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Modeling within-host HIV-1 dynamics and the evolution of drug resistance: Trade-offs between viral enzyme function and drug susceptibility

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Abstract

There are many biological steps between viral infection of CD4⁺ T cells and the production of HIV-1 virions. Here we incorporate an eclipse phase, representing the stage in which infected T cells have not started to produce new virus, into a simple HIV-1 model. Model calculations suggest that the quicker infected T cells progress from the eclipse stage to the productively infected stage, the more likely that a viral strain will persist. Long-term treatment effectiveness of antiretroviral drugs is often hindered by the frequent emergence of drug resistant virus during therapy. We link drug resistance to both the rate of progression of the eclipse phase and the rate of viral production of the resistant strain, and explore how the resistant strain could evolve to maximize its within-host viral fitness. We obtained the optimal progression rate and the optimal viral production rate, which maximize the fitness of a drug resistant strain in the presence of drugs. We show that the window of opportunity for invasion of drug resistant strains is widened for a higher level of drug efficacy provided that the treatment is not potent enough to eradicate both the sensitive and resistant virus.

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Keywords: HIV-1; Drug resistance; Viral fitness; Mathematical model; Viral dynamics

1. Introduction

Mathematical models have proven valuable in the understanding of human immunodeficiency virus type 1 (HIV-1) dynamics, disease progression and antiretroviral responses (see reviews in Nowak and May (2000), Perelson (2002), Callaway and Perelson (2002), Perelson and Nelson (1999, 2002)). Many important insights into the host–pathogen interaction in HIV-1 infection have been derived from mathematical modeling and analyses of changes in the level of HIV-1 RNA in plasma when antiretroviral drugs are administered to perturb the equilibrium between viral production and viral clearance in infected individuals (Ho et al., 1995; Perelson et al., 1996, 1997; Wei et al., 1995).

In a basic HIV model that has been frequently used to describe virus infection, there are three variables: uninfected CD4⁺ T cells, productively infected T cells, and free virus (Nowak and May, 2000; Perelson et al., 1996). In this model, infected cells are assumed to produce new virions immediately after target cells are infected by a free virus. However, there are many biological processes between viral infection and subsequent production within a cell. For example, after viral entry into the host cell, the viral RNA genome is reverse transcribed into a complementary DNA sequence by the enzyme reverse transcriptase (RT). The DNA copy of the viral genome is then imported into the nucleus and integrated into the genome of the lymphocyte. When the infected lymphocyte is activated, the viral genome is transcribed back into RNA. These RNAs are translated into proteins that require a viral protease to cleave them into active forms. Finally, the mature proteins assemble with the viral RNA to produce new virus particles

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that bud from the cell. The portion of the viral life cycle before production of virions is called the eclipse phase. Several mathematical models have been developed that either introduce a constant (discrete) delay (Culshaw and Ruan, 2000; Dixit and Perelson, 2004; Herz et al., 1996; Nelson et al., 2000) to denote the eclipse phase, or assume that the time delay is approximated by some distribution functions (e.g., a gamma distribution) (Mittler et al., 1999; Nelson and Perelson, 2002). The introduction of a time delay in models of HIV-1 primary infection to analyze the viral load decay under antiretroviral therapy has refined the estimates of important kinetic parameters, such as the viral clearance rate and the mortality rate of productively infected cells (Nelson et al., 2000; Nelson and Perelson, 2002). Some more complex models, including age-structured models, have been employed to study virus dynamics (Nelson et al., 2004) and the influence of drug therapy on the evolution of HIV-1 (Kirschner and Webb, 1996; Rong et al., 2007a).

It should be noted that the above-mentioned age-structured models essentially treat the transition of a cell from the uninfected state to the productively infected state as a deterministic process by taking into account the time delay that occurs between various steps in the virus life cycle within a target cell. In contrast, in this study we incorporate an eclipse stage to describe the stage of an infected cell between viral attachment and generation of new virus. The present stage-structured model implicitly treats the progression of an infected cell from the initial infection to subsequent reproduction as an exponentially distributed process. We have chosen to adopt the stage-structured approach because it allows us to explore mechanistically biological trade-offs between protein functions and drug resistance while avoiding the complications of time delay models.

The advent of highly active antiretroviral therapy (HAART) has been an important breakthrough in HIV-1 treatment, resulting in a great reduction in the morbidity and mortality associated with HIV infection (Simon and Ho, 2003). However, the clinical benefits of combination therapy are often compromised by the frequent emergence of drug resistance driven by the within-host selective pressure of antiretroviral drugs (Clavel and Hance, 2004). In addition, the persistence of viral reservoirs, including latently infected resting memory CD4⁺ T cells that show minimal decay even in patients on HAART up to many years (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997; Zhang et al., 1999), has been a major obstacle to the long-term control or eradication of HIV-1 in infected individuals.

Drug resistance results from mutations that emerge in the viral proteins targeted by antiretroviral agents. Most of our knowledge regarding resistance comes from the genotypic analysis of virus isolates from patients receiving prolonged drug treatment (Larder, 1996). Important insights into the mechanisms underlying the evolution of drug resistant viral strains have also been derived from

mathematical modeling of virus dynamics and antiretroviral responses (Bonhoeffer and Nowak, 1997; Kirschner and Webb, 1997; Nowak et al., 1997; Ribeiro and Bonhoeffer, 2000; Ribeiro et al., 1998; Stilianakis et al., 1997). Both deterministic and stochastic modeling approaches suggest that treatment failure is mostly likely due to the preexistence of drug resistant strains before the initiation of therapy rather than the generation of resistant virus during the course of treatment (Bonhoeffer and Nowak, 1997; Ribeiro and Bonhoeffer, 2000). The evolution of HIV resistance is associated with selective pressures exerted by drug treatments that are not potent enough to completely suppress the viral replication. The longer the drug efficacy remains in the intermediate range, the greater the possibility that drug resistant virus variants will arise during therapy (Mugavero and Hicks, 2004). Nonetheless, the conditions of mutant selection are very complex in treated patients due to time-dependent intracellular drug concentrations *in vivo* (Dixit and Perelson, 2004; Huang et al., 2003) and spatial heterogeneity (Kepler and Perelson, 1998). The management of such patients requires a careful understanding of the mechanistic evolution of HIV-1 variants during treatment.

The evolution of resistant strains in the presence of drugs is thought to depend on inherent trade-offs that exist between the proper functioning of HIV's RT and protease enzymes and their reduced susceptibility to antiretroviral regimens in their mutated forms. Indirect evidence for such trade-offs is found in the observation that there is a reduction in replication capacity for drug resistant virus variants in the absence of drug therapy (Clavel et al., 2000; Nijhuis et al., 2001). These trade-offs not only help explain that even after drug resistance arises viral load often remains partially suppressed below pre-therapy levels but also could be potentially exploited in order to better manage the evolution of drug resistance within a patient.

The main purpose of this study is to develop a mathematical framework that can be used to formalize and examine simple hypotheses about the life-history trade-offs that allow drug-resistant viral strains within a patient to persist in the presence of drug therapy. We incorporate the eclipse phase of viral replication into a mathematical model to characterize the stage during which infected CD4⁺ T cells have not yet started to produce new virus. The inclusion of the progression of infected cells from this eclipse phase to the productive stage enables us to capture more variability in HIV dynamics. We observe that the strain of virus with a faster progression rate essentially has a quicker process of reverse transcription of RNA into DNA and integration of the DNA into the chromosome, which gives rise to an increased chance for that viral strain to persist. More importantly, our approach allows us to link drug resistance to RT inhibitors to the progression of the eclipse phase and identify the optimal evolutionary strategy for the drug resistant strain under some simple assumptions. It is widely believed that most HIV drug resistance mutations affect highly conserved amino acid

residues that are thought to be important for optimal enzyme functions, and thus for the full replicative potential of virus (Clavel et al., 2000). Consequently, we assume that in the absence of drug therapy the wild-type strain will evolve to replicate as fast as possible and produce as many new virions as possible. Thus, a viral strain with a slower progression rate, which is operating suboptimally, will possibly have a higher level of resistance to antiretroviral drugs, creating a trade-off between the progression rate and the drug efficacy of RT inhibitors. In addition, there are trade-offs between the viral production rate and the clearance rate of productively infected cells (De Paepe and Taddei, 2006), and between the viral production rate and the drug efficacy of protease inhibitors (see the last section for more discussions). We will investigate how these trade-offs may affect the fitness of drug-resistant viral strains in the presence of drugs at different concentration levels. The optimal progression rate and the optimal viral production rate are derived by maximizing the viral fitness of drug-resistant strains. An invasion criterion of resistant strains is also obtained in the presence of drug therapy. Both analytical results and numerical simulations suggest that with a more effective drug treatment (yet not potent enough to eradicate the virus), a wider range of drug-resistant strains will be able to invade in response to the selective pressure of drugs.

2. Model formulation

A basic mathematical model has been widely adopted to describe the virus dynamics of HIV-1 infection *in vivo* (see Perelson et al. (1996) and reviews in Nowak and May (2000), Perelson (2002), Perelson and Nelson (1999)). Important features of the interaction between virus particles and cells have been determined by fitting the model to experimental data. In this paper, we extend the basic model by including a class of infected cells that are not yet producing virus and two viral strains to study the evolution of drug resistant strains.

