Contrasting the epidemiological and evolutionary dynamics of influenza spatial transmission

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In the past decade, rapid increases in the availability of high-resolution molecular and epidemiological data, combined with developments in statistical and computational methods to simulate and infer migration patterns, have provided key insights into the spatial dynamics of influenza A viruses in humans. In this review, we contrast findings from epidemiological and molecular studies of influenza virus transmission at different spatial scales. We show that findings are broadly consistent in large-scale studies of inter-regional or inter-hemispheric spread in temperate regions, revealing intense epidemics associated with multiple viral introductions, followed by deep troughs driven by seasonal bottlenecks. However, aspects of the global transmission dynamics of influenza viruses are still debated, especially with respect to the existence of tropical source populations experiencing high levels of genetic diversity and the extent of prolonged viral persistence between epidemics. At the scale of a country or community, epidemiological studies have revealed spatially structured diffusion patterns in seasonal and pandemic outbreaks, which were not identified in molecular studies. We discuss the role of sampling issues in generating these conflicting results, and suggest strategies for future research that may help to fully integrate the epidemiological and evolutionary dynamics of influenza virus over space and time.

1. Introduction

With the rapid development and reduced cost of next-generation genome sequencing technologies, gene sequence data for human influenza A virus have become increasingly available at detailed spatial and temporal scales [1,2]. These molecular data have provided important insights into key patterns of influenza viral migration and disease persistence in different geographical regions and at different epidemiological scales. While it has long been known that influenza viruses circulate globally, the existence, location and determinants of a common ‘source’ population from which genetic and antigenic variants might emerge remains a topic of great debate, as does the extent of viral persistence between epidemic seasons within individual localities [3–7].

In parallel to progress in the generation and availability of molecular data, there is a long history of fitting spatial transmission models to influenza epidemiological data, and improvement in computational power has allowed simulation of disease spread at increasingly detailed spatial scales [8–17]. High-resolution disease data now available from non-traditional surveillance sources, such as web searches, electronic medical claim records, cell phone usage and tweets, are becoming essential to validate detailed spatial models [18–20]. In addition, the emergence and rapid global spread of the A/H1N1 pandemic virus in 2009 provide a unique case study to elucidate long-standing epidemiological questions, such as the role of population movements and children on influenza spatial transmission, and to directly compare the findings derived from large-scale epidemiological and molecular data.
The aims of this article are (i) to review efforts to decipher and model the spatial–temporal patterns of influenza virus, using epidemiological and molecular data and (ii) to compare and contrast the findings of these parallel fields. We first explore the spatial dynamics of seasonal influenza based on the increasing wealth of viral gene and genome sequence data, particularly for the A/H3N2 subtype for which most data are available. In comparison, we highlight the insights provided by epidemiological diffusion models. We then discuss the most salient discrepancies between molecular and epidemiological studies, particularly within the context of the 2009 pandemic, and suggest areas for future research to reconcile some of the conflicting findings. Finally, we discuss the implications of spatial studies for public health and control strategies.

2. Spatial dynamics of human influenza based on molecular data

The availability of large-scale gene sequence datasets of human influenza A viruses has provided several key insights into the spatial dynamics of virus transmission at global, national and community scales. The increased number of publicly available human influenza sequences on GenBank (figure 1), from only two whole-genome sequences (and less than 1000 partial haemagglutinin (HA) sequences) in 2000 to more than 6500 genomes (and more than 27 000 partial HA sequences) in 2012, has been instrumental in refining our understanding of the global ecology and migration patterns of influenza, viral persistence between seasonal epidemics and how co-circulating genetic diversity complicates tracing spatial patterns at restricted spatial scales.

