

ON THE EVOLUTIONARY DYNAMICS OF VIRULENCE

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ABSTRACT. The aim of these notes is to demonstrate, by way of examples, how the techniques of Adaptive Dynamics can be used to study the evolutionary dynamics of infectious diseases. We focus on evolution of a single phenotypic trait, namely the disease induced death rate, or virulence. In a series of worked-out examples, we introduce the basic notions of Adaptive Dynamics and follow (some of) the development of evolutionary epidemiology through the years. We begin with the so called single infection model, discuss the conventional evolutionary wisdom and the trade-off hypothesis. Later on, we focus on the role of multiple infections in the evolution of infectious diseases. We investigate in more detail a superinfection model and discuss how the details of the superinfection process shape the course of evolution. In the last part of these notes, we introduce an example of a nested model that explicitly links the epidemiological dynamics at the host population level to the dynamics of infection in a single infected host. Such a nested model allows us to derive the precise form of the superinfection probability from the underlying mechanistic submodel of within-host dynamics.

1. INTRODUCTION

In 1973, the Russian evolutionary biologist Theodosius Dobzhansky wrote in one of his essays: “*Nothing in biology makes sense except in the light of evolution*” [21]. This is especially true for microorganisms, such as bacteria and viruses, for which it is now clear that evolution occurs not only on ecological time scales, but even during a course of an infection of a single infected host. For HIV-1 virus, for instance, the mutation rate per base pair is of the order of 10^{-5} to 10^{-4} . It is estimated that 10^9 replication cycles occur per day within a single infected individual, which means that a tremendous selection pressure is exerted on the virus even during the course of a single infection [45, 46, 50]. Another example of the rapid evolution of pathogens are bacteria which have developed resistance to antibiotics, e.g. Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA) or Vancomycin-resistant *enterococcus* (VRE) [6, 9, 12].

Natural selection acts on pathogens on several different levels. At the host population level, for instance, pathogens compete for susceptible hosts. These can either be uninfected, or, if multiple infections are possible, can also include already infected hosts. At the within-host level, pathogens compete for, for instance, uninfected target cells and even for resources within a single cell. These different levels of selection are interrelated and ideally, evolution of pathogens should be investigated by taking the different levels into account. However, this very quickly leads to complicated models. Traditionally, mathematical models explore selection pressures only at the host population level [5, 8, 13, 14, 26, 33, 34, 37, 40, 42, 47, 48, 51], but the importance of the so called nested (or embedded) models has increasingly been realized in the recent years [1, 2, 7, 11, 29, 39]. In these notes, we start simple by investigating the selection pressures at the host population level (i.e., by ignoring within-host dynamics). Later on, we present an example of a nested model that explicitly links the within-host pathogen dynamics to the traits that determine the spread of the infection at the host population level (e.g. virulence and transmissibility).

It is well documented that selection acts not only on different levels but also on different pathogen traits, such as for instance, the rate of pathogen reproduction within a host, the rate of evasion

from the immune system, the infection induced death rate [32, 43, 52, 54]. In the first part of these notes we focus only on evolution of the disease induced death rate (virulence). When an explicit model of within-host dynamics is embedded into the epidemiological model, virulence will naturally be related to within-host production rate of the pathogen and we will thus focus on evolution of intra-host production rate.

Clearly, some pathogens (such as the virus causing the common cold) are virtually avirulent, while others (for instance, the *ebola* virus) are almost always lethal. What are the factors that determine the levels of virulence of various pathogens? Extensive studies of different aspects of pathogen dynamics have shown that several mechanisms may explain the very different evolutionary paths of pathogens. Transmission mode (i.e., the way in which the pathogen is transmitted from one host to another) and host population regulation, for example, are known to play an important role in the evolution of pathogens [8, 18, 23, 24]. Another factor that plays a significant role, and one that we shall investigate in more detail in these notes, are multiple infections of the host. In general, a host that is already infected by some strain is not completely immune to infections by different strains. It seems reasonable to expect that the success of a reinfecting strain depends on several things, for instance, the difference in within-host competitive ability with the resident strain, the reinfection dose or the host susceptibility to another infection (this can either be reduced due to some partial immunity the first strain confers or increased because the host's immune system is weakened by the first infection). It is thus important to understand how different assumptions regarding the reinfection process shape the course of pathogen evolution.

Apart from a few introductory examples we mainly focus on the role of reinfection (in particular superinfection). We refer the reader to [18, 8, 23, 24, 47] and the references therein for some studies of other aspects of the evolutionary dynamics of infectious diseases.

Throughout the notes we use the tools of Adaptive Dynamics [27, 28, 19]. The reader who is not familiar with the terminology of Adaptive Dynamics can consult the boxes, where the basic notions are explained.

2. THE BASIC MODEL

We base the examples on a simple SI (Susceptible - Infected) model. Our basic assumptions are:

- (i) The population birth rate is constant and is denoted by b . All newborns are susceptible.
- (ii) Susceptible individuals die at a constant per capita rate d .
- (iii) Infected individuals die at an increased per capita rate $d + \alpha$.
- (iv) New infections occur according to the Law of Mass Action. That is, the rate at which an infected individual infects susceptible hosts is proportional to the abundance of susceptible hosts in the population, βS .
- (v) Infected hosts become infectious at the moment of infection.

The disease induced death rate α is often called *virulence* (see however [10, 53] for other meanings of the term virulence). Note that assumptions (i) and (iv) imply that the pathogen is transmitted only horizontally (i.e. from one host to another) and not vertically (from the mother to a newborn child). Note also that we did not include any recovery which means that we limit ourselves to chronic pathogens (see [47] for a comparable study in the context of an SIR model).

If we denote by S and I the abundance of, respectively, susceptible and infected hosts, we can translate the above assumptions into the following system of ODEs,

$$(1) \quad \begin{aligned} \frac{dS}{dt} &= b - \beta SI - dS, \\ \frac{dI}{dt} &= \beta SI - (d + \alpha)I. \end{aligned}$$

System (1) has two equilibria: the infection free steady state,

$$\bar{S} = \frac{b}{d}, \quad \bar{I} = 0,$$

and the endemic steady state given by

$$(2) \quad \hat{S} = \frac{d + \alpha}{\beta}, \quad \hat{I} = \frac{b}{d + \alpha} - \frac{d}{\beta}.$$

The endemic equilibrium is biologically meaningful only when it is positive. This is the case precisely when the *basic reproduction ratio*, \mathcal{R}_0 , is larger than 1. The basic reproduction ratio is defined as the expected number of new infections caused by a single infected host in an otherwise uninfected population [20]. In this case, \mathcal{R}_0 can easily be determined: since each infected individual is expected to live $\frac{1}{d+\alpha}$ units of time and is in that time expected to infect $\beta \frac{b}{d}$ new individuals, we find that

$$\mathcal{R}_0 = \frac{b\beta}{d(d + \alpha)}.$$

The basic reproduction ratio also determines stability of the two equilibria. If $\mathcal{R}_0 < 1$, the infection free steady state is the only biologically meaningful steady state and it is globally stable: when every infected individual produces, on average, less than one new infection, then every introduction of the infection will inevitably die out. If, on the other hand, $\mathcal{R}_0 > 1$, the endemic steady state is globally stable, while the infection free steady state is unstable (cf. [20], Exercise 3.11).

Our aim now is to investigate how virulence α changes in the course of evolution. To keep things simple, we shall assume that the host does not coevolve with the evolving pathogen. That is, the host parameters b and d are assumed to be fixed.

