

# Modelling the role of immunity in reversion of viral antigenic sites

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## Abstract

Antigenic sites in viral pathogens exhibit distinctive evolutionary dynamics due to their role in evading recognition by host immunity. Antigenic selection is known to drive higher rates of non-synonymous substitution; less well understood is why differences are observed between viruses in their propensity to mutate to a novel or previously encountered amino acid. Here, we present a model to explain patterns of antigenic reversion and forward substitution in terms of the epidemiological and molecular processes of the viral population. We develop an analytical three-strain model and extend the analysis to a multi-site model to predict characteristics of observed sequence samples. Our model provides insight into how the balance between selection to escape immunity and to maintain viability is affected by the rate of mutational input. We also show that while low probabilities of reversion may be due to either a low cost of immune escape or slowly decaying host immunity, these two scenarios can be differentiated by the frequency patterns at antigenic sites. Comparison between frequency patterns of human influenza A (H3N2) and human RSV-A suggests that the increased rates of antigenic reversion in RSV-A is due to faster decaying immunity and not higher costs of escape.

*Keywords:* viral evolution, antigenic selection, back mutation, cost of escape, epidemiological model

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## 1. Introduction

Viral evolution is shaped by both epidemiological effects on population dynamics, and molecular effects of mutations in the viral genome [1]. The combination of these effects generates distinctive dynamics at antigenic sites of viral proteins, which are the targets of host immune recognition. Selection for strains carrying antigenic changes that evade immune recognition result in elevated rates of non-synonymous substitution. It is unclear, however, why different dynamics of forward or reverse substitution are observed. Antigenic reversion has been reported frequently in viruses such as HIV [2, 3, 4], respiratory syncytial virus (RSV) [5] and hepatitis C [6, 7], and less frequently in other viruses such as influenza [8, 9], parvovirus [10], hepatitis A [11] and polio [12]. Various explanations for occurrence of reversion have been proposed, such as changing immunity [5], a limited antigenic repertoire [5, 9], or constraints on function [11, 7, 8], but it is not understood how the relative influence of these effects can generate differences in observed rates of reversion.

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12 The difficulty in evaluating the contribution of selective mechanisms is due to the lack of methods that model  
13 both epidemiological and molecular dynamics. Phylodynamic approaches [13] incorporating epidemiological  
14 models into a coalescent framework have provided insight into the origins and spread of novel pathogens.  
15 However, they assume that molecular changes do not affect epidemiological dynamics, and are uninformative  
16 about selection. In contrast, codon-based approaches [14, 15] aim to identify sites that contribute to the  
17 adaptation of a virus, but they assume that the population size is constant and that the selection coefficient  
18 is constant at each site. Various modifications of the substitution model allow for different selective effects  
19 based on directionality or target residue [16, 2], but retain the assumption that substitution occurs as a time-  
20 homogeneous process which is not affected by population dynamics. To understand how the probability of  
21 reversion at antigenic sites is affected by both selective constraint against molecular changes and selection to  
22 evade immune recognition, there is a need to incorporate the time-dependence imposed by epidemiological  
23 dynamics into the substitution process.

24 Models of pathogen dynamics have shown that reversion probabilities are affected by fitness costs [17, 18,  
25 3, 19], at both the within-host and between-host level, and the availability of susceptible hosts [3], at the  
26 between-host level. However, these models were developed in the context of HIV escape mutations. HIV  
27 infects host chronically, with host susceptibility determined by human leukocyte antigen (HLA) type, which  
28 does not vary over time. Due to these infection dynamics the prevalence of each strain changes relatively  
29 slowly, and is expected to eventually stabilise [3]. In contrast, for acute infections such as human influenza and  
30 RSV where transmission occurs frequently and host immunity can last for much longer than the duration of  
31 the infection, the structure of host immunity can vary rapidly over time. Due to differences in the dynamics of  
32 selection, we expect antigenic selection to have qualitatively different effects on sequence changes at antigenic  
33 sites compared to constant selective pressure [1].

34 Here, we examine the probability of antigenic reversion in an epidemiological model, which describes the  
35 complex ecology of multiple viral strains with cross-immunity competing for susceptible hosts. This model  
36 allows us to quantify the relative advantage of an antigenically novel mutation, compared to a reversion which  
37 may be antigenically less advantageous, but improves transmission. Using both a simple three-strain model and  
38 simulations with multiple codon sites, we examine the effect of the duration of host immunity, selective costs,  
39 population size, and the basic reproductive ratio. We show that these effects lead to distinctive dynamics in  
40 the frequencies of derived amino acids, which is informative about the duration of host immunity and strength  
41 of selective constraint. Time-structured sequence data from influenza and RSV are compared to simulated  
42 sequences, and we discuss what these results imply about the relative effects of host immunity and functional  
43 constraint.

## 44 2. Methods

### 45 2.1. Simple analytical model for antigenic reversion

46 The simplest model containing reversion is a system where the population has mutated away from the  
 47 ancestral state, and potentially can mutate either back to the ancestral state (reversion) or to a novel state  
 48 (forward substitution). In an epidemiological context, we consider a viral population described by a three-  
 49 strain SIRS model [20]. The viral population is initially of strain 0 (ancestral state), which is then replaced  
 50 with strain 1, and can subsequently be replaced by either strain 0 (reversion) or strain 2 (forward substitution).

51 We assume a large host population of constant size  $N$ , with homogeneous mixing, so that the dynamics  
 52 of the number of hosts which are susceptible  $S_i$ , infected  $I_i$ , and recovered with immunity  $R_i$ , for strains  
 53  $i = 0, 1, 2$  can be described by

$$\frac{dI_i}{dt} = \beta_i \frac{S_i}{N} I_i - \delta I_i, \quad (1)$$

$$\frac{dR_i}{dt} = \delta I_i - \gamma R_i, \quad (2)$$

54 with transmission rate  $\beta_i$ , recovery rate  $\delta$ , and immunity that decays at rate  $\gamma$ . Interactions between strains  
 55 are described by the implicitly defined term  $S_i$ , which is the number of hosts susceptible to strain  $i$ . Assuming  
 56 that each host can only be infected by a single strain at a time, and prior infection with strain  $j$  reduces  
 57 susceptibility to strain  $i$  by a factor  $\sigma_{ij}$ , the relationship between susceptible and immune hosts is given by

$$S_i = N - \sum_j I_j - \sum_j \sigma_{ij} R_j, \quad (3)$$

58 with the constraint that  $S_i > 0$  for any strain  $i$ . All uninfected hosts ( $N - \sum_j I_j$ ) can be categorised as either  
 59 susceptible ( $S_i$ ) or immune ( $\sum_j \sigma_{ij} R_j$ ) to strain  $i$ . The similarity between this model and the status-based  
 60 model with polarised immunity developed by Gog and Grenfell [21] becomes evident when we differentiate  
 61 Equation (3) to give

$$\begin{aligned} \frac{dS_i}{dt} &= - \sum_j \left( \frac{dI_j}{dt} + \sigma_{ij} \frac{dR_j}{dt} \right), \\ &= - \sum_j \beta_j \frac{S_j}{N} I_j + \sum_j \delta (1 - \sigma_{ij}) I_j + \sum_j \gamma \sigma_{ij} R_j. \end{aligned} \quad (4)$$

62 The main difference is that we retain the history of infections accumulated across the population through  
 63 the additional set of variables,  $R_i$ . This allows us to obtain analytical expressions for the number of hosts  
 64 susceptible to all strains as functions of the same set of variables, as shown in Equation (3). In contrast to the  
 65 Gog and Grenfell [21] model assuming polarised immunity, we assume a model of partial additive immunity.  
 66 A host that was infected twice with strain  $i$  at times  $t_1$  and  $t_2$  will contribute  $r = \sigma_{ii} e^{-\gamma(t-t_1)} + \sigma_{ii} e^{\gamma(t-t_2)}$  to  
 67  $R_i$  at time  $t$ . This additive structure can be easily generalised to incorporate multiple strains. However, our  
 68 model allows a host to contribute  $r > 1$  after multiple re-infections, so we tend to inflate  $R_i$ . The effect of this  
 69 approximation is examined in greater detail for a single strain system in Appendix A.1. Overall, the effect

70 of the approximation is to reduce  $I$ , but leave  $S$  unchanged. The approximation also tends to have minimal  
71 effect when  $\sigma$  is small, or when the rate of immune decay varies between hosts.

