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## Lethal mutagenesis in a structured environment

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### Abstract

We analyze how lethal mutagenesis operates in a compartmentalized host. We assume that different compartments receive different amounts of mutagen and that virions can migrate among compartments. We address two main questions: 1. To what extent can refugia, i.e., compartments that receive little mutagen, prevent extinction? 2. Does migration among compartments limit the effectiveness of refugia? We find that if there is little migration, extinction has to be achieved separately in all compartments. In this case, the total dose of mutagen administered to the host needs to be so high that the mutagen is effective even in the refugia. By contrast, if migration is extensive, then lethal mutagenesis is effective as long as the average growth in all compartments is reduced to below replacement levels. The effectiveness of migration is governed by the ratio of virion replication and death rates,  $R_0$ . The smaller  $R_0$ , the less migration is necessary to neutralize refugia and the less mutagen is necessary to achieve extinction at high migration rates.

### Keywords

lethal mutagenesis; mutagen; drug refugia; structured host

## 1 Introduction

Lethal mutagenesis is a promising new antiviral therapy in which viruses are driven to extinction by chemically increasing their mutation rate (Freistadt et al., 2004). Lethal mutagenesis is appealing because it has the potential to lead to a broad-spectrum antiviral drug. Unlike other antiviral drugs, which generally have to be tailored to a specific virus, chemical mutagens such as nucleoside analogs tend to work on large classes of viruses. In the laboratory setting, lethal mutagenesis has been achieved with a variety of different viruses (Loeb et al., 1999; Sierra et al., 2000; Grande-Pérez et al., 2002; Pariente et al., 2001, 2003; Ruiz-Jarabo et al., 2003; Grande-Perez et al., 2005; Ojosnegros et al., 2008). In the clinical setting, an effective broad-spectrum antiviral drug is ribavirin (Fernandez et al., 1986; Pawlotsky, 2003), and one of its modes of action is mutagenesis (Crotty et al., 2000; Graci and Cameron, 2006).

The idea of killing a virus by mutagenesis is not new; experimental investigations date back at least twenty years (Holland et al., 1990). Yet a systematic theoretical investigation of lethal

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mutagenesis has begun only recently (Bull et al., 2007; Zeldovich et al., 2007; Bull and Wilke, 2008). The key insight of the recent works is that a theoretical description of lethal mutagenesis requires a model that keeps track of absolute virion frequencies (Bull et al., 2005; Wilke, 2005; Bull et al., 2007). Models that work with relative virion frequencies, such as the ones commonly studied in the quasispecies literature (Swetina and Schuster, 1982; Eigen et al., 1989; Campos and Fontanari, 1998; Woodcock and Higgs, 1996; Tannenbaum et al., 2004; Tannenbaum and Shakhnovich, 2004; Wilke, 2005; Saakian and Hu, 2006; Saakian et al., 2006; Stich et al., 2007; Takeuchi and Hogeweg, 2007), provide limited insight into the conditions under which viral extinction takes place.

Here, we extend the recent modeling work to the question of how lethal mutagenesis acts in a structured environment. We envision the situation of a patient suffering from a viral infection that has spread to multiple tissues, and ask how effective lethal mutagenesis will be if the mutagen penetrates certain tissues more readily than others and if there is migration of virus among the tissues. Specifically, we are asking to what extent refugia, i.e., tissues that admit only very little mutagen, interfere with successful lethal mutagenesis.

## 2 Model

### 2.1 Model assumptions

We model an infected host consisting of  $k$  compartments. The compartments reflect different tissues in the host. We assume that viruses replicate with rate  $r$  and die with rate  $d$ . Virus death may be caused by the immune system, by spontaneous decay of virus particles, or by other mechanisms. We do not make any specific assumptions about the causes of virus death. Upon replication, the virus can mutate. We assume that all mutations are either neutral or lethal. We keep track of only the lethal mutations.

We further assume that the viral infection in the host is being treated with a mutagen, which however does not penetrate all compartments equally well. We denote the efficiency with which a tissue absorbs mutagen by the mutagen absorption coefficient  $\alpha_i$ ;  $\alpha_i$  is a number between 0 and 1. The overall amount of mutagen administered to the host is proportional to  $U$ . We choose the units of  $U$  such that the mean number of lethal mutations per virion per round of replication in compartment  $i$  is  $\alpha_i U$ . We assume that the total amount of mutagen administered to the host is proportional to  $U$ , and we neglect all mutations that arise as a consequence of the virus's baseline mutation rate. Therefore, in compartment  $i$ , the mutagen attenuates virus growth by a factor of  $g_i = e^{-\alpha_i U}$  (Bull et al., 2007).

We denote the absolute number of virions in compartment  $i$  by  $n_i$ , and the total virus population size by  $N = \sum_{i=1}^k n_i$ . We assume that virions migrate among compartments with rate  $m$ . The change in the number of virions in compartment  $i$  is given by

$$\frac{dn_i(t)}{dt} = (rg_i - d - m)n_i(t) + m \sum_{j \neq i} c_{ji} n_j(t). \quad (1)$$

The matrix  $(c_{ji})$  describes the connectivity graph among compartments. The elements of  $(c_{ji})$  are between 0 and 1, indicating what fraction of virions that migrate from compartment  $j$  will end up in compartment  $i$ . Note that  $\sum_{i=1}^k c_{ji} = 1$ . Further, we assume that there is one compartment whose  $\alpha_i$  is smaller than all other  $\alpha_i$  values. Without loss of generality, we number compartments such that  $g_1 > g_2 \geq g_3 \geq \dots \geq g_k$ .

