Complementation and Epistasis in Viral Co-infection Dynamics

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Running Head:
Interaction of Complementation with Epistasis in Viruses

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Abstract

Co-infection in RNA virus populations results in two important phenomena, complementation and recombination. Of the two, complementation has a strong effect on selection against deleterious mutations as has been confirmed in earlier studies. As complementation delays the purging of less-fit mutations, co-infection may be detrimental to the evolution of a virus population. Here we employ both deterministic modeling and stochastic simulation to explore the mechanisms underlying the interactions between complementation and other evolutionary factors, namely, mutation, selection, and epistasis. We find that strong complementation reduces slightly the overall fitness of a virus population but substantially enhances its diversity and robustness, especially when interacting with selection and epistasis.
1 Introduction

RNA viruses infect many organisms. They have high mutation rates, compact segmented genomes, very short generation times, huge population sizes and substantial diversity within populations (Chao, 1988; Wilke and Novella, 2003; Froissart et al., 2004; Elena et al., 2006). These properties enable RNA viruses to change continuously and adapt easily to adverse environments, posing continual challenges for anti-viral therapies and vaccinations. Current research on RNA viruses has turned to theoretical population genetics and evolutionary theory for novel and practical approaches for management of viral diseases (e.g., Turner and Chao (1999); Burch et al. (2003); Turner and Chao (2003); Burch and Chao (2004); Dennehy and Turner (2004); Moya et al. (2004); Bretscher et al. (2004); Sanjuán et al. (2005); Boni et al. (2006)).

Co-infection, where multiple viruses infect a cell simultaneously, is important in the life cycle of RNA viruses. During co-infection, haploid viral genomes act in a manner similar to those of diploid organisms, exchanging genetic material randomly or preferentially. Reassortment of genome segments may create severely deleterious mutational combinations, thus speeding up the elimination of mutational load. Co-infection may, however, result in complementation, where viruses carrying different deleterious mutations may benefit from the normal products that each can produce, so that both types of viruses can be represented in the offspring. In contrast to recombination, complementation weakens the selection against deleterious mutations. In this way, it contributes to the stability of the whole virus population as it maintains a high level of diversity without sacrificing the overall fitness of the population.

Froissart et al. (2004) investigated whether complementation or random reassortment would have the stronger effect on selection against deleterious mutations in viral life cycles. They proposed a simple model combining the processes of viral replication and free recombination and, via both experiments and simulation, confirmed the major role of complementation in reducing the selective pressure against deleterious mutations. However, their model assumes only two independent loci and does not accommodate such key evolutionary factors as mutation or higher-order epistasis. They simplified their model by ignoring mutation. In reality, as RNA viruses are highly muta-
ble, a viral population does not completely eliminate deleterious mutations after a period of time, contrary to what appears to be the case in Froissart et al. (2004), but reaches a dynamical balance between selection and mutation. When studying the long run evolution of a viral population, mutation becomes a key factor that should be taken into account appropriately. Epistasis is another important evolutionary factor that affects many evolutionary processes (Desai et al., 2007). Epistasis may be classified as synergistic or antagonistic, which may act on the mutation-selection balance in different ways and also interact with different levels of complementation. Epistasis can also be classified as magnitude and sign epistasis, where magnitude epistasis at a locus indicates that the magnitude of fitness change (loss or gain) of a mutation depends on the genotypic background; sign epistasis exists if the sign, as well as the magnitude, of the fitness cost of a mutation is affected by the genotypic background (Weinreich et al., 2005). To date, the roles of these types of epistasis interacting with co-infection have not been studied systematically. In modeling viral evolution, it may be important to incorporate epistasis and, in particular, to study how the effects exerted by complementation interact with different types of epistasis.

Here we extend Froissart’s model to allow for multiple loci as well as mutation, and complex interactions among mutations. We investigate how complementation interacts with epistasis and how this interaction affects the evolutionary dynamics of viruses. We utilize both deterministic modeling and stochastic simulation to investigate these interactions between complementation and mutation, selection, and epistasis. To introduce mutation, we divide the process of viral replication into three stages, prereplication preparation, replication, and assembly. For each segment of the viral genome, complementation and epistasis determine the strength of selection, and these occur before replication. Mutation occurs during replication, and random reassortment take place after replication during assembly. Mutation and random reassortment can be considered as a time-reversible process as the process of replication of one segment multiple times and then randomly choosing one copy for each assembly is equivalent to randomly choosing one of the two parental viral segments and replicating that segment, and repeating these two steps multiple times. Therefore, it is reasonable to consider mutation as a separate step. For epistasis, we employ the model
of interactions similar to that used by DESAI et al. (2007), including higher order epistasis. Our results indicate that although it has only moderate effects on the overall mean fitness, complementation interacting with epistasis has a strong effect on the composition and diversity of the virus population.

2 Model

We first consider an RNA virus population evolving under selection, complementation, random reassortment, and epistasis. Suppose the virus is haploid with $L$ segments. Without complementation, we assume each segment carrying one or more deleterious mutation incurs a fitness cost $s$ and $s \ll 1$. In this setting, following the multiplicative selection model proposed by DESAI et al. (2007), a virus with $k$ mutated segments has fitness $e^{-ks} \approx 1 - ks$.

