Influenza emergence in the face of evolutionary constraints

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Different influenza subtypes can evolve at very different rates, but the causes are not well understood. In this paper, we explore whether differences in transmissibility between subtypes can play a role if there are fitness constraints on antigenic evolution. We investigate the problem using a mathematical model that separates the interaction of strains through cross-immunity from the process of emergence for new antigenic variants. Evolutionary constraints are also included with antigenic mutation incurring a fitness cost. We show that the transmissibility of a strain can become disproportionately important in dictating the rate of antigenic drift: strains that spread only slightly more easily can have a much higher rate of emergence. Further, we see that the effect continues when vaccination is considered; a small increase in the rate of transmission can make it much harder to control the frequency at which new strains emerge. Our results not only highlight the importance of considering both transmission and fitness constraints when modelling influenza evolution, but may also help in understanding the differences between the emergence of H1N1 and H3N2 subtypes.

Keywords: influenza; fitness costs; emergence; transmission; antigenic drift

1. BACKGROUND

The two influenza A subtypes common in humans, H1N1 and H3N2, frequently escape population immunity by changing their antigenic properties. Between 1983 and 2009, H3N2 fixed more amino acid changes in antigenic sites than H1N1 [1–4], and vaccines against H3N2 were updated more often than vaccines against H1N1 [5,6]. The higher rate of appearance and fixation of non-synonymous mutations in H3N2 could be owing to a combination of factors, presumably including effective virus population size, and selective pressures according to host prior immunity. However, the root cause of the epidemiological and evolutionary differences between subtypes of influenza in humans remains poorly understood [7,8].

Transmissibility, or more specifically the basic reproductive ratio $R_0$, the average number of secondary cases generated by the average infective individual in a naïve population, has been much studied for influenza [9,10]. The probability that a strain will become established is determined by its effective reproductive ratio, which depends on $R_0$ and the level of population immunity. Seasons in which H3N2 was the dominant subtype have been associated with a higher effective reproductive ratio [11,12]; there are also typically more deaths from pneumonia and influenza in seasons where H3N2 is the dominant strain [13]. Transmissibility must be therefore considered as a possible mechanism behind the difference in antigenic drift speeds between the two subtypes.

One strain of influenza may confer partial immunity to another [14], and multi-strain models can be used to study the evolutionary dynamics of the disease [15–17]. Here, we define a new strain to be a virus in which the surface proteins have undergone sufficient change, as a result of mutation, to affect people who were previously immune to it. We will use such a model to examine the role that transmission plays in the emergence of new strains.

While multi-strain models are well established, few allow mutation to directly affect aspects of virus phenotype other than antigenicity. Although partially explored in individual-based simulations [1,18], a previous study [19] introduced a model to investigate the behaviour of influenza evolution when mutation affected the ability of a virus to transmit between hosts.

In studies in vitro, single nucleotide mutations in other RNA viruses have resulted, on average, in a fitness reduction [20–22]. If random mutation carries a fitness cost, there is a possibility that mutations associated with antigenic change also affect the phenotype in some other way, reducing viral fitness of antigenic escape mutants through antagonistic pleiotropy [23]. Alternatively, or additionally, ‘deleterious hitchhikers’—mutations elsewhere on the genome that happen to be picked up in the process of emergence of the antigenic escape mutant—could have a negative effect (M. Lässig 2011, personal communication). The ill-fated side branches of influenza's evolutionary tree could be the result of costs incurred by either mechanism (M. Lässig 2011, personal communication; [24]). However, new influenza strains appear every few years [25]; this ongoing antigenic mutation and survival could be possible through subsequent compensatory mutations [26,27].

Gog [19] developed a simple model of these processes to show that a high-level fitness loss could in fact stop viral evolution ('strain lock'), and that vaccination could also nudge the system into this state. In this paper, a two-tier framework is used to separate the interaction of strains via population immunity, modelled deterministically, and the emergence of new strains, treated as a stochastic process. This improves the realism of the fitness loss and compensatory mutation processes, and makes

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the model computationally far more tractable, allowing
the examination of a variety of assumptions.

We see that although the rate of emergence increases
almost linearly with transmission in the absence of fitness
constraints, when mutation incurs a cost, small changes in
the $R_0$ of the fully fit virus can have a large impact on
emergence. This is an example of phylodynamics [7],
the relationship between epidemic and evolution
dynamics.