3. EVOLUTION OF VIRULENCE IN THE CONTEXT OF A SINGLE INFECTION MODEL

In order to study the competition of multiple pathogen strains (characterized by different values of α) in a populations of hosts, we have to specify assumptions about how multiple strains are handled within a single infected host. We begin with the simplest possible assumption, namely that a host infected by one strain is completely protected from further infections (in other words, we assume complete cross immunity). This yields the so called *Single Infection Model*. In a special case where only two strains (characterized by virulence values α_1 and α_2) circulate in the population, we can describe the dynamics by

$$(3) \quad \begin{aligned} \frac{dS}{dt} &= b - \beta S I_1 - \beta S I_2 - dS, \\ \frac{dI_1}{dt} &= \beta S I_1 - (d + \alpha_1) I_1, \\ \frac{dI_2}{dt} &= \beta S I_2 - (d + \alpha_2) I_2. \end{aligned}$$

Suppose that a mutant strain α_m is introduced into a population in which the resident strain α_r is endemic. The mutant strain grows (or declines) according to

$$\frac{dI_m}{dt} = (\beta S - (d + \alpha_m)) I_m.$$

Box 1. Some basic notions of Adaptive Dynamics

The **invasion exponent** $r(x, y)$ is defined as the growth rate of a mutant population with trait y in the environment set by the resident population with trait x . If the invasion exponent is differentiable as a function of y in the point $y = x$, then the sign of the **selection gradient**

$$\left. \frac{\partial r}{\partial y} \right|_{y=x}$$

determines the direction of evolution from the resident trait x . If it is positive, the trait will (at least locally) increase in the course of evolution and if it is negative, the trait will (locally) decrease in the course of evolution. **Singular strategies** are trait values in which the selection gradient vanishes, i.e.

$$\left. \frac{\partial r}{\partial y} \right|_{y=x} = 0.$$

A singular trait x^* is called **convergence stable**, if a nearby strategy can be invaded (only) by traits that are nearer to x^* . That is, if $x < x^*$, then $r(x, y) > 0$ for $x < y < x^*$, while for $x > x^*$ the invasion exponent is positive when $x^* < y < x$. Convergence stable strategies are thus (local) attractors for monomorphic evolutionary dynamics.

An **evolutionarily stable strategy (ESS)** is a strategy that cannot be invaded by neighbouring traits. That is, x^* is an ESS if $r(x^*, y) < 0$ for $y \in (x^* - \varepsilon, x^* + \varepsilon)$ with some $\varepsilon > 0$. Despite the enticing ‘stable’ in its name, an ESS may not be an evolutionary attractor. If it is, it is called a **continuously stable strategy (CSS)**.

An **evolutionary branching point** is a singular strategy that is convergent stable, but not an ESS. If the invasion exponent is differentiable twice, then the second partial derivatives allow us to classify the singular points. In particular, if

$$\left. \frac{\partial^2 r}{\partial y^2} \right|_{y=x=x^*} < 0,$$

the point x^* is an ESS. If

$$\left. \frac{\partial^2 r}{\partial y^2} \right|_{y=x=x^*} < \left. \frac{\partial^2 r}{\partial x^2} \right|_{y=x=x^*},$$

then x^* is convergence stable. A singular point for which

$$\left. \frac{\partial^2 r}{\partial x^2} \right|_{y=x=x^*} > \left. \frac{\partial^2 r}{\partial y^2} \right|_{y=x=x^*} > 0$$

is a branching point.

Two basic assumptions of Adaptive Dynamics are that (i) mutants are introduced in small numbers and (ii) mutations are rare on the ecological time scale. The first assumption allows us to view the abundance of susceptibles S as depending only on the resident strain, α_r . That is, the mutant is so rare that it initially doesn’t influence the environment into which it is introduced. The second assumption allows us to presume that the resident population has reached an equilibrium, $\hat{S}(\alpha_r)$.

The invasion criterion can thus be formulated in terms of the *invasion exponent*: if the per capita growth rate of a mutant α_m that is introduced into the resident population infected with α_r ,

$$(4) \quad s(\alpha_r, \alpha_m) = \beta \hat{S}(\alpha_r) - (d + \alpha_m)$$

is positive, the mutant will invade, while the invasion fails if $s(\alpha_r, \alpha_m)$ is negative. This is indeed the case when we model invasions deterministically. If the model was stochastic, the invasion would still fail when the growth rate of the mutant is negative. With a positive growth rate, however, the mutant succeeds only with some positive (but smaller than 1) probability since the mutant may go extinct because of demographic stochasticity while it is still rare.

Box 2. Pairwise invasibility plots

Pairwise invasibility plots (PIPs) are a handy way of representing graphically the ability of a mutant trait to grow in the resident community. A PIP is constructed by plotting the sign of the invasion exponent $r(x, y)$ for all feasible pairs (x, y) of (resident, mutant) trait values.

If the resident population is at a stable equilibrium, then $r(x, x) = 0$ and so the zero contour lines contain at least the main diagonal. The shapes of other zero contour lines, if there are any, contain important information about the course of evolution. In particular, singular points are found as intersections of zero contour lines with the main diagonal. If we now imagine that black and white regions in the PIP represent the regions where the invasion exponent is, respectively, negative and positive, then the ‘character’ of a singular strategy can easily be recognized from a pairwise invasibility plot. Namely, if x^* is to be an ESS, and hence uninvadable by the neighbouring strategies, the straight vertical line through (x^*, x^*) must lie, at least locally, in the region where the invasion exponent is negative, i.e. in the black region. The singular trait x^* is convergence stable when the regions left of (x^*, x^*) are, at least close to the diagonal, white above the diagonal and black below the diagonal (i.e. x^* is locally attracting from the left), while the regions right of the point (x^*, x^*) are (at least close to the diagonal) black above the diagonal and white below the diagonal (in other words, x^* is locally attracting from the right).

We can reformulate the invasion criterion in terms of the basic reproduction ratio: the mutant α_m invades if the basic reproduction ratio of the mutant in the environment set by the resident,

$$(5) \quad \mathcal{R}_0(\hat{S}(\alpha_r), \alpha_m) = \frac{\beta \hat{S}(\alpha_r)}{d + \alpha_m}$$

exceeds 1, while the invasion fails if $\mathcal{R}_0(\hat{S}(\alpha_r), \alpha_m) < 1$.

3.1. Conventional wisdom. It was believed for a long time that all pathogens would eventually evolve to be benign to their hosts. The words of the French-American microbiologist René Dubos (1965) reflect this, in that time widely accepted, idea: “*Given enough time, a state of peaceful coexistence eventually becomes established between any host and parasite.*”

In the context of our model, evolution to avirulence is certain if we assume that transmissibility β and virulence α are independent of one another. Using (2) and (4) we can then write the invasion exponent as

$$(6) \quad s(\alpha_r, \alpha_m) = \beta \hat{S}(\alpha_r) - (d + \alpha_m) = \alpha_r - \alpha_m.$$

Hence, mutants that decrease virulence are successful, while those that increase it are not. Assuming that mutualism is not possible (that is, α is always nonnegative), we conclude that evolution indeed drives virulence towards zero. We thus recover the so called *conventional evolutionary wisdom*: pathogens evolve to become avirulent. Figure 1 shows the (very trivial) corresponding pairwise invasibility plot.

3.2. The trade-off hypothesis. Supporters of the avirulence hypothesis argued that the reason we still observe virulent microorganisms today is simply that the process of pathogen adaptation to their hosts has not been long enough. However, there are many examples of host-parasite systems with very long coevolutionary history in which pathogens have not evolved towards avirulence [25, 31].

