Evolutionary dynamics of virulence

Andrea Pugliese
Dipartimento di Matematica
Università di Trento

1 Introduction

The evolution of virulence is a very active area of research; it is impossible to cite here all the publications, or even the very relevant ones. I just suggest the edited book (Dieckmann et al., 2002) as a suitable reference for much of the relevant literature up to its publication year, Ewald (1994) and Stearns (1999) as passionate advocates of the relevance of evolutionary thinking for medicine of infectious diseases, as well as sources of much empirical information. Reference to the more recent literature will be given in the sections, when discussing extensions of the basic model. Beyond the theoretical interest of the subject, it is thought that this research area may provide advice in the design of therapy and vaccines (Williams and Nesse, 1991, Bull, 1994, Dieckmann et al., 2002, Gandon and Day, 2007).

Virulence may have different meanings (Bull, 1994); here I (like many of the cited authors) restrict myself to a precise definition: 'virulence' is the parasite-induced host mortality (which is not the same as case mortality or other measures of lethality (Day, 2002)). Note that in the plant pathology literature 'virulence' has often a rather different meaning (CastagnoneSereno et al., 2007), implying the ability of a pathogen to cause a susceptible response on a host plant carrying a given resistance gene; recent publications (Sacristan, 2008), however, adhere to the meaning of 'virulence' used here.

A parasite (here, the terms 'parasite' and 'pathogen' will be used interchangeablely, without referring to any specific life form) may also decrease a host's fertility or well-being, but, as long as this does not affect its transmissibility, this does not do any harm to a parasite. On the other hand, a parasite that kills its host, kills also itself and prevents further transmission; from this observation, the view (named 'the common evolutionary wisdom' by Anderson and May (1982)) emerged that high virulence is maladaptive: parasites evolve to be relatively benign, since they have no interest in killing their host; parasites are virulent to a host only when their interaction has not been long enough for adaptation. Support for this view came from the devastating effects of some introduction of parasites into new hosts (*Myxoma* virus, Dutch elm disease), although Ebert and Hamilton (1996) argue that these examples are rather the exception than the rule in the introduction of parasites.

In general, there is now good empirical evidence (Read, 1994) for virulence to persist in long-term parasite-host associations. Furthermore, in the case of the *Myxoma* virus in Australia, it is well documented that selection did not proceed all the way to leave only avirulent strains, but stabilized instead at an intermediate level of virulence (Fenner and Ratcliffe, 1965, Fenner, 1983). Actually, later measurements suggest a reincrease of virulence, perhaps in response to acquired resistance in rabbits, although several hypotheses are consistent with the data (Sabelis and Metz, 2002).

Indeed, it is clear (Poulin and Combes, 1999, Day et al., 2007) that virulence is a property of a host-parasite interaction, and not simply of the parasite. From this point of view, it would be appropriate to study the evolution of virulence in a coevolutionary setting, where host resistance is also considered. However, modelling coevolution is much more complex, and except for a pioneering paper (Andreasen and Christiansen, 1993), only recently some papers (Gilchrist and Sasaki, 2002, Dieckmann, 2002, Boots and Bowers, 2003) have started to address models for host-parasite coevolution. Here, as most authors, I limit the analysis

to the evolution of virulence, as if it would depend on the pathogen only. A justification for this is that, usually, parasites have a much shorter generation time than their hosts. A time-scale argument lets us consider, as a first approximation, the case where only parasites can evolve.

A breakthrough in the understanding of the evolution of virulence occurred in the early 80's with the help of simple mathematical models, thanks to the seminal papers of Levin and Pimentel (1981), Anderson and May (1982), and Ewald (1983). Their main argument is based on the existence of a 'trade-off' between virulence and infectivity. The best empirical evidence for the existence of such a trade-off comes from mouse malaria (Mackinnon and Read, 1999, Ferguson et al., 2003, de Roode et al., 2005); some evidence for a trade-off is given also by Fraser et al. (2007) for HIV, while several manipulative experiments (Ebert, 1998, Boots and Mealor, 2007, de Roode et al., 2008) suggest that virulence increases when selection on between-host transmission is released. A trade-off function is explicitly built from the experimental data on *Myxoma* in Dwyer et al. (1990).

Ebert and Bull (2003) question the generality of the trade-off approach, and suggest that virulence may not be directly linked to transmissibility, but rather depend on specific features of the pathogen-host interactions, providing several examples of parasite evolution that appear independent or even inconsistent with the virulence–transmissibility trade-off. A recent review paper (Alizon et al., 2009) discusses in detail the issue, giving a more balanced view; my conclusion is that the trade-off assumption can be used as a sound basis for the analysis, while recognizing that each specific system needs to be analyzed, before applying ideas resulting from the general model.

I will base the following analysis on the existence of a virulence–transmissibility trade-off, following a narrow route through the vast range of issues and models of virulence. The main questions I will address are the expected path of virulence evolution, and the possibility for several parasite strains, differing only quantitatively in virulence, to coexist at an evolutionarily stable state.

The analysis of this chapter will be mainly set in the framework of adaptive dynamics (Metz et al., 1996, Geritz et al., 1997) as presented in previous chapters. I will especially exploit 'pairwise invasibility plots' showing the sign of the invasion coefficients $s_x(y)$ where x and y represent the virulences of different parasite strains.

In Section 2, I will examine the basic model of Anderson and May (1982), restating their results in this framework. The transmissibility-virulence trade-off might be mediated through the speed of pathogen replication: some authors (Gilchrist and Sasaki, 2002, André et al., 2003, Alizon and van Baalen, 2005) have shown that a trade-off function emerges naturally out of standard models for the dynamics of within-host interactions between pathogens and host cells; this idea is illustrated at depth in Section 3 following Gilchrist and Sasaki (2002). In Section 4, I will investigate models with superinfections, where it is possible that a strain can infect also hosts that are already infected with another strain. Finally in Section 5, some other approaches and open problems will be briefly examined.

2 The basic epidemic model

Density vs. numbers... The starting point in the adaptive dynamics approach is a model describing the interaction of different parasite types. This model will be a modification of a standard models for epidemics (Bailey, 1975, Hethcote, 1976) with a single type of parasites. I will discuss here only SIR models: hosts are born susceptible (S) to infection, then may become infected (I) and, if so, they either die or recover and become immune (R) (see Box 1 for more explanations on epidemic models). The model includes disease-related deaths, since the interest lies in the lethal effects caused by the pathogen: thus host population density N will be a dynamic variable instead of a fixed constant.

Box 1. Epidemic models.

Epidemic models generally divide the host population into individuals that are currently infected and infectious (I), individuals that are not infected but are susceptible to the infection (S) and often individuals that are recovered from the disease and immune to further infection (R); the dynamics of the parasites is described simply as the dynamics of infected hosts. Within-host dynamics, in particular, are not modelled explicitly.

A lucid presentation of basic epidemic models is given, for instance, by Hethcote (1989); here I just outline the main ideas. If the infection confers permanent immunity, the dynamics of the infection is $S \to I \to R$ (susceptible individuals become infected and then immune). Without immunity the dynamics of the infection is $S \to I \to S$ (susceptible individuals become infected and then susceptible again). A latency period after infection is often modelled by introducing the class of exposed (E), individuals that have been infected but are not yet infectious, obtaining a $S \to E \to I \to R$ dynamics.

New infections are assumed to occur because of encounters between infectious and susceptible individuals; the standard assumption is a mass-action law for encounters, so that the rate of new infections is βSI. There is not a general agreement on what the variables S, $I, \ldots$ should represent, whether numbers or spatial densities; among others, Jong et al. (1995) discuss the evidence, together the related issue of whether β should vary when population density N is variable; they argue that usually it will be $\beta = \frac{\lambda}{N}$ (they call this 'true mass-action law') where λ is the average number, assumed to be constant, of individuals contacted by one individual in unit time. Here I will consider S, $I, \ldots$ as densities over some area in which homogeneous mixing is plausible, and stick to the assumption of constant β ('standard mass-action law'), mostly for consistency with previous analyses on virulence. Use of the 'true mass-action law' would change some algebraic details, but not the qualitative results for competition among strains (O'Keefe, 2005).

In the simple models discussed here, one needs also to consider rates of birth (all individuals are supposed to be born susceptible), of death, and of recovery.

The equations (the underlying assumptions are presented in Box 1) are

$$\begin{aligned} \frac{dS}{dt} &= b(N)N - dS - \beta SI \\ \frac{dI}{dt} &= \beta SI - dI - \alpha I - \gamma I \\ \frac{dR}{dt} &= \gamma I - dR \end{aligned} \tag{1}$$

where $N = S + I$, β is the contact rate (see Box 1), α parasite-induced death rate (= 'virulence'), γ recovery rate , d natural death rate and $b(N)$ the birth rate, assumed to be density-dependent. In absence of the epidemic, the population size would converge to its carrying capacity K, which is found by solving the equation $b(K) = d$. The assumption of density-regulation through hosts' fertility is the same as in Bremermann and Thieme (1989) and differs from the simpler assumption of a constant per capita birth rate b (Anderson and May, 1982) which entails exponential growth in absence of the epidemic.

The behaviour of system (1) can be understood in terms of the 'basic reproductive ratio' R_0 of the parasite, representing the expected number of new infections caused by a single infected individual when the whole population is susceptible (see Box 2 for details). In our case, R_0 is given by

$$R_0 = \frac{\beta K}{d + \alpha + \gamma}. \tag{2}$$

It is clear that, if each infective host infects, on average, less than one new host ($R_0 < 1$), the epidemic will fade out, since not enough new cases are produced. If, on the contrary, an infective host infects on average more than one other host ($R_0 > 1$), the epidemic will spread, at least as long as the number of susceptibles is large enough. For system (1), the epidemic will then settle at a globally attractive endemic equilibrium with a total host population $\bar{N}$ and a susceptible population $\bar{S}$. At the equilibrium each infective will on average infect one susceptible over its expected life time, so that

$$\frac{\beta \bar{S}}{d + \alpha + \gamma} = 1. \tag{3}$$

Box 2. Computation of the basic reproductive ratio R_0.

The basic reproductive ratio R_0 is the average number of individuals infected by a single infective under some given and constant environmental conditions (often an equilibrium population with all susceptibles).

If the rate at which an infectious individual infects other individuals (the 'effective contact rate') does not depend on the time elapsed since infection, R_0 can be obtained simply from multiplying the 'effective contact rate' (β times the absolute number of susceptibles) with the expected time spent as infective.

In models (such as those consisting of ordinary differential equations) where future dynamics depends on present state only, and not on past history, the time spent in any state (for instance as an infective) will follow an exponential distribution; thus, the expected time spent as infective is simply the inverse of the exit rate from that state. If exits for different causes are possible (recovery, 'natural' death, parasite-induced death), their rates have to be added, yielding the total exit rate.

For instance in model (1), natural mortality rate d, disease-related death rate α and recovery γ have to be added, giving an expected time as infected equal to $1/(d+\alpha+\gamma)$. When examining whether a parasite is able to establish itself, I consider a completely susceptible population at carrying capacity K, thus obtaining (2).

When discussing the success of the invasion of a parasite 2 in a population where parasite 1 is already established, then, in the case of complete and instantaneous cross-immunity, the environment for parasite 2 is the population at the endemic equilibrium with parasite 1, so that the number of susceptibles is $\bar{S}_1$; the expected time as infective is as before $1/(d+\alpha_2+\gamma_2)$, so that (6) is obtained.

The invasion of an established epidemics by a 'new' parasite type can be studied by extending model (1) to allow for two different types of infected individuals I_1 and I_2. While other assumptions are possible (see Section 4), I assume here that an infected individual cannot be further infected. This leads to the following system of differential equations

$$\begin{aligned}
\frac{dS}{dt} &= b(N)N - dS - \beta_1 S I_1 - \beta_2 S I_2 \\
\frac{dI_1}{dt} &= \beta_1 S I_1 - dI_1 - \alpha_1 I_1 - \gamma_1 I_1 \\
\frac{dI_2}{dt} &= \beta_2 S I_2 - dI_2 - \alpha_2 I_2 - \gamma_2 I_2 \\
\frac{dR}{dt} &= \gamma_1 I_1 + \gamma_2 I_2 - dR
\end{aligned} \tag{4}$$

with $N = S + I_1 + I_2 + R$.

