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Heterogeneity in antibody range and the antigenic drift of influenza A viruses

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ABSTRACT

In this paper we explore the consequences of a heterogeneous immune response in individuals on the evolution of a rapidly mutating virus. We show that several features of the incidence and phylogenetic patterns typical of influenza A may be understood in this framework. In our model, limited diversity and rapid drift of the circulating viral strains result from the interplay of two interacting subpopulations with different types of immune response, narrow or broad, upon infection. The subpopulation with the narrow immune response acts as a reservoir where consecutive mutations escape immunity and can persist. Strains with a number of accumulated mutations escape immunity in the other subpopulation as well, causing larger epidemic peaks in the whole population, and reducing strain diversity. Overall, our model produces a modulation of epidemic peak heights and patterns of antigenic drift consistent with reported observations, suggesting an underlying mechanism for the evolutionary epidemiology of influenza, in particular, and other infectious diseases, more generally.

1. Introduction

The persistence of influenza A virus as a human pathogen relies on its ability to evade antibodies specific to the hemagglutinin (HA), the glycoprotein responsible for attachment to cellular receptors. The virus undergoes a process of "antigenic drift" by sequential amino acid substitutions in HA1, the immunogenic domain of HA (Both and Sleigh, 1981; Both et al., 1983). Analysis of historical data on worldwide circulation of strains (Shih et al., 2007) as well as molecular evolution studies (Fitch et al., 1991) have shown that this antigenic drift is driven by positive immune selection: the immune system pressure seems to drive the fixations of the mutations that occur during viral replication (Shih et al., 2007). Five major antigenic sites (A–E) have been described on the HA1 domain of the hemagglutinin (Caton et al., 1982; Wilson and Cox, 1990). A mutation in one of these antigenic sites corresponds to the generation of a new strain: whether the new strain can escape immunity depends on the specificity of the immune response. Escape mutants have been selected by monoclonal antibodies (Yewdell et al., 1979), however, to escape the polyclonal spectrum of antibody specificities typically found in human sera is much more demanding. Antigenic variation in a single antigenic site, shown to occur with an average frequency of 10^{-5}, cannot escape neutralization by an immune response directed against multiple antigenic sites. This would require simultaneous change in multiple antigenic sites, which is estimated to occur at prohibiting low frequencies.

The range of specificities of the antibodies produced by an immune response differs among individuals (Nakajima et al., 2000; Wang et al., 1986). Natali et al. (1981) compared the reaction of sera from children and adults with mutants from a specific strain, and found that children have a more limited antibody repertoire with respect to adult individuals, deducing that this could cause the spread of new antigenic variants. Similarly, it was found (Oxford et al., 1981) that cross-immune responses are typical of adult individuals, but not of children, with young children up to 5 years of age reacting more specifically to a limited region of the HA protein and facilitating the emergence of drift variants (Sato et al., 2004). This increase in cross-reactivity with age is ubiquitous among antigenically diverse pathogens and can be attributed to acquired immunity following sequential exposures or to immune maturation. Here we assume the latter and explore the consequences for the epidemiology of the disease and the evolution of the virus.

For concreteness, we construct a minimal model where immunologically immature individuals mount a narrow antibody response that is essentially monoclonal and heterogeneous among individuals, enabling the virus to accumulate a spectrum of antigenic changes in a sequential manner. As individuals mature, their antibody responses become polyclonal (Haaheim et al., 2006). Concurrently, they also build their antibody repertoir to some extent due to sequential exposures to different viruses.

Various models of epidemic dynamics have been developed in recent years to take into account diversity and immune selection of

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pathogens, including both population-based (Andreasen et al., 1997; Gomes et al., 2002; Gog and Grenfell, 2002; Boni et al., 2004, 2006; Kucharski and Gog, 2011; Kryazhimskiy et al., 2007; Koelle et al., 2006, 2010) and individual-based (Ferguson et al., 2003; Girvan et al., 2002; Tria et al., 2005; Roche et al., 2011) formulations. Here we use an individual-based description which has the advantage of following closely the immune history for each individual in a heterogeneous host population, at the expense of computational ease. The model we build encapsulates the described heterogeneity in the range of antibody specificities and gives rise to the epidemic and evolutionary patterns typical of influenza A, providing a parsimonious explanation of possible mechanisms behind these patterns.

2. Model

We consider a population consisting of two types of hosts with different, age-related immune responses: children up to an average of 5 years of age (monoclonal); and older children and adults (polyclonal). Demography, transmission through social contacts and recovery from the disease are modeled as in traditional *SIR* compartmental models. The immune response is modeled to match the antigenic structure of the surface protein hemagglutinin of the influenza A virus, for which different antigenic sites have been identified (Yewdell et al., 1979; Caton et al., 1982; Both and Sleigh, 1981; Both et al., 1983; Wilson and Cox, 1990; Shih et al., 2007).

Analytical studies of hemagglutination inhibition assays for influenza concluded that the antigenic space of influenza A has dimension (Lapedes and Farber, 2001); on the other hand Smith et al. (2004) produced antigenic maps of influenza assuming a two-dimensional antigenic space. To be conservative regarding dimensionality we have implemented the model so that each host may develop antibodies to five antigenic sites of an infecting virus, although it would be interesting to explore, within our model framework, other assumptions regarding this dimension as described in Section 5.

Consider a monoclonal host that has a potentially infectious contact with an individual infected with strain s, characterized by five antigenic sites $a_i(s)$, $i = 1,\ldots,5$. We will take the probability of fending off the attempted infection to be proportional to the number of matching antibodies in the host's immune repertoire for the five antigenic sites. This corresponds to the idea that matching antibodies act cooperatively and so that full protection is conferred by five matching antibodies only. Upon infection, a monoclonal host will develop antibodies to one and only one of the antigenic sites that is not already represented in its immune repertoire, say $a_i(s)$, and will transmit the strain s through social contacts until recovery from the disease. The antibody against $a_i(s)$ becomes part of the immune repertoire of the host until his/her death, but despite this permanent immune memory, the monoclonal immune response has limited efficacy, in the sense that up to five consecutive reinfections by the same strain s are possible, although with decreasing probability.