Equation (1) describes a system in which virus can grow without bound. Clearly, unbounded growth is not realistic. A more realistic model would include tissue-specific saturation terms  $-n_i^2(t)/K_i$ , where  $K_i$  is the carrying capacity of the  $i^{\text{th}}$  compartment. In this case, we would replace Eq. (1) with

$$\frac{dn_i(t)}{dt} = (rg_i - d - m)n_i(t) + m \sum_{j \neq i} c_{ji}n_j(t) - n_i^2(t)/K_i. \quad (2)$$

The saturation terms dramatically alter the model dynamics for large viral population sizes, when  $n_i(t) \sim K_i$ . Near extinction, however, we have  $n_i(t) \ll K_i$  and the saturation terms are negligible. In the present work, we are primarily interested in lethal mutagenesis and the mutation rate necessary to achieve it. Therefore, we neglect the saturation terms in what follows and focus exclusively on Eq. (1). Because of this modeling decision, all results we report are only valid far from saturation.

## 2.2 Solution

We can rewrite Eq. (1) in vector notation as  $d\mathbf{n}(t)/dt = \mathbf{G}\mathbf{n}(t)$ , where  $\mathbf{n} = (n_1, \dots, n_k)$  and  $\mathbf{G} = (G_{ij})$  is given by

$$G_{ij} = (rg_i - d - m)\delta_{ij} + c_{ij}m(1 - \delta_{ij}). \quad (3)$$

Here,  $\delta_{ij}$  is the Kronecker Delta; it is equal to 1 if  $i = j$  and 0 otherwise.

Given the initial state of the virus population at time  $t = 0$ ,  $\mathbf{n}(0)$ , we can write  $\mathbf{n}(t)$  as

$$\mathbf{n}(t) = \sum_{i=1}^k \beta_i \phi_i e^{\lambda_i t}. \quad (4)$$

$\phi_i$  and  $\lambda_i$  represent the  $i^{\text{th}}$  right-eigenvector and eigenvalue of  $\mathbf{G}$ , respectively, and  $\beta_i$  is chosen to satisfy the initial conditions of  $\mathbf{n}(0)$ .

Without loss of generality, we order eigenvalues by declining real part. In other words, we assume that  $\Re(\lambda_i) \geq \Re(\lambda_j)$  for all  $i < j$ . The asymptotic behavior of  $\mathbf{n}(t)$  for  $t \rightarrow \infty$  is determined by  $\Re(\lambda_1)$ . The total virus population size  $N(t)$  will decrease over time if  $\Re(\lambda_1) < 0$ . Thus, lethal mutagenesis will be successful for all mutation rates  $U > U_{\text{crit}}$ , where  $U_{\text{crit}}$  satisfies  $\Re[\lambda_1(U_{\text{crit}})] = 0$ . For simplicity, in the following we will only consider cases in which  $\lambda_1$  is real and non-degenerate. The Frobenius-Perron theorem guarantees that these two conditions are satisfied if  $(c_{ji})$  is primitive (Varga, 2000).

## 3 Results

### 3.1 Two compartments

We first analyze the case of two compartments,  $k = 2$ . For this special case, we can calculate  $\lambda_1$  exactly. Because migration from one compartment must lead to the other, we have  $c_{ji} = 1 - \delta_{ij}$ . The matrix  $\mathbf{G}$  reads

$$\mathbf{G} = \begin{pmatrix} rg_1 - d - m & m \\ m & rg_2 - d - m \end{pmatrix}. \quad (5)$$

The principal eigenvalue of  $\mathbf{G}$  is given by

$$\lambda_1 = \frac{1}{2} \left[ r(g_1 + g_2) - 2d - 2m + \sqrt{(rg_1 - rg_2)^2 + 4m^2} \right]. \quad (6)$$

If there is no migration,  $m = 0$ , the principal eigenvalue is  $\lambda_1 = rg_1 - d$ . This result makes intuitive sense. In the absence of migration, we expect the asymptotic growth of the virus population to be determined by the growth in the compartment that receives less mutagen. (Recall that  $g_1 > g_2$  by earlier assumption.) Consequently, the population will go extinct if and only if it goes extinct in the compartment that absorbs less mutagen.

By contrast, for large amounts of migration,  $m \rightarrow \infty$ , the principal eigenvalue approaches  $r(g_1 + g_2)/2 - d$ . Thus, for a sufficiently large migration rate, the viral population behaves like a homogeneous population growing in a single tissue whose effective mutagen absorption coefficient is  $\alpha_{\text{eff}} = (-1/U) \ln[(g_1 + g_2)/2]$ .

### 3.2 Multiple compartments

We now consider the case of an arbitrary number of compartments. For this case, a general analytic expression for  $\lambda_1$  does not exist, but we can use perturbation theory (Mathews and Walker, 1970) to investigate the limits of either very little migration ( $m \approx 0$ ) or extensive migration ( $m \rightarrow \infty$ ). To keep the analytic expressions manageable, we only treat the special case of uniform migration among all compartments,  $c_{ij} = (1 - \delta_{ij})/(k - 1)$ .

We begin with the case of small  $m$ . We write  $\mathbf{G}$  as the sum of two matrices  $\mathbf{G}_0$  and  $\mathbf{H}$ ,  $\mathbf{G} = \mathbf{G}_0 + \mathbf{H}$ . We choose the matrices  $\mathbf{G}_0$  and  $\mathbf{H}$  such that we know the spectrum of  $\mathbf{G}_0$  and that we can consider  $\mathbf{H}$  a small perturbation to  $\mathbf{G}_0$ . We use

$$\mathbf{G}_0 = \text{diag}(rg_1 - d - m, \dots, rg_k - d - m) \quad (7)$$

and

$$(\mathbf{H})_{ij} = (1 - \delta_{ij}) \frac{m}{k - 1}. \quad (8)$$

Clearly, for sufficiently small  $m$ ,  $\mathbf{H}$  is negligible compared to  $\mathbf{G}_0$ . Using standard non-degenerate perturbation theory (Mathews and Walker, 1970), we obtain to second order in  $m$ :

$$\lambda_1 = rg_1 - d - m + \sum_{j \neq 1} \frac{m^2}{(k - 1)^2 r(g_1 - g_j)} + O(m^3). \quad (9)$$

For large  $m$ , we follow the same procedure. We write  $\mathbf{G} = m(\mathbf{G}_\infty + \mathbf{H}') - d\mathbf{I}$ , where  $\mathbf{I}$  is the  $k$ -by- $k$  identity matrix,

$$(\mathbf{G}_\infty)_{ij} = (1 - \delta_{ij}) \frac{1}{k - 1} - \delta_{ij}, \quad (10)$$

and

$$\mathbf{H}' = (r/m)\text{diag}(g_1, \dots, g_k). \quad (11)$$

We apply perturbation theory to the matrix  $\mathbf{G}_\infty + \mathbf{H}'$ . Clearly,  $\mathbf{H}'$  is negligible compared to  $\mathbf{G}_\infty$  if  $r \ll m$ .

