figure 3: **Overview table of fewest loci with mutational effect size needed to explain directionality and repeatability for collateral sensitivity and cross-resistance** depending on drug dose, cost, and selection regimes. Some scenarios are excluded: repeatable in one direction, not in the other, as 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, mutation-MIC shift tables are shown, columns represent mutable loci; rows indicate drug-specific effects, drug A, pink top row, and drug B, green bottom row (+ is resistance, ++ is greater resistance and - is sensitivity compared to wild type).

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 2b, summarised in Figure 3c, 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 wild type and double mutant (e.g. grey mutant selection window in Figure 2b).

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 wild type 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 3c 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.

5 Temporal dynamics of collateral effects

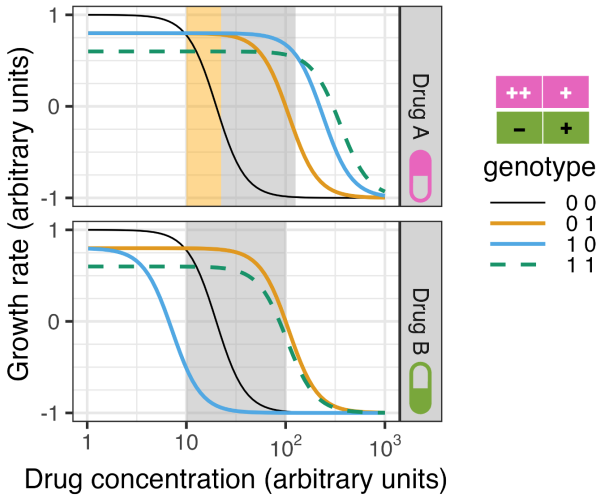


Figure 4: **Mixed cross-resistance and collateral sensitivity can lead to transient collateral effects.** We show dose-response curves for two drugs. The grey mutant selection window denotes drug concentrations that can lead to collateral sensitivity in one direction (A → B) (blue, 10 genotype) and will lead to cross-resistance in the other direction (B → A) (orange, 01 genotype) (reasoning is found in Section 4.2). The orange window can produce collateral sensitivity, cross-resistance, or both phenotypes coexisting (A → B). In the mutation-MIC shift table (top right), columns represent mutable loci; rows indicate drug-specific effects (+ is resistance, ++ is greater resistance, - is increased susceptibility).

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 4 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 4). 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.

6 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 2a and Section 4.2. 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 wild type, 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 2a, 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 2a and 2b). 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 Section 4.3, 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 Section 4.2 becomes bidirectional, repeatable cross-resistance.

7 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 3. We show that these patterns can change with drug concentration and selection regime. Furthermore, we demonstrate that dose-response curves for a combinatorially complete set of genotypes, measured across different single-drug environments, reveal how specific drug concentrations and selection regimes determine the emergence of various collateral effects of drug resistance patterns: repeatability, directionality, and temporal dynamics.

Limitations of this study include not considering complex epistatic patterns and not linking our genetic architecture to the molecular mechanisms of resistance and collateral effects. We also did not consider a changing environment where trade-offs between periods of presence and absence of drug, could define the fittest genotype.

Nevertheless, 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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8 Supplementary material

The code used to create the illustrations is found in the following link: `https://github.com/Mayiiiiii/CollateralEffectsDrugResistance`.

S1.1 Minimal examples of special cases

There are several special, but important cases that were not discussed in the main text due to space limitations.

Bidirectional, repeatable in one direction, non-repeatable in the other cross-resistance can be explained by assuming that there are two possible loci that can be mutated. Both mutated loci confer resistance to drug A, but only one confers resistance to drug B. This corresponds to the dose-response curves in Figure 2b without the third locus. Following the same reasoning and assumptions as Section 4.3, 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.

A special case of competition that leads to unidirectional cross-resistance (Section 4.2) occurs when the low-resistance mutant grows so slowly at a given concentration that it takes a long time to become established. During this extended period, there is a high probability that a high-resistance mutant will emerge from the remaining wild type sub-population and outcompete the low-resistance mutant. This scenario is only likely if the wild type subpopulation has a very slow net death rate and some growth. If the high-resistance mutant appears first, it can quickly become fixed. If the low-resistance mutant appears first, the slow population dynamics mean that the high-resistance mutant is still likely to arise and then outcompete the low-resistance mutant.