72 Our model of partial additive immunity generates similar dynamics to the Gog and Grenfell model [21].  
73 From Equation (4), it can be seen that hosts infected with strain  $j$  are removed from the susceptible class  $S_i$ ,  
74 and then a proportion  $1 - \sigma_{ij}$  of all infected hosts are returned to the susceptible class on recovery, so that  
75 the overall contribution of immunity is  $\sigma_{ij}\beta_j S_j I_j / N$ , which is similar to the  $\sigma_{ij}\beta_j S_i I_j / N$  term in the Gog and  
76 Grenfell [21] model. The difference in the  $S_i$  and  $S_j$  term arises because in the Gog and Grenfell [21] model,  
77 immunity arises from exposure, but in our model, immunity is only generated when infection occurs.

78 The strict exclusion of co-infection involves a second approximation, where an infection by any strain  $j$   
79 will always be removed from  $S_i$  but not from  $R_i$  [first term in Equation (4)]. This occurs because while it is  
80 possible to distinguish between  $S_i$  and  $R_i$  at the time of infection from strain  $j$ , it is not possible, at the time  
81 of recovery from strain  $j$ , to determine whether the host was previously susceptible or immune to strain  $i$ .  
82 Our approximation leads to an underestimation of  $S_i$ . We expect this to have a small effect as the bias lasts  
83 only for the duration of the infection. In addition, strains which are closest to the current circulating strain  $j$   
84 will not be heavily affected ( $\sigma_{ij} \approx 1$ ); the most heavily affected strains are those distant from strain  $j$  which  
85 are likely to be no longer circulating.

86 Using this model, we examine the effect of cross-immunity  $\sigma_{ij}$ , immunity duration  $\gamma$  and selective costs  
87 incurred by antigenic escape  $s$ . The rate of immune decay  $\gamma$  includes the loss of immunity by the death  
88 and migration of immune hosts as well as the loss of immunity in individual hosts. The selective cost is  
89 parametrized through a reduction in the strain-specific transmission rate so that  $\beta_0 = \beta$ ,  $\beta_1 = \beta(1 - s)$  and  
90  $\beta_2 = \beta(1 - s)^2$ . To understand the effect of these parameters, we first characterise the number of susceptible  
91 hosts to each strain at equilibrium, and use this to determine probabilities of fixation, assuming a single strain  
92 appears at a time.

93 We assume the population is initially infected with only strain 0, which is maintained at equilibrium until  
94 strain 1 emerges at time  $t_1$ . Strain 1, then replaces strain 0 and equilibrates until time  $t_2$ , when a third strain  
95 (either strain 0 or strain 2) emerges and can potentially replace strain 1. These equilibrium assumptions  
96 allow us to characterise host immunity accumulated due to infection by strain 0 at  $t_1$  (denoted  $R_0^*$ ), and host  
97 immunity accumulated due to infection by strain 1 at  $t_2$  (denoted  $R_1^*$ ), which then allows us to evaluate the  
98 probability of strain 0 or 2 emerging at time  $t_2$ .

99 The equilibrium is obtained by setting the derivative of  $S_i$  and  $I_i$  to zero. When the viral population  
100 consists of only one strain, the endemic equilibrium, which is asymptotically, locally stable when the basic  
101 reproductive ratio  $\beta_i/\delta > 1$  [20], is given by

$$S_i^* = \frac{\delta}{\beta_i} N, \quad (5)$$

$$I_i^* = \frac{\gamma N}{\delta\sigma_{ii} + \gamma} \left(1 - \frac{\delta}{\beta_i}\right). \quad (6)$$

102 We assume that at time  $t_1$ , when strain 1 emerges, the population remains close to equilibrium. As strain

103 1 has only just emerged and strain 2 has not yet occurred, the cross-immunity terms in Equation (3) can be  
 104 ignored so that it contains only terms of subscript  $i = 0$ . Substitution of Equations (5) and (6) into Equation  
 105 (3) gives

$$R_0^* = \frac{\delta N}{\delta\sigma_{00} + \gamma} \left(1 - \frac{\delta}{\beta_0}\right). \quad (7)$$

106 Now, consider a later time  $t_2$ , when a third strain (either 0 or 2) emerges and can potentially replace strain 1.  
 107 Again, we assume that strain 1 remains close to equilibrium and that the third strain has had negligible effect  
 108 on immunity. In addition, we assume that immunity due to infection by strain 0 has decayed exponentially  
 109 since time  $t_1$ , so that Equation (3) can be approximated as

$$S_1^* = N - I_1^* - \sigma_{11}R_1^* - \sigma_{10}R_0^*e^{-\gamma(t_2-t_1)}. \quad (8)$$

110 Substituting Equations (5) and (6) into (8) then gives

$$R_1^* = \frac{\delta N}{\delta\sigma_{11} + \gamma} \left(1 - \frac{\delta}{\beta_1}\right) - \frac{\delta\sigma_{10}N}{\sigma_{11}(\delta\sigma_{00} + \gamma)} \left(1 - \frac{\delta}{\beta_0}\right) e^{-\gamma(t_2-t_1)}. \quad (9)$$

111 Having obtained an expression for  $R_0^*$  and  $R_1^*$ , we can now compute the proportion of hosts that are susceptible  
 112 to each strain,  $p_i(\tau) = S_i(\tau)/N$ , where  $\tau = t_2 - t_1$  is the time since the emergence of strain 1. Thus,

$$p_0(\tau) = 1 - \frac{I_1^*}{N} - \frac{\sigma_{01}}{N}R_1^* - \frac{\sigma_{00}}{N}R_0^*e^{-\gamma\tau}, \quad (10)$$

$$p_2(\tau) = 1 - \frac{I_1^*}{N} - \frac{\sigma_{21}}{N}R_1^* - \frac{\sigma_{20}}{N}R_0^*e^{-\gamma\tau}, \quad (11)$$

113 which can be written in the form

$$p_i(\tau) = A + B_i e^{-\gamma\tau}, \text{ for } i = 0, 2. \quad (12)$$

114 Assuming that cross-immunity is additive with respect to the number of antigenic differences ( $\sigma_{ii} = \sigma$ ,  $\sigma_{01} =$   
 115  $\sigma_{10} = \sigma_{21} = \sigma/2$  and  $\sigma_{20} = 0$ ), the coefficients simplify to

$$A = 1 - \frac{\delta\sigma + 2\gamma}{2(\delta\sigma + \gamma)} \left(1 - \frac{\delta}{\beta_1}\right), \quad (13)$$

$$B_0 = -\frac{3\delta\sigma}{4(\delta\sigma + \gamma)} \left(1 - \frac{\delta}{\beta_0}\right), \quad (14)$$

$$B_2 = \frac{\delta\sigma}{4(\delta\sigma + \gamma)} \left(1 - \frac{\delta}{\beta_0}\right). \quad (15)$$

116 Note that we expect that prior immunity reduces infection against an unmutated strain at appreciable levels  
 117 ( $\sigma \gg 0.1$ ) and that immunity lasts for much longer than the infection duration ( $\gamma \ll \delta$ ). Within the parameter  
 118 range of interest, the fractional terms containing  $\delta$ ,  $\sigma$  and  $\gamma$  in Equations (13–15) approach constants, so that  
 119  $A$  is approximately a function of only  $\beta_1/\delta$  and  $B_0$  and  $B_2$  are approximately functions of only  $\beta_0/\delta$ .

120 We calculate the probability of a strain generated by reversion or forward mutation at time  $t_2$  giving rise  
 121 to a new epidemic by approximating the emergence of a new strain as a linear birth-death process. Ignoring  
 122 initial changes in host susceptibility, the probability that a new strain reaches fixation [22] is given by

$$f_i = \begin{cases} 1 - \frac{1}{r_{e,i}}, & \text{if } r_{e,i} > 1 \\ 0, & \text{otherwise} \end{cases} \quad (16)$$

123 where  $r_{e,i} = \beta_i p_i / \delta$  denotes the effective reproductive ratio of the new strain  $i$  at the time of emergence. Using  
 124 Equations (12–15), at time  $\tau$  after strain 1 has reached equilibrium, we compute the probability of fixation for  
 125 strain 0 (reversion) and strain 2 (forward substitution) to be

$$f_i(\tau) = \begin{cases} 1 - \frac{\delta}{\beta_i(A+B_i e^{-\gamma\tau})}, & \text{if } \tau > t_{c,i} \\ 0, & \text{otherwise} \end{cases} \quad (17)$$

126 where the threshold  $t_{c,i}$  is given by

$$t_{c,i} = \begin{cases} -\frac{1}{\gamma} \log \left( \frac{\delta}{\beta_0 B_0} - \frac{A}{B_0} \right), & \text{if } i = 0 \\ 0, & \text{if } i = 2 \end{cases} \quad (18)$$

127 The probability of reversion given fixation is therefore

$$\rho(\tau) = \frac{f_0(\tau)}{f_0(\tau) + f_2(\tau)}. \quad (19)$$

128 The probability of reversion is low immediately after the strain 0 has been replaced; in fact from Equations  
 129 (17–19), it is zero for  $\tau < t_{c,0}$ . Asymptotically, if all prior immunity against strain 0 has decayed, then the  
 130 exponential term in the denominator of Equation (17) approaches zero, thus giving

$$\rho_\infty = \frac{\beta_0 A - \delta}{2\beta_0 A - \delta(1 + \frac{\beta_0}{\beta_2})} \quad (20)$$

$$= \frac{\frac{1}{2} \left[ \frac{\beta}{\delta} - (1-s)^{-1} \right] - 1}{\frac{\beta}{\delta} - (1-s)^{-1} - (1-s)^{-2} - 1}. \quad (21)$$

131 In summary, Equation (19) describes the combined effect of immunity  $\gamma$  and functional constraint  $s$  on the  
 132 probability of reversion at some time  $\tau$  after immunity has begun to wane from equilibrium levels. Whereas  
 133 the long-term asymptote  $\rho_\infty$ , given by Equation (21), shows the effect of functional constraint in the absence  
 134 of immunity.