2.1. Inclusion of cells in the eclipse phase

After a virus enters a target CD4⁺ T cell, there are a number of biological events before the production of new virions: reverse transcription from viral RNA to DNA, integration of the DNA copy into the DNA of the infected cell (the integrated viral DNA is called the provirus), transcription of the provirus and translation to generate viral polypeptides, cleavage of polypeptides by the HIV protease, assembly and budding of new virus. Perelson et al. (1993) examined a model for the interaction of HIV with CD4⁺ T cells that considers a class of infected T cells, which contain the provirus but are not producing virus. In this work, we begin with a modification of the model in Perelson et al. (1993), and then incorporate antiretroviral effects to study the evolution of drug resistance.

As suggested in Zack et al. (1990), when a virus enters a resting CD4⁺ T cell, the viral RNA may not be completely reverse transcribed into DNA. If the cell is activated shortly following infection, reverse transcription can proceed to completion. However, the unintegrated virus harbored in resting cells may decay with time and partial DNA transcripts are labile and degrade quickly (Zack et al., 1992). Hence a proportion of resting infected cells can revert to the uninfected state before the viral genome is integrated into the genome of the lymphocyte (Essunger and Perelson, 1994). To model these events, we include a class of infected cells in the eclipse stage of viral replication, i.e., the stage between the initial infection and subsequent viral production. Thus, a portion of infected cells in the eclipse phase can revert to the uninfected class. Let $T(t)$, $T_E^*(t)$, $T^*(t)$ and $V(t)$ denote the concentrations of uninfected CD4⁺ T cells, infected cells in the eclipse stage, productively infected cells, and free virus particles at time t , respectively. The model can be described by the following equations:

$$\frac{d}{dt} T(t) = \lambda - dT - kVT + bT_E^*,$$

$$\frac{d}{dt} T_E^*(t) = kVT - (b + \phi + \delta_E)T_E^*,$$

$$\frac{d}{dt} T^*(t) = \phi T_E^* - \delta T^*,$$

$$\frac{d}{dt} V(t) = pT^* - cV, \tag{1}$$

where λ is the recruitment rate of uninfected T cells, d is the per capita death rate of uninfected cells, k is the rate constant at which uninfected cells get infected by free virus. δ is the per capita death rate of productively infected cells, p is the viral production rate of an infected cell, and c is the clearance rate of free virus. Cells in the eclipse phase revert to the uninfected T class at a constant rate b . In addition, they may alternatively progress to the productively infected class T^* at the rate ϕ , or die at the rate δ_E . Note that our model assumes that the expected residence time of a cell in the eclipse phase is exponentially distributed, and the parameter ϕ is determined, in part, by the activity of RT. For example, if reverse transcription is quick, then ϕ will be large and the infected cells in the eclipse phase will progress to the productively infected state with a high probability, i.e., $\phi/(b + \phi + \delta_E)$.

As with the basic HIV model, there are two possible steady states of model (1). One steady state is the infection-free steady state, the other is the infected steady state.

If we define

$$\mathcal{R}_0 = \frac{k\lambda p\phi}{(b + \phi + \delta_E)dc\delta}, \tag{2}$$

then it can be shown in Appendix A that the infected steady state exists if and only if $\mathcal{R}_0 > 1$. In fact, \mathcal{R}_0 can be written as the product of $k\lambda p/(dc\delta)$ and $\phi/(b + \phi + \delta_E)$.

Obviously, $k\lambda p/(dc\delta)$ is the basic reproductive ratio of the standard model without the eclipse phase. $\phi/(b + \phi + \delta_E)$ is the probability that an infected T cell survives the eclipse phase. Therefore, \mathcal{R}_0 in (2) defines the basic reproductive ratio for model (1). It is further shown that \mathcal{R}_0 determines whether the virus population dies out or persists. The infection-free steady state \bar{E} is locally asymptotically stable (l.a.s.) if $\mathcal{R}_0 < 1$ and unstable if $\mathcal{R}_0 > 1$. The infected steady state \bar{E} is l.a.s. whenever it exists, i.e., when $\mathcal{R}_0 > 1$.

It is clear that \mathcal{R}_0 defined in (2) is an increasing function of the progression rate ϕ (a larger value of ϕ corresponds to quicker reverse transcription) and a decreasing function of the mortality rate δ_E . Thus, with all else equal, we expect that the viral strain that can complete reverse transcription more quickly is more likely to lead to a more severe infection (e.g., viral persistence at a higher infection level). This is supported by numerical simulations (Figs. 1 and 2).

In Fig. 1, \mathcal{R}_0 is plotted as either a function of ϕ or a function of δ_E . In Fig. 1(a), $\delta_E = 0.7 \text{ day}^{-1}$ (or $\ln 2/\delta_E = 1 \text{ day}$ (Zack et al., 1990)) is fixed. It shows that $\mathcal{R}_0 > 1$ for $\phi > 0.23 \text{ day}^{-1}$, in which case the viral load will converge to the infected steady state, and that $\mathcal{R}_0 < 1$ for $\phi < 0.23 \text{ day}^{-1}$, in which case the virus population will die out (the infection-free steady state). In Fig. 1(b), $\phi = 1.1 \text{ day}^{-1}$ (or $1/\phi = 0.9 \text{ days}$ (Perelson et al., 1996)) is fixed. Other parameter values are chosen from the literatures: $k = 2.4 \times 10^{-8} \text{ ml day}^{-1}$ (Perelson et al., 1993); $\lambda = 10^4 \text{ ml}^{-1} \text{ day}^{-1}$ (Dixit and Perelson, 2004); $d = 0.01 \text{ day}^{-1}$ (Mohri et al., 1998); $c = 23 \text{ day}^{-1}$ (Ramratnam et al., 1999); $\delta = 1 \text{ day}^{-1}$ (Markowitz et al., 2003). The viral production rate p can be written as $N\delta$, where N (burst size) is the total number of virus particles released by a productively infected cell over its lifespan (Perelson et al., 1996). The estimate of burst size varies from 100 to a few thousands (Haase et al., 1996; Hockett et al., 1999) and possibly could be significantly larger (Yuan Chen et al., submitted for publication). Here, as an example, we choose $N = 4000$. Thus, $p = 4000 \text{ day}^{-1}$. Because only a small fraction of cells in the eclipse phase will revert to the

uninfected state (Essunger and Perelson, 1994), we assume that $b = 0.01 \text{ day}^{-1}$.

Fig. 2 demonstrates the dynamic behavior of the viral load for different progression rate ϕ or mortality rate δ_E . We observe that there is a viral peak followed by an oscillatory approach to a set-point value. As ϕ increases, the time needed to reach the peak viral load is shortened, while the amplitude of the peak and the subsequent set-point value are increased (Fig. 2(a)). We observe similar behaviors as the mortality rate δ_E decreases (Fig. 2(b)). The steady state of the viral load is presented as either a function of ϕ ($\delta_E = 0.7 \text{ day}^{-1}$ is fixed, see Fig. 2(c)) or a function of δ_E ($\phi = 1.1 \text{ day}^{-1}$ is fixed, see Fig. 2(d)). These results show that the viral strain that has a larger progression rate ϕ or a smaller mortality rate δ_E will have a higher viral steady state level, and thus is more likely to induce faster disease progression.

2.2. The model with two strains

To study the invasion of drug-resistant mutant variants into an environment in which the wild-type strain is already established, we incorporate both drug-resistant and drug-sensitive strains in the model (1) and get the following two-strain model:

$$\frac{d}{dt} T(t) = \lambda - dT - k_s V_s T - k_r V_r T + b_s T_{Es}^* + b_r T_{Er}^*,$$

$$\frac{d}{dt} T_{Es}^*(t) = k_s V_s T - (b_s + \phi_s + \delta_{Es}) T_{Es}^*,$$

$$\frac{d}{dt} T_s^*(t) = \phi_s T_{Es}^* - \delta_s T_s^*,$$

$$\frac{d}{dt} V_s(t) = p_s T_s^* - c_s V_s,$$

$$\frac{d}{dt} T_{Er}^*(t) = k_r V_r T - (b_r + \phi_r + \delta_{Er}) T_{Er}^*,$$

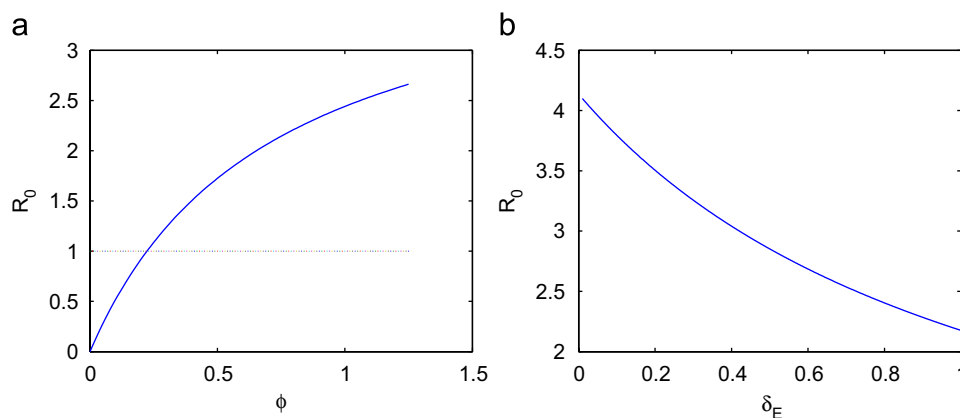


Fig. 1. (a) Plot of the basic reproductive ratio \mathcal{R}_0 in (2) as a function of the progression rate ϕ for a fixed mortality rate of exposed cells $\delta_E = 0.7 \text{ day}^{-1}$. (b) Plot of \mathcal{R}_0 as a function of δ_E for a fixed value of $\phi = 1.1 \text{ day}^{-1}$. Other parameter values are given in the text.

