Host Phylogeny Constrains Cross-Species Emergence and Establishment of Rabies Virus in Bats

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For RNA viruses, rapid viral evolution and the biological similarity of closely related host species have been proposed as key determinants of the occurrence and long-term outcome of cross-species transmission. Using a data set of hundreds of rabies viruses sampled from 23 North American bat species, we present a general framework to quantify per capita rates of cross-species transmission and reconstruct historical patterns of viral establishment in new host species using molecular sequence data. These estimates demonstrate diminishing frequencies of both cross-species transmission and host shifts with increasing phylogenetic distance between bat species. Evolutionary constraints on viral host range indicate that host species barriers may trump the intrinsic mutability of RNA viruses in determining the fate of emerging host-virus interactions.

In recent decades, cross-species transmission (CST) of RNA viruses has resulted in a range of disease emergence outcomes (*1*), from single infection "spillover" events such as rabies virus infections in humans (*2*), to transient outbreaks bound for extinction such as Nipah virus (*3*), to sustained epidemics with the potential for endemic establishment such as the SARS coronavirus (*4*). Although they are critical to anticipating the impact of viral emergence on human and animal health, the factors that determine the frequency and outcome of CST remain obscure. In RNA viruses, evidence for high mutation rates and occasional human epidemics originating from distantly related species have popularized the view that rapid evolution allows these viruses to overcome host-specific barriers in cellular, molecular, or immunological defenses (*5*). Consequently, it has been argued that RNA viruses emerge primarily between species with high contact rates (*6*–*8*). An alternative explanation posits that innate similarity in the defenses of closely related species may favor virus exchange by flattening the fitness valley that viruses traverse during adaptation to new hosts (*9*).

Identifying the most important determinants of viral emergence requires considering how the ecological dynamics of CST interact with evolutionary factors to shape replicated patterns of viral establishment in natural communities. Rabies, a ubiquitous, multihost viral zoonosis, provides this opportunity. In the United States, bats (*Chiroptera*) are the most common source of indigenously acquired human rabies infections, and approximately 2000 rabies-positive bats are collected annually after humans or domesticated animals have been exposed to them (*10*). Transmission occurs mainly by bat bite, and infection causes encephalitis with behavioral and motor abnormalities before death (*11*). The phylogeny of rabies virus in North American bats is structured by host species, reflecting an evolutionary history of host shifts followed by predominately within-species transmission (*12*, *13*). This species association of viral lineages enables identification of the species origins of relatively rare CST events from bats to humans or domesticated animals or within the bat community (*10*). Because North American bats span evolutionary divergences of approximately 3 million to 60 million years, a substantial range of ecological and physiological differences exists among species that might influence viral emergence (*14*).

We sequenced the nucleoprotein gene of 372 rabies viruses from 23 bat species collected across the continental United States over a 10-year period (Fig. 1A and table S1). Bayesian and maximum likelihood (ML) analyses (*15*) revealed 18 phylogenetic lineages of rabies virus that were statistically compartmentalized to particular bat taxa (Fig. 1B and table S2). New viral lineages were discovered in *Lasiurus intermedius floridanus* (LiV), *L. seminolus* (LsV), and *Myotis yumanensis* (MyV), establishing each as an independent rabies virus reservoir. The host-specificity of most viral lineages allowed us to infer the species origin of 360 infections in the data set after confirming the taxonomic identities of bats with mitochondrial DNA sequencing (table S3). Forty-three unambiguous CST events were observed, involving 15 bat species and 26 different species pairs. Nearly all viruses from cross-species infections were tightly nested within source clades and were no more genetically divergent than donor-lineage viruses (table S4), suggesting that they were more likely to be dead-end infections than infections occurring within stuttering chains of transmission in the recipient species (*15*).