The first breakthrough in our understanding of why this could be the case came in the early 1980’s with the work of Anderson and May [3, 4, 36, 37] and Levin and Pimentel [34]. What they suggested was a *trade-off* between virulence and transmissibility, essentially reflecting the idea that

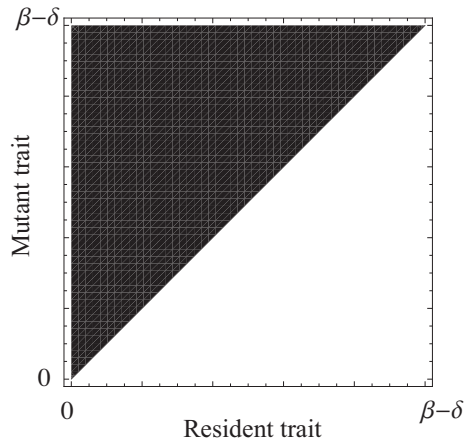


FIGURE 1. Pairwise invasibility plot corresponding to the invasion exponent in (6). White represents the regions where the mutant can invade, while the invasion fails in black regions. Pathogens can persist in the population when $\mathcal{R}_0 > 1$, which is equivalent to $\alpha < \beta - \delta$. Evolution drives the pathogens towards avirulence.

‘you don’t get something for nothing’: pathogens aim to increase transmission to new hosts, but cannot do so without simultaneously harming the host, i.e. increasing the host’s death rate.

The trade-off hypothesis was doubted at first, mainly due to the lack of empirical support. However, there is now good experimental evidence that such trade-offs exist [16, 17, 22, 35, 41, 55]. Note, however, that the lack of empirical support for the existence of a trade-off between transmissibility and virulence in any particular case may simply be due to the fact that the harmful effects of the pathogen on the host manifest themselves in some other form, for instance in decreasing host’s fecundity [44, 49].

So let us suppose that there exists a trade-off between virulence and transmission rate and let us see what this means for the evolution of the pathogen. A mutant strain α_m can invade when

$$(7) \quad s(\alpha_r, \alpha_m) = \beta(\alpha_m)\hat{S}(\alpha_r) - (d + \alpha_m) > 0$$

where

$$\hat{S}(\alpha) = \frac{d + \alpha}{\beta(\alpha)}.$$

Thus, the mutant succeeds if it decreases $\hat{S}(\alpha)$ set by the resident. Since $s(\alpha_r, \alpha_m) > 0$ implies that $s(\alpha_r, \alpha_m) < 0$ (i.e., the resident cannot invade back), the evolution proceeds, in a series of trait substitutions, towards a local minimum of $\hat{S}(\alpha)$. The traits that (locally) minimize the steady state abundance of susceptible hosts are necessarily uninvadable and thus represent the possible end points of evolution, i.e., the continuously stable strategies.

The ultimate evolutionary winner is thus the strain that is able to persist in the worst possible environment, i.e. with the least amount of susceptible hosts. This is sometimes called the *pessimization principle* [19]. Note, incidentally, that minimization of \hat{S} is equivalent to maximization of the basic reproduction ratio. The evolutionary winner is therefore the trait that (locally) maximizes \mathcal{R}_0 .

The precise conclusions about the outcome of evolution will depend on the shape of the trade-off function $\beta(\alpha)$. If the trade-off is concave then there exists a single maximum of \mathcal{R}_0 . If $\beta(\alpha)$ is convex, then there are no maxima of \mathcal{R}_0 . There may, however, exist a single minimum. This minimum is an evolutionary repeller and represents a separating point for evolutionary outcomes: if the starting virulence is below the value that minimizes \mathcal{R}_0 , evolution will drive virulence towards

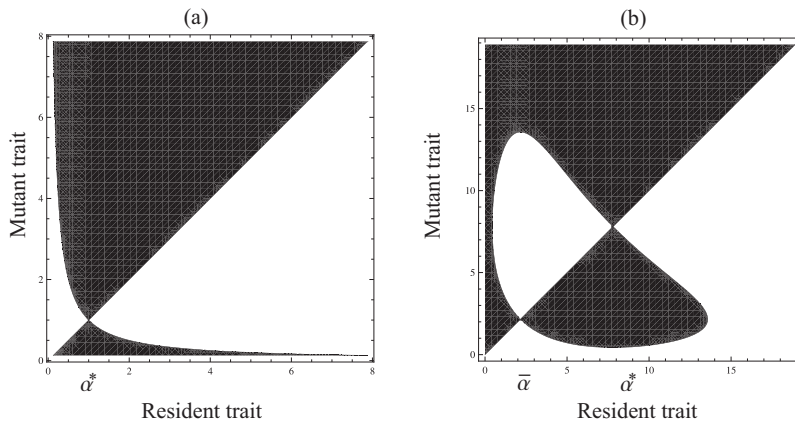


FIGURE 2. PIPs corresponding to (7) and (a) a concave trade-off function $\beta(\alpha) = \frac{10\alpha}{\alpha+1}$, (b) a convex-concave trade-off function $\beta(\alpha) = \frac{A}{B+(1-B)e^{-C\alpha}}$ with $A = 2, B = 0.1, C = 0.4$. In (a), evolution drives virulence to some intermediate value, denoted by α^* . In (b), the point $\bar{\alpha}$ is an evolutionary repeller: traits starting below this value evolve towards zero, traits above evolve towards the continuously stable strategy α^* .

zero, while a starting point above the threshold virulence level means that virulence increases indefinitely. In Figure 2 we present the pairwise invasibility plots for two choices of $\beta(\alpha)$.

Remark 1. Evolution acts as an optimization only in a very special case, when the dimension of the environment equals one [38]. This is the case here where the only environmental variable for the pathogens is the abundance of susceptible hosts, \hat{S} . This simplicity comes not only because of the assumption of complete cross-immunity between strains but also because of the very simple demography of the host population. Once more realistic assumptions of density dependence in birth or death rates are included, the evolutionary dynamics becomes richer and we no longer find optimization. We refer the reader to the studies of Pugliese [48] and Svernungsen and Kisdi [51], where density dependence was taken into account. This shows that demography plays an important role in the evolution of pathogens.

4. THE SUPERINFECTION MODEL

In this section we relax the assumption of complete cross-immunity and consider the possibility of reinfections.

Multiple infections within a single host can be modeled in different ways. *Superinfection models* assume that within-host dynamics is fast compared to processes at the host population level. If an individual infected by strain α_1 is reinfected by another strain α_2 , then the better within-host competitor immediately ousts the other strain and takes over the host (here we have in mind a very simplistic within-host scenario where, in the long run, only one strain can persist inside a host). *Coinfection models*, on the other hand, incorporate also the transient dynamics where the host harbors both strains α_1 and α_2 .

Since there will always be a period in which a reinfected host harbours more than one strain, coinfection models may be argued to be more realistic than superinfection model. The added realism, however, does not come for free and the models very quickly become untractable when the number of strains increases. In these notes we limit ourselves to studying the superinfection models and refer the reader to [40, 18] for a study of virulence evolution in the context of a coinfection model and for a derivation of superinfection as the limiting case of a coinfection process.

To include the possibility of superinfections we extend the single infection model with two strains to

$$(8) \quad \begin{aligned} \frac{dS}{dt} &= b - \beta(\alpha_1)SI_1 - \beta(\alpha_2)SI_2 - dS, \\ \frac{dI_1}{dt} &= \beta(\alpha_1)SI_1 + \beta(\alpha_1)\psi(\alpha_2, \alpha_1)I_1I_2 - \beta(\alpha_2)\psi(\alpha_1, \alpha_2)I_1I_2 - (d + \alpha_1)I_1, \\ \frac{dI_2}{dt} &= \beta(\alpha_2)SI_2 + \beta(\alpha_2)\psi(\alpha_1, \alpha_2)I_1I_2 - \beta(\alpha_1)\psi(\alpha_2, \alpha_1)I_1I_2 - (d + \alpha_2)I_2, \end{aligned}$$

where ψ denotes the *superinfection function*. More precisely, we define

$$\psi(\alpha_1, \alpha_2) := \text{the probability that, upon reinfection, the newly infecting strain } \alpha_2 \text{ takes over the host that is already infected with } \alpha_1.$$

Remark 2. The model could in principle include a rather more general description of a superinfection. For instance, instead of $\beta(\alpha_2)\psi(\alpha_1, \alpha_2)$, one could write $\beta(\alpha_2)\psi(\alpha_1, \alpha_2)\sigma(\alpha_1)$ to take into account the fact that already infected individuals may differ in their susceptibility to an infection from uninfected individuals. If $0 < \sigma(\alpha_1) < 1$, then infection with α_1 has conferred some partial immunity and the individual is less susceptible to infection with α_2 than an uninfected host. If $\sigma(\alpha_1) > 1$, on the other hand, the host resistance to infection by α_2 has decreased because of the existing infection by α_1 . Such modifications would be easy to include, however, to keep the presentation simple, we choose not to do so.