(a) Viral persistence

The study of influenza virus persistence is perhaps the most straightforward utilization of molecular data, and confers one of the most obvious advantages over epidemiological data alone. Studies of intensively sampled localities in temperate regions of the Northern and Southern hemispheres (specifically New York State, USA, Australia and New Zealand) revealed that the A/H3N2 influenza virus rarely persists (if at all) between seasonal epidemics in these locales, suggesting that long-distance, bi-hemisphere migration is essential to sustain global transmission in humans [21,22]. Hence, winter influenza epidemics are not due to the ‘re-igniting’ of viral lineages that survived over the course of the summer. The strong seasonal bottlenecks observed in Bayesian skyline and skyride plots of influenza virus in New York State (USA) and New Zealand (figure 2), which depict changing levels of relative genetic diversity to the next seasonal epidemic in temperate areas, as viruses present at the end of an influenza season are generally genetically different (i.e. fall into different

Figure 1. Map of the total number of viral gene sequences available in the public domain for influenza A/H3N2 (n = 42 075), 2009 pandemic A/H1N1 (42 246), seasonal influenza A/H1N1 (n = 16 998), influenza B (n = 9964), by country. The map serves to illustrate the geographical gaps in sampling of molecular data, especially in Africa. Note also the unbalanced sampling frequency by subtype, in particular the intense collection efforts for pandemic A/H1N1 virus over a short period of time (3 years at the time of this study), relative to the other subtypes that have circulated for over 30 years. Numbers are coded according to the scale on the bottom of the figure, increasing from yellow to dark red. White areas denote countries with no data. Maps are based on a GenBank search performed on 10 July 2012 with the keywords ‘human’, ‘type A, H3N2’, ‘type A, H1N1 (excluding 2009 pandemic H1N1)’, ‘type B’ and ‘type A, 2009 pandemic H1N1 only’.during summer months in temperate countries, including the USA. As such, the more intensive sampling that took place during the 2009 influenza A/H1N1 pandemic provided a unique opportunity to explore viral persistence patterns in (summer) months that are not traditionally sampled. For example, an unseasonal transmission chain of A/H3N2 influenza virus was identified through 1 June 2009 in New York State and may have persisted later in the summer after sampling was halted [24]. Although of small scale, this study suggests that limited influenza transmission may occur during non-epidemic months and go undetected by traditional surveillance systems. However, limited transmission chains in summer do not significantly contribute genetic diversity to the next seasonal epidemic in temperate areas, as viruses present at the end of an influenza season are generally genetically different (i.e. fall into different...
Adapted from Bahl (New York) and Southern (New Zealand) Hemispheres compared with the lower genetic diversity (age of the tree linking these sequences, whereas the green). Localities are arranged from top to bottom by latitude. The genetic diversity in A/H3N2 influenza virus from 2002 to 2006 in New York State, Bayesian skyride analysis depicting the fluctuating levels of relative genetic diversity in A/H3N2 influenza virus from 2002 to 2006 in New York State, USA (blue), Hong Kong (red), Southeast Asia (pink) and New Zealand (NZ, green). Localities are arranged from top to bottom by latitude. The x-axis shows time from youngest sampled sequence to the lower 95% credible interval of the age of the tree linking these sequences, whereas the y-axis depicts relative genetic diversity ($N_e t$), where $N_e$ is the effective population size, and $t$ is the generation time. Note the marked seasonal bottlenecks in the Northern (New York) and Southern (New Zealand) Hemispheres compared with the lower and more constant levels of genetic diversity observed in more tropical regions. Adapted from Bahl et al. [6] with permission.

Phylogenetic lineages) from those that seed the epidemic in the following winter [21,22]. In addition, all phylogenetic studies undertaken to date have revealed that multiple lineages co-circulate within a single geographical locality during a specific influenza season, indicative of multiple viral introductions [7,21,22,25].

Although some global phylogenetic analyses have suggested that a stable influenza virus source population could exist in tropical regions such as East and Southeast Asia [3,4], more recent regional studies focused on Hong Kong and southern China have not yielded convincing evidence of viral persistence in these localities, nor of the consistently higher levels of circulating genetic diversity expected of a source population [6,7] (figure 2). Finally, while small chains of influenza A/H1N1 transmission persisted between the summer and autumn waves of the 2009 pandemic in the UK [26], the time interval between waves was unusually short, such that they are unlikely to represent a general phenomenon.

