Chapter 5

Viral superinfection inhibition and the evolution of virulence

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5.1 Abstract

The competition of multiple pathogen strains within a single host is strongly affected by the replication rate and virulence of the competing strains. Strains with a higher replication rate and, hence, a higher virulence will typically have a competitive advantage. For this reason, many models for the evolution of virulence under superinfection assume that more virulent pathogens are better protected against superinfection. However, this expectation is reversed in a broad range of benign viral systems, which deploy molecular superinfection inhibition mechanisms in order to win within-host competition. Superinfection inhibition mechanisms can lead to an increased rate of superinfection with increased virulence of the first infecting virus. By means of an evolutionary model that includes the molecular mechanisms of superinfection inhibition of bacteriophage λ we show that superinfection inhibition can lead to novel evolutionary dynamics like the evolutionary coexistence of virulent and non-virulent strains. Molecular mechanisms of superinfection inhibition also occur in other
benign viruses of different origins like phage M13, Hepatitis B Virus or Foamy Virus. Neglecting the mechanisms can lead to erroneous predictions on the outcome of viral evolution. In fact, these mechanisms might be crucial for the maintenance of viral benignity.

5.2 Introduction

The genomes of cellular organisms are interspersed by large numbers of viral genomes that reside in a dormant state. For example, 8% of the human genome consists of sequences of retroviral origin (Lander et al. 2001). Likewise, the average number of dormant pro-phage in all sequenced bacterial genomes is 2.6 and some bacterial genomes contain up to seventeen dormant viruses that constitute 10% of their total genome (Casjens 2003). It is still largely unknown why that many viruses remain dormant, while a more virulent strategy seems to provide obvious fitness benefits.

The evolution of virulence is affected by between-host and within-host competition. Between-host competition may reduce virulence when the reduction of host density, caused by virulence, hampers transmission (Anderson and May 1982; Ewald 1983). In contrast within-host competition is generally thought to favor increased virulence since rapidly replicating virulent strains have a competitive advantage over more slowly replicating variants (May and Nowak 1995; Nowak and May 1994; van Baalen and Sabelis 1995b; Frank 1996; Gandon et al. 2001; de Roode et al. 2005). The relative importance of within-host and between-host processes is still under debate. It has been argued (e.g. Ebert and Bull 2003) that direct within-host competition should typically dominate over between-host competition that is mediated by more indirect processes like the reduction of host density. If this is indeed the case an explanation has to be given how within-host competition can lead to reduced virulence and even viral dormancy.

The prediction that within-host processes should generally select for increased virulence are usually based on simple conceptual models that
do not consider mechanistic details of the competitive interactions of viral strains during co-infection. Yet, such details may be of crucial importance. An example are defective interfering particles (DIPs) that parasitize on the protein production of wildtype virus (Turner and Chao 1999; Chao et al. 2000; Dennehy and Turner 2004). By exploiting the wildtype, DIPs can spread, thereby reducing the overall production of viable viruses and, hence, virulence. However, DIPs can never spread to fixation, since they remain dependent on a co-infecting virulent wild-type strain. Accordingly, DIPs can partially explain a reduction of viral virulence, but not the widespread evolution of viral dormancy.

Here we discuss another mechanism that can yield such an explanation. In order to protect themselves from within-host competition, many RNA and DNA viruses of bacteria, plants and animals have evolved so-called superinfection inhibition that prevents the infection of already infected cells by other viral strains of the same viral species (Hutchison and Sinsheimer 1971; Susskind et al. 1974; Mcallister and Barrett 1977; Kliem and Dreiseikelmann 1989; Christen et al. 1990; Simon et al. 1990; Karpf et al. 1997; Ellenberg et al. 2004; Lee et al. 2005; Nethe et al. 2005; Huang et al. 2008). The mechanisms underlying viral superinfection inhibition are well studied. They often involve the repression of the replication machinery of the superinfecting virus. In many cases the molecular mechanisms that repress a co-infecting strain also limit self-replication of the resident virus and, accordingly, its virulence. Hence, reduced virulence might be a side effect of superinfection inhibition.

