# An exploration of viral evolution following zoonosis with suggestions for avoiding future pandemics

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## Abstract

The significant death toll from major viral outbreaks during the past century highlights the importance of understanding the factors that determine whether zoonotic spillovers escalate into full-blown human epidemics. Hundreds of thousands of humans are made sick by animal-derived viruses such as Lassa, Nipah, Marburg, and Ebola every year. This includes thousands of fatalities. Moreover, many viruses, including the four pathogens mentioned above, exhibit human-to-human spread. Given this information, an explanation is needed for why human viral pandemics caused by animal viruses are relatively rare. This review proposes that variations in the fidelity of viral replication play an important role and that low-fidelity replication creates a barrier to extended human-to-human transmission. After a zoonotic transfer to humans, a virus will be subject to unusually strong selection, and full adaptation to the new host would typically require multiple mutations. A rapid route for accumulating such mutations involves minor alterations to the virus's polymerase that increase its mutation frequency, allowing more rapid adaptation. I refer to this route as the mutation-induced enhanced mutability pathway (MIEMP). However, the cost of this route may be the loss of the virus's long-term viability when harmful mutations accumulate. Recombination with a strain with a high-fidelity polymerase could, however, restore fidelity and longevity. This would typically require another virus jump from the original animal reservoir to an infected human. We might expect the MIEMP pathway to be active after zoonosis and also when a virus is subjected to strong selective pressures. There is some evidence from the COVID-19 pandemic to support this scenario: firstly, COVID-19 epidemics in several countries showed rapid rises followed by sudden collapses. Some lineages appeared to lose viability throughout large geographical areas very suddenly, which may have been a result of low fidelity. This is compatible with a MIEMP origin. Secondly, several SARS-CoV-2 variants, including the Omicron variants, appeared with a jump in the number of mutations compared to previous lineages, which is compatible with MIEMP followed by recombination. The same variants also had many mutations in the Spike gene but fewer in the rest of the genome. Moreover, an anomalously low proportion of the mutations in the Spike of Omicron (and other variants) were C-to-T nucleotide "transitions." Many coronaviruses have an excess of C-to-T transitions (often caused by host modifications of viral RNA). In contrast, low-fidelity polymerases are expected to generate all nucleotide exchanges randomly. This mutational pattern, which is otherwise difficult to explain, is therefore consistent with recombination events, where much of the right-hand-end "structural protein" sections of the genomes of these variants, including the Spike genes, came from error-prone partners, while much of the non-structural protein sections, including the polymerases, came from high-fidelity partners. These conclusions lead to recommendations for avoiding conditions that might allow dangerous recombination between well-adapted strains and high-fidelity spillovers.

## Introduction

In the last 100 years, seven pandemics caused by viruses have killed more than 10,000 people. These were 1957/58 Asian flu, 1968/69 Hong Kong flu, 1977 Russian flu, HIV/AIDS (1981-present), 2009 swine flu, the 2013-16 Ebola outbreak, and COVID-19. Except for Russian flu, which is thought to

have reached the public via a laboratory accident or live-vaccine trial escape, all involved animal viruses. Some were spillovers from animals to humans (HIV/AIDS and Ebola), while in other cases (Hong Kong, Asian and swine flu) animal viruses recombined with pre-existing human viruses. The details of the origin of COVID-19 are unclear, but one or more bat viruses were involved [Balloux et al. 2022]. Numerous animal viruses have been observed to spill over to humans without causing major epidemics. Several can cause hemorrhagic fevers that are often fatal, including the Lassa, Lujo, Nipah, Marburg, Ebola, Bolivian, Crimean-Congo, Omsk, and Rift Valley hemorrhagic fevers. At least the first five in this list have been seen to spread from person to person. Some of these cause hundreds or thousands of deaths each year. For example, Lassa fever is caused by a virus spread to humans in the urine and feces of the multimammate mouse, which is present in and around villages in West Africa. Roughly 80% of infections are asymptomatic or mild, but a 2005 study found the virus caused around 400,000 illnesses every year, with 5,000 deaths. Since hundreds of thousands of individuals are infected by animal viruses every year, and since human-to-human transmission is observed, the reason why so few pandemics emerge is unclear. This review suggests an explanation that is compatible with current scientific observations.

## Viral adaptation

In the medium term, all biological entities need relatively low mutation rates, because excessive mutation would result in fatal "error catastrophes." For example, it has been suggested that coronaviruses require their complex RNA polymerase assemblies, which include an unusual error-correction function, to maintain their extra-large RNA genomes [Minskaia et al., 2006]. The mutation rate of SARS-CoV-2 is estimated to be $10^{-5}$ to $10^{-4}$ substitutions per base per transmission event [Van Egeran et al., 2021]. However, if one or more deleterious mutations in the polymerase complex were to reduce fidelity to, say, $1\times10^{-3}$, the higher mutation rate would not be fatal immediately because harmful mutations would take time to accumulate. On the other hand, a virus that begins to replicate in a new host would experience strong selective pressures, and a higher mutation rate would allow it to adapt to its new environment more rapidly than a virus with a standard polymerase.

Figure 1 shows a simple thought experiment, featuring a hypothetical virus with seven genes as it adapts to a new host, such as humankind after a zoonotic spillover. The model shown suggests that the second option (Panel B), the mutation-induced enhanced mutability pathway (MIEMP), is likely to provide more rapid adaptation, but the cost would be reduced replicative fidelity. However, in the event of repeated spillovers of viruses from the original to the new host, a recombination event (Panel C) might establish a well-adapted high-fidelity strain, creating a lineage with pandemic potential. A graphical view of the same process, focussing on how mutations might accumulate over time, is shown in Figure 2.