We start with Froissart’s model combining complementation, selection, and reassortment, and also assume that the frequency of co-infection with more than two virions per cell is negligible (OLKKONEN and BAMFORD, 1989; TURNER et al., 1999; FROISSART et al., 2004). The effect of interactions among mutations is similar to DESAI et al. (2007), but with additional higher order interactions. We assume that double mutants have fitness $e^{-2s(1+\epsilon)}$ and triple mutants have fitness $e^{-3s(1+\epsilon+\eta)}$, where $\epsilon$ is the first-order epistasis parameter and $\eta$ is the second-order epistasis coefficient. For each viral life cycle, we define $F_0$ to be the frequency of the wild type viruses in the population at the beginning of this cycle and $F_{(i,j,\ldots,k)}$ to be the initial frequency of viruses with mutations on segments $i, j, \ldots, k$, where $1 \leq i, j, \ldots, k \leq L$. As in our model mutations are deleterious, we assume viruses carrying four or more deleterious mutations are lethal, i.e., the frequencies of haploids carrying more than three mutations are negligible, i.e., $F_{i,j,k,m} \approx 0$. We denote $F_0^*$ and $F_{(i,j,\ldots,k)}^*$ as the frequencies of wildtype and mutants, respectively, after complementation, epistasis, selection, and reassortment. Denote $p_c$ as the fraction of co-infections out of the total number of infections occurring per generation. Then the wildtype genotype frequency $F_0^*$ after the
action of these four forces is:

\[
\bar{w} F^*_0 = (1 - p_c) F_0 + p_c \left\{ F_0^2 + \sum_i \frac{2}{2} F_0 F_i + \sum_{i,j;i<j} \frac{2}{2} F_0 F_{i,j} + \sum_{i,j;i<j} \frac{2}{2} F_i F_j + \sum_{i,j,k;i<j<k} \frac{2}{2} F_0 F_{i,j,k} \right. \\
+ \sum_{i,j,k;i\neq j,k;k<j} \frac{2}{2} F_i F_{j,k} + \sum_{i,j,k,m;i\neq j,k,m;k<j<m} \frac{2}{2} F_i F_{j,k,m} I_{\{L\geq 4\}} \\
+ \sum_{i,j,k,m;i\neq j,k,m;k<j<m} \frac{2}{2} F_{i,j} I_{\{L\geq 4\}} \\
+ \sum_{i,j,k,m,i\neq j,k,m;i<j;k<m} \frac{2}{2} F_{i,j} F_{k,m} I_{\{L\geq 5\}} \\
+ \sum_{i,j,k,m,n;i\neq j,k,m,n;i<j;k<m<n} \frac{2}{2} F_{i,j} F_{k,m,n} I_{\{L\geq 6\}} \left\} \right.
\]

(1)

where \( I_{\Phi} \) is an indicator function and is equal one if \( \Phi \) is true; otherwise, it is zero. The complicated formulas of frequencies \( F^*_0, F^*_i, \) and \( F^*_{i,j} \) are given in Appendix. Summing these frequency formulas, we obtain the mean fitness \( \bar{w} \).

### 2.1 Mutation

For viruses, mutations mostly occur during the replication processes. Selection, as a result of complementation and epistasis, mainly occurs during protein synthesis before replication. Random reassortment occurs during assembly of replicates. In our simulation, we can consider mutation and random reassortment as reversible. We can reverse the process of replication of one segment and then randomly choosing one copy by randomly choosing one of the two parental viral segments and then replicating the chosen segment, since the two processes are, indeed, equivalent. Thus it is reasonable to separate mutation from the other events. (We also show in Appendix that this separation step does not alter the resulting frequencies, but speeds up the calculation). Denote \( F'_0, F'_i, F'_{i,j}, \) and \( F'_{i,j,k} \) as the frequencies of haploids carrying zero, one, two, and three mutations, respectively, after mutation. We now assume that in each segment the wild type allele can mutate to the deleterious allele at rate \( \mu \) per individual per generation. We further assume no back mutation occurs and ignore the terms with higher orders of \( \mu \) as \( \mu \ll 1 \). This produces the following
recursions.
\[
F'_0 = (1 - L\mu)F^*_0,
\]
\[
F'_i = \mu F^*_0 + (1 - (L - 1)\mu)F^*_i,
\]
\[
F'_{i,j} = \mu(F^*_i + F^*_j) + (1 - (L - 2)\mu)F^*_{i,j},
\]
\[
F'_{i,j,k} = \mu(F^*_i + F^*_j + F^*_k) + F^*_{i,j,k}.
\]  

(2)

3 Simulation

As these recursions cannot be solved analytically, we conducted both deterministic and stochastic simulations to study the stationary distribution of allele frequencies. For deterministic simulations, we iterated the recursions (1), (2), and (A-1) - (A-3) 5000 times (corresponding to 5000 generations) with different values of the parameters. For stochastic simulations, we simulated 100 lineages of haploid viruses, with three segments in the genome, independently over 5000 generations. For generation \( t \) in lineage \( l \) (\( t = 1, 2, \ldots, 5000; \ l = 1, 2, \ldots, 100 \)), we used the following sampling procedure:

- According to FROISSART et al. (2004), the proportions of bacteria infected by zero, one, and two viruses follow a Poisson distribution with mean equal to the multiplicity-of-infection (MOI; ratio of phages to bacterial cells). We denote by \( Pr(0) \), \( Pr(1) \), and \( Pr(2) \) the Poisson probabilities that a cell is infected by zero, one, or two viruses, respectively. Assuming no more than two viruses affect one cell simultaneously, the expected proportion of co-infection with two viruses out of the total number of infections is \( Pr(\text{Co-infection}) = \frac{Pr(2)}{1 - Pr(0)} = p_c \).

Thus each cell is co-infected with probability \( p_c \) or has a single infection with probability \( Pr(\text{Infection}) = \frac{Pr(1)}{1 - Pr(0)} = 1 - p_c \). The frequency of viruses that co-infect is \( f_{co} = 2Pr(\text{Co-infection})/(2Pr(\text{Co-infection}) + Pr(\text{Infection})) = \frac{2p_c}{1+p_c} \). Suppose we have \( N_0 \) viruses at the beginning of generation \( i \) and whether a virus infects or co-infects occurs as a series of Bernoulli trials with the probability of success equal to \( f_{co} \), i.e., we sample the number of co-infecting viruses \( k_{co} \) from a Binomial distribution \( Binom(f_{co}, N_0) \) and need to ensure it is an even and positive number (If an odd number is sampled, we do an extra
sampling step from the uniform distribution on [0,1]. If the sampled value is less than 0.5, we deduct one from the odd number; otherwise, we add one to this odd number to make it an even number. We can also discard this odd number and continue to sample until we find an even number.). Within the group of the $k_{co}$ chosen co-infecting viruses, we randomly group them into pairs by iterating the following steps:

1. Sample an individual virus with probability $\frac{1}{k_{co}}$.
2. Sample the other virus of this pair with probability $\frac{1}{k_{co} - 1}$.
3. Let $k_{co} = k_{co} - 2$.
4. Repeat step 1 and 2 until $k_{co} = 2$.

