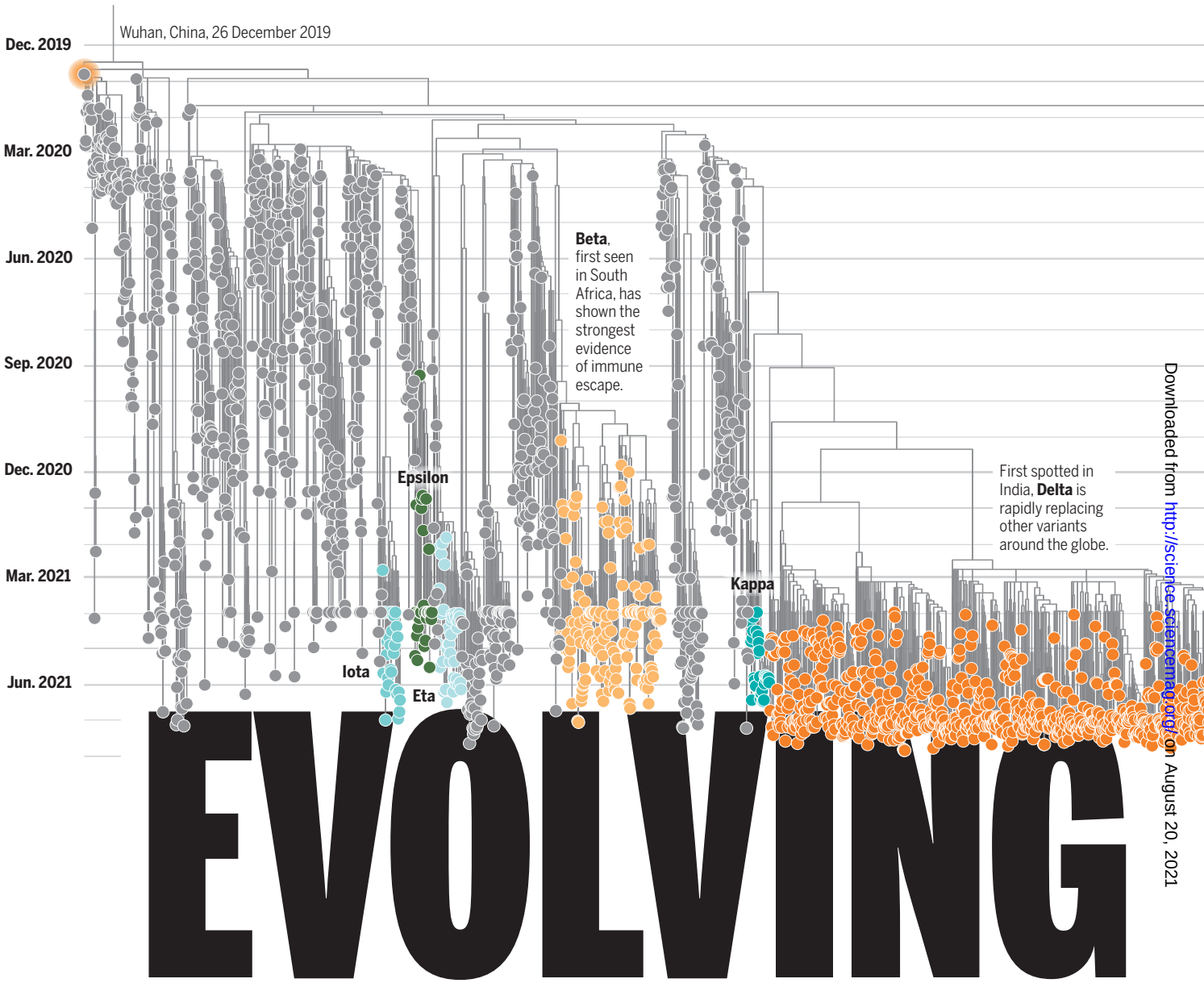
FEATURES



EVOLVING

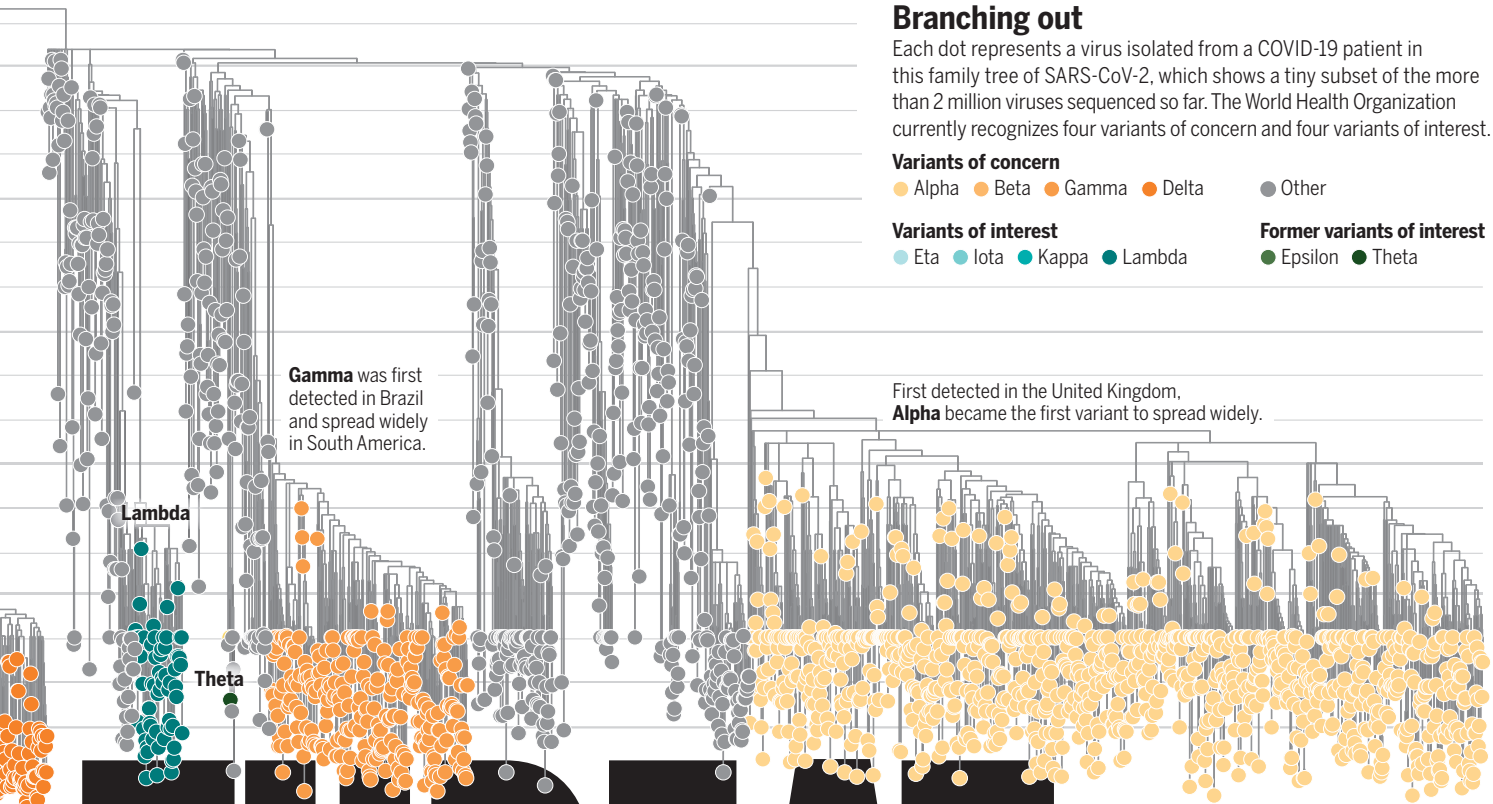
CREDITS: (GRAPHIC) N. DESAI/*SCIENCE*; (DATA) NEXTSTRAIN; GISAID

Edward Holmes does not like making predictions, but last year he hazarded a few. Again and again, people had asked Holmes, an expert on viral evolution at the University of Sydney, how he expected SARS-CoV-2 to change. In May 2020, 5 months into the pandemic, he started to include a slide with his best guesses in his talks. The virus would probably evolve to avoid at least some human immunity, he suggested. But it would likely make people less sick over time, he said, and there would be little change in its infectivity. In short, it sounded like evolution would not play a major role in the pandemic's near future.

"A year on I've been proven pretty much wrong on all of it," Holmes says.

Well, not all: SARS-CoV-2 did evolve to better avoid human antibodies. But it has also become a bit more virulent and a lot more infectious, causing more people to fall ill. That has had an enormous influence on the course of the pandemic.