(b) Global ecology

Identifying a geographical source of new antigenic and genetic variants of influenza virus could potentially improve early detection and prediction of new vaccine strains. As a consequence, this question has stimulated great interest. Early phylogenetic analyses of global influenza datasets led to the hypothesis of a viral source population for influenza A/H3N2 located in the tropics, particularly East and Southeast Asia [3,4]. In particular, influenza viruses from East and Southeast Asia were found to be the shortest distance from the main ‘trunk’ of the tree in a global phylogenetic analysis of the HA1 region, suggesting that they represent a viable source population [4]. However, subsequent analyses of longer time-series have revealed that other regions contribute to global diversity, including the USA [5]. Further, recent studies focused on Southeast Asia suggest that influenza viruses in this region do not consistently fall at trunk locations and do not exhibit the patterns of persistence and genetic diversity expected of a global source population [6,7] (figure 2). Instead, these phylogenetic data are suggestive of a dynamic ‘metapopulations’ model of global influenza ecology, such that a multitude of geographical regions contribute to global viral diversity, and that contributing populations change with time. The sheer number of subpopulations that exist in the densely populated, highly connected, and climatically variable regions of East and Southeast Asia may therefore explain the periodic emergence of new virus variants in this region. It is clear that a complete understanding of influenza metapopulation dynamics will require deeper sampling from understudied tropical and subtropical regions, notably in Africa, India and Latin America, and from seasonal influenza A/H1N1 and influenza B virus, for which there is limited information (figure 1). Overall, the debate over the existence and location of a global source population highlights the dangers of drawing strong conclusions from limited sampling [27].

(c) Pandemic influenza

The 2009 influenza A/H1N1 pandemic presented a unique opportunity to integrate molecular and epidemiological analyses of global and regional spread patterns and epidemic growth dynamics (summarized in table 1). Using molecular data from the early phase of the pandemic in Mexico, Fraser et al. [28] estimated the basic reproduction number ($R_0$) of the epidemic to be 1.22 (95% CI: 1.05–1.60), compatible with epidemiological estimates ranging between 1.3 and 1.6. The date of onset of the outbreak in the first weeks of 2009 as inferred from molecular clock-based analyses of viral sequence data was also in broad agreement with the first clinical case documented in La Gloria, Mexico, on 15 February 2009 [28,31].

Importantly, the statistical inference of viral migration pathways and the selection of spatial diffusion models are now possible with newly developed Bayesian phylogeographic methods, which also account for phylogenetic uncertainty [36,37]. Such methods were instrumental in tracking how the pandemic unfolded across the globe and in confirming the Mexican origin of the most recent common viral ancestor in January–February 2009 [32]. In particular, analysis of viral gene flows suggests an early diffusion from Mexico into several US states during March and April 2009, with the strongest support for migrations between Mexico and Texas (where some of the earliest clinical cases were documented). From the USA, the A/H1N1 virus continued to spread to Europe and Asia, and the reconstructed global pandemic spread provides an interesting opportunity for dynamic visualization using sophisticated tools such as Google Earth [32]. Overall, these early phylogeographic studies of viral gene flow are in broad agreement with the
sequence of pandemic activity experienced by different regions of the world, and with air travel fluxes originating from Mexico [38].

(d) Local transmission
Although molecular data have the potential to reveal detailed spatial patterns defined by individual lineages, the number of influenza viruses that co-circulate in a given locality complicates the study of spatial dissemination at a local scale [39]. Indeed, one of the most consistent and striking observations from studies of localized influenza spread is that even relatively small and isolated US communities harbour multiple geographical lineages, thereby illustrating the fluidity of human movement patterns [25,33]. During the early stage of the 2009 A/H1N1 pandemic in the USA, clear spatial patterns could be detected, as strong founder effects resulted in genetically distinct viral clades sweeping through New York State and Wisconsin in April–May 2009 [33]. However, such spatial structure was broken down by extensive global mixing during the autumn pandemic wave, which was dominated by a single clade that was prevalent in spring in New York City [33]. A slightly different phylogeographic pattern was observed in Scotland, where the spring 2009 pandemic wave was characterized by the expansion of a major clade originating from Birmingham, England, together with multiple introductions from both international and other UK sources [34]. By contrast, the autumn pandemic wave in Scotland was more genetically diverse with several co-dominant lineages in individual locations, indicative of widespread gene flow [34]. The examples of the USA and Scotland during the 2009 A/H1N1 pandemic suggest that spatial patterns may vary substantially between temperate locations.

