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THE EVOLUTIONARY EPIDEMIOLOGY OF MULTILOCUS DRUG RESISTANCE

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The evolution of resistance to drugs is a major public health concern as it erodes the efficacy of our therapeutic arsenal against bacterial, viral, and fungal pathogens. Increasingly, it is recognized that the evolution of resistance involves genetic changes at more than one locus, both in cases where multiple changes are required to obtain high-level resistance, and where compensatory changes at secondary loci ameliorate the costs of resistance. Similarly, multiple loci are often involved in the evolution of multidrug resistance. There has been widespread interest recently in understanding the evolutionary consequences of multilocus resistance, with many empirical studies documenting extensive patterns of genetic interactions (i.e., epistasis) among the loci involved. Currently, however, there are few general theoretical results available that bridge the gap between classical multilocus population genetics and mathematical epidemiology. Here, such theory is developed to shed new light on these previous studies, and to provide further guidance on the type of data required to predict the evolution of pathogens in response to drug pressure. Our results reveal the importance of feedbacks between the epidemiological and evolutionary dynamics, and illustrate how these feedbacks can be exploited to control resistance. In particular, we show how interventions such as social distancing and isolation can influence rates of recombination, and how this then can slow the spread of multilocus resistance and increase the likelihood of reversion to drug sensitivity once drug therapy has ceased.

KEY WORDS: **Antibiotic resistance, compensatory mutation, disease, epistasis, pathogen.**

The evolution of drug resistance in pathogens often involves genetic changes at more than one locus. This can occur when multiple changes are required to obtain high-level resistance, when compensatory changes at secondary loci can ameliorate the costs of resistance at a focal locus, or when resistance to multiple drugs evolves (Maisnier-Patin and Andersson 2004; Weinreich et al. 2005; Poelwijk et al. 2007; Yeh et al. 2009; Andersson and Hughes 2010; MacLean et al. 2010). Interestingly, recent studies have also begun to document extensive patterns of genetic interaction (i.e., epistasis) across multiple loci in a variety of microbes, from antibiotic resistance in bacteria (Weinreich et al. 2006; Trindade et al. 2009; Wiesch et al. 2010; Hall and MacLean 2011; Salverda et al. 2011) to drug resistance in HIV (Hinkley et al. 2011), to antigenic escape in influenza (Kryazhimskiy et al. 2011), to evolutionary responses to new conditions (Chou et al. 2011; Kvitek and Sherlock 2011; Khan et al. 2011; Rokyta et al. 2011). Together, these findings call for a better understanding of multilocus evolutionary dynamics within the context of infectious disease epidemiology. There has been considerable work in the population-genetic literature on understanding the evolutionary dynamics of populations when multiple loci affect the trait of interest (Crow and Kimura 1970; Bürger 2000; Kirkpatrick et al. 2002), but surprisingly little has been done integrating multilocus evolutionary dynamics with mathematical models for the dynamics of infectious diseases.

Here, we bridge this gap by developing theory for multilocus population genetics within the context of commonly used compartment models in mathematical epidemiology (Hethcote

2000). This not only allows us to use insights from the population genetic literature to better understand the evolution of multilocus drug resistance, but it also introduces some novel aspects to the evolutionary dynamics of multilocus genotypes, arising from evolutionary-epidemiological feedbacks. Our focus is on developing relatively generic models for the spread of drug resistance, with the view that the very same processes elucidated here will play out in models for specific diseases as well. Furthermore, although our focus is on models for epidemiological dynamics at the population level, the results are equally applicable to the evolution of drug resistance within single, infected, individuals as has been explored in many HIV studies (Bretscher et al. 2004; Althaus and Bonhoeffer 2005; Fraser 2005; Carvajal-Rodríguez et al. 2007).

In what follows, we first present the generic compartment model that forms the epidemiological basis of our analysis, and we then develop the multilocus population-genetic dynamics within this context. After having described this complete evolutionary-epidemiological system, we then examine the role of recombination, epistatic interactions, and heterogeneity in selection (both in space and time), on the evolution of multilocus drug resistance. In particular, we allow selection coefficients for each locus to depend on both parasite transmission and recovery rates, in treated and untreated environments. This yields a general theoretical framework that can be used to shed light on many different scenarios of drug resistance evolution. As will be shown, it also suggests some novel ways in which interventions can be used to control the evolutionary dynamics of resistance.

Evolutionary Epidemiology of Drug Resistance

EPIDEMIOLOGY

We begin by describing the relatively simple epidemiological model on which our results are based. The epidemiological dynamics are described by a classical susceptible-infected-susceptible compartment model (Hethcote 2000; Anderson and May 2001). The susceptible population (whose density is denoted by S) is maintained by a constant influx of new individuals balanced by a constant per capita mortality rate (see Table 1 for notation). Susceptible individuals become infected according to a law of mass action, and if they recover from infection they are once again susceptible. Such a model is applicable to several infectious diseases including most bacterial infections. Upon infection, each individual has a probability τ of being treated with a drug (e.g., an antibiotic), although we will also use the same model for situations in which there are two drugs available, and τ is then the probability that an infected individual is administered drug 1 (as opposed to drug 2).

Allowing for genetic variation in the pathogen in terms of transmission and recovery, the epidemiological dynamics for the total numbers of susceptible, S, infected, I, and treated, T, individuals are given by (Appendix A; Table 1):

$$\begin{aligned}\frac{dS}{dt} &= \theta - \mu S - (\bar{\beta} I + \bar{\beta}^T T)S + (\bar{\gamma} I + \bar{\gamma}^T T)\\ \frac{dI}{dt} &= (1-\tau)(\bar{\beta} I + \bar{\beta}^T T)S - (\mu + \alpha + \bar{\gamma})I \\ \frac{dT}{dt} &= \tau(\bar{\beta} I + \bar{\beta}^T T)S - (\mu + \alpha + \bar{\gamma}^T)T,\end{aligned} \tag{1}$$

where θ is a constant immigration rate of susceptible hosts, μ is the per capita mortality rate of hosts in the absence of infection, β is the transmission rate of the infection from infected to susceptible hosts, γ is the per capita rate of clearance of the infection, and α is the pathogen-induced host mortality rate (i.e., virulence). The superscript "T" indicates parameters specific to the treated subpopulation. For example, drug treatment might decrease transmission and/or increase recovery rate for a given genotype of pathogen. Furthermore, this effect of treatment will typically be different for different genotypes; and thus, the "overbars" in equation (1) indicate averages over all pathogen genotypes.

EVOLUTION WITH RECOMBINATION

Model (1) specifies how the total number of susceptible, infected, and treated hosts change over time. To track the evolutionary dynamics of the parasite, we now need to describe the underlying genetic variation in the pathogen population. For simplicity, we suppose that drug resistance in the pathogen population is governed by two diallelic loci, and denote the four relevant alleles by a/A and b/B. We assume that treatment affects transmission and recovery only, and thus the genotype of the pathogen affects these quantities as well. We denote the effect, on transmission, of carrying the "A" allele instead of the "a" allele, by $\Delta\beta_A$, in an untreated host. Likewise we use $\Delta\beta_B$ for the effect of carrying the "B" allele instead of the "b" allele, and $\Delta\beta_E$ to denote the extra effect (in addition to $\Delta\beta_A$ and $\Delta\beta_B$) of carrying both alleles (i.e., the epistasis in transmission rate). Thus, a pathogen of genotype "AB" has a transmission rate of $\beta + \Delta\beta_A + \Delta\beta_B + \Delta\beta_E$, where β is the baseline transmission rate. Analogous definitions hold for the recovery rate, γ, as well as for these parameters in treated individuals (Table 2).

In order for recombination to occur, a host must first be infected by more than one pathogen strain, and we incorporate this into the model using a superinfection approach (Nowak and May 1994). This approach posits that, upon coinfection, competitive exclusion occurs rapidly relative to the timescale of the epidemiological dynamics at the population level. During this

Table 1. Table of notation
(in all cases a superscipt "*T*" denotes the value in treated hosts).

