measured by the locomotor activity and pupil size, differed at all three hierarchical levels.

Bugeon *et al.* performed a principal component analysis — an approach that reduces the dimensionality of large data sets by identifying their most important features (named principal components). The analysis revealed a single transcriptomic principal component that could predict how the activity of different cell subtypes would be modulated by brain states. This transcriptomic axis correlates with specific physiological properties[10] of the cells, such as their membrane time constant (a measure of the time taken for a change in electrical potential across the cell membrane) and rheobase (a measure of neuronal excitability). It also correlates with the level of expression of genes that encode cholinergic receptor proteins, which are involved in modulating neuronal activity. These findings will help us to better understand the complex roles that diverse inhibitory neurons have in circuit function, and how different subtypes of neuron interact with one another.

This study begins to address a long-standing question in neuroscience — why are there so many subtypes of inhibitory (and excitatory) neuron in the cortex? Although the results are encouraging, there is still a long way to go to fully answer this question and understand whether different cell subtypes all have unique roles.

Bugeon *et al.* deployed a limited set of visual stimuli while the animal was in one of just a few simple states. Future studies that use more diverse and naturalistic visual stimuli, and engage animals in behavioural and learning tasks, are likely to reveal more differences in how neurons respond to visual cues during different phases of behaviour at the type and possibly subtype levels[11,12]. For instance, it is likely that more-complete analysis of size and contrast tuning in vision will differentiate types within each subclass. This in turn will enable a deeper examination of the relationship between a cell's behaviour and its gene expression.

The approach Bugeon *et al.* have taken is generalizable, and so can be applied to other regions of the brain — researchers should now take the opportunity to extend Bugeon and colleagues' work to all cell types in all layers of the visual cortex. Finally, combining their method with in-depth morphological, connectional and physiological characterization of cells[1] could enable researchers to explore how cell types interact to form the circuits that govern complex processes such as sensory perception, behaviour and learning.

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The authors declare no competing interests.
This article was published online on 6 July 2022.

Virology

100-year-old pandemic flu viruses yield new genomes

Martha I. Nelson & Elodie Ghedin

Lung samples housed in medical archives have yielded three genomes for the influenza A virus that caused the 1918 global pandemic. The sequences reveal mutations that might have triggered the pandemic's devastating second wave.

The last time a mysterious respiratory virus brought the world to its knees was in 1918, when a pandemic caused by an influenza A H1N1 virus spread around the globe. The evolutionary steps taken by that H1N1 virus are of particular interest today, as the COVID-19 pandemic continues. To investigate the virus's evolution, Patrono *et al.*[1] scoured museum archives in Europe for century-old lung samples containing RNA from the 1918 virus. Writing in *Nature Communications*, the researchers describe the fruits of their labour — genome sequences for three 1918 H1N1 viruses. Their work suggests that the 1918 flu virus acquired mutations during the first wave of the pandemic in the Northern Hemisphere's spring of 1918 that might explain why the second wave in autumn was so deadly.

"The authors' work indicates that pandemic viruses evolve dynamically during the early stages."

It took almost a decade to complete the first genomic analysis of a 1918 influenza virus, which was obtained in the 1990s from the lung tissue of a woman buried in permafrost in Brevig Mission, Alaska[2]. This analysis revealed that the 1918 pandemic virus came from a flu virus circulating in birds, and later allowed scientists to reconstruct the virus to study the immune responses that it triggered in animal models[3]. A second full genome was generated in the years that followed[4]. But both of these reconstructed genomes came from people who died in the United States during the deadly autumn wave of the virus — only short RNA fragments have been available for viruses from Europe[5] and from the pandemic's first wave. This lack of information has made it difficult to piece together the strain's evolution.

Since the 1990s, there have been huge advances in sequencing technology. These improvements enabled Patrono *et al.* to efficiently sequence one complete genome and two partial genomes from 1918 viruses found in human lung samples that were preserved in formalin in museum archives in Berlin (Fig. 1). Two of these genomes came from people known to have died in June 1918, before the pandemic's second wave, enabling the authors to study changes in the virus over time.