We shall in fact assume that the probability of superinfection depends only on the difference of the two strains. That is, we shall write

$$\psi(\alpha_1, \alpha_2) = \phi(\alpha_2 - \alpha_1)$$

and, to shorten the notation, we define

$$(9) \quad \Phi(\alpha_1, \alpha_2) := \beta(\alpha_2)\phi(\alpha_2 - \alpha_1) - \beta(\alpha_1)\phi(\alpha_1 - \alpha_2).$$

The invasion exponent now takes the form

$$(10) \quad \begin{aligned} r(\alpha_r, \alpha_m) &= \beta(\alpha_m)\hat{S}(\alpha_r) - (d + \alpha_m) + \Phi(\alpha_r, \alpha_m)\hat{I}(\alpha_r) \\ &= s(\alpha_r, \alpha_m) + \Phi(\alpha_r, \alpha_m)\hat{I}(\alpha_r), \end{aligned}$$

where s is the invasion exponent from the single infection model. Note that the resident equilibrium is the same as in the basic model since the resident is assumed to consist of one strain only and thus no superinfections take place.

The assumptions regarding the superinfection function are now crucial and, as we shall see below, different choices can lead to very different evolutionary outcomes. Note that, since mutations are assumed to be small, the outcome of invasion relies only on the behaviour of ϕ in the vicinity of zero (the shape of ϕ away from zero, however, plays a role in global and in polymorphic dynamics; see [8]).

We assume that the superinfection function is a nonnegative, increasing function and consider the following three classes of superinfection functions:

- (A) $\phi(\alpha) = 0$ for $\alpha \leq 0$, ϕ has a jump discontinuity in $\alpha = 0$,
- (B) $\phi(\alpha) = 0$ for $\alpha \leq 0$, ϕ is continuous in $\alpha = 0$ and is differentiable twice in zero from the right with $\phi'_+(0) > 0$,
- (C) $\phi(0) > 0$, ϕ is differentiable.

Selection gradient exists in cases (B) and (C). We find that a singular strategy α^* satisfies

$$(11) \quad \left. \frac{\partial r}{\partial \alpha_m} \right|_{\alpha_m = \alpha_r = \alpha^*} = \left. \frac{\partial s}{\partial \alpha_m} \right|_{\alpha_m = \alpha_r = \alpha^*} + \Phi_0 \hat{I}(\alpha^*) = \beta'(\alpha^*)\hat{S}(\alpha^*) - 1 + \Phi_0 \hat{I}(\alpha^*) = 0,$$

where

$$\Phi_0 = \begin{cases} \beta(\alpha^*)\phi'_+(0), & \text{in case (B)} \\ \beta'(\alpha^*)\phi(0) + 2\beta(\alpha^*)\phi'(0), & \text{in case (C)}, \end{cases}$$

Since $\beta(\alpha)$ is assumed to be increasing we see that $\Phi_0 > 0$ in both case (B) and case (C). It is then clear from (11) that superinfections drive virulence beyond the point that maximizes \mathcal{R}_0 . In other words, when a host can be superinfected, pathogens evolve to be more virulent than when there is complete cross-immunity between strains.

The three classes of superinfection functions give very different evolutionary outcomes. In the examples that follow we give some biological motivation for a particular class and study the corresponding adaptive dynamics.

4.1. Case A. We first consider the case where ϕ is zero on $(-\infty, 0]$ and moreover has a jump discontinuity at zero. One may, for instance, have in mind

$$(12) \quad \phi(\alpha) = \begin{cases} 1, & \alpha > 0 \\ 0, & \alpha \leq 0, \end{cases}$$

which corresponds to the deterministic description of an invasion: reinfection with a more virulent strain succeeds with probability 1, while a reinfection with a less (or equally) virulent strain always fails.

The discontinuity at the origin implies that even a slightly larger mutant strain will successfully invade the resident strain in the population and that the less virulent strain can never invade back. Indeed, for $\alpha_m = \alpha_r \pm \varepsilon$, the term $\Phi(\alpha_r, \alpha_m)$ in (10) is $\mathcal{O}(1)$, while $s(\alpha_r, \alpha_m)$ is of the order $\mathcal{O}(\varepsilon)$, and so it is the sign of $\Phi(\alpha_r, \alpha_m)$ that determines the outcome of an invasion. Evolution thus increases virulence with every successful mutation. In the long run, therefore, virulence increases either indefinitely or to the maximum virulence level that still allows the infection to persist in the population. As we have seen in the previous section, the convexity of the trade-off determines which of the two scenarios applies.

The evolution of virulence in the context of a superinfection model with a discontinuous superinfection function has been studied already in the 1990's. In their 1994 paper [42], Nowak and May consider the superinfection functions of the form $c\phi$ with ϕ in (12) and some $c > 0$. They find that, assuming that mutations are generated uniformly on some interval $[\alpha_{\min}, \alpha_{\max}]$, a continuum of strains can coexist on the evolutionary time scale.

Adaptive Dynamics, on the other hand, assumes more realistically, that the mutants arise locally around the strains that are already present in the population. In Figure 3, we present the pairwise invasibility plots corresponding to the superinfection function 2ϕ with ϕ as in (12). We moreover consider two choices of β : in the top row (Figures 3a and 3b) we consider a constant transmissibility β while in the bottom row (Figures 3c and 3d) we take $\beta = \frac{10\alpha}{\alpha+1}$.

As predicted, we observe increase of virulence in the course of evolution via a series of trait substitutions. For comparison, we note that in the case of a constant transmission rate, the single infection model predicts evolution towards avirulence (i.e. $\alpha = 0$; see Figure 1), while the second choice of β would lead to some intermediate level of virulence (cf. Figure 2a). When virulence evolves into vicinity of α_{\max} (the maximum virulence value that still allows persistence of infection in the population), however, some pairs of strains (α_1, α_2) are *mutually invadable*, which means that both $r(\alpha_1, \alpha_2)$ and $r(\alpha_2, \alpha_1)$ are positive (i.e., both (α_1, α_2) and (α_2, α_1) fall into the white region of the PIP; see Figures 3b and 3d). In the case of constant β , this region is more easily accessible by small mutations around the boundary α_{\max} .

Thus, we find coexistence of two strains. What happens with dimorphisms on the evolutionary time scale? Since the environment the pathogens experience has now become three dimensional (set by $\hat{S}, \hat{I}_{\alpha_1}, \hat{I}_{\alpha_2}$) it is now in principle possible that a third strain could coexist with α_1 and α_2 .

