Chapter 2

Evolution of viral development of RNA phage MS2 under experimentally disturbed gene balance

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2.1 Abstract

Viral development depends on the careful timing and balance of viral gene production. To understand the role of gene balance for viral development and evolution we over-expressed each of the four genes of RNA phage MS2 during the infection cycle, and evolved the phage under these conditions of disturbed gene balance. Even though overexpression provides viral gene products ‘for free’, this disturbance reduced viral burst size by orders of magnitude and altered the timing of lysis. Phage MS2 was able to adapt its gene regulation to this disturbance in some directions, but failed for others. Adaptation to overexpression of the coat gene led to a burst size above that of the ancestor in the absence of overexpression, but increased lysis time. Adaptation to replicase overexpression shortened lysis time, but resulted in a lower burst size. Viral strains that had adapted to lysis
gene overexpression had only half the lysis time of the ancestor in the absence of overexpression. Surprisingly this had no cost for burst size. The lysis time of MS2 seems therefore to be constrained by lysis efficiency. Our results demonstrate that within limits the shape of the viral growth curve can respond to selection and becomes the target of evolution itself.

2.2 Introduction

Viruses are ideal model systems to study the adaptation of organisms to changes in the environment. The adaptation of the viral life cycle to environmental challenges depends on the availability of genetic variation in viral gene regulatory processes and the biochemistry of the host cell. Optimality models aiming to predict adaptation of the viral life cycle to the environment rarely incorporate such mechanistic details (Bull et al. 2004). A notable exception is the study of bacteriophage lysis timing that incorporates the expression of lysis genes into models for the optimal timing of the viral life cycle (Wang et al. 2000; Young et al. 2000; Heineman et al. 2005; Wang 2006). Here we take this approach one step further and investigate the role of other viral genes for the shape of the viral growth curve and viral lysis timing.

Theory of lysis timing predicts that viruses should evolve a lysis time that balances the increase of viral fecundity due to prolonged within-host replication against the decrease of viral fecundity due to delayed release of viral particles (Abedon 1989; Wang et al. 1996). The optimal lysis time is determined by two factors; the increase of viral particles during replication within the host, given by the viral growth curve, and the average time required to spread to a new host, given by the host density. Optimal viral life history therefore depends on both, the environment and the viral growth curve. Experiments have demonstrated the evolution of lysis time in response to the environment (Abedon et al. 2001), but studies that address the effect of changes in the viral growth curve are lacking.
In virtually all models of viral life history evolution the viral growth curve plays a central role. The viral growth curve integrates the complex dynamics of viral development into a small number of phenotypic parameters (most notably lysis time and burst size) that can be quantified by the classical one-step growth curve essay (Gong et al. 1996; Alexander et al. 1998). These parameters are determinants of viral fitness and therefore crucial ingredients in models that relate viral lysis timing to the evolution of viral virulence (Bull 2006).

However, the shape of the viral growth curve is an evolvable feature itself. In fact, the shape of the viral growth curve reflects the interplay of viral nucleotide replication and the production of viral structural and lysis proteins during intracellular replication. These processes are subject to a number of unavoidable constraints and trade-offs. First, viral genome replication and translation occur in opposite direction along the genome, and can therefore not occur simultaneously (Eigen et al. 1991; Regoes et al. 2005). Second, the packaging process interferes with viral genome replication (Krakauer & Komarova 2003). Additionally, the early stages of development can exhaust resources that are required for later development (Katanaev et al. 1996). Viral gene regulation is predestined to create a balance that optimizes viral growth given these constraints and trade-offs. However, it is largely unknown to what extent the limitations of intra-cellular development constrain the evolution of viral gene regulation and the viral life cycle.

