

domains at the surface of suspended cells maintained Rac1 localization at the plasma membrane (Fig. 4A) and PAK activation (Fig. 4B). Control TfR-beads bound to cells but had no effect. CTxB-beads had no effect on Rac1 GTP loading (fig. S5B). Additionally, no integrin β 1 staining was detected at the bead surface (fig. S5C). These experiments demonstrate that loss of G_{MI} -containing domains from the cell surface in suspended cells is required for the loss of Rac1 targeting and subsequent effector activation.

Rac1 association with the plasma membrane and activation of effectors require membrane binding sites that are controlled by integrins (3, 4). These binding sites are components of cholesterol-rich membrane domains. Integrin-mediated adhesion maintains membrane domains at the plasma membrane. When cells are detached, domains are cleared from the cell surface through internalization. Preventing internalization maintains Rac1 plasma membrane localization and Rac1 signaling in suspended cells. Although selectivity of Rac1 for membrane domains is unexpectedly determined to some extent by the state of the lipids themselves, it is unlikely that lipids alone completely account for this effect. This effect may also provide a means by which adhesion can influence many growth factor pathways that are dependent on integrins (1) to confer anchorage dependence of growth. Local regulation of membrane domains by integrins may explain their ability to locally regulate Rac1 targeting (4) and to recruit many signaling proteins thought to associate with domains (23). Regulation of Rac1 localization by integrins is likely to be important for cell migration and polarity in many systems where precise spatiotemporal control of guanosine triphosphatase function is crucial. Although fibroblasts provide a good model for integrin signaling in anchorage-dependent cells, Rac1 binding sites in specific membrane domains may diverge between epithelial, mesenchymal, and hematopoietic cells, where Rac1 function can also differ (24).

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Large Shifts in Pathogen Virulence Relate to Host Population Structure

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Theory on the evolution of virulence generally predicts selection for an optimal level of virulence determined by trade-offs with transmission and/or recovery. Here we consider the evolution of pathogen virulence in hosts who acquire long-lived immunity and live in a spatially structured population. We show theoretically that large shifts in virulence may occur in pathogen populations as a result of a bistability in evolutionary dynamics caused by the local contact or social population structure of the host. This model provides an explanation for the rapid emergence of the highly virulent strains of rabbit hemorrhagic disease virus.

Over the past 30 years, emerging diseases have caused unexpected and, in some localities, significant human mortality (1). This increase in the prevalence of novel diseases has generally been associated with anthropogenic changes of the environment, such as a change in farming practices or urbanization, as well as the zoonotic transfer of pathogens from wildlife to humans (2). However, there is increasing concern that some pathogens may emerge as a consequence of evolutionary changes in virulence. Here we show that rapid evolution of virulence can occur as a consequence of bistability in the evolutionary dynamics of pathogens associated with changes in host social structure. Evidence from molecular epidemiology studies leads us to suppose that this may have occurred in the emergence of the virulent pathogen rabbit hemorrhagic disease virus (RHDV).

General theory on the evolution of virulence (the death rate due to infection) is focused on the maximization of the epidemiological basic reproductive number of the pathogen R_0 (3); single infections in completely mixed host populations should evolve in a manner that maximizes R_0 . In turn, this suggests that the evolutionarily stable (ES) transmission rate will be the maximum possible, the recovery rate the lowest possible, and the ES virulence the minimum possible. Virulence can, therefore, be seen as a consequence of trade-offs with transmission and recovery, due to underlying mechanisms associated with factors such as pathogen replication rates (4). Specifically, a finite ES virulence will occur when fitness benefits to the parasite in terms of increased transmission or decreased recovery rates become increasingly costly in terms of increased virulence. Evolution toward an evolutionarily stable strategy (ESS) with higher virulence (determined by a trade-off relationship) would typically be gradual, and we would not expect the rapid emergence of a highly virulent strain.

