Information Loss: Potential for Accelerating Natural Genetic Attenuation of RNA Viruses

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Abstract

Loss of information is not always bad. In this paper, we investigate the potential for accelerating the genetic degeneration of RNA viruses as a means for slowing/containing pandemics. It has previously been shown that RNA viruses are vulnerable to *lethal mutagenesis* (the concept of inducing mutational degeneration in a given pathogen). This has led to the use of lethal mutagenesis as a clinical treatment for eradicating RNA virus from a given infected patient. The present study uses numerical simulation to explore the concept of accelerated mutagenesis as a way to enhance natural genetic attenuation of RNA viral strains at the epidemiological level. This concept is potentially relevant to improved management of pandemics, and may be applicable in certain instances where eradication of certain diseases is sought.

We propose that mutation accumulation is a major factor in the natural attenuation of pathogenic strains of RNA viruses, and that this may contribute to the disappearance of old pathogenic strains and natural cessation of pandemics. We use a numerical simulation program, Mendel's Accountant, to support this model and determine the primary factors that can enhance such degeneration. Our experiments suggest that natural genetic attenuation can be greatly enhanced by implementing three practices. (1) Strategic use of antiviral pharmaceuticals that increase RNA mutagenesis. (2) Improved hygiene to reduce inoculum levels and hence increase genetic bottlenecking. (3) Strategic use of broad-spectrum vaccines that induce partial immunity. In combination, these three practices should profoundly accelerate loss of biological information (attenuation) in RNA viruses.

Key words: mutation accumulation, lethal mutagenesis, error catastrophe, mutation meltdown, pandemic, Mendel's Accountant

Introduction

The concept of lethal mutagenesis has been put forward as a strategy for controlling pathogens [1, 2]. The idea of lethal mutagenesis is to enhance the mutation rate of the pathogen, thereby accelerating mutation accumulation and leading to mutational meltdown and extinction of the pathogen within a specific host individual. The concept of mutation accumulation in RNA viruses has been explored

in biological experiments involving bacteriophage [3], tobacco etch virus [4], poliovirus [5], vesicular stomatitis virus [6, 5], and HIV [7–10]. All these researchers report rapid fitness declines of viral strains as deleterious mutations accumulate, often leading to the actual extinction of some strains. This strongly contradicts claims that RNA viruses are somehow robust against the accumulation of deleterious mutations [11–13]. Lethal mutagenesis is considered a potential antiviral therapy for infected patients and is also recognized as having relevance to management of pandemics [2].

RNA viruses are excellent candidates for genetic degeneration because they typically have an extraordinarily high mutation rate [14]. The higher mutation rate of RNA viruses is a consequence of the novel mechanisms required for RNA replication, which are especially prone to mutation, and the lack of effective repair enzymes for RNA replication. Even in RNA viruses with relatively small genomes, there appear to be as many as 0.1 to 1.0 new mutations per virus per replication cycle [15]. The mutation rate in RNA viruses is so high that it becomes difficult to speak of a given viral "strain", because any genotype quickly mutates into a complex of genotypes, such that any patient is soon infected with a "viral swarm". With such a high mutation rate, the large majority of viral genotypes in a patient must carry many deleterious mutations, and so will be inferior to the original infecting genotype. This implies the lack of a realistic mechanism to preserve a "standard genotype", and all RNA viral swarms should typically be on the verge of mutational meltdown.

When a virus is transmitted from one individual to the next, the first individual harbors a viral swarm. The second individual becomes infected by a random subset of that swarm (conceivably a single genotype). With this type of bottlenecking, the "best" viral genotypes within the first swarm have a small probability of being transmitted to the next host. This probability becomes especially small when infection arises from a single viral particle. Given a high mutation rate and regular bottlenecks, the operation of Muller's Ratchet becomes quite certain, which should result in a continuous ratchet-like mutational degeneration of the viral genome [6].