The Delta strain circulating now—one of four "variants of concern" identified by the World Health Organization, along with four "variants of interest"—is so radically different from the virus that appeared in Wuhan, China, in late 2019 that many countries have been forced to change their pandemic planning. Governments are scrambling to accelerate vaccination



THREAT

New variants have changed the face of the pandemic. What will the virus do next?

By **Kai Kupferschmidt**

programs while prolonging or even reintroducing mask wearing and other public health measures. As to the goal of reaching herd immunity—vaccinating so many people that the virus simply has nowhere to go—"With the emergence of Delta, I realized that it's just impossible to reach that," says Müge Çevik, an infectious disease specialist at the University of St. Andrews.

Yet the most tumultuous period in SARS-CoV-2's evolution may still be ahead of us, says Aris Katzourakis, an evolutionary biologist at the University of Oxford. There's now enough immunity in the human population to ratchet up an evolutionary competition, pressuring the virus to adapt further. At the same time, much of the world is still overwhelmed with infections, giving the virus plenty of chances to replicate and throw up new mutations.

Predicting where those worrisome factors will lead is just as tricky as it was a year and a half ago, however. "We're much better at explaining the past than predicting the future," says Andrew Read, an evolutionary biologist at Pennsylvania State University, University Park. Evolution, after all, is driven by random mutations, which are impossible to predict. "It's very, very tricky to know what's possible, until it happens," Read says. "It's not physics. It doesn't happen on a billiard table."

Still, experience with other viruses gives evolutionary biologists some clues about

where SARS-CoV-2 may be headed. The courses of past outbreaks show the coronavirus could well become even more infectious than Delta is now, Read says: "I think there's every expectation that this virus will continue to adapt to humans and will get better and better at us." Far from making people less sick, it could also evolve to become even deadlier, as some previous viruses including the 1918 flu have. And although COVID-19 vaccines have held up well so far, history shows the virus could evolve further to elude their protective effect—although a recent study in another coronavirus suggests that could take many years, which would leave more time to adapt vaccines to the changing threat.

EXPLAINING THE PAST

Holmes himself uploaded one of the first SARS-CoV-2 genomes to the internet on 10 January 2020. Since then, more than 2 million genomes have been sequenced and published, painting an exquisitely detailed picture of a changing virus. "I don't think we've ever seen that level of precision in watching an evolutionary process," Holmes says.

Making sense of the endless stream of mutations is complicated. Each is just a tiny tweak in the instructions for how to make proteins. Which mutations end up spreading depends on how the viruses carrying those tweaked proteins fare in the real world.

The vast majority of mutations give the virus no advantage at all, and identifying the ones that do is difficult. There are obvious candidates, such as mutations that change the part of the spike protein—which sits on the surface of the virus—that binds to human cells. But changes elsewhere in the genome may be just as crucial—yet are harder to interpret. Some genes' functions aren't even clear, let alone what a change in their sequence could mean. The impact of any one change on the virus' fitness also depends on other changes it has already accumulated. That means scientists need real-world data to see which variants appear to be taking off. Only then can they investigate, in cell cultures and animal experiments, what might explain that viral success.

The most eye-popping change in SARS-CoV-2 so far has been its improved ability to spread between humans. At some point early in the pandemic, SARS-CoV-2 acquired a mutation called D614G that made it a bit more infectious. That version spread around the world; almost all current viruses are descended from it. Then in late 2020, scientists identified a new variant, now called Alpha, in patients in Kent, U.K., that was about 50% more transmissible. Delta, first seen in India and now conquering the world, is another 40% to 60% more transmissible than Alpha.

Read says the pattern is no surprise. "The only way you could not get infectiousness rising would be if the virus popped into humans as perfect at infecting humans as it could be, and the chance of that happening is incredibly small," he says. But Holmes was startled. "This virus has gone up three notches in effectively a year and that, I think, was the biggest surprise to me," Holmes says. "I didn't quite appreciate how much further the virus could get."

Bette Korber at Los Alamos National Laboratory and her colleagues first suggested that D614G, the early mutation, was taking over because it made the virus better at spreading. She says skepticism about the virus' ability to evolve was common in the early days of the pandemic, with some researchers saying D614G's apparent advantage might be sheer luck. "There was extraordinary resistance in the scientific community to the idea this virus could evolve as the pandemic grew in seriousness in spring of 2020," Korber says.