A complete analysis of (4) is presented by Bremermann and Thieme (1989) for n competing strains. Here I summarise the relevant results, with some intuitive explanations. It must be kept in mind that, while the present analysis relies on local stability analysis, the global picture is the same (Bremermann and Thieme, 1989), so that, in this case, consideration of pairwise invasibility provides all necessary information for predicting outcomes of competition between parasite strains.

The invasion fitness $s_x(y)$ can be computed linearizing (4) at the endemic equilibrium for only one strain; letting $s_1(2)$ denote the invasion coefficient of parasite 2 (with parameter values α_2, $\beta_2 \ldots$) into a population at equilibrium with parasite 1, we have

$$s_1(2) = \beta_2 \bar{S}_1 - (d + \alpha_2 + \gamma_2). \tag{5}$$

It is more transparent, however, to use a suitable reproductive ratio: specifically, let $R_0^1(2)$ be the expected number of new infections caused by a single individual infected with strain 2 when the host population is at its endemic equilibrium for strain 1. Strain 2 will invade into a population at equilibrium with strain 1 if $R_0^1(2) > 1$; it cannot do so if $R_0^1(2) < 1$. This is proved in a general setting, where

the definition of R_0 is more complex, by Diekmann and Heesterbeek (2000); they also show that the condition on R_0 is equivalent to a condition on invasion fitness.

Following the computations outlined in Box 2, the invasion condition can be written as

$$R_0^1(2) = \frac{\beta_2 \bar{S}_1}{d + \alpha_2 + \gamma_2} > 1. \tag{6}$$

Note from (5) that, in this case, a very simple relation holds between $R_0^1(2)$ and $s_1(2)$: $s_1(2) = (d + \alpha_2 + \gamma_2)(R_0^1(2) - 1)$.

In order to compare different strains, it is convenient to define a 'standardized reproductive potential'

$$\varphi_i = \frac{\beta_i}{d + \alpha_i + \gamma_i}. \tag{7}$$

Since the 'standard mass-action law' (Box 1) is assumed, the basic reproductive ratio of strain i when there are S susceptibles in the population is $\varphi_i S$. Under a 'true mass-action law' (Box 1), the basic reproductive ratio of strain i with S susceptibles in a population of size N would be $\varphi_i \frac{S}{N}$.

Now (6) reads $\varphi_2 \bar{S}_1 > 1$, while (3) for $\bar{S}_1$ yields $\varphi_1 \bar{S}_1 = 1$. Hence, the invasion of strain 2 will be successful if $\varphi_2 > \varphi_1$.

2.1 Optimal virulence

I now turn to the main topic of this chapter, the evolution of virulence, identifying, as discussed in the Introduction, virulence with parasite-induced death rate α. Several conclusions can be drawn from the fact that $s_2(1) > 0$ if and only if $\varphi_2 > \varphi_1$.

First of all, we see that, if $\varphi_2 > \varphi_1$, strain 2 can invade strain 1 but strain 1 cannot invade strain 2; thus mutual invasibility is impossible and coexistence may occur only as a transient or in the infinitely unlikely case where $\varphi_1 = \varphi_2$. Second, strain 1 cannot be invaded by any other parasite type if φ_1 is larger than φ_i for any other feasible strain i; in other words, an evolutionarily uninvadable state will be found at the state that maximizes $\dfrac{\beta}{d + \alpha + \gamma}$ among all feasible states.

It is clear from (7) that if two types differ only in their virulence, the less virulent type will have a larger R and will thus outcompete the other type. Therefore, we would expect an evolutionary trend toward a decreased virulence, recovering the 'conventional wisdom'.

However, matters are different if one assumes, following Anderson and May (1982), a trade-off between contact rate β and virulence α, i.e. a relation that gives the contact rate β as a function of virulence α (see a) and c) of Fig. 1). The existence of such a trade-off is a basic tenet of the current theory on the evolution of virulence, as discussed in the Introduction.

Since β is now envisaged as a function of α, while γ is taken as a constant independent of α (other assumptions are certainly possible, see Alizon (2008)) and d does not depend on parasite strategy, the quantity φ of (7) can be written as a function of virulence α

$$\varphi(\alpha) = \frac{\beta(\alpha)}{d + \alpha + \gamma}. \tag{8}$$

The invasibility condition $\varphi_2 > \varphi_1$ shows that an evolutionarily uninvadable state will be found at a maximum of the function $\varphi(\alpha)$. This result is a consequence of the fact that, in this model, for a parasite the environment is one-dimensional: number of susceptibles S; hence, a maximization principle holds (Chapter ??).

The qualitative conclusions depend on the shape of the function $\beta(\alpha)$: if it is a concave function (like in Fig. 1a), which seems likely because of the 'law of diminishing returns' (Lipsitch et al., 1995), there exists a single maximum of $\varphi(\alpha)$. Simple arguments from one-dimensional adaptive dynamics show that, in this case, the value of α at which φ is maximized is a final state for evolutionary dynamics (a 'continuously stable strategy' (Eshel, 1996)). Often, this strategy will correspond to an intermediate virulence (as shown in Fig. 1a)-b)), but it is also possible to have the maximum at $\alpha = 0$ ('avirulence').

If the function $\beta(\alpha)$ is convex, there are no maxima of the 'basic reproductive ratio' R, but often a single minimum; this, in the framework of adaptive dynamics,

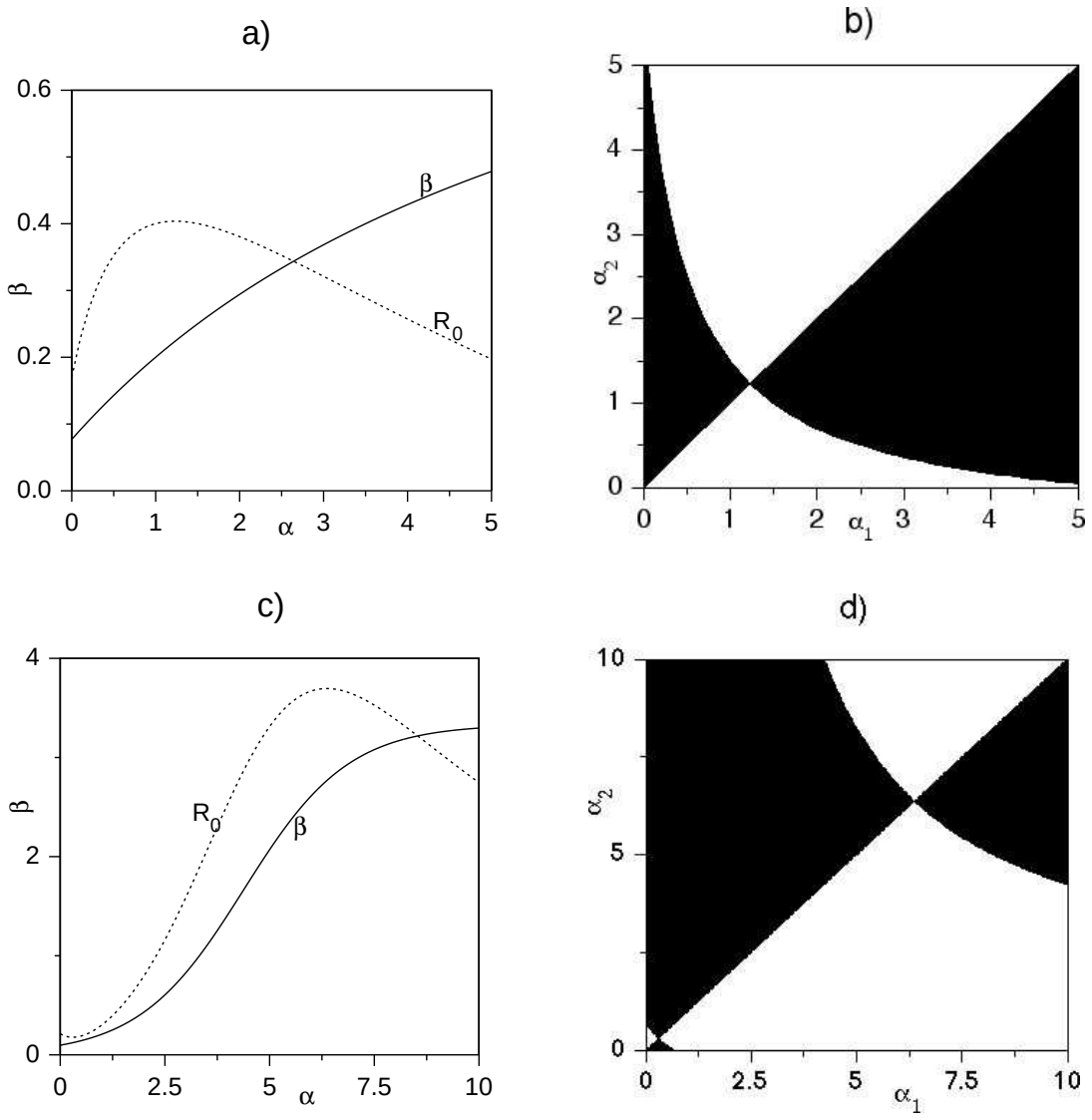

Figure 1: a) An example of a concave function: $\beta(\alpha) = \frac{C\alpha}{A+\alpha} + B$ and the corresponding $\varphi(\alpha)$;
b) the 'invasibility plot' for the functions in a); α_2 can invade α_1 when (α_1, α_2) is in the black region;
c) an example of a concave–convex function: $\beta(\alpha) = \frac{1}{A+Be^{-C\alpha}}$, and the corresponding $\varphi(\alpha)$; Parameter values are $A = 0.5,\ B = 10,\ C = 0.8$;
d) the 'invasibility plot' for the functions in c).

is an evolutionary repeller, i.e. a separating point for evolutionary trajectories: if the initial virulence α is lower than a threshold value, evolution will drive virulence to 0; if it is above, virulence will increase forever in the course of evolution. A more reasonable assumption is that $\beta(\alpha)$ is convex only within a certain range of virulence, and concave beyond that (like in Fig. 1c). In this case, evolution could tend toward high or low virulence, depending on initial conditions.

Note that I use here the terms 'concave' and 'convex' following the standard mathematical use. Often, in the biological literature, 'convex' is used for a function like that in Fig. 1a), which is called 'concave' here.

As mentioned in the Introduction, a trade-off function has been fitted to data on *Myxoma* virus, to study whether the evolutionary path could be interpreted as maximizing the function $\varphi(\alpha)$. More often, the principle has been tested by indirect methods, by showing that a parameter change resulted in a consistent shift in virulence. In order to see the effect of parameters on the optimal value of virulence (in the case of a concave trade-off function), one can note that a maximum of R must satisfy

$$\beta(\alpha) = \beta'(\alpha)(d + \alpha + \gamma). \tag{9}$$

Equation (9) can be expressed in graphical form, as shown, for instance in (van Baalen, 2002). It easily follows that an increase of the natural mortality d should result in a virulence increase. This prediction, that intuitively can be explained by saying that a host's value for a parasite is lower if its expected lifespan is anyway low, has been experimentally confirmed (Ebert and Mangin, 1997).

Along a different, an increase or decrease of transmissibility (due, for instance, to different climatic conditions or hygiene procedures) does not change the optimal level of virulence, as long as the increase [or decrease] is proportional for all levels of α. On the other hand, if the shape of the trade-off function $\beta(\alpha)$ changes, it is clear from (9) that this will affect the selected value of α. Ewald (1994) discusses, through several empirical examples, how this should change with mode of

transmission, and other factors of the infection process.