This type of genetic degeneration happens independently of specific virulence factors. A viral strain may have a few high-impact beneficial mutations that affect "virulence" (i.e., compatibility with a specific host), yet at the same time that same strain can be accumulating large numbers of low-impact mutations throughout its genome, which should systematically degrade function and reduce net fitness. Therefore such a strain can be undergoing genetic degeneration, even while it retains (or gains) favorable virulence factors.

In this light, it appears very likely that RNA viruses should have a strong. tendency to undergo what we will call "natural genetic attenuation". This should

happen within the individual host organism as the mean fitness of the viral swarm continues to diminish with every replication cycle. This should happen even more dramatically as the viral swarm undergoes recurrent bottlenecking, as it passes from host individual to host individual. Such natural genetic attenuation should logically contribute to the transient nature of RNA viral infections within the individual, as well as the transient nature of pandemics caused by RNA viruses.

Historical evidence that RNA viruses undergo natural genetic attenuation

Dengue type-2 virus (DENV), a mosquito-borne, positive-sense, single-strand RNA virus, caused an epidemic in several Pacific Islands from 1971 to 1974. A recent paper [16] studied the epidemiological, clinical and biologic observations recorded during this time. The authors note that the time period, population dynamics and isolation of this epidemic gives a unique opportunity to study virus evolution minus many confounding factors. The initial outbreak of the disease, on Fiji and Tahiti, caused severe clinical symptoms, while the final outbreak on Tonga produced mild symptoms and near-silent transmission. Sequence and phylogenetic analysis showed that the outbreaks were genetically related and all due to a single introduction. Also these analyses placed the Tongan viral isolates in a single clade, with some unique site substitutions compared to viral isolates early in the epidemic. It is these deleterious genetic changes that Steel *et al.* [16] believe was responsible for the reduced epidemic severity on Tonga in 1973/1974.

Severe acute respiratory syndrome (SARS) caused by an animal-derived coronavirus appeared in the human population of Guangdong Province of China in late 2002. Sixty-one viral isolates from humans were sequenced from the early, middle and late phases of the outbreak in this region and were compared to animal derived viral sequences [17]. This epidemic was characterized by its sudden appearance, its extreme virulence, its rapid spread, and the rapid collapse of the pandemic after just two months [17]. This dramatic collapse cannot reasonably be attributed to human intervention. Given that SARS in man appears to have an inordinately high mutation rate of roughly 3 mutations per replication [18], and given that during this very short-term pandemic 291 mutations accumulated in the virus, it seems most reasonable to conclude that the outbreak ended prematurely because the virus underwent mutational degeneration and natural genetic attenuation.

Similarly, Ebola outbreaks have emerged explosively, initially being extremely virulent and extremely contagious, but very quickly they became self-contained apart from human intervention. While the Ebola virus appears to have an extremely wide host range, it has been almost impossible to find it in the natural fauna of the

relevant regions [19]. This can most reasonably be explained by self-containment of the virus due to high mutation rates and natural genetic attenuation. Bowen *et al*. [20] cite the World Health Organization's report suggesting that such attenuation occurred after just 10–11 passages within the human population.

Influenza A virus causes respiratory infections in mammals and birds. In humans, this virus causes a yearly epidemic and an occasional pandemic. It appears that influenza strains are continuously going extinct at a high rate. The actual precursor strains of the H1N1 strain that caused the disastrous 1918 pandemic are unknown, and can be presumed to be extinct [i.e., 21, 22]. The H1N1 strain itself appears to have gone extinct in the mid-twentieth century, and apparently was inadvertently re-introduced from a researcher's lab freezer in 1977 [23, 24]. During the 2009 H1N1 pandemic, one of two original strains went extinct [25]. Given the global nature of influenza spread and distribution, it can very reasonably be asked - why does the previous year's strain of the flu routinely disappear so quickly? Why do most strains of influenza appear to routinely go extinct? The most reasonable answer would seem to be natural genetic attenuation due to mutation accumulation.