The mechanisms underlying the trade-off between superinfection inhibition and virulence are well studied in bacteriophage λ (Figure 1) (Bailone and Devoret 1978; Susskind and Youderain 1983; Oppenheim et al. 2005) and other benign viruses (Kliem and Dreiseikelmann 1989; Christen et al. 1990; Simon et al. 1990; Ellenberg et al. 2004; Lee et al. 2005; Nethe et al. 2005). Phage λ is a temperate bacterial virus that can propagate vertically, integrated as a pro-phage into the genome of its
host, or horizontally by initiation of replication and host lysis. Since horizontal transmission requires host lysis it inevitably causes virulence. In contrast, during vertical transmission the pro-phage maintains a stable dormant state and virulence is low. The genetic mechanisms that repress the switch to the lytic cycle therefore control virulence. In phage λ, this switch to the lytic cycle is achieved by binding of the virulence repressor cI to the $p_{LP_R}$ promoter that controls the viral lysis and replication genes (Johnson et al. 1981; Ptashne 2004; Oppenheim et al. 2005). By the same mechanism the cI repressor also provides the host cell with superinfection inhibition against a second phage, when the cI repressor binds to the $p_{LP_R}$ promoter of the superinfecting phage (Figure 1A). Yet, this inhibition can be avoided by mutations in the the $p_{LP_R}$ promoter of the superinfecting phage (Figure 1A). These mutants however lose the ability to control their own virulence and are therefore termed ultra-virulent (Bailone and Devoret 1978).

Mechanisms as the one described above have not yet been incorporated into models of pathogen evolution. Generally superinfection models assume a decrease in the susceptibility to superinfection with increasing virulence of the resident pathogen (Mosquera and Adler 1998; Pugliese 2002; Boldin and Diekmann 2008), as has been demonstrated for malaria pathogens (de Roode et al. 2005) (Figure 1B). As the example of phage λ and other viral pathogens shows, it is by no means clear whether, and to what extent, this assumption is generally valid. It is therefore not self-evident that within-host competition should generally lead to higher virulence.
Figure 1: Virulence regulation and super-infection inhibition in phage λ. (A) A resident phage stays in the lysogenic state when the virulence repressor cl is bound to its pLpR promoter (1), thereby suppressing the resident’s lysis and replication genes. By the same mechanism cl binds to the pLpR promoter of a superinfecting phage (2), thereby preventing its replication. This inhibits superinfection. Hence, viruses that produce a low level of repressor cl are more virulent and at the same time more susceptible to superinfection. (B) The mechanisms above create a positive association between virulence of the resident phage and the susceptibility to superinfection. This is in contrast to pathogens like malaria parasites where more virulent strains are less susceptible to superinfection.
In order to study the effect of these mechanisms on the evolution of viral virulence, we developed an evolutionary model that integrates biochemical mechanisms into a population model which considers the invasion of a rare viral mutant into an established ecological equilibrium of a resident virus. By this approach we aim to integrate the biochemical, ecological and evolutionary scales of superinfection and to provide a mechanistic view on the evolution of viral virulence under superinfection inhibition.

5.3 A model for the evolution of virulence under superinfection inhibition

We consider a virus that spreads by vertical and horizontal transmission between individual host cells. For simplicity we assume that the virus is highly infective and that, correspondingly, virtually all host cells are infected. Therefore a viral mutant can only increase in frequency when it is able to superinfect a host cell that is already infected by a resident virus. We will focus on the situation where co-infection eventually leads to take-over of the host cell by one of the competitors. The competition between the resident and a mutant strain of a virus can then be studied by following the dynamics of host cells that are infected by either type of virus (Nowak and May 1994). We represent this situation by two coupled differential equations that describe the density of hosts infected by the resident virus $y_R$ and hosts infected by a mutant $y_M$, respectively:

$$\frac{dy_R}{dt} = ry_R \left( 1 - y_R - y_M \right) - \alpha_R y_R + \beta \left( \alpha_R \Phi_{MR} - \alpha_M \Phi_{RM} \right) y_R y_M$$  

$$\frac{dy_M}{dt} = ry_M \left( 1 - y_R - y_M \right) - \alpha_M y_M + \beta \left( \alpha_M \Phi_{RM} - \alpha_R \Phi_{MR} \right) y_R y_M$$