Researchers had never watched a completely novel virus spread so widely and evolve in humans, after all. "We're used to dealing with pathogens that have been in humanity for centuries, and their evolutionary course is set in the context of having been a human pathogen for many, many years," says Jeremy Farrar, head of the Wellcome Trust. Katzourakis agrees. "This may have affected our priors and conditioned many to think in a particular way," he says.

Another, more practical problem is that real-world advantages for the virus don't always show up in cell culture or animal models. "There is no way anyone would have noticed anything special about Alpha from laboratory data alone," says Christian Drosten, a virologist at the Charité University Hospital in Berlin. He and others are still figuring out what, at the molecular level, gives Alpha and Delta an edge.

Alpha seems to bind more strongly to the human ACE2 receptor, the virus' target on the cell surface, partly because of a mutation in the spike protein called N501Y. It may also be better at countering interferons, molecules that are part of the body's viral immune defenses. Together those changes may lower the amount of virus needed to infect someone—the infectious dose. In Delta, one of the most important changes may be near the furin cleavage site on spike, where a human enzyme cuts the protein, a key step enabling the virus to invade human cells. A mutation called P681R in that region makes cleavage more efficient, which may allow the virus to enter more cells faster and lead to greater numbers of virus particles in an infected person. In July, Chinese researchers posted a preprint showing Delta could lead to virus levels in patient samples 1000 times higher than for previous variants. Evidence is accumulating that infected people not only spread the virus more efficiently, but also faster, allowing the variant to spread even more rapidly.

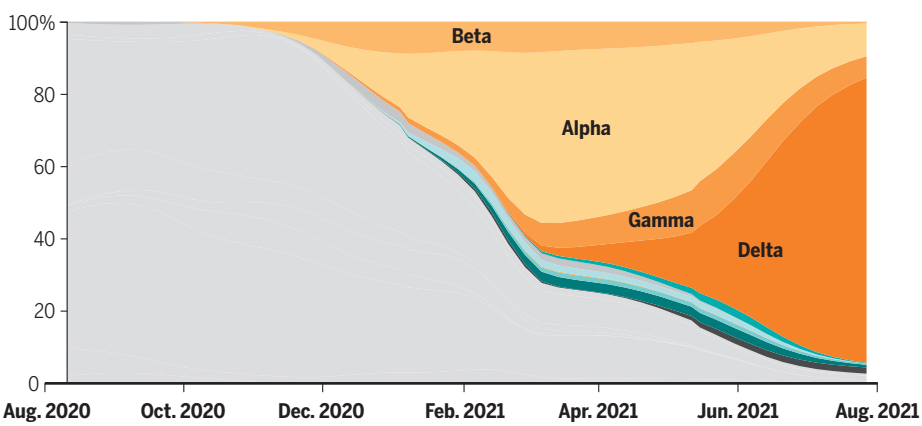
DEADLY TRADE-OFFS

The new variants of SARS-CoV-2 may also cause more severe disease. For example, a study in Scotland found that an infection with Delta was about twice as likely to lead to hospital admission than with Alpha.

It wouldn't be the first time a newly emerging disease quickly became more serious. The 1918–19 influenza pandemic also appears to have caused more serious illness as time went on, says Lone Simonsen, an

Hostile takeovers

SARS-CoV-2 variants began to emerge in 2020. Alpha surged in many countries in early 2021, then was largely replaced by Delta. Two other variants of concern, Beta and Gamma, account for a smaller number of cases.



CREDITS: (GRAPHIC) N. DESAI/*SCIENCE*; (DATA) NEXTSTRAIN; GISAID

epidemiologist at Roskilde University who studies past pandemics. "Our data from Denmark suggests it was six times deadlier in the second wave."

A popular notion holds that viruses tend to evolve over time to become less dangerous, allowing the host to live longer and spread the virus more widely. But that idea is too simplistic, Holmes says. "The evolution of virulence has proven to be quicksand for evolutionary biologists," he says. "It's not a simple thing."

Two of the best studied examples of viral evolution are myxoma virus and rabbit hemorrhagic disease virus, which were released in Australia in 1960 and 1996, respectively, to decimate populations of European rabbits that were destroying croplands and wreaking ecological havoc. Myxoma virus initially killed more than 99% of infected rabbits, but then less pathogenic strains evolved, likely because the virus was killing many animals before they had a chance to pass it on. (Rabbits also evolved to be less susceptible.) Rabbit hemorrhagic disease virus, by contrast, got more deadly over time, probably because the virus is spread by blow flies feeding on rabbit carcasses, and quicker death accelerated its spread.