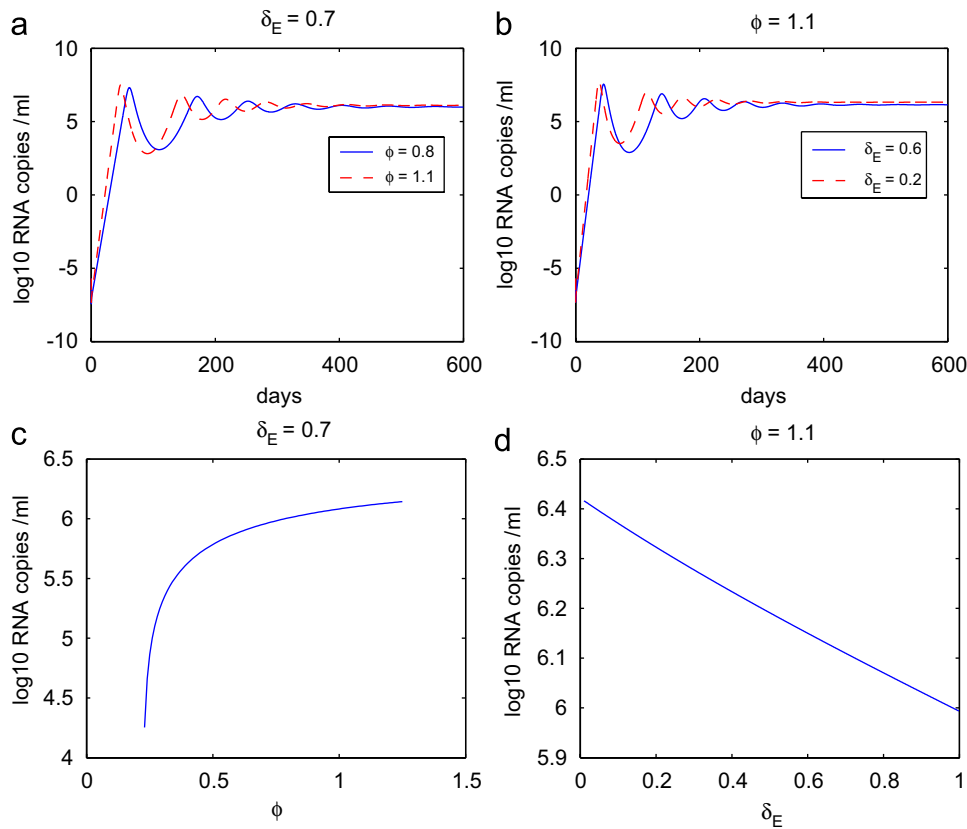


Fig. 2. Time plots for the virus dynamics of model (1) for different ϕ or for different δ_E : (a) $\phi = 0.8$ or 1.1 day^{-1} , $\delta_E = 0.7 \text{ day}^{-1}$; (b) $\delta_E = 0.2$ or 0.6 day^{-1} , $\phi = 1.1 \text{ day}^{-1}$. The values of other parameters are the same as those in Fig. 1. The initial values for T , T_E^* , T^* and V are 10^6 ml^{-1} (Perelson et al., 1993), 0, 0, and 10^{-6} ml^{-1} (Stafford et al., 2000), respectively. The steady state of the viral load is plotted as a function of ϕ or δ_E in (c) and (d). When $\phi < 0.23$, the virus population dies out.

$$\frac{d}{dt} T_r^*(t) = \phi_r T_{Er}^* - \delta_r T_r^*,$$

$$\frac{d}{dt} V_r(t) = p_r T_r^* - c_r V_r, \quad (3)$$

where the subscripts s and r represent the drug sensitive and resistant strains, respectively.

For each strain, we obtain the corresponding reproductive ratio, which is given by

$$\mathcal{R}_i = \frac{k_i \lambda p_i \phi_i}{(b_i + \phi_i + \delta_{Ei}) d c_i \delta_i}, \quad i = s, r. \quad (4)$$

Let \tilde{E}_s denote the steady state in which only the drug-sensitive strain is present and \tilde{E}_r denote the steady state in which only the drug-resistant strain is present. We prove in Appendix B that each steady state is biologically feasible if and only if the reproductive ratio for the corresponding strain is greater than 1. Furthermore, if $\mathcal{R}_r > \mathcal{R}_s > 1$, then \tilde{E}_s is l.a.s. and \tilde{E}_r is unstable. If $\mathcal{R}_r > \mathcal{R}_s$, then \tilde{E}_s is unstable and \tilde{E}_r is l.a.s. Therefore, the resistant strain cannot invade the sensitive strain if $\mathcal{R}_r < \mathcal{R}_s$. If $\mathcal{R}_r > \max(\mathcal{R}_s, 1)$ then the resistant strain is able to invade and out-compete the sensitive strain. We will apply this result to determine the criterion for invasion and to examine how the resistant

virus may evolve to optimize its fitness in the presence of antiretroviral treatment.

2.3. The model with drug therapy and resistance

We modify model (3) by incorporating combination antiretroviral therapy. Currently, a combination of reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) is commonly used in the treatment of HIV infection. RTIs interfere with the process of reverse transcription and prevent the infection of new target cells. PIs prevent infected cells from producing new infectious virus particles (Nowak and May, 2000). To incorporate these drug effects into our model, we define ε_{RTI} and ε_{PI} to be the efficacies of RTIs and PIs for the wild-type strain, respectively. We define these constants relative to the impact of the drugs on the most susceptible genetic variants of RT and protease. As a result, $\varepsilon_i = 0$ ($i = RTI$ or PI) implies that the inhibitor is completely ineffective against wild-type virus, while $\varepsilon_i = 1$ implies that the inhibitor is 100% effective against them. Note that in reality 100% effectiveness may not be clinically feasible due to problems with drug delivery or absorption.

When ε_{PI} is say 0.7, this implies that 70% of the wild-type virus particles produced are non-infectious due to the

action of the protease inhibitor. This population of virions has previously been denoted V_{NI} (Perelson et al., 1996). The remaining 30% of particles are assumed not to be affected by the PI and contain the same population of virions as in an untreated patient. Although this population has a mixture of infectious and non-infectious virions, it has been previously denoted V_I (Perelson et al., 1996) and for simplicity called the infectious population. A more precise definition would call V_I the virions not made non-infectious by the protease inhibitor. In the model below we will follow the drug sensitive and drug resistant forms of the V_I population only, and denote them V_s and V_r , respectively. The equations for the drug sensitive and resistant virion populations corresponding to V_{NI} will be ignored as they can be decoupled from the system (see (5)).

To model the reduced susceptibility of drug-resistant virus variants to antiretroviral agents, we assume that the drug efficacies of RTIs and PIs for the resistant strain are reduced by factors σ_{RTI} and σ_{PI} , respectively. σ_{RTI} and σ_{PI} are between 0 and 1. Therefore, $\varepsilon_{RTI}\sigma_{RTI}$ and $\varepsilon_{PI}\sigma_{PI}$ are the drug efficacies of RTIs and PIs for the resistant strain. $\sigma_i = 1$ ($i = RTI$ or PI) corresponds to the completely drug-sensitive strain while $\sigma_i = 0$ corresponds to the completely drug-resistant strain.

In order to focus on the role of the progression rate of cells in the eclipse phase, ϕ , and the viral production rate, p , on the evolution of drug-resistant virus, we assume that all other parameters for the two strains in model (3) are equal, i.e.,

$$k_i = k, \quad b_i = b, \quad \delta_{Ei} = \delta_E, \quad c_i = c, \quad \text{where } i = s, r.$$

The cost of drug resistance will be discussed later.

The modified model including antiretroviral drugs and resistance can be described by the following equations:

$$\begin{aligned} \frac{d}{dt} T(t) &= \lambda - dT - kV_s T - kV_r T + bT_{Es}^* + bT_{Er}^*, \\ \frac{d}{dt} T_{Es}^*(t) &= kV_s T - (b + \phi_s(1 - \varepsilon_{RTI}) + \delta_E)T_{Es}^*, \\ \frac{d}{dt} T_s^*(t) &= \phi_s(1 - \varepsilon_{RTI})T_{Es}^* - \delta_s T_s^*, \\ \frac{d}{dt} V_s(t) &= p_s(1 - \varepsilon_{PI})T_s^* - cV_s, \\ \frac{d}{dt} T_{Er}^*(t) &= kV_r T - (b + \phi_r(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r)) + \delta_E)T_{Er}^*, \\ \frac{d}{dt} T_r^*(t) &= \phi_r(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r))T_{Er}^* - \delta_r(p_r)T_r^*, \\ \frac{d}{dt} V_r(t) &= p_r(1 - \varepsilon_{PI}\sigma_{PI}(p_r))T_r^* - cV_r. \end{aligned} \quad (5)$$

In the above model, the progression rate of cells in the eclipse class to the productively infected state is reduced due to the effect of RTIs and the viral production rate of infectious virus is reduced due to the effect of HIV protease

inhibitors. As noted above, the equations for the non-infectious particles generated by the PI decouple from the system. These equations are

$$\begin{aligned} \frac{d}{dt} V_s^{NI}(t) &= p_s \varepsilon_{PI} T_s^* - cV_s^{NI}, \\ \frac{d}{dt} V_r^{NI}(t) &= p_r \varepsilon_{PI} \sigma_{PI}(p_r) T_r^* - cV_r^{NI}. \end{aligned}$$

The total drug sensitive and drug resistant populations are then $V_s + V_s^{NI}$ and $V_r + V_r^{NI}$, respectively.

