How to be good at being a virus

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Chapter 6
Opposing selection pressures on receptor destroying enzymes of influenza virus limit viral adaptation and tissue specificity

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6.1 Abstract:

Opposing selection pressures on a viral protein can impose severe limits on viral evolution. The receptor destroying enzyme neuraminidase of influenza virus is an intriguing example. During late viral development destruction of the viral target receptor is required to release newly formed viral particles from the host cell. Early in the viral life cycle, however, receptor destruction can abort viral infection in the stage of host cell attachment. It is therefore not straightforward to predict the evolution of neuraminidase activity in different host and

tissue environments. By means of a viral life history model we demonstrate that optimal viral adaptation to an environment requires specialization in the rate of receptor destruction that can prohibit viral replication in other hosts or tissues. Adaptation of the receptor destroying activity to local conditions can therefore provide a plausible explanation for viral host and tissue specificity and, as a consequence, differences in viral transmissibility and virulence.

6.2 Introduction

Viral reproduction requires the balancing of many genetic and biochemical processes acting at various stages in the viral life cycle. Evolutionary adjustment of this balance is often constrained by conflicting selection pressures that arise when a change that is advantageous during one stage of the viral life cycle is disadvantageous at another stage. In lytic bacterial viruses, for example, reduced lysis time is coupled to a reduction in viral burst size (Wang et al. 1996; Bull et al. 2004); and in polio virus there is a trade-off between the rates of viral genome replication and viral genome encapsidation (Krakauer & Komarova 2003). When two fitness determining components of the life cycle are linked through a trade-off the outcome of evolution cannot be predicted on the basis of each component independently. Instead, an evolutionary analysis requires the integration of fitness effects over the whole viral life cycle (Stearns 1992; Caswell 2001).

Receptor destroying enzymes are an important class of viral proteins that exemplify such opposing selection pressures. These enzymes play an important role in the life cycle of a large family of human and animal pathogens including *Ortho-* and *Paramyxoviridae* (influenza, para-influenza, Newcastle disease virus, mumps and measles) and *Corona-* and *Toroviridae* (Smits et al. 2005; de Groot 2006). A well-studied representative of these families is influenza virus. Influenza virus contains two major surface proteins: the receptor binding protein

hemagglutinin (HA) and the receptor destroying enzyme neuraminidase (NA). HA and NA are essential for viral reproduction and determine important viral properties like host specificity, tissue tropism, virulence and transmissibility (Baigent & McCauley 2003). Yet, the HA and NA proteins counteract each other at several points of the viral life cycle. Whereas the binding of HA to the host sialic acid (SA) receptors establishes viral attachment to the host cell, NA destroys these SA receptors and therefore potentially hampers virus-host attachment in the early viral life cycle. Receptor destruction therefore 'does not seem like a good idea', was it not that the receptor destroying activity of NA is indispensable in at least three other steps of the life cycle: NA activity prevents the aggregation of viral particles in the mucus layer of epithelia in the lung and the intestine (Matrosovich & Klenk 2003; Matrosovich et al. 2004), it enhances the passage of viral particles through the endosome of the host cell (Suzuki et al. 2005) and it prevents the accumulation of newly formed viral particles on the host cell surface after viral budding from infected cells (Palese et al. 1974). Nevertheless, due to its detrimental effect on virus-host attachment, the receptor destroying activity of NA remains a double edged sword and needs to be carefully balanced in accordance with the strength of HA binding and the properties of the available binding receptors (Mitnaul et al. 2000; Wagner et al. 2000; Wagner et al. 2002; Bin et al. 2005).

The environment of a virus is determined by the availability and properties of viral binding receptors in a specific tissue and host or tissue. Accordingly, host and tissue characteristics markedly affect the optimal balance of receptor binding and receptor destruction. The occurrence of cell mucus is one of these characteristics. Mucus contains decoy SA receptors that act as surrogate binding targets for viral HA and immobilize and inactivate viral particles. Liberation from mucus attachment requires the destruction of mucus SA receptors by viral NA activity (Matrosovich & Klenk 2003). In addition to the occurrence and density of mucus, the variation in the binding efficiency of SA receptors also plays an important role. Hosts and tissues differ, for

example, in their concentration and relative frequency of 2,3-α-gal-SA and 2,6-α-gal-SA receptors, and avian and mammalian influenza virus differs markedly in the affinity of HA and NA with both types of receptor (Gambaryan et al. 2006).

Due to the multiple effects of receptor destroying enzymes on the viral life cycle, the evolution of NA activity is a complex process. A quantitative understanding of the evolution of NA activity requires the integration of costs and benefits of receptor destruction in a life history model for the viral life cycle (Stearns 1992; Caswell 2001). Such a model describes the transitions between the various stages of the life cycle by a life cycle graph that corresponds to a system of differential equations. It is then straightforward to derive viral fitness from the properties of the stage transition matrix. Obviously, viral fitness reflects the properties of host and tissues. Hence, a life history models not only allows to determine the optimal level of activity of receptor destroying enzymes, but also the degree to which a virus with an enzyme that is adapted to one particular host or tissue can survive in a different host or tissue. Here we study the evolution of NA activity and HA avidity of influenza virus in such a life history model. We derive the NA activity and HA avidity that maximize the overall growth rate of the viral population. Our emphasis is on the host and tissue dependence of these viral properties and the question whether, and to what extent, local adaptation can provide and explanation for viral host and tissue specificity.

