The generation of influenza outbreaks by a network of host immune responses against a limited set of antigenic types

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It is commonly believed that influenza epidemics arise through the incremental accumulation of viral mutations, culminating in a novel antigenic type that is able to escape host immunity. Successive epidemic strains therefore become increasingly antigenically distant from a founding strain. Here, we present an alternative explanation where, because of functional constraints on the defining epitopes, the virus population is characterized by a limited set of antigenic types, all of which may be continuously generated by mutation from preexisting strains and other processes. Under these circumstances, influenza outbreaks arise as a consequence of host immune selection in a manner that is independent of the mode and tempo of viral mutation. By contrast with existing paradigms, antigenic distance between epidemic strains does not necessarily accumulate with time in our model, and it is the changing profile of host population immunity that creates the conditions for the emergence of the next influenza strain rather than the mutational capabilities of the virus.

evolution | population dynamics | infectious disease | epidemic | immunity

The human influenza virus has been shown to be capable of evading population-level host immunity by altering its surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Pandemic influenza is thought to involve the introduction of genetically divergent HA and NA sequences into human influenza populations, achieved by the reassortment of viral genomic segments during coinfection with other influenza viruses (termed "antigenic shift"). By contrast, annual seasonal outbreaks of interpandemic influenza are believed to rely on the process of "antigenic drift," whereby the gradual accumulation of mutations in HA (and to a lesser extent NA) eventually gives rise to a viral strain that is sufficiently antigenically distinct to be no longer vulnerable to preexisting host immunity (1).

The prevailing view of interpandemic influenza evolution (which informs influenza vaccine composition) conceptualises the virus population as being driven by the appearance, spread, and accumulation of mutations, through a largely unoccupied "antigenic space" in a directional and usually irreversible fashion (2–12). However, mathematical models (4–10) reveal that such a process can lead to observed epidemic patterns of influenza only under certain specific conditions. The crux of the problem is that each influenza epidemic appears to comprise a virus population of very limited genetic diversity. Quantitative models based on the above paradigm must therefore impose some check on the evolutionary process to avoid a multiplicity of viral types simultaneously breaching the barriers of existing herd immunity. This is achieved by either restricting the mode of mutation (for example to "nearest neighbors" within a one-dimensional linear or circular antigenic space) or by invoking some form of short-term suppression of viral transmission. It has been shown that short-term strain-transcending immunity can, in a spatially heterogeneous model, result in a single strain giving rise to an epidemic within the window of opportunity created by the rapid decay of cross-immunity and its rapid reestablishment upon reinfection (7). A recent alternative model (10) plausibly argues that such periods of suppression arise from the relationship between the virus' genotype and its phenotype. The model posits that influenza does not incrementally acquire the ability to evade population-level immunity; rather, a number of antigenically neutral mutations are required before this can be achieved, thereby extending the period that host cross-immunity is effective. Such models, which explictly represent mutation as a process with many degrees of freedom, are, by nature, nonetheless required to make assumptions about the manner and rate at which viral genetic diversity is generated.

Here, we propose a model that requires none of these assumptions and yet reliably generates single-strain epidemics through the agency of long-term epitope-specific host immunity acting on a limited set of antigenic types. The "antigenic space" of our model is explicitly defined by all combinations of possible epitopes, and no restrictions are placed on the rate at which antigenic types are generated by mutation from preexisting types, by genetic exchange, or by gene flow and immigration. Thus, we demonstrate that the successive emergence of unique antigenic types can occur within a simple framework in a manner that does not rely on the mode and tempo of mutation and that these results can be applied in the context of empirical data to provide an alternative perspective on the evolutionary dynamics of influenza.