Violating the assumptions in Section 3 can still give the patterns we describe. Additive effects of multiple mutations on cost and MIC shift, as well as constant steepness (κ) between mutants, are not the only conditions that allow for the reasoning we used in Section 4 to describe the collateral effect patterns. For example, unidirectional cross-resistance (Section 4.2), for the drug where adaptation does not lead to cross-resistance, requires:

1. a low- and high-resistance mutant. The low one with cross-resistance, and the high one without.

2. It also requires one of two things:

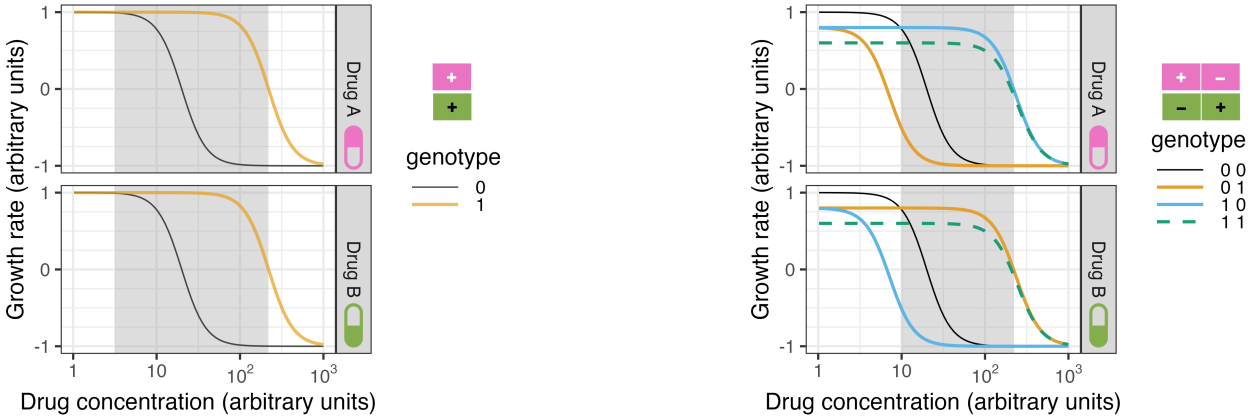
   (a) If the double mutant shows cross-resistance, then the double mutant should be less fit than the high-resistant mutant for some concentration range.

      i. This is the case when the cost of resistance increases when combining these mutations (Figure 2a). However, this cost increase need not be additive.

      ii. Another option is a less steep dose-response curve for the double mutant (an example is found in Figure S2: the same reasoning from Section 4.2 can be applied).

   (b) Alternatively, it requires that the combination of those low- and high-resistance mutations does not lead to cross-resistance, through some epistatic effect.

S1.2 Supplementary figures



(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 the 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 is 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 wild type population adapted to either drug A (blue, 10 genotype) or drug B (orange, 01 genotype).

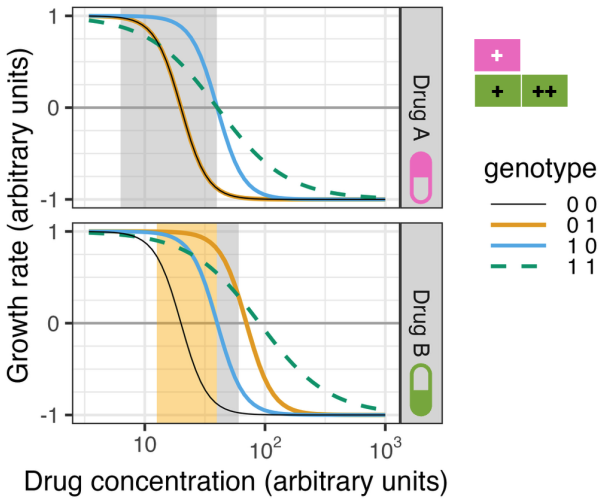


Figure S2: **Unidirectional, repeatable cross-resistance.** Dose-response curve example for two drug environments with corresponding mutation-MIC shift. Mutation-MIC shift: the mutation effect at this locus (column) in drug A (pink top row) and drug B (green bottom row). + means resistance compared to the wild type. ++ refers a larger increase in MIC than +. The grey mutant selection 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). κ is 4 for all genotypes but 1.5 for the double mutant. The first locus shifts the MIC in both drugs by 20, the second locus shifts the MIC in drug B only by 50. There is no cost of resistance.

S2 Supplementary tables

ψ_{max}	1
ψ_{min}	-1
κ in Figure 2a	4
κ in all other figures	3
cost per locus	0.2
$MIC_{\text{wild type}}$	20
MIC shift of low benefit loci in Figure 2a	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.