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immunity effect of vaccinating school children against influenza (18) would be extremely valuable in this context. Not only would the study test an important prediction for seasonal influenza, but it would also provide information about whom to prioritize for vaccination when vaccine is scarce, as it could be during a pandemic, and it also could provide repeated data sets for model testing.

A Final Caveat

We do not know that a human-adapted H5 will have similar epidemiology to human H3. We do know that when in humans, avian H5 infections currently have a substantially different clinical picture than that of human-adapted H3 infections. Hence, even a perfect epidemiological model for human H3 might be a poor model for human H5. Nevertheless, a well-understood and accurate human H3 model would be extremely valuable in its own right and would be a good starting point for any reparameterization necessary for a human H5 epidemiological model once the characteristics of the virus are known.

As we focus attention and funds on preparedness for a pandemic, we must not lose sight of the necessity for medium-term and longer term investments to expand basic understanding of influenza viruses at the molecular, immunological, evolutionary, epidemiological, and ecological levels. Such increases in our basic understanding, some already within reach, will increase our options both to control seasonal influenza and our ability to predict and thus increase preparedness for the next influenza pandemic—regardless of whether it is imminent or many years away.

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PERSPECTIVE

Host Species Barriers to Influenza Virus Infections

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Most emerging infectious diseases in humans originate from animal reservoirs; to contain and eradicate these diseases we need to understand how and why some pathogens become capable of crossing host species barriers. Influenza virus illustrates the interaction of factors that limit the transmission and subsequent establishment of an infection in a novel host species. Influenza species barriers can be categorized into virus-host interactions occurring within individuals and host-host interactions, either within or between species, that affect transmission between individuals. Viral evolution can help surmount species barriers, principally by affecting virus-host interactions; however, evolving the capability for sustained transmission in a new host species represents a major adaptive challenge because the number of mutations required is often large.

The highly pathogenic avian influenza H5N1 virus is just one example of a zoonotic pathogen capable of transmission from animal reservoir species to humans (1). If we are to contain and eradicate such emerging infectious diseases (EIDs), we need to understand how and why some pathogens

become capable of infecting and being maintained in novel host species. Here, we review the interaction of factors that collectively limit the transmission of an infection from a donor host species to a recipient species and that constitute the host species barrier. We discuss these factors specifically as they apply to influenza, but the underlying principles apply to any EID.

The host species barrier is not a simple concept; the likelihood of a virus becoming endemic in a new host species depends on the interaction of three sets of processes (Fig. 1): interspecific interactions between hosts of the donor and recipient species, host-virus interactions within individual hosts of the recipient

species, and host-host interactions within the recipient species. For any type of species transfer, there must be sufficient contact between donor and recipient species and enough compatibility between the virus and the new host to allow replication and the possibility of transmission to other members of the recipient species. If this transmission can occur, the contact network structure of the recipient species, together with variations in transmission through this network, are critical in determining whether the virus will persist or die out. As the history of influenza pandemics and epidemics illustrates, viral evolution can help considerably in lowering the species barrier. However, we argue below that the relative rarity of successful species jumps testifies to the complex adaptations often required to achieve sustained transmission in a new species. We also review the components and evolutionary dynamics of the species barrier as they apply to influenza and then suggest areas for future work.

The Virus-Host Interaction: Within-Host Barriers

Cell entry-exit and receptor biology. For a virus shed by one host to infect another, it must breach entry barriers (e.g., mucus, alveolar macrophages, and epithelium) and find its way to tissues in which it can replicate. For example, chimpanzees are relatively resistant to experimental respiratory exposure to human influenza viruses, possibly because their respiratory tract secretions contain mucins that can specifically bind viruses before they reach airway epithelial cells (2). Once in appropriate

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tissues, a virus must attach to and enter cells before it can replicate. The specificity of receptor molecules governs virus entry into cells (3). For example, hemagglutinin molecules on the viral coats of avian influenza viruses preferentially bind to one form of molecule in the host cell membrane [sialic acid (SA)- α -2,3-Gal-terminated saccharides], whereas the hemagglutinins on human influenza viruses prefer another (SA- α -2,6-Gal-terminated saccharides) (4). This difference, together with the predominance of SA- α -2,6-Gal-terminated saccharides in the human trachea, may explain why replication of avian influenza viruses in humans generally tends to be restricted (5). The rare occurrences of fatal pneumonia in humans infected with the current H5N1 virus from Asia (6) and the H7N7 virus from the Netherlands in 2003 (7) are likely due to the ability of these viruses to attach to and replicate in lower respiratory tract cells, which do have SA- α -2,3-Gal-terminated saccharides (8, 9).