- Each cell, either infected by a wild-type virus or co-infected by two viruses that can complement each other’s defects, produces approximately the same number of offspring, which can be treated as a normal random variable $m \sim N(\delta, \sigma^2)$, where $\delta$ is the expected number of offspring produced by a wildtype virus and $\sigma^2$ is its variance. If a virus carrying one, two, or three mutant segments infects a cell, the number of offspring produced follows $m \sim N(\delta e^{-s}, \sigma^2 * e^{-2s})$, $N(\delta e^{-2s(1+\epsilon)}, \sigma^2 * e^{-4s(1+\epsilon)})$, and $N(\delta e^{-3s(1+\epsilon+\eta)}, \sigma^2 * e^{-6s(1+\epsilon+\eta)})$, respectively, where $s$ is the selection coefficient, $\epsilon$ is the pairwise epistasis coefficient and $\eta$ is the second order epistasis coefficient. Similarly, if two mutant viruses co-infect a cell with one, two, or three mutant segments in common, they will produce a number of progeny similar to the case of a single infection above.

- When viruses replicate genetic material in co-infected cells, assuming random reassortment, each virion randomly inherits a segment from one of its parents, i.e., each segment is selected from one of its parents with chance 0.5.

- During the replication, we assume that deleterious mutations take place at $\mu = 0.01$ per segment per virus generation. According to Drake and Holland (1999) and Drake (2006), the median spontaneous mutation rate of RNA viruses is approximately 0.76 per
genome per replication cycle. Thus it is reasonable to assume a deleterious mutation rate 0.01 per segment per generation after accounting for advantageous mutations. We can sample mutations according to this probability and keep track of them. Each mutation occurs on a wildtype segment and may be inherited by offspring. All mutations suffer the same fitness loss $s$. If a mutation takes place on a mutant segment, we assume this will not have any additional effect on the overall fitness of a virus.

- As the virus population size increases exponentially, a sampling step is necessary to keep a constant sample size that is computationally tractable, namely, 10,000. That is if the population size is greater than 10,000, we apply a Bernoulli sampling process without replacement to generate a sample of 10,000.

4 Results

To evaluate the effects of interactions between complementation and other factors, we ran both deterministic and stochastic simulation at five levels of co-infection (i.e., $p_c \in \{0.1, 0.3, 0.5, 0.7, 0.9\}$) under nine genetic scenarios (see Table 1). For stochastic modeling, we averaged results across 100 lineages and assessed the variation between lineages. The results from deterministic iterations are mostly similar to those from the stochastic simulation and both are shown in supplemental materials. Here we focus mainly on the stationary distribution using the stochastic simulation (as the viral system might get stuck in an unstable equilibrium in the deterministic model as discussed in Discussion section), and also show the three-way interactions among complementation, selection, and epistasis based on deterministic modeling.

4.1 Interaction of complementation with mutation

We modified the complementation model proposed by Froissart et al. (2004) to take mutation dynamics into account. In reality, RNA viruses mutate at such high rates that mutations cannot be neglected even over a short time period and, in the long run, a virus population reaches a mutation-
selection balance instead of completely eliminating deleterious mutations from the population. The life cycle of an RNA virus is typically composed of five steps, absorption and penetration, synthesis of viral proteins, RNA replication, assembly, and release. Complementation and selection take place during the period of viral protein synthesis, and reassortment happens just before the replication of each segment, whereas nucleotides mutate during the replication process. Therefore, it is reasonable to separate mutation from the other processes in our model.

In the absence of mutation, the virus population clears all the initial polymorphisms efficiently at all levels of co-infection, as illustrated in Figure 1(A1-A5) and Supplemental Figures S1 and S6. With a mutation rate of 0.01 per generation per segment, mutations always exist in the population and the mean fitness is about 0.03 lower when the population reaches the mutation-selection equilibrium than in the no mutation case. This is reasonable; according to classical population genetic theory the expected mean fitness of a viral population at the mutation-selection balance is \(1 - L\mu = 0.97\). We observed drastic changes in the patterns of frequencies of wildtype, single mutants, double mutants, and triple mutants. With no mutation, the wildtype’s frequency is fixed at one whereas mutants’ frequencies quickly drop to zero. Generally the frequencies of mutants decrease faster with low co-infection percentages. When mutation occurs, the wildtype frequency increases until it reaches a limit and this stationary frequency decreases as \(p_c\) increases. The stationary wildtype frequency drops more at high levels of co-infection than at low co-infection levels. The frequencies of single mutants decrease from one to a lower level under low co-infection rates than with high co-infection, and the difference between two consecutive co-infection levels remains approximately the same irrespective of the co-infection rate. Both double and triple mutants show a similar frequency pattern with their equilibrium frequencies substantially higher with frequent co-infections than with rare co-infections.

### 4.2 Interaction of complementation with selection

We then investigated how complementation affects the evolutionary dynamics of virus populations when the selection pressure varies. From the mean fitness plot (see Figure 1(B1-B5) and Sup-
plemental Figures S2 and S7), different selection effects drive the virus population to a similar equilibrium of mean fitness, but the composition of this population varies markedly when the selection coefficient increases from 0.1 to 0.2. When selection becomes stronger, more mutants are eliminated from the population; thus the balanced frequencies of wildtype viruses generally increase for all co-infection levels and this increase is larger and more obvious at a high co-infection level. The opposite pattern is observed for single mutant frequencies which drop more with rare co-infection than with frequent co-infection. Both double and triple mutants show a drastic frequency drop at high co-infection levels and their frequencies remain close to zero with low co-infection rates when the selection parameter increases to 0.2. We observed that the virus system reaches the equilibrium state faster under stronger selection, which implies that increasing selection pressure will weaken the effect of complementation on maintaining population diversity, and drive the population to purge mutations faster.