## 135 2.2. Multi-site simulation model

136 To verify our theoretical model, and to examine the impact of increasing the antigenic space, we develop  
 137 a stochastic computer simulation model where each infection is associated with a sequence of antigenic sites.  
 138 Population dynamics are similar to the analytical model (see Table 1 for a complete list of parameters), but  
 139 in the multi-site simulation, we explicitly model the mutation process. In the analytical model, we assumed  
 140 the emergence of three strains at specified times, and calculated the probability that these strains would reach  
 141 fixation. In contrast, for the simulation model, we allow mutations to occur stochastically at any antigenic  
 142 site throughout the simulation; thus, new strains may emerge before the old strain reaches equilibrium and  
 143 even favourable mutations may be lost due to stochasticity.

144 We implement two models using different representations of the antigenic space. The first model uses a  
 145 bit-string representation so that each of the  $L_a$  antigenic sites can take values of  $\mathbf{v} = \{0, 1\}$ , and a change at  
 146 any site away from the ancestral state (0) will reduce transmissibility. The bit-string model with two sites

147 has a antigenic space similar to the analytical model. In the second model, we use a more realistic codon  
148 representation. Sites can mutate to any one of the 64 possible codons, but viral fitness is only affected by  
149 non-synonymous changes (i.e.,  $\mathbf{v}$  consists of the 20 amino acids). Specifically, any amino-acid change will affect  
150 cross-immunity, but only changes from the ancestral amino acid to a derived state will reduce transmissibility.  
151 The ancestral codon sequence is determined at the beginning of each simulation by randomly sampling  $L_a$   
152 non-terminating codons with uniform probability.

153 Throughout the simulation, we track the number of infected hosts  $I$ , the genotype of each infection, and the  
154 immune status of the host population. The last variable is stored in the immunity matrix consisting of  $2 \times L_a$   
155 elements for the bit-string model, or  $20 \times L_a$  for the codon model, where each element  $r_{v,j}$  stores the number  
156 of people with immunity to a value of  $v$  at site  $j$ . That is,  $r_{v,j}$  stores the site-specific immunity accumulated  
157 across the whole population, and we compute the immunity against any viral genotype by summing across  
158 these values (described below).

159 The multi-site model is implemented as a discrete time simulation [22], with a time-step of one day. The  
160 system is initialised with a naive population ( $r_{v,j} = 0$  for all  $v$  and  $j$ ) and an infected host which carries the  
161 ancestral strain. At each time-step, the population changes according to SIRS dynamics, with the following  
162 events occurring:

- 163 1. Mutation: The number of mutations that occur in the viral population in each time-step is drawn from  
164 a Poisson distribution with mean  $\mu L_a$ , where  $\mu$  is the mutation rate per site per time-step, and occur  
165 uniformly across all sites and all individuals. For the codon model, the probability of any codon occurring  
166 at the mutated site is specified by the Kimura two-parameter model [23] with a transition-transversion  
167 rate of  $\kappa = 3$ .
- 168 2. Transmission: The number of potential new infections which occur in each time-step is a Poisson random  
169 variable  $X \sim \text{Pois}(\Lambda)$ , where  $\Lambda = \sum_{i=1}^I \beta(1-s)^{k_i}$  is the force of infection. The scaling factors  $(1-s)^{k_i}$   
170 account for the reduction in transmission of genotype  $i$  due to the cost of  $k_i$  changes away from the  
171 ancestral strain. The genotypes of the  $X$  potential infections are determined by multinomial sampling  
172 according to  $(1-s)^{k_i}$ , to account for variation in transmissibility within the viral population. We can  
173 then calculate the probability of each potential infection  $i$  encountering a susceptible host, given by

$$p_i = \frac{N - I - \frac{\sigma}{L_a} \sum_{j=1}^{L_a} r_{v_{ij},j}}{N}, \quad (22)$$

174 where  $r_{v_{ij},j}$  is the level of recognition against a particular antigenic site as described above. Equation  
175 (22) corresponds to Equations (10–11) in the analytical model. The success of the potential infection is  
176 determined using a Bernoulli random variable  $U \sim \text{Bernoulli}(p_i)$ . If  $U = 1$ , a new infection is generated  
177 with a genotype identical to the parent.

- 178 3. Recovery: The number of infected hosts which recover in each time-step is Poisson with mean  $\delta I$  trun-  
179 cated with an upper bound of  $I - 1$ . Each recovered host  $i$  is drawn from the infected population with

180 uniform probability and increases immunity to allele  $v_{ij}$  at site  $j = 1, \dots, L_a$ . That is, for each recovery,  
181 we update  $L_a$  elements of the immunity matrix

$$r_{v_{ij},j} := r_{v_{ij},j} + 1. \quad (23)$$

182 4. Decay of host immunity (across the whole population) is simulated by reducing  $r_{v,j}$  for all antigenic  
183 states  $v \in \mathbf{v}$  at each site  $j = 1, \dots, L_a$  by a binomial random variable,

$$r_{v,j} := r_{v,j} - V, \text{ where } V \sim \text{Binom}(r_{v,j}, \gamma). \quad (24)$$

184 Note that the epidemic is artificially prevented from extinction. The forcing mechanism is necessary as we  
185 have, for simplicity, not included a migration term. In stochastic models of recurrent epidemics, the infection  
186 frequently dies out without re-introduction by migration, particularly in smaller populations [24, 25].

### 187 3. Results

188 Using the analytical and simulation models, we examine how the epidemiology of the virus affects the  
189 probability of reversion at antigenic sites. We first describe the dynamics of the simple three-strain model  
190 (Section 3.1), before examining the time dependence of this system (Section 3.2) and the effect of the epidemi-  
191 ological parameters (Section 3.3). The combined effect of these interacting factors on the observed amino acid  
192 frequencies is described in Section 3.4, and we compare this to sequence data for human influenza A (H3N2)  
193 and RSV-A in Section 3.5.

#### 194 3.1. Dynamics of changing susceptibility

195 To provide some intuition about the process, we show an example of forward substitution and reversion in  
196 the three-strain model (Figure 1). The dynamics of the simulations, where mutations occur stochastically, are  
197 compared to the analytical model by setting  $t_1$  and  $t_2$  to the times at which the strains are observed to emerge  
198 in the simulation. By analogy with the three-strain model, whichever strain that emerges first containing one  
199 mutation (either 01 or 10) is denoted strain 1. For the time interval shown here, only three strains emerge,  
200 but over longer durations, all four strains will typically be observed.

201 For two separate simulations using the two-site bit-string model, we show the number of hosts infected  
202 with each strain  $i = 0, 1, 2$  [panels (a) and (b)], and the corresponding proportion of susceptible hosts  $p_i$   
203 [panels (c) and (d)]. The emergence of the ancestral strain 0 in the initially naive population sharply reduces  
204 the proportion of susceptible hosts to strain 0,  $p_0$ ;  $p_1$  is also slightly reduced due to cross-immunity between  
205 strains 0 and 1, while  $p_2$  is unaffected. When strain 1 emerges and dominates the population, both  $p_0$  and  $p_2$   
206 are temporarily reduced but  $p_0$  slowly increases above its previous equilibrium.

207 In the first simulation [panels (a) and (c)], strain 1 is rapidly replaced with strain 2, so that at the time  
208 of emergence  $t_2$ , susceptibility to strain 0 remains quite low [black line in panel 1(c)]. In this case, forward  
209 substitution is favoured because there is a larger pool of susceptible hosts for strain 2. In contrast, in panels



210 (b) and (d), the interval between  $t_1$  and  $t_2$  (vertical grey lines) is longer than the first simulation, providing  
211 time for  $p_0$  to reach similar levels to  $p_2$  so that reversion can occur.