2.2 The role of density-dependence

The case where death rate d, and not only birth rate, is density–dependent is surprisingly more complex than system (1) (Andreasen and Pugliese, 1995). However, Pugliese (2002) shows that, when the trade-off function $\beta(\alpha)$ is concave, the evolutionary dynamics is similar, with convergence to an evolutionarily uninvadable state, although shrinking dimorphisms may arise in the path to the stable state. On the other hand, it has recently been shown that, if the trade-off function is convex even in a small part of the range, more complex evolutionary dynamics may occur with branching of divergent strains (Svennungsen and Kisdi, 2009)

I restrict here to the concave case, and analyse the properties of the final evolutionary state. Modifying (5) to this case, one obtains

$$s_{\alpha_1}(\alpha_2) = \beta_2 \bar{S}_1 - (d(\bar{N}_1) + \alpha_2 + \gamma_2) \tag{10}$$

where $\bar{N}_1$ and $\bar{S}_1 = \dfrac{d(\bar{N}_1) + \alpha_1 + \gamma_1}{\beta_1}$ are the equilibrium values of N and S in presence of strain 1 alone.

It is possible to define in this case too a 'standardized reproductive potential'

$$\varphi_N(\alpha) = \frac{\beta(\alpha)}{d(N) + \alpha + \gamma}. \tag{11}$$

that will however depend on the population value N at which it is computed. It is then no longer possible to define a strain that maximizes 'a single standardized reproductive potential, since the latter varies with N.

To determine the direction of virulence evolution, the essential quantity is $D(\alpha) = \dfrac{\partial}{\partial \alpha_2} s_\alpha(\alpha_2)|_{\alpha_2=\alpha}$. One finds

$$D(\alpha) = \frac{\bar{S}(\alpha)}{d(\bar{N}(\alpha)) + \alpha + \gamma} \left(\beta'(\alpha) - \frac{\beta(\alpha)}{d(\bar{N}(\alpha)) + \alpha + \gamma} \right). \tag{12}$$

An evolutionarily singular state α^* satisfies $D(\alpha^*) = 0$, and thus

$$\beta'(\alpha^*) - \frac{\beta(\alpha^*)}{d(\bar{N}(\alpha^*)) + \alpha^* + \gamma} = 0. \tag{13}$$

This means that α^* must maximize, over α, the 'standardized reproductive potential' $\varphi_{\bar{N}(\alpha^*)}(\alpha)$ with N fixed at the equilibrium population size for strain α^*. In other words, finding the evolutionary attractor can be seen as a recursive process: choose a potential α^*, find its equilibrium population size $\bar{N}(\alpha^*)$; then, find the maximum of $\varphi_{\bar{N}(\alpha^*)}(\cdot)$: if this maximum is α^*, this is an evolutionary stationary state; otherwise try with a different α^*. Pugliese (2002) shows that there is always a unique solution to this process, and that this is an evolutionary attractor.

Dieckmann (2002) analyses extensions of model (1) where the demographic rates b and d, as well as the contact rate β and 'virulence' α are allowed to depend in various ways on the densities of susceptibles S and infectives I. This gives rise to somewhat richer evolutionary behaviours than have been discussed so far; moreover, he shows that some kinds of density-dependence give rise to results that cannot be interpreted in terms of R_0. While certainly a case can be made for all rates being density-dependent, I believe that system (1) provides a simple, but reasonably realistic, basis for the analysis; the previous example with density-dependence in the death rate shows some possible consequences of allowing for other types of density-dependence.

3 Within-host processes

Several authors in recent years have started tying virulence and infection transmission between hosts to the dynamics of the infection process within a host (see a recent review by Mideo et al. (2008) who favour the name of 'nested models' for this approach). This, on the one hand, connects this type of models to more measurable processes, on the other hand, avoids invoking the 'trade-off' as a first principle but instead obtains it as the result of a 'mechanistic' model.

Box 3. A simple model of immune-pathogen within-host dynamics. Let the within-host state be described through the pathogen load P and the host's level of specific immunity B. The variable B may represent some precise quantity, like the density of specific B-cells or antibodies, or a more generic index related to the different types of immune cells specific for that pathogen agent.
The model studied by Gilchrist and Sasaki (2002) has the structure of a predator-prey model, with pathogens (the prey) replicating at rate r, in absence of immune response, and being killed by the immune system at rate c on encounter, and the immune cells proliferating proportionally (with a proportionality constant a) to the pathogen load. One then obtains

$$\begin{cases} \dfrac{dP}{d\vartheta} &= rP - cBP \\ \dfrac{dB}{d\vartheta} &= aBP \end{cases} \tag{14}$$

where ϑ represents time since infection of the individual host.
Some solutions, with different initial conditions, of system (14) are shown in Fig. 2: $P(\vartheta)$ initially increases (if $r > cB_0$) to a maximum and then declines to 0, while $B(\vartheta)$ increases to an asymptotic level (depending on initial conditions) B_∞.
Recovery is not explicitly modelled in this system, but when an individual reaches a B level close to B_∞, it is effectively immune to further infections.
Many other models for the dynamics of virus-immune interactions can be found in the books by Nowak and May (2000) and Wodarz (2007).

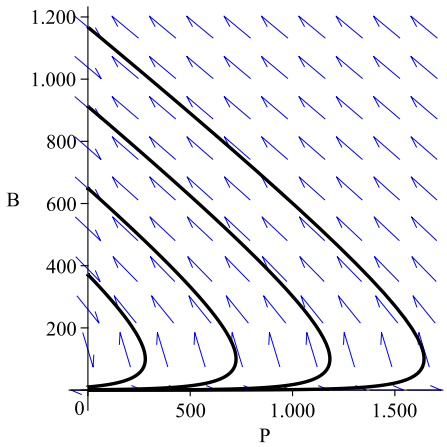

Figure 2: The phase plane of system (14); parameter values are $r = 100$, $a = 0.5$, $c = 1$.

There exists a wide body of literature on the dynamics of within-host infection process, concerning especially the dynamics of HIV (Perelson et al., 1993, Nowak and May, 2000). When within-host dynamics is only a part of a model including between-hosts transmission, models must necessarily be simplified; basically, models can be classified according to two features: the presence or not of an active immune response fighting the pathogen cells; the analysis of the transient phase of the infection process vs. long-term asymptotic state.

The two factors are clearly linked, since, if an active immune response always results in pathogen clearance, only short-term features can be relevant. This is the framework used by Gilchrist and Sasaki (2002) in the first paper that consistently studies pathogen evolution (actually also host-pathogen coevolution) building on a model for within-host dynamics (see Box 3).

The quantity assumed to evolve is the replication rate r, roughly equivalent to virulence. Evolution of c would correspond to the ability of escaping immune recognition, which is something not dealt with here. Evolution of a is discussed in detail by Gilchrist and Sasaki (2002) as a trait controlled by the host, although it certainly depends also on immune recognition, that could depend also on parasite traits. In any case, evolution of a is beyond the scope of this chapter.

In order to study pathogen evolution within the previous framework, it is necessary to connect model (14) for the within-host dynamics to infection transmission, and host mortality. A natural assumption is that transmissibility is proportional to pathogen load; this corresponds to a transmission coefficient depending on age-since-infection $\beta(\vartheta) = \beta_0 P(\vartheta)$. As for hosts' death rate, Gilchrist and Sasaki (2002) assumed that it consists in a basal level (d) plus a component proportional to pathogen replication ($k_1 r P$) plus a component proportional to the proliferation of the immune system ($k_2 a B P$), because of the resource drain; on the whole, the death rate $\alpha(\vartheta)$ depends on age-since-infection as

$$\alpha(\vartheta) = d + k_1 r P(\vartheta) + k_2 a B(\vartheta) P(\vartheta). \tag{15}$$

One can extend the computation of R_0 to the case where parameters depend on age-since infection (see Diekmann and Heesterbeek, 2000), obtaining

$$R_0 = K \int_0^\infty \beta(\vartheta) \exp\{-\int_0^\vartheta \alpha(u)\, du\}\, d\vartheta \tag{16}$$

since the probability of being alive and infectious ϑ time after the infection is

$$\pi(\vartheta) = \exp\{-\int_0^\vartheta \alpha(u)\, du\}. \tag{17}$$

Using the above expressions for β and α, and performing some manipulations, one can write

$$\begin{aligned} R_0 &= \beta_0 K \int_0^\infty P(\vartheta) e^{-d\vartheta} \exp\{-\int_0^\vartheta [k_1 r P(u) + k_2 a B(u) P(u)\, du\}\, d\vartheta \\ &= \beta_0 K \int_0^\infty P(\vartheta) e^{-d\vartheta} \left(\frac{B(\vartheta)}{B_0}\right)^{-k_1 r/a} e^{-k_2(B(\vartheta)-B_0)}\, d\vartheta \\ &= \frac{\beta_0 K}{a} B_0^{k_1 r/a} \int_{B_0}^{B_\infty} e^{-dB^{-1}(u)} u^{-k_1 \frac{r}{a}-1} e^{-k_2(u-B_0)}\, du. \end{aligned} \tag{18}$$

The last equality depends on the fact that, changing ϑ into $-t$, system (14) becomes the classical SIR epidemic model for a closed population, studied by Kermack and McKendrick (1927) and exposed in (Hethcote, 1989). Then one can exploit the prime integral existing for that system; see Gilchrist and Sasaki (2002) for details.

One can also repeat, almost exactly, the computations of Section 2, if host demography satisfies the same assumptions. It turns then out that a pathogen strain characterized by a different value of r can invade if and only if its R_0 is greater than the one of the resident strain. Hence, the principle of maximizing R_0 still holds.

By computing R_0 numerically (see Fig. 3), it has always been found that R_0 has a unique maximum, at an intermediate value of r, except for parameter values that make no biological sense. A rigorous proof of this property of the function R_0 in (18) is however lacking.

In this example, the function R_0 has been computed directly, without defining "virulence", since infection-induced death rate (and transmission) varies, as

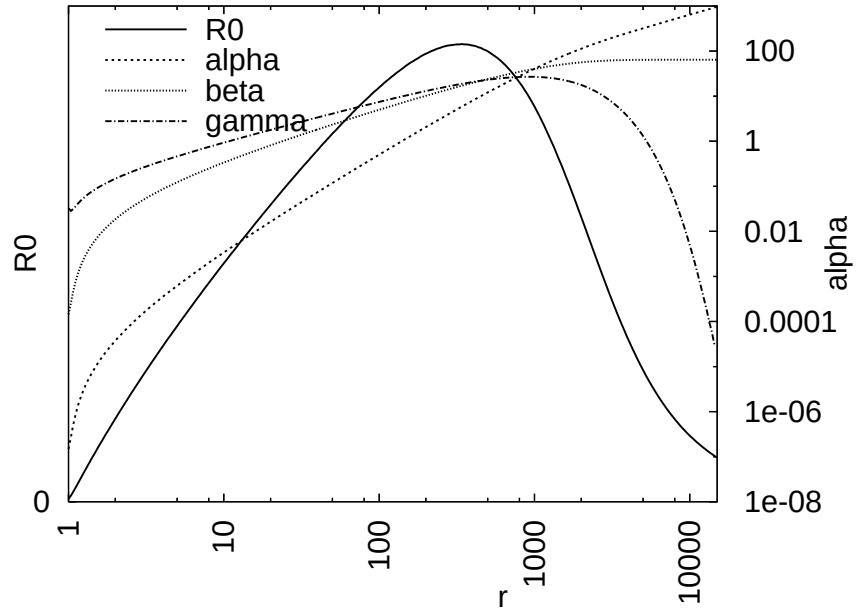

Figure 3: The values of the reproductive ratio R_0, from (18), and the average virulence, transmissibility and recovery rate, as defined in (22), for different values of r. The scale for R_0 is on the left, that for the rates on the right (in logarithmic scale). Parameter values are $a = 1$, $c = 1$, $P_0 = 10^{-3}$, $B_0 = 1$, $d = 10^{-4}$, $k_1 = 10^{-4}$, $k_2 = 10^{-6}$, $\bar{\beta}K = 13.33$

shown in (15), with time-since-infection ϑ; clearly the instantaneous death rate $\alpha(\vartheta)$ increases linearly with r, but the indirect effects through pathogen load $P(\vartheta)$ at different ϑ are not easy to grasp. To place this result in the general context of the previous sections, I find it useful to define 'average' virulence, recovery and transmission rates corresponding to this case; a similar procedure has been adopted by André and Gandon (2006). To this purpose, I compute three quantities that summarize the features of the infection in this detailed model, and equal them to the corresponding quantities in a simple $S \to I \to R$ model.