S	Number of susceptible hosts.
I	Number of infected, untreated, hosts.
T	Number of infected, treated, hosts.
τ	Probability of treatment upon infection.
θ	Immigration rate of susceptible hosts.
μ	Host natural mortality rate.
α	Pathogen-induced mortality (e.g., virulence)
β	Transmission rate ($\Delta\beta_i$ defined in Table 2, $\bar{\beta}$ = average over all pathogen genotypes).
γ	Recovery rate ($\Delta\gamma_i$ defined in Table 2, $\bar{\gamma}$ = average over all pathogen genotypes).
σ	Relative efficiency of superinfection.
p_i	Frequency of allele i.
D	Linkage disequilibrium, LD, between loci.
$\delta^T_{j\|i}$	Probability that second contains allele j given the first contains i; $\delta^T_{j\|i} = (p^T_i\, p^T_j + D^T)/p^T_i$.
r	Recombination rate.
s_i	Selection coefficient i where $i \in \{A, B, E\}$; $s_i = ((1-\tau)S + \sigma I/2)\Delta\beta_i - \Delta\gamma_i$.
s^T_i	Selection coefficient i in treated hosts; $s^T_i = (\tau S + \sigma T/2)\Delta\beta^T_i - \Delta\gamma^T_i$.
$\hat{s}_i$	s_i in absence of recombination; that is, $\hat{s}_i = ((1-\tau)S + (1-r)\sigma I/2)\Delta\beta_i - \Delta\gamma_i$.
$\hat{s}^T_i$	s^T_i in absence of recombination; that is, $\hat{s}^T_i = (\tau S + (1-r)\sigma T/T2)\Delta\beta^T_i - \Delta\gamma^T_i$.
f_{ij}	Frequency of untreated infections with genotype ij.
$\Delta^T_{p_i}$	Allele i frequency difference between pool of propagules coming into the treated hosts from untreated infections, and this frequency in treated infections; that is, $\Delta^T_{p_i} = p_i\frac{\beta_i}{\bar{\beta}} - p^T_i = (p_i - p^T_i) + p_i(\frac{\beta_i}{\bar{\beta}} - 1)$, where $\beta_i = \Sigma_j \frac{f_{ij}}{p_i}\beta_{ij}$ is the average transmission rate of allele i infections.
Δ^T_D	Difference in LD between pool of propagules coming into the treated hosts from untreated infections, and that of the existing treated population; that is, $\Delta^T_D = (\frac{f_{AB}\beta_{AB}}{\bar{\beta}}\frac{f_{ab}\beta_{ab}}{\bar{\beta}} - \frac{f_{Ab}\beta_{Ab}}{\bar{\beta}}\frac{f_{aB}\beta_{aB}}{\bar{\beta}}) - D^T$.

Table 2. Summary of the transmission and clearance rates of each possible genotype in treated and untreated hosts.

		Parasite traits			
		Transmission		Clearance	
		Untreated	Treated	Untreated	Treated
Parasite genotypes					
Wild type	ab	β	β^T	γ	γ^T
Single mutants	Ab	$\beta + \Delta\beta_A$	$\beta^T + \Delta\beta^T_A$	$\gamma + \Delta\gamma_A$	$\gamma^T + \Delta\gamma^T_A$
	aB	$\beta + \Delta\beta_B$	$\beta^T + \Delta\beta^T_B$	$\gamma + \Delta\gamma_B$	$\gamma^T + \Delta\gamma^T_B$
Double mutant	AB	$\beta + \Delta\beta_A + \Delta\beta_B + \Delta\beta_E$	$\beta^T + \Delta\beta^T_A + \Delta\beta^T_B + \Delta\beta^T_E$	$\gamma + \Delta\gamma_A + \Delta\gamma_B + \Delta\gamma_E$	$\gamma^T + \Delta\gamma^T_A + \Delta\gamma^T_B + \Delta\gamma^T_E$

transitory phase of coinfection, the two strains are allowed to recombine with probability r. Thus, after a superinfection event, the strain that occupies the host is either one of the parental strains (each with probability $(1-r)/2$), or it is a recombinant strain with probability r (a simple extension of the model would allow for genotypic differences in within-host competition as well, but we do not pursue these ideas here). We use the parameter σ to denote an infected individual's relative susceptibility to superinfection, with $\sigma = 1$ corresponding to the case where infected hosts are equally susceptible as uninfected hosts, and $\sigma = 0$ corresponding to the case where there is no superinfection (Appendix A).

The evolutionary dynamics are modeled by tracking the allele frequencies at each locus (denoted by p_A and p_B), along with the linkage disequilibrium (LD) or genetic covariance between the two loci (denoted by D). The dynamical equations for the

allele frequencies in the treated population are (Appendix A):

$$\frac{dp_i^T}{dt} = \underbrace{p_i^T\left(1-p_i^T\right)\left(s_i^T+\delta_{j|i}^T s_E^T\right)}_{\text{direct selection}} + \underbrace{D^T s_j^T}_{\text{indirect selection}} + \underbrace{\frac{I}{T}\left(\tau S+\sigma T/2\right)\bar{\beta}\,\Delta_{p_i}^T}_{\text{gene flow}}, \tag{2}$$

where i and j are either allele "A" or "B," s_i^T is the selection coefficient for allele i, s_E^T is the component of the selection due to epistasis (Table 1), and $\delta_{j|i}^T = (p_i^T p_j^T + D^T)/p_i^T$, which is the probability that the allele at the second locus is j, given that the allele at the first locus is i. Finally, $\Delta_{p_i}^T$ is the difference between the frequency of allele i in the pool of propagules coming into the treated hosts from untreated infections, and this frequency in the treated infections (Table 1). The evolutionary dynamics in the untreated population are also as in equation (2) but with the superscripts T removed and the first factor of the third term in equation (2) being $\frac{T}{I}((1-\tau)S+\sigma I/2)$ instead.

The first two terms in equation (2) are the effect of selection on allele i. The factor $p_i(1-p_i)$ is the genetic variance at this locus, and $s_i^T + \delta_{j|i}^T s_E^T$ is the corresponding selection gradient, both through the additive effect of allele i (referred to as direct selection below), as well as through epistatic interactions with allele j at the second locus (referred to as the direct effect of epistasis below). Notice that what matters from the standpoint of evolution is epistasis in fitness which is related, but not equal, to epistasis in the phenotypic traits, β and γ (Table 1). The second term, $D^T s_j^T$, is the indirect effect of selection on allele j at the second locus, on the evolutionary dynamics of allele i at the first locus. Finally, the third term in equation (2) represents gene flow from the untreated to the treated population. The factor $(\tau S+\sigma T/2)\bar{\beta}$ is the sum of the average rate at which new, treated, infections are generated by untreated hosts $\tau S\bar{\beta}$, and the average rate at which already treated, infected, hosts are superinfected by untreated hosts, $\sigma T\bar{\beta}/2$. The quantity $\Delta_{p_i}^T$ is a measure of differentiation and represents the difference in allele frequency between these new infections and the infections already existing in the treated population. This difference can be regarded as being composed of two separate components (Table 1). First, a component $p_i - p_i^T$, that accounts for any difference in allele frequency between the untreated and treated populations. Second, a component $p_i(\beta_i/\bar{\beta}-1)$, that accounts for the fact that the most transmissible genotypes will tend to be the ones most involved in gene flow. This will induce an evolutionary effect even if there is no difference in allele frequency between the two populations, provided that there are genotypic differences in transmission rate (i.e., $\beta_i \neq \bar{\beta}$; Day and Gandon 2006). Finally, I/T scales these effects to account for differences in absolute size between treated and untreated populations.

From equation (2) it can be seen that the evolutionary and epidemiological dynamics interact through the process of gene flow. An examination of the form of the selection coefficients, however, reveals that these dynamics interact through selection as well (Table 1). In fact, the selection coefficients reveal that important differences in the dynamics arise depending on whether pathogen genotypes differ in transmissibility or recovery. If there are genetic differences in transmissibility, then selection coefficients vary with the density of susceptible individuals in the population because a higher availability of susceptible hosts selects more strongly for higher transmission (Day and Proulx 2004; Day and Gandon 2007; Gandon and Day 2007). Notice also that the presence of superinfection (i.e., $\sigma \neq 0$) further increases the strength of selection on transmission because it further increases the size of the pool of hosts that can be infected (Day and Gandon 2006). On the other hand, if pathogen genotype affects the recovery rate only then the selection coefficients are independent of the epidemiological dynamics. Thus, it is important not only to understand how pathogen genotype affects fitness in treated and untreated individuals, but to understand whether these fitness effects act through transmission or clearance (or both).

The equation for the dynamics of LD in the treated population is

$$\begin{aligned}\frac{dD^T}{dt} = &\underbrace{-\sigma r\left(\bar{\beta}^T T+\bar{\beta} I\right)D^T}_{\text{recombination}} \\ &+ \underbrace{\left(\left(1-2p_A^T\right)\hat{s}_A^T+\left(1-2p_B^T\right)\hat{s}_B^T\right)D^T}_{\text{additive selection}} \\ &+ \underbrace{\left(D^T+p_A^T p_B^T\right)\left(\left(1-p_A^T\right)\left(1-p_B^T\right)-D^T\right)\hat{s}_E^T}_{\text{epistasis}} \\ &+ \underbrace{\bar{\beta}\frac{I}{T}\left(S\tau+\frac{\sigma T}{2}(1-r)\right)\left(\Delta_{p_A}^T\Delta_{p_B}^T+\Delta_D^T\right)}_{\text{gene flow}},\end{aligned} \tag{3}$$

where $\hat{s}_i^T$ is the selection coefficient accounting only for infections that do not undergo recombination, and Δ_D^T is the difference between the LD in the pathogen pool that get transmitted from the untreated to the treated population, and that of the existing treated population (Table 1).