Because HIV resistance is usually associated with changes of highly conserved amino acid residues that are believed to be essential for the optimal enzyme function, drug-resistant variants display some extent of resistance-associated loss of viral fitness in the absence of therapy (Clavel et al., 2000; Coffin, 1995). We incorporate this feature in our model by assuming a reduced progression rate ϕ and a reduced viral production rate p for the resistant strain. Based on the arguments given previously, we assume that infected cells of the wild-type strain, the most susceptible strain to drug therapy, have the maximal progression rate, ϕ_s , and the maximal viral production rate, p_s . Therefore, for all resistant strains we have $\phi_r < \phi_s$ and $p_r < p_s$.

We further assume that the resistance factor, σ_{RTI} , is an increasing function of ϕ_r . The justification for this assumption is the following. ϕ_r is mainly determined by the activity of RT, and the more resistant a strain is to an RTI (a smaller σ_{RTI}) the more likely that the RT of that strain functions poorly (a smaller ϕ_r). Thus, we assume that σ_{RTI} is an increasing function of ϕ_r . Similarly, we assume that σ_{PI} is an increasing function of the viral production rate p_r , which reflects the fact that the more drug-resistant a strain is to a protease inhibitor, the more poorly its protease functions and hence the lower the capacity to produce new infectious virus (Clavel et al., 2000; Zennou et al., 1998).

As suggested in Coombs et al. (2003), Gilchrist et al. (2004), the mortality rate of productively infected cells is also an increasing function of the viral production rate. This is because the loss of cell resources utilized to produce virus may impair cell functions. In addition, cell-mediated immune responses are likely to rapidly kill cells expressing more viral proteins.

To summarize, in model (5) we have assumed that $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$ and $\delta_r(p_r)$ are all increasing functions, with $\sigma_{RTI}(\phi_s) = \sigma_{PI}(p_s) = 1$ and $\delta_r(p_r) = \delta_s$ when $p_r = p_s$, i.e., $\delta_r(p_s) = \delta_s$.

3. Results

In this section, we use model (5) and the results in previous sections to investigate the evolution of drug-resistant strains in the presence of antiretroviral treatment. Specifically, we study how the resistant virus evolves to maximize its fitness, and derive the range of drug efficacy in

which the drug-resistant strain will be able to invade and out-compete the wild-type strain.

3.1. Optimal ϕ_r and p_r that maximize viral fitness

Within-host viral fitness has received increasing interest due to its potential clinical implications for viral load, drug resistance, and disease progression (see reviews in Clavel et al. (2000), Nijhuis et al. (2001), Quinones-Mateu and Arts (2001)). The term fitness is commonly used in clinical settings to describe the ability of a virus to effectively replicate in a particular environment. Due to the fact that drug-resistant virus is less susceptible to antiretroviral regimens, the mutant variant is more fit than the wild-type virus in the presence of drug, although resistance mutations may decrease the intrinsic capacity of the virus to replicate. In practice, it still remains unclear which assay is most appropriate to measure the fitness of HIV-1 isolates, and many studies have been performed to test different hypotheses that extend the definition of relative fitness (reviewed in Quinones-Mateu and Arts (2001)). The basic reproductive ratio is a commonly used measure of the absolute fitness of a virus within a host (Gilchrist et al., 2004). In this section we examine the effect of antiretroviral treatment on the HIV-1 fitness of resistant virus by analyzing the reproductive ratio in the presence of therapy.

The reproductive ratio of the resistant strain (in the presence of therapy) for model (5), denoted by \mathcal{R}_r , is given by a function of ϕ_r and p_r (see (4)):

$$\mathcal{R}_r(\phi_r, p_r) = \frac{k\lambda}{dc} F_1(\phi_r)F_2(p_r), \tag{6}$$

where

$$F_1(\phi_r) = \frac{\phi_r(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r))}{b + \phi_r(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r)) + \delta_E},$$

$$F_2(p_r) = \frac{p_r(1 - \varepsilon_{PI}\sigma_{PI}(p_r))}{\delta_r(p_r)} \tag{7}$$

and $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$, and $\delta_r(p_r)$ are increasing functions as mentioned previously.

Using the formulas (6) and (7) we can find the optimal ϕ_r^* and p_r^* that maximize the reproductive ratio $\mathcal{R}_r(\phi_r, p_r)$. Because we assume that ϕ_r and p_r are independent, we can maximize $F_1(\phi_r)$ and $F_2(p_r)$ individually. When specific forms of the functions $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$, and $\delta_r(p_r)$ are given we are able to obtain explicit formulas for ϕ_r^* and p_r^* . Before we discuss some particular forms of these functions, we present the following result in terms of general functions, which provides some convenient criteria for finding the optimal ϕ_r^* and p_r^* . The proof can be found in Appendix C.

Proposition 1. (i) \mathcal{R}_r is maximized at $\phi_r^* \in (0, \phi_s)$ if there exists a unique value ϕ_r^* satisfying

$$1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r^*) - \phi_r^* \varepsilon_{RTI} \sigma'_{RTI}(\phi_r^*) = 0, \quad 0 < \phi_r^* < \phi_s, \tag{8}$$

and

$$\sigma''_{RTI}(\phi_r^*) \geq 0. \tag{9}$$

(ii) \mathcal{R}_r is maximized at $p_r^* \in (0, p_s)$ if there exists a unique value p_r^* satisfying

$$[1 - \varepsilon_{PI}\sigma_{PI}(p_r^*) - \varepsilon_{PI}p_r^* \sigma'_{PI}(p_r^*)] \delta_r(p_r^*) = p_r^* [1 - \varepsilon_{PI}\sigma_{PI}(p_r^*)] \delta'_r(p_r^*) \tag{10}$$

and

$$\sigma''_{PI}(p_r^*) \geq 0, \quad \delta''_r(p_r^*) \geq 0. \tag{11}$$

Proposition 1 suggests that if $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$ and $\delta_r(p_r)$ are concave up functions, then Eqs. (8) and (10) determine the optimal progression rate ϕ_r^* and the optimal viral production rate p_r^* , respectively. It should be noted that the concave up property is not required in Proposition 1, as the four conditions, (8)–(11), involve only ϕ_r^* and p_r^* .

We now consider some specific forms of the increasing functions $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$ and $\delta_r(p_r)$. Noticing that $\phi_r \leq \phi_s$ and $\sigma_{RTI}(\phi_s) = 1$, we choose $\sigma_{RTI}(\phi_r)$ to be a simple power function

$$\sigma_{RTI}(\phi_r) = \left(\frac{\phi_r}{\phi_s}\right)^a, \tag{12}$$

where $a \geq 1$ is a constant, ensuring that the second derivative is nonnegative. From Proposition 1 we get the optimal progression rate $\phi_r^* = \phi_s / \sqrt[a]{\varepsilon_{RTI}(1+a)}$ (see (8)). Therefore, we can establish the following result:

Result 1. Let $\sigma_{RTI}(\phi_r)$ be given in (12). Then

- (i) if $0 < \varepsilon_{RTI} < \frac{1}{1+a}$, then the optimal progression rate ϕ_r^* is ϕ_s ; and
- (ii) if $\frac{1}{1+a} < \varepsilon_{RTI} < 1$, then the optimal progression rate is an intermediate value within $(0, \phi_s)$; i.e., $\phi_r^* = \phi_s / \sqrt[a]{\varepsilon_{RTI}(1+a)} < \phi_s$.

This result suggests that when the drug efficacy ε_{RTI} is low, the best strategy for a resistant strain to achieve the maximal viral fitness is unchanged from the non-treatment scenario, i.e., infected cells in the eclipse phase need to progress to the productively infected state as soon as possible. When the drug efficacy is high, the optimal viral fitness is achieved at an intermediate value $\phi_r^* = \phi_s / (2\varepsilon_{RTI})$ (in the case of $a = 1$), instead of the maximal progression rate ϕ_s .

To examine the optimal production rate p_r^* , we assume that the drug resistance factor $\sigma_{PI}(p_r)$ is a linear function of p_r , and that the death rate of productively infected cells of the resistant strain follows a non-linear relationship between the cell death and viral production as examined in Coombs et al. (2003); i.e.,

$$\sigma_{PI}(p_r) = \frac{p_r}{p_s} \quad \text{and} \quad \delta_r(p_r) = \left(\frac{\beta}{2}\right) \left(\frac{p_r}{p_s}\right)^2 + m, \tag{13}$$

where $p_r \leq p_s$, β is a constant and m is a fixed background mortality rate. Since we require that the function value $\delta_r(p)$ evaluated at $p = p_s$ (the wild-type strain) is exactly the constant δ_s , β can be chosen to be $2(\delta_s - m)$. Then, using (10) we obtain the following result for the optimal production rate p_r^* .

Result 2. Let $\sigma_{PI}(p_r)$ and $\delta_r(p_r)$ be given by (13). Then the optimal production rate p_r^* determined by Eq. (10) is

$$p_r^* = \frac{\sqrt{4m^2\varepsilon_{PI}^2 + 2m\beta} - 2m\varepsilon_{PI}}{\beta} p_s. \quad (14)$$

Moreover, if $\delta_s < 2m$, then $\tilde{\varepsilon}_{PI} = 1 - \frac{\delta_s}{2m}$ provides a threshold such that

- (i) the optimal production rate of resistant virus is p_s if $0 < \varepsilon_{PI} < \tilde{\varepsilon}_{PI}$; and
- (ii) the optimal production rate of resistant virus is an intermediate value given by (14) if $\tilde{\varepsilon}_{PI} < \varepsilon_{PI} < 1$.