Methods and Results

We have conducted a series of numerical simulation experiments using the genetic accounting program Mendel's Accountant (Mendel). This program tracks mutation accumulation over time, as affected by the primary relevant variables such as mutation rate, distribution of mutational effects, selection pressure, and population size [26–31]. Although Mendel has traditionally been used to model higher organisms (e.g., diploid, sexually reproducing species), it has alternative parameter settings that allow us to model populations of organisms with small haploid genomes and which reproduce clonally.

In these experiments, we model a generic RNA virus similar to the influenza virus. We model only a single viral sub-strain, which becomes a viral swarm, which is then transmitted through a single lineage as it moves through a series of 100 individuals during a pandemic lasting 300 days. We model an RNA virus that employs RNA to RNA replication with a viral doubling time of one hour (24 replication cycles per day) [32, 33]. We assume that passage to a new host individual happens every 3 days [34], and that infection in the new host individual involves the transmission of either a low or high level of inoculum, depending on the model run (10 or 1000 viable viral particles randomly sampled from the viral swarm). Following each new host infection, the swarm is allowed to amplify in number until a specified steady-state population size is reached within the individual host. We use a maximal

population size of 10,000 (in our experience, creating populations larger than this has minimal effect on selection efficiency and mutation accumulation, but consistently causes overflow of computer memory). We assume a functional genome size of 10,000 nucleotides, and we assume a starting baseline reference genotype, which we define as our "wild type" (having zero initial mutations by definition). We model 10% of all mutations as being perfectly neutral, with the remainder of mutations being 99% deleterious and 1% beneficial [35]. We model back-mutations based upon mutation rate and the fraction of nucleotides already mutated. We use the well-accepted Weibull distribution for mutation effects (a natural, exponential-type distribution [26]). In this type of distribution, low-impact mutations are much more abundant than high-impact mutations. The lowest impact mutation we model (excluding perfect neutrals) has a fitness effect which is the inverse of the genome size (such a mutation would reduce fitness by one part in 10,000 when arising in a genome of 10,000 functional nucleotides).

In order to be consistent with what is known about deleterious viral mutation distributions, we shape the mutation distribution such that there is a very substantial fraction of all mutations that have a large effect on fitness (10% of the deleterious mutations reduce fitness by 10% or more). We model beneficial mutations to have a similar distribution as deleterious mutations, but with a much narrower range (maximal fitness effect = 0.01). This upper limit excludes major virulence factor mutations, which are outside the scope of these experiments (we wish to study overall fitness, not singular host/pathogen compatibility factors). Viruses are recognized as having a much higher rate of lethal mutations than other organisms [35], and our Weibull distribution does not fully model this. However, since all viral particles with lethal mutations will fail to replicate, they are easily accounted for by simply adjusting the rate of "random deaths". Mutational effects are combined additively within a viral genotype [3].

Mutations were introduced into the viral population at rates ranging from 0.1 to 1.0 mutation per genome per replication [15, 25]. Viral replication was modeled as a simple asexual doubling every replication cycle, causing population size to double. After every replication, we eliminated the surplus population by applying natural selection (partial truncation selection) based upon phenotype, restoring the initial population size. When bottlenecking was modeled, every time a new host was infected the population size was reduced to either 10 or 1000 particles. The population was allowed to undergo rapid growth to restore population size. This was done by temporary partial relaxation of selection, such that roughly 50% of the surplus viral particles were not selected away but were allowed to contribute to population re-growth. As deleterious mutations accumulated to high levels, some viral particles had zero fitness and could not replicate. When there were not enough viable viral particles to repopulate the viral population after each selection cycle, the size of the

viral population necessarily began to shrink each generation. If this continued, the viral population would shrink to zero, causing extinction of the viral swarm.