The first term in equation (3) accounts for how coinfection, and subsequent recombination, randomizes the association between "A" and "B" and thus decreases the LD. The rate at which this happens depends on the rate of superinfection as well as the recombination rate, r. The second term accounts for how selective changes in allele frequency alone can change the LD, provided that it is nonzero. For example, positive LD will be amplified by positive selection acting independently at each locus. The third term accounts for how epistatic selection can alter the association

between "*A*" and "*B*." For example, if epistasis is positive (i.e., $\hat{s}_E^T > 0$) then "*A*" and "*B*" will do "extra well" when together, and this thereby causes descendant pathogens to have "*A*" and "*B*" associated with one another (i.e., to generate positive LD). Finally, the fourth term accounts for how LD changes through gene flow, when the allelic frequencies or disequilibrium in the pool of "migrants" from the untreated population differs from that of the treated population. As described earlier, the Δ_k^T are measures of differentiation in quantity k between the new infections generated by untreated, infected hosts, and hosts already in the treated class (Table 1).

The model given by equations (1)–(3) is a nine-dimensional (9D) system of dynamic variables, tracking the total number susceptible, infected, and treated hosts, along with the allele frequencies and linkage disequilibria in each type of infection. An alternative formulation, and one that is more common in the literature on infectious disease epidemiology, tracks the number of infections of each genotype (see eq. A1, Appendix A). Each formulation represents the same 9D dynamical system expressed in different variables. There are, however, a couple of advantages to the population-genetic formulation of equations (1)–(3). First, it separates the epidemiological dynamics from the evolutionary dynamics and thus allows one to use insights from the literature in evolutionary biology to understand infectious disease evolution. Second, the population-genetic formulation also more readily lends itself to simplifications under certain parameter regimes. For example, if the rate of recombination is high relative to the strength of selection, then the dynamics of LD will occur quickly relative to those of the allele frequencies. In this case, we can use a separation of timescales argument (i.e., a quasi-linkage equilibrium [QLE] approximation; [Crow and Kimura 1970; Kirkpatrick et al. 2002; Otto and Day 2007]), and express both D and D^T as functions of the other variables, thereby reducing the dimensionality of the system by two (see section below on Temporally Heterogeneous Treatment).

Results

We now use the above model to explore a variety of factors that affect the evolution of multilocus drug resistance, paying particular attention to the potential feedbacks that can occur between the epidemiological and evolutionary dynamics. Our analysis leads to two broad kinds of results. First, we show how this theoretical formalism lends new insight into previous studies of multilocus resistance evolution, both in bacteria and in HIV. Second, we show how the understanding provided by this formalism leads to new suggestions for the control of resistance evolution. Throughout we also use the analysis to highlight the kinds of data that are required to adequately predict resistance evolution.

HOMOGENEOUS TREATMENT

The homogeneous treatment case allows us to explore the effects of recombination and epistasis in the absence of the complicating effects of treatment heterogeneity. For homogenous treatment, we set $\tau = 1$, and the number of untreated individuals is zero. Thus, the third term of equation (2) disappears, as does the final term of equation (3). Interestingly, this reveals that if only clearance is affected by genotype, then the evolutionary dynamics are coupled to the epidemiological dynamics only through the dynamics of how LD decays (the first term in eq. 3). If genotype affects transmission rates, however, then the coupling between evolution and epidemiology is more complex as all selection coefficients again depend on host densities. Again this reveals the importance of knowing, not only the fitness consequences of resistance, but also the way in which these effects arise.

With the above-mentioned simplifications to equation (2), we can see that there are three mechanisms that determine the rate at which drug resistance changes in frequency: (1) "direct selection"—larger fitness differences between alleles (i.e., larger selection coefficients, s_i^T) lead to a faster spread of resistance; (2) "direct epistasis"—positive epistasis leads to a faster spread of resistance through the term $\delta_{j|i}^T s_E^T$. In particular, $\delta_{j|i}^T$ is the probability that the allele at the second locus is a mutant, given the focal allele is mutant. In the absence of any LD, this is equal to the frequency of the mutant allele at the second locus (Table 1). More generally, $\delta_{j|i}^T$ is positive regardless of the extent of LD and therefore positive epistasis always enhances the rate of spread of resistance through $\delta_{j|i}^T s_E^T$; and (3) "linkage disequilibrium"—LD affects the evolutionary dynamics, both through indirect selection (the term $D^T s_j^T$) and through its effects on $\delta_{j|i}^T$. It is well known from population genetics (Crow and Kimura 1970) that significant LD is generated in this context (i.e., in a spatially homogeneous, deterministic model) only if there is epistatic selection and if selection is strong relative to recombination. Moreover, the LD that is generated has the same sign as the epistasis. As a result, positive epistasis will engender positive LD, and this will subsequently increase the rate of spread of resistance through indirect selection, $D^T s_j^T$ (provided $s_j^T > 0$; that is, the mutant allele at the second locus is selectively favored), and though the resulting increase in $\delta_{j|i}^T$.

As just described, epistasis plays a central role in the evolutionary dynamics of resistance both directly, and indirectly through its effects of LD. Such epistasis can arise from a number of different mechanisms. For example, it can occur if there are different, interacting, mechanisms of resistance such that the effect of one mechanism depends on the presence of the other. Alternatively, epistasis can arise when one locus is involved in resistance to a drug and the other locus compensates for the cost of the resistance mutation (Weinreich et al. 2005). Yet, another possibility occurs when treatment involves two drugs that interact

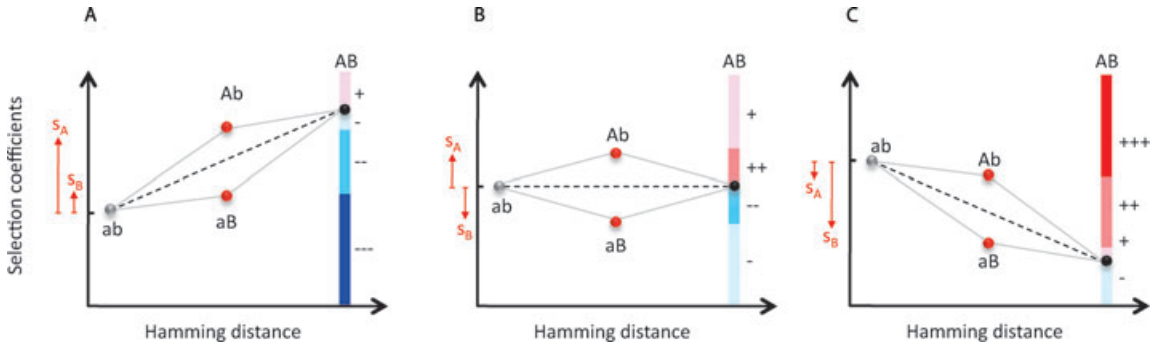

Figure 1. Selection coefficients and different types of epistasis. We plot the fitness of the four different genotypes (see Table 1 for a definition of these selection coefficients) under three different scenarios. We consider that in A both single mutants (the red dot) carry a benefit relative to the wild type (gray dot), in B one single mutant carries a benefit whereas the other single mutant is deleterious and, in C both single mutants are deleterious. In each case, the fitness of the double mutant (black dot) determines if there is epistasis, and the type of epistasis. Any departure from the expectation (dashed line) that the fitness of the double mutant is simply the sum of the effects of each mutant leads to positive (grades of red) or negative (grades of blue) epistasis. Magnitude epistasis (+ or −) implies that the strength of selection at each locus depends on the allele present at the second locus. Single-sign epistasis (++ or −−) implies that the direction of selection on one locus depends on the allele present at the second locus. Finally, reciprocal sign epistasis (+++ or−−−) implies that the direction of selection on both loci depends on the allele present at the other locus.

antagonistically or synergistically, and where resistance to each drug is governed by different loci (Yeh et al. 2009; MacLean et al. 2010). Regardless of the underlying mechanism, however, population geneticists have identified three qualitatively different patterns of epistasis (Weinreich et al. 2005; Poelwijk et al. 2011, Fig. 1): magnitude epistasis, in which the *magnitude* of the selective effect at one locus depends on the allele present at the second locus; single-sign epistasis, in which the *sign* of the selective effect at one locus depends on the allele present at the second locus; and reciprocal (i.e., double) sign epistasis, in which sign epistasis is present for both loci. The latter case is the most extreme possibility and gives rise to multiple peaks in the fitness landscape (Fig. 1).

Interestingly, previous experimental studies have shown that resistance to multiple drugs sometimes evolves more slowly when drug combinations interact antagonistically than when they interact synergistically (Chait et al. 2007; Hegreness et al. 2008). Therefore, these studies suggest that using drugs in a way that generates antagonistic interactions might be beneficial. Yeh et al. (2009) have noted that such antagonistic patterns of drug interaction are analogous to negative epistasis, and therefore equation (2) can help to better interpret these experimental findings, and to pinpoint how and when such novel treatment suggestions might be beneficial. In fact, as described above, the analysis of equation (2) suggests that there are three possible explanations for these experimental findings. Namely that the experimental conditions that generated antagonistic interactions led to (1) weaker direct selection, (2) negative direct epistasis, and/or (3) negative LD. Although existing data do not allow one to distinguish among these three possibilities, there is evidence that (1) alone might be responsible for the results, suggesting that antagonism or epistasis per se need not explain the results (Appendix B).