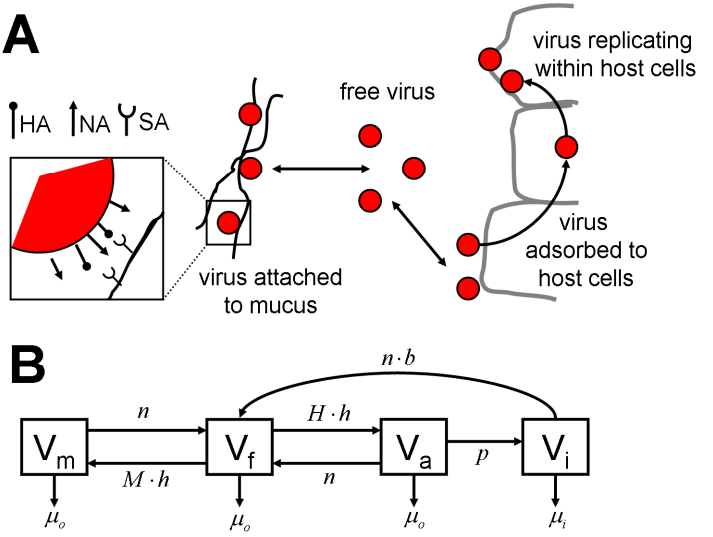

Figure1: The viral life cycle (A) Key processes in the life cycle of influenza virus. The virus can occur in four states: freely moving through the tissue, attached to mucus, attached to a host cell and internalized in a host cell, where it produces offspring virus. Viral attachment to mucus and host cells depends on the density and biochemical properties of sialic acid (SA) receptors and the avidity h of viral hemagglutinin (HA) to these receptors. Release from both, mucus and host cell attachment, to the free stage depends on the receptor destroying activity of the neuraminidase (NA) enzyme. (B) Life cycle graph summarizing the key assumptions of the life history model. The variables V_f, V_m, V_a and V_i denote the concentrations of virus in the four stages free, mucus adsorbed, host adsorbed and internalized. The transition rate from an absorbed to the free state is given by neuraminidase activity n. Adsorption rate to mucus and host cells is given by the product of hemagglutinin avidity h and the concentration H of mucus receptors and M of host cell receptors, respectively. Viruses adsorbed to a host cell are internalized with rate p and newly formed virus particles bud from infected cells at rate b and detach from the cell surface through neuraminidase activity n. Virus particles die at rate μ_o outside the host cell and at rate μ_i inside the host cell.

6.3 A model of HA-NA balance and its evolution

The structure of our model is motivated and summarized in Figure1. We consider four stages of the viral life cycle: free viral particles, mucus-adsorbed particles, particles that are adsorbed to the host cell, and particles that replicate within a host cell. The concentrations of the viral particles in these four stages are represented by the variables V_f, V_m, V_a and V_i, respectively. A virus from the pool of free particles can either enter the pool of mucus-adsorbed virus or host-cell adsorbed virus at a rate that is proportional to the receptor binding avidity h and the abundance of its preferred receptors in the mucus and on the host cell, which are denoted by M and H, respectively. Viral particles destroy receptors and detach from mucus and host cells at a rate determined by the receptor destroying activity n. Thereby the transition from the pool of host-adsorbed virus to free virus represents the abortion of infection, e.g. the negative side effect of receptor destruction. Viral particles that are adsorbed to the host cell penetrate the cell at rate p and enter the stage of replication within the host cell. Replicating particles in V_i produce new viral particles that bud from the host cell at rate b. Budded viral particles accumulate at the cell surface and require receptor destroying activity to detach and enter the pool of free viral particles, thereby finishing their life cycle. The rate of release from each infected cell is therefore proportional to the budding rate b and the detachment of viral particles n. All particles outside of the host cell decay at rate μ_o, while particles inside the host cell decay at rate μ_i. All these assumptions are represented by the life cycle graph in Figure 1B or, equivalently, by the following system of differential equations

$$\frac{dV_m}{dt} = -nV_m + hMV_f - \mu_o V_m \quad (2a)$$

$$\frac{dV_f}{dt} = +nV_m - hMV_f - hHV_f + nV_a + nbV_i - \mu_o V_f \quad (2b)$$

$$\frac{dV_a}{dt} = hHV_f - nV_a - pV_a - \mu_o V_a \quad (2c)$$

$$\frac{dV_i}{dt} = +pV_a - nbV_i - \mu_i V_i \quad (2d)$$

The properties of this linear system are determined by the 4 x 4 matrix of transition rates between states (Caswell 2001). In particular, the asymptotic growth rate λ of the viral population is given by the dominant eigenvalue of this matrix. Explicit calculation of λ, which we will later use as a measure of viral fitness, is intricate and inspires little insight. We therefore simplify the problem by making use of the different time scales of receptor binding and destruction and the process of viral replication. Receptor binding and destruction are spontaneous, fast, and energy-independent processes. In contrast, the processes of host cell penetration, replication within the cell and budding require complex interactions with the host cell and are therefore slow relative to receptor binding and destruction. Therefore, these two types of processes occur on different time scales. This means that on the fast time scale the particles redistribute very rapidly over the free, host-adsorbed and mucus-adsorbed stages, before the total number of viral particles noticeably changes due to the production of new particles. It is therefore reasonable to assume that the particles outside of the host cell reach a quasi-steady-state (Segel 1984) that is characterized by

$$\frac{dV_m}{dt} = \frac{dV_f}{dt} = \frac{dV_a}{dt} = 0 \ . \tag{3}$$

As a consequence the asymptotic growth rate λ of the viral population corresponds to the per capita rate of new infections, which according to (2d) is given by

$$\lambda = \frac{1}{V_i}\frac{dV_i}{dt} = p\frac{V_a}{V_i} - nb - \mu_i \tag{4}$$