Other factors loosen the constraints on deadliness. For example, a virus variant that can outgrow other variants within a host can end up dominating even if it makes the host sicker and reduces the likelihood of transmission. And an assumption about human respiratory diseases may not always hold: that a milder virus—one that doesn't make you crawl into bed, say—might allow an infected person to spread the virus further. In SARS-CoV-2, most transmission happens early on, when the virus is replicating in the upper airways, whereas serious disease, if it develops, comes later, when the virus infects the lower airways. As a result, a variant that makes the host sicker might spread just as fast as before.

EVASIVE MEASURES

From the start of the pandemic, researchers have worried about a third type of viral change, perhaps the most unsettling of all: that SARS-CoV-2 might evolve to evade immunity triggered by natural infections or vaccines. Already, several variants have emerged sporting changes in the surface of the spike protein that make it less easily recognized by antibodies. But although news of these variants has caused widespread fear, their impact has so far been limited.

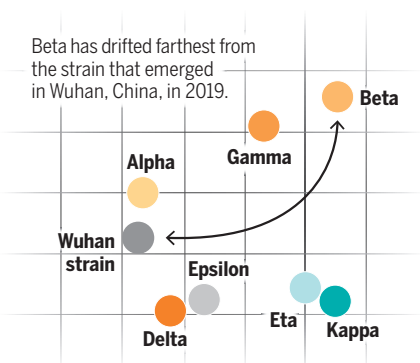
Derek Smith, an evolutionary biologist at the University of Cambridge, has worked for decades on visualizing immune evasion in the influenza virus in so-called antigenic maps. The farther apart two variants are on Smith's maps, the less well antibodies against one virus protect against the other. In a recently published preprint, Smith's group, together with David Montefiori's group at Duke University, has applied the approach to mapping the most important variants of SARS-CoV-2 (see graphic, below).

The new maps place the Alpha variant very close to the original Wuhan virus, which means antibodies against one still neutralize the other. The Delta variant, however, has drifted farther away, even though it doesn't completely evade immunity. "It's not an immune escape in the way people think of an escape in slightly cartoonish terms," Katzourakis says. But Delta is slightly more likely to infect fully vaccinated people than previous variants. "It shows the possible beginning of a trajectory and that's what worries me," Katzourakis says.

The myxoma virus was released in Australia in 1950 to control rabbits after trials at this test site on Wardang Island. It has evolved to become less virulent over time, but not all viruses do.

Viral cartography

On this "antigenic map," produced by Derek Smith, David Montefiori, and colleagues, the distance between two variants indicates how well antibodies against one neutralize the other.



Other variants have evolved more antigenic distance from the original virus than Delta. Beta, which first appeared in South Africa, has traveled the farthest on the map, although natural or vaccine-induced immunity still largely protects against it. And Beta's attempts to get away may come at a price, as Delta has outstripped it worldwide. "It's probably the case that when a virus changes to escape immunity, it loses other aspects of its fitness," Smith says.

The map shows that for now, the virus is not moving in any particular direction. If the original Wuhan virus is like a town on Smith's map, the virus has been taking local trains to explore the surrounding area, but it has not traveled to the next city—not yet.

PREDICTING THE FUTURE

Although it's impossible to predict exactly how infectiousness, virulence, and immune evasion will develop in the coming months, some of the factors that will influence the virus' trajectory are clear.

One is the immunity that is now rapidly building in the human population. On one hand, immunity reduces the likelihood of people getting infected, and may hamper viral replication even when they are. "That means there will be fewer mutations emerging if we vaccinate more people," Çevik says. On the other hand, any immune escape variant now has a huge advantage over other variants.

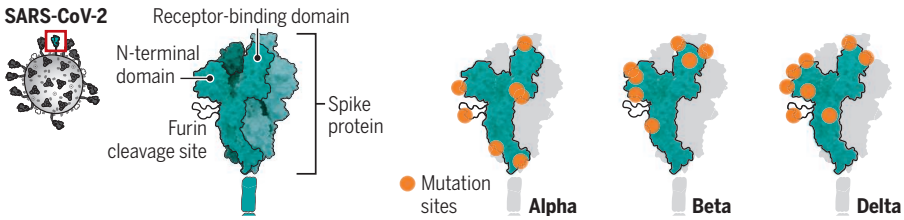
In fact, the world is probably at a tipping point, Holmes says: With more than 2 billion people having received at least one vaccine dose and hundreds of millions more having recovered from COVID-19, variants that evade immunity may now have a bigger leg up than those that are more infectious.