Replication and spread within tissues. Once it has entered a cell of the new host, the virus must successfully co-opt host cell processes to replicate there. Many avian influenza viruses can infect mouse cells but cannot replicate, frequently because amino acid residue 627 of the PB2 protein of the viral polymerase differs between avian and mammalian influenza viruses. In the avian virus, this residue is usually glutamic acid, whereas in mammalian influenza virus it is lysine (4), suggesting that PB2 residue 627 might be important in determining species range. In experimentally infected mice, a glutamic acid-to-lysine mutation at this position in the PB2 protein of H5N1 virus results in increased virulence and the ability to invade extrapulmonary organs (10). It is notable that both H5N1 virus from human patients in Asia (10) and H7N7 virus from a fatal human case in the Netherlands (7) possess a lysine at this site, suggesting rapid evolution within humans of a virus originating in poultry. Strikingly, though, and worryingly, lysine is the PB2 residue in H5N1 viruses isolated from wild waterfowl in mid-2005 from Qinghai Lake, in China (11, 12).

If a virus does succeed in replicating, it needs to be released from the host cell to infect more cells or be shed from the host. In influenza, progeny virus particles are bound to host cell sialosaccharides by their hemagglutinin. Viral neuraminidase cleaves these sialosaccharides, thus releasing newly produced virus from the cell surface. Like the respective hemagglutinins, neuraminidases from avian influenza viruses have a preference for SA- α -2,3-Gal-terminated saccharides, whereas those from many human influenza viruses prefer SA- α -2,6-Gal-terminated saccharides (4). Interestingly, the H2N2 virus that caused the 1957 pandemic initially retained a binding preference for “avian” SA- α -2,3-Gal-terminated

sialosaccharides but switched affinity to “human” SA- α -2,6-Gal-terminated saccharides during subsequent influenza seasons in the human population (13).

Even if progeny virus exits one host cell, host innate immune responses may hinder infection of other cells. Interferons may induce uninfected cells to enter an antiviral state that inhibits viral replication (4). To counter host responses, influenza virus has developed strategies for evading innate immunity: The viral NS1 polypeptide acts as an antagonist of interferon induction in infected cells by sequestration of double-stranded RNA or suppression of host posttranscriptional processing of mRNAs (4). NS1 also may help influenza viruses to replicate in interferon-treated cultured cells, as has been reported for H5N1 virus isolates from 1997 (14); whether currently isolated H5N1 viruses have retained this property remains to be determined.

Sometimes infection is restricted to particular tissues; in other cases, it can be systemic. For influenza virus to spread from the respiratory tract to other susceptible tissues, it needs to enter the lymph and/or blood system, be successfully transported, and exit at tissue-blood junctions (15). In poultry, whether infection is localized or systemic depends on the amino acid sequence at the cleavage site of the precursor hemagglutinin. This cleavage is required for the hemagglutinin to become fully functional. Low pathogenicity influenza viruses require extracellular proteases limited to the respiratory and gastrointestinal tracts to cleave the precursor hemagglutinin, whereas highly pathogenic avian influenza viruses have changes in the cleavage site that allow the precursor hemagglutinin to be processed by ubiquitous intracellular proteases, resulting in fatal systemic infection (4). In mammals, the viral factors de-

termining systemic infection are less clear, although cleavability of the precursor hemagglutinin plays an important role (10).

From their sites of replication, viruses need ultimately to be transmitted to new hosts. In general, dissemination of progeny viruses from the infected host occurs through shedding in respiratory, enteric, or urogenital secretions. Human influenza virus replicates mainly in the upper respiratory tract and is usually readily transmitted via droplets formed during coughing or sneezing (16). By contrast, the H5N1 influenza virus typically infects human cells in the lower respiratory tract (8, 9) and so may be less easily shed from the infected patient; this may partly explain why so far there has been little human-to-human transmission observed.

Host-Host Interactions: Cross-Species Contacts

Human population growth and consumption patterns are now reducing the magnitude of many geographical, environmental, or behavioral barriers that limit contact and potential virus transmission between donor and recipient species. The spread of viral diseases through international travel and trade is a major concern, although natural modes of spread can be important too. H5N1 virus was spread from Southeast Asia to Western Europe in 2005 and 2006, most likely by a combination of migratory wild birds (11, 12) and trade in poultry and poultry products. Even when donor and recipient species live in the same area, spread of infection from the one to the other can be hampered by differences in habitat use or by environmental barriers. The massive increase in poultry production in Southeast Asia has certainly reduced some of the obstacles to interspecific transmission of avian influenza virus.