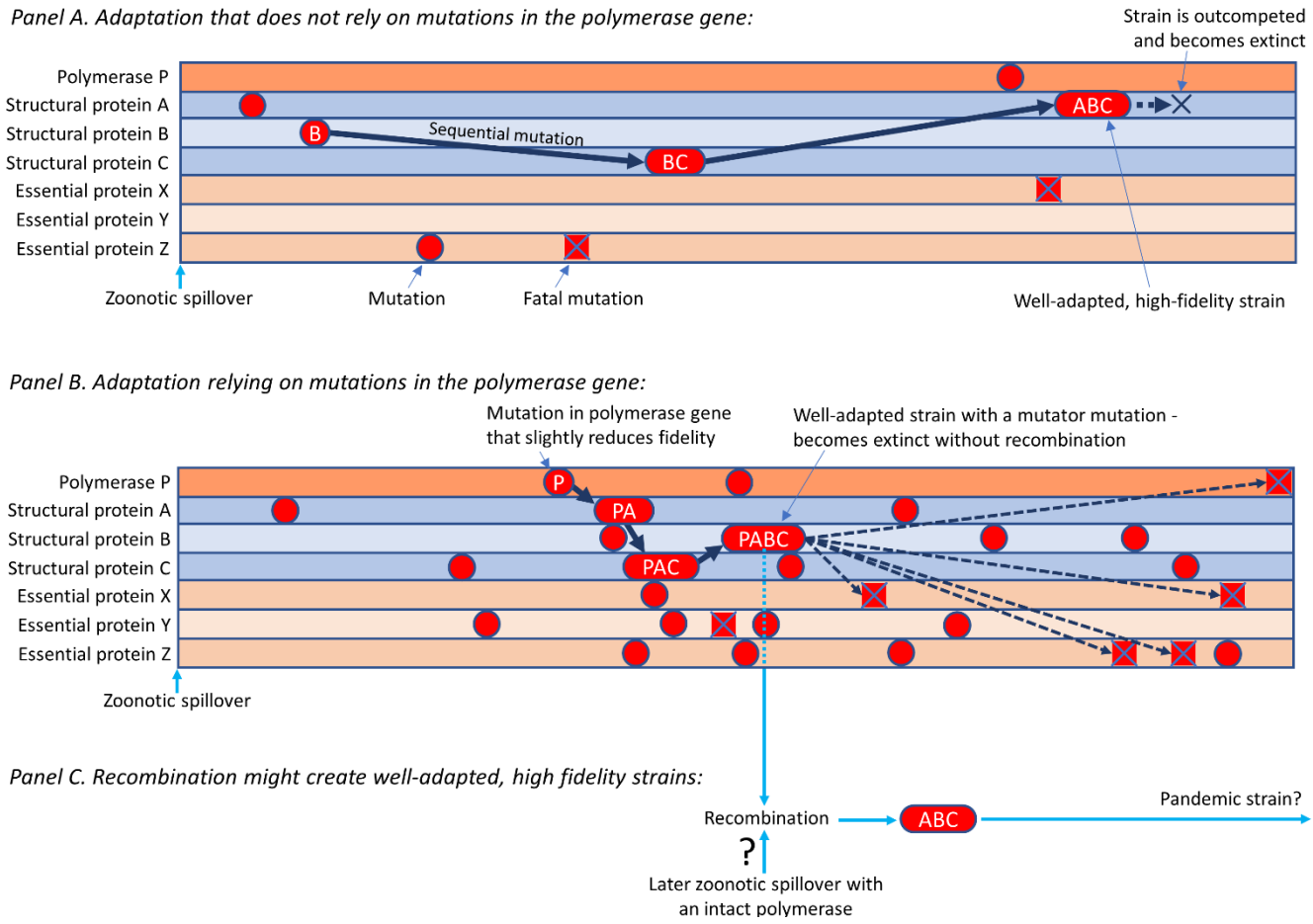


Figure 1. *Conceptual diagram illustrating the suggested role of mutator mutations in facilitating the emergence of well-adapted viral strains and the potential for pandemic strains to arise via recombination.* The model considers a hypothetical virus after a spillover. The virus comprises an RNA or DNA polymerase enzyme, three structural proteins, and three additional proteins that are essential for the virus's replication and assembly. In the figure, circles indicate mutations, and solid arrows sequential mutations within single lineages. Dotted arrows with crosses indicate fatal mutations. Assuming mutations in all three structural proteins are required for adaptation to a new host (such as humankind), two distinct adaptive mechanisms can be envisaged: (1) in a slower, high-fidelity pathway (Panel A), a series of successive mutations occur, delivering the required alterations while maintaining the integrity of the polymerase. (2) In a mutation-induced enhanced mutability pathway (Panel B), a mutation in the polymerase gene first triggers an increase in the viral mutation rate, perhaps by an order of magnitude, resulting in the earlier appearance of beneficial mutations. This mutation, while expedient, results in a virus with lower replication fidelity, which may be unstable and incapable of starting a major epidemic. However, in scenarios where repeated zoonotic spillovers occur, the well-adapted viral strain from the second pathway might acquire a high-fidelity polymerase by recombination, as visualized in Panel C.