### 212 3.2. Time-dependence of the probability of reversion

213 In Figure 2, we show the probability of reversion as a function of  $\tau = t_2 - t_1$ , the interval between the  
214 time of strain emergence (indicated by vertical grey lines in Figure 1). The theoretical probability of reversion  
215 [Equation (19)] is compared to the proportion of reversion events in simulations with a two-site bit-string  
216 model. We compute a proportion by binning substitution events with the same value of  $\log_{10}(\tau)$ , rounded to  
217 two significant figures.

218 To correspond to the analytical model, only substitution events following transitions between strain 0 to  
219 strain 1 are counted. Note that in the analytical model,  $\tau$  is the interval between the times of emergence;  
220 however, in the simulation, it is difficult to determine which of the emerging mutations will reach fixation. As  
221 a proxy for  $\tau$ , the counts from the simulation are binned according to the time between antigenic substitutions  
222 (i.e. the time at which a different antigenic strain becomes the dominant strain in the population).

223 These results confirm that the reversion probability varies with  $\tau$ . The probability of reversion is low if  
224 substitution occurs rapidly, and gradually increases with  $\tau$  until it flattens at the asymptote  $\rho_\infty$ , given by  
225 Equation (21). This asymptotic value represents the probability of reversion in the absence of cross-immunity.  
226 The decay rate of host immunity  $\gamma$  affects the speed at which the asymptotic value is reached, but not the  
227 value of the asymptote.

228 Greater variation is seen in the simulated results for large  $\tau$ , as these represent proportions computed from  
229 a smaller number of more rare events. However, the greatest discrepancy between theoretical and simulated  
230 results occurs near the transition  $t_{c,0}$  [Equation (18)]. At  $\tau = t_{c,0}$ , the theoretical model predicts a sharp  
231 transition away from  $\rho(\tau) = 0$ ; in the stochastic simulations, the transition is more gradual. The reason for  
232 this discrepancy is that the theoretical model assumes that each strain reaches equilibrium before it is replaced.  
233 However, in large viral populations, the mutational input rate can be large enough that strain 1 replaces strain  
234 0 before  $I_0$  can reach equilibrium. In these cases,  $R_0^*$  will be upwardly biased, so that  $\rho(\tau)$  underestimates the  
235 probability of reversion. We confirm this in Figure B10 in Appendix B where a similar plot is shown ignoring  
236 substitution events that occur before equilibrium is reached.

237 Based on the form of  $\rho(\tau)$ , we expect the time-dependent probability to be independent of the viral mutation  
238 rate and population size. Consistent with this, we observe that simulation results for different population sizes  
239 lie on the same curve, with points from small populations (circles) corresponding to large values of  $\tau$  and  
240 points from larger populations (triangles) corresponding to smaller values of  $\tau$ .

### 241 3.3. The effect of epidemiological parameters

242 To examine the effects of viral transmission ( $\beta$ ,  $\delta$ ,  $s$ ) and host immunity ( $\gamma$ ,  $\sigma$ ), we now consider  $\rho$  for  
243 a fixed  $\tau$  in the analytical model [Equation (19)]. For simplicity of notation, we omit the argument  $\tau$  in  
244 this section. Equations (13–15) indicate that the strength of immune protection  $\sigma$  affects  $\rho$  only through the

245 coefficients  $A$ ,  $B_0$ ,  $B_2$ , and is expected to have only a weak effect. In Figure 3, we confirm that the level of  
246 immune protection  $\sigma$  has only a weak effect on  $\rho$  unless the typical duration of the infection  $1/\delta$  [Figure 3(a)]  
247 is as long as the immune duration  $1/\gamma$  [Figure 3(b)], or  $\sigma$  is negligibly small. Throughout the rest of the paper,  
248 we set  $\sigma = 1.0$ .

249 Figure 4(a) shows how the reversion probability varies as a function of the basic reproductive ratio  $\beta/\delta$ , for  
250 various values of selective cost  $s$ , for a fixed level of host immunity ( $\gamma\tau$ ). For sites under no selective constraint  
251 (black line), the probability of reversion increases slightly with  $\beta/\delta$ , but very different effects are observed  
252 for a non-zero selective cost. The effect of a selective cost is strongest for small transmission rates, as slight  
253 decreases in infection rates can have a more detrimental impact on the mutant subpopulation.

254 The interaction between the selective cost  $s$ , and the immunity decay rate  $\gamma$ , is shown for a fixed  $\tau$   
255 [Figure 4(b)]. We showed in Figure 2 that for large  $\gamma\tau$ ,  $\rho(\tau)$  plateaus at  $\rho_\infty$ , which is independent of  $\gamma$ ;  
256 however, when the rate of strain replacement is comparable to the decay rate of host immunity, there are  
257 strong dependencies. The effect of varying  $\gamma$ , in the absence of selective constraint ( $s \approx 0$ ), can be seen in the  
258 difference between  $\rho$  where the curves plateau. Further increases in selective cost leads to a rapid increase in  
259 the probability of reversion, with more rapid increases for longer lasting immunity (solid line).

### 260 3.4. Fluctuating frequencies at antigenic sites

261 In Sections 3.1–3.3, we observed that  $\tau$  had a strong effect on whether reversions occur or not. In fact,  
262 where  $\tau$  is known, no further information on mutation rate  $\mu$  or population size  $N$  is required. However, in  
263 practice this quantity is difficult to measure. It is possible to account for variation in  $\tau$  by integrating over the  
264 distribution of  $\tau$ , but this can remove important information; under certain parameter ranges, the stochasticity  
265 of  $\tau$  is sufficient to cause noticeable variation in reversion probabilities.

266 To observe the effect of fluctuations in  $\rho$ , we measure the frequency of the ancestral allele  $\pi_0$  at each  
267 antigenic site. The frequency of an allele is informative about its fixation probability [26], and the rate of  
268 change in frequency is proportional to the strength of selection  $s$  [27, 17]. Under directional selection, we expect  
269 any allele to eventually reach fixation or extinction. Thus fluctuations between  $\pi_0 = 0$  to  $\pi_0 = 1$  indicates  
270 changes in selection. We measure the frequencies of each antigenic site separately, as immunity against each  
271 site may vary depending on the history of previous circulating strains.

272 In Figure 5, we show frequency trajectories  $\pi_0$ , under conditions of both antigenic selection and selective  
273 constraint, so that antigenic changes away from the ancestral sequence imposes a cost. To account for in-  
274 accuracies due to sampling,  $\pi_0$  was computed from sequences sampled at discrete intervals, and the earliest  
275 sequence sampled after the burn-in period was used as the ancestral sequence. In all panels, we observe fluctu-  
276 ations in frequency levels as reversion probabilities vary due to the stochasticity of the time between antigenic  
277 substitutions, although there is no change in  $\mu$ ,  $N$ , or  $s$  during a simulation. The pattern of fluctuations in  $\pi_0$   
278 differs depending on the host population size  $N$  (varying along columns) or the decay rate of host immunity  
279  $\gamma$  (varying along rows). Faster changes in  $\pi_0$  are observed for larger  $N$  and fixation of the ancestral allele  
280 becomes less likely. Tracking frequency over time also provides information on  $\gamma$  that would not be available

281 in the time-averaged approach. Comparison between columns in Figure 5 indicates that increasing  $\gamma$  tends to  
282 reduce both the frequency and amplitude of  $\pi_0$ . This effect is particularly evident for larger population sizes  
283 [panels (c)–(f)], where the rate of substitution is not limited by the rate of mutational input.

284 The effect of removing the selective cost ( $s = 0$ ) is shown in Figure 6. Although fluctuations can still  
285 occur, the ancestral allele at the antigenic site rarely returns to fixation ( $\pi_0 = 1$ ) and, if so, does not remain  
286 fixed for long. This effect occurs even for small population sizes [panel (a)] which favour reversion. Continual  
287 antigenic selection drives further substitutions to other derived amino acid residues, that have not induced  
288 prior immunity. That is, multiple instances of increasing  $\pi_0$  as an indication of high selective costs  $s$  is robust  
289 to misspecification of the ancestral allele. However, consistently low values of  $\pi_0$  may simply be due to using  
290 an misspecified ancestral allele (an alternative interpretation is that  $\pi_0$  correctly identifies that an unfavoured  
291 amino acid is unconstrained).

### 292 3.5. Application to influenza and RSV

293 In Figure 7, we show  $\pi_0$  changing over time for the human influenza A virus subtype H3N2 and the  
294 respiratory syncytial virus (RSV) subtype A at antigenic and non-antigenic sites. The H3N2 data set consists  
295 of all HA sequences for human H3N2 from the influenza virus database [28] where the year of sampling is  
296 known. The accession numbers surface G protein sequences of RSV-A sequences that we used were listed  
297 in Botosso et al. [5]. In total, we analysed 5831 H3N2 sequence spanning 45 years and 538 RSV sequences  
298 spanning 19 years.