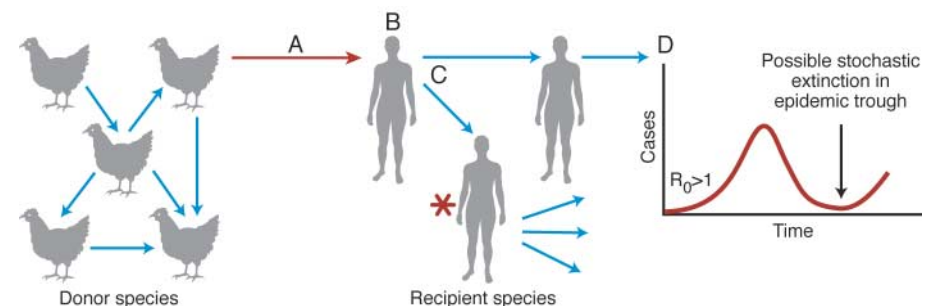


Fig. 1. Schematic illustrating phases in overcoming species barriers. **(A)** Interspecific host-host contact must allow transmission of virus from donor species to recipient species. **(B)** Virus-host interactions within an individual of recipient species affect the likelihood of the virus replicating and being shed sufficiently to infect another individual of recipient species. **(C)** Intraspetic host-host contact in recipient species must allow viral spread ($R_0 > 1$) in the presence of any preexisting immunity. Superspreader events (red asterisk) early in the transmission chain can help this process. **(D)** The pathogen must persist in the recipient species population even during epidemic troughs (after most susceptible individuals have had the disease) so that subsequent epidemics can be seeded: If few susceptibles are left, the virus may (stochastically) go extinct in epidemic troughs. Viral variation and evolution can aid invasion and persistence, particularly by affecting host-virus interactions.

Increasing numbers of poultry are kept in close proximity to wild waterfowl (17) and are brought into contact with other species at live animal markets (18). Even if two host species share the same geographical area and habitat, host behavior may limit pathogen transmission by, for instance, restricting infective contacts. Conversely, certain behaviors can predispose hosts to increased pathogen exposure. For example, consumption of raw poultry products resulted in fatal H5N1 virus infection in both humans and felids (19, 20), and racing greyhounds may have contracted equine

influenza virus A/H3N8 after being fed meat from infected horses (21).

Host-Host Interactions: Intraspecific Contacts in the Recipient Population

Assuming that a virus can be transmitted between individuals of the recipient host species, persistence of infection then depends on how it is spread through the host's contact network, and successful invasion depends on the network structure and the likelihood of particular individuals in the network transmitting the vi-

rus. The basic reproduction ratio of infection, R_0 , represents the number of secondary cases produced when an infected individual is introduced into a well-mixed local population of wholly susceptible individuals (22, 23). It is well established that, as the proportion of susceptibles in the population, s , drops (as individuals become infected, then recover), the number of secondary cases per infection, R , also drops: $R = sR_0$. If $R < 1$, as is currently the case for H5N1 virus in humans, an infection will not cause a major epidemic. But if R is even modestly greater than unity, a novel infection may spread locally, with potential for further spread in the absence of control (24).

For some novel infections that jump the species barriers, there is no preexisting immunity; however, preexisting immune protection can sometimes reduce the number of susceptibles, and hence R . For instance, humans who had previously encountered an influenza virus with the N2 neuraminidase may have been partially protected in the 1968 H3N2 pandemic that followed the global circulation of H2N2 viruses (25). There is also indirect evidence of short-term immunity between subtypes of influenza viruses (24, 26), which could play a role in the early spread of pandemics (24). In addition, cross-reactive T cells also may contribute to heterosubtypic immunity to influenza and reduce viral shedding (27).

The biological characteristics and evolutionary potential of the invading virus can also affect the longer term persistence of the infection. Acute immunizing infections are typified by initial steep increases in the number of infected individuals. This type of epidemic depletes the supply of susceptibles, leading to deep epidemic troughs (Fig. 1D) when local stochastic extinction of infection is possible, especially after a major epidemic typical of an invasion of a new host species. This effect creates a barrier to sustained transmission that applies especially to infections with strong, stable immunity, such as morbilliviruses (28); however, it is less of a handicap to infections such as influenza, in which the viruses can evolve quickly to overcome prevailing herd immunity (29). Teasing out what combination of immune escape and spatial dynamics allows interpandemic influenza to persist, despite its short infectious period and strongly seasonal transmission, is an important area for future work (29).