The formula (14) allows us to study the effect of the background mortality rate, m , on the viral fitness. As $m \rightarrow 0$, the optimal production rate $p_r^* \rightarrow 0$. A straightforward calculation shows that $F_2(p_r^*) \rightarrow +\infty$. This implies that slow production is the best strategy for long-lived

infected cells. This result is consistent with the observation in Coombs et al. (2003).

These results are demonstrated in Fig. 3. Fig. 3(a) illustrates the optimal progression rate ϕ_r^* for the special case $a = 1$ in (12). ϕ_r^* is plotted as a function of the drug efficacy of RTIs, ε_{RTI} . Fig. 3(b) plots the optimal production rate p_r^* as a function of the drug efficacy of protease inhibitors, ε_{PI} . In these graphs, m is chosen to be the same as δ_E , and the values for other parameters are the same as those in Fig. 1.

Fig. 3(c) and (d) plot the reproductive ratios for the drug-resistant strain using the optimal values ϕ_r^* and p_r^* (as shown in the upper panel) and the wild-type strain. The flat surface is constant 1, the upper surface is for the reproductive ratio of the drug-resistant strain (\mathcal{R}_r , r for resistant strain), and the lower surface is for that of the wild-type strain (\mathcal{R}_s , s for sensitive strain). We choose different background mortality rates of infected cells. For example, in Fig. 3(c) $m = \delta_E$ and hence $\beta = 2(\delta_s - \delta_E)$, and in Fig. 3(d) $m = d$ and hence $\beta = 2(\delta_s - d)$. In both cases, the reproductive ratio of the resistant strain (\mathcal{R}_r) is always greater than or equal to that of the sensitive strain (\mathcal{R}_s).

We observe that for a large background mortality rate m (for example, m is equal to the death rate of infected cells in the eclipse phase), \mathcal{R}_r becomes less than one as drug

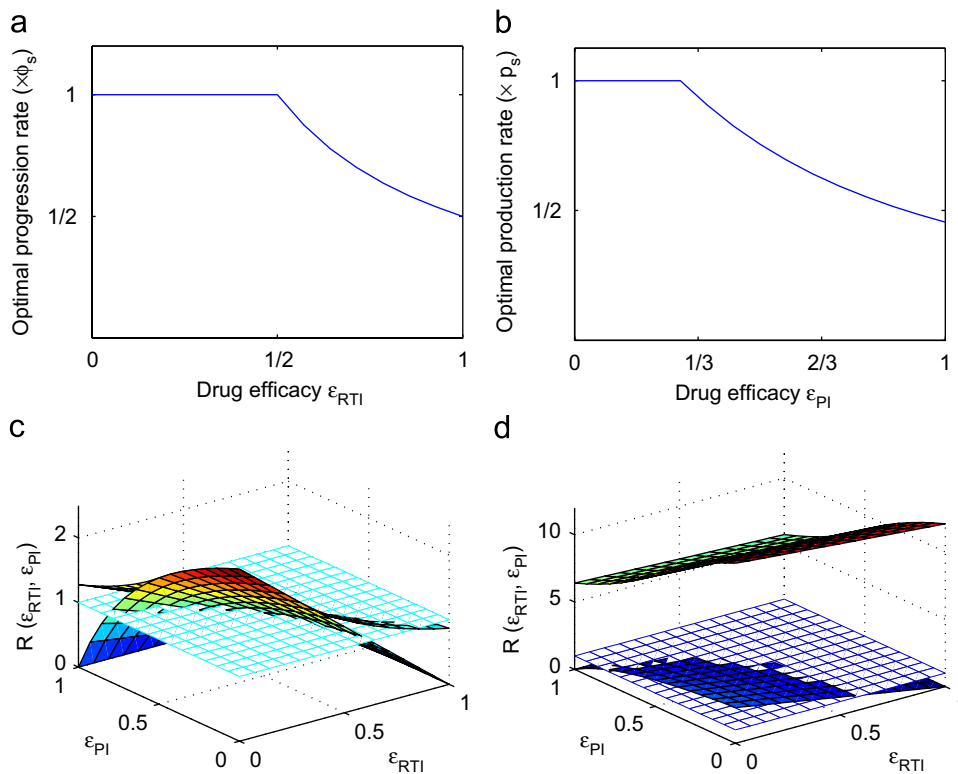


Fig. 3. (a) Plot of the optimal progression rate ϕ_r^* as a function of the drug efficacy of RTIs. (b) Plot of the optimal production rate p_r^* as a function of the drug efficacy of PIs. (c) and (d) are plots of the reproductive ratios of the drug-resistant strain using the optimal ϕ_r^* and p_r^* (as shown in (a) and (b)) and the wild-type strain. In (c) we assume $m = \delta_E = 0.7 \text{ day}^{-1}$; in (d) $m = d = 0.01 \text{ day}^{-1}$. The other parameters are: $k = 2.4 \times 10^{-8} \text{ ml day}^{-1}$, $\lambda = 10^4 \text{ ml}^{-1} \text{ day}^{-1}$, $d = 0.01 \text{ day}^{-1}$, $c = 23 \text{ day}^{-1}$, $b = 0.01 \text{ day}^{-1}$, $\delta_E = 0.7 \text{ day}^{-1}$, $\delta_s = 1 \text{ day}^{-1}$, $\phi_s = 1.25 \text{ day}^{-1}$, $p_s = 4000 \text{ day}^{-1}$. The flat surface is constant 1, the upper surface is for the reproductive ratio of the drug-resistant strain (\mathcal{R}_r), and the lower surface is for that of the wild-type strain (\mathcal{R}_s). We observe that in both cases, \mathcal{R}_r is always greater than or equal to \mathcal{R}_s . In (c), \mathcal{R}_r becomes less than 1 for a high level of drug efficacy, while in (d) \mathcal{R}_r is always greater than 1.

efficacy increases although it is always greater than or equal to \mathcal{R}_s . Thus, in this case both strains of virus will be eradicated for a high drug efficacy (Fig. 3(c)). However, if the background mortality rate is very small (for example, m is equal to the death rate of uninfected T cells), then the threshold value of ε_{PI} corresponding to (14) is $\tilde{\varepsilon}_{PI} = 1 - \frac{\delta_s}{2d}$, which is less than zero. Hence, the optimal production rate p_r^* is always given by the intermediate value determined by (14). In this case, simulation results show that \mathcal{R}_r is always greater than both \mathcal{R}_s and 1 (Fig. 3(d)), and according to the result given in Section 2.2, the drug-resistant strain that evolves with the optimal ϕ_r^* and p_r^* will always be able to invade and out-compete the wild-type strain in the presence of drug therapy.

3.2. Invasion criterion

In the previous section, we have shown that if the drug-resistant strain continuously evolves to adopt the optimal ϕ_r^* and p_r^* that maximize its viral fitness, then the resistant strain will always be expected to emerge and out-compete the established wild-type strain, provided that the antiretroviral treatment is not potent enough to eradicate both strains. Now a natural question arises: if the optimal viral fitness is not achieved, is it possible that the drug-resistant strain can still invade the population of the wild-type virus? If yes, what is the invasion criterion? Below we attempt to address these questions using model (5).

To derive the condition under which a drug-resistant strain (with parameters ϕ_r and p_r , $\phi_r < \phi_s$ and $p_r < p_s$) can invade the sensitive-strain in the presence of drug therapy, we assume that the population of wild-type virus is at the infected steady state. Recall that the infected steady state exists only if the reproductive ratio of the wild-type strain is greater than 1. From Section 2.2, the drug-resistant strain will be able to invade the wild-type strain if the following condition is satisfied:

$$\mathcal{R}_r > \mathcal{R}_s. \tag{15}$$

The reproductive ratio for the drug-resistant strain in the presence of therapy is given by (see (4), (6) and (7))

$$\mathcal{R}_r = \frac{k \lambda}{c d} F_1(\phi_r) F_2(p_r), \quad 0 < \phi_r < \phi_s, \quad 0 < p_r < p_s \tag{16}$$

and the reproductive ratio for the wild-type strain is

$$\mathcal{R}_s = \frac{k \lambda}{c d} F_1(\phi_s) F_2(p_s), \tag{17}$$

where the functions F_1 and F_2 are given in (7). Using the criterion (15) and formulas (16) (17), we can establish the following result. The proof is given in Appendix D.

Result 3. (i) *When both drug efficacies, ε_{RTI} and ε_{PI} , are low then the resistant strain cannot invade the sensitive strain.* (ii) *If the drug efficacies are above certain threshold values then invasion is possible by a resistant strain for which the progression rate ϕ_r and the viral production rate p_r are in some given ranges.*

Clearly, the invasion ranges defined by (37) and (38) in Appendix D depend on the drug efficacies ε_{RTI} and ε_{PI} . In fact, such ranges increase with increasing ε_{RTI} and ε_{PI} (see Fig. 4). Also, if the background death rate m is much smaller than δ_s , then from the formula (38) we can see that $\mathcal{R}_r > \mathcal{R}_s$ for almost all values of p_r such that $p_r < p_s$.