In quasi-steady-state the viral particles distribute over the extra-cellular stages in the proportions

$$\tilde{V}_a = \frac{hH}{n + p + \mu_o}\tilde{V}_f = r_1\tilde{V}_f \tag{5a}$$

$$\tilde{V}_m = \frac{hM}{n + \mu_o}\tilde{V}_f = r_2\tilde{V}_f \tag{5b}$$

$$\tilde{V}_f = \frac{nbV_i}{h\left(M + H\right) - n\left(r_1 + r_2\right) + \mu_o} \tag{5c}$$

The ratios r_1 and r_2 represent the proportions of viral particles on the host cell and in the mucus relative to the amount of free virus

($r_1 = \tilde{V}_a / \tilde{V}_f$, $r_2 = \tilde{V}_m / \tilde{V}_f$). Substituting $\tilde{V}_a / V_i = r_1 \tilde{V}_f / V_i$ into (4), we get an explicit expression for the asymptotic growth rate of the virus:

$$\lambda = \frac{1}{V_i}\frac{dV_i}{dt} = \frac{p r_1 n b}{h(M+H) - n(r_1 + r_2) + \mu_0} - nb - \mu_i \qquad (6a)$$

In the appendix we show that under the assumption $\mu_0^{\,2} << H$ (that we will make from now on) this expression simplifies to

$$\lambda = nb\left[\frac{p}{p + \mu_0\left[1 + \frac{1}{H}\left(M + \frac{n+p}{h}\right)\right]} - 1\right] - \mu_i \qquad (6b)$$

The growth rate λ is a function of viral properties (n, h, p, b) and the conditions of the host tissue environment (M, H). We can therefore use λ to derive the optimal combination of the biochemical strategies, h and n, for a given host tissue that is characterized by M and H.

6.4 Optimal receptor destroying activity

The optimal receptor destroying activity n that maximizes the viral per-capita growth rate $\lambda(n,h)$ can be found by calculating the maximum of $\lambda(n,h)$ in the direction of n.

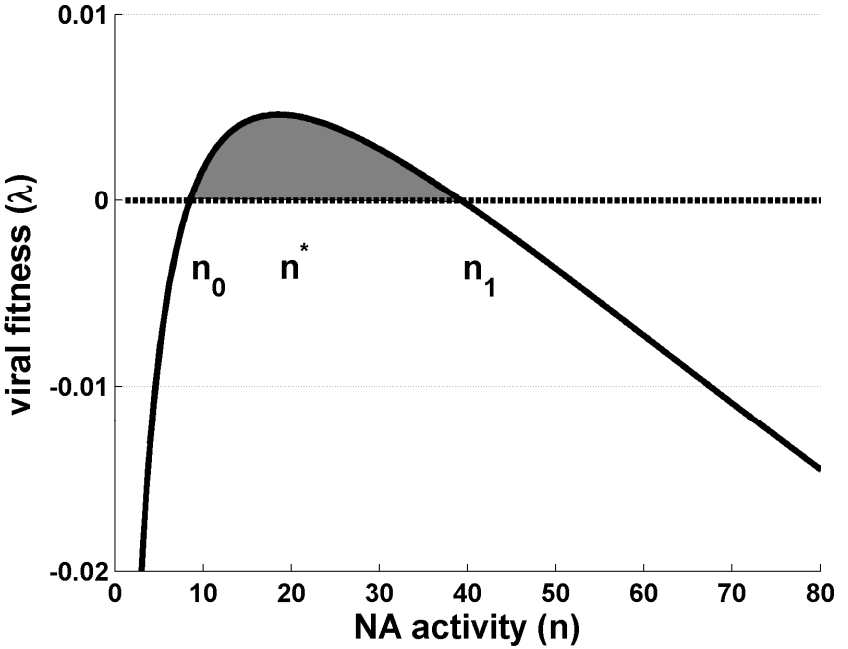

Figure 2: Viral fitness as a function of neuraminidase activity. A virus with a low NA activity $n \approx 0$ tends to accumulate within the host. Therefore it has a negative growth rate that is approximately given by $\lambda = -\mu_i$. A virus with a high NA activity tends to accumulate in the free state. Accordingly, it also has a negative growth rate that is approximated by $\lambda = -\mu_o$. Some host tissues do not allow viral growth irrespectively of the value of n. In the example presented here, there is a tissue specific interval $n_0 < n^* < n_1$ allowing viral growth. Viral fitness (= asymptotic growth rate) is maximized at an intermediate neuraminidase activity n^* (Parameter values: $h = 1$, $b = 3$, $p = 10^{-2}$, $\mu_o = \mu_i = 0.15$, $M = 1$, $H = 1$).