4.3 Interaction of complementation with pairwise epistasis

Synergistic epistasis entails that mutations show a stronger effect in combination than the sum of their individual effects. Antagonistic epistasis ($\epsilon < 0$) makes this effect weaker. When the epistatic effect is moderate, e.g., $\epsilon = \pm 0.3$ as in Figure 1(C1-C5) and Supplemental Figures S3 and S8 (known as magnitude epistasis (WEINREICH et al., 2005)), the overall mean fitness is similar at different levels of co-infection, with slight differentiation among the cases of positive, negative, and no epistasis. Compared with the case of no epistasis, synergistic epistasis increases the frequency of wildtype while antagonistic epistasis reduces its frequency, and their effects are generally enhanced as $p_c$ increases. For single mutants, both types of epistasis have subtle effects in the presence of strong or weak complementation (e.g. 0.9 or 0.1, respectively), but their effects become stronger at intermediate levels of co-infection (e.g., around 0.5). Strong complementation has a much larger impact on the stationary frequencies of double and triple mutants when interacting with both types of epistasis as illustrated in Figure 1 (C4, C5). At high $p_c$, the frequencies of double and triple mutants increase more with antagonistic epistasis than they are reduced with syn-
ergistic epistasis. When the epistatic effect increases dramatically (\(| \epsilon > 0.5 \)|), say ±0.8, the effect of sign epistasis occurs (WEINREICH et al., 2005), i.e., double and triple mutants can have much higher or lower fitness than single mutants, resulting in remarkably variable frequency trajectories before stabilizing (see Figures 1(D1-D5) and 2 and Supplemental Figure S4). When mutations interact synergistically, the mean fitness equilibria shift to just above those without epistasis, and the higher the co-infection rate, the greater the mean fitness. Synergistic interaction affects the patterns of frequencies of wildtype and single mutants in a manner similar to strong selection. But it has a much stronger effect in eliminating double mutants and triple mutants, as illustrated in Figure 2 where their frequencies are seen to decrease dramatically to close to zero. Antagonistic epistasis, in contrast, drives wildtype and single mutants to elimination while double and triple mutants thrive in the population. Strong complementation slows down the process of eliminating the wildtype and single mutants. It is reasonable that double and triple mutants constitute the ultimate population as our simulation starts with pure single mutants, and double and triple mutants are superior to single mutants; random reassortment shuffles these segments and generates a lot more combinations of mutations that accumulate over time, while wildtypes and single mutants are removed. The frequencies of double mutants and triple mutants fluctuate dramatically and it takes a much longer time to reach the balanced state. Frequent co-infection tends to elevate the frequencies of triple mutants over double mutants as triple mutants are easily generated through recombination. As double and triple mutants are frequent and they have a slightly lower fitness than the wildtype, the mean fitness with antagonistic epistasis is approximately 0.02 lower than that with synergistic interaction.

4.4 Interaction of complementation with higher order epistasis

We also compared the results from scenarios VIII and IX (described in Table 1) to assess how higher order epistasis interacts with complementation and how this interaction affects the virus population. Second-order epistasis tends to have a much milder effect on mean fitness and frequencies of wildtype and single mutants (see Figure 1(E1-E5) and Supplemental Figures S5 and S9).
The difference in wildtype proportions and double mutant proportions between positive and negative second-order epistasis becomes slightly apparent as the co-infection fraction increases to 0.9. Second order epistasis has a fairly strong effect on the fraction of triple mutants when the fraction of co-infections is high. This is reasonable as the initial virus population is composed of single mutants, and double mutants are less fit than single mutants or wildtypes. Few double mutants exist, leading to a low number of triple mutants even though they are strongly favored by selection.

4.5 Three-way interaction among complementation, selection, and epistasis

To observe evolutionary trajectories under the simultaneous influence of complementation, selection and epistasis, we iterated deterministically for 5,000 generations, assuming mutation rate 0.01 and initial frequencies as in Table 1, using a series of parameters, \( p_c \in \{0.1, 0.3, 0.5, 0.7, 0.9\} \), \( s \in \{0.0, 0.01, \ldots, 0.49, 0.50\} \) and \( \epsilon \in \{-0.50, -0.48, \ldots, 0.48, 0.50\} \) (only magnitude epistasis is considered here, i.e., fitness decreases monotonically with increasing \( k \), the number of mutant segments in a virus). From Supplemental Figure S10, we observe the overall mean fitness at equilibrium decreases as selection intensity increases and epistasis decreases, and strong complementation tends to weaken the effect of selection and synergistic epistasis on mean fitness. The stationary frequency of wildtype increases gradually with the strength of selection, while frequent co-infection and antagonistic epistasis substantially reduces the speed at which the wildtype frequency increases, as shown in Figure 3. Figure 4 shows an intriguing frequency pattern of single mutants that starts at zero in the absence of selection against deleterious mutations, then sharply increases and attains its maximum when the selection coefficient increases to \( 0.05 \sim 0.1 \), but gradually decreases as selection becomes stronger. Strong complementation and negative epistasis generally slow down the reduction of the single mutant frequencies. Double mutants have an equilibrium frequency pattern similar to that of single mutants, "start low - increase - decrease", though they disappear more quickly as the selection coefficient increases, as in Figure 5. Triple mutants take over the virus population when the selection coefficient is zero, as mutations without fitness cost continually accumulate. Then the frequencies of triple mutants decrease dramatically
as stronger selection is applied (see Figure 6). Even strong complementation and antagonistic epistasis have moderate effects on selection against triple mutants. Through these three-way interaction patterns, we find that both selection and synergistic epistasis tend to eliminate mutations and combinations of mutations, whereas strong complementation and antagonistic epistasis drive the virus population in the opposite direction.