Results and Discussion

Single-Strain Emergence Within Multiepitope Models. We first establish the conditions under which single antigenic types can successively emerge within a general multiepitope model that (*i*) incorporates variation in the number of alleles encoding the dominant epitope regions (hereafter designated loci), and (*ii*) allows cross-immunity to accumulate as hosts experience the different variants (or alleles) that define a particular antigenic type. Fig. 1 captures the essential features of our model for a two-locus system with two alleles (*a* and *b*) at one locus and two alleles (*x* and *y*) at the other. Each antigenic type is designated as "*ij*" by virtue of containing allele *i* at one locus and allele *j* at the other. Within our model, compartment z_{ij} represents the fraction of the population that has been exposed to pathogen type *ij* and is now either infected or recovered; this model compartment can overlap with others, as illustrated in Fig. 1 in the context of an antigenic type *ax*. Fig. 1 *Upper Left* shows the proportion of the population, w_{ax}, that has been exposed to any antigenic type that shares alleles with *ax* (and includes *ax* itself), whereas the compartment x_a (shown in Fig. 1

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Abbreviation: HA, hemagglutinin.

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POPULATION BIOLOGY

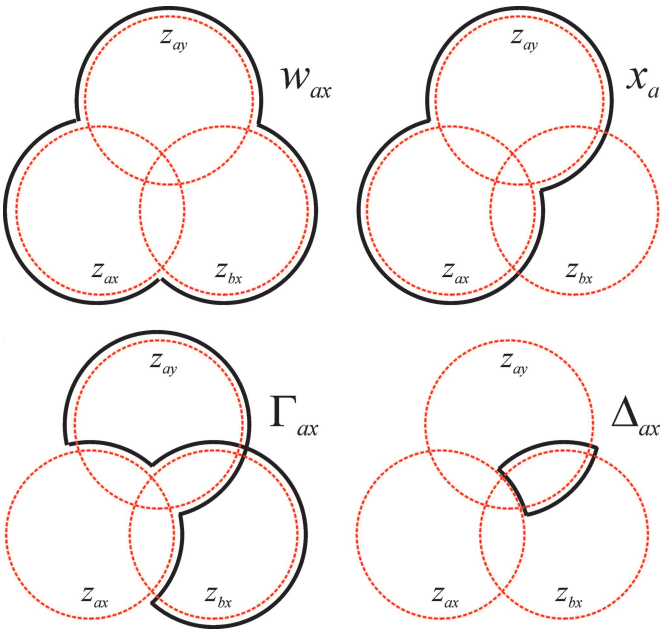

Fig. 1. Model structure and relevant variables are shown with respect to the antigenic type *ax* within a system with two alleles (*a* and *b*) at one locus and two alleles (*x* and *y*) at another (see model description in text for explanation).

Upper Right) includes only those hosts that have been exposed to types sharing allele *a*. Fig. 1 *Lower Left* shows exactly the proportion of the host population, Γ_{ax}, that has been subjected to one of the alleles contained in *ax* but not to both alleles. We assume that these individuals have a lower probability $(1 - \gamma)$ of becoming infectious than those who have never been in contact with *ax* or with any types that share alleles with *ax*. In Fig. 1 *Lower Right*, we indicate another subset, Δ_{ax}, which contains individuals that have been in contact both with types sharing allele *a* and with types sharing allele *x*, but have never been exposed to *ax* itself. These individuals benefit from an additional reduction in the risk of becoming infectious, $(1 - \delta)$, because of the combined exposure to alleles that define *ax*.

The two key immunological parameters involved are thus, γ and δ. The first, γ, is a measure of the cross-immunity that results from previous exposure to at least one allele contained within an antigenic type. The second parameter, δ, is a measure of the additional cross-immunity arising from accumulated exposure to more than one allele.

We ran this model for various numbers of alleles $(n \times m)$ at two loci to determine the occurrence of single strain (antigenic type) epidemics within the (γ, δ) parameter space as indicated by the following measure:

$$\varepsilon = \frac{1}{P} \sum_{i=1}^{P} \frac{y^i_{max} - y^i_{sub}}{y^i_{max}},$$

where P = number of peaks in some defined time interval, y_{max} = the prevalence of peaking strain, and y_{sub} = the prevalence of the strain with second-highest peak.