One important assumption within these classical virulence models is that the host population is free-mixing, whereas in nature hosts typically live in spatially structured

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populations with transmission events occurring locally. Previous work has shown that this population structure is important in the evolution of disease virulence because it sets a limit on the transmission rates of pathogens even without a trade-off with virulence (5, 6), and when such constraints are incorporated we find that local infection processes tend to favor less virulent pathogens (7). However, this spatial theory of the evolution of virulence does not include the consequences of acquired immunity (5–7), which may be particularly important because immune individuals will tend to cluster around infectious ones, and this will have important implications for the invasion of recently evolved pathogen strains and their relative fitness. At a simple level, a pathogen that transmits very quickly may produce local aggregations of not only infected individuals but also immune ones, which will affect the local spread of the disease and the availability of space for the recruitment of susceptible individuals.

To determine the implication of immune (recovered) individuals to the evolution of the pathogen, we build on the existing theory that includes the population structure of susceptible and infected hosts (5–7). We developed a generic susceptible, infected, and recovered (SIR) evolutionary model that examined different degrees of host population structure by varying the proportion of the interactions that occur locally (7). This approach simply captures some key features of the complex variation in spatial structure observed in most vertebrate species, including humans (8). The population structure is modeled using an individual-based, regular lattice where each location may be empty or occupied by an individual in one of three states: susceptible (S), infected (I), or recovered (R) [supporting online material (SOM) Text]. Infection and reproduction can occur locally or globally, and by altering the proportion of these processes we can simulate population structures that vary from freely mixed populations of the classical theory to a rigid spatial population in which all the interactions are between nearest neighbors. We simulated the evolution of pathogens in this model by considering a range of different pathogen strains with different levels of virulence and recovery rates determined by a trade-off relationship. Mutation can occur between the different strains, and the simulations reveal the outcome of evolution over a number of generations.

By including spatial structure and acquired immunity in our model, we find the existence of bistability with avirulent and highly virulent strains, over a wide range of population structures (Fig. 1). The bistability occurs with reasonable parameter estimates with an accelerating trade-off (as virulence increases, recovery

becomes increasingly more difficult, as might be expected) between the virulence (A) and the recovery rate (G). The basic reproductive ratio of the pathogen strain is independent of the case mortality α , such that if there is no trade-off then $R_0 = \beta/(d + \gamma)$. However, with the trade-off assumed in Fig. 1, R_0 attains the maximum ($R_{0|\alpha=0} = 9.09$) by minimizing the case mortality (the lowest R_0 is attained by the lethal pathogen, $R_{0|\alpha=1} = 4.76$). This corresponds to the simulation results for when all the interactions are global, in which the minimum case mortality is globally evolutionarily stable. Once spatial structure is included, such that some of the interactions are local, very strong case mortality can also be locally evolutionarily stable.

The bistability arises because the avirulent strain is favored in a dense viscous population,

whereas the virulent strain is favored in a more open one. Higher virulence may favor a pathogen strain in a sparse population because killing the host prevents immune individuals from blocking the spread of the strain. Once a highly virulent strain is established in a sparse population, a less virulent strain will tend to block itself by producing immune individuals and therefore not replace the resident. In contrast in a dense population, many individuals are already immune due to recovery from avirulent parasite strains. A more virulent strain invading such a population would kill itself but still be blocked by immune individuals created by other strains and, therefore, would not invade. Before any particular population structure is established, an initially virulent strain will tend to create a sparse population due to the mortal-

Fig. 1. An illustration of the evolutionary bistability in pathogen virulence expressed as case mortality over a range of different population structures. The points represent locally evolutionarily stable case mortalities, and the degree of spatial structure is represented as the log of the proportion of global interactions. The arrows indicate the direction of evolution of the pathogen after local mutation. There are 101 pathogen strains with the case mortalities equally divided between the harmless ($\alpha = 0$) and the lethal ($\alpha = 1$). The trade-off between the mean infectious period $1/\gamma$ and the case mortality α is $\gamma = 0.5 + 0.5\alpha$. The mutation rate in the case mortality between adjacent pathogen strains is $u = 0.5$. The mean case mortality at the evolutionary equilibrium is plotted as a function of the fraction P_{Global} of global transmission and reproduction. Host reproductive rate, $r = 5$; natural mortality, $d = 0.05$; transmission rate, $\beta = 5$. The trade-off between the recovery rate $G = \gamma(1 - \alpha)$ and the virulence $A = \gamma\alpha$ is $G + A = \gamma = 0.5 + 0.5A/(G + A)$.