For any  $k$ , the principal eigenvalue of  $\mathbf{G}_\infty$  is 0, and the corresponding normalized eigenvector is  $\phi_1 = (\frac{1}{\sqrt{k}}, \dots, \frac{1}{\sqrt{k}})$ . We obtain the leading correction to the principal eigenvalue of  $\mathbf{G}$  from the expression  $\phi_1 \cdot \mathbf{H}' \cdot \phi_1^T$ . We find:

$$\lambda_1 = \sum_i \frac{rg_i}{k} - d + O(m^{-1}). \quad (12)$$

These approximations recapitulate the results for two compartments: If migration is weak, then the overall growth of the virus population is determined by the growth in the compartment that provides the best environment for replicating viruses, i.e.,  $\alpha_i \ll 1$ . If migration is strong, then the overall growth is determined by the average growth over all compartments. In general, migration tends to reduce the growth rate of the viral population, i.e., the larger  $m$ , the smaller  $\lambda_1$ . Since our approximation to the principal eigenvalue of  $\mathbf{G}$  for large  $m$  is only valid if  $r$  is small compared to  $m$ , migration will be more efficient in reducing the average growth rate of the viral population for smaller  $r$ .

The maximal amount by which migration can reduce the average growth rate depends on the mutagen absorption coefficients  $\alpha_i$ . We see from eqs. (9) and (12) that a system with large differences in absorption coefficients  $\alpha_i$  (hence large differences in growth factors  $g_i$ ) will experience an overall larger effect from migration, because the average  $\sum_i rg_i/k$  will be smaller compared to the maximum value  $rg_1$ .

Next, we determine the level of migration at which the overall viral growth becomes comparable to the average growth over all compartments. We calculate the point  $m^*$  at which the two approximations Eqs. (9) and (12) intersect (Fig. 1), and find

$$m^* = r(g_1 - \sum_i \frac{g_i}{k}). \quad (13)$$

For values of  $m \ll m^*$ , the small- $m$  approximation is appropriate, whereas for values of  $m \gg m^*$ , the large- $m$  approximation is appropriate.

From Eq. (13), we see that  $m^*$  is proportional both to  $r$  and to the difference between the largest  $g_i$  and the mean  $g_i$ . The proportionality to  $r$  simply reflects that  $m$  and  $r$  need to be measured in the same units for meaningful comparison. Migration will generally be small if little migration happens on the time scale on which viruses replicate, and it will be large if little replication happens on the time scale on which viruses migrate. The proportionality to  $g_1 - \sum_i g_i/k$  means that the larger the difference in effective growth rates among compartments, the more migration will be required to reach optimal conditions for lethal mutagenesis. Thus, the condition under which migration reduces overall virus growth the most, large  $g_1 - \sum_i g_i/k$ , is also the condition under which comparatively more migration is needed to achieve the maximum reduction in virus growth.

### 3.3 Sectors

In the previous subsection, we found simple expressions for the overall virus growth in the limiting cases of small or large  $m$ . The advantage of these expressions is that they are asymptotically exact. Their drawback, however, is that they were derived under the assumption of homogeneous migration among all compartments. We will now develop an alternative approximation method that does not require homogeneous migration. The alternative method does not employ an asymptotically correct expansion, but instead relies on a phenomenological description of the system.

We make use of the observation that a system with a large difference in growth rates between compartments will experience a larger effect from migration. We assume that we can subdivide all compartments into two groups, one group in which the growth rate is comparatively high and one group in which it is comparatively low. We assume that there are  $p$  compartments in the first group (small  $\alpha_i$ ) and  $q$  compartments in the second (large  $\alpha_i$ ), with  $p + q = k$ . We refer to these two groups as *sectors*.

We denote by  $c'_{ji}$  the fraction of virions that travel out of sector  $j$  and into sector  $i$ , and we assume that any migration from a sector to itself can be ignored. We then consider a population vector such that the  $i^{\text{th}}$  position corresponds to the total number of virions in sector  $i$ . Our growth matrix  $\mathbf{G}$  takes on the form

$$\mathbf{G} = \begin{pmatrix} rg_1 - d - c'_{12}m & c'_{21}m \\ c'_{12}m & rg_2 - d - c'_{21}m \end{pmatrix}. \quad (14)$$

We determine  $c'_{ji}$  by assuming that we can take the sum of all fractions of virions going from a compartment in sector  $i$  to a compartment in sector  $j$  and then average each of these sums over the total number of compartments in sector  $i$ . In other words,

$$c'_{12} = \frac{1}{p} \sum_{j=1}^p \sum_{i=p+1}^k c_{ji}, \quad (15)$$

$$c'_{21} = \frac{1}{q} \sum_{j=p+1}^k \sum_{i=1}^p c_{ji}. \quad (16)$$

Under the sector approximation,  $\mathbf{G}$  is a  $2 \times 2$  matrix, and we can calculate the principal eigenvalue  $\lambda_1$  exactly. We obtain

$$\lambda_1 = \frac{1}{2} \left[ r(g_1 + g_2) - 2d - m(c'_{12} + c'_{21}) + \sqrt{[m(c'_{12} + c'_{21}) + r(g_1 - g_2)]^2 + 4mrc'_{12}(g_2 - g_1)} \right]. \quad (17)$$