Previous work has shown that often a threshold can
exist where vaccination can slow or pause the emergence
of new strains [19,28,29] and therefore we also look at
how the use of vaccination in slowing antigenic evolution
is affected by change in $R_0$. We show that vaccination can
be far more successful in controlling evolution when fit-
ness costs are present, highlighting the importance of
considering such constraints when modelling influenza
evolution.

2. METHODS
(a) A two-tiered model
A first approach to adding fitness costs to a multi-strain
model, suggested by Gog [19], was to take the one-dimen-
sional line of strains used in many previous models [16,30]
and extend it to a two-dimensional space. Each variant had
an intrinsic fitness, as well as an antigenic type (which chan-
ged in one direction). Mutation to a new antigenic type
causeda reduction in fitness, and compensatory mutations
were included so that strains can also regain fitness lost. A
simple stochastic approximation was included; a test was
imposed on new strains present in less than one individual
to ensure that they did not emerge automatically. This
meant that stochastic effects were present in a basic way,
although the approximation had some drawbacks, which
will be discussed here.

Suppose $R_0$ denotes the basic reproductive ratio of the
fully fit virus, i.e. the top level of strains described by Gog
[19]. This definition of $R_0$ will be used throughout this
paper. A full fitness strain therefore has an effective reproduc-
tive ratio $R = R_0 S/N$, where $S$ is the number of individuals
susceptible to the strain, and $N$ is the population size.
Here, as in Gog [19], we assume that antigenic change
incurs a fixed fitness cost, so a new strain has an effective
reproductive ratio $R_1 = \chi R < R$ initially, where $\chi$ denotes
the relative fitness of the mutant, reducing $R$ by a factor
$0 \leq \chi \leq 1$.

Let $I_0$ be the number of individuals infected with the new
strain. If large numbers are infective with the dominant
strain, even a low rate of mutation will lead immediately to
$I_0 > 1$. Hence it circumvents the stochastic test, and means
a possible ‘overspill’ effect, whereby a circulating strain can
prop up its weaker mutant, allowing it to persist long enough
to undergo a compensatory mutation, despite the fact that
would not necessarily survive if both fitness loss and compen-
sation were modelled as a branching process. Conversely, if
$I_0 < 1$ always, as can happen when the dominant strain is
endemic at low numbers, the stochastic test will not allow
the weaker mutation to ever emerge if it has $R < 1$. However,
it has been shown that if emergence is modelled stochastically,
infections with $R < 1$ may still survive long enough to
subsequently undergo a compensatory mutation [9]. A
further issue, mentioned by Gog [19], is that, for $\chi = 1$,
there are still two versions of each strain circulating, whereas

(b) Emergence process
We consider two routes to emergence, as shown in figure 1.
In particular, we wish to determine whether a strain with
$R < 1$ can mutate before it goes extinct. Similar problems
have been tackled for evolution in the face of selection
pressure [32] and zoonoses [9,33].

First, we outline a useful result that will be used in the full
problem: the probability that a strain with particular fitness
fails to cause an epidemic. We assume that $I_0 \ll S/N$, and each
infective individual can be considered independent. In the
standard susceptible-infective-recovered model, where the effec-
tive reproductive ratio $R$ is constant, the probability a single
infective individual will fail to generate an epidemic is [34]:

$$P = \begin{cases} 
1 & \text{if } R > 1, \\
1 - \frac{1}{R} & \text{otherwise.} 
\end{cases}$$

As transmission by each infective individual can be
considered independent, we have:

$$P = \min\left\{1, \left(\frac{1}{R}\right)^{I_0} \right\}.$$  

Next, this result can be applied to the full evolutionary
process, with compensatory mutation occurring at rate $\mu$.
Let $I_0(\tau)$ be the number of individuals infected with the
reduced fitness strain, which has reproductive ratio $R_1$, and
$I_2(\tau)$ be the number of individuals infected with the compen-
sated strain, with reproductive ratio $R_2 > R_1$. Although this
method will work for more general fitness structures, in
this paper we assume full fitness recovery, i.e. $R_2 = R_0 S_2/N$,
where $S_2$ is the number of individuals susceptible to strain 2.