Precisely, one can define

$$R = \int_0^\infty P(\vartheta)\pi(\vartheta)\,d\vartheta \qquad T_i = \frac{\int_0^\infty \vartheta P(\vartheta)\pi(\vartheta)\,d\vartheta}{R} \qquad L_i = \int_0^\infty \pi(\vartheta)\,d\vartheta. \quad (19)$$

the quantity R is proportional (through quantities independent of r) to the reproduction ratio R_0: indeed $R_0 = \beta_0 K R$. T_i is then the average time after which an

infected individual infects another one; it can be considered as a generation time of the infection. Finally, L_i is the expected life span of an infected individual.

These quantities have been computed numerically for different values of r; practically, to avoid integration over an infinite interval, I found a time τ_i such that $P(t)\pi(t) < \varepsilon$ for all $t > \tau_i$ where ε has been chosen small enough that the probability of infecting an individual after time τ_i becomes negligible. Then, using (15) and (17), I approximated

$$R \approx \int_0^{\tau_i} P(\vartheta)\pi(\vartheta)\,d\vartheta \qquad T_i \approx \frac{\int_0^{\tau_i} \vartheta P(\vartheta)\pi(\vartheta)\,d\vartheta}{R} \qquad L_i \approx \int_0^{\tau_i} \pi(\vartheta)\,d\vartheta + \frac{\pi(\tau_i)}{d} \tag{20}$$

where the last term represents the expected life time after τ_i when infection related deaths no longer occur.

In an $S \to I \to R$ model, the same quantities can be explicitly computed as

$$R = \frac{\beta}{d+\alpha+\gamma} \qquad T_i = \frac{1}{d+\alpha+\gamma} \qquad L_i = \frac{1}{d+\alpha+\gamma} + \frac{\gamma}{d+\alpha+\gamma}\cdot\frac{1}{d}. \tag{21}$$

Equating the quantities in (19) and (21), one obtains

$$\bar{\alpha} = \frac{1-dL_i}{T_i} \qquad \bar{\gamma} = d\left(\frac{L_i}{T_i} - 1\right) \qquad \bar{\beta} = \frac{R}{T_i}. \tag{22}$$

In Fig. 3 the average virulence, transmission and recovery rates are shown as function of the pathogen replication rate r, showing that indeed virulence and transmission increases with r, while γ has a more complicated dependence (it was taken as a constant in the classical trade-off model).

If one plots the obtained $\bar{\beta}$ as a function of $\bar{\alpha}$, one obtains (see Fig. 4, noting the double-logarithmic scale) a concave trade-off curve very similar to those used in Fig. 1a), while $\bar{\gamma}$ first increases then declines with increasing $\bar{\alpha}$.

3.1 Effect of immune system on the optimal virulence

An interesting problem that can be addressed through (14)–(17) is how features of the hosts affect the evolution of virulence. To make the question specific, I studied how the previous results depend on the value of the parameter a, the

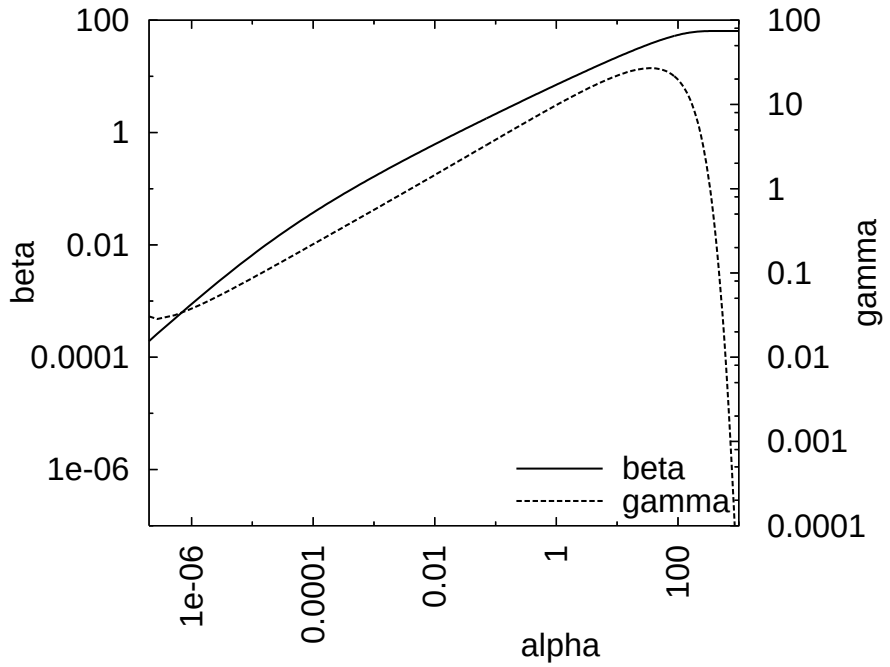

Figure 4: Average transmissibility and recovery rate vs. virulence, as defined in (22). Parameter values as in Fig. 3

replication speed of the immune system in response to the pathogen. a can be considered as an overall measure of the strength of the immune system: a host with a higher a will be more effective against pathogens, at the cost of a higher self-induced death rate, because of the form (15) used for the death rate.

In Fig. 5 I show how the optimal pathogen replication rate r depends on a: it can be seen, as expected, that the higher is a, the higher will be the selected value of r, $r_{\rm opt}(a)$, in a typical arms' race pattern. The dependence appears to follow almost exactly a power law (note the doubly logarithmic scale in Fig. 5). Some insight towards this fact might be obtained by studying the dependence of (18) on a and r, especially in the approximation of $d = 0$.

In the same figure, I plot also the probability of surviving the infection $\pi(\tau_i)$ as a function of a with $r = r_{\rm opt}(a)$; it can be seen that survival initially increases with a but eventually declines. The exact shape of this curve depends on parameter values, but in all examined cases survival eventually decreases with increasing a,

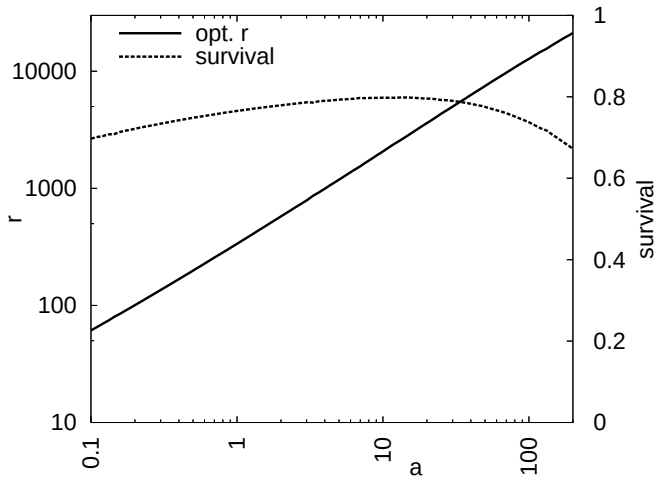

Figure 5: Optimal pathogen replication rate and probability of surviving the infection as a function of a, vs. virulence, a measure of immune system strength (see text for details). Parameter values, except a, as in Fig. 3

as long as $k_2 > 0$, i.e. there is cost for developing the immune response.

3.2 A simpler approach

A simpler approach to derive macroscopic trade-off from a within-host model has been adopted by Alizon and van Baalen (2005) (see also Alizon and van Baalen, 2008, Boldin and Diekmann, 2008). They modify the second equation of (14) to $B' = h + aP - \delta B$. Hence, solutions converge to a stable positive state $(\bar{P}, \bar{B}) = ((\delta r/c - h)/a, r/c)$. Neglecting deaths and infection transmission in the transient before convergence to the equilibrium, they take $\beta = \beta_0 \bar{P}$, $\alpha = k_1 r \bar{P} + k_2 \bar{B}$, obtaining

$$\beta = \beta_0 \frac{dr - hc}{ac} \qquad \alpha = k_1 r \frac{dr - hc}{ac} + k_2 \frac{r}{c}.$$

From the fact that β is a linear function of r, while α is a quadratic, it is easy to see that $R_0(r) = \frac{\beta(r)}{d+\alpha(r)}$ is a function with a unique intermediate maximum; writing instead β as a function of α, one can see that it is a concave function, exhibiting then a 'trade-off' as in Fig. 1a).

This derivation is however based on considering only the asymptotic state of

within-host dynamics, totally neglecting the phase of acute infection. Although this simplification may be justified for some pathogens, it appears unwarranted to me for many infections and parameter values. A reasoning like that leading to (18), although quite laborious and requiring numerical computations, seems necessary for giving a sound basis to the idea of a macroscopic 'trade-off' emerging from a mechanistic model of within-host dynamics.

4 Superinfection

It has been shown (Levin and Pimentel, 1981, Hochberg and Holt, 1990) that one mechanism leading to parasite coexistence is that of superinfection (see Box 4). Specifically, if the strain with lower R_0 is capable of superinfecting the other one more often than vice versa, then coexistence may occur. The models are actually similar to models used for describing coexistence of plant species in patches of suitable habitat (Tilman, 1994).

The evolutionary dynamics of virulence in this context has been presented, mainly through numerical computations, by Adler and Mosquera (1998), who consider coinfection, and superinfection as a limiting case of coinfection. I restrict myself here to the case of superinfection, following the analysis by Pugliese (2002) and especially Boldin and Diekmann (2008).

Coinfection models (see Box 4) are much more complex. In fact, the state space of these models can be very large, since one should allow for hosts being infected with any combination of strains. To cope with these problem while maintaining the possibility of coinfection, some authors (van Baalen and Sabelis, 1995, Mosquera and Adler, 1998) do not allow further infections of doubly-infected hosts.

As in the previous section, I will consider several parasite strains differing in the value of virulence α, i.e. the disease-induced death rate. The density of infected individuals carrying strain α at time t is denoted by $I(t, \alpha)$. The

Box 4. Superinfection and coinfection.

The models of competition between different pathogen strains discussed in the previous sections assume that infection with one strain provides hosts with complete protection against infections by other strains.

Levin and Pimentel (1981) introduced the possibility of superinfections: a host infected with strain 1 may become infected by strain 2 upon contact with a host already infected with strain 2. It is further assumed that, in this case, the host will lose the previous infection with strain 1; thus, no host will be infected with more than one strain at the same time.

Following the same considerations as in Box 1, the rate at which such superinfections of strain 1 by strain 2 occur will be proportional to the product $I_1 I_2$ if I_i is the density of hosts infected with strain i. It is convenient to express the proportionality constant as the product of the contact rate β_2 with a scaling factor ρ_{21}. Thus the rate at which such superinfections of strain 1 by strain 2 occur is assumed to be $\beta_2 \rho_{21} I_1 I_2$. Conversely, superinfections of strain 2 by strain 1 occur at rate $\beta_1 \rho_{12} I_1 I_2$.

Generally, it is assumed that the constants ρ_{ij} are smaller or equal to 1: already infected individuals are not easier to infect than susceptible ones. This constant ρ_{ij} is called the *superinfection factor*.

This model of superinfection consists of several mechanisms at the level of the individual host: necessarily, a host already infected with strain 1 that is attacked by strain 2 will go through a period where both strains are present in its body. Superinfection models assume that this period is so short to be negligible and eventually only one strain will persist.

Coinfection models assume (more realistically) that hosts may be infected with more than one strain at the same time. A host already infected with strain 1 that is attacked by strain 2 will become a host infected with strains 1 and 2; afterwards, it may persist in this state or (because of competition between strains) revert to being infected with only one strain.

assumption that the contact rate β depends on the virulence level according to a given function $\beta(\alpha)$ stays in place. Since also superinfections are now allowed, one needs a function relating virulence to superinfection rates. In the notation of Box 4, one needs to know how the superinfection factor ρ_{ij} depends on the virulence of the infecting strain α_i and of the strain α_j that is being infected. It will be assumed here that there exists a function $\rho(\alpha_2, \alpha_1)$ that gives the superinfection factor of a strain with virulence α_1 from a strain with virulence α_2. Mosquera and Adler (1998) and Boldin and Diekmann (2008) obtain an expression for this function on the basis of a within-host model of strain competition (see Subsection 4.1), but I start here with a general analysis under the assumption that the superinfection factor depends only on the difference in virulence, i.e. $\rho(\alpha_2, \alpha_1) = k(\alpha_2 - \alpha_1)$; this assumption has no particular biological motivation, but simplifies the computations and does not change qualitatively the results.