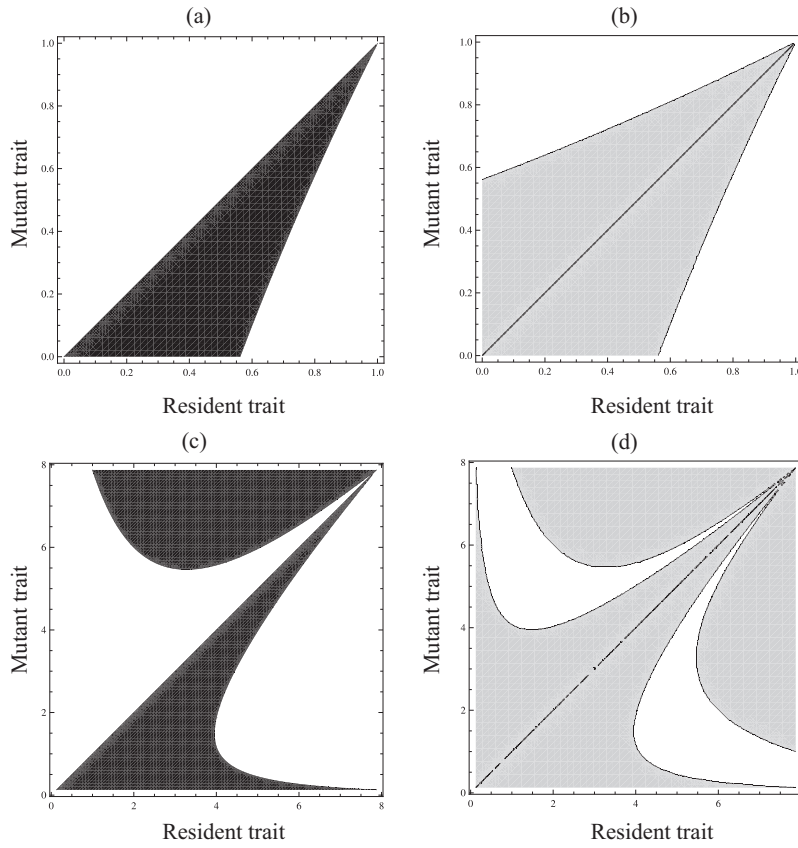


FIGURE 3. Case (A), superinfection function is 2ϕ with ϕ in (12): (a) PIP corresponding to $\beta = 2$, (b) regions of mutual invadability in (a) are depicted in white, (c) PIP corresponding to $\beta(\alpha) = \frac{10\alpha}{\alpha+1}$, (d) regions of coexistence in (c) are depicted in white.

The dimension of the environment then increases to four, so there is a possibility of the fourth strain, and so on. However, the dynamics of polymorphisms has, to our knowledge, never been investigated in the context of this model and we thus end here the discussion of Case A.

4.2. Case B. We now assume that the superinfection function is continuous in $\alpha = 0$. As we shall see in the following section (when the within-host dynamics is taken into account), the continuity of the superinfection function at the origin arises naturally when we consider the reinfection process as a stochastic event.

Even though the superinfection function may not be differentiable, the selection gradient exists and is given by

$$(13) \quad \left. \frac{\partial r}{\partial \alpha_m} \right|_{\alpha_m = \alpha_r} = \left. \frac{\partial s}{\partial \alpha_m} \right|_{\alpha_m = \alpha_r} + \beta(\alpha_r) \phi'_+(0) \hat{I}(\alpha_r),$$

which means that the singular strategies can be determined by calculating the points in which the selection gradient vanishes. Note that, if $\phi'_+(0) = 0$, the singular strategies coincide with the ones obtained from the single infection model.

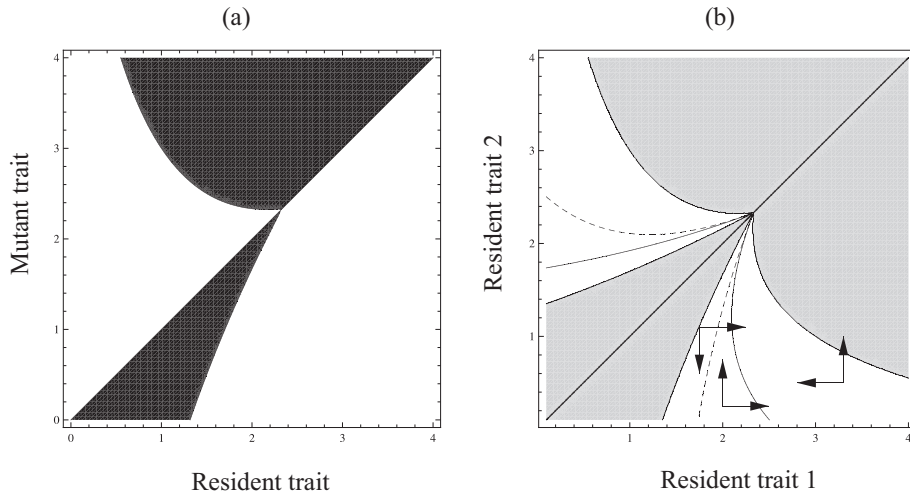


FIGURE 4. (a) Pairwise invasibility plots corresponding to Example 3: $\beta(\alpha) = 10$ and $\phi(\alpha) = \frac{\alpha}{\alpha+2}$ for $\alpha > 0$, (b) The regions of coexistence (depicted in white), along with isoclines that depict the evolution of dimorphisms. The isocline $\frac{\partial R(\alpha_1, \alpha_2, \alpha_m)}{\partial \alpha_m} \Big|_{\alpha_m = \alpha_1} = 0$ is depicted with a full line (in the interior of the white region), while the dashed line represents the isocline $\frac{\partial R(\alpha_1, \alpha_2, \alpha_m)}{\partial \alpha_m} \Big|_{\alpha_m = \alpha_2} = 0$.

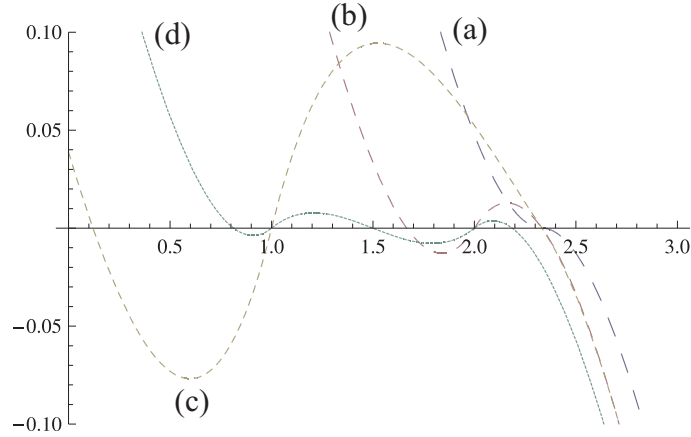


FIGURE 5. Plots of (a) $r(\alpha^*, \alpha_m)$, (b) $R(\alpha^*, 2, \alpha_m)$, (c) $R(\alpha^*, 1, \alpha_m)$ and (d) $R(1, 2, \alpha_m)$.

Since r isn't differentiable twice we cannot characterize the singular strategies in the usual way, however we can write

$$(14) \quad \frac{\partial^2 r}{\partial \alpha_m^2} \Big|_{\alpha_m = \alpha_r = \alpha^*} = \begin{cases} \beta''(\alpha^*)\hat{S}(\alpha^*) + 2\beta'(\alpha^*)\hat{I}(\alpha^*)\phi'_+(0) + \hat{I}(\alpha^*)\beta(\alpha^*)\phi''_+(0), & \alpha > \alpha^* \\ \beta''(\alpha^*)\hat{S}(\alpha^*) - \hat{I}(\alpha^*)\beta(\alpha^*)\phi''_+(0), & \alpha < \alpha^* \end{cases}$$

Expression in (14) allows us to determine whether the singular strategies are invadable or not. The discontinuity of ϕ' at the origin implies that the non-generic type of singular points, which are invadable from one side but uninvadable from the other, may now be the rule rather than the exception. We demonstrate this on two examples.

Example 3. Suppose that the transmission rate β is constant. Singular strategies are then given by

$$\alpha^* = \frac{\beta b}{\frac{1}{\phi_+'(0)} + d} - d.$$

Furthermore, expression (14) simplifies to

$$(15) \quad \left. \frac{\partial^2 r}{\partial \alpha_m^2} \right|_{\alpha_m = \alpha_r = \alpha^*} = \begin{cases} \beta \hat{I}(\alpha^*) \phi_+''(0), & \alpha > \alpha^* \\ -\beta \hat{I}(\alpha^*) \phi_+''(0), & \alpha < \alpha^*, \end{cases}$$

In this case, the singularity will always be invadable from one side but not from the other. The curvature of the superinfection function at the origin determines which of the two sides is invadable: if $\phi_+''(0) > 0$, then the singularity is invadable from above and uninvadable from below, and vice versa if $\phi_+''(0) < 0$.