The system has the following interpretation. In the absence of viral infection, the host grows logistically with intrinsic growth rate $r$ and a carrying capacity that is normalized to 1. This part of the growth equation includes all mortality not induced by the viruses and it
determines the rate of vertical transmission of the viruses. The terms \( \alpha_R y_R \) and \( \alpha_M y_M \) correspond to virus induced mortality. Hence \( \alpha_R \) and \( \alpha_M \) characterize the virulence of resident and mutant, respectively. Virus-induced mortality is associated with cell lysis and viral reproduction. The number of newly produced resident virus is therefore proportional to \( \alpha_R y_R \). The rate at which these viruses attempt to superinfect host cells incorporating the mutant virus is proportional to their abundance \( \alpha_R y_R \) and to the abundance \( y_M \) of mutant-infected hosts. The constant of proportionality \( \beta \) includes the yield of virus production, diffusivity of the medium and the adsorption rate upon encounter with a host cell (which we assume to be the same for both types of virus). The crucial ingredient in our model is the superinfection term \( \Phi_{MR} \), which corresponds to the proportion of superinfection attempts that result in a ‘take-over’ of a mutant-infected host by the resident virus. Hence the rate of recruitment of new resident infected hosts due to successful superinfection by resident viruses is given by \( \beta \alpha_R y_R y_M \Phi_{MR} \). Similarly, the recruitment of new mutant-infected hosts due to successful superinfection by mutant viruses is given by \( \beta \alpha_M y_M y_R \Phi_{RM} \). Notice that the indices in the superinfection terms \( \Phi_{RM} \) and \( \Phi_{MR} \) reflect the order of arrival of the two types of viruses. Hence, \( \Phi_{AB} \) corresponds to the probability that upon adsorption to a host infected by \( A \) a newly formed virus \( B \) takes over this host (Mosquera and Adler 1998).

When we consider the resident virus in the absence of a mutant (i.e. \( y_M = 0 \)), the resident host population reaches the population dynamical equilibrium

\[
y_R^* = \frac{r - \alpha_R}{r}
\]  

(2)
In this resident equilibrium a rare mutant can increase in frequency when its per capita growth rate is positive:

\[
\frac{1}{y_M} \frac{dy_M}{dt} \bigg|_{y_R=y_R^*, y_M=0} = \alpha_R - \alpha_M + \beta \frac{r - \alpha_R}{r} \left( \alpha_M \Phi_{RM} - \alpha_R \Phi_{MR} \right) > 0. \quad (3)
\]

The per capita growth rate of a mutant in the resident equilibrium corresponds to the invasion fitness of the mutant (Geritz et al. 1998) that we will denote by $W$. Equation (3) shows how invasion fitness depends on virulence $\alpha$ and the susceptibility to superinfection $\Phi$ of both, the resident and the mutant virus. Although the rate of superinfection is often directly described as a function of virulence, e.g. $\Phi_{RM}(\alpha_R, \alpha_M)$, it is likely that virulence $\alpha$ and the rate of superinfection $\Phi$ are more indirectly related, e.g. via a correlation to some underlying trait or the concentration of some protein (like the virulence repressor protein cI of phage $\lambda$). In order to allow for both possibilities we choose a general approach in which $\alpha$ and $\Phi$ are functions of some trait $x$ (that we will specify later). In other words we assume that