Fig. 2 records the measure of single-strain dominance (ε) for various $(n \times m)$ combinations. We find, in line with previous observations (13), that at low levels of cross-immunity, γ, all strains coexist at a common level of prevalence, a state of no strain structure (NSS). This state generates no score on the ε scale and corresponds to the white areas on the left hand side of each Fig. 2 plot. A second steady-state involves the stable dominance of a subset of strains (13), referred to as discrete strain structure (DSS), and occurs in regions where either γ or δ is high (top and right-hand white areas of each Fig. 2 plot). Separating NSS and DSS is a region of chaotic or cyclical strain structure (CSS) where dominant types are periodically replaced. Within this region, we identified a central core of chaotic behavior that frequently exhibits sharp epidemics dominated by a single strain (see Fig. 3*a*). For asymmetric $n \times m$ systems (where n is not a multiple of m) the region of single-strain dominance is extended by a number of other behaviors, such as the ordered, alternating appearance of antigenic types of the form $ax \rightarrow by \rightarrow cz \rightarrow aw \rightarrow bx \rightarrow cy$ in a periodic (see Fig. 3*b*) or quasi-periodic (see Fig. 3*c*) manner. See supporting information (SI) for futher information on the dynamical behavior of the model.

Antigenic Evolution of Influenza. We believe that this model can provide a more comprehensive explanation of influenza dynamics than the prevailing conceptual frameworks described above (2–12). It has been proposed that host immunity structure may play an important role in influenza epidemiology (12) but that such models would still need to incorporate continuous incremental antigenic change. Here, we show that a dynamic network of host immune responses against a small number of functionally constrained epitopes can provide an alternative explanation to this type of model of antigenic drift. Our results demonstrate that single-strain epidemic outbreaks can occur across a broad range of conditions describing the degree of immunological cross-protection conferred by previous exposure (defined by parameters γ and δ). These dynamics are most likely to be observed when cross-protection is not complete, and the additive effects of further exposure are low. That γ is likely to take intermediate values for influenza is indicated by the levels of cross-reactivity observed among different strains (14) as well as reinfection patterns (15). Also, the phenomenon of "original antigenic sin" observed in influenza, whereby current exposure selectively boosts the immune response to an earlier infecting antigenic type (16), would reinforce these dynamics by reducing δ.

The observation that amino acid changes within a set of HA codons are associated with influenza isolates that give rise to epidemics in subsequent years (2, 3) is currently interpreted as support for the prevailing antigenic drift paradigm. However, mutation at these highly evolutionarily constrained sites (see SI) could as easily lead to the regeneration of antigenic types within an influenza subtype (such as H3N2), which then emerge epidemically as a result of variant-specific cross-protection. Pleiotropic effects of viral mutations (17) are likely to further restrict the variety of possible epitopes within the HA gene, thus justifying its representation as multilocus system with a limited number of alleles at each locus. Crucially, antigenic distances between successive epidemics (which are determined by only a few nucleotide sites) may contract or remain static while the overall genetic divergence of influenza continues to accumulate linearly through time.

And there is indeed empirical evidence that antigenic distances between influenza epidemics do not always increase with time. The antigenic distances among influenza A H3N2 isolates have recently been measured by using hemagglutination inhibition (HI) assays (18). When these data are transformed onto a two-dimensional antigenic space by using multivariate statistics, the H3N2 population displays a zigzagging, not linear, trajectory (18) (Fig. 4*c*) whose changes in direction cannot be explained by the current antigenic drift paradigm. Furthermore, comparison with our model suggests that the linear component of this movement is, at least in part, due to use of censored data in the multivariate analysis (18) and not to the sequential accumulation of antigenic distance across epidemics. Specifically, we used a five-locus, two-allele system ($2 \times 2 \times 2 \times 2 \times 2$; Fig. 4*a*) to compare the antigenic dynamics of our model with influenza A. Mathematically, the antigenic space of our model is a 5-hypercube whose nodes represent the 32 possible antigenic types. However, if our model population is subjected to the same sampling scheme