As before, it is assumed that $\beta(\alpha)$ is increasing with α (higher virulence allows for higher transmissibility). To clarify the role of super-infection, death rate d is assumed to be density-independent; moreover, the 'per capita' reproductive ratio $\varphi(\alpha)$, defined in (8), has a single maximum $\hat{\alpha}$ (see Fig. 1a,b), so that, without super-infection, evolution would drive α to $\hat{\alpha}$. Furthermore, it seems reasonable to assume that there exists a bounded interval $[\alpha_m, \alpha_M]$ with $0 \leq \alpha_m < \alpha_M$ such that $K\varphi(\alpha) \geq 1$ if and only if $\alpha \in [\alpha_m, \alpha_M]$; these would be the virulence levels that allow the pathogen to persist in the host population.

In conclusion, superinfections of a strain with virulence α_1 from a strain with virulence α_2 occur at rate

$$\beta(\alpha_2)\rho(\alpha_2, \alpha_1)I(t, \alpha_1)I(t, \alpha_2) - \beta(\alpha_2)k(\alpha_2 - \alpha_1)I(t, \alpha_1)I(t, \alpha_2).$$

Differently from the previous Sections, I will not consider here recovery from infection, since superinfection is relevant only for long–lasting infections. Hence, the only exit from the infected state will be through death at rate d (the natural death rate) plus α (the parasite-induced death rate).

One can then write the equations when strains $\alpha_1, \ldots, \alpha_n$ are present, using the abbreviation $I_j(t) = I(t, \alpha_j)$, as

$$\frac{d}{dt} I_j(t) = I_j(t) \left(\beta(\alpha_j) S(t) + \sum_{k \neq j} \delta(\alpha_j, \alpha_k) I_k(t) - d - \alpha_j \right) \qquad j = 1 \ldots n \quad (23)$$

where

$$\delta(\alpha_2, \alpha_1) = \beta(\alpha_2)\rho(\alpha_2, \alpha_1) - \beta(\alpha_1)\rho(\alpha_1, \alpha_2) = \beta(\alpha_2)k(\alpha_2 - \alpha_1) - \beta(\alpha_1)k(\alpha_1 - \alpha_2). \quad (24)$$

The model must be completed by an equation for the susceptibles:

$$\frac{dS}{dt} = b(N)N - dS - \sum_{j=1}^{n} \beta_j S I_j. \quad (25)$$

The dynamics of the resulting model (23)–(25) has been studied by several authors (Levin and Pimentel, 1981, Hochberg and Holt, 1990). Under certain conditions, it allows for the coexistence of several strains at equilibrium; in words, this occur when the strain(s) that has a lower value of R_0 is better able at super-infecting the one(s) with a higher R_0 than vice versa.

The question of the evolutionary dynamics is instead much more complex, and depends strongly on the function k describing super-infection. Generally, this will be an increasing function with values ranging between 0 and 1. Three assumptions concerning its behaviour in 0 lead to rather different conclusions:

(i) k is differentiable with $k(0) \geq 0$ and $k'(0) \geq 0$ (the differentiable case);

(ii) $k(x) = 0$ if $x \leq 0$; k is differentiable from the right in 0 and $k'_+(0) > 0$ (the non-differentiable case);

(iii) $k(x) = 0$ if $x \leq 0$; $\lim_{x \to 0^+} k(x) > 0$ (the discontinuous case).

Nowak and May (1994) used a discontinuous function: $k(x) = s > 0$ if $x > 0$; $k(x) = 0$ if $x \leq 0$. A consequence of the jump of k at 0 is that any resident strain can be invaded by any other strain with infinitesimally larger virulence. In

the framework of adaptive dynamics, this would result in a continuous virulence increase, up to the level α_M where the pathogen would not be able to persist. Assuming instead continuous mutations with arbitrary distribution, it is possible to obtain a continuum of persisting strains (Nowak and May, 1994), with no limit to similarity (see also Kinzig et al., 1999). These consequences may be regarded as being rather pathological.

A model of within-host competition (Mosquera and Adler, 1998, Boldin and Diekmann, 2008) discussed in Subsection 4.1 supports the non-differentiable case, and implies anyway $k(0) = 0$ (i.e., less virulent strains are unable to super-infect individuals infected with more virulent strains). Here I consider briefly both cases (i) and (ii), not necessarily with $k(0) = 0$, using the adaptive dynamics approach.

$s_{\alpha_1}(\alpha_2)$ denotes the rate of increase, when rare, of strain α_2 in a population at equilibrium with strain α_1. By looking at (23) with only two strains, α_1 and α_2, one sees that

$$s_{\alpha_1}(\alpha_2) = \beta(\alpha_2)\bar{S}_1 + \delta(\alpha_2, \alpha_1)\bar{I}_1 - d - \alpha_2 \tag{26}$$

where $\bar{S}_1$ and $\bar{I}_1$ represent the equilibrium levels of susceptibles and infectives when only strain α_1 is present in the population.

$\bar{S}_1$ can be easily computed as

$$\bar{S}_1 = \frac{d + \alpha_1}{\beta(\alpha_1)} = \frac{1}{\varphi(\alpha_1)} \tag{27}$$

while $\bar{I}_1$ can be found only as the solution of an equation that involves the function $b(\cdot)$.

As already discussed, in the adaptive dynamics approach, one needs to compute $D(\alpha) = \dfrac{\partial}{\partial \alpha_2} s_\alpha(\alpha_2)|_{\alpha_2=\alpha}$. One finds

$$D(\alpha) = \frac{\beta'(\alpha)}{\varphi(\alpha)} + d_1(\alpha)\bar{I}(\alpha) - 1 \tag{28}$$

where $d_1(\alpha) = \dfrac{\partial}{\partial \alpha_2}\delta(\alpha_2, \alpha)|_{\alpha_2=\alpha}$.

The derivative $D(\alpha)$ exists even if k is not differentiable in 0. Indeed

$$d_1(\alpha) = \begin{cases} \beta'(\alpha)k(0) + 2\beta(\alpha)k'(0) & \text{if k is differentiable} \\ \beta(\alpha)k'_+(0) & \text{if } k \text{ is non-differentiable.} \end{cases} \tag{29}$$

Note that in the case in which k is differentiable and satisfies $k(0) = 0$, necessarily $d_1(\alpha) \equiv 0$.

An evolutionarily singular state α^* satisfies $D(\alpha^*) = 0$, and thus, with some algebraic manipulations,

$$d_1(\alpha^*)\bar{I}(\alpha^*)\varphi(\alpha^*) + \varphi'(\alpha^*)(d + \alpha^*) = 0. \tag{30}$$

If k is differentiable and satisfies $k(0) = 0$, from the above considerations one sees that necessarily $a^* = \hat{\alpha}$, the maximum of $\varphi(\alpha)$.

Otherwise from (30) one sees that necessarily $\varphi'(\alpha^*) < 0$ so that α^* has to stay to the right of the maximum of the function $\varphi(\alpha)$ (see Fig. 1). In agreement with intuitive expectations, the evolutionarily singular type in the presence of superinfections will thus be more virulent than the optimal type in the absence of superinfections.

A question one may ask is whether an evolutionary singular state always exists and is unique. This is discussed at length in (Pugliese, 2002) where some sufficient conditions are obtained, but also a counterexample, although in a case with extreme parameter values. Here, I do not discuss the issue, and assume that there always exists a unique evolutionary singular state, as generally obtained numerically.

For the classification of singular points, one needs to compute the second derivatives of $s_{\alpha_1}(\alpha_2)$ in the point $\alpha_1 = \alpha_2 = \alpha^*$. In the differentiable case, it was shown by Pugliese (2002) that, under the assumption that the trade-off function $\beta(\alpha)$ is concave, the evolutionary singular state, when unique, is always evolutionary stable. With infinitesimally small mutations, evolution would then move α towards the unique attractor α^*, although transient dimorphisms could arise in the process. Note, however, that in general α^* will be stable against

invasions by nearby mutants, but could be invaded by mutants of significantly lower virulence, possibly leading to evolutionary fluctuating polymorphisms in virulence, if larger mutations occur.

If the trade-off function is not always concave, but is convex in some interval (as in Fig. 1c), the evolutionary dynamics can however be much more complex (Svennungsen and Kisdi, 2009).

In case the function k is non-differentiable at 0, the second derivatives of $s_{\alpha_1}(\alpha_2)$ at $\alpha_1 = \alpha_2 = \alpha^*$ do not exist. Through some computations (see also Boldin and Diekmann, 2008), one can see that strategy α^* can be invaded by close strategies α with $\alpha > \alpha^*$ if

$$D_{2+} = \frac{\beta''(\alpha^*)}{\varphi(\alpha^*)} + \left(2\beta'(\alpha^*)k'_+(0) + \beta(\alpha^*)k''_+(0)\right)\bar{I}(\alpha^*) > 0 \tag{31}$$

and by close strategies α with $\alpha < \alpha^*$ if

$$D_{2-} = \frac{\beta''(\alpha^*)}{\varphi(\alpha^*)} - \beta(\alpha^*)k''_+(0)\bar{I}(\alpha^*) > 0. \tag{32}$$

Here

$$k''_+(0) = \lim_{x\to 0^+} k''(x) = \lim_{x\to 0^+} \frac{k'(x) - k'_+(0)}{x}$$

assumed to exist. Clearly, it is then possible that α^* is invasible from above, and not from below, or vice versa, giving rise to scenarios somewhat different from those generally considered in adaptive dynamics.

In order to look at a simple case that allows for some analytic computations, I assume in what follows that β is constant, independently of virulence α. In absence of superinfections, then, the optimal strategy would be $\hat{\alpha} = 0$. In this case, the quantity D_{2+} and D_{2-} will have opposite signs (depending on the concavity of the function k), so that α^* will necessarily be invasible from one side.

The graph of $s_{\alpha^*}(\alpha_2)$ (as a function of α_2) will look locally as the joining of the two parabolas with opposite signs, like the curves "alpha_hat" in Fig. 6.

If α_1 is close to α^*, by continuity the graph of $s_{\alpha_1}(\alpha_2)$ will be similar, except that $D(\alpha_1) > 0$ for $\alpha_1 < \alpha^*$ while $D(\alpha_1) < 0$ for $\alpha_1 > \alpha^*$. This implies that the

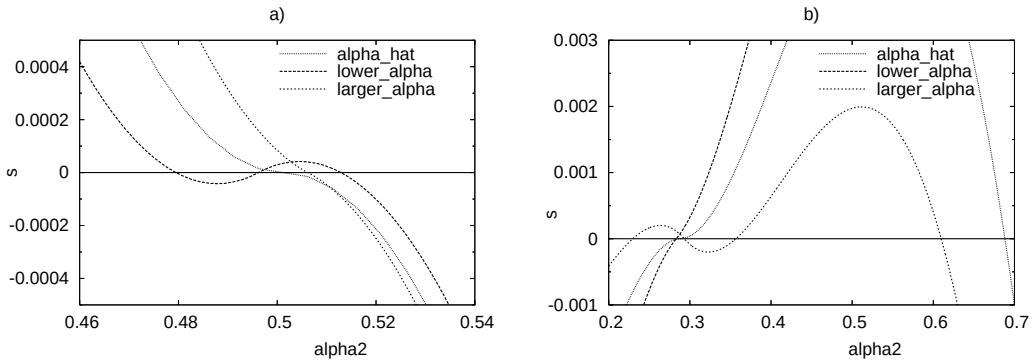

Figure 6: Invasion fitnesses $s_\alpha(\alpha_2)$. In the left panel, $k(x) = 1 - e^{-1.2x}$ for $x > 0$, 0 otherwise; $\alpha = \alpha^* = 0.50124$ (curve "alpha_hat"), $\alpha = 0.496198$ (curve "lower_alpha") and $\alpha = 0.50625$ (curve "higher_alpha"). In the right panel, $k(x) = \dfrac{x^2 + x}{x^2 + x + 1.4}$ for $x > 0$, 0 otherwise; $\alpha = \alpha^* = 0.288242$ (curve "alpha_hat"), $\alpha = 0.283239$ (curve "lower_alpha") and $\alpha = 0.293242$ (curve "higher_alpha"). In both panels $b(N) = b_0 \left(\frac{b_0}{d}\right)^{-\frac{N}{K}}$ with $b_0 = 1$, $d = 0.5$, $K = 1$.

graph of $s_{\alpha_1}(\alpha_2)$ will have a non-zero linear component at $\alpha_2 = \alpha_1$, so that it will look like the curves "lower_alpha" in Fig. 6 for $\alpha_1 < \alpha^*$, and like the curves "higher_alpha" in Fig. 6 for $\alpha_1 > \alpha^*$.