Something similar appears to have happened when a new H1N1 influenza strain emerged in 2009 and caused a pandemic, says Katia Kölle, an evolutionary biologist at Emory University. A 2015 paper found that changes in the virus in the first 2 years appeared to make the virus more adept at human-to-human transmission, whereas changes after 2011 were mostly to avoid human immunity.

It may already be getting harder for SARS-CoV-2 to make big gains in infectiousness. "There are some fundamental limits to exactly how good a virus can get at transmitting and at some point SARS-CoV-2 will hit that plateau," says Jesse Bloom, an evolutionary biologist at the Fred Hutchinson Cancer Research Center. "I think it's very hard to say if this is already where we are, or is it still going to happen." Evolutionary virologist Kristian Andersen of Scripps Research guesses the virus still has space to evolve greater transmissibility. "The known limit in the viral universe is measles, which is about three times more transmissible than what we have now with Delta," he says.

Scratching the surface

Researchers trying to understand which genetic changes make SARS-CoV-2 variants more successful have focused on the spike protein, which studs the viral surface and binds to human cells. Alpha, Beta, and Delta have mutations in three key areas of the protein that may affect the virus' infectiousness and its ability to elude the immune system.



The limits of immune escape are equally uncertain. Smith's antigenic maps show the space the virus has explored so far. But can it go much farther? If the variants on the map are like towns, then where are the country's natural boundaries—where does the ocean start? A crucial clue will be where the next few variants appear on the map, Smith says. Beta evolved in one direction away from the original virus and Delta in another. "It's too soon to say this now, but we might be heading for a world where there are two serotypes of this virus that would also both have to be considered in any vaccines," Drosten says.

Immune escape is so worrying because it could force humanity to update its vaccines continually, as happens for flu. Yet the vaccines against many other diseases—measles, polio, and yellow fever, for example—have remained effective for decades without updates, even in the rare cases where immune-evading variants appeared. "There was big

Do chronic infections breed dangerous new variants?

New variants of SARS-CoV-2 have major impacts around the globe, driving up COVID-19 case and mortality numbers (see main story, p. 844). But each of those viruses picks up its crucial changes as it divides in the cells of an infected human being. The nature of those infections—how fast the virus replicates and for how long—may determine the odds that they will give rise to new and more troublesome mutants, researchers say.

After someone is infected, the virus starts to multiply at a dizzying rate, producing billions of viral particles within days. Because small copy mistakes happen during every replication cycle, a huge variety of slightly different genomes quickly emerges. With SARS-CoV-2's genome spanning just 30,000 nucleotides, and only three ways to change any one position, every possible mutation likely arises in an infected individual.

The vast majority of those changes offer the virus no benefit, and even those that do only have a small chance of being passed on to the next person. A paper published in 2020 estimated that about 1000 viral particles are transmitted when one person infected another, but a reanalysis by Katia Kölle of Emory University and a colleague, published as a preprint in February, concluded that 99% of all successful transmissions come from three or fewer virus particles. A study published in *Science* in April by evolutionary biologist Katrina Lythgoe at the University of Oxford put the number of transmitted virus particles at infection between one and eight.

This means that, unless a mutation arises early and gives the virus so big an advantage that it quickly becomes dominant in the host, it has a low chance of being transmitted, which puts the brake on virus evolution. "It's generally thought that when transmission bottlenecks are tight, that slows adaptive evolution at the population level," Kölle explains.

That may sound like good news for humanity, but it is offset by the huge number of SARS-CoV-2 infections globally, says Jesse Bloom, an evolutionary biologist at the Fred Hutchinson Cancer Research Center. Besides, the virus may have a shortcut. In most people, the immune system curbs the infection within days, but a few develop a chronic infection lasting months. That gives time for mutations to accumulate and become dominant, increasing their chances of transmission. In a short-lived acute infection, evolution is "more like roulette," Kölle says, but in chronic cases, "you have the time needed to adapt to that environment."