We applied Markov chain Monte Carlo (MCMC) simulation to viral genetic data to compare four models of the strength and direction of CST between species pairs: symmetrical bidirectional transmission, asymmetrical bidirectional transmission, and each case of unidirectional transmission (*15*). Models selected by Akaike's information criterion were exclusively asymmetrical and predominately unidirectional (21 out of 26), suggesting unequal probability of infection for a given interspecific contact rate. Using parameters estimated from MCMC simulations (table S5), we quantified the expected number of infections in species i resulting from a single infected individual of species j (the per capita CST rate, R_{ij}) and visualized these in a "transmission web" (Fig. 2). Depending on species, a single rabid bat may infect between 0 and 1.9 heterospecifics, and on average, CST occurs once for every 72.8 within-species transmission events.

We next explored the intensity of CST between bat species pairs as a function of their ecological overlap (i.e., similarity in foraging behavior, roosting strategy, and body length), geographic range overlap, and phylogenetic relatedness, using host trait values estimated from our data and the literature (table S6). The intensity of R_{ij} declined continuously with the genetic distance between donor and recipient species and increased to a lesser extent with the amount of geographic overlap between species (Fig. 3A); however, our ecological proxies of interspecies contact failed to predict CST (tables S7 and S8). Results were robust to exclusion of several viruses for which the taxonomic identity of the host was based on morphology alone ($F_{2,25}$ = 9.38, $P < 0.001$; table S4 lists exclusions). Finally, a reanalysis of the transmission web using a novel metric (*15*) of connectance from food web theory (the proportion of realized interspecific connections in a food web) illustrated that rates of CST were highest to and from bat species that are sympatric with many closely related species but independent of the viral genetic diversity within the donor clade and the sampling effort for each bat species [supporting online text; $F_{2,13}$ = 12.24, coefficient of determination (r^2) = 0.67, P = 0.001]. These results suggest that initial infection of a new species is facilitated by evolutionary conservation of the cellular, immunological, or metabolic traits of hosts, with secondary effects of probabilistic factors, perhaps including exposures involving high viral load, that increase with species' range overlap.

In light of the host specificity implied by compartmentalization tests and phylogenetic analyses (table S2 and Fig. 1B), the high rates of CST shown here indicate that the vast majority of cross-species infections are evolutionary dead ends. Nevertheless, it is clear that rabies virus has successfully established itself repeatedly in North American bat species (*13*). This observation prompts the critical question of what determines whether CST causes a dead-end infection or sustained transmission in recipient species. We tested

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whether historical host shifts share a common phylogenetic constraint to present-day CST, using Bayesian ancestral state estimation of the host species origin of viral lineages (*15*). Nearly all (22 of 23) host shifts occurred between bat species that were more closely related than the median pair, and 66% of host shifts occurred within the top 25% of the most closely related North American bats, which is consistent with a lack of sustained transmission in distantly related species (Fig. 3B).

Phylogenetic signal in pathogen host range has been observed in fungal infections of heterospecific plants (*16*, *17*) and in a database study of parasite community similarity in wild primates (*18*). Although the consistency of host phylogeny as a predictor of emergence has been questioned for RNA viruses because of their potential for rapid within-host adaptation (*8*), sufficient data to test this hypothesis have been unavailable until now. Our study demonstrates that rapid evolution can be insufficient to overcome phylogenetic barriers at two crucial stages of viral emergence: initial infection and sustained transmission.

The decline in CST that we observed among more distantly related bat species might result from lower interspecific contact rates or a reduced probability of infection upon exposure. Although we could examine only a small number of species traits for which data were available, we found no effect of ecological proxies of interspecific contact on CST. This result is surprising given the infectiousness of rabies virus across mammals and abundant opportunities for CST among bats that share roosting and foraging sites. One explanation is that the disorientation and indiscriminate aggression caused by rabies infection (*11*) could limit the selectivity of interspecific contacts, causing their occurrence to depend on the frequency of host species sympatry. Our analysis supported both geographic overlap and host phylogenetic distance as strong predictors of CST. These two factors probably determine the frequency of exposure and the likelihood of infection after exposure, respectively.