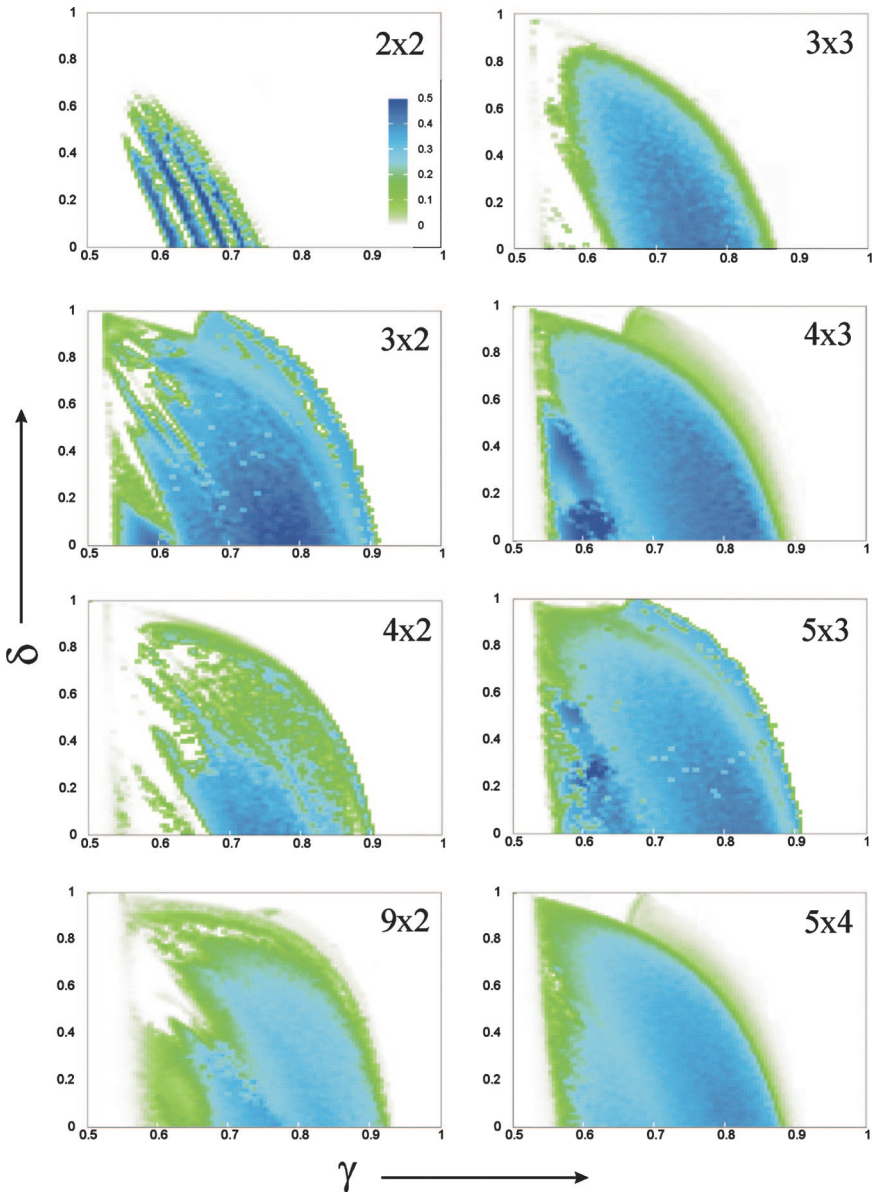

Fig. 2. The intensity of single-strain dominance, ε, is shown for a variety of combinations of numbers of alleles ($n \times m$) at two loci within the (γ, δ) parameter space. See model description in text for definitions of γ and δ. Other model parameters used were $\mu = 0.02$, $b = 40$, and $s = 10$. The number of peaks, P, varied according to the convergence rate of ε.

and multivariate analysis that was applied to empirical influenza data (see *Methods*), then we obtain a very similar zig-zagging trajectory in a two-dimensional antigenic space (Fig. 4*b*). The dynamical switching among antigenic types (i.e., among the 32 vertices of the 5-hypercube) can be clearly seen but now appears to proceed temporally along the vertical axis of the antigenic space. This behavior is due to the fact that, to replicate the structure of the HI data matrix used in ref. 18, antigenic distances between isolates from nonadjacent time points were replaced by censored distances. Our model predicts that this temporal trend would not be observed if accurate distance measures were available for all of the elements of the HI data matrix. We note that a recent HI checkerboard study on subtype 1 (H1) swine influenza viruses (19) from the U.S. that does not contain censored data exhibits marked contractions in antigenic distance over long periods of time in a manner consistent with our model predictions (see SI). In addition, antigenic analysis of recent H5N1 isolates from Asia (20) also demonstrates discordances in cross-reactivity that do not sit comfortably with the continual accrual of antigenic distance through time, these data show strong immunological recognition between a reference chicken isolate from Pennsylvania collected in 1983 with Asian viruses collected between 1999 and 2005, suggesting the reemergence of antigenic types.