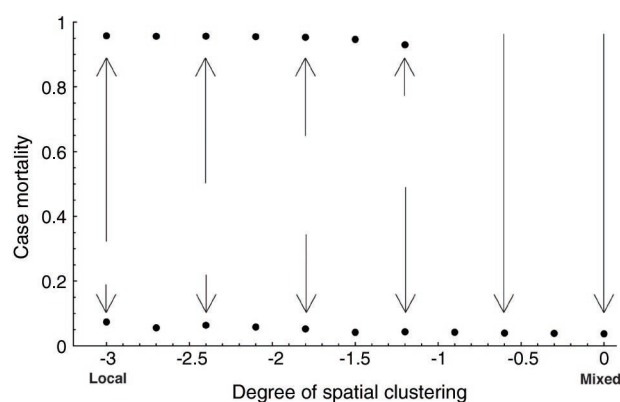
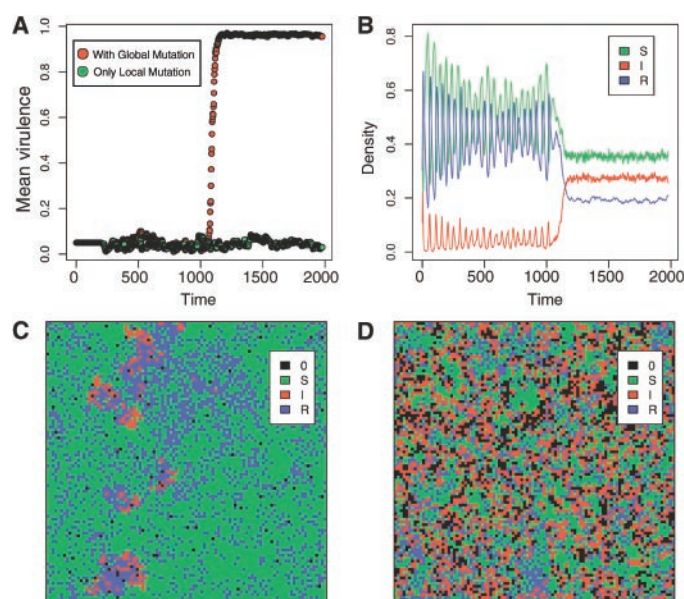


Fig. 2. The temporal and spatial dynamics of the jump from low to high virulence that can occur with global mutation. (A) The evolution of virulence through time in a region of bistability with an established parasite population with low virulence with and without a low (0.0002) frequency of global mutation (occurring at random across the whole virulence space). With the large mutations, there is a shift to the higher virulence. (B) The demographic structure of the population before and after the change, illustrating the differences in susceptible (S), infected (I), and recovered (R) densities in the two states while (o) represents empty space. The spatial structure of the population (C) before and (D) after the shift in virulence illustrates the sparser population that is created, which then stabilizes the highly virulent strain. Other parameters are the same as in Fig. 1.



ity it causes and, therefore, favor even more virulent strains. Conversely, relatively avirulent strains will tend to maintain viscous populations, thereby selecting for less virulence. Because both states are locally evolutionarily stable, the pathogen evolves to either low or high virulence, depending on the initial virulence.