\[
\alpha_R = \alpha(x_R), \quad \Phi_{RM} = \phi(x_R, x_M) \quad (4a)
\]

and

\[
\alpha_M = \alpha(x_M), \quad \Phi_{MR} = \phi(x_M, x_R) \quad (4b)
\]

where $x_R$ and $x_M$ are the trait value of the resident and the mutant virus, respectively. Now, the invasion fitness can be rewritten as

\[
W(x_M, x_R) = \alpha(x_R) - \alpha(x_M) + \beta \frac{r - \alpha(x_R)}{r} \left[ \alpha(x_M) \phi(x_R, x_M) - \alpha(x_R) \phi(x_M, x_R) \right] \quad (5)
\]
Obviously $W(x_R, x_R) = 0$. This makes sense, since the resident should neither grow nor decline in a population of residents. A rare mutant with trait $x_M$ will invade when $W(x_M, x_R) > 0$. The direction of selection (i.e. whether selection favors larger or smaller values of $x$) is therefore given by the selection gradient

$$\frac{\partial W}{\partial x_M} = \alpha(x_R) + \frac{r}{r} \left( \alpha(x_R) \phi(x_R, x_R) - \alpha(x_R) \left( \frac{\partial \phi(x_R, x_R)}{\partial x_M} - \frac{\partial \phi(x_R, x_M)}{\partial x_M} \right) \right)$$

(6)

Of particular importance are those resident strategies $x_R^*$ where there is no directional selection any more (so-called evolutionarily singular strategies; Geritz et al. 1998), i.e. those resident strategies $x_R^*$ for which

$$\frac{\partial W}{\partial x_M} = 0$$

(7)

The resident strategy $x_R^*$ is an evolutionarily stable strategy (ESS) when the invasion fitness $W(x_M, x_R^*)$ has a maximum in the direction of the mutant strategy $x_M$ or

$$\frac{\partial W}{\partial x_M} = 0 \quad \text{and} \quad \frac{\partial^2 W}{\partial x_M^2} < 0.$$  

(8)

At an ESS all mutant traits $x_M$ in the vicinity of $x_R^*$ have a lower fitness than the resident. Accordingly, a resident population with strategy $x_R^*$ is immune by invasion against mutants. An ESS is not necessarily reachable by a series of small gene substitution events
(Geritz et al. 1998). The resident strategy $x_R^*$ is convergence stable (i.e. an evolutionary attractor) if

$$\frac{\partial W}{\partial x_M}_{x_M=x_R^*} = 0 \quad \text{and} \quad \frac{\partial}{\partial x_R} \left[ \frac{\partial W}{\partial x_M}_{x_M=x_R^*} \right]_{x_M=x_R^*} < 0. \quad (9)$$

When $x_R^*$ is convergence stable but not evolutionarily stable, evolution will converge to an evolutionarily unstable strategy; a so-called branching point. In this case, evolution a polymorphism of two (or more) coexisting strategies will result.

### 5.4 Simplified scenarios

#### 5.4.1 Scenario 1: Superinfection directly related to virulence

We will now show that the relation between virulence and susceptibility to superinfection is decisive for the dynamics and outcome of evolution. To this end, we first consider the special case where virulence itself is the target of selection, i.e.

$x_R = \alpha_R$ and $x_M = \alpha_M \quad (10)$

Moreover we make the simplifying assumption that the rate of superinfection only depends on the virulence of the first infecting phage, which is related to its repressor concentration, or

$$\Phi_{RM} = \phi(\alpha_R), \quad \Phi_{MR} = \phi(\alpha_M) \quad (11)$$

Thereby $\phi$ is a function taking on values between 0 and 1 and characterizes the nature of the trade-off between virulence of the first infecting phage and susceptibility to superinfection. (We will elucidate the biochemical link between repressor concentration, virulence and
superinfection in the next section.) Under these assumptions (10) and (11), the invasion fitness is given by

$$W(\alpha_M, \alpha_R) = \alpha_R - \alpha_M + \beta \frac{r - \alpha_R}{r} [\alpha_M \phi(\alpha_R) - \alpha_R \phi(\alpha_M)]$$  \hspace{1cm} (12)