This optimum n^* is given by

$$\left.\frac{\partial\lambda(n,h)}{\partial n}\right|_{n=n^*} = 0 \qquad \text{and} \qquad \left.\frac{\partial^2\lambda(n,h)}{\partial n^2}\right|_{n=n^*} < 0\,. \qquad (7)$$

Let us first try to understand intuitively the effect of receptor destroying activity n on viral growth rate. It is easy to see that neither a very small n nor a very large n are beneficial. For $n=0$, all viral particles accumulate in infected hosts, where they decay at rate μ_i. Therefore the growth rate $\lambda=-\mu_i$ is negative for small n, implying viral extinction. On the other end, for large n, the ratios r_1 and r_2 become small and all viral particles accumulate in the free stage where they decay at rate μ_o. Large n therefore also leads to a negative growth rate $\lambda=-\mu_o$ and, accordingly, also to extinction. It is possible that the host tissue is too hostile to allow viral growth: $\lambda(n,h)<0$ for all n. For other tissues, however, λ is positive for intermediate values of n, i.e. for values from an interval of $n_0<n<n_1$, where n_0 and n_1 reflect the tissue properties M and H and the receptor binding avidity h (Figure 2). As shown in the appendix, the optimal receptor destroying activity of n^* is given by

$$n^* = \frac{h}{\mu_o}\left(\sqrt{pHu}-u\right) \qquad \text{with} \qquad u = pH+\mu_o\left(H+M+\frac{p}{h}\right) \qquad (8)$$

We can conclude that viral growth is negative for extreme values of n and optimal for an intermediate level of receptor destroying activity n^*.

The optimum n^* depends on the receptor binding avidity h and the properties of the target tissue H and M. For this reason differences in H and M between target tissues will lead to different optima in the receptor destroying activity n^* and different regions of positive viral growth $n_0 < n < n_1$. In the next section we will demonstrate how these differences in optimal receptor destroying activity n^* can prevent viral spread between tissues of different receptor availability H and M.

In our model the situation is markedly different for HA avidity h. As shown in the appendix, the asymptotic growth rate of the virus is positively related to the rate of receptor binding avidity h. Therefore h should always evolve towards the maximal attainable value.

6.5 Differences in optimal rates of receptor destruction determine tissue specificity

In order to determine if a virus can spread between different tissues we need to determine whether a virus that is adapted to a tissue of origin X has a positive growth rate in a certain target tissue Y. Within one host organism tissues differ in their relative abundance of receptors in the mucus M and on the host cell H. A virus that is adapted to a tissue X with an optimal strategy n_X^* will be able to spread to a target tissue Y when the growth rate $\lambda\left(n_X^*\right)$ of a virus with NA activity n_X^* is positive in tissue Y. The target tissue Y in turn only provides a positive viral growth-rate for a receptor destroying activity n within the interval $n_{0,Y} < n < n_{1,Y}$. A virus can therefore not spread from tissue X to Y when n_X^* falls outside the interval $n_{0,Y} < n < n_{1,Y}$ (see Figure 3).

Lets now consider which viruses that are adapted to a different environment can invade a given reference environment. To calculate whether a virus from an environment $X = \left(H, M\right)$ can invade the

reference environment Y we calculated the optimal strategy n_X^* and determined whether $n_{0,Y} < n_X^* < n_{1,Y}$. This procedure divides the parameter space into environments X that do not allow viral growth at all [i.e. $\lambda\left(n_X^*\right) < 0$; black region in Figure 4], regions that allow growth in the reference environment Y [i.e. $n_{0,Y} < n_X^* < n_{1,Y}$; white region in Figure 4] and in regions where viruses adapted X cannot grow in Y [gray regions in Figure 4]. For a given value of M viral growth does not occur below a threshold level for the concentration of host receptors H. This threshold increases with M. The minimal level of H that is required for viral growth can be approximated by a linear function of M (see Appendix). Combinations of M and H that allow for viral growth, or $\lambda\left(n_X^*\right) > 0$, fall into two categories: Environments which produce viruses that can invade the environment Y and those which cannot. Intuitively, environments X which are more similar to environment Y can produce viruses that can spread from X to Y. Distance in the direction of M and H has, however, a different effect on tissue specificity. Whereas viruses from an environment with a higher value of H can all invade the reference environment Y, viruses from environment of either very low or very high values of M are not able to invade the reference environment. Accordingly, the mucus concentration M is the primary factor that causes tissue specificity (Figure 4).