In Figure 4a we show the pairwise invasibility plot corresponding to $\phi(\alpha) = \frac{0.5\alpha}{0.5\alpha+1}$ (for $\alpha > 0$ and $\phi(\alpha) = 0$ otherwise). The fact that ϕ is concave implies that the singular strategy $\alpha^* = \frac{7}{3}$ is invadable from below and uninvadable from above. Simple geometric arguments show that there must exist a region of mutual invadability close the singular strategy. Hence, after evolution has brought virulence in the vicinity of α^* the population becomes dimorphic. To decide whether any such dimorphism would be converging or diverging, we calculate the invasion exponent with two resident strategies, α_1 and α_2 , $R(\alpha_1, \alpha_2, \alpha_m)$. Because of continuity of invasion exponent, the graph of R will be (for small perturbations) similar to the graph of r and R will thus in a generic case have three roots. In Figure 5 we show the graphs of R for a few choices of dimorphic residents. The nature of dimorphisms can, however, most easily be determined using the isoclines

$$\left. \frac{\partial R(\alpha_1, \alpha_2, \alpha_m)}{\partial \alpha_m} \right|_{\alpha_m = \alpha_1} = 0 \quad \text{and} \quad \left. \frac{\partial R(\alpha_1, \alpha_2, \alpha_m)}{\partial \alpha_m} \right|_{\alpha_m = \alpha_2} = 0,$$

which we show in Figure 4b. The isoclines, along with the arrows showing the direction of dimorphic evolution, reveal that, in this case, dimorphisms are only of transient nature and eventually all converge to the monomorphic singularity. Further numerical experiments (not shown here) reveal, however, that divergent dimorphisms are possible as well in the context of this model (see also [7]).

Example 4. Let us now consider the trade-off $\beta(\alpha) = \frac{10\alpha}{\alpha+1}$ and the superinfection functions of the form

$$(16) \quad \phi_a(\alpha) = \begin{cases} \frac{a\alpha}{a\alpha+1}, & \alpha > 0 \\ 0, & \alpha \leq 0, \end{cases}$$

for some $a \geq 0$. Note that $\phi_{a+}'(0) = a$ and $\phi_{a+}''(0) = -2a^2$. The parameter a therefore represents the slope of the superinfection function at the origin (to the right). As we shall see in the next section, the slope of ϕ at the origin is related to the reinfection dose, i.e. the number of pathogens of the superinfecting strain. So how do the singular strategies depend on the value of a ?

In Figure 6 we show a series of pairwise invasibility plots corresponding to increasing values of a . Note that the case $a = 0$ corresponds to the single infection model (superinfections are not possible) and we thus recover the PIP in Figure 2a. The singular strategy is a CSS in this case.

When a increases, the singular point increases and moves towards to boundary α_{\max} . In the limit $a \rightarrow \infty$ we should recover the PIP from case (A) since for $a \rightarrow \infty$ the superinfection functions converge to the step function in (12).

Note that, both the trade-off function and the superinfection functions are concave. This implies that the second derivative in (14) is always negative for $\alpha > \alpha^*$, which means that the singular strategy is never invadable from above. For small values of a , the singular strategy is uninvadable

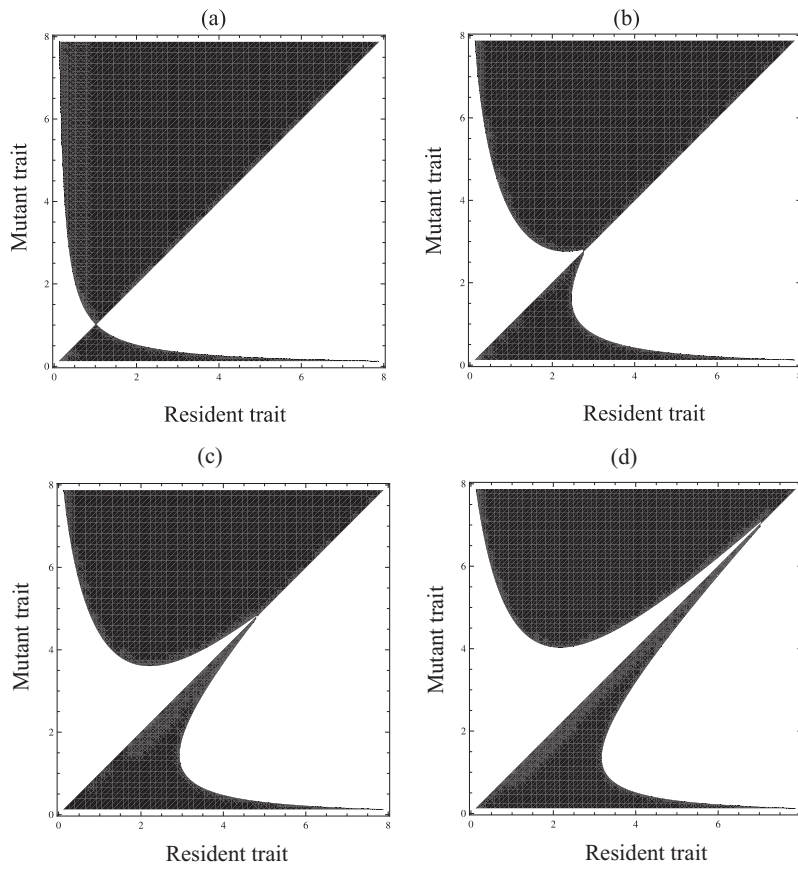


FIGURE 6. Pairwise invasibility plots corresponding to Example 4: $\beta(\alpha) = \frac{10\alpha}{\alpha+1}$ and ϕ_a in (16) with (a) $a = 0.01$, (b) $a = 0.7$, (c) $a = 2$, (d) $a = 20$.

also from below and is thus a CSS. At some critical value \bar{a} a bifurcation occurs and the singular strategy becomes invadable from below (see Figure 7).

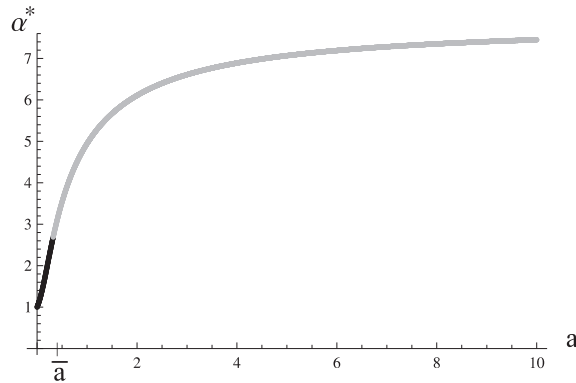


FIGURE 7. Singular strategy α^* as a function of a (Example 4). The black line shows the region where α^* is a CSS. The grey line shows the region where the singular strategy invadable from below, but not from above.

4.3. **Case C.** The case where $\phi(0) > 0$ corresponds to the situation where both the resident and the invading population are assumed to be finite and subject to demographic stochasticity. Indeed,

if N denotes the abundance of the resident strain and n the number of newly introduced pathogens of identical virulence, then the new infection settles with probability $\phi(0) = \frac{n}{n+N} > 0$. In reality, n will typically be very small. On the other hand, N is large and in the limiting case, where N is considered to infinite we obtain $\phi(0) = 0$, as was the case in previous examples. The additional assumption of differentiability in Case (C) is made purely to simplify the analysis.

The case where $\phi(0) > 0$ was studied extensively by Pugliese in [48] and more recently by Boldin et al. in [8] (where, in addition, different assumptions about the host population regulation were investigated). We refer the reader to the papers for more details, here we only summarize the main findings.