Two explanations could account for the elevated frequency of host shifts among closely related bats. First, similarity in the biological barriers and social structure of closely related species could minimize the amount of evolution required to achieve an optimal balance of within-

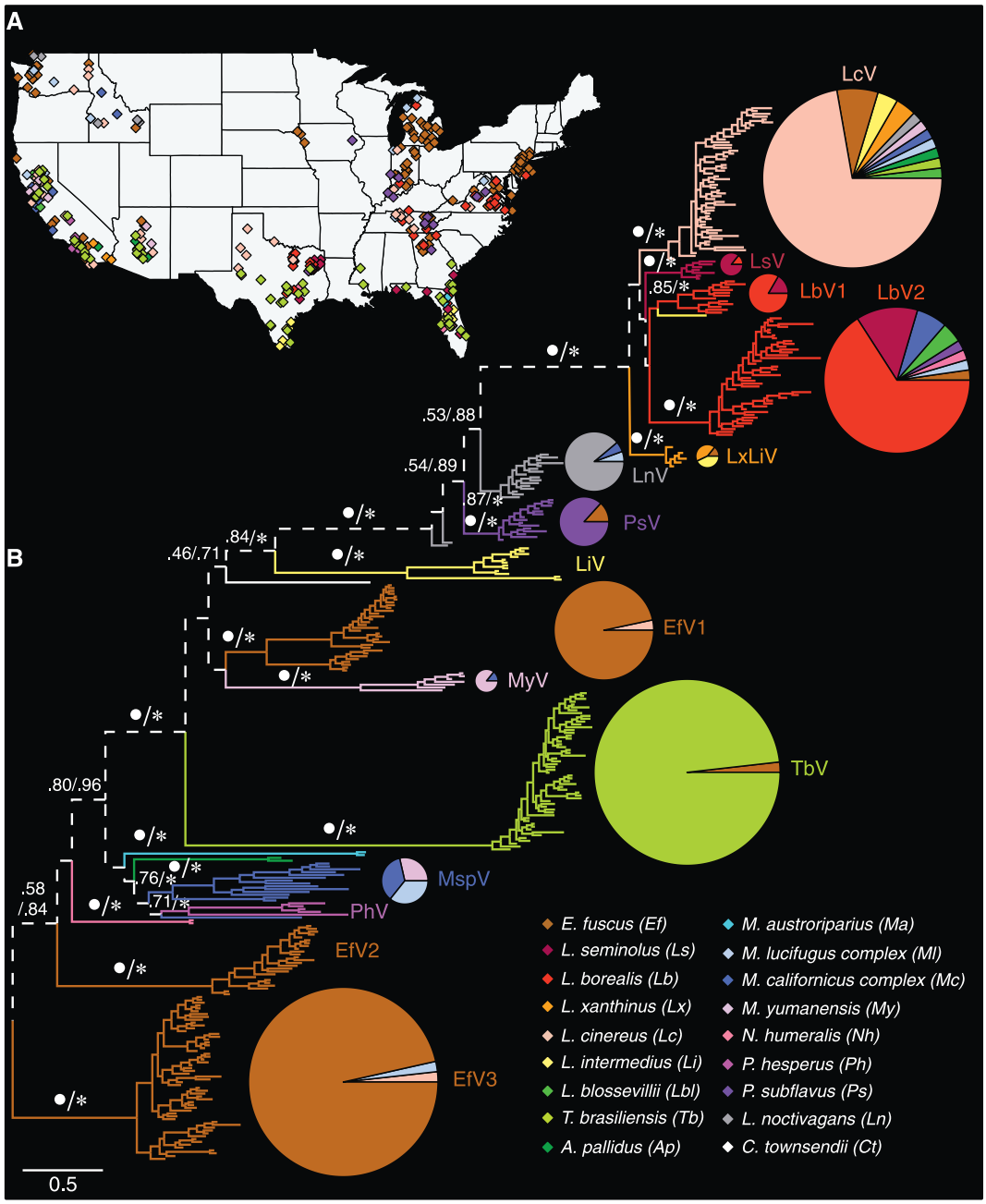

Fig. 1. Geographic origins, phylogenetic relationships, and host range of viral lineages. (**A**) Collection localities for 347 of 372 rabies virus samples; diamonds are jittered randomly to minimize overlap. (**B**) Bayesian phylogenetic tree with viral lineages labeled by donor host (table S3 contains full species names). MspV was associated with various *Myotis* species in the northwestern United States; LxLiV was associated with the western yellow bat (*L. xanthinus*) and the northern yellow bat (*L. i. intermedius*). Pie charts show the host species composition of lineages found in multiple species; the pie diameter is proportional to the number of bats sampled. ML bootstrap values (BVs) > 0.50 and Bayesian posterior probability (PP) values > 0.70 are shown to the lineage level (BV/PP). White circles are BV ≥ 0.90; asterisks are PP ≥ 0.98. The root branch has been removed for clarity; the dashed line indicates the trunk.

host replication and viral shedding (*9*). Although rabies virus uses evolutionarily conserved receptors for cell entry, receptor density on susceptible cells varies among species, leading to variable resistance to infection that maladapted viruses must overcome (*19*). Evolution of optimal virulence through modulation of transcription, gene expression, and replication might also be needed to balance entry into the central nervous system (ultimately leading to host death) with the timing and intensity of viral excretion from the salivary glands (necessary for transmission) to ensure sustained transmission within new host species (*20*). As a second