An evolutionarily singular strategy $\alpha_R^*$ is now given by the condition

$$\frac{\partial W}{\partial \alpha_M} \bigg|_{\alpha_M = \alpha_R^*} = -1 + \beta \frac{r - \alpha_R^*}{r} \left[ \phi(\alpha_R^*) - \alpha_R^* \phi'(\alpha_R^*) \right] = 0$$  \hspace{1cm} (13)

or equivalently

$$\beta \left[ \phi(\alpha_R^*) - \alpha_R^* \phi'(\alpha_R^*) \right] = \frac{r}{r - \alpha_R^*}$$  \hspace{1cm} (14)

In view of

$$\frac{\partial^2 W}{\partial \alpha_M^2} \bigg|_{\alpha_M = \alpha_R^*} = -\beta \frac{r - \alpha_R^*}{r} \alpha_R^* \phi''(\alpha_R^*)$$  \hspace{1cm} (15)

a positive solution $\alpha_R^* > 0$ of (14) is evolutionarily stable whenever $\phi''(\alpha_R^*) > 0$, or in other words whenever the superinfection function is convex in the vicinity of $\alpha_R^*$. Making use of (14), the condition for convergence stability is given by

$$\frac{\partial}{\partial \alpha_R} \left[ \frac{\partial W}{\partial \alpha_M} \right] \bigg|_{\alpha_M = \alpha_R^*} = -\frac{r}{r - \alpha_R^*} - \beta \frac{r - \alpha_R^*}{r} \alpha_R^* \phi''(\alpha_R^*) < 0$$  \hspace{1cm} (16)

This is automatically satisfied whenever $\phi''(\alpha_R^*) > 0$. In other words, and evolutionarily stable strategy is always convergence stable.
However, an evolutionarily unstable strategy can also be convergence stable if $\phi''(\alpha^*_R)$ is not too negative. To be precise, a solution of (14) is a branching point whenever

$$- \frac{r}{\beta \alpha^*_R (r - \alpha^*_R)^2} < \phi''(\alpha^*_R) < 0$$

(17)

Numerical analysis through a pairwise invasibility plot shows that in this case virulence $\alpha^*_R$ either evolves towards the branching point or towards the non-virulent strategy $\alpha^*_R = 0$, depending on the initial level of virulence (Figure 2).

Figure 2: Pairwise invasibility plot for a concave superinfection function. A concave relation between the susceptibility to superinfection and virulence of the first infecting strain ($\phi''(\alpha) < 0$) can lead to three evolutionarily singular points: The ESS $\alpha^* = 0$, an evolutionary branching point for virulence and an evolutionary repellor which separates the two strategies (Superinfection function: $\phi(\alpha) = \alpha / (\alpha + 1)$ Parameters: $\beta = 20$, $r = 1$).
5.4.2 Scenario 2: Virulence and susceptibility to superinfection are determined by repressor binding

In the previous section we showed that a concave positive relation between the rate of superinfection and the virulence of the first infecting virus can lead to evolutionary branching of virulence or the maintenance of a non-virulent population. Yet, we did not elucidate the origins of the relation between the susceptibility to superinfection and virulence. Therefore, in this section, we will derive this relation from the molecular mechanisms of viral virulence and superinfection control, along the example of bacteriophage λ. In λ the virulence \( \alpha \) and the rate of superinfection \( \phi \) are both determined by sigmoidal binding dynamics of the repressor cI to the pLpR promoter (Johnson et al. 1981; Hendrix et al. 1983; Ptashne 2004). Following these binding dynamics the virulence \( \alpha_R \) of the resident phage is determined by the resident repressor concentration \( c_R \) and the resident promoter affinity \( k_R \). The rate of superinfection \( \phi_{RM} \) is in turn determined by the repressor concentration of the resident phage \( c_R \) and the promoter affinity of the super-infecting phage \( k_M \) (see Figure 1A). This way we can describe the proportion \( p \) of host cells, which have a repressor molecule bound to the pLpR promoter, and remain in the lysogenic state, by second order receptor binding kinetics in the form

\[
P = \frac{c^2}{k^2 + c^2}
\]