As in Case (A) and (B), we no longer have optimization in the course of evolution (which can easily be recognized by the loss of skew symmetry in PIPs). However, superinfections do not imply evolutionary coexistence per se. By writing out the second derivative

$$\left. \frac{\partial^2 r}{\partial \alpha_m^2} \right|_{\alpha_m = \alpha_r = \alpha^*} = \beta''(\alpha^*)(\hat{S}(\alpha^*) + \phi(0)) + 2\beta'(\alpha^*)\phi'(0)$$

we observe that the curvature of the trade-off plays a role in the characterization of singular strategies. It was shown in [48] that, if the trade-off function belongs to a certain family of concave functions, the singular strategy is unique and it is always a CSS, which means that dimorphisms, if they occur, are only of transient nature and are eventually resolved in a CSS. As was shown by Boldin et al. in [8], branching points can be found, even among the concave trade-offs. In [8] it was furthermore investigated how the assumptions about the host population regulation influence the occurrence of branching. This was done by investigating in detail three population dynamics regimes, (i) the constant population birth rate (as we assume here), (ii) constant population size and (iii) logistic population growth. Case (i) appears to be the most conducive to branching. Branching is found also in other models, however, the convexity ranges of the trade-offs that yield branching are narrower. Moreover, we found mutual exclusion, which is contrary to the common belief that superinfections promote coexistence. We refer the reader to [8] for some more detailed examples and for a discussion on the dynamics after branching.

Remark 5. In these notes we investigated the adaptive dynamics by choosing a particular trade-off. Using the so called *critical function analysis*, one can turn the question around and ask: which trade-offs lead to a particular outcome (for instance, branching)? We shall not go into the details of critical function analysis here but refer the interested reader to [8, 51] for examples.

5. LINKING POPULATION DYNAMICS TO THE DYNAMICS WITHIN THE HOST

Even though transmissibility β as well as virulence α are likely to be related to the individual's within host state of infection (such as for instance, the amount of viruses the individual harbors), our modeling thus far ignored a detailed description of the infection within a host. As a consequence, we had to settle with some phenomenological trade-off $\beta = \beta(\alpha)$ and superinfection function ϕ .

In this section we introduce an explicit model of within-host pathogen dynamics that follows the time evolution of target cells and free pathogens. This will allow us to make more natural (and ultimately more easily tested by experiments) assumptions about how transmissibility and virulence depend on individual's infection state. Moreover, such description will allow us to derive the superinfection function from the mechanistic intra-host submodel.

This section is based on the modeling and analysis presented in [7].

5.1. A model of within-host pathogen dynamics. We describe the dynamics inside a single host using three variables: T, T^* and V represent, respectively, the number of uninfected and infected target cells and the number of free pathogens. We assume that

- (i) In the absence of infection, target cells are produced at a constant rate λ and die at a constant per capita rate δ .
- (ii) Free pathogens inside a host die at per capita rate c .
- (iii) Infections of uninfected target cells are described by the mass action term kVT . That is, the rate at which pathogens find uninfected target cells, successfully bind to the surface of the cell and/or enter the target cell, is proportional to the product of the numbers of uninfected target cells and free pathogens. Upon infection, the uninfected target cell and the pathogen that infected it, form an infected cell.
- (iv) Infected target cells produce free pathogens at a rate p . This production comes at a cost, namely, it increases the death rate of infected target cells by $\mu(p)$. We assume that μ is a nonnegative, increasing function of the production rate p .

These assumptions yield the following system of ODEs,

$$(17) \quad \begin{aligned} \frac{dT}{dt} &= \lambda - kVT - \delta T \\ \frac{dT^*}{dt} &= kVT - (\delta + \mu(p))T^* \\ \frac{dV}{dt} &= pT^* - kVT - cV. \end{aligned}$$

System (17) has two equilibria: the infection free steady state,

$$(18) \quad \bar{V} = \bar{T}^* = 0 \quad \text{and} \quad \bar{T} = \frac{\lambda}{\delta}$$

and the nontrivial equilibrium given by

$$(19) \quad \begin{aligned} \hat{T} &= \frac{c}{k(\mathcal{B}_0(p) - 1)} \\ \hat{T}^* &= \frac{\mathcal{B}_0(p)}{p} \left(\lambda - \frac{cd}{k(\mathcal{B}_0(p) - 1)} \right) \\ \hat{V} &= \frac{\lambda}{c} (\mathcal{B}_0(p) - 1) - \frac{\delta}{k}. \end{aligned}$$

Here, \mathcal{B}_0 stands for the so called *burst size*, i.e. the expected number of pathogens produced by one infected target cell. If pathogens are produced at rate p , then

$$\mathcal{B}_0(p) = \frac{p}{\delta + \mu(p)}.$$

The nontrivial steady state given by (19) is biologically meaningful only when all three components in (19) are positive. This is the case when the within-host basic reproduction ratio of a pathogen, \mathcal{R}_0^w (the superscript w serves to distinguish it from the pathogen's basic reproduction ratio at the host population level) exceeds one. \mathcal{R}_0^w is defined as the expected number of new pathogens produced by a single pathogen introduced into a virgin cell environment. Since free pathogens need to enter uninfected target cells in order to reproduce and since the probability with which the pathogen enters a target cell in a virgin environment equals $\frac{k\lambda}{k\lambda + dc}$, the within-host basic reproduction ratio of a pathogen with trait p equals

$$\mathcal{R}_0^w(p) = \frac{k\lambda}{k\lambda + \delta c} \mathcal{B}_0(p).$$

When the nontrivial equilibrium exists, it is locally asymptotically stable, while the infection free steady state is unstable in that case (see [15] for a global stability result).

We now consider the rate of pathogen production p as the (only) trait that is subject to natural selection. All the other parameters in the within-host model will be kept constant throughout. We furthermore assume for simplicity that, if a target cell is infected with one trait, it is protected from further infections. In other words, we do not consider superinfections or coinfections at the cell level. Since this assumption implies that the pathogens compete within a host for only one resource, i.e. uninfected target cells, the evolutionary dynamics at the within-host level is very simple. Namely, when a mutant trait, say q , is introduced into a host where the trait p is resident, the mutant is successful (according to the deterministic model) if and only if it exploits the resource better than the resident, i.e. when $\hat{T}(q) < \hat{T}(p)$. Note, incidentally, that minimization of \hat{T} is equivalent to maximization of \mathcal{R}_0^w and also to maximization of \mathcal{B}_0 .

5.2. The superinfection model revisited. We can now rewrite the superinfection model in the form

$$(20) \quad \begin{aligned} \frac{dS}{dt} &= b - \beta(p)SI_p - \beta(q)SI_q - dS \\ \frac{dI_p}{dt} &= \beta(p)SI_p + \Phi(q, p)I_pI_q - (\alpha(p) + d)I_p \\ \frac{dI_q}{dt} &= \beta(q)SI_q + \Phi(p, q)I_pI_q - (\alpha(q) + d)I_q, \end{aligned}$$

where now

$$\Phi(p, q) = \beta(q)\phi(p, q) - \beta(p)\phi(q, p)$$

and the superinfection function $\phi(p, q)$ is defined as

$$\begin{aligned} \phi(p, q) &:= \text{probability that the reinfecting strain } q \text{ wins the within-host competition} \\ &\quad \text{with strain } p \text{ and takes over the host that is already infected by } p. \end{aligned}$$

As before, we could now investigate how different choices of superinfection function shape the course of evolution. However, the mechanistic submodel of within-host dynamics now allows us to derive explicit expression for the superinfection probability directly from the underlying branching process. We now present the derivation.