Attempted infection of a polyclonal host will elicit an immune response that is less specific (Haaheim et al., 2006) and hence less sensitive to the multiplicity of matching antibodies: a single matching antibody provides full protection. Thus, if the polyclonal host already has antibodies for any of the five antigenic sites of the strain, the virus will be eliminated. Otherwise the virus will replicate, causing infection in the host with the production of antibodies against all five antigenic sites of s. These antibodies will become part of the immune repertoire of the host until his/her death, and in particular they will be active during infection against mutants of s generated in viral replication. Therefore, these mutants would have no selective advantage inside the host and would be eliminated by the same mechanism that enables polyclonal hosts to fend off attempted infections by strains which have at least one antigenic site represented in their immune repertoire. Contrary to monoclonal hosts, in our model, infected polyclonal hosts can only transmit the strain they were infected with.

During infection by a given strain, random mutations may occur with equal probability in every antigenic site. We assume no structure in the virus strain space, so that all mutations are equivalent from the point of view of the basic fitness of the mutant. Different strains compete only through cross-immunity at the individual and population levels. Demographic processes and contact structure are implemented whereby individuals within each subpopulation interact preferentially with each other, and less with individuals of the complementary subpopulation (Mossong et al., 2008; Medlock and Galvani, 2009). We assume no further structure in the host population.

3. Methods

We construct a stochastic model of a rapidly mutating virus circulating in a population with a heterogeneous immune response. The two types of individuals introduced earlier form two subpopulations of sizes N_1 and N_2, respectively, coupled in the first place by demography. Newborns are monoclonal: they mature into polyclonal at a rate μ_1, and polyclonals are subject to natural mortality at a rate μ_2. Deaths are compensated by births to insure that the total population, $N = N_1 + N_2$, remains constant. The ratio of the initial values for N_1 and N_2 verifies the equilibrium condition:

$$\frac{N_1}{N_2} = \frac{\mu_2}{\mu_1} \tag{1}$$

We implement transitions between compartments using constant rates. Other transition models are possible, but in the absence of empirical data on the distribution of times spent in each immunogical compartment, we opted for the approach that would allow more direct comparison with population-based formalisms. We use an agent-based description where individuals store information regarding their age-class, epidemiological status and immunological memory. A virus strain is a bit of information that is transmitted among individuals in infection events and is formed by a 5-tuple of integer numbers $k = (k_1, k_2, k_3, k_4, k_5)$, each number abstractly representing a particular conformation of an antigenic site. A mutation in one of the antigenic sites increases the corresponding integer number by one unit. For instance, a strain represented by the 5-tuple (3,2,7,2,6) mutating in the antigenic site associated with k_2 will always generate the strain (3,3,7,2,6). Mutations always increase the integers representing the conformation of the antigenic sites. A mutation associated with a decrease in one of these integer numbers would correspond to a mutation that reverts the conformation to a previous one. Although this is a possible mutation, its occurrence is unlikely and will not be considered here.

The epidemiological status of each individual host can be either susceptible or infected. Upon recovery from infection by a particular strain, individuals return to a susceptible state that has been modified by the acquisition of specific antibodies during infection. To record immunity, each individual has 5 pools, $\lambda_1, \ldots, \lambda_5$, representing sets of antibodies induced by an entire history of previous infections. When an individual infected with the virus represented by the 5-tuple $k = (k_1, \ldots, k_5)$ acquires immunity to the antigenic site j, the value k_j is added to the set of values in λ_j. Monoclonal responses are modeled by the addition of a single new conformation k_j selected at random, while in the case of

polyclonal responses all $k_1, \ldots, k_5$ are added to the corresponding memory pools.

A susceptible host with a given infection history can be infected by a virus transmitted through an infectious contact. However, infection only occurs after verifying the viability of the virus against the host's immune repertoire. The set of antigenic sites of the virus, represented by a sequence of integers $k = (k_1, k_2, k_3, k_4, k_5)$, is compared with the hosts's immune repertoire represented by the sets $\lambda_1, \ldots, \lambda_5$. This comparison is different for monoclonal and polyclonal hosts. In the monoclonal response, the protection conferred by the immune repertoire is proportional to the number of matching antibodies for the antigenic sites $(k_1, k_2, k_3, k_4, k_5)$. In practice, this is implemented by selecting a particular k_j, and checking whether this value is stored in the corresponding list λ_j. If so, the strain will be eliminated and infection does not occur, otherwise infection develops and k_j is added to the hosts' set λ_j. In polyclonal hosts, infection will be prevented if any one of the k_j is already stored in the hosts' corresponding set λ_j. Otherwise, infection will develop and the five conformations k_j that characterize the strain will be added to the corresponding sets λ_j, $j = 1,\ldots,5$.

All hosts recover naturally from the disease at an average rate γ and become susceptible again. In Appendix A, we explore the influence of gamma distributed recovery profiles on strain drift and diversity by considering multiple infective classes as in Lloyd (2001). The number of infective classes L interpolates between the usual exponential recovery for $L = 1$ and well defined recovery times for large L.

Potentially infectious contacts, that is, contacts between infected and susceptible individuals, occur at an average rate β. Since monoclonal hosts are conceived as being mainly children under 5 years of age, we structure contacts according to preferential mixing within age groups. A parameter α is introduced to account for this preference, so that the infection within each age group occurs at a rate $\alpha\beta$, while infection across age groups occurs at a rate $(1-\alpha)\beta$. In all simulations we assume $\alpha = 0.8$, which is consistent with recent estimates (Mossong et al., 2008). In Appendix B, we perform a sensitivity analysis to this parameter.