Well-known population-genetic results can also be called upon to understand the evolutionary consequences of recombination on the spread of drug resistance (Crow and Kimura 1970). In particular, as can be seen from equations (2) and (3), recombination affects the spread of alleles at each locus only through the dynamics of LD. In the absence of epistasis, LD will never be generated when treatment is homogenous and therefore we expect resistance alleles at each locus to spread independently of one another. On the other hand, when there is epistasis, LD will build up and recombination will affect resistance evolution through its effect on the dynamics of LD. Negative magnitude epistasis will generate negative LD, and equation (1) shows that evolution at a given locus will be affected by indirect selection at the other locus, and through a change in the magnitude of $\delta^T_{j|i}$. In particular, the negative value of D^T will slow the spread of resistance alleles through both of these effects. In this case, heightened recombination will enhance the spread of resistance alleles at each locus by decreasing the magnitude of D^T. Conversely, positive magnitude epistasis will generate positive D^T, and in this case, heightened recombination will then hinder the spread of resistance alleles at each locus.

The above results are well known in the population genetic literature, but the fact that the epidemiological dynamics can affect the decay of LD in equation (3) introduces some novel effects. Specifically, it demonstrates that the rate at which recombination erodes LD is proportional to the prevalence of infection. If infection prevalence is high, then recombination will be high and vice versa. Combining this finding with the above-mentioned results from population-genetics, we arrive at the following prediction: if epistasis is negative, then populations with high disease prevalence will show a faster spread of drug resistance alleles than

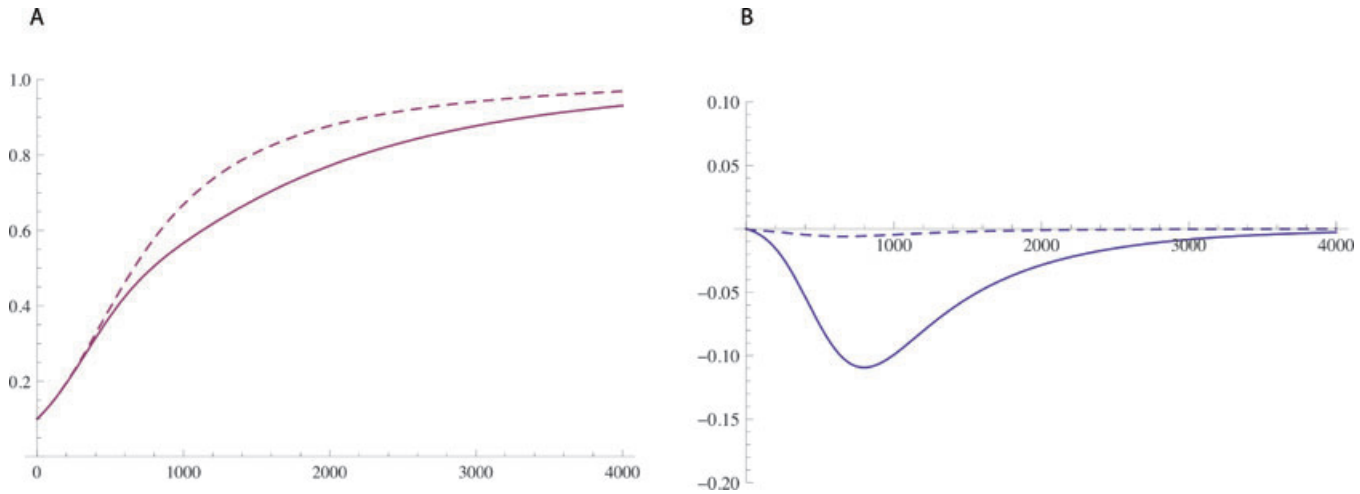

Figure 2. **Evolutionary dynamics of drug resistance under negative epistasis for two different disease prevalences (obtained by varying θ), assuming resistance acts through clearance rates only. Epidemiological dynamics were allowed to reach equilibrium before introducing the drug resistance alleles. Solid: $\theta = 5.25$. Dashed: $\theta = 25$. Other parameter values: $r = 0.5$, $\mu = 0.01$, $\beta^T = 0.00004$, $\Delta\beta_A^T = \Delta\beta_B^T = \Delta\beta_E^T = 0$, $\gamma^T = 0.01$, $\Delta\gamma_A^T = \Delta\gamma_B^T = -0.0045$, $\Delta\gamma_E^T = 0.004$, $\alpha = 0$, $\sigma = 1$. (A) Allele frequency dynamics. (B) Linkage disequilibrium dynamics. Linkage disequilibrium is always negative when epistasis is negative, and this hinders the spread of resistance alleles at each locus. Populations with high disease prevalence (i.e., high θ), however, have higher levels of recombination. This reduces the magnitude of linkage disequilibrium and thereby increases the rate of spread of resistance alleles. The opposite occurs under positive epistasis (not shown).**

populations with low disease prevalence (Fig. 2). Theoretical results based upon Fisher's geometrical model of adaptation as well as empirical studies (Martin et al. 2007; Chou et al. 2011; Khan et al. 2011; Rokyta et al. 2011) suggest that negative epistasis should be common during the spread of drug resistance, implying that we might generally expect a positive correlation between the prevalence of a disease and the rate at which multilocus resistance spreads when treatment is homogeneous. This suggests a novel way in which such feedbacks might be exploited. Interventions such as social distancing and isolation will reduce the occurrence of multiple infection and thus will reduce the rate of recombination. When epistasis is negative, this will slow the spread of multilocus resistance.

SPATIALLY HETEROGENEOUS TREATMENT

We now consider a case where treatment levels are intermediate and constant in time to illustrate how heterogeneity in selection affects evolution. We focus primarily on heterogeneity arising from the occurrence of treated and untreated subpopulations, but the results apply equally to heterogeneity arising from the use of different drugs in each of the subpopulations instead. For simplicity, we refer to all such heterogeneity as spatial heterogeneity although the subpopulations need not occupy different geographic locations. The main difference from the homogeneous treatment case is that, even in the absence of epistatic selection, LD can now be generated by the effect of gene flow between the two subpopulations (the last term in eq. 3). As a result, this can affect the rate at which resistance spreads as well as the effect of recombination.

Let us first consider the case where mutations affect only recovery rates. In this case, a key component of the evolutionary dynamics of LD is the product of the differentiation between new, "immigrant," infections and existing infections at each locus; that is, $\Delta_{p_A}^T \Delta_{P_B}^T$ in the final term of equation (3). One can view this product as a measure of the covariance in selection across the two loci (Lenormand and Otto 2002). It will be positive, and thus generate positive LD, whenever the differentiation between new, immigrant, infections and existing infections is in the same direction at each locus.

As an example, positive covariance in selection will occur if both allele *A* and allele *B* are selectively advantageous under drug treatment and disadvantageous in untreated hosts (e.g., because of the cost of resistance). Such a scenario is very likely if multiple loci contribute to resistance toward a single drug. In such cases, because of the positive LD generated by the spatially heterogeneous selection, recombination will tend to decrease the rate at which such resistance alleles spread through the population. On the other hand, the differentiation among subpopulations will be in the opposite direction at each locus if different drugs are used in each of the two subpopulations and if there is a cost to resistance against each. In such situations, allele *A* might confer resistance to drug A and allele *B* to drug B. As a result, the product $\Delta_{p_A}^T \Delta_{P_B}^T$

will tend to be negative, and recombination will tend to enhance the rate at which the resistance alleles spread.

Again this suggests novel strategies for control. In the absence of epistasis among loci, interventions that decrease the multiplicity of infection (e.g., social distancing and isolation), and thus rates of recombination, should slow the spread of resistance when multiple drugs are being deployed. The converse of this argument is that higher recombination rates are potentially desirable for slowing the spread of multilocus resistance to single drugs; however, the risks associated with achieving this by promoting enhanced multiple infection are likely to outweigh any potential benefits.

If mutations also affect transmission, heterogeneity of treatment may alter the evolutionary dynamics through additional mechanisms. Indeed the fact that the most transmissible genotypes will tend to be the ones most involved in gene flow will affect the Δ_k^T terms in equations (2) and (3). This will affect both allele frequency change and the dynamics of LD. Interestingly, this effect can influence the dynamics of LD even under homogeneous treatment provided there is some heterogeneity in the host population. This recognition sheds some light on the contrasting findings of many studies that have examined the effect of recombination on the within-host evolution of chronic infections such as HIV. For example, fast and slow turnover cells are thought to be important in the within-host dynamics of HIV (Fraser 2005). This heterogeneity in life expectancy between cell types can generate enough differentiation between cells in pathogen genotypes to potentially explain the contrasting effects of recombination documented in previous studies (Bretscher et al. 2004; Althaus and Bonhoeffer 2005; Fraser 2005; Carvajal-Rodríguez et al. 2007; Kouyos et al. 2009; Appendix C).