Small bacteriophages contain only few genes and therefore offer an exquisite possibility to study the evolution viral gene balance and the viral growth curve. One example is the single-stranded RNA phage MS2 that contains a genome of only 5.3kb encoding the four genes maturation, coat, lysis and replicase. Regulatory interactions between these genes are well characterized. Coat represses replicase and the expression of coat and lysis are positively correlated by read-through translation. In contrast, expression of maturation is independent of the
expression of other MS2 genes and it does not affect the balance of other MS2 genes (Poot et al. 1997; Van Duin & Tsareva 2004).

The RNA genome of MS2 folds into an intricate secondary structure that is involved in gene regulation by protein-RNA interactions. A key element in viral gene regulation is the central operator loop of MS2 (pos. 1715-1765) that is part of the overlapping replicase and lysis genes, forms the coat termination hairpin, contains the Shine-Dalgarno sequence and the start-codon of the replicase gene (Olsthoorn et al. 1994). Expression of replicase is controlled by two mechanisms: the Min-Jou long-range RNA-RNA fold and the binding of the coat protein to the operator (Van Himbergen et al. 1993; Licis et al. 1998). Coat-RNA binding is at the same time the nucleation point of the viral packaging process (Peabody 1997).

Due to these structural constraints and functional overlap it is obvious that regions like the central operator loop are subject to a multitude of pleiotropic effects that strongly limit sequence evolution (Olsthoorn et al. 1994; Licis & Van Duin 2006). By means of an experimental evolution approach we aim at shedding light on the question how these structural constraints affect the evolution of the viral growth curve. To this end we confronted MS2 with a host environment that disturbs the natural balance of MS2 gene expression during the infection cycle. This was achieved by cloning individual genes of MS2 in an expression plasmid and in-trans production of a surplus of these genes during the infection cycle of MS2. By this approach we investigated the immediate physiological effects of viral gene disturbance on the shape of the viral growth curve. Furthermore, we followed the adaptation of MS2 to these conditions of disturbed gene regulation in order to investigate in how far the virus can evolve its own gene regulation to restore the viral growth curve. Knowledge of this evolutionary flexibility of the viral growth curve is essential for predicting viral evolution, since the shape of the viral growth is a key ingredient for models of optimal viral life history.
2.3 Material & Methods

2.3.1 Strains and growth conditions

We acquired MS2 ancestor by heat induction (10 min 42°C) from the MS2 cDNA plasmid pMS2000 that we received as a generous gift from René Olsthoorn. The host E.coli Top10F’ (*Invitrogen*) was used for all experiments under standard growth conditions (37°C, Luria broth LB). The plasmid pBAD and the F’ plasmid, required for MS2 infection, were maintained by Ampicillin (Amp) and Tetracycline (Tet) resistance markers, at 100 µg/ml Amp and 60 µg/ml Tet (LB+Amp+Tet) in all growth media.

2.3.2 Cloning

The ORF’s of the genes maturation, coat, lysis and replicase were PCR amplified from pMS2000 by forward primers that introduce a NcoI restriction site at the start codon and reverse primers that introduce a HindIII restriction site after the stop codon. The PCR products were sequentially digested by NcoI and HindIII (*New England Biolabs*) and ligated into NcoI and HindIII digested plasmid pBAD (*Invitrogen*). All constructs were verified by sequencing. We refer to the plasmids carrying the maturation, coat, lysis and replicase ORF’s as pMat, pCoat, pLys and pRep, respectively. The primers that were used for cloning are summarized in Table 1.

2.3.3 Protein electrophoresis

After induction, the cells carrying pMat, pCoat, pLys and pRep were expected to express an additional protein of molecular weights of 42 kDa, 12 kDa, 8 kDa and 60 kDa (*Katanaev et al. 1996*), respectively. To verify this, cells carrying pMat, pCoat, pLys, pRep and empty pBAD were diluted 1:10 into LB+Amp+Tet and grown for 4h prior to induction with 10 mM Arabinose (ara). Upon induction the cells were grown at 30°C for additional 4h, paletted by centrifugation and boiled
for 5 min in sample buffer (4 % SDS, 10 % 2-Mercaptoethanol, 20 % Glycerol, 0.1 % Broomphenol blue, 100 mM Tris, pH 8.0) prior to electrophoresis on a 4-20% polyacrylamide gradient gel for 2-4h at 300V. For the cells carrying pMat, pCoat, pLys and pRep we observed bands at the expected molecular weights that are absent in the negative control (Figure 1A). Expression of the smaller lysis protein could not be visualized by protein electrophoresis, but could be demonstrated by its phenotype of cell lysis upon ara induction of a pLys-carrying strain (see Figure 1B).