Following Keeling & Rohani [34], if $I_0 = I(0) = 1$, the
probability the reduced fitness strain fails to cause an epi-

demic, $P_1$, can be written as a function of the recovery,
transmission and compensatory mutation processes. $P_2$, the
probability the full fitness strain fails to cause an epidemic,
can be treated the same as $P$ above. Rescaling time by the

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infectious period $1/\gamma$, we have:

$$P_1 = \frac{1}{1 + R_1 + m_\gamma} + \frac{R_1 \gamma}{1 + R_1 + m_\gamma} p_2^2$$

$$+ \frac{m_\gamma}{1 + R_1 + m_\gamma} p_2,$$

where $m_\gamma = m/\gamma$. Taking the smallest root in $[0,1]$ of this equation gives us the probability that the reduced fitness strain fails to cause an epidemic [35],

$$P_1 = \min \left\{ 1, 1 - \frac{(R_1 - 1 - m_\gamma) + \sqrt{(R_1 - 1 - m_\gamma)^2 + 4m_\gamma R_1(1 - P_2)}}{2R_1} \right\}$$

(2.3)

where $P_2 = \min \{1,1/R_2\}$.

If $I_0 \neq 1$ and $I_0 \ll S/N$ still, we can again use independence of interactions to obtain:

$$P \{ \text{fails to emerge} | I(0) = I_0 \} = P_1^{I_0}. \tag{2.4}$$

Also note that if $m_\gamma = 0$ in equation (2.3), we recover the single strain case $P_1 = \min\{0,1/R_1\}$, as expected. This is also true if $R_1 - 1 - m_\gamma \gg 4m_\gamma (1 - P_2) R_1$, implying that if there is little or no fitness cost, with $R_1 > 1$ and $m_\gamma \ll 1$, the probability of emergence behaves as if we had a single strain branching process. Therefore, the step-down and step-up do not have an unnecessary effect if $R_1$ is not reduced by a fitness cost.

We can use these results to approximate the probability a strain will emerge in our full model. Suppose strain $a$ is our frontmost strain (i.e. the one that emerged most recently), at each point in time, we know the prevalence, $I_a$, and susceptibility to a mutant of this strain, $S_{a+1}$. If mutation occurs at a rate $m$, we would expect to see

$$I_0 = L(1 - e^{-m}) \tag{2.5}$$

new mutants appear in a single time step. Using the above results, we can therefore calculate $P_{a+1} = p_{a+1}(R_1, I_0)$, the probability that an infective individual fails to emerge with the new strain $a + 1$, with $R_1 = \chi S_{a+1}/N$, and $I_0$ as above.

Computationally, this only requires the following test to be applied at each time step:

$$I_{a+1} = \begin{cases} 0 & \text{if } P_{a+1} > \text{uniform random variable } X \in (0,1), \\ 1 & \text{else.} \end{cases} \tag{2.6}$$

Although we have two routes to emergence (figure 1), and hence two possible values of $R$ for $I_{a+1}$, it is reasonable to assume that if the virus does cause an epidemic, its numbers will end up sufficiently large for it to undergo the compensatory mutation during this epidemic [26,36]. $I_{a+1}$ therefore represents individuals infective with the full fitness strain.

As in Gog [19], unrealistic strain survival is avoided by setting any strain that decreases in prevalence to $I < 1$, with an $R < 1$, equal to zero. This avoids the decay to infinitesimal levels (i.e. attostains [37]), and potential re-emergence, which can occur in purely deterministic systems.

(c) Epidemiological process

The second tier of the model keeps track of how immunity changes during epidemics at the population level. To do this, we use a status-based model with one level of fitness only [16], with $S_a$ denoting the number of hosts susceptible to (at least) strain $a$; $I_a$ the number of hosts infectious with strain $a$; and $A_a$ the force of infection of strain $a$. We will use a discrete formulation, as it will make it easier to deal with the mutation step at each point in time (which is based on the random number tests in §2b). Our system is therefore:

$$S' = S_a(1 - e^{-\mu}) + S_a e^{-\mu I_a}$$

and

$$I' = S_a(1 - e^{-\beta_a I_a}) + I_a e^{-\gamma},$$

(2.7)

where

$$A_a = \sum_k \beta_a \epsilon_a I_k.$$

Here $S_a'$ and $I_a'$ represent the values of $S_a$ and $I_a$ at the next time step. Each time step represents one day, and our parameters are scaled as such. We assume a fixed population size $N$, so $\mu$ acts as both the birth and death rate. $\beta_a$
represents the transmission rate for strain \( a \). Recovery is at a fixed rate \( \gamma \). Cross-immunity is given by the term \( c_{ka} \). We set this to decay exponentially with antigenic distance, and we assume that strains give complete immunity to themselves, so \( c_{ka} \) must be such that \( c_{aa} = 1 \). Table 1 gives the values of the parameters used in the model. The infectious period in §2b was given by \( 1/\gamma \), so here \( \gamma \) includes both recovery from infection and natural death. However, for typical human influenza parameters, the death rate makes a negligible contribution to \( \gamma \).