If a change to a much higher virulence occurs in a parasite in a population where there are susceptible hosts, the virulent strain creates vacant space as it spreads through the population, owing to its high mortality. This sparser population structure would then favor the more virulent strain. A large change in virulence, which may be generated through, for example, recombination, along with the availability of susceptible hosts would therefore allow the pathogen population to move rapidly from one evolutionarily stable state to the other (Fig. 2). Because major changes in virulence are often likely to be associated with an antigenic shift, new highly virulent strains will often meet populations where there are many individuals that, although immune to the resident strain, are susceptible to it.

Obtaining empirical information on evolutionary changes in virulence is notoriously difficult and usually requires detailed long-term studies. However, there is evidence to suppose that RHDV has shown sudden shifts in virulence and that both host and pathogen exhibit characteristics which fit the models presented here. Highly virulent RHDV emerged on an aeroplane of domestic rabbits that were flown from Germany to China in 1984 (9). The virus spread through captive rabbit populations, causing massive mortality, initially in the Far East but then throughout much of Europe, and also invaded the free-living populations of rabbits, causing widespread and massive mortality in Australia, New Zealand, and some European countries. The impact caused by this virulent strain was unprecedented and would no doubt have been recorded in domestic rabbits had it occurred previously. Interestingly, recent molecular epidemiological research has shown that antibody to RHDV and nucleotide sequences of the capsid gene were present in healthy rabbits back to 1955 in the United Kingdom and many years before the appearance of the virulent strain (10). Assessment of comparative sequence data and phylogenies that examined tree congruency supported by boot-scanning analysis provides good evidence of recombination amongst strains of RHDV and provides a parsimonious explanation for the sudden appearance of virulent RHDV in 1984 (11). As such, RHDV is a highly virulent disease that emerged rapidly from what appears to be one or more avirulent strains and is a possible example of a pathogen switching from a locally evolutionarily stable, relatively avirulent state to a highly virulent state. Furthermore rabbits, in both domestic and natural conditions, live in

highly structured social populations that our model identified could have provided the conditions suitable for a bistability in virulence. Without bistability, a very different strain with such high virulence brought about by recombination should be selected against and lost.

Given the increasing long-distance movement of people and domestic animals around the modern world, our results have important implications for emerging diseases in general. Recombination amongst avirulent (and therefore possibly previously undetected) strains of viruses and other pathogens may produce new virulent strains that may spread through vertebrate host populations because they have shifted to a new evolutionarily stable state.

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In Vivo Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2

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MDM2 binds the p53 tumor suppressor protein with high affinity and negatively modulates its transcriptional activity and stability. Overexpression of MDM2, found in many human tumors, effectively impairs p53 function. Inhibition of MDM2-p53 interaction can stabilize p53 and may offer a novel strategy for cancer therapy. Here, we identify potent and selective small-molecule antagonists of MDM2 and confirm their mode of action through the crystal structures of complexes. These compounds bind MDM2 in the p53-binding pocket and activate the p53 pathway in cancer cells, leading to cell cycle arrest, apoptosis, and growth inhibition of human tumor xenografts in nude mice.

The tumor suppressor p53 is a potent transcription factor that controls a major pathway protecting cells from malignant transformation (1, 2). As such, it is the most frequently inactivated protein in human cancer (3). In response to stress, the cellular level of p53 is elevated by a posttranslational mechanism, leading to cell cycle arrest or apoptosis. Under nonstressed conditions, p53 is tightly controlled by the MDM2 protein through an autoregulatory feedback loop (4–8). p53 can

activate MDM2 expression which, in turn, leads to the repression of p53 by three mechanisms. First, MDM2 binds p53 at its transactivation domain and blocks its ability to activate transcription. Second, it is involved in the nuclear export of p53. Third, MDM2 serves as a ubiquitin ligase that promotes p53 degradation (9).

The *mdm2* gene has been found amplified or overexpressed in many human malignancies (10, 11). Therefore, activation of the p53 pathway through inhibition of MDM2 has been proposed as a novel therapeutic strategy (12–14). Several recent studies have shown that disruption of the p53-MDM2 interaction by different macromolecular approaches or by the suppression of MDM2 expression can lead to the activation of p53 and tumor growth inhibition

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