5 Discussion

Co-infection generates two important phenomena in the viral life cycle, reassortment and complementation. It is commonly acknowledged that reassortment benefits the virus population by generating combinations of deleterious mutations, thus accelerating the removal of these mutations, whereas complementation is detrimental to the overall fitness of the group as it allows the population to harbor some deleterious mutations for long periods of time. Froissart et al. (2004) showed that complementation, rather than reassortment, is a major force in the evolution of viruses. What is the real role of complementation in viral evolution that allows viruses to sacrifice higher mean fitness, especially when complementation interacts with mutation, selection, or epistasis?

Here we extend the complementation model by Froissart et al. (2004) to multiple loci and incorporate the key evolutionary factors, mutation and epistasis, into the model. This more realistic model enables the assessment of the evolutionary impact of the interactions between complementation and mutation, selection, epistasis as well as higher order epistasis. Our simulation has confirmed Froissart’s result that in the absence of mutation, a high co-infection ratio delays the clearance of deleterious mutants in a virus population. We also find that with mutation, the virus population achieves almost the same overall mean fitness at equilibrium irrespective of the levels of co-infection. Scrutinizing the population composition, we observe that strong complementation lowers the wildtype frequencies and increases the frequencies of mutants. Similar results are found in the case of selection and epistasis; complementation has a moderate effect on the overall ability of the virus population to reproduce, but plays a major role in the population composition. In this
way, complementation increases the diversity and stability of a virus population substantially while maintaining similar or slightly lower fitness, which in turn improves the chance of survival of a virus population when coping with adverse environments.

In our modeling of recombination, we assume implicitly that reassortment is always random during replication and segments of two viruses are randomly incorporated into offspring genomes with probability 0.5 when co-infecting a cell. These assumptions are not necessarily met in reality. Preferential associations among RNA segments have been observed in several types of RNA viruses (Graham et al., 1987; Urquidi and Bishop, 1992). And the abundance of offspring segments may also vary due to the lengths of virus segments, the concentration of RNA polymerase, etc. Though the effect of recombination is critical when interacting with epistasis, as shown in Bretsch er et al. (2004), we focus here on the interaction between complementation and other factors, thus simplifying our model but without loss of generality. We also assume a fixed selection coefficient for all mutations in each simulation scenario, which, although not necessarily true in reality, helps to reveal the mechanism underlying the interaction between complementation and different types of epistasis.

To model sign epistasis, which has been detected in RNA viruses (e.g., HIV-1 (Mammano et al., 2000)) and is mostly induced by antiviral drugs, we modify the epistasis model of Desai et al. (2007) by taking the product of the number of mutations and epistatic effect \((e^{-sk(1+\epsilon)})\) instead of raising the number of mutations to the power of epistatic effect \((e^{-sk^{1+\epsilon}})\). This enables us to contrast magnitude epistasis with sign epistasis. In the deterministic simulation with sign epistasis, we observe that the frequencies of the wildtype and single mutants reach an equilibrium above zero when co-infection rates are above 0.7, as illustrated in Supplemental Figure S4, while in the stochastic simulation, these frequencies decrease to zero. We conducted further simulations to investigate this phenomenon under different initial conditions. We observe that initial frequencies affect the equilibrium states of the deterministic system (see Supplemental Figure S11). This is probably because the deterministic system has more than one equilibrium states when \(p_c\) is greater than 0.7. In the stochastic simulation, many small perturbations occur, easily driving the virus
population to leave one balance state and enter into another stable state, as is shown in Figure 2. We also observe that it takes a much longer time for a virus system to reach an equilibrium with antagonistic sign epistasis in the stochastic simulation than in the deterministic model. This may result from the strong fluctuations produced in the stochastic case that add lots of variation to the frequencies of double and triple mutants. The variance of frequencies among those 100 simulated lineages is generally quite low (data not shown), probably owing to the congruent initial frequencies for all simulation replications.

Complementation with synergistic epistasis functions differently from that with antagonistic epistasis. Synergistic epistasis tends to strengthen the selection against the combinations of less-fit mutations, while frequent co-infection generally weakens the selection against all mutants, with the result that wildtypes and single mutants constitute a major proportion of the virus population, and the stronger the complementation, the lower the frequency of wildtypes. In the presence of antagonistic epistasis, the pattern of frequency variation becomes complex. With magnitude antagonistic epistasis, (i.e., the magnitude of their effect may depend on the genetic background although the sign of fitness effect of mutations is unconditional (WEINREICH et al., 2005)), wildtypes and single mutants are slightly advantageous over double mutants. At equilibrium, wildtype, single mutants, and double mutants all constitute a major fraction of the population when co-infection is frequent. With sign epistasis, i.e., both wildtype and combinations of mutations are more advantageous than single mutants in terms of selection (WEINREICH et al., 2005), at the beginning we observe a sharp increase in the frequencies of both wildtype and double mutants then a quick drop in single mutants through random reassortment and selection. As mutations accumulate over time in the population, the number of wildtypes decreases gradually until it is close to zero. The frequency of single mutants continues to drop as these are the least fit genotypes. The number of double mutants increases to a peak and decreases sharply as co-infection begins to play a major role, which generates a sharp rise in the frequency of triple mutants, although double mutants are slightly superior to triple mutants. The fluctuations in the frequencies of double and triple mutants before equilibrium are mainly due to stochasticity, which does not appear in the deterministic simulation.
co-infection rates lead to higher frequencies of triple mutants at stationarity, because with high $p_c$, triple mutants have a greater chance of sharing normal gene products and transmitting their mutant genes to offspring; thus the viral system tends to produce more triple mutant offspring. Therefore, the overall mean fitness of the population reaches almost the same equilibrium value at all levels of co-infection with antagonistic epistasis.