Mutations occur at rate m_0 in infected hosts. When this occurs, a new strain is generated in an infected host by mutating one of the 5 antigenic sites of the virus as previously explained. As circulating strains can generate mutants at constant rate, the effective mutation rate m, defined as the frequency of mutants generated per infection, depends on the recovery rate as $m = m_0/\gamma$. The new strain will only be viable if the mutation occurs in the antigenic site(s) against which the immune response of the host is already acting, and if the new mutation evades the immune response of the host. As polyclonal immunity is directed toward all 5 antigenic sites, no mutant strain is viable in the subpopulation of individuals capable of mounting such broad responses. By contrast, under monoclonal immunity a fraction of mutant strains is viable, and we assume that viable mutations become predominant in their hosts by effectively substituting the original strain. The new strain acts as a novel infection and elicits a new immune response.

Simulations start with a population of individuals fully susceptible to all strains. A small fraction (1 in 100,000 of the population) is then infected by the same initial seed strain. Immunity develops in individuals and in the population as the strain and its descendants spread. To limit the memory requirements of the simulations, every few timesteps we remove all immunity data referring to strains that cannot circulate anymore because none of the existing strains could have them in their descent. The large amount of memory required to store the individual immune repertoire and to keep track of the circulating strains in a population as large as possible led us to avoid fast but memory-intensive Gillespie algorithms and to choose a slower but more memory conservative (and inherently simpler) algorithm, following a scheme similar to Verdasca et al. (2005). The algorithm uses a fixed timestep: at each Monte Carlo step N status updates are attempted. At each attempt an event is chosen with probability $r\,\Delta t$, with r being the characteristic rate of the event $(\alpha\beta, (1-\alpha)\beta, \mu_1, \mu_2, \gamma,$ or, $m_0)$. This sets the optimal value for Δt to that for which the sum of all these probabilities is 1: a higher value is not allowed, while a lower value would be valid but inefficient. The dynamics then proceeds by selecting an individual and attempting the event as explained in Verdasca et al. (2005). Note that in our case recovery is not deterministic, hence it is treated by the algorithm as another stochastic event. The use of this algorithm allowed us to follow tens of thousands of strains in a populations of 8 million individuals, during a simulation time corresponding to 50 years.

4. Results

The simulations were performed using realistic parameter values for human influenza A whenever there was available information. The demographic rates were set to $\mu_1 = 1/5$ years^{-1}, $\mu_2 = 1/65$ years^{-1}, corresponding to an average of 5 years to mature from monoclonal to polyclonal immunity, and an average human lifespan of 70 years. The average recovery rate was set to $\gamma = 1/4$ days^{-1}. The transmission rate $\beta = 136.5$ years^{-1} corresponds to a basic reproduction number of roughly $R_0 = 1.5$ which is consistent with recent literature (Medlock and Galvani, 2009; Fraser et al., 2009). The nominal mutation rate used for these parameters was $m_0 = 0.5$ years^{-1}, and corresponds to the minimum level of mutation required for a significant part of the simulations (50%) not to become extinct in a 50 years time span. It is worth noting that almost all extinctions occur during the first year and a half, implying that extinction is strongly influenced by the way we implement the initial condition. Although the value assigned to m_0 is arbitrary in this sense, its choice will be reflected in the rate of antigenically relevant mutations obtained in the simulations, and this is comparable with available data.

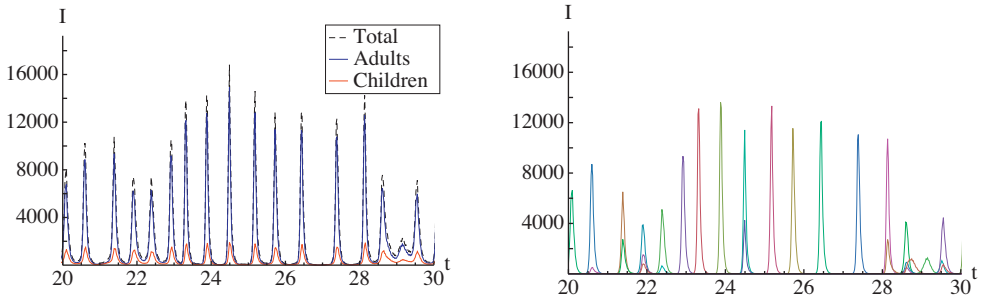

Fig. 1. (Left) Weekly incidence of infection per 100,000 individuals: the infective patterns for adults and children are coupled, but large peaks are the result of a sharp increase of infected adults. Time is in years. (Right) Contribution of distinct strain to the overall prevalence: large peaks are due to an extremely limited number of strains becoming dominant. Parameters as described in Section 4, $L = 5$, and $\alpha = 0.8$. Time is in years.

Fig. 1 shows the weekly infective's incidence as a function of time: the incidence follows a sequence of peaks that should be interpreted as a modulation of influenza epidemics. Note that no seasonality is present in the model. The figure further shows the contribution of the monoclonal and polyclonal subpopulations to the overall infection. The dynamics in the two subpopulations are almost synchronous although they play different roles in the propagation of influenza as we will see further on. The right panel of Fig. 1 shows how different strains enter and exit circulation. The total number of circulating strains for the time span shown in the figure is over 3000: more than 15,000 strains circulate during the 50 years time span of the simulation, however, only a few strains are visible as the majority of strains have very low prevalence and undergo rapid extinction. The mutation rate m_0 must be large enough to guarantee the generation of new strains during these periods of low incidence. The large peaks correspond to strains that have mutated in all antigenic sites with respect to the previous large prevalence peak and are able to spread even in the polyclonal subpopulation.