Strictly speaking, Eqs. (14) and (17) assume that all compartments within each sector have the same  $\alpha_i$ . But the sector approximation works also if there is variation in absorption coefficients  $\alpha_j$  within sectors. To model this variation, we use an appropriate effective absorption coefficient  $\alpha_i$  for each sector. We calculate our effective absorption coefficients as follows:

$$\tilde{\alpha}_1 = \frac{1}{p} \sum_{i=1}^p \alpha_i, \quad (18)$$

$$\tilde{\alpha}_2 = \frac{1}{q} \sum_{i=p+1}^k \alpha_i. \quad (19)$$

A priori, it is not necessarily clear into which sector a specific compartment should be grouped. Through extensive numerical simulation, we determined the following rule of thumb. If

$\alpha_i < \frac{1}{2} [\alpha_{max} + \alpha_{min}]$ , compartment  $i$  can be put into the high growth rate sector and vice versa for the low growth rate sector, where  $\alpha_{max}$  and  $\alpha_{min}$  are the maximum and minimum absorption coefficients, respectively, of our system.

We find that the sector approximation loses accuracy for very low levels of migration and a large spread in absorption rates  $\alpha_i$  within sectors. Nonetheless, by and large, the sector approximation provides a useful and reliable description of the viral growth rate under mutagenesis and migration. See Fig. 2.

### 3.4 The critical mutation rate

We now turn our attention to the critical mutation rate  $U_{crit}$ , i.e., the minimum mutation rate sufficiently large to guarantee viral extinction. The critical mutation rate is simply the root of the equation  $\lambda_1(U_{crit}) = 0$ . Since this equation is transcendental in the present context, we cannot derive an exact analytical expression for  $U_{crit}$ . Instead, we employ further approximations.

We will again take advantage of the fact that migration has a more pronounced effect when the difference between absorption rates is large for different compartments, and will assume that either  $\alpha_1 \ll \alpha_2$  (for  $k = 2$ ) or  $\alpha_1 \ll \alpha_2$  (for the sector approximation). We first consider the case  $k = 2$ .

In Eq. (6), we omit  $g_2$ , set  $\lambda_1(U_{crit}) = 0$ , and find that

$$U_{crit} = \frac{1}{\alpha_1} \ln \frac{(d+m)r}{d(d+2m)}. \quad (20)$$

When  $m = 0$ , this expression simplifies to  $U_{crit} = (1/\alpha_1) \ln R_0$ , where  $R_0 = r/d$  is the basic reproduction number. As  $m$  approaches  $\infty$ , this expression simplifies to  $U_{crit} = (1/\alpha_1)[\ln R_0 - \ln 2]$ . Eq. (20) loses validity as  $R_0$  approaches 2, but serves as a faithful approximation as long as  $R_0 \gg 2$ . See Fig. 3.

Using these two limiting cases, we can calculate the maximum percent reduction in  $U_{crit}$  caused by migration:

$$\% \text{ reduction} = 1 - \frac{U_{crit}(m=\infty)}{U_{crit}(m=0)} = \frac{\ln 2}{\ln R_0}. \quad (21)$$

The percent reduction depends only on the ratio  $R_0$ . The larger  $R_0$ , the smaller the percent reduction. In other words, if a virus population has a very large  $R_0$ , then migration does not

substantially alter the value of the critical mutation rate. However, the ratio  $R_0$  enters Eq. (21) only logarithmically. For realistic values of  $R_0$ , which will probably not exceed  $10^3$  and will usually be smaller, Eq. (21) predicts at least a 10% reduction in the critical mutation rate.

We now consider the sector approximation. Once again, we omit  $g_2$  and set Eq. (17) equal to 0. We find

$$U_{\text{crit}} = \frac{1}{\tilde{\alpha}_1} \ln \frac{r(d+mc'_{21})}{d^2+dm(c'_{12}+c'_{21})}. \quad (22)$$

This approximation works well if the mutagen absorption coefficients within each sector are homogeneous (Fig. 4A), but it loses accuracy if the absorption or replication coefficients vary substantially (Fig. 4B).

When  $m = 0$ , we have  $U_{\text{crit}} = (1/\tilde{\alpha}_1) \ln R_0$ . As  $m$  approaches  $\infty$ , we have

$U_{\text{crit}} = (1/\tilde{\alpha}_1)[\ln R_0 - \ln(1+c'_{12}/c'_{21})]$ . Therefore, the maximum percent reduction in this case is

$$\% \text{ reduction} = \frac{\ln(1+c'_{12}/c'_{21})}{\ln R_0}. \quad (23)$$

Eq. (23) reduces to Eq. (21) if  $c'_{12}=c'_{21}$ , i.e., if the two sectors have equal sizes, so that the amount of virions flowing into a sector is exactly balanced by the amount of virions flowing out. If  $c'_{12}>c'_{21}$ , more virions flow out of sector 1 than into sector 1. As a consequence, the maximum percent reduction caused by migration is larger than for even-sized sectors. If  $c'_{12}<c'_{21}$ , on the other hand, the maximum percent reduction caused by migration is less than for even-sized sectors. As with our approximation for  $k = 2$ , we have an analogous restriction that  $R_0 \gg (1+c'_{12}/c'_{21})$  for the approximation to be valid.