299 We computed  $\pi_0$  for antigenic sites which have been identified by experimental methods, as sequence-based  
300 methods are also designed to identify sites with variation in amino acid composition. For H3N2, we used the  
301 seven sites (145, 155, 156, 158, 159, 189, 193) listed in a recent study [29] which used antigenic cartography  
302 which integrates information over multiple pairs of antigen and antisera in order to evaluate overall antigenic  
303 change [30]. For RSV-A, experimental studies with monoclonal antibodies have identified a large number  
304 of sites which react to different monoclonal antibodies [31, 32]. More recent studies have used phylogenetic  
305 analysis of natural isolates to identify potential antigenic sites [33, 34]. Note that there is an ascertainment  
306 bias in using sites identified on the basis of frequent amino acid changes. Here, we have restricted the analysis  
307 to eight sites (225, 226, 233, 237, 244, 274, 280, 290) which were identified as reducing antigenic recognition  
308 in multiple studies [33, 34, 31, 32], with at least one being experimental [31, 32]. Including a larger number of  
309 sites does not affect the results, but will obscure features of distinct trajectories.

310 For both viruses, we obtain oscillating patterns of  $\pi_0$  that are consistent with our expectations for antigenic  
311 sites evolving under both immune selection and functional constraint. Non-antigenic sites [Figure 7(c) and (d)]  
312 generally do not exhibit these fluctuations, but some non-antigenic sites in RSV-A may experience frequency  
313 fluctuations due to linkage to antigenic sites [Figure 7(d)]. Patterns of frequency change in H3N2 and RSV-  
314 A differ considerably from each other. H3N2 frequencies have sharper and slower oscillations, which are  
315 suggestive of both a smaller population size and longer lasting immunity. At least four antigenic sites in H3N2  
316 revert and fix at the ancestral state which indicates very strong selective constraint. RSV-A shows more rapid

317 oscillations, suggesting faster decaying immunity and moderate selective constraint. The relatively short time  
318 that the ancestral allele is at high frequencies suggests that selective constraint has a smaller influence than  
319 for H3N2.

#### 320 4. Discussion

321 We have shown that for acute, recurrent infections, the probability of reversion at antigenic sites depends  
322 on the interaction between the cost of immune escape and the duration of host immunity. Similar to models  
323 for HIV [17], we find that a higher cost of immune escape increases the probability of antigenic reversion. The  
324 impact of the cost of immune escape on the reversion probability is greater when the basic reproductive ratio  
325 is low, as small reductions in transmissibility have a more detrimental effect. This is in agreement with a  
326 previous study on the effect of selective constraint on antigenic drift [35]. In addition to these two parameters,  
327 we find that longer lasting immunity can also reduce the probability of reversion, but the precise extent of this  
328 reduction depends on the time between antigenic substitutions.

329 The time between antigenic substitutions, which is inversely proportional to the viral population size and  
330 mutation rate, is closely related to the rate of mutational input  $\theta$ , a parameter commonly used in population  
331 genetics to describe the time-scale of selection and drift. In the epidemiological model, it affects the balance  
332 between selective constraint and antigenic selection by determining the extent to which prior immunity has  
333 decayed. When the interval between antigenic substitutions is small, immunity against the ancestral strain  
334 remains high at the time of substitution so that antigenic selection reduces the reversion probability. For larger  
335 intervals between antigenic substitutions, prior immunity will have decayed to a greater extent and the basic  
336 reproductive ratio and cost of immune escape become stronger determinants of the reversion probability. In the  
337 context of phylodynamic models,  $\theta$  is also the parameter which is used to link the coalescent to epidemiological  
338 models [36].

339 Previous studies have described varying levels of reversion in a range of viruses and speculated on the  
340 influence of host immunity [5, 2, 8], but it has been unclear how the level of reversion should be quantified  
341 and how these results should be interpreted. In contrast to previous studies [5, 37] based on phylogenetic  
342 methods, we propose using temporal patterns of frequency change to quantify reversion. Where sequence data  
343 from multiple time-points is available, a frequency-based approach can more easily show the time-dependent  
344 effect of antigenic selection. Simulation results predict that varying parameters controlling population size,  
345 transmission rate, immunity decay and selective constraint have qualitative effects on the frequency of the  
346 ancestral allele  $\pi_0$  which are consistent with the analytical model, providing a means for interpretation. As  
347 our approach uses site-frequency data rather than a phylogeny, it is amenable to the application of large  
348 time-structured data sets, but is also more sensitive to effects such as biased sampling and spatial structure.

349 In this paper, we compared patterns of  $\pi_0$  for two viruses that induce acute respiratory infection which  
350 recurrently infect human populations and induce long-term immunity: influenza A (H3N2) and RSV-A. For  
351 both viruses, we observed fluctuations in frequency at antigenic sites suggesting the presence of both immune

memory and selective constraint. Without the continuously changing balance between these two effects, we would expect an allele for a particular site to reach fixation and remain in that state [27]. While RSV has been reported to experience high levels of reversions [5], previous phylogenetic studies have not identified reversion in H3N2. However, a recent study [8] showed that changes at antigenic sites in H3N2 occur as cycles in a genotype network; that is, mutations to multiple states occur before reversion to the ancestral allele, so that the reversion is not identifiable along a the phylogeny.

Our model suggests that the higher rates of reversion in RSV-A compared to H3N2 is due mainly to more rapidly decaying immunity rather than stronger selective constraint. Fluctuations in frequency are more rapid and complete fixation of the ancestral amino acid does not occur for most antigenic sites of RSV-A. In contrast, for H3N2, we observe multiple occasions where a fixation of the ancestral amino acid occurs, and long periods where  $\pi_0 = 1$  is maintained, suggesting strong selective constraints. This is consistent with the location of the sites within the receptor binding region of the HA gene, so that any antigenic change is also likely to affect viral transmissibility [29]. Comparison between the frequency of the oscillations also suggests that H3N2 induces more long-lasting immunity than RSV-A. RSV-A exhibits more rapid fluctuation while several of the antigenic sites in H3N2 were fixed for long periods ( $> 10$  years) at a derived amino acid, supporting the hypothesis that immune pressure against reversion is maintained for long periods. Frequency patterns of H3N2 frequency patterns are consistent with multi-site codon simulations (Figure 5) with host immunity decay rate  $\gamma$  on the order of  $10^{-4}$ , whereas a value of  $\gamma \approx 10^{-3}$  is more compatible with frequency patterns for RSV-A. These values are in agreement with reinfection experiments which estimate immunity for H3N2 lasting 8 years ( $\gamma = 3 \times 10^{-4} \text{ day}^{-1}$ ) [38] compared to 1.8 years ( $\gamma = 1.5 \times 10^{-3} \text{ day}^{-1}$ ) for RSV-A [39].

Our study shows that the frequency of the ancestral allele,  $\pi_0$ , which can be easily calculated for time-stamped viral sequences, is informative about the immune dynamics and cost of escape. In particular, sharp fluctuations in frequency is indicative of immune selection occurring at a comparable time-scale to substitutions at antigenic sites. However, a small number of linked sites may also display similar patterns as they co-segregate with antigenic sites. That is, frequency patterns should not be used as a method to identify antigenic sites; but where the antigenic sites are known, frequency patterns provide information about the epidemiology of the virus as a whole.

The approach outlined here provides a qualitative description rather than estimates of the epidemiological parameters. Analytical expressions, relating the probability of reversion to the parameters underlying the viral dynamics for the three-strain model, rely on the assumption that each strain reaches equilibrium before it is replaced. This assumption tends to be violated when population sizes and mutation rates become large, so that we generally underestimate the probability of reversion. To address the restrictions of the equilibrium assumptions and the assumption of only three strains, we used computer simulations describing sequence dynamics in a multi-site model. Formal inference using a complex computational model is a challenge for future research. Despite the simplicity, our approach is useful in providing a scheme to consider both epidemiological and molecular effects simultaneously. As such, it is complementary to both coalescent approaches [40, 41, 42] which assume epidemiological dynamics are largely unaffected by molecular changes, and to codon-based

389 methods [14, 15] which assume that substitution occurs instantaneously as a time-homogeneous process along  
390 branches of the phylogeny.

391 Our model highlights the importance of understanding the interaction between epidemiological and molec-  
392 ular effects. The results imply that different evolutionary trajectories are expected in viral populations with  
393 the same distribution of fitness effects but differing population size and contact rates. In particular, we expect  
394 that viral populations in larger cities with denser populations undergo less reversion and are more likely to  
395 generate antigenically novel variants.

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Table 1: Table of parameters used in the multi-site simulation model.