A moving strain space, as outlined by Gog [19], is also used to hasten computation. At each point in time all strains with \( I > 0 \) are identified. By the nature of mutation in the model, these cluster together and hence form a set of adjacent strains on our line. This set is active in the model, in that the above equations only use the variables corresponding to these prevalent strains. We update this active set at each time step. If the backmost (i.e. least recent) strain in the set falls below one infective, then we drop that variable, and if a new strain appears (see §2b), we add new variables. There is also a buffer in practice: if a new strain emerges, we create variables associated to the next strain on the line, in anticipation of its emergence.

We approximate the new level of susceptibility by multiplying the immunity with the previous strain by the reduction owing to the imperfection of cross-immunity:

\[
S_{a+1} = (N - S_a) e^{-\alpha}.
\]

This approximation underestimates immunity (in the form of reduced transmissibility) slightly [19]; this could affect accuracy under a high mutation rate, as more frequent appearance of mutants would mean that the strain space moves faster. However, it is less of an issue for rates similar to those considered in this paper.

Drift speed, used to measure the speed of evolution in our model, is defined as the rate of emergence: the mean number of strains that appear per year, over twenty 50-year simulations. If the system goes extinct, we only look at the behaviour up to the point of extinction. Over many runs, this rate approaches a fixed value for each \( R_0 \) and \( \chi \). We focus on \( \chi \in [0.6,1] \), however, as below these values a tendency to lock or go extinct reduces the accuracy of the mean.

3. RESULTS

In a manner similar to that of Gog [19], our model exhibits three dynamical behaviours: **locked**, where only one strain circulates; **drifting**, with the continuing emergence of strains; and **extinction**, where no strains remain. Once drift is established, the system is more likely to continue drifting than switch to the other two, especially when \( \chi \) is larger. As \( \chi \) is reduced, a drifting system will have an increasing tendency to fall into a locked state, or go extinct.

First, to check the basic drift dynamics when there is no fitness loss (\( \chi = 1 \)), this model is compared with a more basic deterministic model [16] using a continuous version of equations (2.6) and (2.7). In this simpler model, the authors could use an analytical method to calculate drift speed \( \epsilon \), and it was shown that:

\[
\epsilon = \min_{\lambda} \left\{ \frac{\beta - \gamma - \mu + 2m(\cosh \lambda - 1)}{\lambda} \right\}.
\]

Such a result is not possible for a stochastic system of equations, but using \( R_0 = \beta/\gamma \), we can compare the above theoretical result with the speed observed in our model (using the same values of \( \gamma \), \( \mu \) and \( m \)). Simulations were run for several values of \( R_0 \), with the model deliberately started in a drifting state, and calculated rate of emergence over twenty 50-year runs. This gives a measure of the speed of antigenic drift.

Figure 2 shows that the qualitative relationship between \( R_0 \) and speed of emergence is very similar, with the rate of emergence dependent on \( R_0 \in [1.4,3] \) in a near-linear way. Although the model presented in this paper evolves at a slower rate, this is most probably owing to the stochastic step that tempers emergence.

However, when fitness costs are introduced we see something quite different. The left-hand side of figure 3 shows that when \( \chi < 1 \), the impact of \( R_0 \) can become
A drop-off appears, along which small changes in transmission can have a disproportionate effect on the speed at which strains appear. Moreover, at the base of this ‘drift cliff’, the relationship is much flatter, and \( R_0 \) has even less effect on evolution than it did at when there were no fitness constraints.

It also shows that, if \( \chi \) and \( R_0 \) do not vary much between strains, the appearance of a more fit strain with larger \( R_0 \) could immediately impact the rate of emergence, as any subsequent antigenic mutant will find it much easier to establish itself.

We can elucidate this result by considering the role of the stochastic step in the rate of emergence. Figure 4 shows the probability a strain emerges for a particular effective reproductive ratio, \( R_1 = \chi R_0 S/N_0 \), equal to \( 1 - P \), in equation (2.3). As \( R_1 \) decreases towards 1, it becomes less likely a new strain will emerge at that step. If \( S \) were fixed this would imply that, for each \( R_0 \), there is a critical value of \( \chi \) below which the appearance of strains is relatively rare. Although a subsequent compensatory mutation and emergence event can still occur, as shown by the solid line in figure 4, this is rare for \( R_1 \) < 1 and the long wait for the appearance of a new strain slows down the rate of emergence.