limiting factor, even if the likelihood of viral establishment is independent of host phylogeny, viruses might still shift disproportionately between close relatives because of the greater frequency of CST. Although our results imply that rabies virus host shifts followed common rather than rare CST, the overwhelming support for host phylogenetic distance as the principal predictor of initial infection argues more strongly for intrinsic features of the host-virus interaction as the primary barrier to emergence.

The repeated failure of a notoriously generalist virus to colonize bat species that are capable of enzootic maintenance highlights the limitation of viral evolution to overcome host species barriers within a mammalian order. Similar effects could be critical determinants of the host range of other infections of public health or veterinary concern, such as lentiviruses in primates or morbilliviruses in carnivores. Nonetheless, the ultimate goal for predicting viral emergence is to understand drivers across varying taxonomic scales. Future studies of viral host range could examine whether the phylogenetic barriers that are evident at relatively shallow evolutionary distances dissipate for more distantly related taxa, where all emergence events might be equally improbable and driven by the frequency of interspecific contact.

Finally, we outlined a general framework to identify the origins of host shifts and quantify CST in complex multihost communities. A similar approach could be applied to any host-associated pathogen for which molecular sequence data are attainable. Quantification of per capita rates of pathogen transmission between species will be particularly useful to parameterize predictive models of viral emergence, which have traditionally ignored the process of CST despite its importance as the defining feature of zoonoses (*1*). Models incorporating such information will be