Our first experiment was a preliminary Mendel run using very conservative parameters. This was designed as a base-line for minimal genetic attenuation of our model RNA virus, as would occur during a 300-day pandemic. We used a low mutation rate of 0.1 mutations per virus particle per replication cycle. In every replication cycle the number of viral particles was allowed to double, and we modeled zero random death (zero percent of the viral particles were randomly lost). Phenotypic selection was applied (partial truncation), to eliminate all of the surplus population (50%), such that the initial population size was restored. In this first experiment, we did not model any population bottlenecks. The results of this experiment are summarized in Figure 1. we see that even using highly favorable assumptions and intense selection, the simulation failed to prevent mutation accumulation. After 7200 replication cycles, each virus accumulated an average of 235 deleterious, 74 neutral, and 9.4 beneficial mutations. There were 523 polymorphic mutant alleles segregating in the population, meaning that it was a very genetically diverse viral swarm. Although 7 beneficial mutations went to fixation within the swarm, these carried with them 180 deleterious mutations that also went to fixation. Fitness declined 16% in just 300 days. By the end of the experiment deleterious mutation count per virus was increasing at an essentially constant rate, and mean viral fitness was declining at nearly a constant rate. These results indicate the presence of strong forces working to attenuate any given strain, even when conditions for maintenance of the virus are optimal.

We then conducted a series of four simulations wherein we modeled the effect of factors that might accelerate natural genetic attenuation. Figure 2 summarizes the fitness decline seen in these experiments. In the first of these experiments, we introduced a realistic, but modest degree of random loss of viral particles (25%), as might be expected due to chance and various host defense mechanisms. Simultaneously, we introduced a very weak, and recurrent bottlenecking of population size (1000 viral particles/infection), corresponding to high inoculum levels during viral transmission to new host individuals. The result of this second experiment was a very slight acceleration in the rate of genetic attenuation compared to Figure 1 (final mean fitness was reduced from 0.84 to 0.82, see Figure 2).

In the second simulation, we tested the effect of increasing the random loss of viral particles as might arise, for example, due to host RNase activity, or as a result of antiviral pharmaceuticals, or as might arise due to partial immunity within the host. We eliminated 40% of all viral particles by random death, thus reducing the viral surplus population from 50% to 10%. This effectively reduces selection intensity. The result was another very slight acceleration of fitness decline (final mean fitness declined to 0.79, see Figure 2).