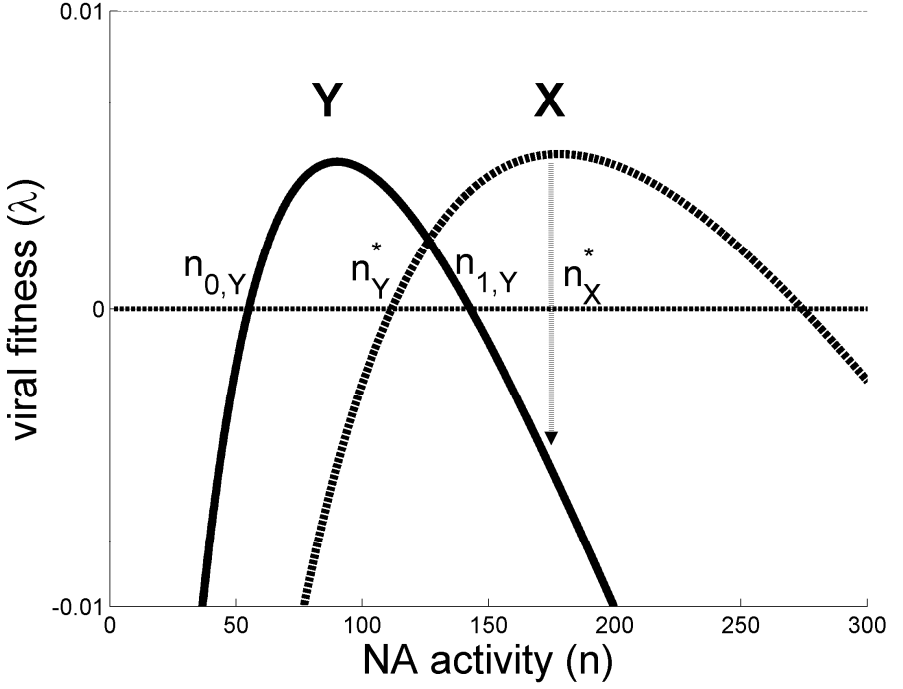

Figure 3: Tissue specificity of viral adaptation. Dependence of viral fitness (=asymptotic growth rate) on neuraminidase activity for two tissues X and Y. The tissues differ in the concentration of SA receptors on the host cell ($H_X = 3.7$, $H_Y = 2$) and in the surrounding mucus ($M_X = 200$, $M_Y = 50$). In environment Y, viral growth is possible for n-values between $n_{0,Y}$ and $n_{1,Y}$. The virus optimally adapted to environment X, does not satisfy this requirement (since $n_X^* > n_{1,Y}$) and hence has a negative growth rate in environment Y (see dotter arrow). Other parameters: $h = 1$, $b = 3$, $p = 10^{-2}$, $\mu_o = \mu_i = 0.15$.

6.6 Discussion

Conflicting selection forces can severely hamper viral adaptation. Here we studied the example of the receptor destroying enzyme neuraminidase (NA) of influenza virus to establish a framework that integrates conflicting enzymatic effects into the viral-life cycle. We furthermore use this model to predict viral adaptation to the environment. Our model demonstrates that viral adaptation to the prevailing tissue environment requires specialization of receptor destroying activity that can prohibit spread of the virus to other tissues. This specialization in receptor destroying activity could provide an additional mechanism for viral tissue and host specificity, next to other mechanisms, like the presence of suitable receptors and necessary proteases.

Viral fitness in the situation of an *in vivo* infection is affected by multiple factors like the viral replication rate, the ability to avoid the immune system and the rate of transmission between host organisms. In our model we focus on a single aspect of viral fitness which is its replication rate. Viral replication rate is generally strongly related to fitness. Maximal fitness can, however, deviate from the maximal replication rate, when viral replication has a negative side effect on other components of viral fitness. Viral replication can for example increase host mortality and therefore negatively affect viral transmission (Ewald 1994; van Baalen & Sabelis 1995); but see also (Ebert & Bull 2003). Furthermore, viral replication can have negative consequences for the avoidance of the specific immune system. Rapidly replicating viral mutants, for example, create a higher antigen dose and therefore a stronger specific immune response. In principle, this coupling between replication, antigen dose and immune response can select against strains with highest replication rate and create a rareness advantage of slow replicating mutants (Nowak et al. 1991). In some cases replication rate and immune avoidance can also be determined by a single mutation (Both et al. 1983). In our model, we do not address the action of a specific immune response. Instead, we

represent a non-specific immune response in terms of the decay parameters μ_o and μ_i. In principle the negative side effects of viral

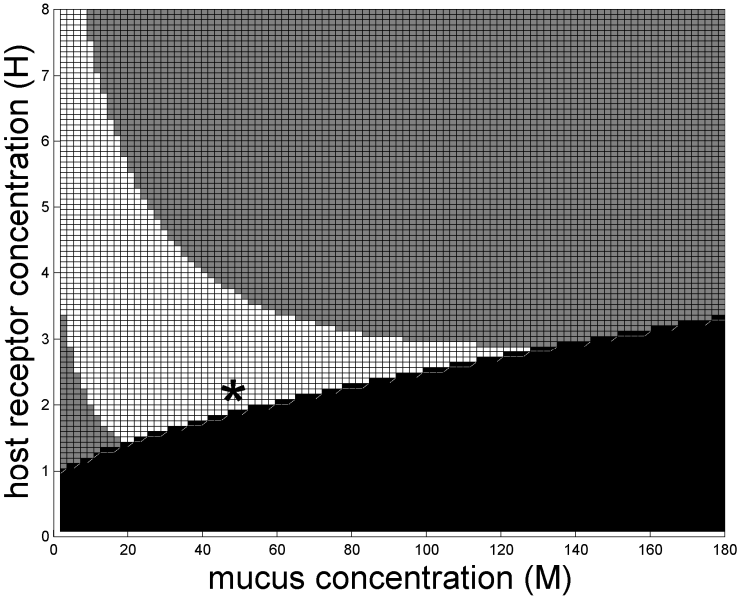

Figure 4: Parameter dependence of viral extinction and tissue specificity. Viral growth strongly depends on the tissue characteristics H (= concentration of SA receptors on host cells) and M (= receptor concentration on mucus). The black region corresponds to those tissues where viral growth is impossible irrespective of the value of neuraminidase activity n. The star marks a specific reference environment characterized by $M = 50$ and $H = 2$. The white region corresponds to those tissues where the virus optimally adapted to the given tissue is able to grow in the reference environment. Viruses derived from tissues in the grey parameter region cannot grow in the reference environment. Other parameters: $h = 1$, $b = 3$, $p = 10^{-2}$, $\mu_o = \mu_i = 0.15$.

replication onto future transmission and on immune avoidance can create other interesting trade-offs in addition to the here described conflicting selection on receptor destroying activity, which are

addressed elsewhere (Both et al. 1983; Nowak et al. 1991; Ewald 1994; van Baalen & Sabelis 1995). Assuming that the specific immune system is not affecting the early stages of viral infection we can nevertheless use the maximization of replication as a predictor for viral fitness as has been done by previous models for within-host viral growth [e.g. (Regoes et al. 2005)].