(18)

In turn, the proportion of cells that switch to the lytic cycle is \( 1 - p \). Since the switching rate to the lytic cycle is equivalent to virulence \( \alpha \), we can write

\[
\alpha_R(c_R,k_R) = 1 - p_R = \frac{k_R^2}{k_R^2 + c_R^2},
\]

(19a)

and
\[ \alpha_M(c_M, k_M) = 1 - p_M = \frac{k_M^2}{k_M^2 + c_M^2}. \] 

(19b)

On the other hand the susceptibility to superinfection is determined by the repressor concentration of the resident phage \( c \) and the promoter affinity of the super-infecting phage \( k \), or

\[ \phi_{RM}(c_R, k_M) = \gamma \frac{k_M^2}{k_M^2 + c_R^2} \] 

(19c)

and

\[ \phi_{MR}(c_M, k_R) = \gamma \frac{k_R^2}{k_R^2 + c_M^2} \] 

(19d)

where \( \gamma \) is the relative binding efficiency between self and foreign promoter binding.

By the use of \( \alpha_R(c_R, k_R) \), \( \alpha_M(c_M, k_M) \), \( \phi_{RM}(c_R, k_M) \) and \( \phi_{MR}(c_M, k_R) \) we can now rewrite the system of two competing strains (1a, 1b) in terms of the underlying biochemical properties. This way we can represent the two trade-offs between virulence and superinfection inhibition, and the avoidance of superinfection inhibition and virulence, directly by the biochemical properties \( c \) and \( k \). We can follow the evolution of the biochemical properties \( c \) and \( k \) by the numerical method of an evolutionary walk. Thereby, we introduce a rare mutant of \( c_M \) or \( k_M \) into a resident population \( c_R \) and \( k_R \) and integrate the system (1a, 1b) given the relations (8, 9). When a mutant increases in frequency and displaces the resident, the mutant becomes the new resident and the evolutionary path makes one step. By iteration of this process we can follow the correlated evolution of \( c \) and \( k \) and its effect on virulence \( \alpha \) and the rate of superinfection \( \phi \).
The evolution of virulence for the biochemical model (1a, 1b, 8, 9) leads to evolutionary branching in virulence $\alpha$, but evolution in the underlying parameters now proceeds in two dimensions (Figure 3). This leads to an adaptation process in several steps. At first virulence $\alpha$ decreases through directional selection towards lower receptor affinity $k$ until it approaches the branching point. Close to the branching point the repressor concentration $c$ increases until the population divides into two strategies: Low receptor affinity and low virulence and high receptor affinity and high virulence (Figure 3A). This way a single ancestor strategy can evolve into two coexisting strategies: A defense specialist that is analogous to the lysogenic phage $\lambda$ wildtype and an attack specialist that is analogous to ultra-virulent mutants of phage $\lambda$. This example provides a mechanism by which virulent and non-virulent viruses can stably coexist in a natural virus population.