### 3.5 The effect of replication and absorption abilities on the system

In the previous analysis, we have assumed that viruses have constant replicating and infecting ability in every compartment. In a real infection, this is not necessarily the case, and we will now explore the consequences of variations in viral replication rate among compartments. Suppose we take  $r$  to be a base replication rate and denote the deviation of replication or infecting ability in compartment  $i$  from this base rate as  $\rho_i$ , so that the growth rate in compartment  $i$  becomes  $r\rho_i$ . We expect  $\rho_i$  to be of order one, but in principle it could be much larger or much smaller than this value. (We assume  $\rho_i > 0$ .) The system of differential equations describing viral growth then becomes

$$\frac{dn_i(t)}{dt} = (r\rho_i g_i - d - m)n_i(t) + m \sum_{j \neq i} c_{ji} n_j(t). \quad (24)$$

All our previous results carry through if we replace all occurrences of  $g_i$  by  $\rho_i g_i$ . In our expressions for the critical mutation rate, Eqs. 20 and 22, we have to use  $r\rho_1$  in place of  $r$ . Thus, in principle, the inclusion of varied replication and infecting ability can substantially alter the phenomenology of the system. For example, a compartment could absorb only a small amount of mutagen (i.e.,  $g_i \approx 1$ ) yet hinder replication dramatically (i.e.,  $\rho_i \ll 1$ ). In this case, the



effective viral growth rate in the compartment,  $\rho_i g_i$ , would be small. Similarly, a compartment could absorb a large amount of mutagen (i.e.,  $g_i \approx 1$ ) but facilitate replication substantially (i.e.,  $\rho_i \gg 1$ ). In this case, the effective viral growth rate in the compartment would be large. We will briefly discuss these effects in a two-compartment system.

Suppose we have a two-compartment system with  $R_0 = 20$ ,  $m = 10$ ,  $\alpha_1 = 0.1$ , and  $\alpha_2 = 1$ . We assume that  $\rho_2$  is fixed at  $\rho_2 = 1$ , and investigate the effect of  $\rho_1$  on the system behavior. In this case, the value of  $\rho_1$  directly determines whether or not compartment 1 can serve as a refugium for the virus. Alternatively, if we keep  $\rho_1$  fixed at  $\rho_1 = 1$  and vary  $\alpha_1$ , then the value of  $\alpha_1$  directly determines whether or not compartment 1 can serve as a refugium for the virus. Fig. 5 visualizes both scenarios. It shows that the critical mutation rate does not respond to absorption and replication in the same way. In the regime in which the first compartment can act as a refugium, for  $\alpha_1 \ll 1$ , we find that even a small reduction in  $\alpha_1$  results in a substantial increase in  $U_{crit}$ . By contrast,  $\rho_1$  needs to increase by orders of magnitude to have a similar effect on the critical mutation rate. We can explain this result by calculating an approximate expression for the critical mutation rate in a two-compartment system which contains a well protected compartment (i.e.  $\rho_1 g_1 \ll \rho_2 g_2$ ). We obtain this expression by writing  $r\rho_1$  in place of  $r$  in Eq. (20):

$$U_{crit} = \frac{1}{\alpha_1} \ln \frac{(d+m) r \rho_1}{d(d+2m)}. \quad (25)$$

We see that the critical mutation rate is proportional to the logarithm of  $\rho_1$  and inversely proportional to  $\alpha_1$ . Thus, a small reduction in  $\alpha_1$  has a large effect on  $U_{crit}$ , but a large increase in  $\rho_1$  has only a small effect on  $U_{crit}$ . Since it is unlikely that a specific tissue will speed up viral replication by many orders of magnitude, we conclude that differences in replication rate among different compartments have only a minor effect and can be neglected to first order.

## 4 Discussion

We have developed a model of lethal mutagenesis in a structured host with limited migration among compartments and with uneven mutagen penetration of compartments. We have found, not surprisingly, that in the absence of migration virus extinction is determined by the compartment that is the least susceptible to mutagen. The virus population will go extinct if and only if it goes extinct in this specific compartment.

Migration generally increases the efficiency of mutagenesis. The more migration, the lower the critical mutation rate at which the virus population goes extinct. But there is a limit to the extent to which migration can reduce the critical mutation rate. In general, in the limit of infinitely fast migration, the total virus population grows with the average growth rate of all compartments, and the critical mutation rate in this limit is the mutation rate at which this average growth rate turns negative. Consequently, migration will affect the extinction threshold considerably if the average growth rate is substantially lower than the growth rate in the compartment least affected by the mutagen.

The effect of migration on the extinction threshold is largely determined by the ratio  $R_0$ . This ratio interacts with migration in two ways. First, the large- $m$  approximation is valid for  $m \gg R_0$ . Thus, the smaller  $R_0$ , the less migration is needed to achieve the maximum reduction in  $U_{crit}$ . Second, the size of the maximum reduction also depends on  $R_0$ . The smaller  $R_0$ , the larger the reduction in  $U_{crit}$  as we move from  $m = 0$  to  $m = \infty$ . In this context, we have to emphasize that  $R_0$  is not the epidemiological basic reproductive ratio, which describes the average number of newly infected cases arising from each infected individual in an epidemic. Instead, here

$R_0$  is the within-host basic reproductive ratio and describes the average number of newly infected host cells arising from every infected host cell.

In practice, we will be interested in the question under what circumstances a tissue that is not well penetrated by mutagen can serve as a refugium for the virus and can prevent successful lethal mutagenesis. Our results suggest that if the tissue is small and there is significant migration into and out of the tissue, it will not hinder lethal mutagenesis much. However, if the tissue is large and/or well isolated, then the amount of mutagen required to clear the virus may be substantial.

The method of lethal mutagenesis we studied here was using a mutagen to drive the viral replication rate below replacement level. Another method of lethal mutagenesis is lethal defection, whereby defective interfering particles accumulate and lead to the collapse of the virus population (Iranzo and Manrubia, 2009). Lethal defection is reliant on coinfection of viable and non-viable virions and has to date only been observed in systems that show persistent infections (Grande-Perez et al., 2005) or that have been artificially enriched with defective sequences (González-López et al., 2004). Whether lethal defection could occur in lytic viruses, in which we expect the multiplicity of infection to drop below one near the extinction threshold, remains an open question.

