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Viral evolution from one generation of human influenza infection to the next

Key Messages

1. In a sub-tropical epidemic, most of the apparent household secondary cases are actually secondary infections.
2. The consensus sequence for the entire influenza virus genome is not usually identical within the same household sample. Rather, there are commonly one or two nucleotide changes.
3. These results hint at an obvious generational threshold for adaptation at the level of the consensus sequence.

Introduction

Phylodynamics describes the complex interaction between the evolution of a pathogen and its transmission dynamics between hosts as a single system.[1] Influenza presents unique patterns of evolution and infection. Explicit theoretical models have been used to explain the two time scales of antigenic drift and antigenic shift of human influenza infection.[2,3] In addition, implicit phylodynamic models are often used to interpret the results of phylogenetic analysis of influenza. For example, the apparent exchange of internal genes in human influenza A samples taken from New York between 1999 and 2004 suggests that co-infection and subsequent recombination in humans is more common than previously thought.[4] The scales at which phylodynamic models (both implicit and explicit) can be applied to uncover fundamental interactions between a pathogen and its host depend on the resolution of available data. Sequence data from only the neuraminidase gene for many samples over 30 years were used to infer the presence of a short-term, non-specific immune response generated by one influenza strain against all influenza strains.[3] The entire genomes of 156 samples taken over 2 years were used to describe frequent recombination.[4] The robust statistical incorporation of pathogen evolution dynamics into more detailed transmission models requires more finely resolved genetic data than has been used to date.

We suggest that there exists an intuitive fundamental unit for phylodynamic analysis of human influenza infection, namely, the expected evolutionary distance between virions isolated from a typical infector and infectee. This generational unit of viral evolution can be used in academic and public health investigations of infectious disease outbreaks. Laboratory techniques for isolation and sequencing of viruses continue to improve. Therefore, evolutionary data on viral infections may be incorporated into routine epidemiological analysis in the same way as for bacterial infections. However, strains captured by global surveillance systems are not useful for the study of smaller-scale patterns of influenza transmission and evolution. Most strains captured by surveillance systems come from clinical settings. Because many influenza infections are either mild or entirely asymptomatic, very few sequential infections are captured. However, viral samples obtained from intensive household-based transmission studies enable investigation of smaller-scale evolutionary patterns of influenza in humans. We present the full genome sequences of viral samples from such a study. We compare the distribution of genetic distances between isolates from the same household and isolates from different households. Also, we use the dates of isolation of samples to construct a timed evolutionary tree, with which the 2007 influenza A H3N2 season was compared with a set of global samples.

Methods

This study was conducted from January to December 2009. Viral samples from households with apparent transmission during a trial of non-pharmaceutical interventions were used.[5] For the intervention study, individuals who were at least 2 years old, exhibited two or more symptoms of influenza-like illness (ILI), and were living in a household with at least two other individuals who had not reported ILI symptoms in the previous 2 weeks were recruited at clinics.

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Participants were tested with the QuickVue Influenza A + B rapid test. If the test was positive, they and their household members were followed up immediately (same or next day) and then on the 3rd, 6th, and 9th days. Nose and throat swabs were taken at each household visit, and each swab was cultured for influenza A and B. Standard cultures were obtained from all swabs.

Viral RNA was extracted directly from cell culture using the QIAamp viral RNA minikit (Qiagen Inc). Complementary DNA was synthesised by reverse transcription reaction with gene amplification performed by polymerase chain reaction (PCR) using specific primers for each gene segment. The PCR products were purified with the QIAquick PCR purification kit (Qiagen Inc) and sequenced by synthetic oligonucleotides. Following the manufacturer's instructions, reactions were performed with a Big Dye-Terminator v3.1 Cycle Sequencing Reaction Kit on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). All sequences were assembled and edited with Lasergene version 8.0 (DNASTAR). Phylogenetic trees were inferred using the neighbour-joining distance method, with genetic distances calculated by total distance. Temporal phylogenies and rates of evolution were inferred using a relaxed molecular clock model that allows rates to vary among lineages within a Bayesian Markov chain Monte Carlo framework.[6] This was used to sample phylogenies and the dates of divergences between viruses from their joint posterior distribution, in which the sequences were constrained by their known date of sampling and a codon-position-specific HKY1C substitution model was used.