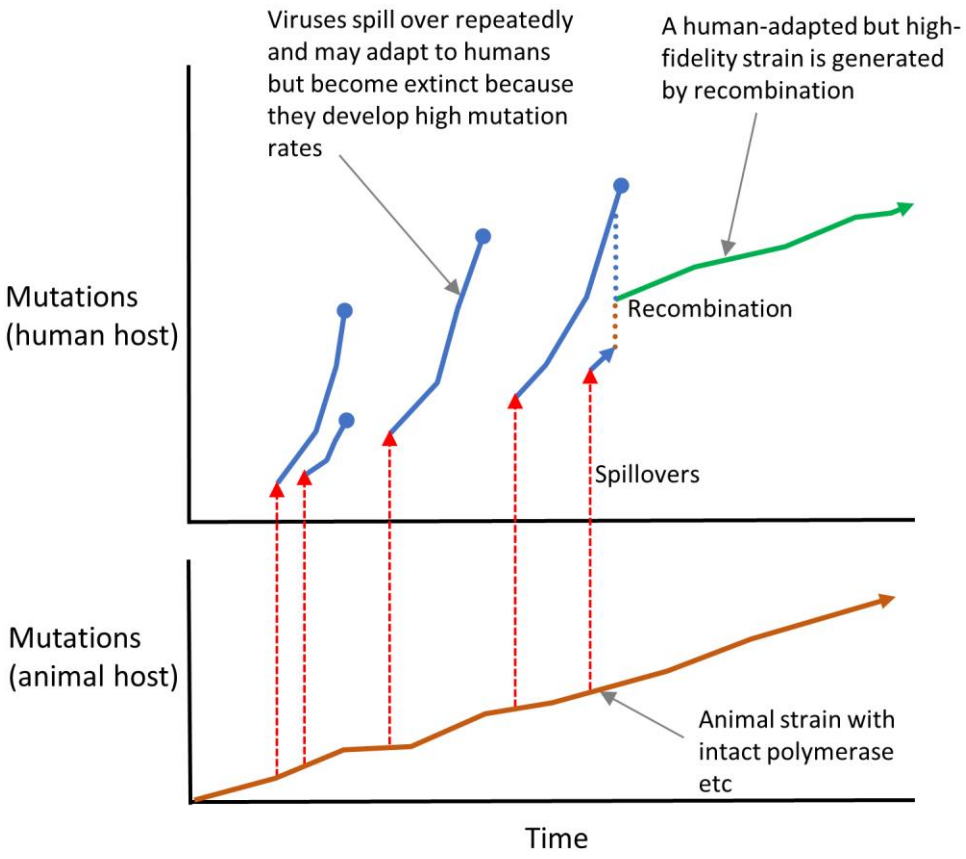


Figure 2. *A schematic view of how a MIEMP adaptation route followed by recombination might generate stable lineages in the event of repeated zoonotic spillovers.* An animal virus is shown that slowly accumulates mutations as it adapts to its environment and evades its host's immune response (brown line). Suppose several viruses now spill over to a new host, such as humankind, and begin to spread. In that case, they immediately experience increased selective pressure (because a greater proportion of random mutations are now beneficial), and they can benefit in the short term from a higher mutation rate. Therefore, they tend to adapt by the MIEMP route (blue lines). However, their higher mutation rate usually makes these lineages unstable, resulting in their extinction. In conditions that allow repeated zoonotic spillovers, however, a high-fidelity lineage and a well-adapted one may co-infect a single human, allowing recombination and the generation of a stable lineage with pandemic potential (green line).

## Comparison with prokaryotes

Viruses are relatively simple entities. Bacteria and archaea are more complex and, therefore, capable of more complex responses. The mutation rate of the bacterium E. coli was shown to increase when it was subjected to nutritional stress by limiting phosphate, carbon, or iron [Maharjan & Ferenci, 2017]. This raises the intriguing possibility that bacteria may possess mechanisms that rapidly but temporarily increase their mutation rate in response to stress so that they can "evolve their way out of trouble" using the MIEMP route. In principle, this effect might be reversible – when the stress is removed, the mutation rate might return to normal (without alteration of the genes encoding polymerases and related proteins). Further studies are needed. (Experiments with lineages that pass through repeated bottlenecks of single individuals might be necessary to distinguish reversible fluctuations from the natural selection of genotypes affecting fidelity at the population level following recombination.) Such mechanisms are not expected to be available to viruses, highlighting the importance of recombination to restore fidelity.