More generally, heterogeneity in epistasis may also affect the build up of LD. This variation in epistasis might occur, for instance, if one locus compensates for the cost of drug resistance at the other locus. This compensation might be active in only one environment (treated or untreated hosts), or in both environments (Wiesch et al. 2010). In particular, one can imagine situations in which epistasis has opposite signs in the two environments, which could lead to LD of opposite signs in the two populations. In this case, recombination would have opposite effects in the two types of hosts on the speed of evolution. Considerations of these more complicated scenarios are beyond the scope of the present study, but the theoretical framework presented here reveals the kinds of information that are required to make accurate evolutionary predictions in these cases.

TEMPORALLY HETEROGENEOUS TREATMENT

In this final section, we consider the case where treatment level varies over time. For simplicity, we assume that, at any given time, treatment is either 100% or zero. As such there are two different ways in which treatment might vary. The first is simply where the treatment fraction, τ, varies over time between zero and one. In this case, although the treatment fraction is never intermediate, treated and untreated hosts will nevertheless coexist temporarily. For example, after switching from $\tau = 1$ to $\tau = 0$, all newly infected individuals will be untreated, but some previously treated individuals will nevertheless still be circulating in the population until they either die or recover. As a result, this form of temporal heterogeneity generates the type of spatial heterogeneity considered in the previous section. The second possibility is that, upon changing the treatment fraction, τ, all currently infected individuals also have their treatment status altered as well. This will not generate any spatial heterogeneity in treatment and all the results derived for homogeneous treatment will then apply within any given treatment epoch. For simplicity, in all of the analyses below, we employ the first kind of temporal heterogeneity.

One of the most significant questions related to temporally varying drug treatment is the extent to which evolutionary reversion to drug sensitivity occurs when drug pressure is removed (Wijngaarden et al. 2005; Andersson and Hughes 2010; Wiesch et al. 2010). Alleles conferring resistance to drugs often suffer a fitness cost in the absence of treatment (Maisnier-Patin and Andersson 2004; Andersson and Hughes 2010), and therefore once treatment stops, we might expect resistance alleles to decrease in frequency. It is widely appreciated, however, that mutations at secondary loci can sometimes compensate for this cost of resistance, and these compensatory mutations might thereby inhibit reversion to drug sensitivity, even once treatment has stopped. The theoretical framework presented here helps to better understand the conditions under which reversion is likely to occur.

Whether reversion occurs, and the way in which this happens, depends critically on the fitness effects of the different alleles and the pattern of epistasis in the treated and untreated environments. The most extreme case is reciprocal sign epistasis (Weinreich et al. 2005, 2006; Poelwijk et al. 2011). For example, reciprocal sign epistasis in untreated individuals occurs if both resistance alleles are individually selected against, but if each is selectively advantageous whenever the other resistance allele is present. Such patterns of sign epistasis are typical when there is compensation. In this case, there will be two peaks in the fitness landscape in the absence of treatment, one corresponding to genotype ab and the other corresponding to genotype AB.

Previous studies have explored the evolutionary dynamics of such systems in the absence of recombination and found that reversion always occurs provided that the double mutant (e.g., the compensated, resistant, genotype) is less fit than the wild type in the absence of treatment (Wiesch et al. 2010). This has provided a relatively simple criterion for understanding whether we might expect reversion. The framework presented here reveals, however, that this conclusion is fundamentally altered once recombination

is allowed. In particular, if the recombination rate is large relative to selection, then a bistability occurs. To understand why, a QLE approximation (Crow and Kimura 1970; Kirkpatrick et al. 2002; Otto and Day 2007) can be used to show that LD equilibrates at $D \approx \frac{1}{r\sigma\beta\tilde{I}} p_A(1-p_A)p_B(1-p_B)\hat{s}_E$. Substituting this into equation (2) gives $dp_i/dt \approx p_i(1-p_i)\left(s_i + p_j s_E\right)$ to first order in the strength of selection. Thus, the rate of change of allele frequency is negative (i.e., $dp_i/dt < 0$) when both alleles are rare (i.e., when $p_A \approx 0$, $p_B \approx 0$), and it is positive (i.e., $dp_i/dt > 0$) when both are common (i.e., when $p_i \approx 1$, $p_i \approx 1$). Biologically, when recombination rates are high, even though the wild-type genotype has a fitness advantage in the absence of treatment, it cannot increase in frequency when rare because recombination with the resident double mutant continually breaks it down. If the wild-type genotype is frequent enough, however, then it is able to further increase in frequency because most recombination events are then with other wild-type genotypes. Conversely, when recombination rates are low relative to the strength of selection, the selectively advantageous wild-type genotype can remain intact long enough for selection to drive it to fixation regardless of its starting frequency (see also Fraser 2005).

The theoretical framework presented here can also be used to find the critical recombination rate, r_c, above which fixation of the double mutant is locally stable in the absence of treatment:

$$r_c = \frac{s_A + s_B + s_E}{\sigma\tilde{I}\,(\beta + (\Delta\beta_A + \Delta\beta_B + \Delta\beta_E)/2)}, \qquad (4)$$

where $\tilde{I}$ is the equilibrium density of infected hosts when the parasite population is fixed for the double mutant (i.e., the compensated, resistant, genotype). Expressions analogous to (4) have been derived previously in the population-genetic literature (e.g., Park and Krug 2011 and references therein), but expression (4) differs from these previous results in that the denominator of (4) accounts for the effect of epidemiological feedbacks. This feedback operates in two ways. First, even if there is no genetic variation in transmission rate (i.e., $\Delta\beta_j = 0$ for $j = A, B, E$), the effective rate of recombination still depends on the occurrence of multiple infections, and thus the critical recombination rate in (4) is a decreasing function of disease prevalence, as $\beta\sigma\tilde{I}$ still appears in the denominator. Second, if genetic differences in transmission do exist, then the critical recombination rate is further modified by the term $(\Delta\beta_A + \Delta\beta_B + \Delta\beta_E)\sigma\tilde{I}/2$, reflecting the fact that the most transmissible genotypes are more often involved in recombination.

The epidemiological feedback documented in equation (4) again suggests novel strategies for increasing the likelihood of reversion once treatment has ceased, and thus for maintaining the effectiveness of a drug. For example, after stopping the use of the drug, an effort to reduce transmission through social distancing, isolation, and/or other hygiene measures will reduce both the transmission rate and the density of infected hosts (i.e., it will reduce $\tilde{I}$) and thereby increase the critical recombination rate (Fig. 3).

It is also interesting to note that even in the absence of recombination, where reversion always occurs if the wild type is the most fit genotype, this reversion can happen in different ways depending on the nature of epistasis. For example, with some patterns of epistasis, both the resistance allele and the compensatory allele spread under treatment, and when treatment is stopped, the loss of both alleles occurs (as in Fig. 3). Under other patterns of epistasis, somewhat paradoxically, the compensatory allele spreads only once treatment has ceased (T. Day and S. Gandon, unpubl. data). As a result, in this second scenario, the compensated, resistant, genotype is present in significant frequency only once treatment has ended.

Discussion

The evolutionary dynamics of multilocus genetic systems are well studied in the population-genetic literature. Likewise, the dynamics of infectious diseases are well studied in the literature on mathematical epidemiology. The theoretical results presented here bridge the gap between these two important areas of research, to better understand the processes involved in the evolution of multilocus drug resistance. Central to our findings is the importance of feedbacks between evolutionary and epidemiological dynamics. We use this framework to shed new light on previous empirical and theoretical studies of multilocus resistance, and to reveal some new possibilities for the control of resistance based on manipulations of recombination.

Our general analysis revealed three important factors governing the spread of drug resistance when treatment is homogenous in time and space: (1) direct selection, (2) direct epistasis, and (3) LD. Spatial heterogeneity, in the form of different drug treatments for different individuals, or the existence of some untreated individuals, introduces an additional factor arising from the effects of gene flow between subgroups. Such gene flow affects the evolutionary dynamics of allele frequencies directly through mixing, and it also can affect the evolutionary dynamics of LD. This then can impose an indirect effect of gene flow on the allele frequency dynamics as well. Temporal heterogeneity can introduce further complications, although we illustrated how some forms of temporal heterogeneity can be understood via the results from the homogenous and spatially heterogeneous cases.