Chronic infections may explain why the Alpha variant, first seen in the United Kingdom in late 2020, appeared to emerge with a slew of mutations all at once. In theory, Alpha could have picked up those changes one by one before arriving in the country, says Andrew Rambaut of the University of Edinburgh, but the fact that most of its genome resembles other U.K. viruses at the time suggests instead that a local virus underwent extended evolution in a single patient. "I am still reasonably confident that a chronic infection is the best explanation," Rambaut says.

COVID-19 treatments may accelerate evolution in chronic patients. In July, researchers in Germany published data on six immunocompromised patients treated with a monoclonal antibody that targeted SARS-CoV-2. In five of them, the virus acquired E484K, a mutation known to help it elude the immune system, and the virus rebounded in all five patients.

Still, the evidence that chronic patients are the source of new variants is circumstantial, Bloom cautions. People who don't develop chronic infections but do take longer than average to clear SARS-CoV-2 could also generate and spread mutants, Lythgoe says—and they are more numerous. "Are these the infections that really drive the evolution of acute viruses like SARS-CoV-2? There's really interesting questions there." —*K.K*

alarm around 2000 that maybe we'd need to replace the hepatitis B vaccines," because an escape variant had popped up, Read says. But the variant has not spread around the world: It is able to infect close contacts of an infected person, but then peters out. The virus apparently faces a trade-off between transmissibility and immune escape. Such trade-offs likely exist for SARS-CoV-2 as well.

Some clues about SARS-CoV-2's future path may come from coronaviruses with a much longer history in humans: those that cause common colds. Some are known to reinfect people, but until recently it was unclear whether that's because immunity in recovered people wanes, or because the virus changes its surface to evade immunity. In a study published in April in *PLOS Pathogens*, Bloom and other researchers compared the ability of human sera taken at different times in the past decades to block virus isolated at the same time or later. They showed that the samples could neutralize strains of a coronavirus named 229E isolated around the same time, but weren't always effective against virus from 10 years or more later. The virus had evidently evolved to evade human immunity, but it had taken 10 years or more.

"Immune escape conjures this catastrophic failure of immunity when it is really immune erosion," Bloom says. "Right now it seems like SARS-CoV-2, at least in terms of antibody escape, is actually behaving a lot like coronavirus 229E."

Others are probing SARS-CoV-2 itself. In a preprint published this month, researchers tinkered with the virus to learn how much it has to change to evade the antibodies generated in vaccine recipients and recovered patients. They found that it took 20 changes to the spike protein to escape current antibody responses almost completely. That means the bar for complete escape is high, says one of the authors, virologist Paul Bieniasz of Rockefeller University. "But it's very difficult to look into a crystal ball and say whether that is going to be easy for the virus to acquire or not," he says.

"It seems plausible that true immune escape is hard," concludes William Hanage of the Harvard T.H. Chan School of Public Health. "However, the counterargument is that natural selection is a hell of a problem solver and the virus is only beginning to experience real pressure to evade immunity."

And the virus has tricks up its sleeve. Coronaviruses are good at recombining, for instance, which could allow new variants to emerge suddenly by combining the genomes—and the properties—of two different variants. In pigs, recombination of a coronavirus named porcine epidemic diarrhea virus with attenuated vaccine strains of another coronavirus has led to more virulent variants of PEDV. "Given the biology of these viruses, recombination may well factor into the continuing evolution of SARS-CoV-2," Korber says.

Given all that uncertainty, it's worrisome that humanity hasn't done a great job of limiting the spread of SARS-CoV-2, says Eugene Koonin, a researcher at the U.S. National Center for Biotechnology Information. Some dangerous variants may only be possible if the virus hits on a very rare, winning combination of mutations, he says. It might have to replicate an astronomical number of times to get there. "But with all these millions of infected people, it may very well find that combination."

Indeed, Katzourakis adds, the past 20 months are a warning to never underestimate viral evolution. "Many still see Alpha and Delta as being as bad as things are ever going to get," he says. "It would be wise to consider them as steps on a possible trajectory that may challenge our public health response further." ■

Residents line up outside a vaccination center in Sydney, where a rapidly growing outbreak of the highly contagious Delta variant of SARS-CoV-2 led officials to order a new lockdown in June.

PHOTO: SAEED KHAN/AFP/GETTY IMAGES

Evolving threat

Kai Kupferschmidt

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