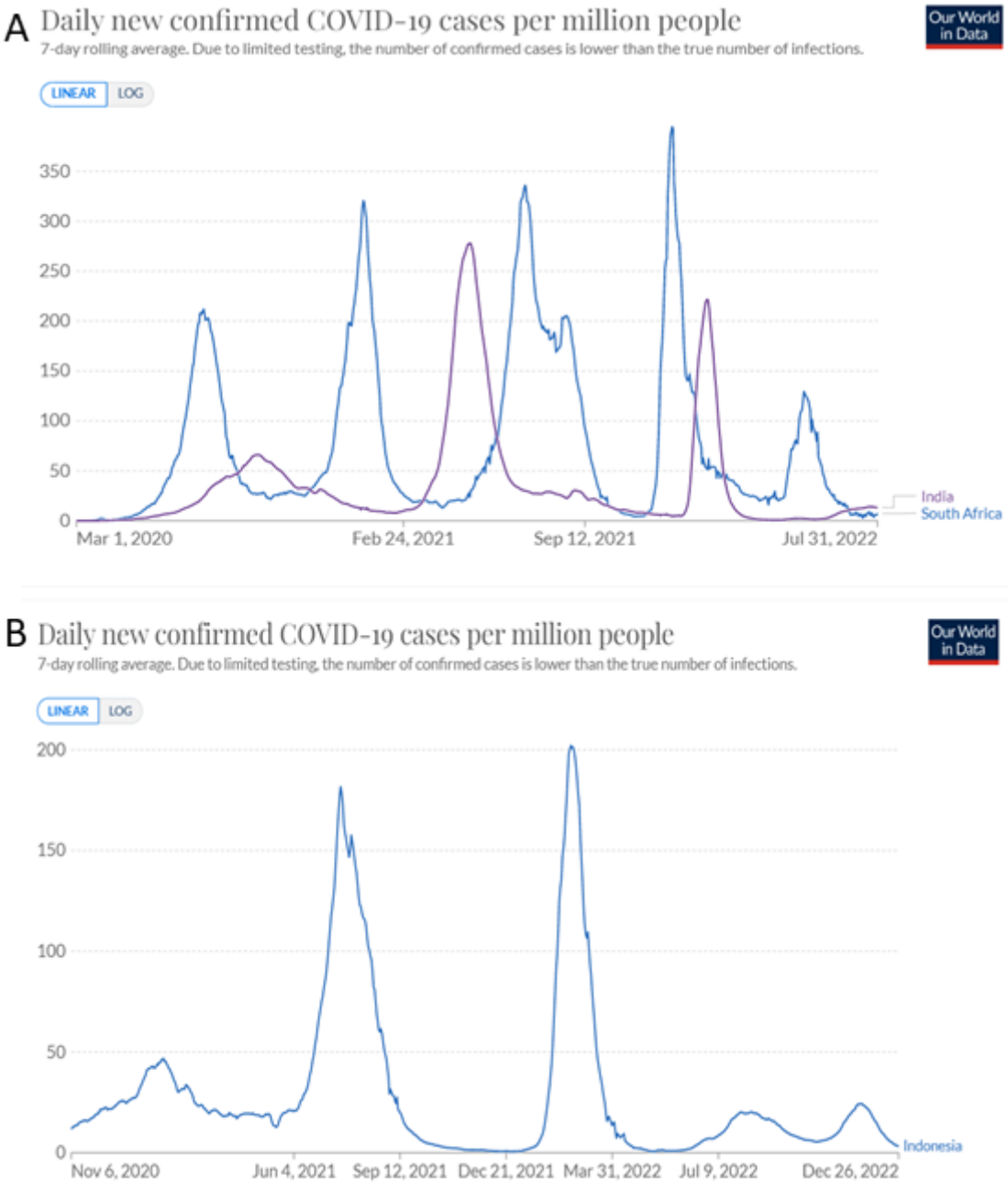


Figure 3. COVID-19 epidemics in three countries where rapid surges in cases were followed by sudden collapses. Panel A shows several epidemics in South Africa and India that displayed unusually sharp peaks. Several peaks had almost perfectly straight sides. Panel B shows two epidemics in Indonesia with similar sharp peaks.