Results

A total of 79 samples from 38 individuals in 17 households were positive for either influenza A or B based on the rapid test (Table). Influenza B samples and samples from which successful cultures could not be obtained were excluded, as were all samples from household in which secondary transmission of influenza A H3N2 was not confirmed by culture. Therefore, the current study was based on the full genome sequences of 31 samples of influenza A H3N2 obtained from 23 individuals in 11 households during 2007.

To visualise total nucleotide differences between 13 apparent transmission events in 10 households, a total distance neighbour-joining tree was constructed (Fig 1a). The households from the Hong Kong non-pharmaceutical intervention study did not form a single monophyletic group. However, in all but one case, viruses sequenced from the same household clustered together. Household clusters were distributed throughout the tree, with most closely related isolates from North America and, in one instance, Taiwan.

The samples from the intervention study were grouped into three clades. Out of 35 possible pair-wise comparisons of full viral genomes within study households, 13 pairs were identical, 14 pairs differed by a single nucleotide, five pairs by two nucleotides, one pair by three nucleotides, and two pairs by 39 nucleotides (Fig 1b). The latter arose from household 111. Two samples from the index case were identical: one was taken during the baseline visit and the second during the first follow-up visit. However, a third virus isolated from a second household member during visit 2 differed by 39 nucleotides. In contrast, the most similar pair of viral isolates from different households in the intervention study differed by 30 nucleotides.

To make full use of dates of isolation and sequence data, a temporal phylogeny of the full genomes of all viruses in our sample is shown (Fig 2). The grouping of the Hong Kong samples into three clades was preserved. The most recent common ancestor for samples in household 111 was estimated with narrow confidence bounds to be ~1 month prior to the recruitment of the household into the study. The phylogenetic relationship between full genome sequences from a study of seasonality in Managua, Equador[5] contrast sharply with the samples from the Hong Kong intervention study; the Managua samples form a monophyletic group.

Conclusions

By obtaining full genetic sequences for 31 samples from 17 household outbreaks, the degree of adaptation that occurs in that setting was quantified. Most pair-wise comparisons between consensus sequences showed three or fewer nucleotide changes. These results extend an earlier study in which the haemagglutinin genes were identical in all household outbreaks.[6]

The 17 household outbreaks appeared to be a result of between-household transmission, rather than within-household transmission. A single seed did not initiate the 2007 Hong Kong transmission season of H3N2 influenza. In contrast, the 2007 outbreak of influenza in Managua, Equador was very likely initiated by a single introduction. These patterns are consistent with Hong Kong acting as

Table. Properties of samples included

Properties of samples	Households	Individuals	Samples
Laboratory confirmed apparent secondary cases in the Hong Kong non-pharmaceutical intervention study[5]	17	38	79
Influenza B on culture	-3	-7	-13
All apparent secondary infections negative on culture	-3	-8	-20
Other negatives on culture	-0	-0	-13
Included	11	23	31