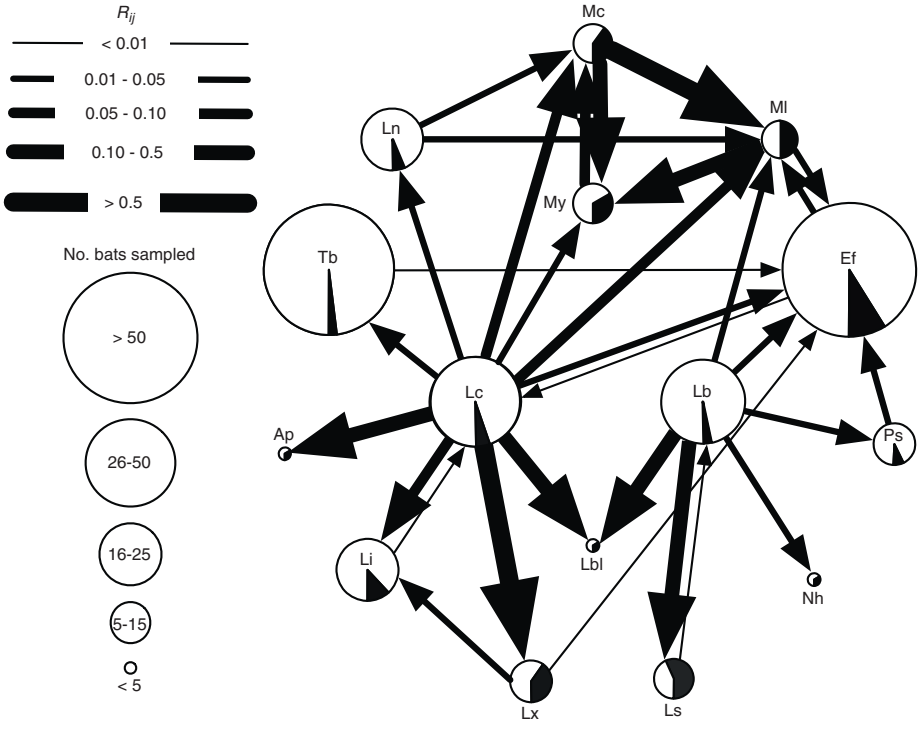

Fig. 2. Transmission web for 15 bat species. Pie charts describe the observed proportion of each species infected by CST. Arrows show the direction of transmission between species; the arrow width indicates per capita transmission rate (R_{ij}). Abbreviations for bat species names follow Fig. 1.