We made several simplifying assumptions in our model, to keep the mathematics tractable. First, we used a deterministic model. Since lethal mutagenesis is to first order a deterministic process that will work in arbitrarily large populations (Bull et al., 2007), this assumption seems reasonable. However, with a deterministic model, we do not take into account fluctuations in the viral population size. When viral population numbers are low, i.e. near extinction, the rate of deleterious mutation fixation increases (Lynch et al., 1993). In addition, stochastic fluctuations in a small population can drive a population to extinction even when the mutation rate is below the deterministic threshold (Demetrius et al., 1985). Thus, we would expect that the critical mutation rate of a finite population is lower than our deterministic calculation predicts.

Second, we assumed that all mutations are either neutral or lethal, and we kept track of only the lethal mutations. The resulting growth rate within a compartment,  $R_0 e^{-\alpha_i U}$ , is identical to the equilibrium growth rate in a model that takes into account arbitrary deleterious mutations (Kimura and Maruyama, 1966; Bull et al., 2007). Therefore, our predictions will be approximately correct for any virus that sits at the top of a local fitness optimum. Some deviations from our predictions will arise because the virus population is not at equilibrium at the beginning of the mutagenic treatment. However, as long as beneficial mutations can be neglected, as will be the case for a virus at the top of a fitness optimum, the out-of-equilibrium mean fitness of the viral population will exceed  $R_0 e^{-\alpha_i U}$ . Consequently, for any  $U$  smaller than the  $U_{\text{crit}}$  predicted based on the equilibrium theory the viral population will survive at all times, and for any  $U$  larger than  $U_{\text{crit}}$  the population fitness will decline until the population goes extinct. Thus,  $U_{\text{crit}}$  remains the same even if the population is initially out of equilibrium. Additional deviations from our predictions will arise if beneficial mutations increase in frequency and effect as the mutation rate increases and fitness decreases, as we would expect near extinction via lethal mutagenesis (Silander et al., 2007). The Kimura-Maruyama formula  $R_0 e^{-\alpha_i U}$  holds only approximately in this case. By contrast, if the virus initiating the infection is far from a fitness optimum or if new fitness optima become available under mutagenesis, then our predictions will likely not match experimental outcomes. A comprehensive treatment of beneficial mutations is beyond the scope of this study and we leave this topic for future work.

Third, our model does not include any delay in migration. Thus, we assume that migration from one tissue to another is immediate. We believe that neglecting delays is a reasonable assumption for vertebrates with a cardiovascular system because virus dispersal through the blood will happen much faster than viral replication. In an average-sized human body, the blood turns over about once per minute (Jacob et al., 1982). By contrast, viral replication time is on the order of hours to days. For example, HIV-1 *in-vivo* generation time is estimated to be 2 days (Markowitz et al., 2003).

Fourth, we assumed that the multiplicity of infection (m.o.i.) remains low at all times, so that viral coinfection can be ignored. Low m.o.i. is a reasonable assumption for a viral population close to extinction.

In this study, we introduced the sector approximation as a way of analytically analyzing lethal mutagenesis in structured systems with multiple compartments. The sector approximation subsumes all compartments into two classes (sectors), compartments that allow a lot of viral replication and compartments that do not. While the sector approximation did not necessarily achieve high numerical accuracy in all cases, it captured the qualitative behavior of all multi-compartment systems to which we applied it. Therefore, the sector approximation will likely be a powerful tool for future studies that aim to remove some of the other simplifying assumptions we made here.

## Acknowledgments

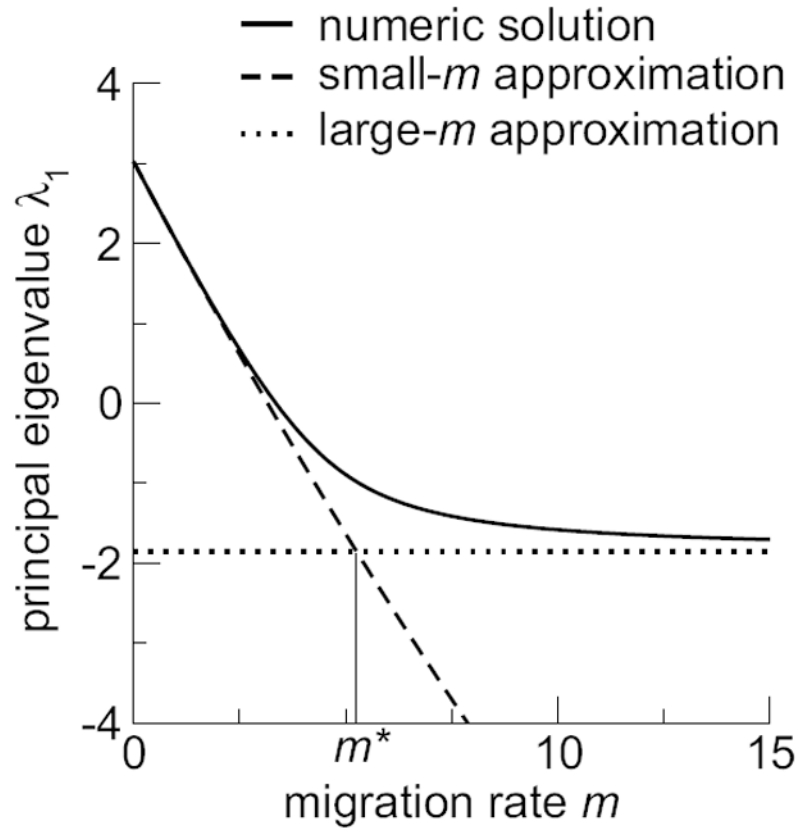
This work was supported in part by NIH grant AI 065960 and by NSF grant EF-0742373.