We will see that the stark incidence variation is the result of the interplay of monoclonal and polyclonal infected individuals and of the evolution dynamics of polyclonal hosts that select specific mutants. The addition of seasonal forcing to the model might stimulate seasonal influenza epidemics each year (Dushoff et al., 2004).

4.1. The role of monoclonal and polyclonal hosts

The role of monoclonal and polyclonal hosts can be clarified further if we consider two extreme cases: one case where all individuals show a polyclonal response since birth, and one of a whole population that remains monoclonal throughout the entire lifespan.

In a host population with a homogeneous polyclonal immune response, any mutation will be recognized by the immune system of the infected host and no mutant will be able to escape, resulting in early extinction once immunity to the initially circulating strain is large enough in the population. A way out of this extinction bottleneck would be for the virus to improve its fitness through mutations that increase its transmissibility, rather than investing on antigenic escape, offering a plausible scenario for the establishment of highly transmissible and antigenically conserved viruses associated with childhood diseases (Frank and Bush, 2007).

In contrast, the results for a fully monoclonal population, shown in Fig. 2, are crucial in a strategy of antigenic escape. In this case, most mutants will be able to escape immunity and will infect other hosts. Strain competition is present through cross-immunity, giving newly evolved strains a temporary advantage and thus driving viral evolution. In the absence of an additional selection mechanism that favors one strain over the others, all strains are equivalent in the long term, and most of them manage to survive because they may cause up to five repeat infections in any host. Hence the result is a relatively high level of infectives and a large set of circulating strains where no strain dominates.

To describe the strain behavior resulting from our simulations we use measures and representations that are standard in the analysis of genetic sequence data, namely divergence, genealogy, and diversity. Divergence measures the cumulative number of mutations from the initial strain. This information is obtained after

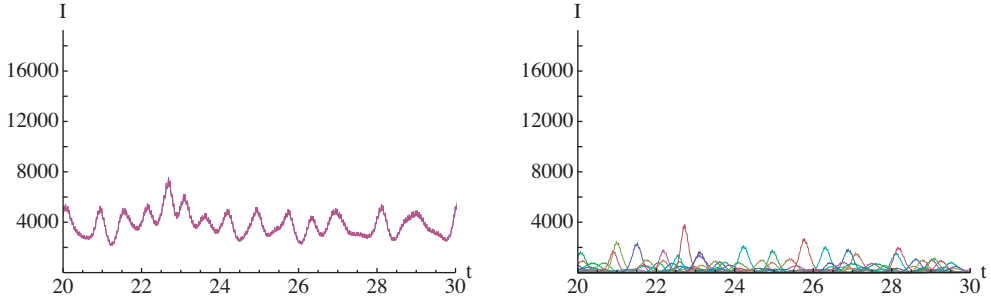

Fig. 2. (Left) Weekly incidence of infection per 100,000 individuals for a population of monoclonal individuals only, showing a relatively high level of infectives at any time. Time is in years. (Right) Contribution of distinct strains to the overall prevalence: in the absence of polyclonal individuals many strains co-circulate with similar prevalence levels. Parameters as described in Section 4, $L = 5$, and $\alpha = 0.8$. Time is in years.

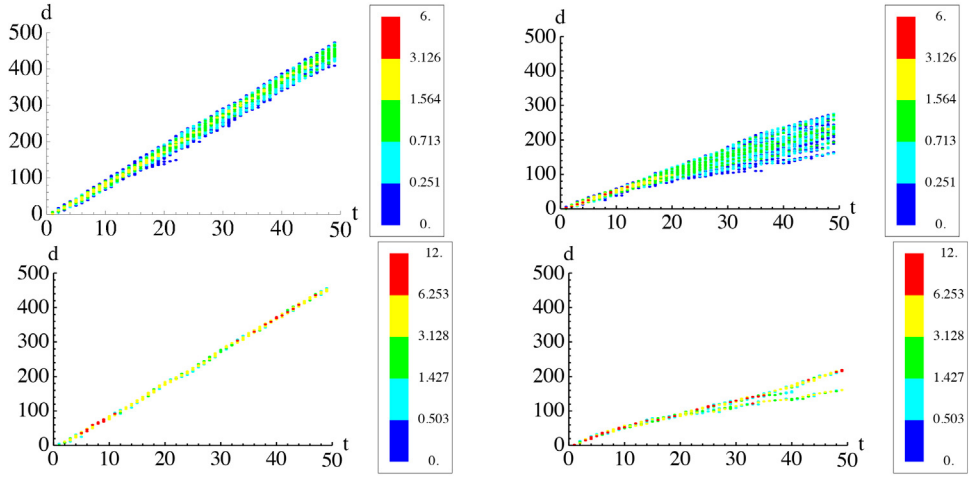

Fig. 3. Divergence from the original strain for: (left) full model; (right) monoclonal-only population. Plots were obtained by yearly sampling strains according to their prevalence. On the top we show averages over 20 simulations: the color code represents the average number of different strains found at the same distance from the original strain. On the bottom we show results for two representative simulations: the color code represents the number of different strains found at the same distance from the original strain. Parameters as described in Section 4, $L = 5$, and $\alpha = 0.8$.