Table 1: Primers used for cloning of the four MS2 genes

**Maturation**

<table>
<thead>
<tr>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>5’ GCGCGCCATGG CACGAGCTTTTAGTACCCTT</td>
<td>5’ GCGCGAAGCTT CTA TCTAGAGAGCGTTCCTG</td>
</tr>
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**Coat**

<table>
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<tbody>
<tr>
<td>5’ GCGCGCCATGG CAGCTTCTAACTTTACTCAGTT</td>
<td>5’ GCGCAAGCTT CTA TTAGTAGATGCCGGAGT</td>
</tr>
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**Lysis**

<table>
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<th>Reverse</th>
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<tr>
<td>5’ GCGCGCCATGG CAGAAACCCTGATTCCCTCAG</td>
<td>5’ GCGCAAGCTT TTA AGTAAGCAATTGCTGTAAG</td>
</tr>
</tbody>
</table>

**Replicase**

<table>
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<tr>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ GCGCGCCATGG CATCGAAGACAACAAAGAAGTT</td>
<td>5’ GCGCAAGCTT CTA CCGAGGAGAGCTCG</td>
</tr>
</tbody>
</table>
Figure 1: Demonstration of gene expression in host cells carrying plasmids with an MS2 gene. (A) SDS-PAGE of total protein of cells carrying the plasmids pMat (M), pCoat (C), pLys (L) and pRep (R) after 2h of induction with 10 mM arabinose (ara). In case of pMat, pCoat, and pRep (R) an extra band is visible that corresponds to the expected size of the maturation (42 kDa), coat (12 kDa) and replicase (60 kDa) proteins, respectively. These bands are absent in cells carrying the empty pBAD plasmid (Con-). (B) The lysis protein (expected size 8 kDa) could not be visualized by SDS-PAGE. Instead expression of pLys was demonstrated by a drop of density in the bacterial growth curve after induction with 10 mM ara.
2.3.4 One-step growth curve assays

With the help of an automated setup (Figure 2) we were able to perform fine-scale measurements of the one-step growth curve of 8 viral strains in parallel. The strains were diluted to a titer of $10^6$ pfu/ml. The titers were confirmed by plating and plaque count. Host cell cultures were inoculated in 10 ml LB+Tet+Amp from 100 µl frozen aliquots (in 40% Glycerol at -80°C) and grown aerated in exponential phase for 6 h until OD600=1 (~$10^7$ cfu/ml). With one exception, plasmid expression was induced 30 min prior to infection by 10 mM ara. In the case of pLys the time for induction was reduced to 5 min prior to infection, since after 30 min of induction of pLys most cells were already lysed (Figure 1B) (The latter effect was not yet known when the experiment underlying Figure 3B was performed. For this reason, the induction time of pLys was 30 min). Induced cells were infected at a multiplicity of infection smaller than one (MOI < 1) and incubated for 20 min at 37°C without shaking. The infected host cells were applied to a filter-holder that carries a 0.2 µm PVDF membrane (Millipore) and pre-rinsed by 20 times the volume of the filter chamber (20 x 0.2 ml) in order to remove free phage. Subsequently, we rinsed the infected cells by a constant flow (8 volume changes per 10 min) of growth medium and collected the filtered fractions in a 96-well block. The 96 phage fractions thus obtain a stable titer since the infected host cells are retained on the filter. The rate of phage production per time interval for each of the 8 growth curves was determined by top-agar plating and plaque count of all 96 obtained fractions (12h incubation at 37°C and saturated humidity).