Overall, the likelihood of an EID persisting in a new species depends in a complex way on the population sizes and the degrees of mixing of donor and recipient host species as well as the values of R_0 in each (30). At every geographical level, whether local, regional, or global, the rate and the pattern of disease spread depend on the spatial distribution and mixing of the host population. Long-range spatial spread is in general facilitated by high

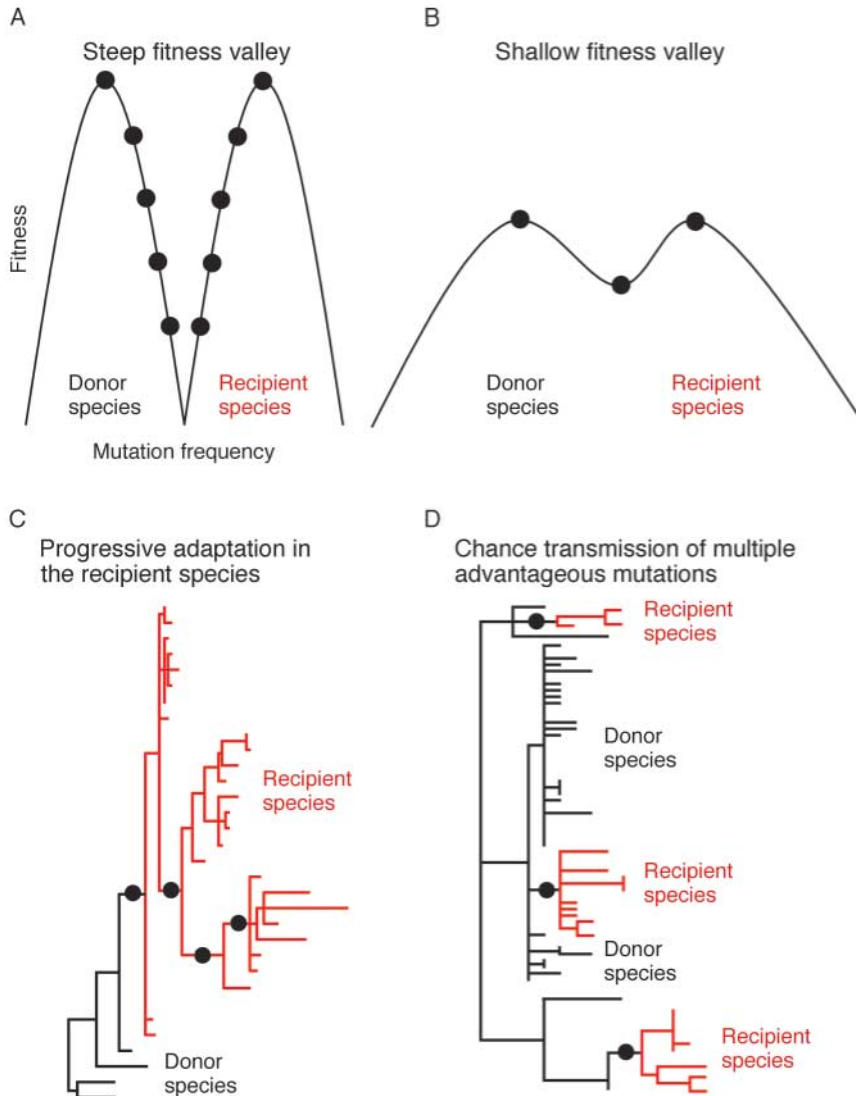


Fig. 2. Evolutionary models for the cross-species transmission of pathogens. (A) The donor and recipient species represent two distinct fitness peaks separated by a steep fitness valley. Multiple adaptive mutations (solid circles) are therefore required for the virus to successfully establish onward transmission in the recipient host species. (B) The donor and recipient species are separated by a far shallower fitness valley. This facilitates successful cross-species transmission because only a small number of advantageous mutations are required. (C) When multiple mutations are required for a virus to adapt to a new host, these may evolve progressively in the recipient species. However, this necessarily requires some onward transmission. (D) It is also possible that many of the mutations required to allow adaptation to a new host species were preexisting in the donor species and transmitted simultaneously. This will accelerate successful viral emergence.