Looking at the graphs of Fig. 6, the pairwise invasibility plot must necessarily look like in Fig. 7, so that there must be a region of mutual invasibility for $\alpha_1 < \alpha^* < \alpha_2$.

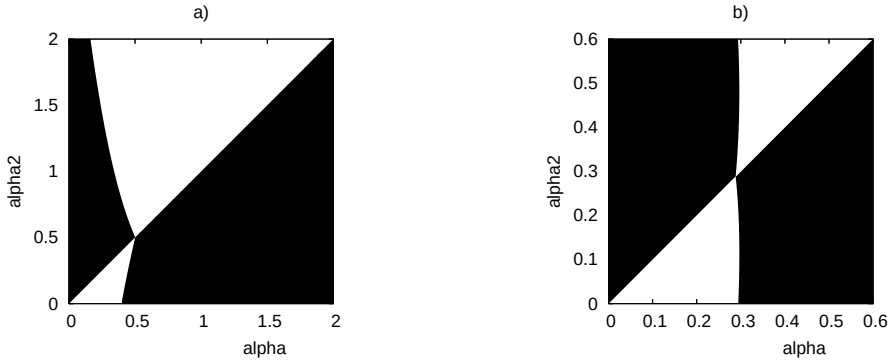

Figure 7: Pairwise invasibility plots in the super-infection model. Parameters as in Fig. 6.

Functions k with opposite concavity in 0 (left or right panel of Figs. 6 and 7) yield essentially the same invasibility diagrams, if one exchanges α larger than α^* with those smaller than α^*.

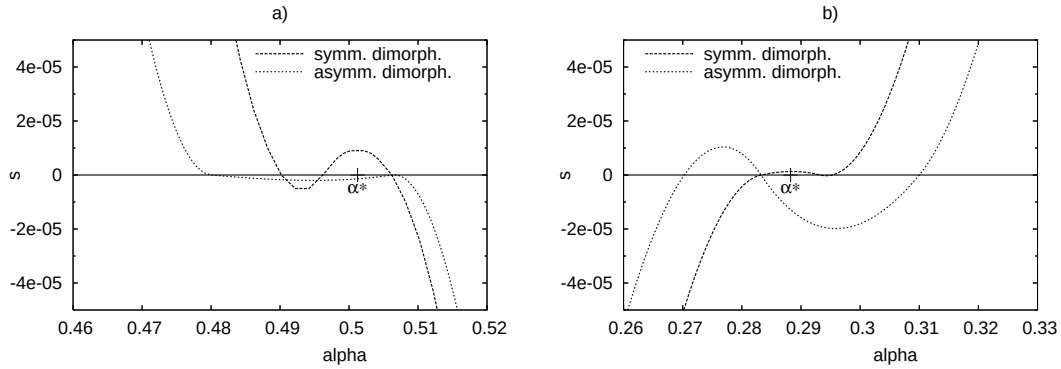

Figure 8: Invasion fitnesses $s_{(\alpha_1,\alpha_2)}(\alpha)$. In the left panel, $\alpha_1 = 0.496198$, $\alpha_2 = 0.50625$ (curve "symm. dimorph."); $\alpha_1 = 0.48$, $\alpha_2 = 0.50625$ (curve "asymm. dimorph."). In the right panel, $\alpha_1 = 0.283239$, $\alpha_2 = 0.293242$ (curve "symm. dimorph."); $\alpha_1 = 0.283239$, $\alpha_2 = 0.31$ (curve "asymm. dimorph.") Parameters as in Fig. 6.

We can then study the invasion fitnesses $s_{(\alpha_1,\alpha_2)}(\alpha)$ when the resident population is at a dimorphic equilibrium (α_1, α_2) with $\alpha_1 < \alpha^* < \alpha_2$. Some examples are shown in Fig. 8: there are always three zeroes near α^*, as can be deduced from the fact that $s_{(\alpha_1,\alpha_2)}(\alpha)$ has to be close to $s_{\alpha^*}(\alpha)$, and so has to change sign moving from α much lower than α^* to α much larger than α^*; moreover, by definition, $s_{(\alpha_1,\alpha_2)}(\alpha_1) = s_{(\alpha_1,\alpha_2)}(\alpha_2) = 0$, so that it has at least two zeroes; generically, hence, there will be three zeroes close to α^* (where $s_{\alpha^*}(\alpha)$ is close to 0).

From Fig. 8, it can also be seen that the pattern of the sign of $s_{(\alpha_1,\alpha_2)}(\alpha)$ appears to differ according to how α_1 and α_2 are located. When they are symmetric with respect to α^*, $s_{(\alpha_1,\alpha_2)}(\alpha) > 0$ for $\alpha_1 < \alpha < \alpha_2$: this appears to imply that the dimorphism shrinks towards α^*; however, the dimorphism can be invaded also by strategies to one side (of lower virulence in panel a), of higher virulence in panel b), as long as they are a little farther away. An invasion of this type may lead to

an asymmetric dimorphism (i.e. one in which a strain is much closer to α^* than the other), which then could be invaded only from strains more extreme than the coexisting ones, and not by intermediate ones. Asymmetric dimorphisms appear then to be branching dimorphisms.

It is not clear to me whether precise assumptions about the stochastic mutation process are needed to reach a conclusion about the expected outcome, or whether the use of more advanced tools in adaptive dynamics can provide a clearcut prediction (the recent paper by Boldin et al. (2009) shows how critical function analysis may help in obtaining necessary conditions for evolutionary branching in a superinfection model with differentiable trade-off). It appears plausible that evolution would go through a number of converging dimorphisms before a sufficiently asymmetric dimorphism arises, and this gives rise to a branching of the population. It can also be noted that, in both examples, one of the two regions where $s_{(\alpha_1,\alpha_2)}(\alpha) > 0$ (for $\alpha > \alpha_2$ in panel a), for $\alpha < \alpha_1$ in panel b) is extremely small (almost invisible in panel a)). One can extrapolate that one of the branches in the dimorphism will not have much room for evolving (the upper one in panel a), the lower one in panel b), while the other branch will, through subsequent invasions, change significantly in virulence. Indeed, such a behaviour has been found through simulations by Boldin and Diekmann (2008) in a similar example.

In conclusion, the properties of evolutionary dynamics with super-infection depend essentially on the properties of the function k near 0, and also on the assumptions about mutations, i.e. whether only infinitesimally small mutations are allowed (according to the paradigm of adaptive dynamics, used in this chapter) or whether larger mutations occur (this would often allow invasion by strains much less virulent than the resident ones).

Using the adaptive dynamics methods, it has been seen here that, in the discontinuous case, virulence would increase to infinity, unless physiological limits or pathogen extinction stop the increase; on the other hand, in the differentiable

case, one always obtains a convergent stable strategy, which, if $k(0) = 0$ is the same as that of the model without superinfection, otherwise it will correspond to higher virulence (Pugliese, 2002); finally, in the non-differentiable case, it is possible that branching points arise with a consequent divergence of several strains (Boldin and Diekmann, 2008).

4.1 Building superinfection function from within-host competition

Since it seems difficult to design experiments that allow to discriminate empirically such fine properties of the super-infection function, it is worth relating the function k to more mechanistic models of strain competition. One such example has been provided by Boldin and Diekmann (2008), building on the work by Mosquera and Adler (1998).

They have based their work on a model of the interaction between T cells and HIV virus particles, studied for instance by Perelson et al. (1993). Considering only one viral strain, the variables of the model are T, the density of healthy T-cells, Y, the density of infected T-cells, and P, the density of free virus. The equations can be written as

$$\begin{cases} T' &= h - \delta T - kPT \\ Y' &= kPT - (\delta + \mu(r))Y \\ P' &= -kPT + rY - mP \end{cases} \tag{33}$$

In system (33) h represents the rate at which healthy T-cells are generated, and δ the rate at which they die. Hence, in absence of virus $\hat{T} = h/\delta$ is the equilibrium density of healthy T-cells. m is the rate at which free viral particles die, and k is the rate at which they attack healthy T-cells (it is implicitly assumed that infected T-cells are protected from further infection).

Finally, r and $\mu(r)$ may have different interpretations, according to the life cycle of the virus: assuming a continuous release of virus from an infected cell, viral cells are released one at a time at rate r from infected T-cells, which suffer an increased (relative to healthy cells) death rate equal to $\mu(r)$. Instead, in case

of a lytic cycle, N viral cells are released from each infected cell at its death, occurring at rate η, giving $r = N\eta$; assuming that infected cells may also die, before the copies of the viral genetic material have been completed, at rate $\delta + \zeta$, one has $\mu(r) = \eta + \zeta$. The notation $\mu(r)$ implies that death rate of infected cells will be higher the higher is the rate, r, at which new viral particles are released (whatever be the mechanism).

Let

$$R_0^w = B(r)\frac{k\hat{T}}{k\hat{T}+m} \quad \text{with} \quad B(r) = \frac{r}{\mu(r)+\delta}. \tag{34}$$

Beyond the infection–free equilibrium $(\hat{T}, 0, 0)$, system (33) has, if $R_0^w > 1$, a positive equilibrium (T^*, Y^*, P^*) with

$$T^* = \frac{m}{k(B(r)-1)} \quad Y^* = \frac{\delta(m+k\hat{T})}{k(\mu(r)+\delta)(B(r)-1)}(R_0^w - 1)$$
$$P^* = \frac{\delta(m+k\hat{T})}{km}(R_0^w - 1). \tag{35}$$

$B(r)$ represents the mean number of free viral particles produced by one virus that has infected a cell, while R_0^w (which represents the *within host* reproduction number) is equal $B(r)$ times the probability (for a free virion) to infect a cell before dying. It is possible to prove (Perelson et al., 1993) that the positive equilibrium is globally attractive if $R_0^w > 1$. All the strains considered in what follows will be assumed to satisfy $R_0^w > 1$, which implies $B(r) > 1$.

To complete the model, one needs to relate the 'macroscopic' parameters (between-host transmission β and disease-induced death rate α) to within-host process. Boldin and Diekmann (2008) have chosen the relation, similarly to the method discussed in Subsection 3.2, on the basis of the asymptotic values as $\beta = \beta(T^*, Y^*, P^*)$, $\alpha = \alpha(T^*, Y^*, P^*)$; in numerical computations they use $\beta = P^* + Y^*$, $\alpha = kP^*/h$ as an example, but other choices may be justified.

Ignoring superinfections, one could then find the value of r (if that is assumed to be the parameter subject to evolution) that maximizes R_0. However, the aim of this section is to relate the superinfection factor ρ (see Box 4) to the values of

α (or r) through an extension of model (33).

Consider in fact the introduction of a few virions of a different viral type 2 into a host that is at equilibrium with viral strain 1; the dynamics can be described similarly to (33), considering now two strains of free virus and infected T-cells and using a stochastic description, because of the small number of particles of type 2 in the initial period after introduction. In this first phase, variations in healthy and 1-infected T-cells, as well as in viral cells of type 1, can be ignored, and one can describe the dynamics of type 2 cells as a Markov branching process with rates described in the derivation of (33).

The aim of the following analysis is to compute the probability of extinction of the descendants of an initial inoculum before the numbers become large enough to allow the use of a deterministic model. Hence, we can (see e.g. Haccou et al., 2007) use the embedded Galton-Watson process, ignoring the time variable. At this point, virus with a lytic cycle, and those with a continuous release of virus have to be distinguished.