The intermediate optimum for receptor destroying activity has profound consequences for viral evolution. Most importantly, the adaptation to specific receptor destroying activity can prevent the spread of virus between different hosts and tissues. Yet, one has to consider the role of different optima in receptor destroying enzymes in relation to other mechanisms that determine viral tissue tropism. Viral spread between tissues is, for example also affected by the availability of cellular proteases that cleave the HA precursor HA0 to the active form of HA. For seasonal human influenza viruses and low pathogenic avian viruses the required proteases are expressed tissue-specifically. In contrast, highly pathogenic viruses, of avian origin (HPAI), contain a multibasic cleavage site which allows cleavage of HA_0 to its active form by ubiquitous proteases. Therefore, replication of HPAI does not require the presence of proteases that are specific for lung tissues. Consequently, avian HA_0 can be cleaved in tissues outside the lung which enables unrestricted viral spread and systemic infection. In avian hosts, the occurrence of a multibasic cleavage site shows a strong correlation with tissue tropism and virulence (Horimoto & Kawaoka 1994). In contrast, in mammalian hosts this correlation is weaker (Steinhauer 1999). In mammalian hosts, tissue differences in optimal receptor destroying activity could therefore be an important factor for tissue specificity, next to HA cleavability. Ultimately, the role of different optima of receptor destroying activity for tissue specificity remains, however, an empirical question.

Inhibitors of the receptor destroying activity of NA are currently the only possible pharmaceutical intervention for an influenza infection.

Obviously, NA inhibiting drugs disturb the balance of viral attachment and receptor destruction and therefore inhibit viral replication. Adaptation to NA-inhibitors can, however, readily occur through restoration of the attachment/detachment balance (Gubareva et al. 2001; Gubareva 2004; Reece 2007). Quantitative understanding of the evolutionary limitations of receptor destroying enzymes is an important tool to understand the limitations of viral adaptation to NA inhibitors. For example, different optima for receptor destroying activity between tissues predict situations in which the application of inhibitors of receptor destroying enzymes (NA-inhibitors) actually can promote the spread of virus between tissues. This undesired effect of NA-inhibitors can occur when a virus, that is adapted to an environment with a high optimum of receptor destroying activity, enters a tissue or host organism that requires a low rate of receptor destroying activity. In this case, reduction of NA activity by NA inhibitors moves the receptor destroying activity closer to the optimal level in the new environment and increase viral replication. Even worse, when the difference of optimal receptor destroying activity prevents viral spread between tissues, NA inhibitors could facilitate spread to new tissues and promote systemic infection. NA inhibitors have not yet been shown to increase viral replication directly. However, mutations that decrease the receptor destroying activity can indeed increase viral replication (Bin et al. 2005). It is therefore plausible that reduced receptor destroying activity, can increase viral replication in tissues with a low optimal receptor destroying activity and facilitate the spread from mucus to non-mucus tissues, regardless whether receptor destroying activity is reduced by NA-inhibition or directed mutations.

Tissue specific optima of receptor destroying activity imply that the adaptation to one environment worsens the success in another environment. When viral tissue specificity affects virulence and transmission, this effect can create a trade-off between viral virulence and transmission. As mucus-adapted viruses will be more successful in the mucus tissues which form the entrance route of infection, mucus

adaptation should promote transmission between host organisms. The virulence of a viral strain in turn depends on its ability to spread to mucus free tissues within one host orgamism, causing systemic infection. Even though viruses that are adapted to mucus tissues should transmit readily between host individuals, they are maladapted for growth in mucus free tissues and should therefore show low virulence. In turn, viruses that are adapted to mucus free tissues should be virulent but poorly transmitting. There is some evidence that highly virulent viral strains, indeed, transmit poorly in experimental transmission experiment even when the contact rate of animals is very high (Yen et al. 2007).

The trade-off between the specialization onto mucus containing and mucus free tissues, respectively, poses the question whether a generalist virus can evolve, which is able to reproduce in both types of environments and is therefore highly virulent and transmittable at the same time. Considering the evolutionary limitations on receptor destroying activity the occurrence of such a highly transmissible and highly virulent mutant might be less likely then previously thought. Evaluation of this possibility should focus on the measurement of HA avidity and NA activity as quantitative kinetic parameters in various tissues and host species.