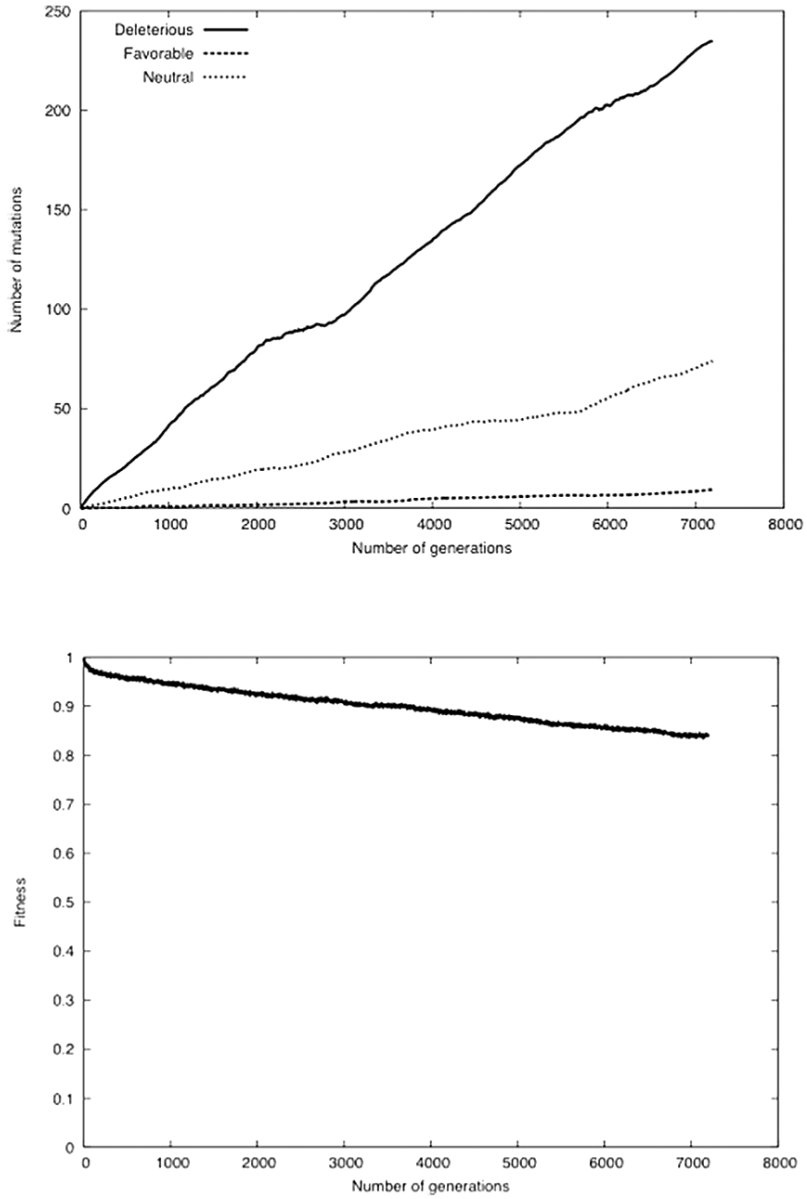

Fig.1. A preliminary numerical simulation experiment with parameters optimized for slowing genetic degeneration of a model RNA virus. Mutation rate = 0.1/genome/replication (89% of mutations deleterious, 10% neutral, and 1% beneficial). Partial truncation selection was employed (50% selective elimination, every replication cycle). No random death and no bottlenecking. Mean mutation count per virus over time (figure above), and fitness decline over time (figure below).

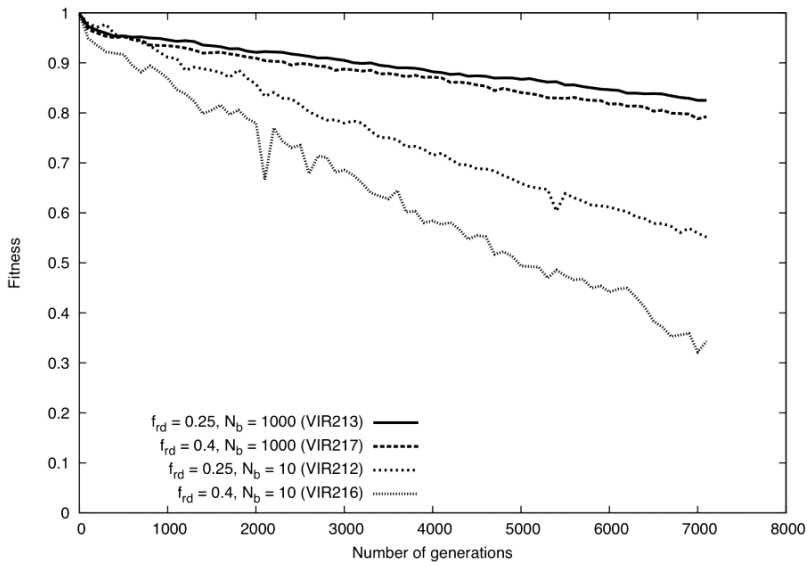

Fig. 2. Four simulations demonstraing the effect of bottlenecking and random death on fitness degradation during an RNA virus pandemic. VIR213 = minimal bottlenecking and modest random loss, VIR217 = more random loss, VIR212 = more severe bottlenecking, VIR216 = combination of more random loss and more severe bottlenecking. N_b is the population size during the bottleneck. F_{rd} is the fractional occurrence of random death.

In the third simulation, we tested the effect of much more severe bottlenecking, with just 10 viable viral particles per new infection. This might be clinically achieved either by use of antiviral pharmaceuticals or through better hygiene. We see that when we have strong bottlenecking, selection is significantly less effective and genetic attenuation is much faster. Fitness declined 45% in 300 days (final mean fitness was 0.55, see Figure 2). After 7200 replication cycles, each virus accumulated an average of 356 deleterious, 77 neutral, and 9.9 beneficial mutations. There were only 81 segregating polymorphic alleles in the population, reflecting the homogenizing effect of recurrent bottlenecking. Although 9 beneficial mutations went to fixation, along with them 338 deleterious mutations went to fixation.