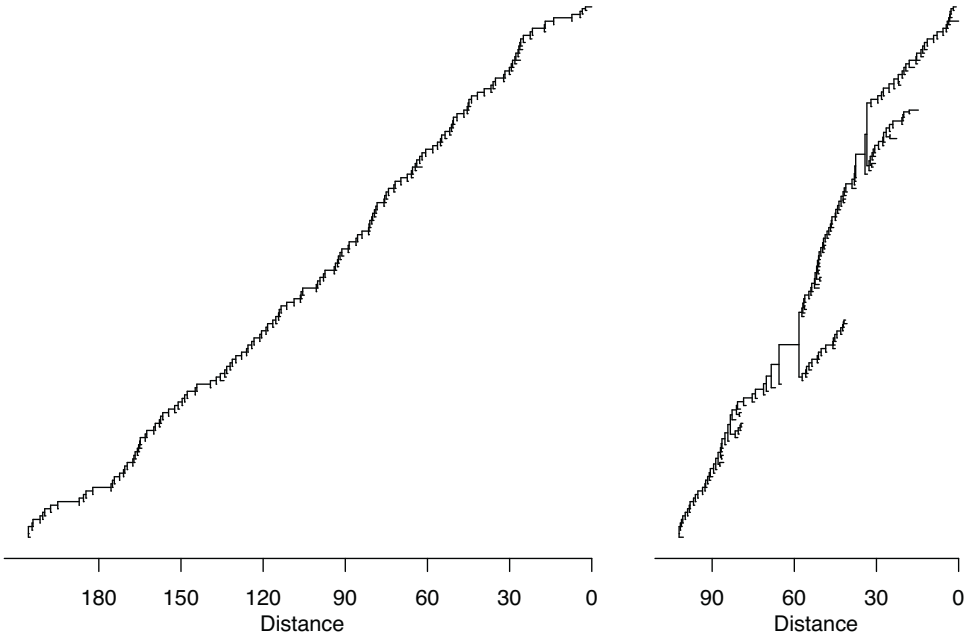

Fig. 4. Phylogenetic trees for representative simulations of the full model (left) and a monoclonal-only population (right).

selecting each year a set of strains, each chosen with a probability proportional to its prevalence in the population during that year. We select 100 strains per year which, considering that we use a population size of 8 million individuals, is comparable to the sampling ratio of many cities in available influenza databases. The distance from the original strain which, in our model, is given simply by $\sum_{j=1}^{5}(k_j - k_j^0)$, with k_j and k_j^0 being, respectively, the numerical indices of the 5 antigenic sites of the sampled strain and those of the original strain, is then calculated for all sampled strains: in general there are several different strains associated with the same distance value. Averaging over multiple realizations, we obtain for the heterogeneous immune response model the plots shown in Fig. 3 (left) and for the fully monoclonal population the results of Fig. 3 (right). In both panels, each point at a given distance is color coded according to the number of different strains found at that distance, so that the figures contain information about the drift rate in antigenic space and also qualitative information about the strain diversity, or number of representative co-circulating strains. Comparing the two scenarios, we see that the selection mechanism introduced by the polyclonal subpopulation has two effects: it increases the drift rate because it enhances the selection for specific mutants, and it reduces the spread in antigenic space mainly because strains that have been circulating for long times are unable to invade the polyclonal subpopulation and instead become extinct.

Phylogenetic trees were generated for the same sets of simulated data, giving rise to Fig. 4. The neighbor-joining trees were built from a sample of 150 strains taken at randon, with weights to reflect the abundance of each strain in the host population. It is interesting to compare the two simulated tree genealogies: one obtained from a realization of the full model and shown in Fig. 4 (left); and the other from the corresponding realization in a population with monoclonal hosts only and shown in Fig. 4 (right). Consistently with the observations in Fig. 3, the introduction of polyclonal hosts increases the drift rate and, in addition, shortens secondary branches in a way that is highly suggestive of what differentiates the evolution of human influenza A viruses from that of its close relatives human influenza B (Hay et al., 2001) and swine influenza A (de Jong et al., 2007).

Fig. 5 shows, averaged by prevalence, the yearly strain diversity index of order two (Hill, 1973; Jost, 2007; Tuomisto, 2010): $D = 1/\sum_j p_j^2$. where p_j is the relative abundance of strain j. The figure shows this diversity index for a simulation of the full model and for a simulation of monoclonal hosts only as a function of time. The plots show that strain diversity is more important in the monoclonal subpopulation.

Overall, these observations clarify the role of the two subpopulations in strain dynamics. Monoclonal hosts are the source of new strains as they allow mutations to survive and diffuse in the population, increasing the overall strain diversity.

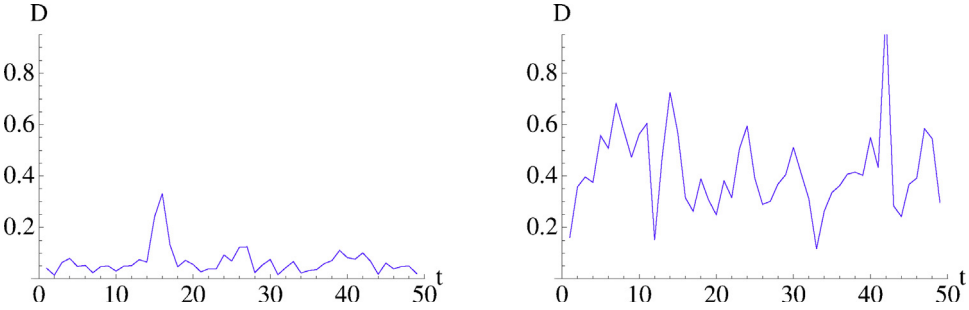

Fig. 5. Prevalence-averaged strain diversity plotted for a simulation of a full model (left) and monoclonal-only population (right) as a function of time.

Polyclonal hosts on the other hand operate by assigning an additional selective advantage to some of the mutants that emerge from monoclonal hosts, allowing only particular strains to become dominant, and thus restricting strain diversity while accelerating the rate of evolution. The resulting behavior is a balance of these two competing effects.

5. Discussion

The simulations presented here explore the epidemiological and evolutionary consequences of established notions regarding antibody response to viruses. Viruses have on their surface proteins specialized in attaching and entering susceptible host cells. Being essential to viral success, it is not surprising that the same proteins constitute a target for host immunity. As the host produces antibodies with increasing affinity to those antigens, it optimizes its ability to neutralize the virus. Finally, errors inherent to viral replication make it theoretically possible that selected mutants escape antibody recognition, infect other hosts and antigenic evolution ensues. Whether this occurs in practice depends on a few numbers.