The examples above demonstrate that biochemical constraints between different parts of the viral life cycle can be valuable to illuminate the limitations of viral evolution. Although biochemical conflicts in viral adaptation are likely to be ubiquitous, models that derive evolutionary limitations directly from the underlying biochemistry are scarce, probably because they require virus specific models. Current biochemical models of the viral life cycle focus on generic processes of the viral replication, like the dynamics of viral genome replication and protein production. For example, Krakauer & Komarova (2003) have focused on within cell processes like the encapsidation of viral genomes and its effect on viral genome replication. Regoes and

colleagues (2005) have investigated the optimal ratio of positive and negative RNA strands that maximizes the replication of polio virus. The example of receptor destroying enzymes demonstrates that mechanisms for conflicting selection lure also in other parts of the viral life cycle besides genome replication and packaging, like the attachment and detachment processes. Even though the mechanisms that constrain the evolution of receptor destroying activity are rather specific, these constraints have implications for a large group of animal pathogens.

6.7 References

Baigent, S. J. and J. W. McCauley. 2003. Influenza type A in humans, mammals and birds: determinants of virus virulence, host-range and interspecies transmission. *Bioessays,* 25: 657-671.

Bin, L., H. Zhou, D. Ye, G. Kemble and H. Jin. 2005. Improvement of influenza A/Fujian/411/02 (H3N2) virus growth in embryonated chicken eggs by balancing the hemagglutinin and neuraminidase activities, using reverse genetics. *Journal of Virology,* 79: 6763-6771.

Both, G. W., C. H. Shi and E. D. Kilbourne. 1983. Hemagglutinin of swine influenza-virus - a single amino-acid change pleiotropically affects viral antigenicity and replication. *PNAS,* 80: 6996-7000.

Bull, J. J., D. W. Pfennig and I. N. Wang. 2004. Genetic details, optimization and phage life histories. *Trends in Ecology & Evolution,* 19: 76-82.

Caswell, H. 2001. *Matrix Population Models : Construction, Analysis, and Interpretation*. Sinauer Associates, Sunderland, MA.

de Groot, R. J. 2006. Structure, function and evolution of the hemagglutinin-esterase proteins of corona- and toroviruses. *Glycoconjugate Journal,* 23: 59-72.

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

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

Gambaryan, A., A. Tuzikov, G. Pazynina, N. Bovin, A. Balish and A. Klimov. 2006. Evolution of the receptor binding phenotype of influenza A (H5) viruses. *Virology,* 344: 432-438.

Gubareva, L. V. 2004. Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. *Virus Research,* 103: 199-203.

Gubareva, L. V., L. Kaiser, M. N. Matrosovich, Y. Soo-Hoo and F. G. Hayden. 2001. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *Journal of Infectious Diseases,* 183: 523-531.

Horimoto, T. and Y. Kawaoka. 1994. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza-A virus. *Journal of Virology,* 68: 3120-3128.

Krakauer, D. C. and N. L. Komarova. 2003. Levels of selection in positive-strand virus dynamics. *Journal of Evolutionary Biology,* 16: 64-73.

Matrosovich, M. and H. D. Klenk. 2003. Natural and synthetic sialic acid-containing inhibitors of influenza virus receptor binding. *Reviews in Medical Virology,* 13: 85-97.

Matrosovich, M. N., T. Y. Matrosovich, T. Gray, N. A. Roberts and H. D. Klenk. 2004. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *Journal of Virology,* 78: 12665-12667.

Mitnaul, L. J., M. N. Matrosovich, M. R. Castrucci, A. B. Tuzikov, N. V. Bovin, D. Kobasa and Y. Kawaoka. 2000. Balanced hemagglutinin and neuraminidase activities are critical for efficient replication of influenza A virus. *Journal of Virology,* 74: 6015-6020.

Nowak, M. A., R. M. Anderson, A. R. Mclean, T. F. W. Wolfs, J. Goudsmit and R. M. May. 1991. Antigenic diversity thresholds and the development of Aids. *Science,* 254: 963-969.

Palese, P., K. Tobita, M. Udea and R. W. Compans. 1974. Characterization of temperature sensitive influenza-virus mutants defective in neuraminidase. *Virology,* 61: 397-410.

Reece, P. A. 2007. Neuraminidase inhibitor resistance in influenza viruses. *Journal of Medical Virology,* 79: 1577-1586.

Regoes, R. R., S. Crotty, R. Antia and M. M. Tanaka. 2005. Optimal replication of poliovirus within cells. *American Naturalist,* 165: 364-373.

Segel, L. A. 1984. *Modeling Dynamic Phenomena in Molecular and Cellular Biology*. Cambridge University Press, Cambridge.

Smits, S. L., G. J. Gerwig, A. L. W. van Vliet, A. Lissenberg, P. Briza, J. P. Kamerling, R. Vlasak and R. J. de Groot. 2005. Nidovirus sialate-O-acetylesterases - Evolution and substrate specificity of coronaviral and toroviral receptor-destroying enzymes. *Journal of Biological Chemistry,* 280: 6933-6941.

Stearns, S. C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

Steinhauer, D. A. 1999. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology,* 258: 1-20.

Stevens, J., O. Blixt, T. M. Tumpey, J. K. Taubenberger, J. C. Paulson and I. A. Wilson. 2006. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science,* 312: 404-410.

Suzuki, T., T. Takahashi, C. T. Guo, K. I. P. J. Hidari, D. Miyamoto, H. Goto, Y. Kawaoka and Y. Suzuki. 2005. Sialidase activity of influenza A virus in an endocytic pathway enhances viral replication. *Journal of Virology,* 79: 11705-11715.

van Baalen, M. and M. W. Sabelis. 1995. The scope for virulence management - a comment on Ewald's view on the evolution of virulence. *Trends in Microbiology,* 3: 414-416.

van Riel, D., V. J. Munster, E. de Wit, G. F. Rimmelzwaan, R. A. M. Fouchier, A. D. M. E. Osterhaus and T. Kuiken. 2006. H5N1 virus attachment to lower respiratory tract. *Science,* 312: 399.