Indirect evidence for contraction of antigenic distance may be obtained also from epidemiological studies. For example, the reinfection rate for a particular H3N2 variant that caused an epidemic in 1984–85 in Texas was, paradoxically, twice as high among individuals who were infected in 1982–83 with H3N2 than among those who had been infected in 1980–81 (21). Such studies however typically focus on clinical immunity (i.e., how many people had symptoms) rather than the degree of immunological inhibition of previously circulating strains among individuals. The latter can be reliably documented only

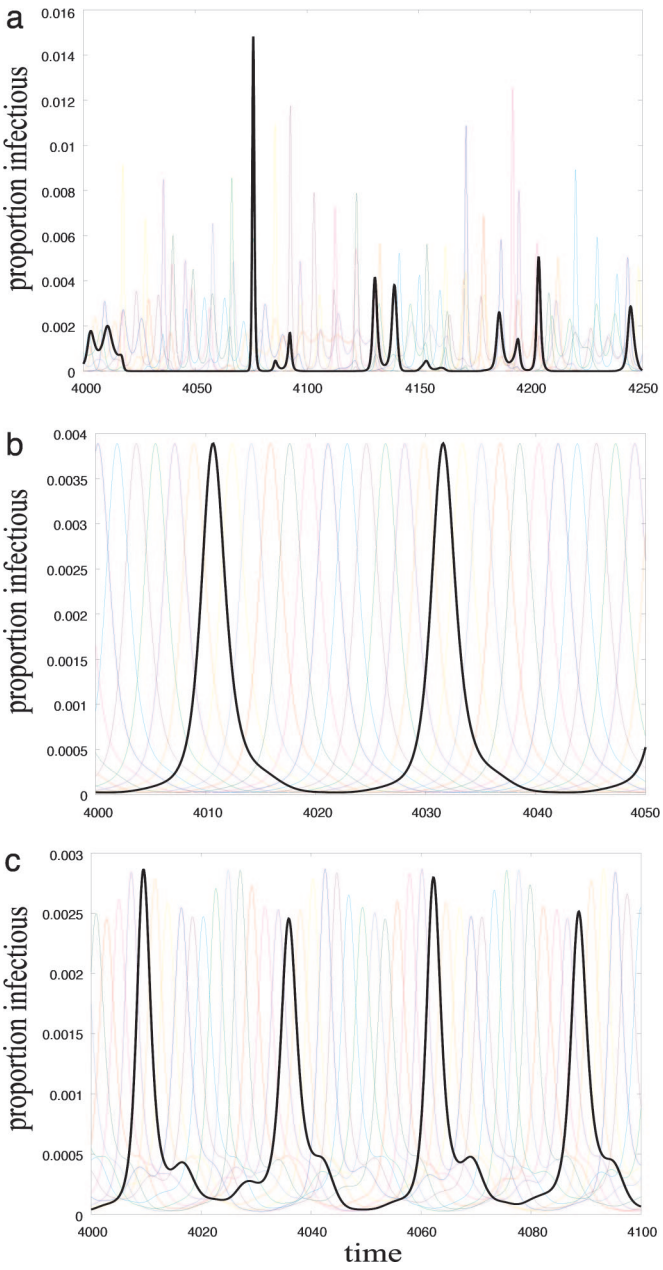

Fig. 3. Changes in the proportions of hosts that are infectious for the 12 different strains within a (3 × 4) system for various values of (γ, δ). (*a*) $\gamma = 0.75$, $\delta = 0.2$. (*b*) $\gamma = 0.6$, $\delta = 0.0$. (*c*) $\gamma = 0.58$, $\delta = 0.4$. Model parameters used were $\mu = 0.02$, $b = 40$, and $s = 10$. In each case, the black line tracks the fate of one particular (possibly hypervirulent) strain.

by use of a combination of serological assays within communities that are regularly naturally challenged with influenza and for whom archived influenza samples are available; we hope that the testable hypotheses generated by our model will encourage more activity in this area.