IMPLICATIONS FOR UNDERSTANDING PREVIOUS STUDIES

It is well known from population genetics that patterns of epistasis play a critically important role in the evolutionary dynamics of

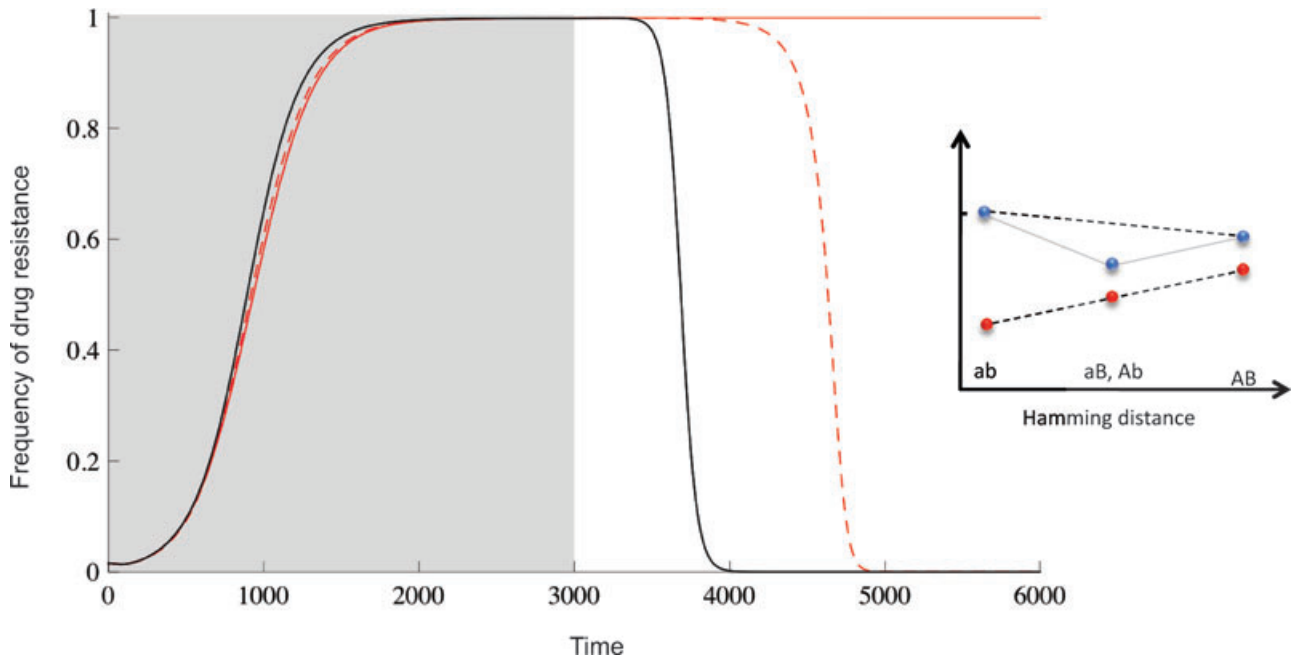

Figure 3. **Evolution of drug resistance during and after the use of a drug when there is reciprocal sign epistasis. The use of the drug selects for resistance at both loci (gray zone). When the drug is removed, the frequency of resistance drops rapidly if the parasite is asexual (black line) or remains stuck near fixation if the parasite undergoes high levels of recombination (red line). Dashed red line shows how interventions that decrease transmission rate (and thus decrease the extent of recombination) drive reversion to drug sensitivity. Parameter values for dashed red line are identical to those for solid red line except that the overall transmission rate has been decreased by 20%. Inset shows pattern of epistasis in fitness in the presence (red) and absence (blue) of drug treatment.**

multilocus systems (Crow and Kimura 1970), and our findings echo this importance. This is particularly evident in the results for homogeneous treatment, where we demonstrate how epistasis generates linkage disequilibria, and how recombination breaks down these genetic associations. Empirical studies (Chait et al. 2007; Hegreness et al. 2008) have shown that interactions between drugs can influence the dynamics of resistance evolution and it has been suggested that these effects are analogous to epistasis (Appendix B). The theoretical results presented here shed new light on these findings and reveal that the above three factors (i.e., direct selection, direct epistasis, and LD) can each potentially explain these results. Although existing data do not yet allow one to distinguish among these possibilities, there is strong evidence that differences in direct selection are an important component of the explanation for these experimental findings.

The results for spatially heterogeneous treatment also demonstrate that the importance of LD is not restricted to instances where there is epistasis. Rather, any genetic differentiation at both loci among subpopulations will generate LD through gene flow (Slatkin 1975; Otto and Lenormand 2002). Our results also show that heterogeneity in the host population can lead to such genetic differentiation, and thus we often expect such LD to arise in many cases where there are different kinds of susceptible individuals. These findings provide further insight into contrasting results found in several previous studies that have examined the role of recombination for the within-host evolution of multilocus drug resistance in HIV (Appendix C).

Extreme forms of epistasis, such as reciprocal sign epistasis, can further complicate the evolutionary outcome by generating two peaks in the fitness landscape. Reciprocal sign epistasis can arise for a variety of reasons, not the least of which is when compensatory mutations at secondary loci ameliorate the costs of drug resistance. Previous studies (Wiesch et al. 2010) have explored the evolutionary consequences of such compensation on the evolutionary reversion of drug resistance in the absence of treatment. A key finding has been that reversion always occurs if the double mutant (i.e., the compensated, resistant, genotype) is less fit than the wild type in the absence of treatment. The results presented here reveal more clearly why this happens, and they demonstrate that this prediction is fundamentally altered by recombination. In particular, a critical recombination rate exists (eq. 4) above which reversion to the drug-sensitive, wild-type, genotype can fail to occur even when it has a higher fitness than the double mutant.

IMPLICATIONS FOR NOVEL THERAPEUTIC INTERVENTIONS

In addition to the above-described insights, the theoretical results present here suggest how the feedback between epidemiology and

evolution might be manipulated to help control the spread of drug resistance. Our focus has been on the feedback between disease transmission and the rate of recombination that arises through its effect on multiplicity of infection. On purely epidemiological grounds, interventions that reduce transmission are typically desirable, and therefore we explored how such interventions might also be evolutionarily beneficial through their effects on reducing the rate of recombination.

Although we have not conducted an exhaustive analysis, we have identified three potential scenarios in which interventions that reduce transmission (e.g., social distancing, isolation, increased hygiene) will be evolutionarily beneficial through their effects on reducing recombination: (1) when treatment is homogenous and there is negative epistasis among loci; (2) when different drugs are deployed in different individuals and the loci in question confer resistance to each drug independently; and (3) when reciprocal sign epistasis exists (e.g., through compensation of the costs of resistance) and drug treatment is removed to select for reversion to sensitivity.

All three of the above effects arise as a result of the intervention decreasing recombination and thereby encouraging the maintenance of high levels of LD. In each scenario, however, this LD is beneficial for different reasons. In scenario (1), negative epistasis among loci will generate negative LD, and this disequilibrium will slow the spread of resistance. Indeed, recent empirical studies have demonstrated that such interference among selectively advantageous alleles is widespread in virus and bacteria (Martin et al. 2007; Chou et al. 2011; Khan et al. 2011; Rokyta et al. 2011). Thus, any intervention that encourages the maintenance of this LD (e.g., by reducing recombination) will slow the spread of resistance. In scenario (2), even though epistasis is absent, different alleles are selectively advantageous in different subpopulations. As a result, genetic differentiation will arise between the two, ultimately leading to negative LD through gene flow. Just as in scenario (1), this ought to then slow the spread of resistance, and therefore reducing the rate of recombination will be beneficial. In scenario (3), positive LD will arise because the wild type and the double mutant each occupy a local fitness peak. If the wild type is most fit once treatment has ceased however, then interventions that reduce recombination will encourage the maintenance of this positive LD, thereby allowing the wild type to competitively exclude the double mutant.

IMPLICATIONS FOR FUTURE EMPIRICAL STUDIES

Although our results reveal that the evolution of multilocus drug resistance can be quite complicated, they also identify the kinds of information that are required to better understand the underlying dynamics and, consequently, to make predictions. There has been widespread recent interest quantifying patterns of epistasis among loci, with the recognition that this will greatly affect the evolutionary dynamics of drug resistance. The framework presented here helps to put these ideas into context through its direct connection with classical results from population genetics, and it more clearly reveals how the extent of recombination then interacts with this epistasis to affect the evolutionary dynamics.

Importantly, however, the theoretical results also reveal other important kinds of information that are required. For example, we not only need to know the fitness effects of different alleles, but we also need to know how these fitness effects are manifested in the disease life-history traits of the genotypes. If fitness effects occur through recovery rates only, then the epidemiological and evolutionary dynamics are not as tightly coupled because the selection coefficients are then independent of the epidemiological dynamics. In such cases, the epidemiology drives evolution only through the effects of gene flow between treated and untreated individuals, and through the dynamics of LD (as a result of its effects on recombination). On the other hand, if fitness effects also occur through transmission rates, then a coupling arises through the selection coefficients as well. To date, the effects of drug resistance mutations on these disease life-history traits are largely unknown for most pathogens, and we hope that our highlighting the significance of these biological details will motivate further experimental work in this direction.