5.5 Discussion

Within-host competition between parasites strains has fundamental consequences for the evolution of pathogen virulence (Pugliese 2002; Boldin and Diekmann 2008; de Roode et al. 2005; Nowak and May 1994; Gandon et al. 2001; Adler and Mosquera 2000). The competitive interactions of pathogen strains during co-infection are complex. A common approach to simplify these competitive interactions is the introduction of a superinfection function which describes the rate at which a first infecting pathogen is out competed and replaced by a second pathogen from its host cell. The superinfection function can be interpreted as a limiting case of co-infection with an instantaneous replacement of pathogens through competition.
Figure 3: Evolutionary branching of virulence reflects the biochemical details of phage λ repressor binding. (A) Correlated evolution of repressor concentration and receptor affinity determine the evolution of virulence. Selection towards lower virulence decreases receptor affinity (= stronger repressor binding) until the branching point is reached. In the branching point repressor concentration continues to increase until a level is reached at which the population divides into two strategies: Low receptor affinity and low virulence and high receptor affinity and high virulence. These strategies
are equivalent to lysogenic and ultra-virulent mutants of phage λ. (B) Accordingly, evolution decreases virulence until the branching point is reached and disruptive selection leads to the co-existence of virulent and non-virulent population. (Parameters $\beta = 0.8$, $\gamma = 4$, $c_0 = k_0 = 0.5$).

Viral superinfection inhibition, as described in the case of phage λ has important consequences for the properties of the superinfection function and the evolution of virulence. First, in a host population which is fully infected by pathogen that deploys superinfection inhibition the relative benefit of horizontal transmission is reduced. Second, in phage λ, the molecular link between increased viral virulence and increased susceptibility to superinfection create a cost for virulence, since a more virulent virus has a higher probability to be displaced from its host cell by a superinfecting competitor. These two aspects of superinfection inhibition increase the relative benefits of vertical transmission and therefore enable the persistence of a fully vertical transmitting viral population or the co-existence of horizontal and vertical transmission strategies. The increase in the benefit of vertical transmission allows for the ecological specialization and co-existence of a non-virulent vertically transmitting ‘defense’ strategy and horizontally transmitting ‘attack’ strategy. Under these conditions a non-virulent virus can persist in the presence of a virulent counterpart.

The co-existence of virulence strategies has been described earlier. Two main properties of the superinfection function are decisive for the possible co-existence of virulence strategies. These are the slope of the superinfection function and the smoothness of the superinfection function, e.g. its behavior in the point of equal virulence of resident and superinfecting pathogen (Pugliese 2002; Boldin and Diekmann 2008). Most superinfection models assume a superinfection function that decreases with resident virulence and focus on the effect of non-smoothness around the point of equal virulence between resident and superinfecting pathogen. When the superinfection function has a strong non-smoothness in the origin, e.g. is a step function of virulence, a
large number of virulent strains can coexist around a virulence optimum (Nowak and May 1994). This is however an extreme case since a step-wise superinfection function enables mutants with infinitesimally small differences in virulence to enter and persist in the population (Pugliese 2002). A more realistic approach is the use of a piece-wise differentiable superinfection function \( \phi_{RM}(\alpha_R, \alpha_M) \) that is \( \phi_{RM} = 0 \) zero for \( \alpha_R > \alpha_M \) and steadily increasing for \( \alpha_M > \alpha_R \). This less extreme non-smooth behavior of a piece-wise differentiable function can promote ecological and evolutionary and coexistence (Pugliese 2002; Boldin and Diekmann 2008).

The example of phage \( \lambda \) differs from these previous models in two important aspects. First, in contrast to previous models of superinfection the example of phage \( \lambda \) considers simultaneous horizontal and vertical transmission. Second, the mechanisms of superinfection inhibition introduce interference competition next to competition for host resources. Under these conditions the evolutionary coexistence of horizontal and vertical transmission strategies as well as the stable persistence of a fully vertically transmitting host population can occur even with a smooth superinfection function, under the premise that virulence and the susceptibility to superinfection show a positive (concave) relation.

Due to its stabilizing effect on vertical transmission strategies superinfection inhibition might play an important role in the maintenance of benignity in viral systems in general and therefore create an alternative explanation for the evolution towards lowered levels of virulence. Currently the major factor for the evolution of reduced virulence is seen in the ecological feedback of virulence on between-host transmission. Selection towards lowered virulence due to ecological feedback of virulence onto transmission might however be relatively weak in comparison to selection for increased virulence during within host competition during co-infection (Ebert and Bull 2003). In the absence of ecological feedback, other mechanisms are
required to enable the persistence of benign viruses. Superinfection inhibition might be an important mechanism for the maintenance of low virulence and should therefore be a common characteristic of benign viruses.