2.3.5 Estimation of burst size and lysis time

We estimated burst-size as the ratio of the phage fraction with the highest titer and the titer in the initial fraction. Lysis time was estimated by the time point after which the slope of the one-step-growth curve starts to decrease. The reference values of lysis time and burst size of
the ancestor in the absence of overexpression were based on the data underlying Figure 3A (Burst-size 1.6*103 pfu, lysis time 65min).

**Figure 2: Automated setup for the measurement of the one-step growth curve.** Infected host cells are immobilized on 0.2 µm filters that retain infected bacteria but allow newly produced phage to pass through the filter. With the aid of a multi-channel peristaltic pump, sterile medium is pumped through the filters and all newly produced phage are washed through the filter into the tubes of a 96-well fraction collector. The fraction collector is moved to a new position at constant time intervals. The collected fractions of phage lysate per time interval can be used to determine the rate of phage production by plating and counting plaque forming units. By this method the growth curves of 8 viral strains can be assayed in parallel under identical environmental conditions.
2.3.6 Conditions of experimental evolution

Host strains were daily inoculated from frozen aliquots and grown in 6 ml LB in exponential phase for 3h and induced with 10 mM ara, 30 min prior to infection with MS2. As explained above, hosts that carry pLys, were induced 5 min prior to infection. Infected cells were grown for 6h at 37°C. At the end of the growth cycle the remaining host cells were killed by the addition of chloroform and vortexing. Phage were transferred to freshly grown and induced cultures daily, with the aid of sterile toothpicks.

2.3.7 Estimation of viral fitness

A virus that grows with burst size $B$ and generation time $\tau$ can go through $\frac{1}{\tau}$ generations per time interval and therefore produces $B^{1/\tau}$ offspring. We take the logarithm of this as our fitness measure

$$W = \log \left( B^{1/\tau} \right) = \frac{1}{\tau} \log(B).$$

The values in Figure 5 are represented relative to the fitness of the ancestor in the absence of overexpression

$$W_A = \frac{1}{65} \log(1.6 \cdot 10^3).$$

2.4 Results

2.4.1 Immediate effect of MS2 gene overexpression

The overexpression of all four viral genes negatively affected the growth of MS2, but the magnitude strongly differed between genes (see Figure 3). Whereas the overexpression of coat, replicase and lysis reduced the burst size by two or three orders of magnitude (factor of 1500, 400 and 1000 respectively), the overexpression of maturation
reduced phage production only by a factor 3. As indicated above, this mild effect of *maturation* is not surprising, since overexpression of maturation has no known regulatory consequences for other MS2 genes (Poot et al. 1997).

### 2.4.2 Evolutionary adaptation to overexpression

We evolved viral populations on host cells that carry pRep, pCoat, pLys for 20 consecutive transfers in batch culture, with 4 replicates per treatment. Evolution to pMat-carrying cells was omitted, since expression of maturation showed little effect on the viral growth curve and will therefore only create weak selection. We referred to the 12 evolutionary lines as R1, R2, R3, R4, C1, C2, C3, C4 and L1, L2, L3, L4, respectively. To indicate the cycle numbers we use a notation like C1-20 for transfer 20 of line C1.

Within the first five days of experimental evolution the populations L2 and R2 went extinct. This suggests that selection was stringent. In order to enable survival of the remaining lines in the lysis overexpression treatment (L1, L3, L4) we reduced the induction of pLys from 30 min to 5 min prior to infection (equivalent conditions are used in Figure 4B).