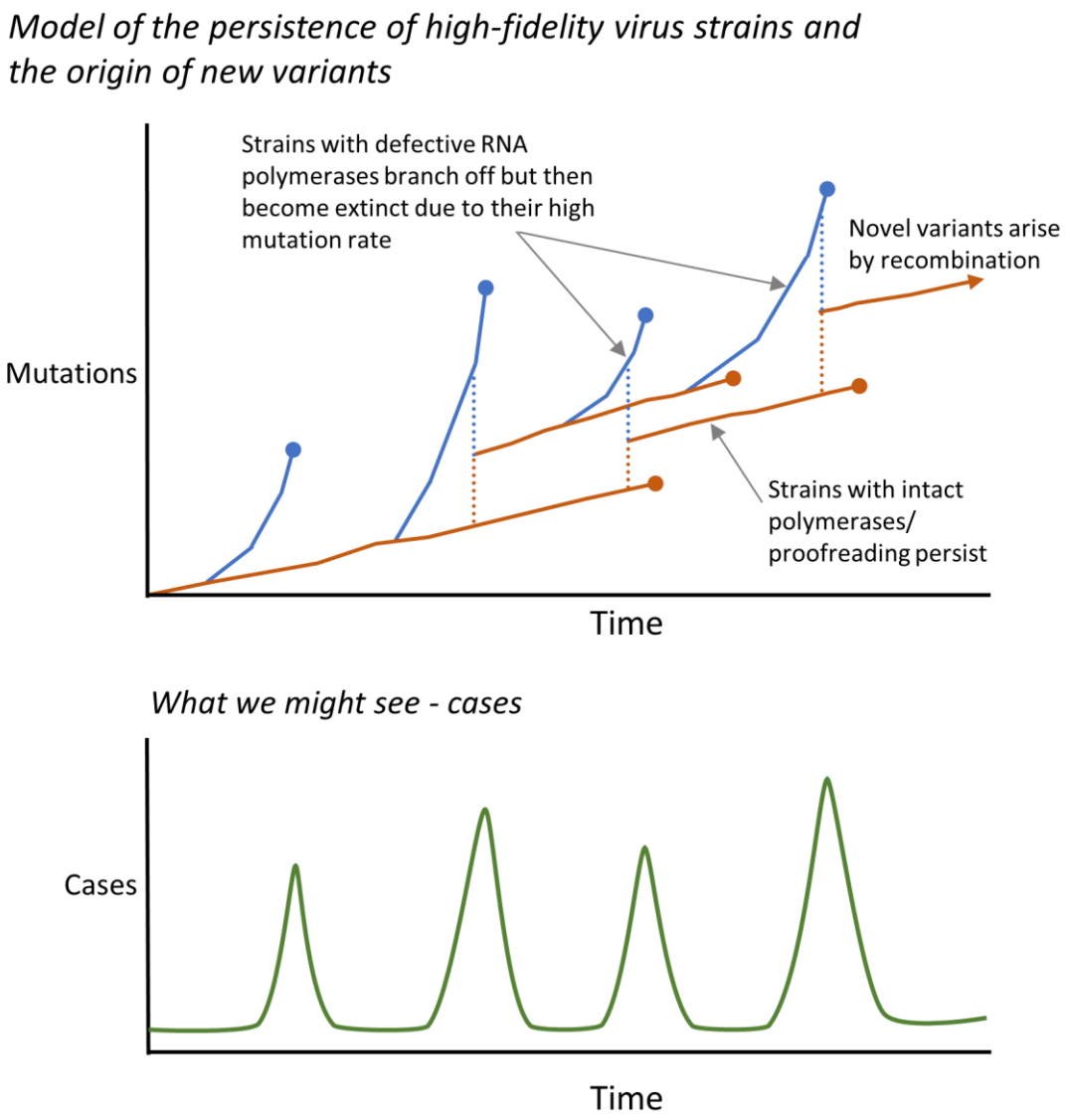


Figure 4. *A schematic illustration of the mechanism where the MIEMP pathway generates sharp peaks in case numbers and how MIEMP combined with recombination might generate new variants.* The sharp peaks occur when MIEMP generates short-lived variants that subsequently collapse (blue lines). Jumps in the number of mutations on the main tree occur when the MIEMP pathway generates well-adapted lineages that recombine (dotted lines) with more stable lineages, giving rise to well-adapted and stable variants (brown lines). Note that sequences corresponding to the blue lines would not normally appear in online databases such as Nextstrain because these lineages have more mutations than usual (see Figure 7 below).

## Epidemiological evidence

If strong selective pressures encourage the rapid emergence of low-fidelity strains, we would expect such strains to appear in human viral epidemics even without zoonosis. COVID-19 is the epidemic studied in the greatest detail, and its epidemiology displays several anomalous features that are compatible with the MIEMP pathway and recombination. Firstly, cases rose rapidly in several countries before collapsing suddenly (Figure 3). This resulted in sharp triangular peaks rather than the flattened curves predicted by conventional models - in which the proportion of susceptible individuals in a population gradually decreases. Note that if surges were to arrive in separate geographical regions of large countries such as India, South Africa and Indonesia at different times,

the overall profile would be rounded or irregular. Hence, these sharp profiles suggest simultaneous spreading and collapse in many regions. These sharp peaks can be explained by the mechanism shown in figure 4: lineages with reduced replicative fidelity evolve rapidly, but subsequently suffer error catastrophes and become extinct. Recombination between these lineages and more slowly evolving lineages can, however, generate well-adapted lineages with high fidelity (Figure 4). Changes in population mobility could not explain the collapse in cases on at least one occasion: Figure 5 shows that cases do not align with changes in personal mobility in South Africa in December 2021. Influenza epidemics often show similar, very sharp, peaks. Figure 6 shows the eight largest influenza outbreaks in the UK town Cirencester, recorded by Edgar Hope-Simpson. Several severe outbreaks developed in just 2–3 weeks and abruptly terminated in a similar period, resulting in the sharp peaks shown. Figure 7 confirms that the observed genomic epidemiology of SARS-CoV-2 matches the general form predicted by the mechanism shown in Figure 4.


Figure 5. COVID-19 cases and personal mobility in South Africa in December 2021. It can be seen that cases stopped rising and began to fall rapidly on 18 December despite activity in retail and recreation continuing to increase for another seven days. Therefore, the collapse in cases does not seem to be triggered by changes in host mobility. Case numbers and

estimated R-values were obtained from Our World in Data (https://ourworldindata.org) and mobility values from Google COVID-19 Community Mobility Reports (https://www.google.com/covid19/mobility).