Genetic Diversity of Influenza. Our model also helps clarify the often misunderstood relationships among influenza genetic diversity, phylogenetic tree shape, and antigenic diversity. Because our model does not explicitly model the mutational process, it demonstrates that the dynamic processes that generate cyclical epidemics and antigenic type switching can be uncoupled from those that generate genetic diversity and divergence at the vast majority of sites; such sites are genetically linked to antigenically selected sites but are not themselves under immune selection. There is therefore no reason to equate the linear accrual of overall genetic distance through time with a hypothesized accumulation of antigenic divergence. Hence, a phylogeny estimated from full-length HA gene sequences will accurately represent the shared ancestry among the sampled sequences but not necessarily their antigenic relationships. In the context of a different model Koelle *et al.* (10) make a similar point but go even further in suggesting that sites that can contribute to antigenic change sometimes evolve neutrally as a result of the network structure that maps HA genotype to antigenic phenotype. In both cases, the pattern of shared ancestry among the sampled sequences (i.e., the shape of the estimated phylogeny) will depend on the population dynamics of each antigenic type, which in turn depend on the dynamics of host cross-immunity. For our model, this situation can be formally represented by a population-genetics model in which the epidemic is represented as a metapopulation structured by antigenic type, with the population dynamics of each subpopulation determined by the network of host immune responses and by the parameters γ and δ (i.e., a structured coalescent process (22, 23) generalized to nonequilibrium deme sizes); mutation and recombination among antigenic types is exactly represented by the movement of sampled lineages among subpopulations. Work remains to be done on the implementation of this framework to establish whether our model can produce the same level of restriction of viral genetic diversity as achieved by selective sweeps that accompany "cluster" transitions within the neutral network model of Koelle *et al.* (10) or by short-term strain-transcending immunity as suggested by the results of Ferguson *et al.* (7). As mentioned, our model has the advantage of being robust to rate of generation of genetic diversity [whether by mutation, gene flow, or the reassortment of genomic segments (24)] and thus has the potential to generate the characteristic phylogenetic tree shape of type A influenza under a minimum of assumptions.

Pathogenicity and Vaccine Development. For pathogens among which only certain antigenic types cause disease (that is, if certain antigens are determinants of virulence or are genetically linked to such), our model can easily explain why certain pathogenic forms emerge at irregular intervals, as illustrated in Fig. 3 by the highlighting of a particular antigenic type through time. There is ample evidence that the HA molecule of the H5 subtype qualifies as a virulence determinant in its avian hosts (25, 26). It is therefore tempting to speculate that the particular H5N1 strain that is currently causing deaths within humans is a hypervirulent member of a larger network of H5N1 antigenic types circulating in avian populations that has recently risen in frequency and may, as shown in Fig. 3, undergo a natural decline. Infection of humans by H5N1 Avian influenza is clearly rare (27), although in certain transmission settings, such as among poultry market workers, seropositivity may be as high as 10% (28, 29). Importantly, the observation that the latter are mainly asymptomatic suggests that prior exposure to other H5 antigenic types may confer protection from severe disease. Cross-protection from illness and death after lethal challenges with a heterologous H5N1 virus has also been demonstrated in ferrets immunized with A/Duck/Singapore/3/97 H5N3 (30) and also among mice vaccinated with A/Duck/Pottsdam/1042–6/86 H5N2 (31). These results give hope that the effects of a newly emergent pandemic H5N1 strain may be mitigated by vaccination even if the vaccine strain is not antigenically identical to the pandemic strain.