Wagner, R., M. Matrosovich and H. D. Klenk. 2002. Functional balance between haemagglutinin and neuraminidase in influenza virus infections. *Reviews in Medical Virology,* 12: 159-166.

Wagner, R., T. Wolff, A. Herwig, S. Pleschka and H. D. Klenk. 2000. Interdependence of hemagglutinin glycosylation and neuraminidase as regulators of influenza virus growth: a study by reverse genetics. *Journal of Virology,* 74: 6316-6323.

Wang, I. N., D. E. Dykhuizen and L. B. Slobodkin. 1996. The evolution of phage lysis timing. *Evolutionary Ecology,* 10: 545-558.

Yen, H. L., A. S. Lipatov, N. A. Ilyushina, E. A. Govorkova, J. Franks, N. Yilmaz, A. Douglas, A. Hay, S. Krauss, J. E. Rehg, E. Hoffmann and R. G. Webster. 2007. Inefficient transmission of H5N1 influenza viruses in a ferret contact model. *Journal of Virology,* 81: 6890-6898.

6.8 Appendix

6.8.1 Existence of a region of positive viral growth

In view of (6) and the definition of r_1 and r_2 in (5) the asymptotic growth rate of the virus is given by

$$\lambda = \frac{1}{V_i}\frac{dV_i}{dt} = nb\left[\frac{phH}{\left(n+p+\mu_0\right)\left[h\left(M+H\right)-n\left(\frac{hH}{n+p+\mu_o}+\frac{hM}{n+\mu_o}\right)+\mu_o\right]}-1\right]-\mu_i \tag{A1}$$

This can be rewritten as

$$\lambda = nb\left[\frac{p}{p+\mu_0+\frac{\mu_0}{H}\left[M+\frac{n+p}{h}\right]+\frac{\mu_0^2}{H}\left[\frac{M}{n+p}+\frac{1}{h}\right]}-1\right]-\mu_i \qquad \text{(A2)}$$

For large n the quotient in (A2) converges to zero. Accordingly, the term in brackets, and with it the per capita growth rate λ, becomes negative for large values of n and converges to $\lambda = -\mu_o$. On the other end $\lambda = -\mu_i$ for $n = 0$. Therefore there are two scenarios: either λ is negative for *all* values of *n* (implying that the virus cannot persist), or λ is positive for an interval $n_0 < n < n_1$. An optimal n^* that supports viral growth, exists in this interval, when $\lambda(n)$ is concave and $\lambda(n^*) > 0$. To simplify the analysis we assume that ${\mu_0}^2 << H$ allowing us to drop the small term $\frac{\mu_0^2}{H}\left[\frac{M}{n+p}+\frac{1}{h}\right]$ from (A2) to arrive at

$$\lambda = nb\left[\frac{p}{p+\mu_0\left[1+\frac{1}{H}\left(M+\frac{n+p}{h}\right)\right]}-1\right]-\mu_i \qquad \text{(A3)}$$

It is now easy to see that λ is positive when

$$\frac{1}{H}\left(M+\frac{n+p}{h}\right) > x_0 = \frac{p}{\mu_0}\left[\frac{nb}{\mu_i+1}-1\right]-1 \qquad \text{(A4)}$$

or, equivalently

$$H > \frac{1}{x_0}\left(M+\frac{n+p}{h}\right) \qquad \text{(A5)}$$

Correspondingly λ is positive when

$$h > \frac{n+p}{x_0 H - M} \qquad \text{(A6)}$$

We can conclude from (A5) that for $\mu_0^{\,2} << H$ the minimal H permitting viral growth is approximately linearly related to M (see also Figure 4A) and from (A6) we can see that the minimal h that enables viral growth asymptotically approaches infinity for $M \to x_0 H$ (see Figure 4B). Therefore at the critical mucus concentration $M_c = x_0 H$ no further increase in h can support viral growth.

6.8.2 Optimal NA activity and HA avidity

In order to calculate the optimal NA activity n^* we solve the equation $\partial\lambda / \partial n = 0$ which yields

$$n^* = \frac{h}{\mu_0}\left(\sqrt{pHu} - u\right) \quad \text{with} \quad u = pH + \mu_0\left(H + M + \frac{p}{h}\right) \tag{A7}$$

Furthermore, n^* is a local maximum when λ is concave in n or

$$\frac{\partial^2 \lambda}{\partial n^2} < 0 \qquad \Leftrightarrow \qquad \mu_0\left(H + M + p\right) + hpH > 0 \tag{A8}$$

This implies that n^* is always a local maximum for positive values of μ_0, h, p, H, M.

In contrast to the selection for intermediate for NA activity n^*, HA avidity h should evolve to its attainable maximum. This can be seen directly from (A3). When $h \to \infty$ the viral growth rate $\lambda(h)$ increases until it asymptotically reaches a maximum

$$\lambda_{\max} = nb\left[\frac{pH}{p + \mu_0\left(1 + \frac{M}{H}\right)} - 1\right] - \mu_i \tag{A9}$$

For high values of h the asymptotic growth rate is therefore insensitive to changes in h.