OTHER APPLICATIONS AND EXTENSIONS

Another question that has been the subject of considerable investigation is whether, given the availability of two drugs, these drugs should be used simultaneously or in a cyclic fashion to control the spread of resistance (Bonhoeffer et al. 1997; Bergstrom et al. 2004). In most previous studies, the focus has been on single-locus resistance and therefore the impact of recombination has been largely ignored. Bergstrom et al. (2004) do demonstrate, however, that a cycling strategy is expected to lead to a lower rate of emergence of multiple resistance because it tends to ensure that there are only high frequencies of resistance to one drug at any given time. This effect of "segregation in time" of the single mutants adapted to each drug can be obtained using the present formalism as well (results not shown). However, a full analysis of the consequences of mixing versus cycling on the evolutionary dynamics of multilocus resistance remains to be done.

Our analysis has focused on a somewhat generic form of recombination between loci without explicitly saying much about what this process represents. In fact, pathogens differ in the process of genetic mixing from the organisms that are typically the focus of population-genetics studies of recombination (Awadalla 2003). For example, influenza undergoes a process of genetic mixing referred to as reassortment. This differs from true recombination in that the viral genome is segmented, and coinfecting viral strains containing different segments can then give rise to novel, mixed, genotypes. Indeed this how new influenza subtypes

arise. The formalism presented here can nevertheless be used to model this process by interpreting the parameter r as reflecting the extent of reassortment rather than it being a reflection of physical proximity of the loci within a genome. As such, the theoretical framework presented might shed interesting new light on a variety of questions. For example, it has been suggested that drug resistance in influenza has spread in the absence of treatment through a process of hitchhiking with selectively advantageous antigenic escape mutants (Simonsen et al. 2007). The approach presented here provides an explicit, quantitative, framework for exploring the plausibility of this argument. Likewise, it can be used to explore a variety of other questions including the joint evolution of virulence and transmission when they are encoded by different loci and the joint evolution of virulence and antigenicity.

There are also a number of interesting avenues for further theoretical development. For example, the present results consider only two loci, but many more mutations can be involved in drug resistance. Consequently, an extension to the present analysis to an arbitrary number of loci might be worthwhile, particularly if one is interested in making quantitative predictions. For simplicity, we have also employed a deterministic model, but it is known that stochasticity can affect the evolutionary dynamics of multilocus systems through the build up of negative LD (Hill and Robertson 1966). This has already been explored through simulations in the context of the within-host evolution of drug resistance in HIV (see Appendix C) but extensions to our model could provide a theoretical foundation for these results. Lastly, we have made the simplifying assumption of superinfection, meaning that each infected host contains only a single-pathogen genotype at any given time. It would be interesting to relax this assumption because many infections (e.g., HIV, malaria) are known to harbor multiple strains for extended periods of time. Furthermore, such an extension would open the door to exploring the consequences of complementation, in which mutant alleles in one pathogen can be masked by the presence of a wild-type allele in a coinfecting pathogen strain (see Gao and Feldman 2009 and references therein).

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Appendix A: Derivation of the Main Equations

The model of the text assumes that there are four genotypes of pathogen. We first derive the dynamics of the number of hosts infected with each of these four genotypes, and we then change variables to those used in the text.

DYNAMICS OF GENOTYPE DENSITIES

We use β_{ij} and γ_{ij} to denote the transmission and recovery rates, respectively, of genotype ij in untreated hosts, where $i \in \{a, A\}$ and $j \in \{b, B\}$ (the model can be extended to allow for an effect on virulence as well, using an analogous approach). We use a superscript, "T" to indicate these same parameters in treated hosts. The assumptions laid out in the main text then lead to the following differential equations for each of the pathogen genotypes:

$$\begin{aligned}
\dot{S} &= \theta - \mu S - \left(\bar{\beta} I + \bar{\beta}^T T\right) S + \left(\bar{\gamma} I + \bar{\gamma}^T T\right) \\
\dot{I}_{ij} &= (1-\tau)\left(\beta_{ij} I_{ij} + \beta_{ij}^T T_{ij}\right) S - \left(\mu + \alpha + \gamma_{ij}\right) I_{ij} \\
&\quad - \sigma\left(\bar{\beta} I + \bar{\beta}^T T\right) I_{ij} + \sigma \pi_{ij} \\
\dot{T}_{ij} &= \tau\left(\beta_{ij} I_{ij} + \beta_{ij}^T T_{ij}\right) S - \left(\mu + \alpha + \gamma_{ij}^T\right) T_{ij} \\
&\quad - \sigma\left(\bar{\beta} I + \bar{\beta}^T T\right) T_{ij} + \sigma \pi_{ij}^T
\end{aligned} \tag{A1}$$

where σ is a parameter that scales the efficiency of superinfection (see main text), $I = \sum_{i,j} I_{ij}$ denotes the total density of untreated infected hosts, and the term $\sigma(\bar{\beta} I + \bar{\beta}^T T)$ represents the loss of infections through superinfection. The quantities $\bar{\beta} = \sum_{i,j} p_{ij}\beta_{ij}$ and $\bar{\gamma} = \sum_{i,j} p_{ij}\gamma_{ij}$ denote the average transmission and recovery rates in untreated hosts, where $p_{ij} = I_{ij}/I$ is the frequency of genotype ij in untreated hosts. Similarly, in treated hosts, we define $T = \sum_{i,j} T_{ij}$, $\bar{\beta}^T = \sum_{i,j} p_{ij}^T \beta_{ij}^T$, $\bar{\gamma}^T = \sum_{i,j} p_{ij}^T \gamma_{ij}^T$, and $p_{ij}^T = T_{ij}/T$. Note that equations (A1) consist of a total of nine differential equations describing the evolutionary-epidemiological dynamics.

The parameter π_{ij} (π_{ij}^T) in equation (A1) denotes the rate of production of strain I_{ij} (T_{ij}) through all superinfection occurring in the population. These can be derived by considering all the

ways in which secondary infection generates type I_{ij} (T_{ij}), giving

$$\pi_{ij} = \frac{\sigma}{2}\left(I_{ij}h + I h_{ij}\right) + \eta^i_j \frac{\sigma r}{2}\left(I_{12}h_{21} + I_{21}h_{12} - I_{11}h_{22} - I_{22}h_{11}\right)$$

$$\pi^T_{ij} = \frac{\sigma}{2}\left(T_{ij}h + T h_{ij}\right) + \eta^i_j \frac{\sigma r}{2}\left(T_{12}h_{21} + T_{21}h_{12} - T_{11}h_{22} - T_{22}h_{11}\right),$$

where $h = \bar{\beta}I + \bar{\beta}^T T$, $h_{ij} = \beta_{ij}I_{ij} + \beta^T_{ij}T_{ij}$, and

$$\eta^i_j = \begin{cases} -1 & i \neq j \\ 1 & i = j \end{cases}.$$

With the above model, we can then perform a change of variables to obtain the equations of the text. In particular, in addition to S, I, and T defined above, we also define the six new variables

$$p_A = \frac{I_{Ab} + I_{AB}}{I},\ p_B = \frac{I_{aB} + I_{AB}}{I},\ D = \frac{I_{ab}I_{AB}}{I^2} - \frac{I_{aB}I_{Ab}}{I^2}$$
$$p^T_A = \frac{T_{Ab} + T_{AB}}{T},\ p^T_B = \frac{T_{aB} + T_{AB}}{T},$$
$$D^T = \frac{T_{ab}T_{AB}}{T^2} - \frac{T_{aB}T_{Ab}}{T^2}.$$

These represent allele frequencies in the two different kinds of hosts (p_i and p^T_i), along with the linkage disequilibrium (LD) in each (D and D^T). Rewriting equation (A1) in terms of these new nine variables then yields equations (1), (2), and (3) of the main text.

Appendix B: Drug Interactions, Epistasis, and the Evolution of Resistance

In recent years, there has been an increased interest in using combination drug therapy as a means of reducing the spread of resistance. One reason why multidrug treatment guards against resistance is that the probability of a mutant arising that is resistant to multiple drugs is greatly reduced. Another potential benefit of using multiple drugs, however, is that drugs can interact in ways that reduce the spread of resistance alleles once they arise.

In a series of elegant papers (Chait et al. 2007; Hegreness et al. 2008; Michel et al. 2008; Yeh et al. 2009; Torella et al. 2010), Kishony and colleagues have argued, both theoretically and experimentally, that antagonistic drug interactions can have just this effect. An antagonistic interaction is said to occur when two drugs in combination have a lesser effect on suppressing pathogen growth than would be predicted by their individual effects alone. In the extreme case, the antagonism can be so strong that one drug can completely lose its effectiveness if the concentration of the second drug is high enough (and vice versa). Chait et al. (2007) and Hegreness et al. (2008) have shown experimentally that such antagonistic interactions can slow the spread of drug resistance (reviewed in Yeh et al. 2009).