Consider a free viral particle of type 2 in the continuous-release case: with probability $m/(m+kT_1^*)$, it will die before infecting a T-cell; here T_1^* represents the number of healthy T-cells at equilibrium with strain 1; hence, using (35), $\dfrac{m}{m+kT_1^*} = \dfrac{B(r_1)-1}{B(r_1)}$. If it infects a T-cell (probability equal to $1-\dfrac{B(r_1)-1}{B(r_1)} = \dfrac{1}{B(r_1)}$), this, with probability

$$\frac{\delta+\mu(r_2)}{\delta+\mu(r_2)+r_2} = \frac{1}{B(r_2)+1},$$

will die before producing any virus; with the complementary probability $\dfrac{B(r_2)}{B(r_2)+1}$ it will produce at least one free virus. At the moment of releasing a free virus, the probability remains the same, since the process is Markovian, so that the number of descendants follows a geometric distribution. Precisely, the number of descendants will be

$$n \quad \text{with probability} \quad \pi_n = \frac{1}{B(r_1)} \left(\frac{B(r_2)}{B(r_2)+1} \right)^n \frac{1}{B(r_2)+1},\ n \geq 1$$

$$0 \quad \text{with probability} \quad \pi_0 = \frac{B(r_1)-1}{B(r_1)} + \frac{1}{B(r_1)} \frac{1}{B(r_2)+1}.$$

The probability of extinction of the descendants of a single free viral particle is (Haccou et al., 2007) the smallest solution in $(0, 1]$ of the equation $G(z) = z$ where $G(z) = \sum_{j=0}^{\infty} \pi_j z^j$ is the probability generating function.

One can compute

$$G(z) = \frac{B(r_1)-1}{B(r_1)} + \frac{1}{B(r_1)} \frac{1}{1+B(r_2)(1-z)}.$$

Hence, with some algebra, one obtains that the probability of extinction is

$$p_e = \begin{cases} 1 - \frac{B(r_2)-B(r_1)}{B(r_1)B(r_2)} & \text{if } B(r_2) > B(r_1) \\ 1 & \text{if } B(r_2) \leq B(r_1). \end{cases} \tag{36}$$

If the initial inoculum consists of n cells, by independence of the branching process, it is clear that the probability of extinction will be p_e^n.

If a strain 2 such that $B(r_2) > B(r_1)$ avoids early extinction and reaches a sizeable number, the deterministic model extending (33) shows that strain 1 will decrease to extinction. It is then possible to conclude that the super-infection factor ρ_{21} will be equal to 1 minus the extinction probability, i.e.

$$\rho(r_2, r_1) = \begin{cases} 1 - \left(1 - \frac{B(r_2)-B(r_1)}{B(r_1)B(r_2)}\right)^n & \text{if } B(r_2) > B(r_1) \\ 0 & \text{if } B(r_2) \leq B(r_1) \end{cases} \tag{37}$$

where n represents the typical number of cells in an inoculum into a new host. More accurately, one should take a probability distribution for n and take the expectation of (37) relatively to that distribution, but this seems unnecessary for a qualitative picture.

To put (37) as a function of α_1 and α_2, one needs only to invert the relation connecting r to α. In any case, it is clear that (37) belong to the non-differentiable case discussed in the previous Section, although not of the form $k(\alpha_2 - \alpha_1)$.

Consider now a virus with a lytic cycle producing N_i (depending on viral strain) cells at the death of an infected T cell. Now $r_i = \eta_i N_i$ where η_i is the rate

at which infected T cells rupture releasing viral cells. Again, a free viral cell of type 2 will die before infecting a T-cell with probability $\frac{B(r_1)-1}{B(r_1)}$. If it infects a T-cell, this, with probability

$$\eta_2/(\delta+\zeta_2+\eta_2)=\frac{B(r_2)}{N_2}$$

will complete the cycle and release N_2 viral cells; with the complementary probability $1-\frac{B(r_2)}{N_2}$, the infected cell will die before the viral copies are ready, and the viral cell will leave no descendants. In this case, hence

$$\pi_{N_2}=\frac{1}{B(r_1)}\frac{B(r_2)}{N_2},\ \pi_0=\frac{B(r_1)-1}{B(r_1)}+\frac{1}{B(r_1)}\left(1-\frac{B(r_2)}{N_2}\right),\ \pi_n=0 \text{ otherwise.}$$

Then the generating function is

$$G(z)=\frac{B(r_1)-1}{B(r_1)}+\frac{1}{B(r_1)}\left(1-\frac{B(r_2)}{N_2}(1-z^{N_2})\right). \tag{38}$$

The mean of the distribution $G'(1)=B(r_2)/B(r_1)$ is, as expected, the same as in the continuous release case. However, the probability of extinction, for $B(r_2)>B(r_1)$, i.e. the solution in $(0,1)$ of $G(z)=z$ will be different from (36), though it cannot be computed explicitly, let it be denoted as $\bar{z}(r_2,r_1,N_2)$.

In this case, hence, the superinfection factor will be

$$\rho(r_2,r_1)=\begin{cases}1-(\bar{z}(r_2,r_1,N_2))^n & \text{if } B(r_2)>B(r_1)\\ 0 & \text{if } B(r_2)\leq B(r_1).\end{cases} \tag{39}$$

Once the value of N_i are given and their relation with r_i (remember in the lytic case $r_i=\eta_i N_i$), and the laws connecting r to α, one can compute the function $\rho(\alpha_2,\alpha_1)$ and study the evolutionary dynamics.

Here, I wish only to remark that within-host models can, through several assumptions that are indeed debatable, yield the superinfection function $\rho(\alpha_2,\alpha_1)$; this will depend on the virus life cycle, but will always be of the non-differentiable type.

5 Conclusion

In this chapter I have considered the evolution of virulence assuming the existence of a fundamental trade-off between transmissibility and virulence, generally with transmissibility an increasing and concave function of virulence. Using the adaptive dynamics framework (Metz et al., 1996), the evolutionary dynamics in the simple model of Anderson and May (1982) is well understood: convergence to the strategy α that maximizes $\varphi(\alpha)$ (the 'per capita' reproductive ratio). Andreasen and Pugliese (1995) considered a variant of that model in which density-dependence acted on mortality instead of on fertility. Then, transient dimorphisms are possible; under the assumptions of adaptive dynamics, however, these contract towards the unique continuously stable strategy.

A complete analysis of the model with superinfection is still missing. Preliminary results suggest that, when the function k relating virulence to superinfection rates is differentiable, a unique continuously stable strategy for virulence α^* exists; in this model too, dimorphisms are only transients. However, the evolutionary dynamics may depend on the size of mutations, since α^* is only locally uninvadable. When the function k is not differentiable, evolutionary dynamics can be more complex, since branching points may exist.

A relevant emphasis has been given in this Chapter to how trade-off functions can be built from models describing within-host pathogen dynamics ((see also the recent review by Mideo et al., 2008). In principle, these models can help overcoming one of the main problem of the model for superinfection discussed here, i.e. that infected individuals are assumed to be infected with a single pathogen strain. The approximation of quick replacement of the original strain with the superinfecting strain seems questionable when the virulence of competing strains is very similar, as they must be when analysing evolutionary stability. At the moment, however, models allowing for host coinfection and considering also transient dynamics at the within-host level, seem to be beyond the power

of analytical methods, except for very simplified cases (Coombs et al., 2007, Alizon and van Baalen, 2008); perhaps more general cases could be addressed only through simulation studies.

As stated at the beginning, the evolution of virulence is very actively studied, both experimentally (Ebert and Mangin, 1997, Ebert, 1998, Ferguson et al., 2003, Koella and Agnew, 1999) and theoretically. I have completely disregarded, for lack of space and of the necessary competence, several fundamental problems, some of which I wish to quote here, together with some references to see for further information.

All models considered here follow the adaptive dynamics paradigm, i.e. that mutations are rare enough that population dynamics has reached an equilibrium, before the following advantageous mutation arises. This assumption appears questionable for many pathogens, especially RNA viruses. A different approach, described by Day and Proulx (2004), is to assume that mutations are frequent enough to describe, at each time, a population as a continuum in genetic space, whose dynamics is described through mutation-selection equations. In the simplest case (corresponding to the model studied in Section 2, they obtained evolution to a stationary distribution (in virulence space) centred at a point very close (but not identical) to the continuously stable strategy found through adaptive dynamics. In this case it would be possible that the evolutionary equilibrium is approached in an oscillating way. It would be interesting to extend this approach to more complex cases.

An aspect that has received a great attention in the ecological literature (Dieckmann et al., 2000) but has been ignored here is the spatial structure of the host population. Several papers (Haraguchi and Sasaki, 2000, Boots and Sasaki, 1999, Webb et al., 2007) have studied the evolution of virulence in a spatially structured population; the qualitative predictions of these models, i.e. that virulence should be lower when most interactions are local, have also received experimental support (Boots and Mealor, 2007).

All aspects of pathogen evolution concerning avoidance of recognition by the immune system, or more generally adaptation to hosts' defenses, have not been considered here. While generally these topics have been studied with a variety of approaches (Andreasen et al., 1997, Recker et al., 2004, Restif and Grenfell, 2006), a paper using adaptive dynamics in this area too has recently been published (Adams and Sasaki, 2007).

Finally, as discussed in the beginning, virulence is actually a property of the interaction between host and pathogen, rather than of pathogen itself. There exists by now an abundant literature on the evolutionary dynamics of host resistance and tolerance (Boots and Bowers, 1999, Roy and Kirchner, 2000, Restif and Koella, 2004, Miller et al., 2005). Clearly, a coevolutionary treatment would be needed for a proper understanding of the topics; while there exist several papers (Andreasen and Christiansen, 1993, Sasaki, 2000, Bennett and Bowers, 2008) dealing with polymorphic (sometimes with a complete genetical model) hosts and pathogens, mainly with the aim of explain evolutionary cycles, to my knowledge, only the papers by Gilchrist and Sasaki (2002) and by Dieckmann (2002) discuss virulence evolution within this setting.

I hope the present review may help in setting a basis on which much more complex problems can be studied.

Acknowledgments

I wish to thank Samuel Alizon, Barbara Boldin, Odo Diekmann, and the two editors of the book, Ulf Dieckmann and Hans Metz, for a careful reading of previous versions of this Chapter, and for suggesting several improvements and references. All inaccuracies are of course mine.

References

B. Adams and A. Sasaki. Cross-immunity, invasion and coexistence of pathogen strains in epidemiological models with one-dimensional antigenic space. *Math. Biosci.*, 210:680–699, 2007.

S. Alizon. Transmission-recovery trade-offs to study parasite evolution. *Amer. Nat.*, 172:E113–E121, 2008.

S. Alizon and M. van Baalen. Multiple infections, immune dynamics and virulence evolution. *Amer. Nat.*, 172:E150–E168, 2008.

S. Alizon, A. Hurford, N. Mideo, and M. van Baalen. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.*, 22:245–259, 2009.

S. Alizon and M. van Baalen. Emergence of a convex trade-off between transmission and virulence. *Amer. Nat.*, 165:E155–E167, 2005.

R.M. Anderson and R.M. May. Coevolution of hosts and parasites. *Parasitology*, 85:411–426, 1982.

J.-B. André and S. Gandon. Vaccination, within-host dynamics, and virulence evolution. *Evolution*, 60:13–23, 2006.

J.-B. André, J.-B. Ferdy, and B. Godelle. Within-host parasite dynamics, emerging trade-off, and evolution of virulence with immune system. *Evolution*, 57 (7):1489–1497, 2003.

V. Andreasen and F.B. Christiansen. Disease-induced natural selection in a diploid host. *Theor. Pop. Biol.*, 44:261–298, 1993.

V. Andreasen and A. Pugliese. Pathogen coexistence induced by density dependent host mortality. *J. theor. Biol.*, 177:159–165, 1995.

V. Andreasen, J. Lin, and S.A. Levin. The dynamics of cocirculating influenza strains conferring partial cross-immunity. *J. Math. Biol.*, 35:825–842, 1997.

N. T. J. Bailey. *The mathematical theory of infectious diseases and its applications*. Griffin, London, 1975.

R. Bennett and R. G. Bowers. A baseline model for the co-evolution of hosts and pathogens. *J. Math. Biol.*, 57:791–809, 2008.