Indeed mechanisms that link viral virulence to superinfection inhibition which are similar to the mechanisms of phage λ can be pointed out in several other viral systems. Taking a closer look at other examples of benign DNA and RNA viruses, next to bacteriophage λ, we can see that mechanisms which relate of virulence to superinfection are indeed, common. Even though the genome organization of benign DNA and RNA can be vastly different these mechanisms show a certain ‘core theme’: Gene products that are involved in the limitation of viral self-replication are often used to suppress competing viruses. This regulatory core theme seems to have emerged multiple times. The single stranded DNA (ssDNA) phage M13, for example, causes a chronic infection of its host E.coli with a relatively mild effect on host mortality. In order to achieve this low level of virulence, M13 produces large amounts of protein P5 that on the one hand covers the single stranded form of M13 to prevent a conversion to the double stranded DNA (dsDNA) replicative form (RF) and on the other hand P5 inhibits the P2 gyrase that is required for the RF rolling circle replication. This way the replication repressor P5 limits the intra-host replication of M13 and, at the same time, blocks the replication initiation of a super-infecting M13 (Baas 1985). Therefore virulence and superinfection resistance are also tightly coupled in M13, although the mechanism is very different from virulence repression in phage λ. Another example is the retro-virus Hepatitis B (HBV), which causes a chronic liver infection. In order to escape immune suppression the HBV strictly limits its intra-cellular replication. It achieves this regulation by auto-repression of its reverse transcription protein P. This way reverse transcriptase P activity is high in the initial phase of infection but is repressed when protein P accumulates (Cao and Tavis 2006). When any competing HBV therefore enters an infected cell as a pre-genomic
RNA stage at a late stage of infection, its reverse transcription is also blocked by high levels of protein P that are produced by the residing virus, and superinfection is prevented. This way self-repression and superinfection inhibition are directly coupled in HBV.

Another class of retro-viruses, the Foamy Viruses use a system that is more similar to the one of phage λ. Foamy viruses cause benign infection. The genome of Foamy Virus contains next to the \textit{gag}, \textit{pol} and \textit{env} proteins, the genes \textit{tas} and \textit{bet} that control the switch between latent, chronic viral infection and lytic viral replication. Thereby \textit{tas} stimulates a switch to lytic replication, whereas \textit{bet} represses the internal promoter and therefore suppresses a switch to the lytic stage. Strikingly, the expression of \textit{bet} in Foamy Virus free cells has also been shown to provide resistance to superinfection by other Foamy Viruses (Nethe et al. 2005). The mechanisms of coupling between virulence and superinfection inhibition is therefore analogous to the \textit{cro} and \textit{cI} system in phage λ.

These examples suggest that genetic mechanisms that link virulence with increased susceptibility to superinfection, have evolved multiple times and could therefore be of major importance for the maintenance of viral benignity. The interpretation of the genetic coupling of increased virulence with increased susceptibility to superinfection might have analogies to the cost of ‘cheater’ strategies in kin selection theory that are required to maintain cooperation. Increased sensitivity to superinfection seems to be an intrinsic cost for high virulence. These associated costs of virulence are often required to stabilize the persistence of prudent host exploitation, i.e. low virulence against the invasion of virulent mutants (van Baalen and Sabelis 1995a; West et al. 2006). This way the genetic coupling of virulence and susceptibility to superinfection can be seen as a self applied limitation mechanism for the levels of virulence, which is ‘hard-wired’ in the viral gene regulation scheme. More attention should therefore be given to the evolution of mechanisms of viral superinfection inhibition as they have
the propensity to be at the core of the maintenance of low virulence in viruses.

5.6 References