Partial cross-protection against disease is also clearly a feature of human influenza types (14, 15) and constitutes an

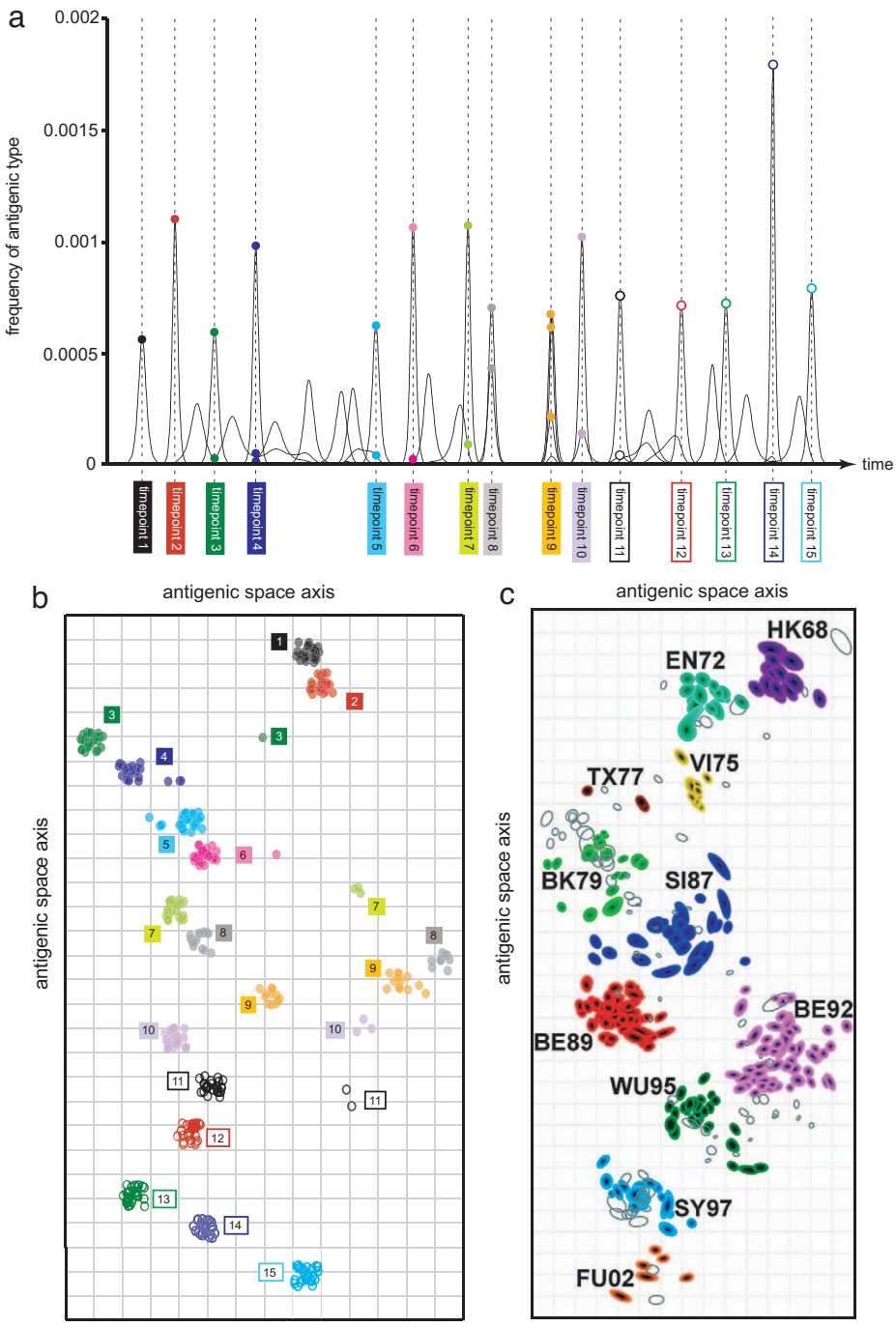

Fig. 4. Antigenic map of influenza. (*a*) Changes in the proportions of hosts that are infectious for the 32 different strains within a ($2 \times 2 \times 2 \times 2 \times 2$) system with $\delta = 0$ and $\gamma = 0.8$. Other model parameters used were $\mu = 0.014$, $b = 400$, and $s = 100$. The superimposed time series are not labeled by strain; however, this information is provided in SI. Twenty-five infected individuals were randomly sampled from the model population at 15 time points, corresponding to the peaks of 15 successive epidemics (dotted lines). Circles highlight the relative frequencies of the strains that were sampled at each time point. (*b*) The antigenic map of the sampled isolates, calculated by using multivariate analysis (see *Methods* for further details). Each circle represents one of the 375 sampled infected individuals, colored and labeled by time point. Each point was subjected to a small amount of random noise to simulate measurement error. (*c*) The antigenic map of human influenza A isolates sampled between 1968 and 2002 (adapted from ref. 18 with permission), calculated by using the same multivariate statistical analysis (see *Methods*).

important option in the prevention of such serious consequences as the mortality imposed by the 1918 H1N1 strain, should this, or any other, highly virulent strain reemerge. With regard to current epidemic influenza, our model suggests that the prediction of future antigenic types is not feasible because of the chaotic nature of their emergence, even though they may be retrospectively seen to arise in a manner that locally maximizes antigenic distance (2, 11). On the other hand, the fact that the actual set of antigenic types is limited affords the possibility of comprehensive vaccine coverage against all variants. However, as with other antigenically diverse pathogens, the boosting of host responses against more conserved, and less naturally im-

munogenic, determinants (14, 32) remains the more promising strategy.

Methods

Transmission Dynamics. The two-locus model is governed by the following set of coupled differential equations for each antigenic type ij (defined as allele i at one locus and allele j at the other):