Parameter	Description
$\beta$	Transmission rate per time-step
$\delta$	Recovery rate per time-step
$\gamma$	Decay rate of host immunity per time-step
$\sigma$	Strength of immune protection
$\mu$	Mutation rate per site per time-step
$s$	Cost of immune escape
$L_a$	Number of antigenic sites
$N$	Host population size

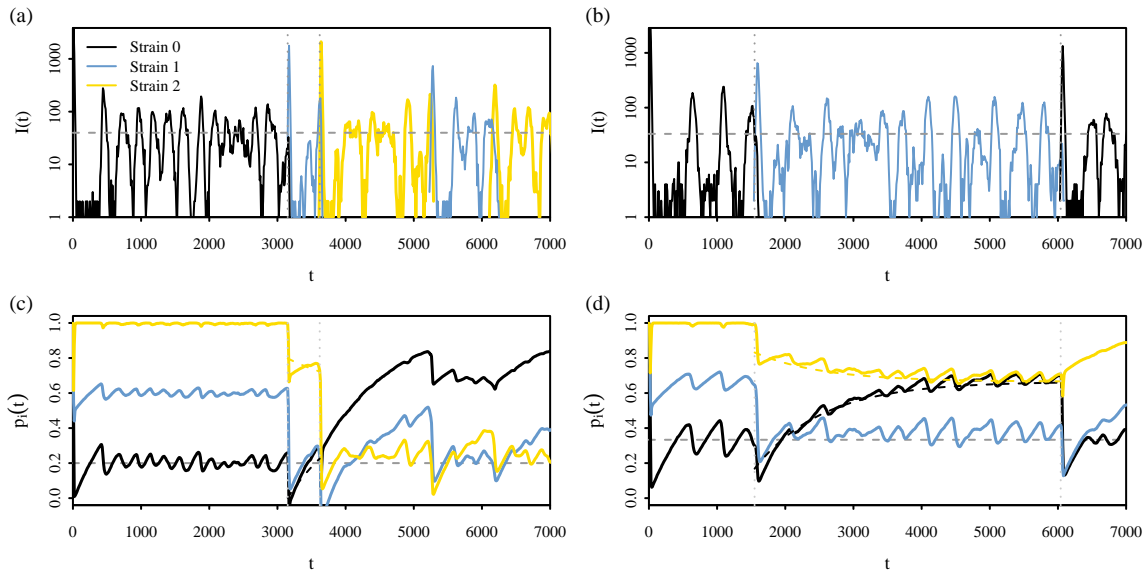


Figure 1: An example of forward substitution [panels (a) and (c)] and reversion [panels (b) and (d)] in the two-site bit-string epidemiological model. Solid lines show the trajectory from a single simulation of the number of hosts [panels (a) and (b)] infected by, and the proportion of hosts susceptible [panels (c) and (d)] to strains 0, 1 and 2. Simulations are initialised with a small number of hosts infected with strain 0 which tend towards the equilibrium [horizontal grey dashed lines; Equations (5–6)]. At time  $t_1$ , strain 1 emerges and dominates the population until time  $t_2$  when a third strain (either strain 0 or 2) emerges. Times  $t_1$  and  $t_2$  are indicated by vertical dotted grey lines. Between  $t_2$  and  $t_1$ , the expected proportion of susceptible hosts [Equations (12–15)] is shown by dashed lines. Simulations were run with parameters (a)  $\beta = 1.0 \text{ day}^{-1}$  and (b)  $\beta = 0.6 \text{ day}^{-1}$  and in both panels,  $N = 10^4$ ,  $\delta = 0.2$ ,  $\gamma = 10^{-3} \text{ day}^{-1}$  and  $s = 0.1$ .

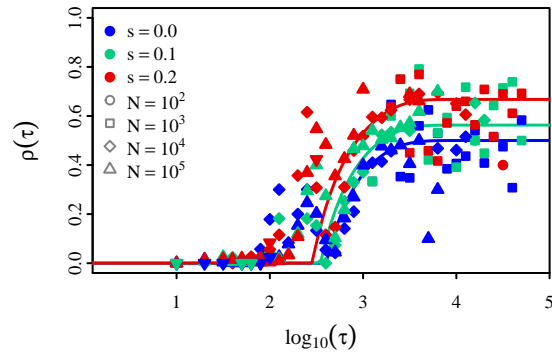


Figure 2: The probability of reversion, as a function of the time between strain emergence  $\tau$ . The blue line shows the reversion probability [Equation (19)] of an unconstrained antigenic site as immunity decays, whereas the green ( $s = 0.1$ ) and red ( $s = 0.2$ ) lines show the combined effect of selective cost and immunity. Points show the proportion of reversion events observed from simulations of the two-site bit-string model. The proportion was computed from the binned number of substitution events that occurred immediately after a transition from strain 0 to strain 1, using the observed time between antigenic substitutions as a proxy for  $\tau$ . Simulations were run for  $10^5$  time-steps, with a time-step of one day, with 1000 replicates for each parameter combination of  $s$  and  $N$ . All other parameters were set to immune decay:  $\gamma = 10^{-3} \text{ day}^{-1}$ , mutation rate:  $\mu = 10^{-5} \text{ site}^{-1} \text{ day}^{-1}$ , recovery rate:  $\delta = 0.2 \text{ day}^{-1}$  and transmission rate:  $\beta = 0.6 \text{ day}^{-1}$ .

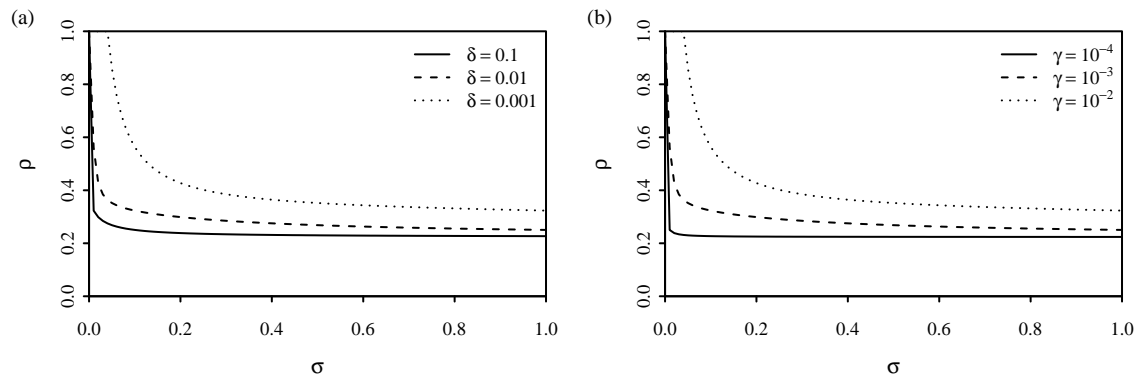


Figure 3: A comparison of the sensitivity of the reversion probability [Equation (19)] to the strength of immunity  $\sigma$  for different (a) rates of recovery  $\delta$  and (b) rates of immunity decay  $\gamma$ . Unless otherwise specified, parameters were set to  $\gamma = 10^{-3} \text{ day}^{-1}$ ,  $\delta = 0.1 \text{ day}^{-1}$ ,  $\beta/\delta = 5$ ,  $\gamma\tau = 0.5$  and  $\mu = 10^{-5} \text{ site}^{-1} \text{ day}^{-1}$ .

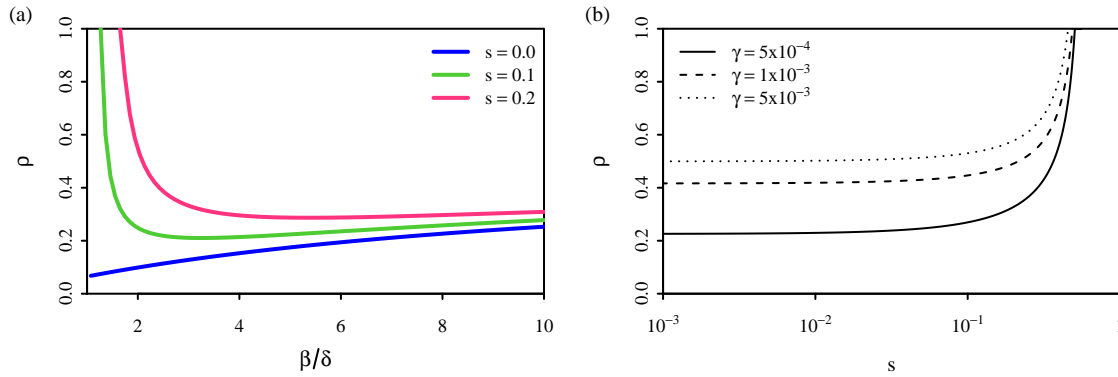


Figure 4: The effect of the cost of immune escape  $s$  and decay rate of immunity  $\gamma$  on the probability of reversion [Equation (19)]. In (a), we hold the level of immunity ( $\gamma\tau = 0.5$ ) constant to show how varying basic reproductive ratio  $\beta/\delta$  changes the effect of  $s$ . In (b), we show the effect of varying  $s$  for different values of  $\gamma$  with a fixed time between strain emergence  $\tau = 3 \times 365$  days and  $\beta/\delta = 5$ . Other parameters were set to  $\delta = 0.2 \text{ day}^{-1}$  and  $\mu = 10^{-5} \text{ site}^{-1} \text{ day}^{-1}$ .