In Fig. 4, the reproductive ratios \mathcal{R}_s and \mathcal{R}_r are plotted either as a function of ϕ_r (Figs. 4(a) and (b)) or as a function of p_r (Figs. 4(c) and (d)) for different values of ε_{RTI} or ε_{PI} . For example, Figs. 4(a) and (b) are for $\varepsilon_{RTI} = 0.4$ and $\varepsilon_{RTI} = 0.5$, respectively, for fixed values of $\varepsilon_{PI} = 0$ and $a = 3$ (see Eq. (12)). We observe that the range in which $\mathcal{R}_r > \mathcal{R}_s$ is bigger for a larger value of ε_{RTI} , suggesting that for a more effective drug therapy, the resistant strain can invade the sensitive strain at a smaller progression rate ϕ_r .

Figs. 4(c) and (d) are for $\varepsilon_{PI} = 0.5$ and $\varepsilon_{PI} = 0.6$, respectively, for a fixed value of $\varepsilon_{RTI} = 0$. We have assumed that the background mortality rate m is equal to δ_E , hence $\beta = 2(\delta_s - \delta_E)$. We observe again that the range in which $\mathcal{R}_r > \mathcal{R}_s$ is bigger for a larger value of ε_{PI} . Therefore, for a higher protease inhibitor drug efficacy, the resistant strain can invade the sensitive strain at a smaller production rate p_r .

4. Discussion and conclusion

Advances in the development of potent combination antiretroviral therapy have dramatically reduced HIV-related morbidity and mortality in the developed world. However, increasing emergence of resistance to antiretroviral drugs could challenge this achievement. The rapid development of drug resistant HIV variants is due to the high turnover of HIV—approximately 10 billion new virus particles are produced per day in the average mid-stage HIV-infected untreated patient (Perelson et al., 1996)—and the exceptionally high error rate of HIV reverse transcriptase (RT). This leads to a high mutation rate and constant production of new viral strains, even in the absence of drug therapy. Understanding the evolution of viral resistance during therapy has far-reaching implications in predicting treatment outcomes and designing treatment strategies employed in clinical practice.

In this work, we have developed a mathematical model to explore the initial constraints that may shape the evolution of viral resistance to antiretroviral drugs. We focused on the interactions between two classes of drugs (reverse transcriptase inhibitors (RTIs) and protease inhibitors) and the enzymes they target, and the trade-offs that are likely to result from such interactions. For RT and its inhibitor we assumed that there is a trade-off between the efficiency of RT and its susceptibility to the inhibitor. Our rationale was as follows: within-patient selection should favor the virus that maximizes its burst size N , the total number of virions made by an infected cell during its lifetime (Gilchrist et al., 2004). The burst size is a function of the lifespan of the infected cell, with longer living cells

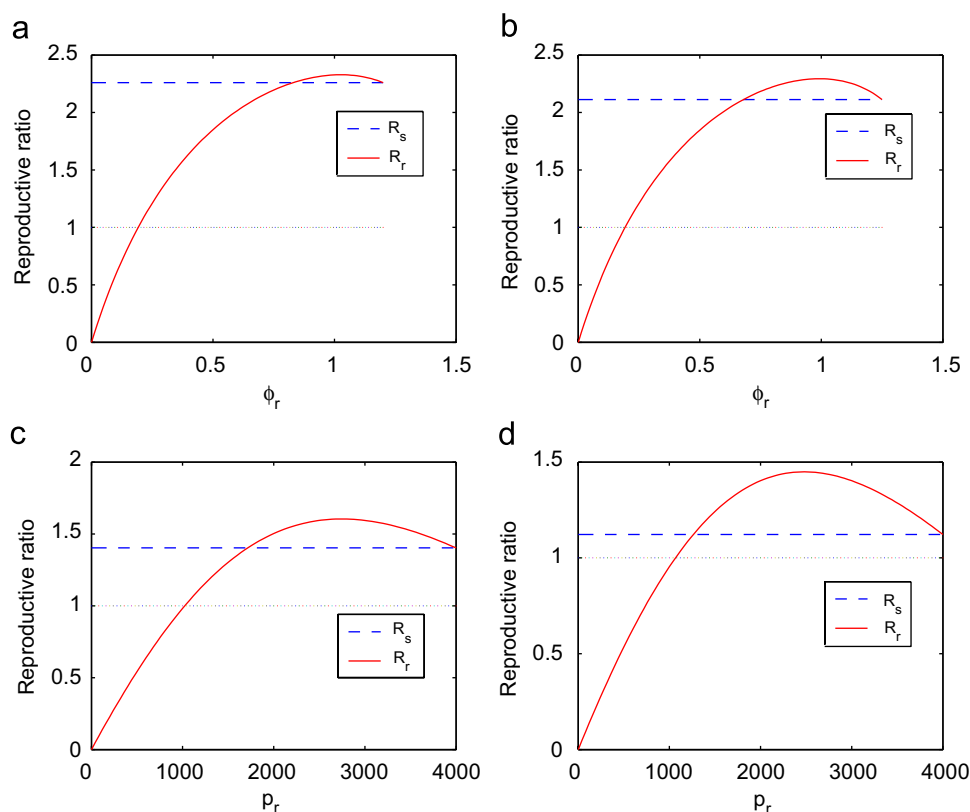


Fig. 4. Plots of \mathcal{R}_s and \mathcal{R}_r for different values of ε_{RTI} or ε_{PI} . The long dashed line is for \mathcal{R}_s , and the solid curve is for \mathcal{R}_r : (a) $\varepsilon_{RTI} = 0.4$; (b) $\varepsilon_{RTI} = 0.5$ ($\varepsilon_{PI} = 0$ and $a = 3$ for both (a) and (b)); (c) $\varepsilon_{PI} = 0.5$; (d) $\varepsilon_{PI} = 0.6$ ($\varepsilon_{RTI} = 0$ for both (c) and (d)). It is shown that the range in which $\mathcal{R}_r > \mathcal{R}_s$ becomes bigger for larger values of ε_{RTI} or ε_{PI} .

potentially able to make more virions. Due to the mortality rate of an infected cell the contribution of virion production to N is effectively discounted as the infected cell ages. In addition, viral mRNA is susceptible to attack by host nucleases once it enters the cell. As a result, within-host selection will inherently favor the virus with an RT that can rapidly reverse transcribe the virus' genome and integrate it into the host's genome. Because we expect these forms of RT to be favored by within-host selection we also expect them to be the most susceptible to inhibition by drugs designed to interfere with their activity. Along the same line of reasoning, other forms of RT that have low activity levels are expected to have low frequencies within the host, maintained primarily by drift and mutation. However, the very genetic changes that confer low activity levels to these RT variants are also likely to confer some resistance to the drugs designed to target RT with high activity levels. As a result we posit that there is likely a simple trade-off between RT activity and susceptibility to RT inhibitors.

The HIV protease also plays a critical role in the virus' life cycle by converting a viral polypeptide into mature and functional viral proteins necessary for viral infectivity. Because mutations associated with the emergence of drug resistance to protease inhibitors modify some key viral proteins (Barrie et al., 1996; Winslow et al., 1995), the virus

forced to develop resistance under drug pressure is thought to have a substantial impairment in its replicative capacity (Clavel and Hance, 2004) even though some additional mutations can compensate for this impaired viral replication potential (Nijhuis et al., 2001). We thus expect that there is a trade-off between the efficacy of protease inhibitors and the viral production rate for the drug-resistant virus variants selected during therapy.

Once a cell begins actively producing virions it becomes highly susceptible to attack by the patient's immune response and viral cytopathic effects. Viral cytopathicity and cell-mediated immune responses are assumed to depend on the rate of viral production. If the mortality rate of infected cells is a concave up function with respect to the viral production rate, then the optimal viral production rate is likely to be at some intermediate level below its physiological maximum (Gilchrist et al., 2004). Under such conditions, an intermediate production rate will maximize the within-patient viral fitness by maximizing the burst size N . This is consistent with our findings when drug resistance to antiretroviral regimens is considered in the model. It should be mentioned that our model assumes that the viral production rate is time independent. When the production rate is allowed to vary with time during infection, the optimal production schedule to maximize the burst size is still to produce virus at a constant rate

(Coombs et al., 2003). More results on the optimal viral production schedule from the perspective of virus can be found in Coombs et al. (2003).

Taken together, the model developed here allows us to investigate the fitness of different HIV variants taking into account the trade-offs between the progression of infected cells in the eclipse phase and resistance to RT inhibitors, between viral production and cell mortality, and between viral production and resistance to protease inhibitors. The model predicts that when the drug efficacy is not high enough to exert sufficient selective pressure (the threshold values in our example are $\varepsilon_{RTI} = 0.5$ and $\varepsilon_{PI} = 1 - \frac{\delta_i}{2m} \sim 0.3$), the resistant strain will be unable to invade the established sensitive strain. For a more effective drug therapy (but not potent enough to eradicate both the wild-type and resistant strains), a wider range of resistant virus variants can invade and out-compete the drug-sensitive strain.

In the present model, the efficacies of antiretroviral drugs are assumed to be constant. However, this assumption may not be realistic because drug concentrations in the blood and in cells continuously vary due to drug absorption, distribution and metabolism. There are some existing models that use time-varying drug concentrations to determine the efficacy of antiviral treatment (Dixit and Perelson, 2004; Huang et al., 2003; Wahl and Nowak, 2000; Wu et al., 2005). The pharmacokinetic model developed by Dixit and Perelson (2004) was also employed to determine drug efficacies for both the sensitive and resistant strains (Rong et al., 2007b). They showed that using the average drug efficacy can still give a good prediction of the long-term outcome of therapy although the viral load displays frequent oscillations when the time-varying drug efficacy is employed.