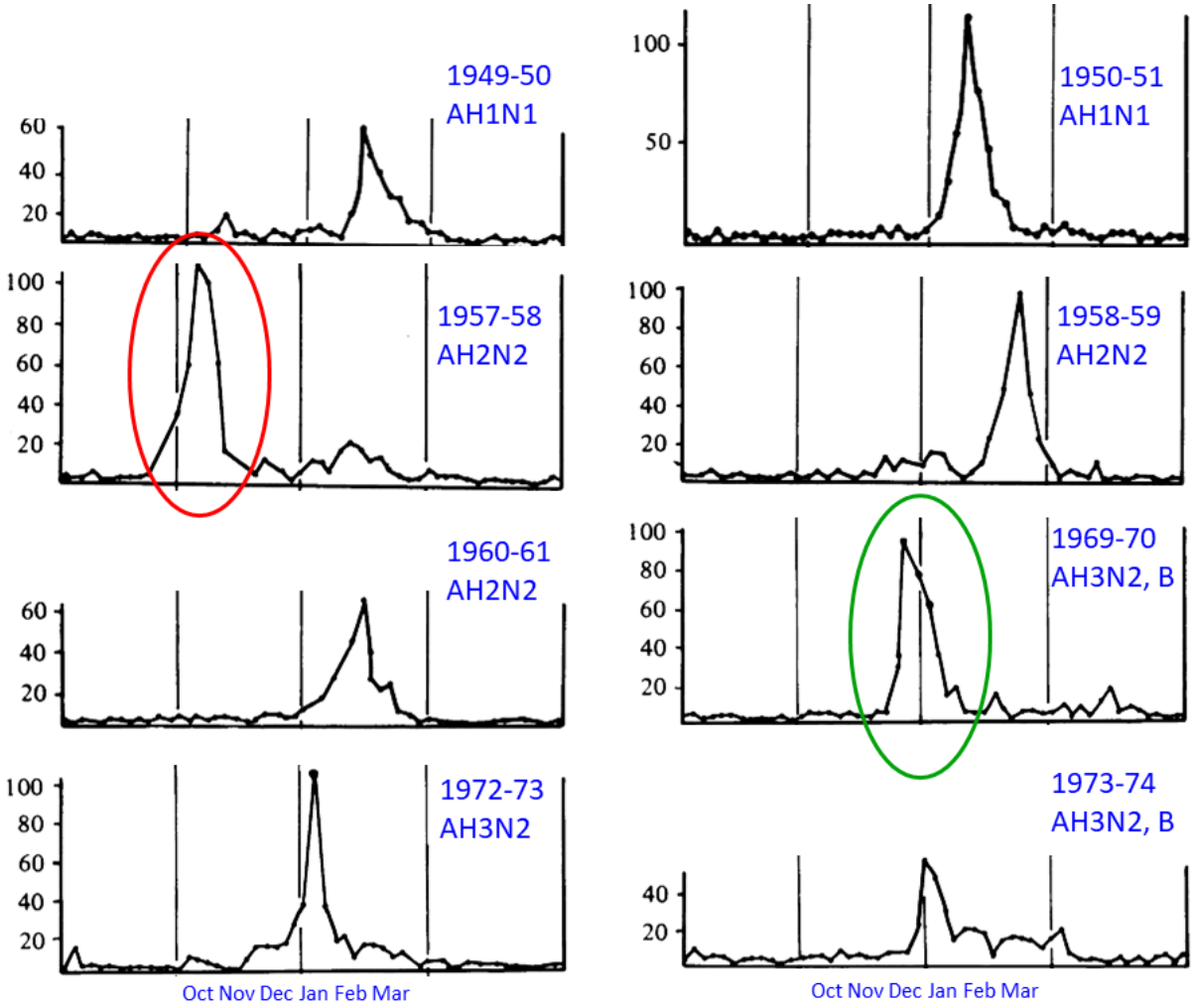


Figure 6: The eight largest influenza outbreaks in Cirencester, UK, 1946–1974. The graphs show the number of patients treated for acute febrile respiratory illness. Several outbreaks showed sharp peaks that are similar to those seen for COVID-19 in figure 3. The red oval shows the arrival of Asian flu, green, the first major epidemic of Hong Kong flu (which arrived the previous year). Adapted from Epidemiology & Infection, 1981 Feb;86(1):35-47.

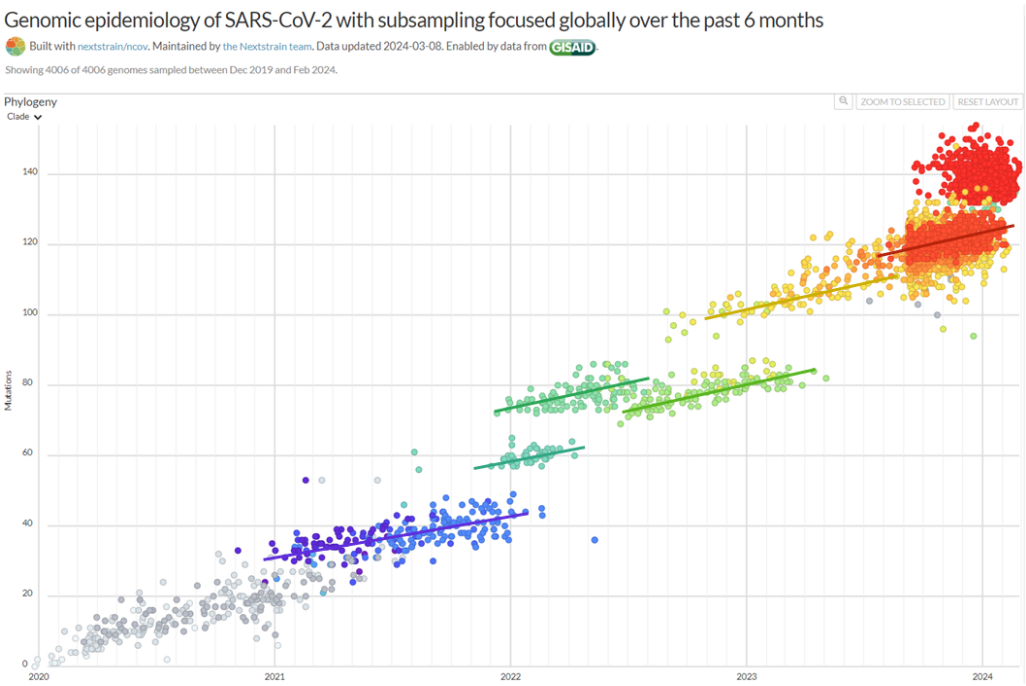


Figure 7. *The genomic epidemiology of SARS-CoV-2, as visualized by the nextstrain.org website.* The number of mutations with respect to a reference sequence is plotted against the date of sample collection. I have added rough regression lines by eye. It can be seen that all variants evolve at a roughly constant rate of around 10 mutations per year, but there is typically a substantial jump in the number when new variants appear. Note that sequences corresponding to the blue lines in Figure 5 would not be expected to appear on this plot because Nextstrain has a policy of excluding any sequence that has more than the normal number of "private mutations", which are mutations that are not shared with the most similar node of the reference tree.