When complementation, selection, and epistasis interact simultaneously, the overall mean fitness decreases as selection, i.e., fitness cost, increases. Increase in the co-infection rate and decrease of the epistasis coefficient also reduce the mean fitness since both co-infection and antagonistic epistasis ($\epsilon < 0$) result in accumulation of mutations, and double and triple mutants have inferior fitness compared with wildtype or single mutants although the effects are greater than additive. The "low-high-low" trajectories of single mutant frequencies along the selection axis occur because when selection against deleterious mutations is very weak, e.g. 0.01, triple mutants become a major part of the population. As the selection strength increases to about 0.1, the fitness of single mutants is slightly less than that of wildtype but the fitness cost increases exponentially with the number of mutations; thus double and triple mutants are inferior and the frequency of single mutants reaches a peak. High levels of co-infection tend to expand this stage until the selection coefficient increases to 0.2, as complementation and random reassortment can mitigate the increased fitness cost for single mutants. Strong negative epistasis retards the increase in frequency of single mutants as it reduces the fitness cost of double and triple mutants. As selection strength continues to increase, the wildtype becomes much more superior to single mutants, resulting in a gradual reduction in single mutant frequencies. The frequency pattern of double mutants is similar to that of single mutants although double mutants are more sensitive to the increase of either selection strength or synergistic epistasis. Triple mutants start at the highest frequency with weak selection and then their frequency drops sharply as selection increases. Co-infection and strong negative epistasis tend to exert mild effects on the decrease of triple mutant frequencies.

We anticipate that our results will have broad implications in viral disease management. Strong complementation results in weaker selection against deleterious mutations generally, and may even
favor mutants against wildtype when complementation interacts with antagonistic epistasis. A large number of mutations persist in the population, and through random reassortment, multiple combinations of mutant segments can be generated. Both magnitude and sign antagonistic epistasis can exist (Weinreich et al., 2005; Mammano et al., 2000), which substantially reduces the efficacy of antiviral drugs. Therefore, reducing the co-infection ratio is critical in controlling the spread and variation of viruses. Isolation of virus-infected patients or organisms and alternating drug usage over a period of time are commonly used to reduce the co-infection level. We also suggest using multiple antiviral drugs simultaneously instead of one at a time. As antiviral drugs are mainly designed according to the structure of viral proteins, there is a greater chance that not all viruses are eliminated in a population with high $p_c$ when these kinds of therapies are applied, as some mutated viral molecules will not be affected by these specifically-designed drugs. In addition, as viruses mutate with high frequency and have short generation times, these drug resistant viruses will, with high probability, either mutate into fitter individuals or recombine with those carrying strongly favored genes, thus spreading their drug resistant genes rapidly. This implies that using a single type of antiviral therapy will not work efficiently. It is more rational to design antiviral drugs for both wildtype proteins and potential mutant proteins and apply them simultaneously. In this way, the number of viruses will be reduced dramatically even if it is a stable system with strong complementation. When the density of viruses is very low, the chance of co-infection becomes minimal and the effect of complementation in reducing the selection against deleterious mutations is ameliorated.
6 Acknowledgement

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APPENDIX: Details of Deterministic Model

1. Frequencies of Mutants

The single mutant frequency $F^*_i$ after the action of complementation, epistasis, selection, and reassortment is:

$$
\bar{w} F^*_i = (1 - p_c) F_i e^{-s} + p_c \{ F_i^2 e^{-s} + F_0 F_i + \sum_{j:j \neq i} F_i F_{i,j} e^{-s} + \sum_{j:j \neq i} \frac{1}{2} (F_i F_j + F_i F_{i,j}) \\
+ \sum_{j,k,j,k \neq i,j<k} \frac{1}{2^2} (F_i F_j + F_0 F_{i,j,k}) + \sum_{j,k,l,j,k \neq i,l<j<k} \frac{1}{2} (F_i F_{i,k} + F_i F_{i,j,k}) e^{-s} \\
+ \sum_{j,k,m,j,k \neq m,j<k<l} \frac{1}{2} F_{i,j,k} + \sum_{j,k,l,m,j,k \neq m,j<i,j<k} \frac{1}{2} F_{i,j,k} F_{i,m} e^{-s} I_{\{L \geq 4\}} \\
+ \sum_{j,k,m,j,k \neq m,i,j<k<m} \frac{1}{2} F_{i,j,k} F_{m} I_{\{L \geq 4\}} \\
+ \sum_{j,k,l,m,j,k \neq l,j<k,l<m} \frac{1}{2} F_{i,j,k} F_{i,m} e^{-s} I_{\{L \geq 5\}} \\
+ \sum_{j,k,l,m,j,k \neq l,j<k,l<m} \frac{1}{2} F_{i,j,k} F_{i,m} I_{\{L \geq 5\}} \\
+ \sum_{j,k,l,m,j,k \neq l,j<k,l<m} \frac{1}{2} F_{i,j,k} F_{k,l,m} I_{\{L \geq 5\}} \\
+ \sum_{j,k,l,m,j,k \neq l,j<k,l<m} \frac{1}{2} F_{i,j,k} F_{l,m,n} I_{\{L \geq 6\}} \}.
$$