5.3. The dynamics in the initial stages of a superinfection. In the initial stages of a superinfection, the invading trait q is likely to be present only in small quantities. Hence, even when $\hat{T}(q) < \hat{T}(p)$ (and so the newly introduced trait has the potential to outcompete the resident trait), trait q may go extinct due to demographic stochasticity in the initial stages of a superinfection, when it is still rare. We thus describe the initial stages of an invasion as a stochastic birth-and-death process. At this point, lytic viruses have to be distinguished from the non-lytic (or budding) viruses. Here we present the derivation of the superinfection probability only for non-lytic viruses and refer the reader to [47] for a similar analysis of lytic viruses.

Suppose first that only one free pathogen with trait q is introduced into a host that is already infected by trait p . If we assume that the trait p resides at a stable equilibrium, then the new trait q is introduced into an environment given by the steady state value of $\hat{T}(p)$,

$$(21) \quad \hat{T}(p) = \frac{c}{k(\mathcal{B}_0(p) - 1)}.$$

The probability that the clan of this initially introduced pathogen survives in an already infected host, is given as the smallest fixed point of a generating function [30]. In order to compute it, we must first derive the probabilities π_n with which one free pathogen with trait q will produce n new pathogens.

In order to reproduce, a pathogen must bind to an uninfected target cell. This happens with probability

$$\frac{k\hat{T}(p)}{k\hat{T}(p) + c}.$$

When the pathogen enters a target cell, its survival relies on the survival of the target cell that hosts it. The life span of a target cell infected with trait q is exponentially distributed with parameter $(\mu(q) + d)$. The infected target cell produces free pathogens according to a Poisson process with parameter q . So the probability density that an infected target cell lives t units of time and in that time produces n offspring equals

$$(\delta + \mu(q))e^{-(\delta + \mu(q))t} e^{-qt} \frac{q^n t^n}{n!}.$$

Accounting for all possible times t , we arrive at the following expression for π_n ,

$$(22) \quad \pi_n = \frac{k\hat{T}(p)}{k\hat{T}(p) + c} \int_0^\infty (\delta + \mu(q))e^{-(\delta + \mu(q))t} e^{-qt} \frac{q^n t^n}{n!} dt,$$

which is valid for $n \geq 1$. For the probability of having no offspring at all, however, we have to take into account that the pathogen may never reproduce simply because it never enters an uninfected target cell. Since the probability with which the pathogen dies before it binds to a cell equals $\frac{c}{k\hat{T}(p) + c}$, we obtain the following generating function $G(z)$,

$$G(z) = \frac{c}{k\hat{T}(p) + c} + \sum_{n=0}^{\infty} \pi_n z^n,$$

which, by using (22) and interchanging the order of summation and integration, can be written as

$$(23) \quad G(z) = \frac{c}{k\hat{T}(p) + c} + \frac{k\hat{T}(p)}{k\hat{T}(p) + c} \cdot \frac{1}{1 + \mathcal{B}_0(q)(1 - z)}.$$

The probability with which the clan of the invading pathogen goes extinct is given as the smallest solution of $G(z) = z$ (cf. [30]). Whether this solution lies in $[0, 1]$, depends on the value of $G'(1)$, which equals the invaders within-host reproduction ratio in the environment set by the resident,

$$\mathcal{R}_0^w(\hat{T}(p), q) = \frac{k\hat{T}(p)}{k\hat{T}(p) + c} \mathcal{B}_0(q).$$

If $\mathcal{R}_0^w(\hat{T}(p), q) \leq 1$, the clan will go extinct with certainty. If, on the other hand, $\mathcal{R}_0^w(\hat{T}(p), q) > 1$, the invasion will be successful with nonzero probability.

Let $P(p, q)$ denote the probability of extinction of trait q , following an introduction of a single free pathogen into an environment set by the resident trait p . Using (23) and (21) we obtain

$$P(p, q) = \min \left\{ 1, \frac{c}{c + k\hat{T}(p)} + \frac{1}{\mathcal{B}_0(q)} \right\} = \min \left\{ 1, 1 - \frac{1}{\mathcal{B}_0(p)} + \frac{1}{\mathcal{B}_0(q)} \right\}.$$

Thus, the invading trait has a nonzero probability of success only when its burst size exceeds the burst size of the resident trait p . We also observe that (i) $P(p, p) = 1$, as it should be since the resident trait resides at a stable equilibrium, and (ii) when $\mathcal{B}_0(q) \rightarrow \infty$, the invading trait will survive with certainty, provided that the pathogen initially introduced makes it to an uninfected target cell. The probability of extinction must therefore equal the probability with which the pathogen dies before it enters a target cell. And indeed,

$$\lim_{\mathcal{B}_0(q) \rightarrow \infty} P(p, q) = \frac{c}{k\hat{T}(p) + c}.$$

The complementary probability

$$\phi_1(p, q) = 1 - P(p, q)$$

is the probability that the clan of one free pathogen with trait q survives in the environment set by the resident trait.

When n pathogens are introduced, therefore, the probability of survival equals

$$(24) \quad \phi_n(p, q) = \begin{cases} 1 - P^n(p, q), & \mathcal{B}_0(p) < \mathcal{B}_0(q) \\ 0, & \text{otherwise} \end{cases}$$

or, rewritten in terms of $\hat{T}(p)$ and $\hat{T}(q)$,

$$(25) \quad \phi_n(p, q) = \begin{cases} 1 - \left(1 - \frac{k\hat{T}(p)}{c + k\hat{T}(p)} + \frac{k\hat{T}(q)}{c + k\hat{T}(q)}\right)^n, & \hat{T}(q) < \hat{T}(p) \\ 0, & \text{otherwise} \end{cases}$$

We observe that the superinfection functions are continuous, but not differentiable in $q = p$. The fact that they are increasing as functions of $\mathcal{B}_0(q)$, implies that the traits that significantly increase the burst size also have a better chance of surviving in the host than the traits which are only slightly better within-host competitors than the resident.

Note also that $\{\phi_n\}$ is an increasing sequence for every resident strategy, that is,

$$\phi_0(p, q) < \phi_1(p, q) < \phi_2(p, q) < \dots$$

for every p . Thus, if p and q are given, the larger the reinfection dose is, the better the chances of survival of the mutant are.

In the limit, when the number of initially introduced pathogens goes to infinity, we have

$$\lim_{n \rightarrow \infty} \phi_n(p, q) = \begin{cases} 1, & \hat{T}(q) < \hat{T}(p) \\ 0, & \text{otherwise,} \end{cases}$$

i.e., the superinfection function is a discontinuous function which furthermore doesn't discriminate among the winning strategies: every trait that reduces the steady state level of target cells to a lower level than the resident trait succeeds with probability one. Hence, when infinitely many pathogens with trait q are introduced, the deterministic description gives the full story: if the newly introduced trait goes extinct, it is because it loses the competition within the host and not due to bad luck while still rare. In this deterministic description, an even slightly better within-host competitor will outcompete the resident strain and evolution will drive p towards the within-host optimum. Assuming small mutational steps, therefore, the outcome of evolution at the population level will be the same as in a single infected host. Contrary to the within-host evolution, however, we do not have an optimization model at the population level (we refer to [7] for details). For comparison, we note that while the basic superinfection model predicted ever increasing virulence, the superinfection model with a nested within-host submodel predicts evolution towards the within-host optimum.

If, on the other hand, n approaches zero, the chance of a successful invasion becomes virtually zero. In this case, therefore, superinfections play a negligible role. In the limit $n = 0$ we end up with the single infection model.

For intermediate levels of n , the singular strategy lies inbetween the within-host optimum and the optimum of the single infection model. With increasing n , the singular strategy moves from the optimum of the single infection model towards the optimum of the within-host model. Depending on the trade-off and the reinfection dose n , the convergence stable strategies can either be uninvadable or invadable. However, because of the fact that the superinfection function is merely continuous, we may again get singular points that are invadable from one side only. If branching occurs, one of the two strains has very little room to evolve and remains virtually constant through the course

of evolution. We refer the reader to [7] for examples and more details.

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