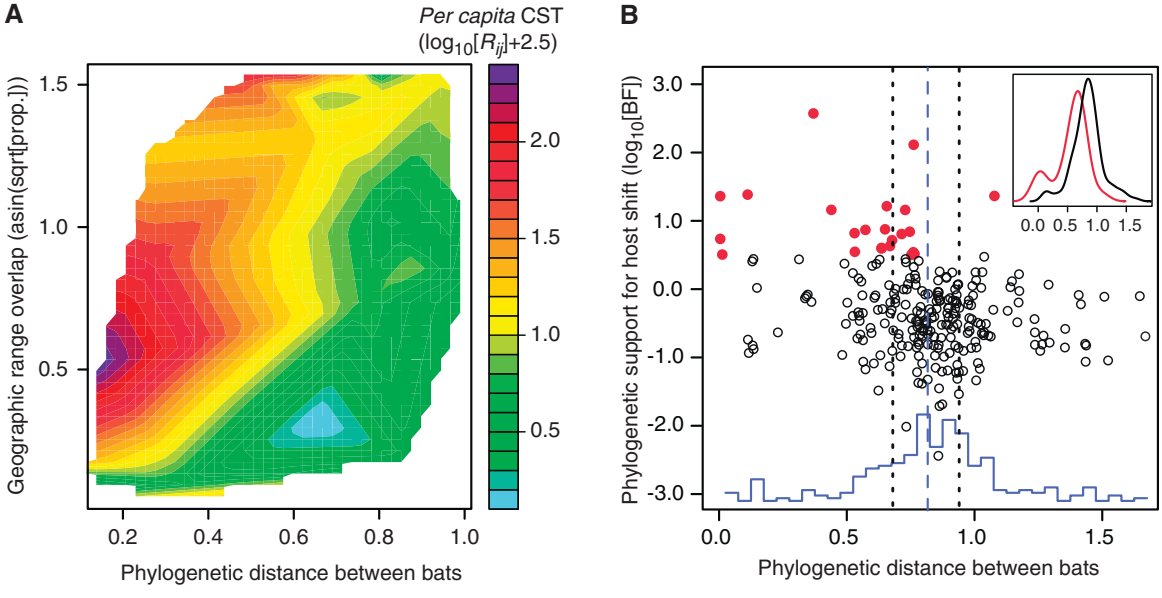

Fig. 3. Predictors of two stages of viral emergence. (**A**) Per capita CST declines with the genetic distance (substitutions per site) between bat species (slope = −3.93, $F_{1,28}$ = 21.89, P < 0.001) and increases with the proportion of their shared geographic range (slope = 1.21, $F_{1,28}$ = 8.22, P = 0.008; Full model: n = 31 bat species pairs, r^2 = 0.44; P < 0.001). Values for the contour plot were generated by bivariate interpolation. (**B**) The Bayes factor (BF) statistic describes the relative support for models containing versus lacking epidemiological linkages (i.e., historical host shifts) between each pair of viral lineages. Red circles indicate host shifts supported by ancestral state estimations (BF > 3), and open circles indicate host shifts that were inconsistent with phylogenetic data (BF < 3). Vertical lines show the median (blue) and lower and upper 25% limits (black) of the distribution of pairwise genetic distances between bats (blue histogram). Inset densities show the distributions of pairwise genetic distances for bat species implicated (red) or not implicated (black) in host shifts (*t* test for difference of means: t = 4.57, P < 0.0001).

critical to test the efficacy of specific disease prevention strategies applied not only within donor and recipient communities, but also in the realm where they intersect.

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Supporting Online Material

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Materials and Methods
SOM Text
Fig. S1
Tables S1 to S9
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An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species

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White-nose syndrome (WNS) is an emerging disease affecting hibernating bats in eastern North America that causes mass mortality and precipitous population declines in winter hibernacula. First discovered in 2006 in New York State, WNS is spreading rapidly across eastern North America and currently affects seven species. Mortality associated with WNS is causing a regional population collapse and is predicted to lead to regional extinction of the little brown myotis (*Myotis lucifugus*), previously one of the most common bat species in North America. Novel diseases can have serious impacts on naïve wildlife populations, which in turn can have substantial impacts on ecosystem integrity.

Emerging infectious diseases are increasingly recognized as direct and indirect agents of extinction of free-ranging wildlife (*1*–*4*). Introductions of disease into naïve wildlife populations have led to serious declines or local extinctions of different species in the past few decades, including amphibians from chytridiomycosis (*5*, *6*), rabbits from myxomatosis in the United Kingdom (*7*), Tasmanian devils from infectious cancer (*3*), and birds in North America from West Nile virus (*8*). Here we demonstrate that white-nose syndrome (WNS), an emerging infectious disease, is causing unprecedented mortality among hibernating bats in eastern North America and has caused a population collapse that is threatening regional extinction of the little brown myotis (*Myotis lucifugus*), a once widespread and common bat species.

WNS is associated with a newly described psychrophilic fungus (*Geomyces destructans*) that grows on exposed tissues of hibernating bats, apparently causing premature arousals, aberrant behavior, and premature loss of critical fat reserves (*9*, *10*) (Fig. 1). The origin of WNS and its putative pathogen, *G. destructans*, is uncertain (*9*). A plausible hypothesis for the origin of this disease in North America is introduction via human trade or travel from Europe, based on recent evidence that *G. destructans* has been observed on at least one hibernating bat species in Europe (*11*). Anthropogenic spread of invasive pathogens in wildlife and domestic animal populations, so-called pathogen pollution, poses substantial threats to biodiversity and ecosystem integrity and is of major concern in conservation efforts (*1*, *2*).

WNS has spread rapidly and now occurs throughout the northeastern and mid-Atlantic regions in the United States and in Ontario and Québec provinces in Canada and currently affects at least seven species of hibernating bats (Fig. 2). Many species of bats in temperate North America hibernate in caves and mines (*12*) in aggregations of up to half a million individuals in a single cave (*13*). In late spring, these winter aggregations typically disperse into smaller sex-segregated groups of conspecifics, when adult females form maternity colonies and adult males mostly roost alone (*14*, *15*). From August to October, females and males assemble at hibernacula or swarming sites to mate before hibernating (*16*, *17*). The mechanisms for the persistence and transmission of *G. destructans* during summer and fall months are unknown, but spread of the fungus to new geographic regions and to other species may result from social and spatial mixing of individuals across space and time.

During the past 4 years, WNS has been confirmed in at least 115 bat hibernacula in the United States and Canada and has spread over 1200 km from Howe Cave near Albany, New York, where it was first observed in February

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