The double mutant frequency $F^*_{i,j}$ after the action of complementation, epistasis, selection,
\[
\bar{w} F_{i,j}^* = (1 - p_c) F_{i,j} e^{-2s(1+\epsilon)} + p_c \{ F_{i,j}^2 e^{-2s(1+\epsilon)} + \frac{1}{2} F_{i,j} F_i F_j + F_i F_j + F_j F_i \} F_{i,j} e^{-s} \\
+ \sum_{k:k\neq i,j} \frac{1}{2} F_{i,j} (F_{i,k} + F_{j,k}) e^{-s} + \sum_{k:k\neq i,j} \frac{1}{2} (F_{i,k} F_{i,j} + F_{j,k} F_{i,j} + F_{j,k} F_{i,k}) F_{i,j} e^{-s} \\
+ \sum_{k:k\neq i,j} \frac{1}{2} (F_{i,j} F_k + F_{i,j} F_{j,k} + F_{j,k} F_{i,j}) e^{-s} + \sum_{k,m:k\neq i,j} \frac{1}{2} (F_{i,m} F_{j,m}) F_{i,j} e^{-s} I_{(L \geq 4)} \\
+ \sum_{k:k\neq i,j} F_{i,j} e^{-2s(1+\epsilon)} + \sum_{k,m:k\neq i,j,k < m} \frac{1}{2} F_{i,j} F_{i,j,m} e^{-2s(1+\epsilon)} I_{(L \geq 4)} \\
+ \sum_{k,m,j:k\neq m\neq i,k < m} \frac{1}{23} (F_{i,j} F_{k,m} + F_{i,k} F_{j,m}) I_{(L \geq 4)} \\
+ \sum_{k,m,j:k\neq m\neq i,k < m} \frac{1}{23} (F_{i,j} F_{k,m} + F_{i,k} F_{j,m}) I_{(L \geq 4)} \\
+ \sum_{k,l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{24} (F_{i,j,k} F_{i,l,m} + F_{i,k} F_{j,l,m}) e^{-s} I_{(L \geq 4)} \\
+ \sum_{k,l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{24} F_{i,j,k} F_{i,l} I_{(L \geq 4)} \\
+ \sum_{k,l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{24} (F_{i,j,k} F_{i,l,m} + F_{i,j} F_{j,l,m}) I_{(L \geq 4)} \\
+ \sum_{k,l,m,n,j:k\neq l\neq m\neq n\neq i,l < m < n} \frac{1}{2^n} F_{i,j,k} F_{i,l,m} I_{(L \geq 0)} \}.
\]

(A-2)

The triple mutant frequency \( F_{i,j,k}^* \) after the action of complementation, epistasis, selection, and reassortment is:

\[
\bar{w} F_{i,j,k}^* = (1 - p_c) F_{i,j,k} e^{-3s(1+\epsilon + \eta)} + p_c \{ F_{i,j,k}^2 e^{-3s(1+\epsilon + \eta)} + \frac{1}{2} (F_i + F_j + F_k) F_{i,j,k} e^{-s} \\
+ \frac{1}{2} (F_i F_j + F_j F_k + F_k F_{i,j} + F_0 F_{i,j,k}) + \frac{1}{2} (F_{i,j} F_i + F_{i,j} F_j + F_{i,j} F_{j,k}) e^{-s} \\
+ (F_{i,j} + F_{i,k} + F_{j,k}) F_{i,j,k} e^{-s(1+\epsilon)} + \sum_{m:m\neq i\neq j\neq k} \frac{1}{27} (F_{i,m} F_{j,m} + F_{j,m} F_{i,m}) F_{i,j,k} e^{-s} I_{(L \geq 4)} \\
+ \sum_{m:m\neq i\neq j\neq k} \frac{1}{27} (F_{i,j,m} F_{i,k} + F_{i,j,m} F_{j,k} + F_{i,j,m} F_{j,k,m}) e^{-s} I_{(L \geq 4)} \\
+ \sum_{m:m\neq i\neq j\neq k} \frac{1}{27} (F_{i,j,m} F_{i,k} + F_{i,j,m} F_{j,k} + F_{i,j,m} F_{j,k,m}) F_{i,j,k} e^{-s(1+\epsilon)} I_{(L \geq 4)} \\
+ \sum_{l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{2^4} (F_{i,j} F_{k,m} + F_{i,k} F_{j,m} + F_{j,k} F_{i,m} + F_{i,j,k} F_{i,m}) I_{(L \geq 4)} \\
+ \sum_{l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{2^4} (F_{i,j} F_{k,m} + F_{i,k} F_{j,m} + F_{j,k} F_{i,m} + F_{i,j,k} F_{i,m}) I_{(L \geq 4)} \\
+ \sum_{l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{2^4} (F_{i,j} F_{k,m} + F_{i,k} F_{j,m} + F_{j,k} F_{i,m} + F_{i,j,k} F_{i,m}) I_{(L \geq 4)} \\
+ \sum_{l,m,j:k\neq l\neq m\neq i,l < m} \frac{1}{2^n} F_{i,j,k} F_{i,l,m} I_{(L \geq 6)} \\
+ \sum_{l,m,n,j:k\neq l\neq m\neq n\neq i,l < m < n} \frac{1}{2^n} (F_{i,j,l} F_{k,m,n} + F_{i,k} F_{j,m,n} + F_{j,k} F_{i,m,n}) I_{(L \geq 0)} \}.
\]

(A-3)
2. Integrated Deterministic Model

We propose a model to integrate the effects of complementation, epistasis, selection, mutation, and random recombination and show it is equivalent to the previous deterministic model with the separation step. We denote the mean fitness of viral populations using this integrated model $\bar{w}^{**}$ and frequencies of mutants with $F^{**}$. The frequency of wildtype genotype at the end of viral replicating cycle is:

\[
\bar{w}^{**}F_0^{**} = (1 - L\mu)(1 - p_c)F_0 + (1 - L\mu)p_c\{F_0^2 + \sum_i F_0 F_i + \sum_{i,j;i<j} \frac{1}{2} F_0 F_{i,j} \\
+ \sum_{i,j;i<j} \frac{1}{2} F_i F_j + \sum_{i,j,k;i<j<k} \frac{1}{2} F_0 F_{i,j,k} \\
+ \sum_{i,j,k;i\neq j,k; j<k} \frac{1}{2} F_i F_{j,k} + \sum_{i,j,k,m;i\neq j,k,m;j<k<m} \frac{1}{2} F_i F_{j,k,m}I_{\{L\geq 4\}} \\
+ \sum_{i,j,k,m;i\neq j,k;m;j<k<m} \frac{1}{2} F_{i,j} F_{k,m}I_{\{L\geq 4\}} \\
+ \sum_{i,j,k,m,n;i\neq j,k,m,n;j<k<m<n} \frac{1}{2} F_{i,j} F_{k,m,n}I_{\{L\geq 5\}} \\
+ \sum_{i,j,k,l,m,n;i\neq j,k,m,n;l<k<m<n} \frac{1}{2} F_{i,j,k} F_{l,m,n}I_{\{L\geq 6\}} \}
\]

\[
= (1 - L\mu)\bar{w} F_0^*.
\]