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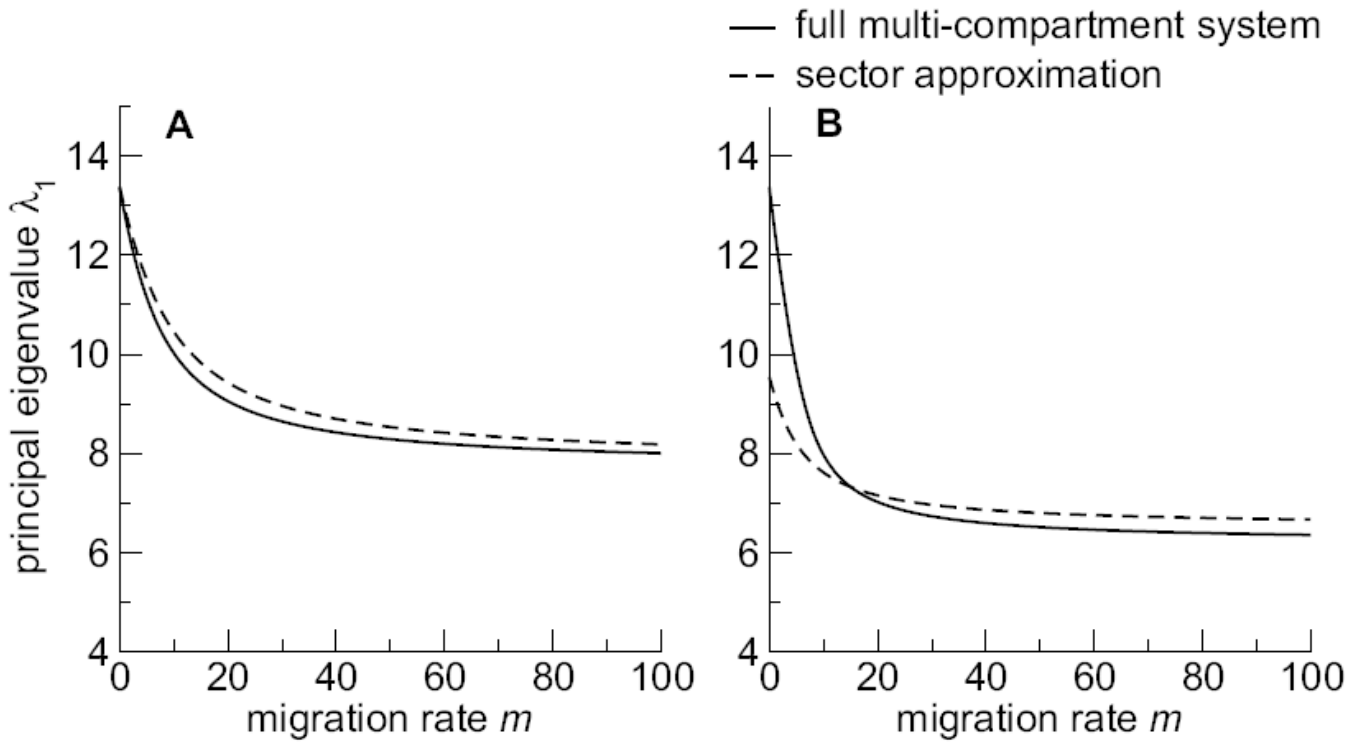
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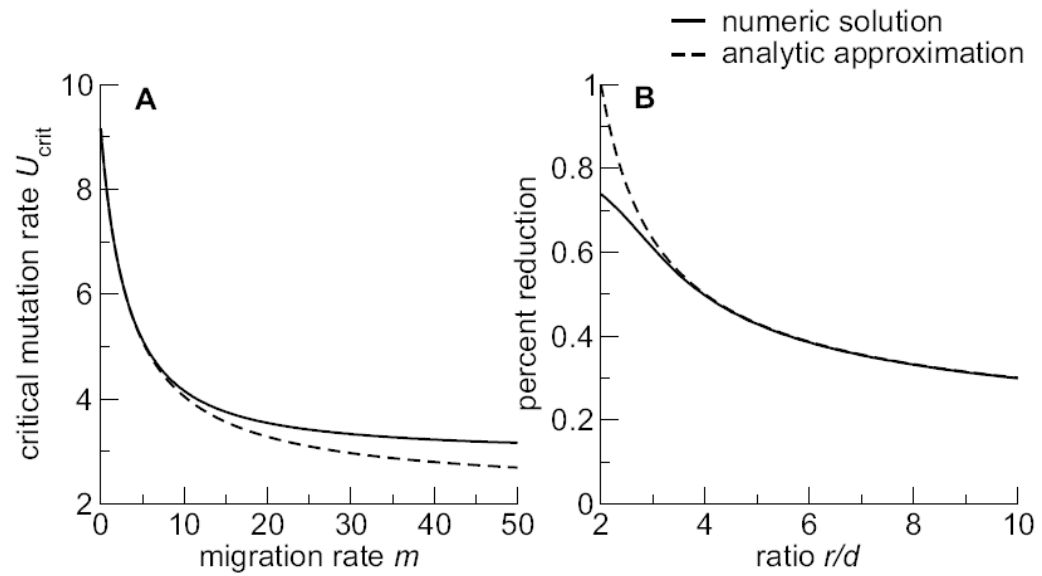
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**Fig. 1.** Analytic approximations to the principal eigenvalue  $\lambda_1$ . The dashed line corresponds to Eq. (9), and the dotted line corresponds to Eq. (12). Parameter values are  $k = 15$ ,  $\alpha_1 = 0.3$ ,  $\alpha_2 = \dots = \alpha_8 = 0.7$ ,  $\alpha_9 = \dots = \alpha_{15} = 1.0$ ,  $r = 20$ ,  $d = 3$ , and  $U = 4$ .