A common observation from numerous community-level studies of genetic diversity during the 2009 pandemic A/H1N1 was the co-circulation of multiple viral clades, which again impeded the detailed analysis of the patterns and dynamics of spatial diffusion [26–28,31–34,36–46]. In Hong Kong, extensive genetic diversity was detected not only at the community level, but also within households and individuals [46]. In the most detailed molecular study of community transmission of influenza virus to date, pandemic A/H1N1 viruses circulating among students at the University of California-San Diego were shown to exhibit little geographical clustering by student residence or city zip code, suggesting that most viral transmission occurred off-campus [35]. Taken together, these findings illustrate the remarkable spatial fluidity of the virus within the community, probably explained by a high degree of human mixing. Taken together, the complex patterns of viral genetic diversity within individual localities—especially the presence of multiple co-circulating lineages—and lack of sufficient sampling depth have prevented more precise spatial analyses. While major advances in the methods of phylogeographic inference [36,37,47] have facilitated the effective use of gene sequence data to compare competing spatial models in other disease systems (such as animal rabies virus or swine influenza [48–50]), small-scale spatial patterns have not yet been identified for human influenza.

3. Spatial dynamics of human influenza based on epidemiological data

(a) Global spread
Large-scale mathematical and computational transmission models have been used to simulate, in detail, the global spread of pandemic viruses and assess the effectiveness of control measures [11–17,51]. These models typically integrate...
information on air travel volumes with local work commute patterns, together with population demographics and seasonality factors. Such large-scale modelling efforts predicted that an early peak of pandemic influenza A/H1N1 virus activity would occur in October/November 2009 in the Northern Hemisphere, several weeks before vaccination campaigns could be carried out, and that antiviral use could delay peak pandemic timing. Further, modelling studies have concluded that substantial reductions in air travel would provide only a short delay in pandemic progression [51,52].

(b) Regional and local spread of influenza

(i) Seasonal influenza

Analysis of influenza epidemiological data at the scale of a country has highlighted the importance of ‘hierarchical’ spread between large population centres of temperate areas. Seminal studies of influenza diffusion in Iceland in the spread between large population centres of temperate areas. The 2009 A/H1N1 pandemic provides an opportunity to undertake a detailed validation of existing diffusion models, aided by high-resolution epidemiological data. Accordingly, the spring wave of the pandemic in the USA was characterized by sporadic outbreaks in northeastern and midwestern

(ii) Pandemic influenza

The 1918 pandemic presents a particularly interesting case study owing to its unusual severity, making it relatively straightforward to characterize spatial patterns in epidemiological data. Detailed studies of disease diffusion in rural and urban areas of England and Wales have highlighted the rapid spread of the lethal autumn wave of 1918, with larger and more urban population areas experiencing earlier disease onset, reminiscent of the spread of seasonal epidemics in the USA [10,63]. Pandemic diffusion was much slower and less synchronous in the USA than in the UK, probably owing to less intense population mixing in the USA [54] and geographical differences in non-medical interventions between US cities [64,65]. With only 16 partial haemagglutinin HA1 virus sequences available from this historical pandemic to date [66], it is unfortunately impossible to compare diffusion patterns identified in epidemiological data with those derived from analysis of molecular data.