In the fourth of these simulations, we modeled intensified bottlenecking combined with 40% random loss. The result was dramatically accelerated fitness decline (final mean fitness was 0.35, see Figure 2).

As evident from Figure 2, more severe bottlenecking and higher rates of random loss combine synergistically to greatly accelerate both fitness decline and genetic attenuation. More random death by itself had a very small effect, while the effect of bottlenecking by itself was more significant, but still fairly modest. However, a higher rate of random loss (hence lower viral titer) greatly amplified

the bottleneck effect (because after a serious bottleneck, random loss increases the time needed for population size to recover, effectively extending the duration of each bottleneck).

We lastly conducted a series of four simulations wherein we examined the consequence of increasing mutation rate, as might be achieved by using a pharmaceutical such as Ribavirin. We used the most conservative settings shown in Figure 2 (weak bottlenecking and only a moderate rate of random loss), and we then examined the effect of increasing mutation rate from 0.1 to 0.2, 0.4, 0.6 and 0.8. The fitness decline resulting from an elevated mutation rate is shown in Figure 3.

As can be seen, even modest changes in the viral mutation rate had a substantial effect on viral fitness decline. This should not be surprising because it is known that RNA viruses are already near the edge of error catastrophe, due to mutation rates which are already very high. A mutation rate of 0.2 resulted in a final mean fitness of 0.57 (as opposed to a final fitness of 0.82 when the mutation rate was 0.1). A mutation rate of 0.4 caused strain extinction after 5,743 replications (239 days into the pandemic). A mutation rate of 0.6 caused strain extinction after 2,224 replications (93 days into the pandemic). A mutation rate of 0.8 caused strain extinction after 1,003 replications (42 days into the pandemic). As can be seen, even these very modest increases in mutation rate caused very rapid acceleration of fitness decline, due to the mutational meltdown phenomenon.

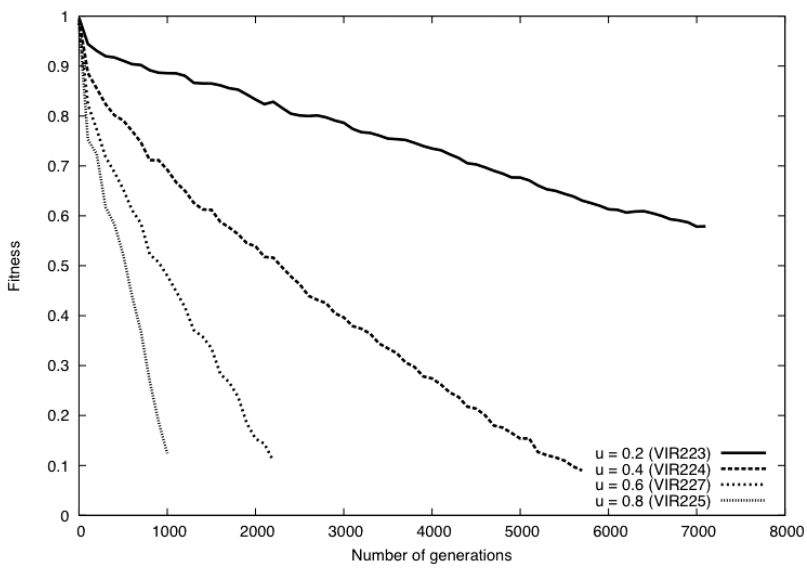

Fig. 3. Effect of mutation rate (u) on fitness degradation over time during an RNA virus pandemic. Four experiments showing that slight increases in mutation rate dramatically shorten pandemic duration. Mutations per virion per replication (u) were 0.2, 0.4, 0.6, and 0.8.