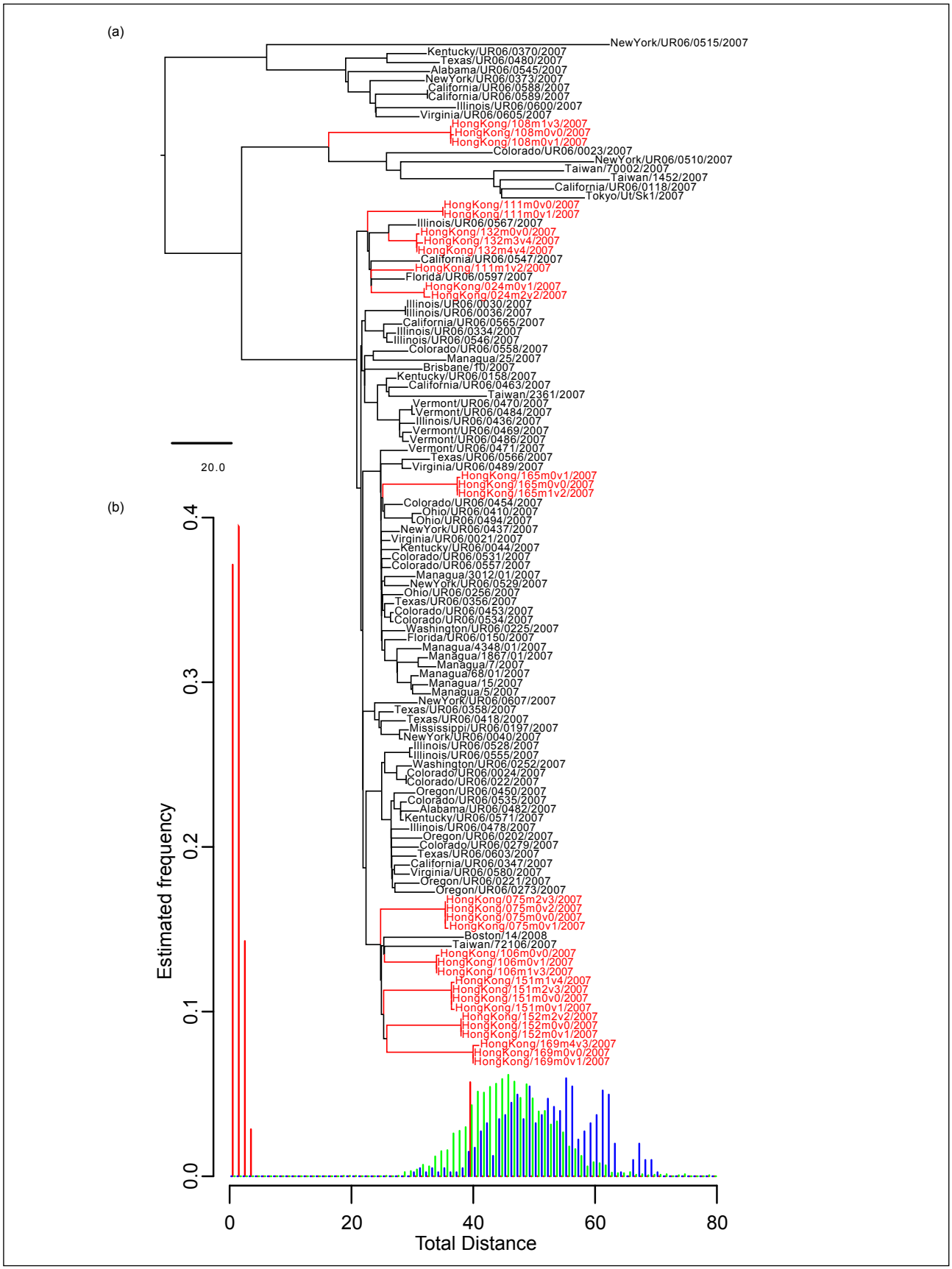

Fig 1. (a) Neighbour-joining total distance tree for 120 of the 153 genomes: the number of taxa are reduced to aid presentation, and all sequences from the Hong Kong study are included. (b) Distribution of pair-wise distances for three groups of samples: between isolates from the same household in the intervention study (red), between isolates from different households in the intervention study (blue), and between samples from the other 2007 samples in Genbank with full sequence and date of isolation (green).