B. Boldin and O. Diekmann. Superinfections can induce evolutionarily stable coexistence of pathogens. *J. Math. Biol.*, 56(5):635–672, May 2008. ISSN 0303-6812.

B. Boldin, S. A. H. Geritz, and É. Kisdi. Superinfections and adaptive dynamics of pathogen virulence revisited: A critical function analysis. *Evol. ecol. Res.*, 11:153–175, 2009.

M. Boots and R. G. Bowers. Baseline criteria and the evolution of hosts and parasites: D_0, R_0 and competition for resources between strains. *J. Theor. Biol.*, 223(3):361–365, Aug 2003. ISSN 0022-5193.

M. Boots and R.G. Bowers. Three mechanisms of host resistance to microparasites - avoidance, recovery and tolerance - show different evolutionary dynamics. *J. theor. Biol.*, 201:13–23, 1999.

M. Boots and M. Mealor. Local interactions select for lower pathogen infectivity. *Science*, 315(5816):1284–1286, Mar 2007. ISSN 0036-8075.

M. Boots and A. Sasaki. 'small worlds' and the evolution of virulence: infection occurs locally and at a distance. *Proc.Roy.Soc. B*, 266:1933, 1999.

H.J. Bremermann and H.R. Thieme. A competitive exclusion principle for pathogen virulence. *J. Math. Biol.*, 27:179–190, 1989.

J.J. Bull. Virulence. *Evolution*, 48:1423–1437, 1994.

P CastagnoneSereno, M Bongiovanni, and E Wajnberg. Selection and parasite evolution: a reproductive fitness cost associated with virulence in the parthenogenetic nematode meloidogyne incognita. *Evolutionary Ecology*, 21:259–270, 2007.

D. Coombs, C.L. Ball, and M.A. Gilchrist. Evaluating the importance of within- and between- host selection pressures in the evolution of chronic pathogens. *preprint*, 2007.

T. Day. On the evolution of virulence and the relationship between various measures of mortality. *Proc. Roy. Soc. B*, 269:1317–1323, 2002.

T Day and SR Proulx. A general theory for the evolutionary dynamics of virulence. *Amer. Nat.*, 163:E40–E63, 2004.

T. Day, A. Graham, and A.F. Read. Evolution of parasite virulence when host responses cause disease. *Proceedings of the Royal Society of London, B*, 274: 2685–2692, 2007.

J. C. de Roode, R. Pansini, S. J. Cheesman, M. E. H. Helinski, S. Huijben, A. R. Wargo, A. S. Bell, B. H. K. Chan, D. Walliker, and A. F. Read. Virulence and competitive ability in genetically diverse malaria infections. *Proc.Nat.Acad.Sci.USA*, 102(21):7624–7628, May 2005. ISSN 0027-8424.

J. C. de Roode, A. J. Yates, and S. Altizer. Virulence-transmission trade-offs and population divergence in virulence in a naturally occuring butterfly parasite. *Proc.Nat.Acad.Sci.USA*, 105(21):7489–7494, May 2008. ISSN 0027-8424.

U. Dieckmann. Adaptive dynamics of host-parasite interactions. In Dieckmann et al. (2002), pages 39–59.

U. Dieckmann, J. A. J. Metz, M. Sabelis, and K. Sigmund, editors. *The adaptive dynamics of infectious disease: in pursuit of virulence management*. Cambridge Univ. Press, 2002.

U. Dieckmann, J. A. J. Metz, M. Sabelis, and K. Sigmund, editors. *The geometry of ecological interactions : simplifying spatial complexity.* Cambridge Univ. Press, 2000.

O. Diekmann and J.A.P. Heesterbeek. *Mathematical Epidemiology of Infectious Diseases.* Wiley, Chichester, New York, 2000.

G. Dwyer, S.A. Levin, and L. Buttel. A simulation model of the population dynamics and evolution of myxomatosis. *Ecol. Mon.*, 60:423–447, 1990.

D. Ebert. Evolution: Experimental evolution of parasites. *Science.*, 282:1432, 1998.

D. Ebert and W. D. Hamilton. Sex against virulence : the coevolution of parasitic diseases. *Trends Ecol. Evol.*, 11:79–82, 1996.

D. Ebert and K. L. Mangin. The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution*, 51:1828–, 1997.

D. Ebert and J. J. Bull. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.*, 11:15–20, 2003.

I. Eshel. On the changing concept of evolutionary population stability as a reflection of a changing point of view in the quantitative theory of evolution. *J. Math. Biol.*, 34:485–510, 1996.

P. W. Ewald. *The evolution of Infectious Disease.* Oxford University Press, 1994.

P.W. Ewald. Host-parasite relations, vectors, and the evolution of disease severity. *Ann. Rev. Ecol. Syst.*, 14:465–485, 1983.

F. Fenner. Biological control, as exemplified by smallpox eradication and myxomatosis. *Proc. R. Soc. London B*, 218:259–285, 1983.

F. Fenner and F. N. Ratcliffe. *Myxomatosis*. Cambridge Univ. Press, 1965.

H. M. Ferguson, M. J. MacKinnon, B. H. Chan, and A. F. Read. Mosquito mortality and the evolution of malaria virulence. *Evolution*, 57(12):2792–2804, Dec 2003. ISSN 0014-3820.

C. Fraser, T. D. Hollingsworth, R. Chapman, F. de Wolf, and W. P. Hanage. Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. *Proc. Natl. Acad. Sci. USA*, 104:17441–17446, 2007.

S. Gandon and T. Day. The evolutionary epidemiology of vaccination. *J. Roy. Soc. Interface*, 4(16):803–817, Oct 2007. ISSN 1742-5689.

S. A.H̃. Geritz, J. A.J̃. Metz, E. Kisdi, and G. Meszena. Dynamics of adaptation and evolutionary branching. *Phys. rev. Letter*, 78:2024–2027, 1997.

M.A. Gilchrist and A. Sasaki. Modeling host-parasite coevolution. *J. theor. Biol.*, 218:289–308, 2002.

P. Haccou, P. Jagers, and V. A. Vatutin. *Branching processes : variation, growth, and extinction of populations*. Cambridge Univ. Press, 2007.

Y. Haraguchi and A. Sasaki. The evolution of parasite virulence and transmission rate in a spatially structured population. *J. theor. Biol.*, 203:85, 2000.

H. W. Hethcote. Three basic epidemiological models. In T.G. Hallam S.A. Levin and L.J. Gross, editors, *Applied Mathematical Ecology*, pages 119–144. Springer Verlag, 1989.

H.W. Hethcote. Qualitative analysis of communicable disease models. *Math. Biosci.*, 28:335–356, 1976.

M. E. Hochberg and R. D. Holt. The coexistence of competing parasites I. the role of cross-species infection. *Amer. Nat.*, 136:517–541, 1990.

M. De Jong, O. Diekmann, and H. Heesterbeek. How does transmission of infection depend on population size? In D. Mollison, editor, *Epidemic models: their structure and relation to data*, pages 84–94. Cambridge University Press, 1995.

W O Kermack and A G McKendrick. A contribution to the mathematical theory of epidemics. *Proceedings of the Royal Society of London A*, 115:700–721, 1927.

A. P. Kinzig, S. A. Levin, and S. Pacala. Limiting similarity, species packing, and system stability for hierarchical competition-colonization models. *Amer. Nat.*, 153:371, 1999.

J.C. Koella and P. Agnew. A correlated response of a parasite's virulence and life cycle to selection on its host's life history. *Journal of Evolutionary biology.*, 12:70, 1999.

S. A. Levin and D. Pimentel. Selection for intermediate rates of increase in parasite-host systems. *Amer. Nat.*, 117:308–315, 1981.

M. Lipsitch, E.A. Herre, and M.A. Nowak. Host population structure and the evolution of virulence. *Evolution*, 49:743–748, 1995.

M. J. Mackinnon and A. F. Read. Genetic relationships between parasite virulence and transmission in the rodent malaria Plasmodium chabaudi. *Evolution*, 53 (3):689–703, Jun 1999. ISSN 0014-3820.

J.AJ. Metz, S.A.H. Geritz, G. Meszena, F.J.A. Jacobs, and J.S. van Heerwaarden. Adaptive dynamics, a geometrical study of the consequences of nearly faithful reproduction. In *Stochastic and spatial structures of dynamical systems, S.J. van Strien and S.M. Verduyn Lunel (eds.)*, pages 183–231. North Holland, Amsterdam, 1996.

N. Mideo, S. Alizon, and T. Day. Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases. *TREE*, 23:511–517, 2008.

M.R. Miller, A. White, and M. Boots. The evolution of host resistance: tolerance and control as distinct strategies. *J. theor. Biol.*, 236:198–207, 2005.

J. Mosquera and F.R. Adler. Evolution of virulence: a unified framework for coinfection and superinfection. *J. Theor. Biol.*., 195:293, 1998.

M. A. Nowak and R.M. May. *Virus dynamics: Mathematical principles of immunology and virology*. Oxford Univ. Press, 2000.

M.A. Nowak and R.M. May. Superinfection and the evolution of parasite virulence. *Proc. R. Soc. London B*, 255:81–89, 1994.

K. J. O'Keefe. The evolution of virulence in pathogens with frequency-dependent transmission. *J. Theor. Biol.*, 233:55–64, 2005.

A. S. Perelson, D. E. Kirschner, and R. De Boer. Dynamics of HIV infection of CD4+ T cells. *Math. Biosci.*, 114:81–125, 1993.

R. Poulin and C. Combes. The concept of virulence: interpretations and implications. *Parasitology Today*, 15:474–475, 1999.

A. Pugliese. On the evolutionary coexistence of parasites. *Math. Biosci.*, 177-178: 355–375, 2002.

A.R. Read. The evolution of virulence. *Trends Microbiology*, 2:73–76, 1994.

M. Recker, S. Nee, P. C. Bull, S. Kinyanjui, K. Marsh, C. Newbold, and S. Gupta. Transient cross-reactive immune responses can orchestrate antigenic variation in malaria. *Nature*, 429:555–558, 2004.

O. Restif and J. C. Koella. Concurrent evolution of resistance and tolerance to pathogens. *Amer. Nat.*, 164(4):E90–E102, Oct 2004. ISSN 0003-0147.

O. Restif and B. T. Grenfell. Integrating life history and cross-immunity into the evolutionary dynamics of pathogens. *Proc. R. Soc. B*, 273:409–416, 2006.

B.A. Roy and J.W. Kirchner. Evolutionary dynamics of pathogen resistance and tolerance. *Evolution.*, 54:51, 2000.

M. W. Sabelis and J. A. J. Metz. Taking stock: relating theory to experiment. In Dieckmann et al. (2002), pages 379–398.

F. Sacristan, S. & Garcia-Arenal. The evolution of virulence and pathogenicity in plant pathogen populations. *Mol. Plant. Biol.*, 9:369–384, 2008.

A. Sasaki. Host-parasite coevolution in a multilocus gene-for-gene system. *Proc.Roy.Soc. B*, 267:2183, 2000.

S. Stearns. *Evolution in health and disease*. Oxford Univ. Press, 1999.

T.O. Svennungsen and E. Kisdi. Evolutionary branching of virulence in a single-infection model. *J. Theor. Biol.*, in press., 2009.

D. Tilman. Competition and biodiversity in spatially structured habitats. *Ecology*, 75:2–16, 1994.

M. van Baalen. Dilemmas in virulence management. In Dieckmann et al. (2002), pages 60–69.

M. van Baalen and M.W. Sabelis. The dynamics of multiple infection and the evolution of virulence. *Amer. nat.*, 146:881–910, 1995.

S. D. Webb, M. J. Keeling, and M. Boots. Host-parasite interactions between the local and the mean-field: How and when does spatial population structure matter? *J. Theor. Biol.*, 249(1):140–152, Nov 2007. ISSN 0022-5193.

G.C. Williams and R.M. Nesse. The dawn of Darwinian medicine. *Quart. Rev. Biol.*, 66:1–22, 1991.

D. Wodarz. *Killer Cell Dynamics. Mathematical and Computational Approaches to Immunology*. Springer, 2007.