Discussion

Numerical simulations support our thesis that RNA viruses should be subject to natural genetic attenuation through mutation accumulation. Using conservative parameter settings for our model RNA virus, we observed continuous increases in the number of deleterious mutations per viral particle, and continuous genetic declines in viral fitness (Figure 1). Across a wide range of parameter settings, we have consistently observed that natural selection fails to remove a large fraction of the deleterious mutations, and that deleterious mutation count per viral particle increases linearly with time.

Our simulations indicate that genetic attenuation was accelerated by any of three factors (Figure 2), including: (1) increased rates of random death of virions (where there is significant loss of virions due either to poor assembly, degradation, or other host defenses); (2) more intense genetic bottlenecking; and (3) elevated mutation rates. Mutation rate had the greatest effect, and random death had the least effect. However, these three factors were most effective, by far, when acting in concert. When combined, these three factors caused very rapid genetic attenuation and would have clearly caused premature collapse of the model pandemic. How much fitness loss is required to stop a pandemic? This is unknown, but certainly fitness does not need to approach zero. The fitness loss in our most conservative case (16% decline, see Figure 1) may be sufficient in itself to explain the natural cessation of most pandemics.

We saw that even slight increases in the mutation rate had a profound effect on the rate of genetic attenuation. Just an 8-fold increase in the mutation rate was enough to cause rapid mutational meltdown and strain extinction after just 42 days (see Figure 3). This is consistent with Domingo *et al.* [5] who claim that even a 2.5-fold increase in mutation rate is sufficient to cause loss of infectivity of both poliovirus and vesicular stomatitis virus. Such elevated mutation rates can be readily achieved using certain pharmaceuticals [36–39, 9, 10]. Indeed, even if only half the infected people employed such antiviral medications, the use of such pharmaceuticals would be expected to increase the average mutation rate very significantly.

Use of mutation-enhancing pharmaceuticals should have the additional benefit of simultaneously reducing viral titers ("random loss"), and increasing the degree of bottlenecking. These benefits, along with an elevated mutation rate, should act synergistically in accelerating genetic attenuation. Better hygiene might also greatly increase bottlenecking, and thus significantly enhance genetic attenuation. Likewise, broad-spectrum vaccines, which help build more general immunity, along with other treatments that reduce viral titers, should also enhance attenuation. In some cases, these combined treatments might even be employed where a RNA virus has been targeted for eradication.

Our results suggest that, while lethal mutagenesis holds promise for treating individuals, a much more significant application may be on the epidemiological level. There appears to be a great potential for more effectively managing pandemics by increasing those factors described above. To the extent that we can significantly increase the mutation rate in RNA viruses, we can clearly accelerate natural genetic attenuation, and in many cases may be able to cause mutational meltdown of a given viral strain in a relatively short period of time. Deployment of better hygiene practices by itself should reduce inoculum levels, which should result in stronger bottlenecking and accelerated decline. Lastly, adding a higher rate of random elimination of viral particles, as might occur due to various factors favorable for viral elimination (i.e., partial immunity, fever, use of complimentary antiviral drugs, etc.), should further accelerate genetic attenuation. It is noteworthy that only 1% of poliovirus released from a host cell are able to complete a full cycle of replication [15]. These three factors (mutation rate, bottlenecking, and various mechanisms that reduce viral load) clearly combine synergistically to accelerate viral degeneration.

We acknowledge that our model virus may not precisely match any known RNA virus, but we feel it provides a reasonable approximation of a typical RNA virus. Our greatest reservation is that no one knows the precise shape of the distribution of mutation effects for a given virus. Our distribution of mutation effects may be skewed too far toward higher-impact mutations (the mean mutation effect in all these experiments, prior to selection, was 3.7% reduction in fitness). This may be causing unrealistically rapid genetic decline, resulting in over-estimation of the rate of fitness decline. Alternatively, our distribution of mutational fitness effects may be too skewed toward low-impact mutations, in which case the simulations would indicate unrealistically slow genetic decline, thereby resulting in under-estimating the rate of attenuation. However, we have consistently observed that when we shift the mutation effect distribution toward mutations with lower impact on fitness, the selection breakdown phenomenon is much more severe, such that a much higher proportion of deleterious mutations escape selection altogether. Shifting the distribution of mutation effects either up or down creates tradeoffs, resulting in only modest changes in the way the genetic damage accumulates. Therefore, we feel our model RNA virus is a useful approximation of how a real RNA virus should respond to the mutation/selection process.