In influenza A viruses, the antigenic domain of the surface protein hemagglutinin consists of 329 amino acid residues, encoded by 987 nucleotides (Wiley and Skehel, 1987). Antibodies are immune-related proteins whose variable ends, consisting of 110–130 amino acids, specialize in binding to antigen, and it is conceivable that multiple antibodies bind simultaneously to the hemagglutinin at specific antigenic sites. This has been confirmed by monoclonal antibody analysis for more than 30 years, and now many viruses have their antigenic structure mapped by structural biology techniques. For example, 5 antigenic sites have been identified in influenza A virus (Caton et al., 1982), 4 in poliovirus (Mateu, 1995), 3 in rhinovirus (Mateu, 1995), while hepatitis A virus appears to have a single immunodominant antigenic site (Ping and Lemon, 1992). More than being descriptive, these numbers appear amongst the most critical determinants in antigenic evolution.

Antigenic drift has been simulated in vitro by growing viruses in the presence of monoclonal antibodies and inspecting for mutants that fail to bind to the antibodies used for selection. In influenza A viruses, this procedure showed occurrence of mutants, at a frequency of 10^{-5}, and independence among the 5 antigenic sites (Yewdell et al., 1979; Caton et al., 1982). Assuming that the normal antiviral activity in vivo comprises antibodies directed against all antigenic sites, multiple mutations would be required to escape neutralization. As the frequency of multiple mutations is too low to account for the regular occurrence of antigenic drift in human influenza A viruses, possible refinements have been proposed Yewdell et al., 1979: in the human respiratory tract intensity parameters such as neutralization or frequency of variation during viral replication might be different; or humans might possess a more restricted antibody repertoire, and this effect is either widespread or limited to a subpopulation. The notion of a subpopulation possessing an antibody repertoire restricted to one or two antigenic sites has been further advocated and elaborated (Nakajima et al., 2000; Wang et al., 1986; Natali et al., 1981; Oxford et al., 1981; Sato et al., 2004; Haaheim et al., 2006).

Serological studies have accumulated in support of a restricted antibody repertoire in young children with respect to the general population (Natali et al., 1981; Sato et al., 2004). Consistently, it was found that young children mount less cross-reactive immune responses (Oxford et al., 1981). This work provides further support to this hypothesis by demonstrating its implications in simulated epidemiological and evolutionary patterns. Our model encapsulates the hypothesis that young children (relatively immature, immunologically) mount a restricted antibody response that is essentially monoclonal, enabling the virus to accumulate a spectrum of antigenic changes in a sequential manner and fueling cyclic epidemics. The polyclonal antibody response associated with the general population is also essential to limit diversity in a pattern that is consistent with the ladderlike appearance of human influenza A phylogenies Smith et al., 2004. More strikingly, when the effects of the polyclonal subpopulation are relaxed the model predicts reduced drift rates and multiple-branch phylogenies as in human influenza B and swine influenza A Hay et al., 2001; de Jong et al., 2007. This is consistent with the notions that influenza B is less common among adults and that domesticated animals have shortened adult lifespans.

Although the model was constructed to resolve an inconsistency in influenza evolution at the level of the individual host, it is interesting to seek agreement with epidemiological and evolutionary patterns at the population level. In their antigenic mapping of influenza A H3N2, Smith et al. (2004) note that antigenic evolution occurs in a punctuated manner and identify clusters that replace each other every few years. Conceivably the epidemic dynamics generated by our model are related to the occurrence of cluster jumps but a more quantitative comparison with the data cannot be made without explicitly representing seasonality in the model.

Aspects where the model most urgently needs improvement are perhaps the demographic and environmental dimensions. We consider the same demography worldwide and no temporal variation in external factors influencing influenza transmission, while realistic variability is likely to slow down slightly the dynamics reported here and allow a thorough inspection into evolutionary patterns.

On a more theoretical note, we propose a mechanism that simultaneously enables the selection of escape mutants at the level of an individual host and offers an alternative framework to investigate the punctuated antigenic evolution described by antigenic mapping (Koelle et al., 2006).

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Appendix A. The role of γ and L

Here we explore the role of the length of the infectious period and of the form of the recovery profile in the behavior of the system (the role of the parameter α that controls the bias toward intra-group contacts is studied in Appendix B). Recalling that the number of mutations per infection is $m = m_0/\gamma$, it is clear that a change in γ has a direct effect on the drift speed. Fig. 6 shows the behavior for different values of γ: when γ decreases (and the effective mutation rate m increases), the drift speed increases, as expected. For values of γ larger than those shown here, the effective mutation rate is too low to sustain infection through the prevalence troughs, and early extinction occurs.

Interestingly, the behavior of the system is also strongly dependent on the distribution of recovery profiles. For $L = 1$, an increase in γ leads from a regime of early extinction to a regime characterized by a large divergence spread. As L is increased to $L = 2$ and higher, an intermediate