infectivity, a long infectious period, and (at least in human influenza) a period of transmission before symptoms become apparent and quarantine measures can be taken. This contrasts with severe acute respiratory syndrome (SARS), in which the period of infectiousness begins with the onset of symptoms, allowing quarantine measures to be taken before maximum infectiousness is attained (31). The fate of an epidemic can also depend strongly on heterogeneities in R_0 , particularly on the role of “superspreaders” early in the epidemic (32). For example, some superspreader individuals may be more infective per contact for some reason; other superspreaders may not have higher per-contact transmission, but have many more contacts and therefore greatly multiply the rate of spread (22). If superspreaders become infected early in an outbreak, the epidemic is more likely to take off, which can have substantial implications for disease control (32).

The Role of Pathogen Evolution

In evolutionary terms and from the perspective of the pathogen, the host species barrier for infection can be thought of as a fitness valley lying between two distinct fitness peaks representing donor and recipient hosts, respectively (Fig. 2). The more mutations required for a virus to move between these peaks, the deeper the valley and the less likely that this can occur in a single step, particularly if adaptation involves changes at multiple loci, as in the case of avian influenza virus transmitting in human populations (33). Such a model has two important implications for our understanding of viral disease emergence.

First, the effective rate of virus adaptation is not simply determined by the overall rate at which mutations arise, but by the fitness of these mutations, particularly the proportion that are advantageous in multiple hosts. Hence, although RNA viruses often show prodigious levels of genetic variation, reflecting their high rates of mutation and immense intrahost population sizes (34), a large proportion of the mutations that arise within an individual viral population will be deleterious or slightly so (35). As a case in point, vector-borne RNA viruses seem to be characterized by a particularly high proportion of deleterious mutations because of the fitness trade-offs that are inherent in replication in hosts as distant as mammals and arthropods (36) and that thereby constitute a major constraint on their adaptability (23). Further, there is growing evidence that mutations, both advantageous and deleterious, often show complex epistatic interactions, which can also have major effects on the rate and the progress of adaptation (37).

Second, the critical parameters determining when successful onward transmission will occur include not only the time it takes to optimize fitness ($R_0 > 1$) in the new host species (38) but

also the probability that the recipient population is exposed to a viral strain that, by chance, already harbors several mutations required for successful onward transmission. For example, of the myriad influenza viruses produced by faulty replication within an individual, some, by chance, may possess those mutations that affect the receptor binding site to alter sialic acid binding capacity. This was highlighted recently (January 2006) in samples from a patient infected with H5N1 virus in Turkey. This individual had a mixed population of viruses, some of which expressed hemagglutinin with an amino acid sequence associated with an increased affinity for SA- α -2,6-Gal-terminated saccharides. Because intrahost genetic diversity has rarely been examined in RNA viruses, it is unclear how much adaptively important genetic variation rests within hosts.

Reassortment and recombination further complicate the picture. These processes will allow some viruses to acquire many of the key adaptive mutations in a single step and hence make a major jump in fitness space. However, whereas reassortment may allow viruses to traverse the adaptive landscape faster than through mutation alone, the optimal epistatic interactions among genes are likely to be broken by reassortment, and most reassortments, like most point mutations, are also expected to be largely deleterious.

Future Research Needs

Although there is a vast body of literature on influenza, only a few studies consider the barriers for influenza viruses to cross from one species to another. There are several important questions in this area that need to be addressed. Which genetic changes would allow the currently circulating H5N1 virus to acquire the characteristic to spread efficiently among humans? Such a study would require a combination of reverse genetics to generate potential virus candidates and a suitable animal model to simulate human-to-human transmission. If such a virus were to evolve, which factors at the population level would allow it to cause a pandemic? Investigating this requires epidemiological models that take into account not only the properties of the donor and recipient populations but also the characteristics of the newly emerged virus. What is the within-host diversity of influenza viruses, and does it include mutants that are able to replicate in a new host species? The current status of viral genome sequencing makes such studies possible, although studies of intrahost viral diversity are notable for their rarity. We have a detailed understanding of the replication cycle of influenza virus in a cell culture system, but what, at the tissue and organ level *in vivo*, are the barriers that limit human influenza virus to the respiratory tract while allowing H5N1 virus to cause systemic disease? To understand this requires a multidisciplinary approach based on a

combination of laboratory investigation and human clinical studies. Apart from the specific application to influenza virus, answering these questions will also result in a better understanding of the general principles that prevent viruses from jumping the species barrier.

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