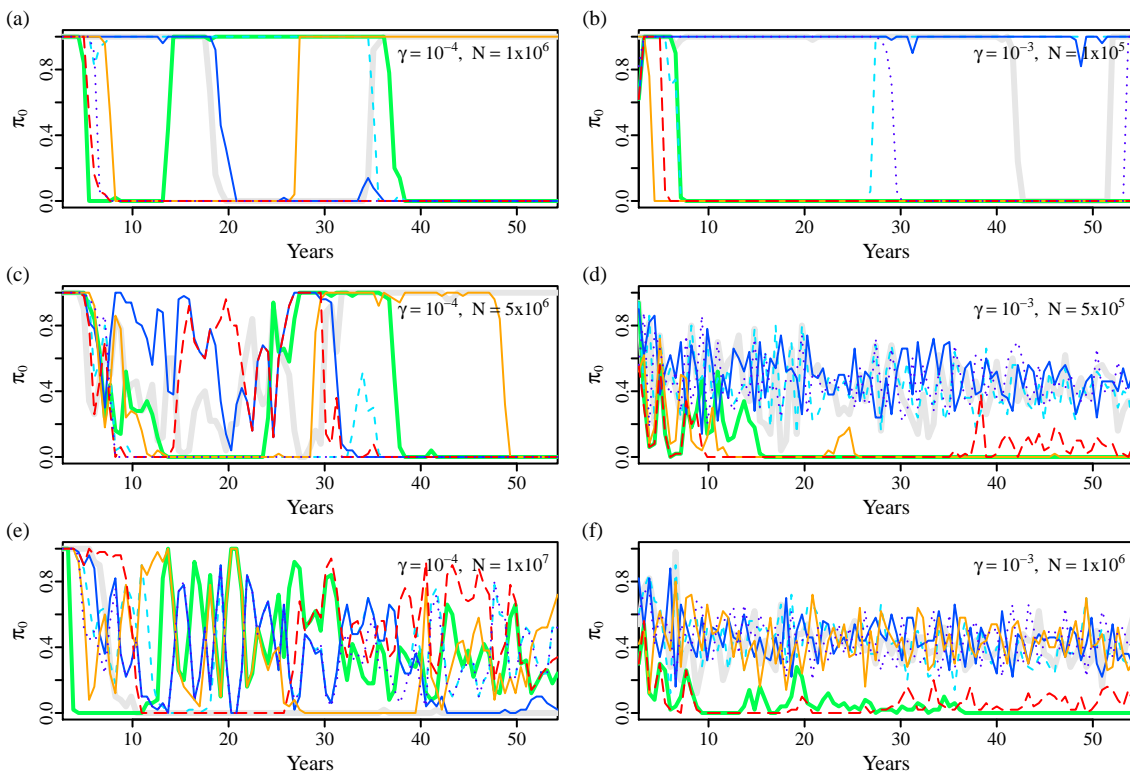


Figure 5: The effect of population size and duration of immunity on the frequency of the ancestral allele  $\pi_0$  at antigenic sites under selective constraint ( $s = 0.2$ ). Each line represents the changing frequencies, at a single antigenic site, of the ancestral allele estimated to be the earliest sampled amino acid residue after the burn-in period (1000 days). For high rates of mutational input [panels (d) and (f)], the earliest sequence may not be the true ancestral sequence (set to be the most transmissible), which in some cases results in low observed values of  $\pi_0$ . Each panel represents the dynamics of a single simulation, with  $\pi_0$  computed from samples of 20 sequences taken every 200 days. All simulations were run with a time-step of one day and parameters  $\beta = 1.0 \text{ day}^{-1}$ ,  $\delta = 0.2 \text{ day}^{-1}$ ,  $\mu = 10^{-5} \text{ site}^{-1} \text{ day}^{-1}$ ,  $L_a = 7$ , to match parameters used for human influenza A (H3N2) [43].

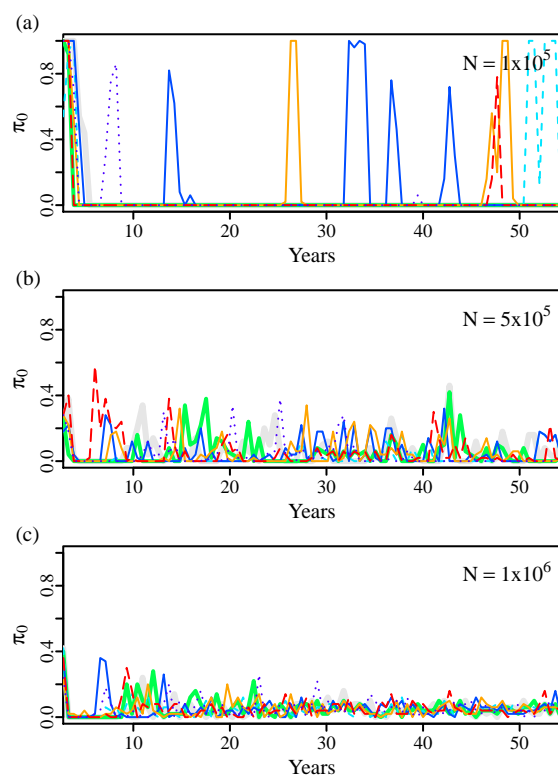


Figure 6: The frequency of the the ancestral allele  $\pi_0$  at antigenic sites under no selective constraint ( $s = 0$ ). Lines in each panel show the changing frequency of the ancestral (earliest sampled) allele at each antigenic site in a single simulation.  $\pi_0$  was computed from samples of 20 sequences taken every 200 days, discarding all sequence data from the burn-in period of 1000 days. All simulations were run with  $\gamma = 1 \times 10^{-3} \text{ day}^{-1}$ ,  $\beta = 1.0 \text{ day}^{-1}$ ,  $\delta = 0.2 \text{ day}^{-1}$ ,  $\mu = 10^{-5} \text{ site}^{-1} \text{ day}^{-1}$ , and  $L_a = 7$ .

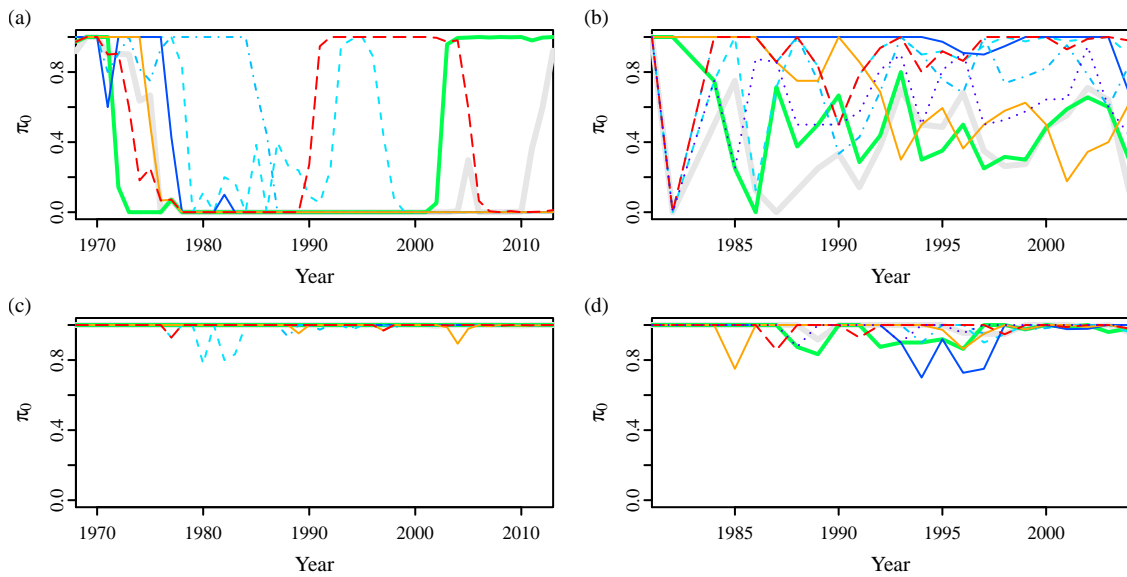


Figure 7: Trajectory of the frequency of the ancestral allele  $\pi_0$  computed at antigenic sites of (a) human H3N2 and (b) human RSV-A show fluctuations which are distinct from randomly chosen non-antigenic sites [panels (c) and (d)]. Frequencies were computed at (a, c) seven sites in the HA segment of H3N2 with A/Aichi/2/1968 as the ancestral strain, and (b, d) eight sites in the C-terminal hyper-variable region of the surface G protein of RSV-A using strain AF065406 (sampled in 1981) as the ancestral strain. Sequences were pooled according to the year of isolation, with years in which fewer than five sequences were sampled were excluded.