Yeh et al. (2009) have noted that patterns of drug interaction are analogous to patterns of allelic epistatis in that the effects can be synergistic (positive epistasis), additive, or antagonistic (negative epistasis; drugs can also vary in concentration, however, and Yeh et al. [2009] review the ways in which this confounding effect can be controlled). Chait et al. (2007) have also noted that the effect of mutant alleles on drug resistance can be viewed, in effect, as simply reducing the effective concentration of the drug in question. Thus, the occurrence of synergistic drug interactions (when properly controlled for concentration) can be viewed as giving rise to positive genetic epistasis (assuming different loci effect resistance to the two drugs separately). Likewise, the occurrence of antagonistic drug interactions can be viewed as giving rise to negative genetic epistasis. As such, the theoretical framework presented here should speak to the experimental differences in resistance evolution that occur under these different regimes (Chait et al. 2007; Hegreness et al. 2008).

Equation (2) of the main text, under the assumption of 100% treatment, can be used to better understand why the evolutionary dynamics of resistance are different under synergistic versus antagonistic drug interactions:

$$\frac{dp^T_i}{dt} = p^T_i\left(1 - p^T_i\right)\left(s^T_i + \delta^T_{j|i}s^T_E\right) + D^T s^T_j. \qquad \text{(B1)}$$

Suppose that antagonistic drug interactions give rise to negative epistasis and synergistic drug interactions give rise to positive epistatis. Equation (B1) then reveals three potential reasons why the spread of resistance might be slower under antagonistic drug interactions when compared with synergistic drug interactions (see also the explanation in the main text).

First, it is possible that the experimental conditions that give rise to antagonism also give rise to a difference in direct selection on the resistant allele in question, when compared with the synergistic case. In other words, although different patterns of drug interaction give rise to corresponding patterns of epistasis, they might also inadvertently give rise to differences in the strength of selection as measured by s^T_i in equation (B1). This would not be an effect of synergy per se, but rather an effect that arises simply from differences in the strength of selection for single-allelic substitutions in the two experimental treatments.

Second, because antagonistic drug interactions generate negative epistasis, s^T_E is negative in this case, and positive in the case of synergistic drug interactions. As a result, whenever the focal resistance allele is paired with the resistant allele at the second locus, the strength of selection on this focal allele will be less in the antagonistic case. Specifically, such a pairing between

resistance alleles occurs with probability $\delta^T_{j|i}$, and because s^T_E is negative under antagonism and positive under synergism, the factor $s^T_i + \delta^T_{j|i} s^T_E$ in (B1) will be smaller in the former case. This will reduce the rate at which resistance spreads, and is termed "direct epistasis" in the main text.

Third, because antagonistic drug interactions generate negative epistasis between resistance alleles at the two loci, this will lead to negative LD, D^T. Conversely, synergistic drug interactions will lead to positive LD. Thus, the final term in (B1) will be negative for antagonistic interactions and positive for synergistic interactions. Moreover, the probability, $\delta^T_{j|i}$, will be smaller for antagonistic interactions that for synergistic interactions. Both effects of the negative LD will reduce the rate at which resistance spreads.

Given the data currently available, it is not possible to unambiguously determine which of the above three factors is most important in explaining the experimental results of Chait et al. (2007) and Hegreness et al. (2008). Nevertheless, there are reasons to suspect that differences in direct selection (i.e., the first factor) is very important. For example, an examination of the fitness landscapes from the studies (e.g., Fig. 2, Chait et al. 2007; Fig. 3, Hegreness et al. 2008) reveal that, if separate loci are indeed responsible for resistance to the two drugs, then there are significant differences in the fitness effects of allelic substitution at a single locus between the antagonistic and synergistic cases. In other words, the value of s^T_i does appear to be smaller in the antagonistic case (Fig. 3B, Hegreness et al. 2008) and even becomes negative under extreme antagonism (Fig. 2C, Chait et al. 2007). It is possible, however, that the second factor, direct epistasis, also played a role. It seems less likely, however, that LD would have been important because the experimental conditions would need to have allowed for genetic associations between loci conferring resistance to the two drugs to build up over time. Although this is possible, there is no evidence reported in the studies to suggest that this is the case.

Appendix C: Effect of Recombination on HIV Drug Resistance

The model developed in the main text is primarily meant to describe a population of hosts infected by a pathogen but it can also be used to shed some light on within-host evolution in chronic infections. In particular, the impact of recombination on drug resistance evolution in HIV has attracted a lot of theoretical attention.

The first attempts to understand the effects of recombination on the within-host evolution of drug resistance used a population-genetics approach to show the impact of epistasis on the build up of LD and how this is altered by recombination. Bretscher et al. (2004) pointed out that combination therapy is likely to generate positive epistasis (because a large increase in fitness is only likely to occur when all the resistance alleles are combined within a single-viral genome). In this case, recombination will slow the emergence of multidrug resistance. Our model can readily be used to show that this argument continues to hold if we allow for dynamically varying viral densities within a host (something that was absent from Bretscher et al. 2004). Indeed, our model can be viewed as a description of the epidemiology and evolution of a virus population infecting a pool of susceptible cells within an infected host. As pointed out in the main text (see also Appendix B), equation (2) can then be used to show that positive (negative) epistasis speeds up (slows down) the spread of multidrug resistance.

Another approach in the literature stems from a detailed description of the within-host dynamics of the virus. Fraser (2005) used a simulation model of within-host HIV dynamics and pointed out the impact of population dynamics on the evolutionary outcome. This model also recovers the impact of epistasis on the effect of recombination. Interestingly, however, in the absence of epistasis, recombination was also shown to slow the spread of multidrug resistance. Kouyos et al. (2009) also obtained this result in a very similar model, and pointed out the importance of the heterogeneity of different types of host cells. Indeed, both the Fraser (2005) model and that of Kouyos et al. (2009) allow for short-lived and long-lived virus-producing cells. Kouyos et al. (2009) showed that this heterogeneity can lead to the build up of strong positive LD. The framework presented here can be used to better understand the importance of this heterogeneity. Indeed, equations (2) and (3) can be modified to describe the evolutionary dynamics when the heterogeneity is not due to treatment, but to differences in the life span of infected cells. Simulations (not shown) reveal that, in the absence of epistasis, the only way to recover an effect of this heterogeneity between cells is when the resistance mutations affect transmission (an assumption made in both Fraser 2005 and Kouyos et al. 2009). Indeed, there is no effect when resistance mutations affect recovery only because, in this case, if one starts with the same genotype frequency in both types of cells, the dynamics are:

$$\frac{dp_i}{dt} = p_i(1-p_i)s_i + Ds_j$$
$$\frac{dp^L_i}{dt} = p^L_i\left(1-p^L_i\right)s_i + D^L s_j,$$

where L refers to long-lived cells and I to short-lived cells. Hence, nothing can generate a difference in the two types of cells. In a similar way, one can show that nothing generates LD.

In contrast, if the mutations affect transmission, then with the very same initial conditions, we obtain:

$$\frac{dp_i}{dt} = p_i(1-p_i)s_i + Ds_j + \frac{L}{I}(\tau S + \sigma I/2)p_i^L\left(\beta_i^L - \bar{\beta}^L\right)$$
$$\frac{dp_i^L}{dt} = p_i^L\left(1-p_i^L\right)s_i + D^L s_j + \frac{I}{L}(\tau S + \sigma L/2)p_i(\beta_i - \bar{\beta}).$$

In this case, the final terms (which are positive because it is assumed that drug resistance increases transmission) will be different between the two types of cells because the ratios L/I and I/L will be different. For example, in the extreme case where the short-lived cells are very short lived, we have $L/I >> I/L$. This will generate an asymmetry in the evolutionary dynamics between the two types of cells, with the change in resistance frequency being faster in short-lived cells. This will result in differentiation between short-lived and long-lived cells, which will subsequently impact the dynamics of allele frequencies and LD. In particular, one can show that LD is going to build up when mutations affect transmission. It will be positive in long-lived cells but it will have a more complex dynamics in short-lived cells. Note that, because the dynamics depend on the ratios between the densities of different cell types, very different dynamics can result from different assumptions regarding the proportion of cells that can become long lived. When this proportion is low, we find that the LD is positive in long-lived cells and drives positive LD in short-lived cells. The overall LD thus becomes positive, which is consistent with what has been found in Kouyos et al. (2009).

Finally, a third factor that has been explored in simulation studies is the impact of stochasticity (Althaus and Bonhoeffer 2005; Carvajal-Rodríguez et al. 2007; Kouyos et al. 2009). Stochasticity affects drug resistance evolution through its impact on LD. As pointed out by earlier population-genetics studies, the interplay between natural selection and genetic drift tends to generate negative LD (Hill and Robertson 1966). In HIV models, demographic stochasticity also results in the build up of LD. Thus, in those situations, even in the absence of epistasis recombination has an effect on the speed of adaptation and tends to speed up the spread of multidrug resistance genotypes. The effect of stochasticity is absent in our model that is fully deterministic.