Although earlier pandemics predate the era of modern virology by several decades, a recent analysis of epidemiological records dating back to the 1889 pandemic have highlighted the rapid regional spread of the disease in Europe and the Americas via the railway network [67]. Unfortunately, no viral specimens have been preserved from this era. While virological and epidemiological surveillance networks were in place during the more recent pandemics of 1957 and 1968, lack of spatially disaggregated data for these relatively mild events has hampered analyses of disease spread. Country-level epidemiological studies have provided evidence for regional differences in the timing and intensity of the 1968 pandemic, potentially linked to differences in circulating viruses and prior immunity [68]. In the early 1960s, mathematical metapopulation models were developed to simulate the spread of influenza in 126 cities of the Soviet Union based on the air traffic transportation network [11]. These models were expanded on a global scale to reproduce broad diffusion patterns of the 1968 pandemic in 52 cities [69]. Recent efforts to incorporate international travel data in disease spread models have built heavily on these seminal efforts, relying on the theoretical concept that speed of spread should increase proportionally to population movements. However, few studies have attempted to match long-term trends in diffusion patterns of influenza epidemics with changes in human mobility, as seen over the past decades [70–73].
4. Discussion

With the availability of increasingly large volumes of molecular and epidemiological data, it has become possible to study influenza migration and transmission patterns at various spatial and temporal scales. Overall, the findings derived from large-scale molecular and epidemiological studies of inter-regional or inter-hemispheric spread in temperate regions are in broad agreement, revealing intense epidemics followed by deep troughs strongly driven by seasonal bottlenecks, multiple viral introductions, and lack of sustained viral persistence between epidemics [3,21,23,76,77]. However, there are important differences in studies conducted at smaller spatial scales, most likely reflecting the inadequate sampling of smaller molecular studies given the number of co-circulating lineages. As a case in point, country-specific epidemiologic studies reveal spatially structured diffusion patterns (e.g. the autumn wave of the 2009 pandemic in the USA [30], seasonal epidemics in Brazil [60]), or hierarchical spread driven by population size and distance (seasonal influenza in the USA [15,54]), which have not yet been clearly observed in molecular data. Such discrepancies prevent the complete integration of epidemiological and evolutionary models for spatial data, as proposed in early visions for the field of phylodynamics [78].

Despite the limitations of current molecular studies, one key advantage they have over epidemiological data is that they allow the exact characteristics of influenza virus to be determined (e.g. specific type, subtype and lineage). Indeed, there are inherent dangers in basing all epidemiological estimates on incidence of influenza-like-illnesses (ILI), which by definition must also contain cases owing to other respiratory pathogens. It is estimated that in a typical season, only one-third of ILI cases are caused by an influenza virus infection, with the highest influenza aetiological fraction observed near the time of the epidemic peak, during more severe outbreaks, and in young and middle-aged adults [29]. During the 2009 A/H1N1 pandemic, which occurred at an unusual time of the year when circulation of other respiratory pathogens was limited, the proportion of ILI caused by an influenza virus reached 40–60% [79,80]. The bias introduced by non-influenza pathogens can be reduced by relying on timing of peak ILI incidence, or on ILI rates in excess of a seasonal baseline, which are highly correlated with viral activity data, especially in influenza A/H3N2 seasons [15,81,82]. However, in contrast to molecular studies, epidemiological data cannot provide more detailed information on the spread of individual viral lineages that may differ in major phenotypic properties such as antigenicity [2,25] and capacity for drug resistance [83,84].

As noted earlier, the most obvious and severe limitation of molecular data is that, although it provides a uniquely powerful retrospective vision on the pathways of viral transmission, it is subject to strong sampling bias, particularly when viruses are collected for the purpose of vaccine strain selection. Although efforts by the National Institute of Allergy and Infectious Diseases-led Influenza Genome Sequencing Project have increased the availability of population-based collections of viral sequences, and specifically those based on complete viral genomes [1,2], the influenza virus specimens collected have tended to come from localities that are able to sample. Hence, there is still limited molecular information from locales that might harbour the most interesting patterns of genetic diversity, particularly in tropical regions. Phylogeographic analyses can draw migrational links only between populations for which samples are available, and hence may provide a false picture of the frequency of connections between undersampled geographical localities. Similarly, there is relatively little effort to collect samples outside the typical influenza season in temperate areas, nor from the full spectrum of clinical cases, although these may be central to understanding spatial viral dynamics. This problem is most acute at smaller spatial scales in which most samples are not given a sufficiently distinct spatial identifier (such as a GPS location) to enable detailed phylogeographic analysis. For the future, it will be essential to undertake both large-scale and structured sampling of influenza virus from diverse geographical locations and from diverse patient groups.