Another important factor that affects drug efficacy is patients' adherence to prescribed regimen protocols. In fact, non-adherence and non-persistence with antiretroviral therapy is the major reason most individuals fail to benefit from their treatments (Becker et al., 2002). A number of mathematical models have been developed to study the effects of non-perfect adherence to drug regimens (Ferguson et al., 2005; Huang et al., 2003; Phillips et al., 2001; Rong et al., 2007b; Smith, 2006; Wahl and Nowak, 2000; Wu et al., 2006). An overview can be found in Heffernan and Wahl (2005). Careful modeling of drug pharmacokinetics and more realistic adherence patterns can provide an important tool in the study of the kinetics of evolutionary adaptation of HIV to drug therapy and ultimately may improve our ability to develop procedures to defeat this deadly virus.

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Appendix A. Stability of steady states of model (1)

The infection-free steady state of model (1) is

$$\bar{E} = (\bar{T}, \bar{T}_E^*, \bar{T}^*, \bar{V}) = \left(\frac{\lambda}{d}, 0, 0, 0 \right). \tag{18}$$

The infected steady state is $\tilde{E} = (\tilde{T}, \tilde{T}_E^*, \tilde{T}^*, \tilde{V})$, where

$$\begin{aligned} \tilde{T} &= \frac{(b + \phi + \delta_E)c\delta}{kp\phi}, & \tilde{T}_E^* &= \frac{k\lambda p\phi - (b + \phi + \delta_E)dc\delta}{kp\phi(\phi + \delta_E)}, \\ \tilde{T}^* &= \frac{\phi}{\delta} \tilde{T}_E^*, & \tilde{V} &= \frac{p\phi}{c\delta} \tilde{T}_E^*. \end{aligned} \tag{19}$$

Using (2), \tilde{T}_E^* can be rewritten as

$$\tilde{T}_E^* = \frac{(b + \phi + \delta_E)dc\delta}{kp\phi(\phi + \delta_E)} (\mathcal{R}_0 - 1).$$

Therefore, the infected steady state exists if and only if $\mathcal{R}_0 > 1$.

Let $\hat{E} = (\hat{T}, \hat{T}_E^*, \hat{T}^*, \hat{V})$ denote a steady state of model (1). Then the characteristic equation at \hat{E} is

$$\begin{vmatrix} -d - k\hat{V} - \zeta & b & 0 & -k\hat{T} \\ k\hat{V} & -(b + \phi + \delta_E) - \zeta & 0 & k\hat{T} \\ 0 & \phi & -\delta - \zeta & 0 \\ 0 & 0 & p & -c - \zeta \end{vmatrix} = 0, \tag{20}$$

where ζ is an eigenvalue. Eq. (20) can be simplified to

$$\begin{aligned} &[(\zeta + d + k\hat{V})(\zeta + b + \phi + \delta_E) - k\hat{V}b](\zeta + c)(\zeta + \delta) \\ &= (\zeta + d)\phi pk\hat{T}. \end{aligned} \tag{21}$$

(i) Let $\mathcal{R}_0 < 1$. Evaluating (21) at the infection-free steady state \bar{E} , we get

$$(\zeta + d)(\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta) = (\zeta + d)\phi pk \frac{\lambda}{d}.$$

Clearly, there is one negative eigenvalue $-d$, and other eigenvalues are determined by

$$(\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta) = \phi pk \frac{\lambda}{d},$$

which can be rewritten as (see (2))

$$(\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta) = \mathcal{R}_0(b + \phi + \delta_E)c\delta. \tag{22}$$

If ζ has a nonnegative real part, then the modulus of the left-hand side of (22) satisfies

$$|(\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta)| \geq (b + \phi + \delta_E)c\delta, \tag{23}$$

which leads to a contradiction in (22) since $\mathcal{R}_0 < 1$. Therefore, all the eigenvalues have negative real parts, and hence \bar{E} is l.a.s.

When $\mathcal{R}_0 > 1$, we define

$$f(\zeta) = (\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta) - \mathcal{R}_0(b + \phi + \delta_E)c\delta.$$

It is clear that $f(0) < 0$ and $f(\zeta) \rightarrow \infty$ when $\zeta \rightarrow \infty$. By the continuity we know there exists at least one positive root. Hence, the equilibrium point \tilde{E} is unstable if $\mathcal{R}_0 > 1$.

(ii) Let $\mathcal{R}_0 > 1$. Substituting the infected steady state \tilde{E} for \tilde{E} in the characteristic equation (21), we have

$$[(\zeta + d + k\tilde{V})(\zeta + \phi + \delta_E) + (\zeta + d)b](\zeta + c)(\zeta + \delta) = (\zeta + d)(b + \phi + \delta_E)c\delta. \quad (24)$$

Obviously, (24) does not have a nonnegative real solution.

From (19) and (2), we can write \tilde{V} in terms of the basic reproductive ratio in the form

$$\tilde{V} = \frac{b + \phi + \delta_E d}{\phi + \delta_E k} (\mathcal{R}_0 - 1).$$

Now we want to prove that (24) does not have any complex root ζ with a nonnegative real part. Suppose, by contradiction, that $\zeta = x + iy$ with $x \geq 0, y > 0$ is a root of (24).

When $\mathcal{R}_0 \rightarrow 1$, Eq. (24) reduces to

$$(\zeta + d)(\zeta + b + \phi + \delta_E)(\zeta + c)(\zeta + \delta) = (\zeta + d)(b + \phi + \delta_E)c\delta. \quad (25)$$

Using the same arguments as in part (i), we can show that (25) does not have any root with a nonnegative real part.

By the continuous dependence of roots of the characteristic equation on \mathcal{R}_0 , we know that the curve of the roots must cross the imaginary axis as \mathcal{R}_0 decreases sufficiently close to 1. That is, the characteristic equation (24) has a pure imaginary root, say, iy_0 , where $y_0 > 0$. From (24), we have

$$[(d + k\tilde{V} + iy_0)(\phi + \delta_E + iy_0) + (d + iy_0)b](c + iy_0)(\delta + iy_0) = (d + iy_0)(b + \phi + \delta_E)c\delta. \quad (26)$$

We now claim that the following inequality holds:

$$|(d + k\tilde{V} + iy_0)(\phi + \delta_E + iy_0) + (d + iy_0)b| > |d + iy_0|(b + \phi + \delta_E). \quad (27)$$

In fact, after straightforward computations, we have

$$\begin{aligned} & |(d + k\tilde{V} + iy_0)(\phi + \delta_E + iy_0) + (d + iy_0)b|^2 \\ & - |d + iy_0|^2(b + \phi + \delta_E)^2 \\ & = y^4 + (d + k\tilde{V})^2 y^2 + (\phi + \delta_E)^2 k\tilde{V}(2d + k\tilde{V}) \\ & \quad + 2bk\tilde{V}(y^2 + (\phi + \delta_E)d) \\ & > 0. \end{aligned}$$

Thus, (27) holds. It follows that

$$|(d + k\tilde{V} + iy_0)(\phi + \delta_E + iy_0) + (d + iy_0)b||c + iy_0||\delta + iy_0| > |d + iy_0|(b + \phi + \delta_E)c\delta.$$

This contradicts (26). Therefore, we conclude that the characteristic equation (24) does not have any root with a

nonnegative real part. Thus, the infected steady state \tilde{E} is l.a.s whenever it exists.

Appendix B. Steady states and stability of model (3)

Assume that $\tilde{E}_s = (\tilde{T}_s, \tilde{T}_{Es}^*, \tilde{T}_s^*, \tilde{V}_s, 0, 0, 0)$ and $\tilde{E}_r = (\tilde{T}_r, 0, 0, 0, \tilde{T}_{Er}^*, \tilde{T}_r^*, \tilde{V}_r)$. We have

$$\begin{aligned} \tilde{T}_i &= \frac{(b_i + \phi_i + \delta_{Ei})c_i\delta_i}{k_i p_i \phi_i}, \quad \tilde{T}_{Ei}^* = \frac{k_i \lambda p_i \phi_i - (b_i + \phi_i + \delta_{Ei})c_i \delta_i}{k_i p_i \phi_i (\phi_i + \delta_{Ei})}, \\ \tilde{T}_i^* &= \frac{\phi_i}{\delta_i} \tilde{T}_{Ei}^*, \quad \tilde{V}_i = \frac{p_i \phi_i}{c_i \delta_i} \tilde{T}_{Ei}^*, \quad i = s, r. \end{aligned} \quad (28)$$

Obviously, each steady state exists if and only if the corresponding reproductive ratio is greater than 1.

If $\tilde{E} = (\tilde{T}, \tilde{T}_{Es}^*, \tilde{T}_s^*, \tilde{V}_s, \tilde{T}_{Er}^*, \tilde{T}_r^*, \tilde{V}_r)$ denotes a coexistence steady state (i.e., $\tilde{V}_s \neq 0$ and $\tilde{V}_r \neq 0$, hence both strains are present), then \tilde{T} satisfies $\tilde{T} = \frac{\lambda}{d} \frac{1}{\mathcal{R}_s} = \frac{\lambda}{d} \frac{1}{\mathcal{R}_r}$. Therefore, \tilde{E} exists only if $\mathcal{R}_r = \mathcal{R}_s$.