We believe there is strong theoretical evidence that RNA viruses should systematically undergo natural attenuation, which in now supported by our numerical simulations. This raises the obvious question – if this is true, why have not all RNA viruses gone extinct? The most likely explanation seems to be that such viruses are preserved in natural reservoirs where they are more stable. The most obvious way for an RNA virus to be more genetically stable is to be

in an environment where they have slower replication, and higher fidelity RNA replication. Since the host provides sub-units for the RNA replicase complex, the host should have very significant impact on both speed of replication and fidelity of replication, and therefore a specific host may foster much greater viral stability for a given virus. In the case of retroviruses, we know they can persist indefinitely in their DNA form (within the host genome). In this form they have very low mutation rates. Other viruses may lay dormant indefinitely in other states and in other types of natural reservoirs. For example, the H1N1 strain of influenza apparently went extinct for 20 years in the mid-twentieth century, but it is thought to have been resurrected from a researcher's freezer in 1977, and is once again circulating globally [23, 24]. The 1918 influenza virus strain (which gave rise to essentially all the current human and pig influenza strains) is assumed to have arisen from the natural reservoir of aquatic birds which harbor influenza viruses. However, there is no clear precursor for the 1918 strain in either bird hosts or other known hosts [i.e., 21, 22], so we can only say modern human/pig influenza emerged from an unknown natural reservoir around the turn of the twentieth century. There may be many ways that an RNA virus may be held in reserve for long periods of time in natural reservoirs.

Conclusions

Our findings are consistent with the idea that there are already very high rates of natural extinction among RNA viral strains, and that the vast majority of RNA viral strains die out naturally due to mutation accumulation. Such mutational degeneration should play a significant role in the natural progression of pandemics, with mutation accumulation causing the natural genetic attenuation of any given RNA viral strain. Our numerical simulations strongly indicate that such natural genetic attenuation can be enhanced during pandemics by: (a) employing strategic use of antiviral pharmaceuticals that increase RNA mutagenesis; (b) increasing genetic bottlenecking by reducing inoculum levels through improved hygiene and other means; and (c) strategic use of broad-spectrum vaccines that induce partial immunity and other means for reducing viral titers.

Addendum — *This study was purely theoretical, based upon biologically realistic numerical simulations. After this chapter was already accepted and finalized, an empirical analysis was initiated of actual mutation accumulation within the H1N1 Influenza viral genome since 1918. The results provided a remarkable validation of the present theoretical study. Within the human lineage, nearly every H1N1 strain that arose very quickly became extinct. All circulating human*

H1N1 strains went extinct in the mid-1950s, but the human H1N1 lineage was re-seeded into the human population in 1976, apparently from a researcher's freezer. The human lineage apparently again went extinct in 2009. During the entire history of H1N1 within man, mutations accumulated in a perfectly linear fashion – exactly as seen in this theoretical study. In the course of 90 years, almost 15% of the viral genome mutated, always at a very constant rate. Viral fitness, as reflected by associated human mortality rates, declined continuously and systematically from 1918 all the way to the apparent extinction of the human H1N1 strain in 2009. Because the publication of these proceedings was significantly delayed, the empirical study was published before the present theoretical study (which spawned the empirical study). See: Carter R.C. & Sanford, J.C. (2012). A new look at an old virus: patterns of mutation accumulation in the human H1N1 influenza virus since 1918. Theoretical Biology and Medical Modeling 9:42doi:10.1186/1742-4682-9-42.

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