520 **Appendix A. Comparison of the compartmental SIRS model with an agent-based model for a**  
521 **single strain**

522 The compartmental SIRS model described in Section 2.1 tracks only the number of hosts with immunity  
523 in the population  $R$ , which is increased with each infection by an increment of  $\sigma$ . The model, however, does  
524 not account for the fact that partially immune hosts which are re-infected cannot gain more than complete  
525 immunity. To examine the effect of this approximation, we implement, for the single strain case, an agent-  
526 based model which tracks the level of immunity  $r_i \in [0, 1]$  in each host  $i$  in the population. The agent-based  
527 variables can be related to the population model [Equation (3)] by summing across all uninfected hosts  $\tilde{I}$ ,

$$\sum_{i \in \tilde{I}} r_i = \sigma R.$$

528 We implement two agent-based simulations which differ in how they model the decay of host immunity. In  
529 model A1, immunity decays deterministically, so there is no variability in the rate of decay between hosts. In  
530 contrast, in model A2, we maximise the variability in the rate of decay by having complete loss of immunity  
531 in a proportion of hosts. In both models, population-wide levels of immunity are reduced, but there are  
532 considerable differences in the variation in levels of immunity between hosts, which is known to affect the  
533 epidemiological dynamics [44].

- 534 1. Transmission : The number of new infections in each time-step is Poisson with mean  $\beta SI/N$ . Newly  
535 infected hosts are randomly drawn with the set of uninfected hosts by multinomial sampling, according  
536 to  $1 - r_i$ . Note that their immunity status is not altered on infection, so they retain immunity obtained  
537 from prior infections, but their contribution is not included into the population variable  $R$ .
- 538 2. Recovery: The number of infected hosts which recover in each time-step is Poisson with mean  $\delta I$  trun-  
539 cated with an upper bound of  $I - 1$ . Each recovered host  $i$  is drawn from the infected population with  
540 uniform probability and their immunity is increased by

$$r_i := \min(1, r_i + \sigma). \tag{A.1}$$

- 541 3. Decay of host immunity is simulated differently between models A1 and A2. In model A1, immunity is  
542 reduced deterministically in each uninfected hosts  $i$

$$r_i := r_i(1 - \gamma), \tag{A.2}$$

543 whereas in model A2,

$$r_i := r_i(1 - U), \text{ where } U \sim \text{Bernoulli}(\gamma). \tag{A.3}$$

544 We compare the agent-based models to the compartmental model implemented in Section 2.2 with no mutation  
545 (single-strain). Importantly, the equilibrium value for  $S$  is largely unchanged between models [Figure A.8(b)],



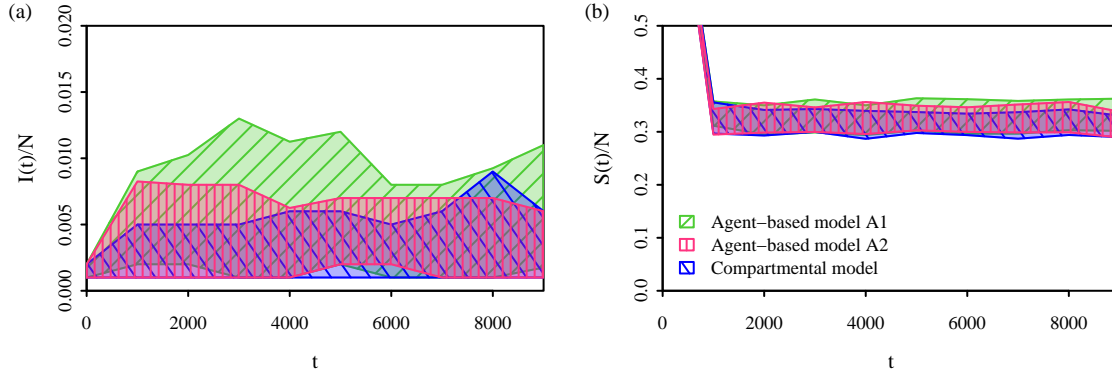


Figure A.8: Comparison of the simulation dynamics between agent-based and compartmental simulations. Shaded areas show the interquartile range for 100 replicates with  $\beta = 0.6 \text{ day}^{-1}$ ,  $\delta = 0.2 \text{ day}^{-1}$ ,  $\gamma = 10^{-3} \text{ day}^{-1}$ ,  $\sigma = 0.8$ ,  $N = 10^3$ .

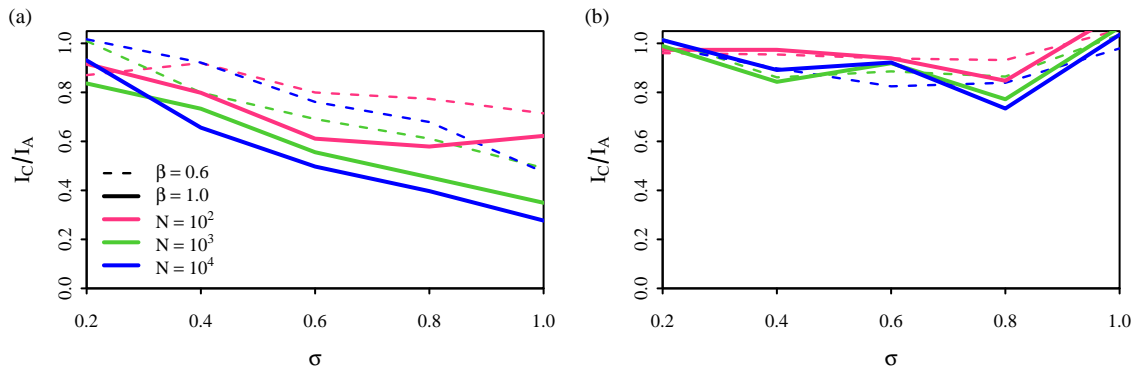


Figure A.9: Underestimation of the size of the infected population in the compartmental model compared to the agent-based models (a) A1 with gradual decay of host-immunity and (b) A2 with sudden decay of host-immunity. The extent of underestimation was computed by taking the mean of  $I(t)$  over 100 simulations over  $10^4$  time-steps for both the compartmental model and the agent-based model, and taking the ratio of the means. Simulations were run with  $\delta = 0.2 \text{ day}^{-1}$  and  $\gamma = 10^{-3} \text{ day}^{-1}$ .

546 suggesting that derivations of  $\rho(\tau)$  remain valid. However, differences can be seen in equilibrium value of  $I$ .  
 547 The compartmental model equilibrates at a lower mean value of  $I$  compared to both agent-based models A1  
 548 and A2, with a larger difference when compared to A1 (Figure A.9). However, this discrepancy is comparable  
 549 to the variance in the of  $I(t)$  over time [Figure A.8(a)]. The difference in  $I$  between simulations is largely  
 550 unaffected by changes in population size  $N$  or transmission rate  $\beta$ , but mainly influenced by  $\sigma$  (Figure A.9).

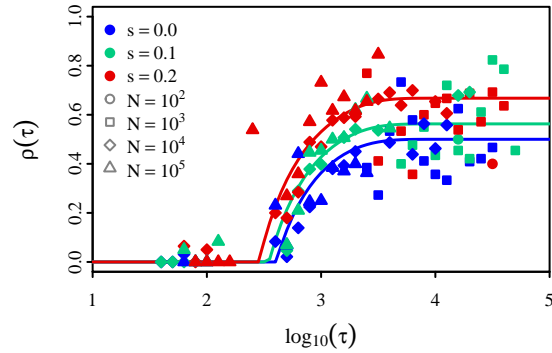


Figure B.10: The probability of reversion, as function of the time between strain emergence  $\tau$ . This is the same as Figure 2, except that substitution events which occur before the population reaches equilibrium are ignored.

## 551 Appendix B. Effect of equilibrium assumption in SIRS model

552 The derivation of the probability of reversion [Equation (19)] depends heavily on the assumption that the  
553 system reaches equilibrium before antigenic substitutions occur. This effect can be seen by comparing Figure  
554 2 to Figure B.10 where substitution events that occur before equilibrium is reached are ignored. The points  
555 with the greatest discrepancy to the theoretical values of  $\rho(\tau)$  for intermediate values of  $\tau$  are not seen in  
556 Figure B.10. There is more variation from theoretical expectations for large  $\tau$ , as more substitution events  
557 have been removed the calculation.