**Fig. 2.**

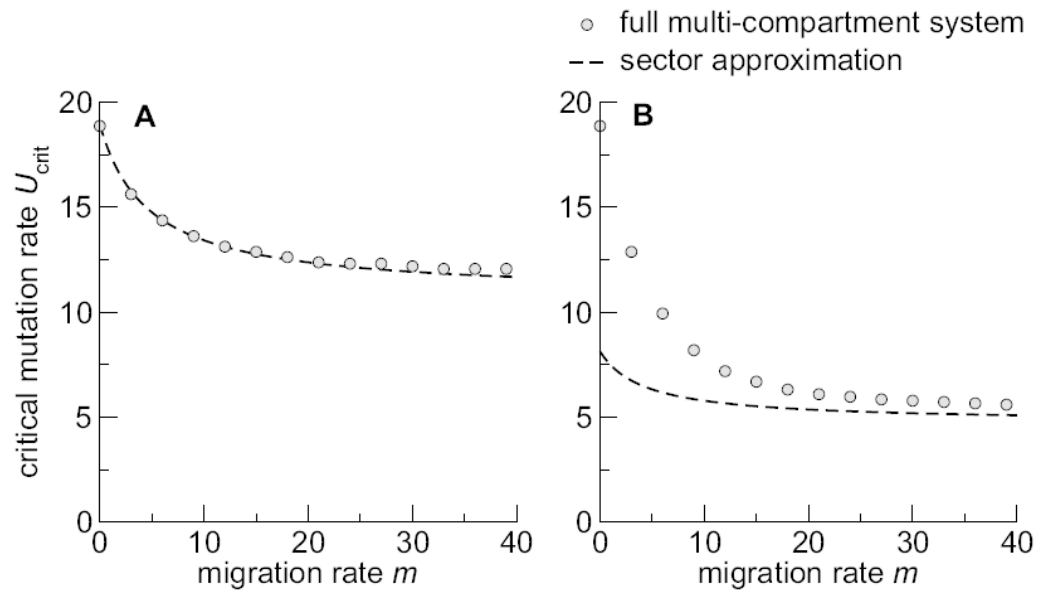
Comparison of sector approximation to the full multi-compartment system. Parameters are  $p = 12$ ,  $q = 8$ ,  $r = 20$ ,  $u = 2$ , and  $d = 3$ . We selected the  $c_{ji}$ 's at random from a uniform distribution on the unit interval, conditional on  $\sum_{i, i \neq j} c_{ji} = 1$ , and set  $c_{jj} = 0$ . In (A), absorption and replication coefficients were uniform within the low- and highabsorption sector ( $\alpha_1 = \dots = \alpha_{12} = 0.1$ , and  $\alpha_{13} = \dots = \alpha_{20} = 1.0$ ). In (B), we used three absorption coefficients in both the low- and the high-absorption sector ( $\alpha_1 = \dots = \alpha_5 = 0.1$ ,  $\alpha_6 = \alpha_7 = 0.4$ ,  $\alpha_8 = \dots = \alpha_{12} = 0.3$ ,  $\alpha_{13} = \alpha_{14} = \alpha_{15} = 0.6$ ,  $\alpha_{16} = \alpha_{17} = 0.7$ , and  $\alpha_{18} = \alpha_{19} = \alpha_{20} = 1.0$ ).



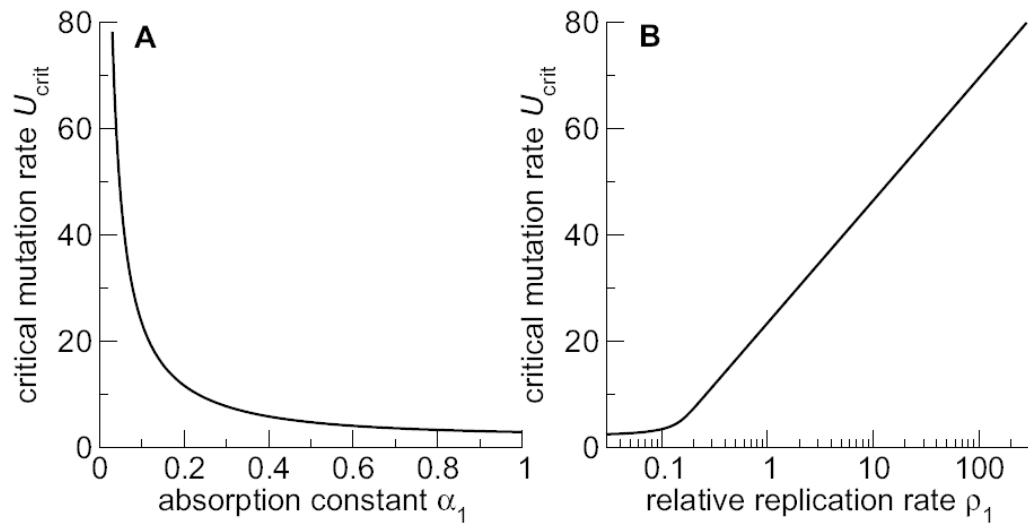
**Fig. 3.**

Effect of migration on the critical mutation rate. (A) Critical mutation rate vs. migration rate  $m$ , for fixed ratio  $R_0 = 5/2$ . The dashed line shows Eq. (20). (B) Percent reduction in the critical mutation rate between no migration and maximum migration, as a function of the ratio  $R_0$ . The dashed line shows Eq. (21). For both (A) and (B), parameters are  $\alpha_1 = 0.1$ ,  $\alpha_2 = 1.0$ , and  $d = 5$ .





**Fig. 4.** Comparison of sector approximation to the full multi-compartment system. Parameters are identical to Fig. 2A and B. The dashed line corresponds to Eq. (22).



**Fig. 5.** Critical mutation rate as a function of mutagen absorption and viral replication rate, as determined by exact numerical solution. Parameters are  $k = 2$ ,  $R_0 = 20$ ,  $m = 10$ ,  $\alpha_2 = 1$ , and  $\rho_2 = 1$ . (A)  $\alpha_1$  varies and  $\rho_1 = 1$ . (B)  $\rho_1$  varies and  $\alpha_1 = 0.1$ .