$$\frac{dz_{ij}}{dt} = \lambda_{ij}(1 - z_{ij}) - \mu z_{ij}, \quad \forall i \in \{a, b, c, \ldots\} \cap j \in \{x, y, z, \ldots\}$$

$$\frac{dw_{ij}}{dt} = \left(\sum_k \lambda_{ik} + \sum_l \lambda_{lj} - \lambda_{ij} \right)(1 - w_{ij}) - \mu w_{ij}$$

$$\frac{dx_i}{dt} = \sum_k \lambda_{ik}(1 - x_i) - \mu x_i$$

$$\frac{dx_j}{dt} = \sum_l \lambda_{lj}(1 - x_j) - \mu x_j$$

$$\frac{dy_{ij}}{dt} = \lambda_{ij}(\Omega_{ij} + (1 - \gamma)\Gamma_{ij} + (1 - \gamma)(1 - \delta)\Delta_{ij}) - \sigma y_{ij}.$$

In the above, z_{ij} is the fraction of the population that has been exposed to pathogen type ij and is now either infected or recovered; y_{ij} is the proportion of the population currently infectious with type ij, consequently, λ_{ij} ($= \beta y_{ij}$) is the force of infection; w_{ij} denotes the proportion of the population that has been exposed to any antigenic type that share alleles with ij (and includes ij itself), whereas x_i and x_j includes only those that have been exposed to types sharing either allele i or allele j, respectively; the average life expectancy is given by $1/\mu$.

Individuals who have never been in contact with type ij or any types that share alleles with ij, here given as Ω_{ij}: $= 1 - w_{ij}$, have no protection and become infectious. Those individuals that have been exposed to antigenic types sharing alleles at one or the other locus, here denoted as

$$\Gamma_{ij} = \sum_{k=i,j} (w_{ij} - x_k),$$

have a lower probability $(1 - \gamma)$ of becoming infectious; and individuals that have been in contact both with types sharing allele i and types sharing allele j, but have never been exposed to ij itself, denoted as Δ_{ij} ($= w_{ij} - z_{ij} - \Gamma_{ij}$) benefit from an additional reduction in the risk of becoming infectious, $(1 - \delta)$, because of the combined exposure to alleles that define ij.

Antigenic Map. Infected individuals were sampled from our model population at the peaks of successive epidemics in proportion to the relative frequency of their respective antigenic types. Antigenic distances among these sampled individuals were calculated as the number of loci at which they differed. Distances were mapped onto a two-dimensional Euclidean space by using the same multidimensional scaling method developed by Smith *et al.* (18) (available from www.antigenic-cartography.org; best-fit result of 25 runs is shown).

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