The Jacobian matrix at \tilde{E}_s is

$$J = \begin{pmatrix} G & * \\ 0 & H \end{pmatrix},$$

where

$$G = \begin{pmatrix} -d_s - k_s \tilde{V}_s & b_s & 0 & -k_s \tilde{T}_s \\ k_s \tilde{V}_s & -(b_s + \phi_s + \delta_{Es}) & 0 & k_s \tilde{T}_s \\ 0 & \phi_s & -\delta_s & 0 \\ 0 & 0 & p_s & -c_s \end{pmatrix}, \quad (29)$$

$$H = \begin{pmatrix} -(b_r + \phi_r + \delta_{Er}) & 0 & k_r \tilde{T}_s \\ \phi_r & -\delta_r & 0 \\ 0 & p_r & -c_r \end{pmatrix} \quad (30)$$

and “*” denotes a 4×3 matrix that does not affect the proof. Notice that the characteristic equation of G is exactly the same equation (20) with the subscript s added. From Appendix A and $\mathcal{R}_s > 1$, all eigenvalues of G have negative real parts. Thus, the stability of \tilde{E}_s is completely determined by the eigenvalues of H .

Suppose ζ is an eigenvalue of H , then ζ satisfies

$$[\zeta + (b_r + \phi_r + \delta_{Er})](\zeta + \delta_r)(\zeta + c_r) = k_r p_r \phi_r \tilde{T}_s. \quad (31)$$

If we define

$$\mathcal{R}_r^\circ = \frac{k_r p_r \phi_r \tilde{T}_s}{(b_r + \phi_r + \delta_{Er})c_r \delta_r}, \quad (32)$$

then (31) can be rewritten as

$$[\zeta + (b_r + \phi_r + \delta_{Er})](\zeta + \delta_r)(\zeta + c_r) = \mathcal{R}_r^\circ (b_r + \phi_r + \delta_{Er})c_r \delta_r. \quad (33)$$

We remark that \mathcal{R}_r° represents the *effective* reproductive ratio for the drug-resistant strain (i.e., the reproductive ratio when the sensitive strain is at its infected steady state). If $\mathcal{R}_r^\circ > 1$, then the resistant strain will be able to invade the established wild-type strain.

Using the same arguments as in Appendix A, we have that \tilde{E}_s is l.a.s. if $\mathcal{R}_r^\circ < 1$ and it is unstable if $\mathcal{R}_r^\circ > 1$. Notice from (28) and (4) that

$$\tilde{T}_s = \frac{\lambda}{d} \frac{1}{\mathcal{R}_s}.$$

Substituting this for \tilde{T}_s in (32) and using (4), we obtain

$$\mathcal{R}_r^\circ = \frac{\mathcal{R}_r}{\mathcal{R}_s}.$$

Thus, $\mathcal{R}_r^\circ > 1$ if and only if $\mathcal{R}_r > \mathcal{R}_s$ and $\mathcal{R}_r^\circ < 1$ if and only if $\mathcal{R}_r < \mathcal{R}_s$. It follows that \tilde{E}_s is l.a.s. if $\mathcal{R}_r < \mathcal{R}_s$, and it is unstable if $\mathcal{R}_r > \mathcal{R}_s$.

From the mathematical symmetry of the two strains we can use the same arguments for the stability analysis of \tilde{E}_r , and show that \tilde{E}_r is l.a.s. if $\mathcal{R}_r > \mathcal{R}_s$ and unstable if $\mathcal{R}_r < \mathcal{R}_s$.

Appendix C. Proof of Proposition 1

(i) We want to find ϕ_r^* that maximizes $F_1(\phi_r)$ (see Eq. (7)). Let

$$f_1(\phi_r) = \phi_r(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r)). \quad (34)$$

Then $F_1(\phi_r)$ is maximized if and only if $f_1(\phi_r)$ is maximized. Notice that (8) holds if and only if ϕ_r^* is a critical point of f_1 on $(0, \phi_s)$. Since $\varepsilon_{RTI}\sigma_{RTI}(\phi_r^*) < 1$, we have

$$\begin{aligned} f_1''(\phi_r^*) &= -2\varepsilon_{RTI}\sigma'_{RTI}(\phi_r^*) - \varepsilon_{RTI}\phi_r^*\sigma''_{RTI}(\phi_r^*) \\ &= -\frac{2(1 - \varepsilon_{RTI}\sigma_{RTI}(\phi_r^*))}{\phi_r^*} - \varepsilon_{RTI}\phi_r^*\sigma''_{RTI}(\phi_r^*) \\ &< -\varepsilon_{RTI}\phi_r^*\sigma''_{RTI}(\phi_r^*). \end{aligned}$$

Hence, $f_1''(\phi_r^*) < 0$ if $\sigma''_{RTI}(\phi_r^*) \geq 0$. It follows that f_1 , and hence \mathcal{R}_r , assumes its maximum at ϕ_r^* if (8) and (9) hold.

(ii) If p_r^* satisfies (10) then we can easily verify that p_r^* is a critical point of $F_2(p_r)$; i.e., $F_2'(p_r^*) = 0$. The second derivative of $F_2(p_r)$ at p_r^* is

$$F_2''(p_r^*) = \frac{[-2\varepsilon_{PI}\sigma'_{PI}(p_r^*) - \varepsilon_{PI}p_r^*\sigma''_{PI}(p_r^*)]\delta_r(p_r^*) - p_r^*[1 - \varepsilon_{PI}\sigma_{PI}(p_r^*)]\delta_r''(p_r^*)}{\delta_r^2(p_r^*)}. \quad (35)$$

It is easy to verify that $F_2''(p_r^*) < 0$ if $\sigma''_{PI}(p_r^*)$ and $\delta_r''(p_r^*)$ are both nonnegative, which implies that $F_2(p_r)$ has a maximum at p_r^* . Therefore, \mathcal{R}_r is maximized at $p_r = p_r^*$. This finishes the proof of Proposition 1.

Appendix D. Proof of Result 3

We prove this result using the specific functional forms for $\sigma_{RTI}(\phi_r)$, $\sigma_{PI}(p_r)$, and $\delta_r(p_r)$ given by (12) (in the case of $a = 1$) and (13).

(i) The invasion condition (15) is equivalent to (see (16) and (17))

$$F_1(\phi_r)F_2(p_r) > F_1(\phi_s)F_2(p_s). \quad (36)$$

From the analysis in Section 3.1, we know that for a low level of drug efficacy ε_{RTI} (e.g., $0 < \varepsilon_{RTI} < 1/2$ when $a = 1$,

see Result 1), the maximum of $F_1(\phi_r)$ can only occur at $\phi_r^* = \phi_s$. Thus, $F_1(\phi_r) < F_1(\phi_s)$ for $\phi_r < \phi_s$. Similarly, from Result 2 we know that the maximum of $F_2(p_r)$ can only occur at $p_r^* = p_s$ if $0 < \varepsilon_{PI} < \tilde{\varepsilon}_{PI}$, where $\tilde{\varepsilon}_{PI} = 1 - \frac{\delta_s}{2m}$. Thus, $F_2(p_r) < F_2(p_s)$ for $p_r < p_s$. Therefore, the invasion condition (36) does not hold for any drug efficacies with $0 < \varepsilon_{RTI} < 1/2$ and $0 < \varepsilon_{PI} < 1 - \frac{\delta_s}{2m}$.

(ii) When $1/2 < \varepsilon_{RTI} < 1$, solving the inequality $F_1(\phi_r) > F_1(\phi_s)$ for ϕ_r , we have

$$\frac{\phi_r \left(1 - \frac{\phi_r}{\phi_s} \varepsilon_{RTI}\right)}{b + \phi_r \left(1 - \frac{\phi_r}{\phi_s} \varepsilon_{RTI}\right) + \delta_E} > \frac{\phi_s(1 - \varepsilon_{RTI})}{b + \phi_s(1 - \varepsilon_{RTI}) + \delta_E},$$

which is equivalent to

$$\phi_r(1 - \varepsilon_{RTI}\phi_r/\phi_s) > \phi_s(1 - \varepsilon_{RTI}),$$

or

$$\varepsilon_{RTI}\phi_r^2 - \phi_s\phi_r + \phi_s^2(1 - \varepsilon_{RTI}) < 0.$$

From the above inequality (and noticing that $\varepsilon_{RTI} > 1/2$), we have

$$\left(\frac{1}{\varepsilon_{RTI}} - 1\right)\phi_s < \phi_r < \phi_s. \quad (37)$$

When $\tilde{\varepsilon}_{PI} < \varepsilon_{PI} < 1$, solving the inequality $F_2(p_r) > F_2(p_s)$ for p_r gives

$$\frac{p_r \left(1 - \frac{p_r}{p_s} \varepsilon_{PI}\right)}{\frac{\beta \left(\frac{p_r}{p_s}\right)^2}{2} + m} > \frac{p_s(1 - \varepsilon_{PI})}{\delta_s},$$

which can be rewritten as

$$[2\delta_s\varepsilon_{PI} + \beta(1 - \varepsilon_{PI})]p_r^2 - 2\delta_s p_s p_r + 2m(1 - \varepsilon_{PI})p_s^2 < 0.$$

Noticing that $\beta = 2(\delta_s - m)$, we can solve the above inequality and obtain

$$\frac{m(1 - \varepsilon_{PI})}{\delta_s - m(1 - \varepsilon_{PI})} p_s < p_r < p_s. \quad (38)$$

Since $\varepsilon_{PI} > \tilde{\varepsilon}_{PI} = 1 - \frac{\delta_s}{2m}$, which guarantees that

$$\frac{m(1 - \varepsilon_{PI})}{\delta_s - m(1 - \varepsilon_{PI})} < 1,$$

we know that (38) defines an interval on which $F_2(p_r) > F_2(p_s)$. Therefore, for (ϕ_r, p_r) in the regions defined by (37) and (38) the invasion condition (36), or equivalently (15), holds.

This finishes the proof of Result 3.

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