## SARS-CoV-2 mutations

Figure 8 shows the nucleotide diversity of the SARS-CoV-2 genome. As predicted by the hypothesis, the region encoding the viral polymerase and proofreading functio shows the lowest diversity, and the region encoding the structural proteins the highest. This is compatible with the MIEMP route with recombination, but it could also result from greater adaptive selection acting on the structural proteins, with greater negative selection in ORF1ab. Analysis of mutations at the nucleotide level can shed light on this issue. The Omicron variant (BA.1) surprised virologists when it appeared because it had 29 non-synonymous mutations in the Spike gene, but only 15 non-synonymous mutations in the whole of the rest of the genome. Moreover, an anomalously low proportion of the mutations in the Spike of Omicron (and other variants) were caused by C-to-T nucleotide transitions. Most C-to-T transitions in SARS-CoV-2 are thought to be generated by host modifications to viral RNA, especially by the APOBEC family of cytidine deaminases [Milewska et al., 2018], and we can get an idea of the underlying frequency of C-to-T mutations by looking at synonymous mutations, which are not expected to be strongly selected. Combining the totals of all synonymous "defining mutations" of Alpha, Beta, Gamma, Delta and Omicron variants, the majority (53%) were C-to-T transitions, as shown on table 2 [data from NextStrain.org]. However, only 11 of 92 non-synonymous Spike mutations (i.e. 12%) were C-to-T transitions [data for Beta, Delta, Lambda and Omicron, private communication from Tony Van Dongen]. This data is summarized in Table 1.

Table 1: Analysis of mutations in SARS-CoV-2 variants

| | Length | Synonymous mutations~ | Of which, C-to-T~ | Synonymous C-to-T mutations, %~ | All mutations* | Of which, C-to-T* | C-to-T mutations, %* |
|---|---|---|---|---|---|---|---|
| ORF 1ab | 21,287 | 19 | 13 | 68% | ? | ? | ? |
| All "structural" proteins | 8,138 | 21 | 8 | 38% | ? | ? | ? |
| Spike only | 3,849 | | | | 92 | 11 | 12% |
| All | | 40 | 21 | 53% | ? | ? | ? |

~From "defining mutations" of Alpha, Beta, Gamma, Delta, Omicron, from Nextstrain.org

*From sequence data from Beta, Delta, Lambda, Omicron

These variants were likely generated by recombination between error-prone strains (which had acquired many beneficial, random, non-C-to-T mutations via defective polymerases) and more stable high-fidelity strains as shown in Figure 5 above. In this case, much of the region of the genome encoding "structural protein" genes near the 3' end of the genomes of these variants, including the Spike genes, would have come from error-prone partners, while much of the non-structural protein sections, including the polymerases, would have come from high-fidelity partners.

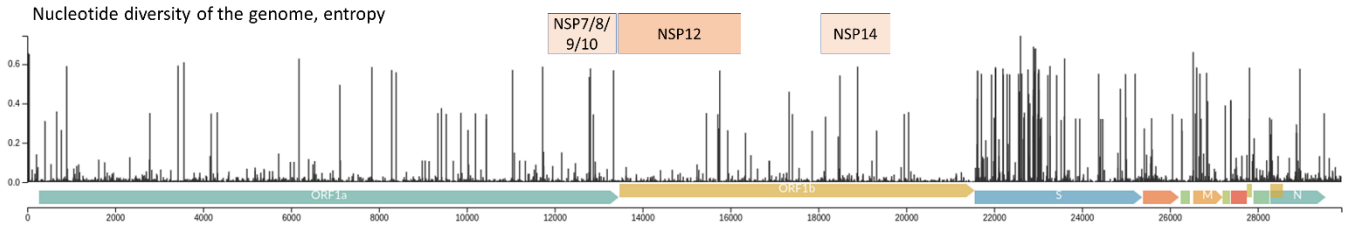


Figure 8: the nucleotide diversity of the SARS-CoV-2 genome, shown as Shannon entropy. The ORF1b region of the genome has lower diversity than the rest of the genome. ORF1b contains several non-structural protein genes, including those encoding the polymerase "core", NSP12, and the error-correction function, NSP14. The nidovirus RdRp-associated nucleotidyltransferase domain (NiRAN), which is close to the N-terminus of NSP12, seems to be particularly highly conserved. ORF1a, which has intermediate diversity, contains other non-structural proteins. The position of NSPs 7-10, which participate in viral RNA replication by interacting with NSP12, is shown. The structural genes, including Spike (S), M and N, have higher diversity. The placement of the genes for the structural proteins, which are particularly immunogenic and therefore subject to strong selection, at the extreme right-hand end of the genome may allow efficient recombination of these genes.