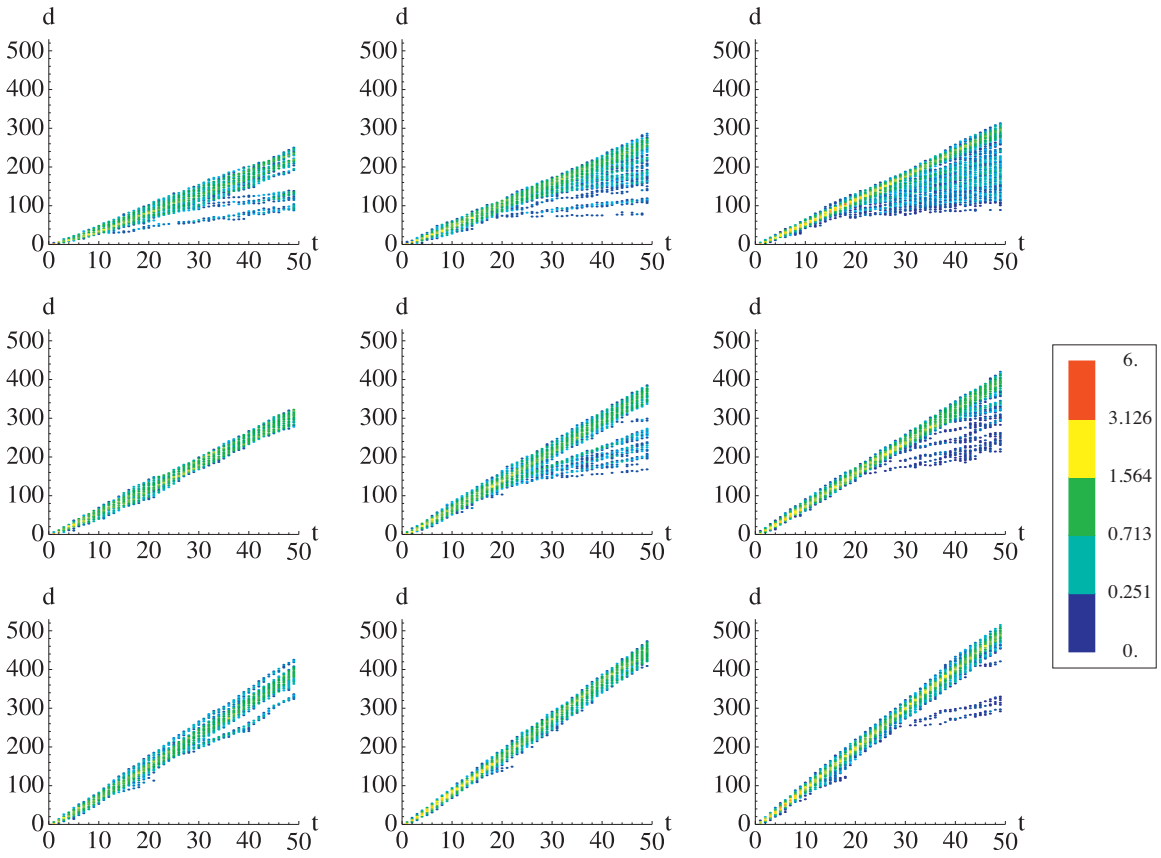

Fig. 6. Distance from the original strain for different values of $\gamma(m)$ and L. From top to bottom: $L = 1$, 2 and 5. From left to right: $1/\gamma = 3.8$ days, 4.0 days, 4.2 days. Data collection and color coding as in Fig. 3.

regime is found where strains drift and co-circulating strains at each time point have similar values of divergence with respect to the original strain. In this regime, the drift speed is very well defined and increases with L. The range of γ values where this intermediate regime holds also increases with L. This behavior is illustrated in Fig. 6. According to our analysis of the role of monoclonal and polyclonal hosts, this is an indication that the selective advantage for some mutants due to cross-immunity in the polyclonal subpopulation increases with the parameter L. We shall see below that this is indeed the case, and that this is due to an increase with L of the size of the epidemic bursts in the polyclonal subpopulation.

Increasing L from $L = 1$ to $L = 2$ and higher corresponds to moving from exponential to unimodally distributed recovery times. It is known (Cox and Miller, 1965) that this change leads to larger, more synchronized epidemics. Large epidemic bursts in the polyclonal subpopulation deplete the pool of susceptibles that can be infected by strains that share antigenic site's configurations with the strain that caused the epidemic, increasing the selective advantage of other mutants. This influences both drift speed, increasing it, and divergence spread, reducing it, much as what happens when a heterogeneous population is compared with a monoclonal population. We have verified by inspection that for $L = 5$ an epidemic due to strain $k = \{k_1, k_2, k_3, k_4, k_5\}$ is most of the times followed by an epidemic due to strain $k' = \{k_1 + 1, k_2 + 1, k_3 + 1, k_4 + 1, k_5 + 1\}$, leading to a predictable sequence of dominant strains that reflects the role of the polyclonal subpopulation. On the contrary, for low values of L, the smaller epidemic does not fully exhaust the pool of susceptibles, so that strains invading the adult population often do not follow this regular pattern. For instance, in a simulation obtained for $L = 1$ an epidemic due to the strain $\{3, 2, 4, 2, 3\}$ was followed by a new epidemic due to strain $\{4, 3, 6, 3, 2\}$, showing that in the previous epidemic immunity to conformation 2 of the fifth antigenic site was acquired by a fraction only of the polyclonal subpopulation. This fraction determines the effective value of R_0 for an invading strain that has kept the same conformation of the fifth antigenic site, and when this value is smaller than but close to one, successful invasion and extinction may both occur with high probability. In this example, invasion by strain $\{4, 3, 5, 3, 2\}$ was avoided, but strain $\{4, 3, 6, 3, 2\}$ managed to invade. The following epidemic was strain $\{5, 4, 5, 4, 4\}$, showing a complex interaction among strains for $L = 1$.

Although occasional departure from a regular evolution pattern can happen for all the values of L we have explored, lower values of L make it much more frequent due to smaller epidemic burst size. In these conditions mutant strains can survive longer, and divergence spread becomes large. Increasing L enhances the role of the polyclonal subpopulation, because larger epidemics in this subpopulation give escape mutants a greater selective advantage.