This review highlights how molecular analyses of influenza dynamics in localized communities have generally failed to provide clear evidence of spatial structure. To be informative, future studies will require greatly expanded and more structured sampling, perhaps by more than an order of magnitude over what is currently available. Inference of spatial structure in phylogeographic methods relies on the occurrence of nucleotide substitutions across the influenza virus genome. The rapid pace of human movement compared with the relatively slow pace of mutational fixation, coupled to the sparseness of sampling, is the most likely reason why there is a disconnect between those spatial patterns inferred from epidemiological and molecular data. However, the exact extent of sampling required to capture spatial patterns at smaller scales remains unclear, even for more remote localities.

For the future, it will also be important to go beyond molecular analyses limited to the HA1 domain of the influenza virus haemagglutinin. Although these are very informative about antigenic evolution, and provide an essential comparative dataset, they often lack the phylogenetic resolution required to precisely infer spatial dynamics, particularly at
localized spatial scales. In addition, segments other than the HA can present different phylogeographic patterns owing to frequent reassortment events, which may be essential to the development of both antigenic diversity [2,3] and drug resistance [83,84]. Hence, the analysis of complete genome sequences should be a priority to improve both the resolution and usefulness of future phylogeographic studies [2]. Deep intra-host sequencing may also prove to be a useful tool in elucidating detailed spatial transmission patterns, such as within-households, revealing transmission linkages that would otherwise not be detected by population consensus sequencing [85,86].

Another interesting area for future research is the issue of viral introductions and how it relates to seasonal drivers of transmission, which remain debated for influenza and other respiratory infections [87–89]. It will be useful to reconcile findings from molecular studies demonstrating multiple viral introductions over long periods of time in individual locations [25,33], with the unimodal and strikingly seasonal shape of influenza epidemics illustrated by epidemiological data. It remains unclear whether seeding is constant throughout the year or highly seasonally dependent, and whether seeding varies with travel volumes globally. Further, the exact relationship between viral dispersal and human mobility remains debated, with some epidemiological studies suggesting that population mixing is high in Northern Hemisphere temperate locations [56,57,73], and that changes in domestic and international connectivity may matter only in extreme situations such as isolated islands [71]. Molecular studies explicitly contrasting travel movements with patterns of viral seeding and migrations in well-sampled global locations would help quantify this relationship with better resolution than in previous studies [32].

Finally, we believe that it will be necessary to heavily sample locations that are strongly connected by human movements but which experience different climatic and influenza seasonality patterns, as these may provide insights into the drivers of influenza seasonality. Some areas of Latin America offer great opportunities in this respect, including the coastal, mountain and jungle areas of Peru [90] and Brazilian states [60], where pronounced geographical structure in timing and seasonal characteristics of epidemics has been identified. Whether these distinct spatial patterns observed in epidemic data can be reconciled with molecular patterns remains unclear.

A better integration of molecular and epidemiological models for the spatial transmission of influenza would benefit public health and help guide intervention strategies. In particular, clarification of the global migration patterns of influenza viruses and the existence of source populations, across different viral subtypes, would be helpful to focus surveillance efforts and improve vaccine design. Further, the projected impact of travel restrictions and border screenings would benefit from a better understanding of the global migration of influenza virus. Similarly, a better understanding of spatial dynamics at a local scale could help identify foci of influenza transmission and fine-tune the effectiveness of localized interventions, such as school closure or household prophylaxis. In conclusion, we believe that synergies between epidemiological and evolutionary studies of influenza spatial transmission will ultimately be helpful to improve predictive models and guide pandemic preparedness plans.

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