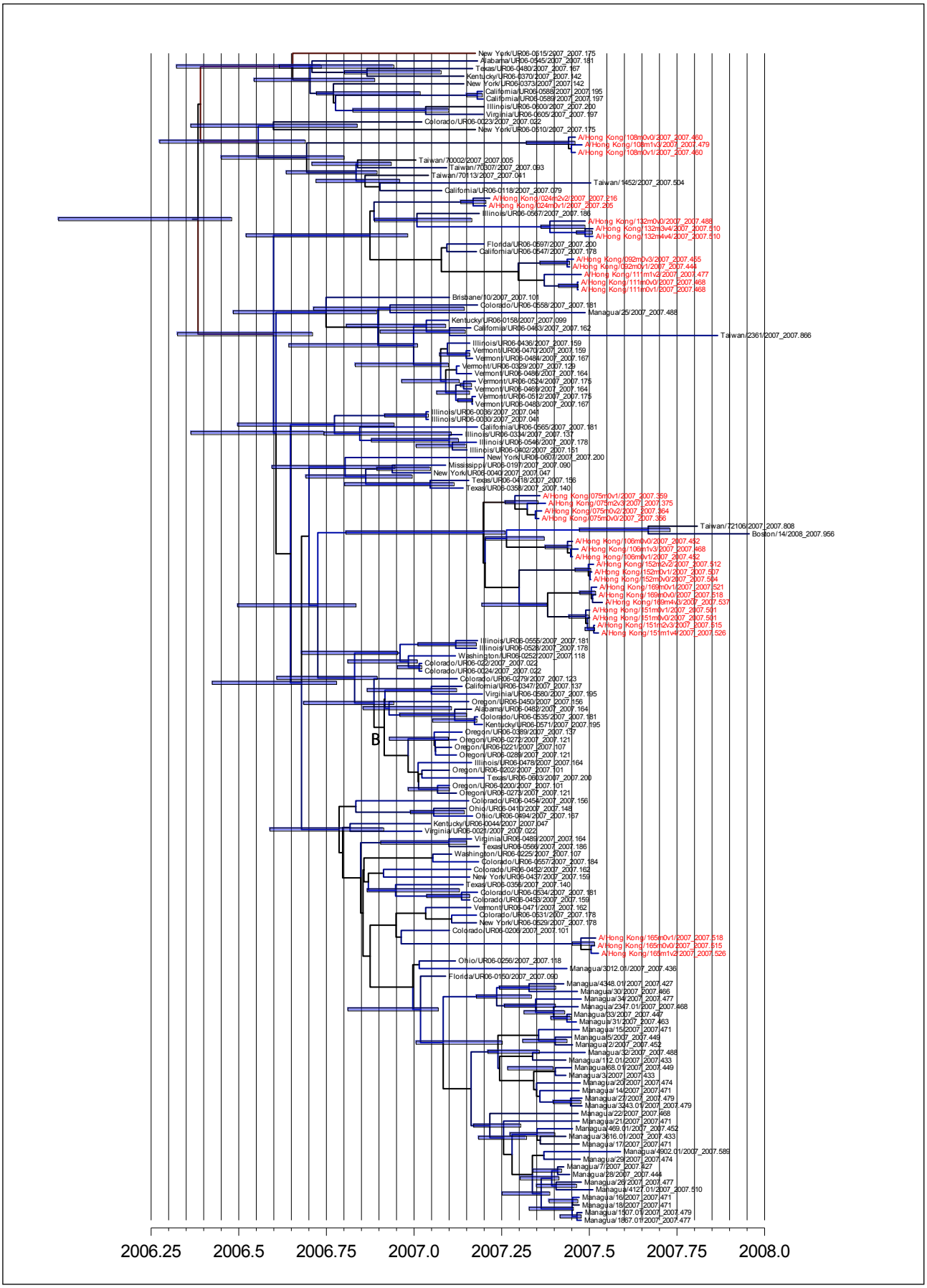

Fig 2. Dated phylogenetic tree of the full genome sequences for this study and all those full genomes for samples collected during 2007 (as available from NCBI).

a highly connected international hub, whereas the study population in Managua was far less connected. Notably, the study in Managua was not in any way intended to be representative of the entire city and was largely restricted to a number of smaller local communities. Thus, the phylogenetic patterns for full genome sequences from Hong Kong and Managua during 2007 are strikingly different, despite differences in recruitment strategies.

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