Adaptation of viral strains to the conditions of overexpression substantially improved viral reproduction with respect to the unevolved ancestor (Figure 4). The effect of adaptation differed between the three overexpressed genes, but was highly consistent within the replicate evolution lines per treatment.
Figure 3: Effect of overexpression of MS2 genes on the one-step growth curve of the wildtype strain. (A) Growth curve of the wildtype strain of MS2 in host cells that carry the plasmids pMat, pCoat, pLys and pRep without the induction of protein expression. Burst size is about $1.6 \cdot 10^3$ pfu, and lysis time is about 65 min. (B) Growth curve of the wildtype strain after induction leading to overexpression of the maturation, replicase, coat and lysis proteins. Overexpression of replicase, coat and lysis proteins leads to a strong reduction in burst size, while there is only a marginal reduction in burst size in case of maturation-overexpression.
Strains that evolved under coat overexpression (Figure 4 A) recovered a burst size that is comparable to the burst size of the ancestor in the absence of overexpression (Table 2 and Figure 5 A). Surprisingly lines C2-20 and C3-20 even evolved a 3 fold higher burst size than that of the wild type in the absence of overexpression. Apparently these two strains evolved a strategy to use the surplus of coat protein. However, the strains that evolved under overexpression of coat show a delay in lysis time by 10-20 min relative to the ancestor in the absence of overexpression (Table 2, Figure 5B). This negative effect of increased lysis time more than compensates for the increase in burst size. Hence after 20 generations of adaptation the overall fitness of the evolved lines was still smaller than the fitness of the ancestor in the absence of overexpression.

Table 2: Lysis time and burst size of evolved strains and wild type

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lysis time</th>
<th>Burst size</th>
<th>95% confidence interval of burst size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancestor</td>
<td>65</td>
<td>1553</td>
<td>± 161</td>
</tr>
<tr>
<td>C1-20</td>
<td>75</td>
<td>983</td>
<td>± 484</td>
</tr>
<tr>
<td>C2-20</td>
<td>85</td>
<td>3196</td>
<td>*</td>
</tr>
<tr>
<td>C3-20</td>
<td>85</td>
<td>5012</td>
<td>± 646</td>
</tr>
<tr>
<td>C4-20</td>
<td>75</td>
<td>1418</td>
<td>± 249</td>
</tr>
<tr>
<td>R1-20</td>
<td>55</td>
<td>417</td>
<td>± 55</td>
</tr>
<tr>
<td>R3-20</td>
<td>55</td>
<td>394</td>
<td>± 59</td>
</tr>
<tr>
<td>R4-20</td>
<td>55</td>
<td>661</td>
<td>± 181</td>
</tr>
<tr>
<td>L1-20</td>
<td>30</td>
<td>1196</td>
<td>± 568</td>
</tr>
<tr>
<td>L3-20</td>
<td>30</td>
<td>905</td>
<td>± 79</td>
</tr>
<tr>
<td>L4-20</td>
<td>30</td>
<td>669</td>
<td>± 111</td>
</tr>
</tbody>
</table>

* no replicate
Figure 4: One-step growth curves after adaptation to overexpression of the viral genes (A) coat, (B) lysis and (C) replicase. (A) Lines that were adapted to the conditions of coat overexpression (C1-20, C2-20, C3-20 and C4-20)
reach a burst size that is three orders of magnitude higher than that of the unevolved ancestor, but show a delayed lysis time. (B) Lines that were adapted to the conditions of lysis overexpression (L1-20, L3-20 and L4-20) reach a larger burst size and shorter lysis time than the unevolved ancestor. Within a short lysis time these strains produce a substantial burst size. (C) Lines that were adapted to the conditions of replicase overexpression (R1-20, R2-20 and R4-20) reach a burst size that is three orders of magnitude higher than the burst size of the unevolved ancestor and show a shortened lysis time.

Strains that evolved under lysis overexpression (Figure 4 B) reached a burst size that is below that of the ancestor in the absence of overexpression (Table 2, Figure 5 A). However, the relatively mild reduction was associated with a strong reduction in lysis time (30 min in strains L1, L3 and L4 vs. 65 min in the ancestor in the absence of overexpression) (Figure 5 B). Considering that this lysis time allows the strains to complete almost two generations in the time of a single generation of the ancestor, the adapted lines have a much higher fitness than the ancestor in the absence of overexpression (Figure 5C). (Note: Here the burst size of the ancestor under lysis overexpression is larger than in Figure 3B since the expression of lysis is milder. See M&M).