## Implications and suggestions for avoiding pandemics

Other factors can influence the chance that pandemics will commence. In particular, the transmission routes used by animal viruses may provide important barriers that prevent sustained human-to-human transmission. For example, ticks are unlikely to bite more than one human in succession, reducing the chance that tick-borne illnesses will spread and cause pandemics. However, the tropism of viruses can change, allowing them to spread by novel routes. For example, wild waterfowl provide a large reservoir of avian influenza A viruses of various subtypes, which often infect these birds' gastrointestinal tracts and are spread by the oral-to-fecal route [Alexander &

Capua, 2008]. In land birds, however, the virus often spreads by the respiratory route [Alexander & Capua, 2008]. SARS-CoV-2 provides another example because the bat viruses from which it is descended are thought to infect the bats' gastrointestinal tracts, but COVID-19 is a respiratory disease in humans, again emphasizing that transmission routes may switch after a spillover [Balloux et al. 2022]. In other cases, viruses spill over to a new species by a route that differs from the route used in the original host, but they revert to the original route in the new host. For example, the progenitor of HIV, the simian immunodeficiency virus, is spread in chimpanzees and monkeys in the same way that it is spread in humans: sexually, through blood contact, and in breast milk [Peeters et al., 2014]. However, it is thought to have spread to humans through hunting chimpanzees for meat [Peeters et al., 2014]. This raises the possibility that one-off transfer mechanisms might introduce viruses to humans that are capable of sustained human-to-human transmission by another route. These examples show that epidemic viruses may reach new hosts by both direct and indirect routes and that indirect routes may circumvent some apparent barriers to transmission.

Table 2 shows various precautions that may reduce the chance of pandemic-capable viruses arising and spreading in humans, including safety measures for hospitals and clinical and virology labs.

Table 2. Anticipated dangers and recommendations for avoiding epidemics caused by animal viruses.

| *Setting/ location* | *Anticipated danger, according to the hypothesis* | *Comments* | *Recommendations* |
|---|---|---|---|
| All locations/ general considerations. | In this table, "simultaneous infection" refers to co-infection of one individual by a human-adapted virus (originating from an earlier spillover) and, simultaneously, by a related animal virus, allowing recombination. Such recombination is normally required to generate viruses that are capable of sustained human-to-human transmission. | Some animal viruses, such as those that cause Chikungunya, yellow fever and Lassa fever, cause symptoms that range in severity from mild to fatal hemorrhagic fevers. Rodents and bats often live in high-density colonies that overlap with human settlements. Rodent and bat colonies can therefore harbor many diverse viruses including some that may infect humans. Infection may occur via contaminated urine and feces, or arthropod vectors may be involved. | Healthcare workers should remember that they may be more susceptible than members of communities with greater exposure to animal viruses. Clinical samples from different patients should be analyzed and sequenced separately to reduce the chance of laboratory workers being simultaneously infected by more than one strain. Stringent precautions should be taken to avoid dangerous viral recombination in cell cultures, especially if a strain is suspected of being human-adapted. |
| Towns, villages and other communities with high exposure to rodents and bats. | Individuals may be infected simultaneously with well-adapted and high-fidelity strains allowing recombination to take place. | Individuals in poor communities may be compelled by their financial situation to work even when they are sick, increasing the chance of simultaneous infection. However, by the time a simultaneous infection occurs, many members of the community may have acquired immunity, which has a good chance of preventing onward spreading. | Serological studies can identify communities at risk. Reducing rodent infestations and avoiding bat excreta can reduce exposure. Health authorities should act with caution to avoid spreading recombinant strains. Quarantining communities experiencing active epidemics may be essential. |

| | | | |
|---|---|---|---|
| Farms. | Chronic infections might allow viral adaptation within one individual, and further contact with animals might allow dangerous simulaneous infection and recombination. | In wealthy communities, people can usually take time off work and avoid social contact when they are sick, so extended undetected human-to-human transmission is unlikely. | Serological studies can identify communities at risk. Cases should be followed up. Samples of strains that may be human-adapted should be handled with care. Advice should be given to avoid exposure. Farm workers should recover fully before returning to work after viral infections. Rodent populations should be controlled. |
| Hospitals and clinical labs. | Human-adapted and high-fidelity strains may be introduced to a hospital by different patients. | | *Patients who may be infected with related strains should be isolated in separate wards to reduce the chance of dangerous recombination.* |
| Laboratories working with animal viruses. | There are multiple dangers in the animal virology lab: (1) a lab may house both human-adapted and related animal viruses. (2) Scientists may deliberately or accidentally introduce high-fidelity polymerases into human-adapted viruses, or they may introduce genes conferring human adaptation to high-fidelity strains. (3) Infected lab workers may be exposed to high-fidelity animal viruses, allowing recombination. | The antagonistic pleiotropy hypothesis suggests that passaging through human cells and humanized animals may generate viruses that cannot transmit efficiently from person to person since functionality essential for transmission and immune evasion may be lost in cell cultures and non-human animals. Moreover, passaging in strongly selective conditions may produce strains with reduced replicative fidelity. | *(1) Human-adapted strains should not be handled in the same lab as wild-type animal strains. (2) Dangerous engineering experiments should be identified and prohibited. (3) Sick lab workers should be strictly quarantined until completely asymptomatic.* |

## Conclusions

Although spillovers of animal viruses to humans are relatively common, pandemics following spillovers are rare, with only a few major pandemics per century. An important reason may be that viruses that spill over are subject to strong selective pressures and, therefore, tend to adapt by the MIEMP pathway, i.e., their adaptation starts with one or more mutations affecting their polymerases or related proteins. This may allow them to adapt to their human host rapidly, but the cost would be a high mutation rate that can only be restored on a useful timescale by recombination with similar strains that possess intact RNA-replicating proteins. This hypothesis explains the rarity of pandemics in the past and provides suggestions for reducing the frequency of future epidemics and pandemics caused by animal viruses. If we can avoid the eventualities shown in Table 2, we may experience fewer pandemics in the next 100 years than the last.

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