(A-4)

Similarly, the frequency of mutants are:

\[
\bar{w}^{**}F_i^{**} = (1 - (L - 1)\mu)\bar{w} F_i^* + \mu \bar{w} F_0^*
\]

\[
\bar{w}^{**}F_{i,j}^{**} = (1 - (L - 2)\mu)\bar{w} F_{i,j}^* + \mu (\bar{w} F_i^* + \bar{w} F_j^*)
\]

\[
\bar{w}^{**}F_{i,j,k}^{**} = \bar{w} F_{i,j,k} + \mu \bar{w} (F_{i,j}^* + F_{i,k}^* + F_{j,k}^*)
\]

(A-5)

After simple arithmetic operations, it is clear that $F^{**}$'s are equal to $F$'s.
References


MAMMANO, F., V. TROUPLIN, V. ZENNOU and F. CLAVEL, 2000 Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: virus fitness in the absence and in the presence of drugs. J. Virol. 74: 8524–8531.


List of Figures

1 Interaction patterns at equilibrium under different levels of co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. The first row of barplots shows the interaction between complementation and mutation ($\mu$) under scenarios I and II, with $s = 0.1$, $\epsilon = 0.0$, $\eta = 0.0$, red representing $\mu = 0.0$ and light blue for $\mu = 0.01$. The second row represents the interaction between complementation and selection ($s$) under scenarios II and III, with $\mu = 0.01$, $\epsilon = 0.0$, $\eta = 0.0$, red for $s = 0.1$ and light blue for $s = 0.2$. The third row shows the interaction of complementation with magnitude epistasis ($\epsilon$) under scenarios II, IV and V, with $\mu = 0.01$, $s = 0.1$, $\eta = 0.0$, red for $\epsilon = 0.3$, yellow for $\epsilon = 0.0$, and light blue for $\epsilon = -0.3$. The fourth row shows the interaction between complementation and sign epistasis ($\epsilon$) under scenarios II, VI and VII, with $\mu = 0.01$, $s = 0.1$, $\eta = 0.0$, red for $\epsilon = 0.8$, yellow for $\epsilon = 0.0$, and light blue for $\epsilon = -0.8$. The fifth row shows the interaction of complementation with second-order epistasis ($\eta$) under scenarios VIII and IX, with $\mu = 0.01$, $s = 0.1$, $\epsilon = 0.1$, red for $\eta = 0.8$ and light blue for $\eta = -0.8$. The first column of barplots represents the pattern of the overall mean fitness under various scenarios, the second column for wildtype frequencies, the third column for single mutant frequencies, the fourth column for double mutant frequencies, and the fifth column for triple mutant frequencies. Note that the vertical scales of the last three columns of barplots are different and vary from 0.02 to 1.0.

2 (a) Trajectories of mean fitness changes under scenarios II, VI and VII using stochastic simulation. (b), (c), (d), and (e) show under scenarios II, VI and VII the frequency trajectories of wildtype, single mutants, double mutants, and triple mutants, respectively. Dashed lines represent the results assuming epistasis coefficient $\epsilon = 0.8$. Dotted lines represent the results assuming no epistasis. Solid lines represent the results assuming epistasis coefficient $\epsilon = -0.8$. Each color represents a co-infection fraction as labeled in the legend.
Wildtype frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors represent the values of wildtype frequency, red= 0.00, yellow= $\frac{1}{3}$, green= $\frac{2}{3}$, and blue= 1.00.

Single mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.

Double mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.

Triple mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.
Figure 1:
Figure 2: (a) Trajectories of mean fitness changes under scenarios II, VI and VII using stochastic simulation. (b), (c), (d), and (e) show under scenarios II, VI and VII the frequency trajectories of wildtype, single mutants, double mutants, and triple mutants, respectively. Dashed lines represent the results assuming epistasis coefficient $\epsilon = 0.8$. Dotted lines represent the results assuming no epistasis. Solid lines represent the results assuming epistasis coefficient $\epsilon = -0.8$. Each color represents a co-infection fraction as labeled in the legend.
Figure 3: Wildtype frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors represent the values of wildtype frequency, red = 0.00, yellow = $\frac{1}{3}$, green = $\frac{2}{3}$, and blue = 1.00.
Figure 4: Single mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.
Figure 5: Double mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.
Figure 6: Triple mutant frequency pattern under the three-way interaction among complementation, selection and epistasis. (a), (b), (c), (d), and (e) show the interaction patterns between selection and epistasis with the co-infection ratios, 0.1, 0.3, 0.5, 0.7, and 0.9, respectively. Colors are as in Figure 3.
### Table 1: Parameter combinations for nine simulation scenarios.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>( L )</th>
<th>( \mu )</th>
<th>( s )</th>
<th>( \epsilon )</th>
<th>( \eta )</th>
<th>Initial Frequencies</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>0.01</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>0.3</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>-0.3</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>0.8</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>-0.8</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>VIII</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>0.1</td>
<td>0.8</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
<tr>
<td>IX</td>
<td>3</td>
<td>0.01</td>
<td>0.1</td>
<td>-0.8</td>
<td>0.0</td>
<td>( F_1 = F_2 = F_3 = \frac{1}{3} )</td>
</tr>
</tbody>
</table>

\( L \) represents the number of segments in a viral genome. \( \mu \) represents mutation rates per generation per segment. \( s \) represents selection coefficients. \( \epsilon \) represents epistasis coefficients. \( \eta \) represents second-order epistasis coefficients. \( F_1, F_2 \) and \( F_3 \) represent the initial frequencies of single mutants.