Appendix B. The role of α

The parameter α accounts for the fact that transmission within age groups is favored, with α being the fraction of transmissions occurring within each age group. Fig. 7 shows the distance from the original strain for different values of α, and Fig. 8 shows representative phylogenetic trees. A decrease of α causes a decrease of the transmission between individuals of the same age group, while increasing inter-age transmission. This causes less frequent transmission within the monoclonal subpopulation resulting in early

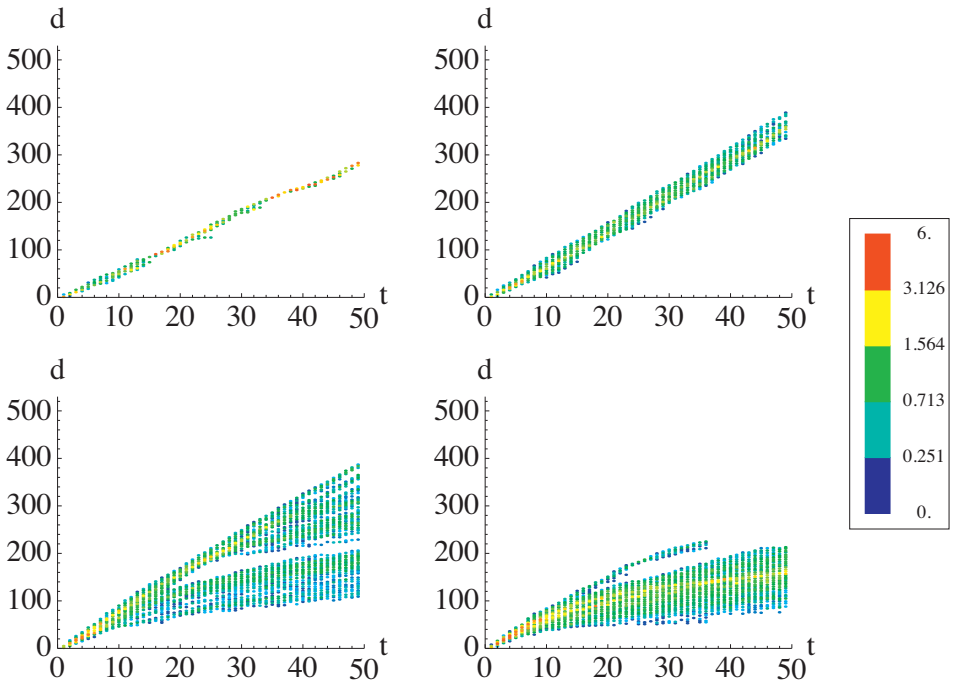

Fig. 7. Distance from the original strain for different values of α. (top left) $\alpha = 0.75$; (top right) $\alpha = 0.8$, (bottom left) $\alpha = 0.85$; (bottom right) $\alpha = 0.9$. Data collection and color coding as in Fig. 3.

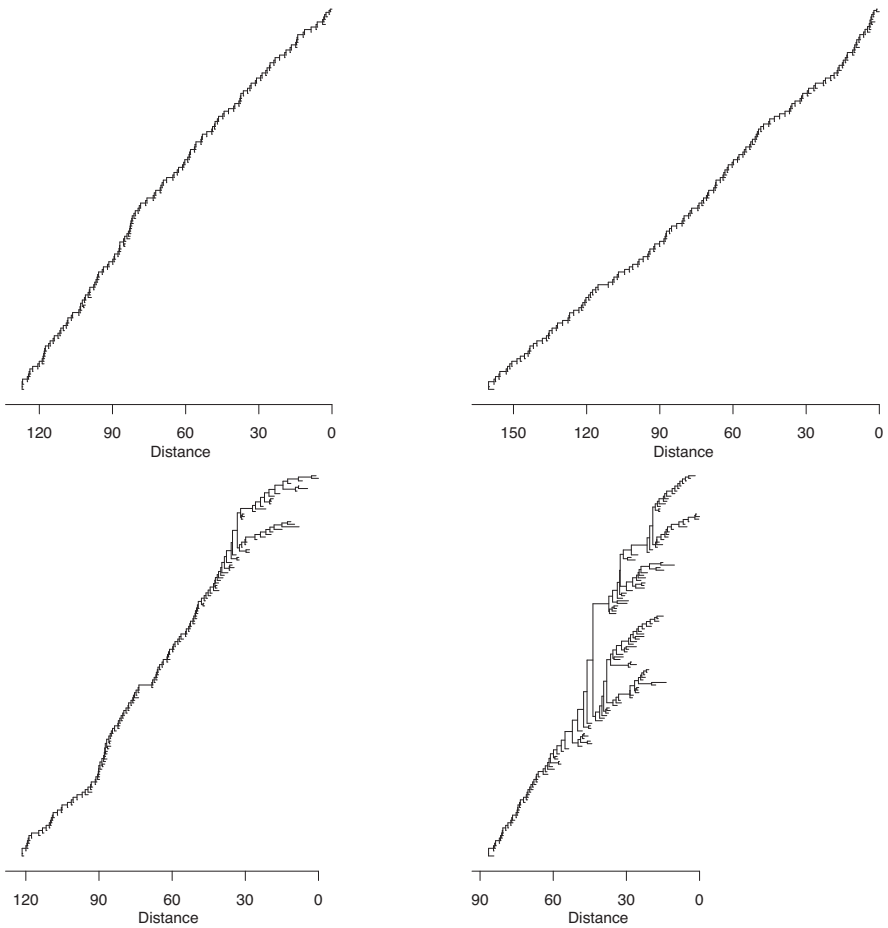

Fig. 8. Phylogenetic trees for different values of α. (top left) $\alpha = 0.75$; (top right) $\alpha = 0.8$, (bottom left) $\alpha = 0.85$; (bottom right) $\alpha = 0.9$.

extinction: below the value $\alpha = 0.75$ no strain survives the first year. On the contrary, an increase of α augments the segregation of the two subpopulations while increasing transmission among individuals belonging to the same age group. The dynamics are dominated by the monoclonal subpopulation where strains evolve and divergence spreads, with occasional invasions of the polyclonal subpopulation. The resulting evolutionary patterns and drift speed approach those found for the monoclonal subpopulation.

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