However, in our model, \( S \) is not fixed because immunity to each strain varies, and depends heavily on other circulating infection. Therefore, we can only estimate this relationship between \( \chi \) and \( R_0 \). Simulations show that susceptibility \( S \) to new strains falls between 0.75 and 0.9, so, by finding the region where \( \chi R_0 S/N_0 < 1 \), this can be used to estimate the location of the drop-off in the rate of emergence. The right-hand side of figure 3 shows that this theoretical result agrees with the simulated behaviour, and hence provides an explanation for the location of the drop-off in emergence.

(a) **Vaccination**

Vaccination will be explored by observing how the effects of pulsed vaccination vary with fitness and transmission. For each \( \chi \) and \( R_0 \), we let the system settle in a drifting state before vaccinating a fixed proportion of the population chosen at random all at once, at time \( T \), with a vaccine most similar to the most recently emerged strain, and cross-reactive with reduced factor \( e^{-\alpha_d} \) for a strain distance \( d \) away. Hence, if \( v \) is the vaccine strain, and we vaccinate a proportion \( p \),

\[
\text{new } S_a = (1 - p e^{-\alpha_d v}) S_a.
\]

The number of strains that emerge in the interval \([T, T + 365]\) is then counted and this number is compared with the expected rate of emergence for that \( \chi \) and \( R_0 \), as calculated in figure 3, to obtain a relative speed between 0 and 1 as a result of vaccination of the population.

When 20 per cent of the population is vaccinated, the rate of emergence of the strain with \( R_0 = 1.6 \) is slowed much more than that of the one with \( R_0 = 2 \). Taking the mean from 200 runs, figure 5 shows that while \( R_0 \) makes little difference when \( \chi = 1 \), for \( \chi < 1 \), the infection with \( R_0 = 2 \) can be far harder to control.

This is because, for a strain with reduced fitness \( R_1 \) barely above 1, vaccination can lower susceptibility and put \( R_1 \)
ing) observed by Gog [19]. As influenza vaccines are selected around nine months under fitness constraints, a small increase in \( R_0 \) can make controlling the rate at which new strains emerge much harder (figure 5), as well as controlling the rate at which they spread. However, it also implies that if strains do lose fitness when escaping immunity, vaccination could have a greater benefit than a simpler model would imply.

We have made several assumptions in our model. We have used status-based variables for tractability, simplifying the immune structure of the population. We have also imposed a one-dimensional strain structure on influenza evolution. Although this reflects the sequential appearance of strains implied by the ladder-like phylogenetic tree of influenza A [7,44], the causes of this phylogeny are likely to be complex, with several explanations having been proposed [1,17,18,45] for the observed evolution. By using a simple line of strains, this paper does not address the issue of strain dimensionality. Instead, it assumes that, in the long term, through some mechanism not included here, strain evolution follows a one-dimensional path through antigenic space, with cross-reactivity between strains decaying exponentially as the distance between them increases.

Like several status-based models, we have also assumed that cross-immunity acts to reduce transmission. We do not consider spatial structure, which may also affect how transmission rates impact on strain emergence [12], nor seasonality: this paper presents a single population with influenza constantly circulating. Our parameters, although chosen to be plausible, are also approximate. In particular, mutation rates can affect the steepness of the drop-off; an increase leads to easier emergence and a slightly flatter relationship, whereas a slower rate hinders the appearance of new strains and accentuates the drop (results not shown). Further, the mechanism of fitness loss and regain, via compensatory mutation, is necessarily approximate: a better understanding, ideally quantitative, of how antigenic escape might incur a fitness cost is needed if the effects of evolutionary constraints are to be modelled more accurately. If it was the case that the process of emergence involved several mutations, we would expect that the \( R_0 = 1 \) boundary is still important in deciding the viability of a new strain. Although simplifications have been made, our framework could easily incorporate new developments in the understanding of influenza or RNA fitness constraints.

Taking a two-tiered approach, we focused on the emergence process and interaction of strains via epidemic dynamics separately, combining a stochastic approach
for the former with an ordinary differential equation model for the latter. This allowed us to not only draw attention to the role $R_0$ can have in dictating a strain’s rate of antigenic escape, but also to show that inclusion of realistic evolutionary constraints, as well as careful consideration of the emergence process, can have implications on the use of vaccination in reducing the rate of such escape.

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