Lines that were adapted to replicase overexpression (Figure 4 C) could partially restore their burst size and showed a shortened lysis time. All the surviving lines R1, R3 and R4 showed a 10 min shorter lysis time than that of the ancestor in the absence of overexpression, at a burst size that is below the burst size of the ancestor in the absence of overexpression (Table 2, Figure 5A). The negative effect on burst size is partially restored by the shortened lysis time and adaption to replicase results in fitness that is comparable to the ancestor in the absence of over-expression (Figure 5C).
2.5 Discussion

The parameters of the viral growth curve are the central ingredients of optimality models that predict the adaptation of the viral life cycle to environmental conditions. The viral growth curve reflects the molecular mechanisms underlying viral development, but the interplay of these mechanisms is largely unknown. Previous experimental work has mainly focused on the effect of lysis gene expression on the timing of lysis (Heineman et al. 2005; Wang 2006). Here we consider the balance of all viral genes that are expressed upstream to the expression of the lysis gene. In order to systematically investigate the fitness consequences of the disturbance of all four genes of the bacteriophage MS2 we overexpressed each of its genes individually and determined the effect of overexpression on the viral growth curve. Furthermore we evolved the virus under conditions of viral gene overexpression enabling us to study viral adaptation to a disturbance of gene balance.

Overexpression of coat, replicase and lysis reduced viral fecundity by orders of magnitude whereas maturation had only a weak effect on viral reproduction. The weak effect of maturation is not too surprising since maturation has no known regulatory interactions with other viral genes (Poot et al. 1997). Overexpression of maturation is therefore not likely to disturb the balance of viral development and has only minor consequences for the viral growth curve. In contrast to maturation the genes coat, replicase and lysis are coupled by mutual regulatory interactions that balance genome replication and protein synthesis. In the natural infection cycle of MS2 the expression of replicase is downregulated by two independent mechanisms. The so-called Min-Jou long-range RNA interaction prevents replicase expression in the absence of coat translation (Van Himbergen et al. 1993). In addition coat protein acts as direct translation repression of replicase by binding to the translation initiation site of replicase (Peabody 1997).
Figure 5: Burst size, lysis time and fitness of evolved lines relative to the ancestor in the absence of overexpression. (ANC represents the ancestor)
This redundancy in the repression of replicase suggests that uncontrolled replicase expression has severe fitness costs. These costs probably arise from a trade-off between the production of viral RNA and the production of viral proteins. Viral RNA replication consumes host resources in the early stage of development that may become limiting during late development. Excessive viral RNA replication would be inefficient since more genome strands would be produced than could be packaged. This effect was demonstrated during the formation of MS2 particles in an in vitro translation system (Katanaev et al. 1996). When increasing amounts of viral RNA are added to an in vitro translation system the production of MS2 particles increases until a maximum in the production of viral particles is reached. When more than this optimal amount of viral RNA was added, the production of viable viral particles sharply declined. In our experiment, the fitness effect of replicase overexpression is likely caused by the same trade-off between the production of viral RNA and proteins. The limited degree of adaptation to replicase overexpression achieved in our experiment indicates that this trade-off cannot easily be compensated by changes in viral gene regulation. In view of these results, stimulation of viral genome replication might offer an interesting anti-viral strategy that is resilient to viral adaptation. This is in striking contrast to many anti-viral strategies that aim at the exact opposite – a reduction of viral genome replication (Xu et al. 2007; Paeshuysse et al. 2007; Kong et al. 2007; Vrancken et al. 2008).

Coat is a direct repressor of replicase (Peabody 1997). Overexpression of coat has therefore been proposed as a potential anti-viral strategy in the MS2-related phage Qβ (Lindemann et al. 2002). Experiments designed to test this idea were, however, not able to maintain viral replication repression, since Qβ could readily adapt to coat overexpression by avoiding the repressive action of the coat protein. In our experiments we found the same rapid adaptation to overexpression of coat in MS2. Interestingly, adaptation to overexpression of coat even led to a burst size that by far exceeds that of the ancestor in the absence
of overexpression (C2-20 and C3-20 in Figure 5A). One explanation is that overexpression of coat provides a protein that is limiting viral growth during late viral development. For normal viral development the coat protein is required in an amount that is 6 to 10 times higher than that of any other viral gene (Beremand & Blumenthal 1979; Katanaev et al. 1996). During late infection coat might therefore be limiting and external production might relax this limitation. Since coat is a repressor of replication the virus nevertheless needs to avoid the repressive action of coat during early infection in order to utilize it during late infection. Evolution of a complete avoidance of coat-RNA binding is not possible, since the coat-RNA interaction is the starting point of viral packaging (Peabody 1997). For this reason residual replicase repression through the utilization of excess coat might explain the delayed lysis time in the phage that evolved to coat overexpression.

Viral packaging might become a limiting factor for intra-cellular viral development also for another reason. It has been proposed that in single-stranded RNA viruses the rate of packaging could exhaust the pool of replicating RNA genomes and push viral replication to a standstill (Krakauer & Komarova 2003). In principle, this might explain the delayed lysis time in the lines adapted to coat overexpression. For two reasons, we consider this explanation unlikely. First, the positive feedback of RNA replication on replicase production leads to an increase of RNA genomes at a rate that is proportional to the square of the RNA concentration, whereas the rate of packaging increases only linearly with the RNA concentration (Eigen et al. 1991). It is therefore not easy to perceive how packaging could outcompete RNA replication. Second, plus-stranded RNA (RNA+) viruses like MS2, copy through a RNA+ to RNA- template mechanism (Regoes et al. 2005). Since the RNA- templates are not packaged they accumulate in excess abundance (about 50 times the amount of RNA+ strands). Replication from RNA- templates will therefore continue even if all RNA+ strands are packaged. Accordingly, the exhaustion of the pool of viral RNA genomes through the packaging process is not likely to slow
down the viral production process in the absence of a repression mechanism for replicase.

Adaptation to lysis overexpression reduces lysis time by half at almost no costs for burst size. Halved lysis time allows the phage adapted to lysis overexpression to go through two generations in the time frame of one ancestral generation in the absence of overexpression. This greatly increases viral fitness. It is therefore an intriguing question why the virus does not reduce its lysis time during a natural infection. The most straightforward explanation is that in MS2 the achievable lysis time is constrained by the expression level and/or the efficiency of the MS2 lysis protein. Indeed, the single-component lysis system of phage MS2 is known to be less efficient than the two-component lysis system of phages of comparable genome size, like DNA phage ΦX174 or the RNA phage f2 (Loeb & Zinder 1961; Wang et al. 2000; Young et al. 2000). The two-component lysis systems of ΦX174 and f2, allow a lysis time of 25 min and 40 min, respectively, with a burst-size comparable to that of MS2 (Loeb & Zinder 1961; Beremand & Blumenthal 1979; Pepin et al. 2006). The relative inefficiency of the lysis system of MS2 is apparently caused by inefficient lysis expression, since artificial expression of the same lysis protein readily lyses host cells within 30 min after induction (Figure 1B). This constraint in lysis time in MS2 contrasts with the general idea that lysis time can evolve freely to match the environmental optimum (Bull et al. 2004).

Our findings demonstrate that the viral life cycle is integrated through the balance between processes during early and late viral development. Both lysis timing and virus production strongly reflect the interplay of viral genome replication and protein production. Models for the evolutionary optimization of the viral life cycle need to consider the finite supply of host resources as well as the trade-offs between replication and protein production. Integration of these molecular trade-offs into current theoretical approaches could provide an important link
between evolutionary modelling and detailed knowledge of viral gene regulation.

2.6 References