The new sequences exhibit several genomic differences compared with the US strains, including in the viral polymerase complex — a set of genes that is key to viral pathogenicity. The authors therefore reconstructed the viral polymerase complex from the European and Alaskan viruses, and compared their function *in vitro*. The complex from the Alaskan virus was twice as active as the European version, suggesting that the observed genomic changes might have optimized the virus for replication in a human host, explaining the autumn wave's increased deadliness.

All human influenza A viruses circulating currently contain genes that trace their origins to the 1918 virus[6]. But the origins of one gene, which encodes the viral H1 protein (the external spike targeted by host immune responses), has been less clear. The influenza A virus genome comprises eight segments that can be swapped in their entirety when two different strains of the virus infect a cell together.

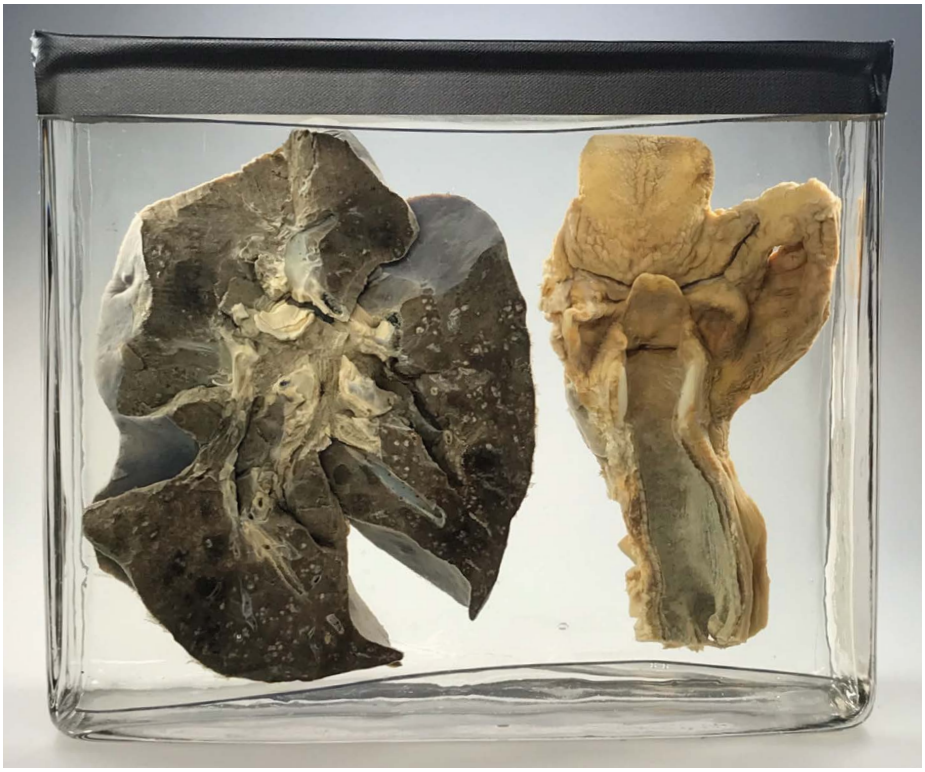
This process, called reassortment, means that genes from the same virus can have markedly different evolutionary histories. Differences in the shape of phylogenetic trees inferred for different influenza A genes previously led researchers to surmise that reassortment occurred between the 1918 H1N1 virus and another virus from which the current H1 protein originated, creating a new, mixed virus that spawned subsequent seasonal outbreaks[7].

However, correctly classifying a virus as having undergone reassortment relies on an accurate phylogenetic tree, which is trickier for influenza A viruses because they evolve at variable rates — more slowly in horses and faster in pigs[8]. To complicate matters, several host switches occurred around the time of the 1918 pandemic. Bayesian statistical methods that harness previous knowledge about the rates at which a virus evolves in different species can be used to correct erroneous trees[9]. But the rate of viral evolution can also vary between lineages that infect people.

Patrono *et al*. observed a faster rate of evolution along one branch of the viral tree than in other regions. This long branch represents a gap of unsampled viruses that circulated between 1918 and the earliest-sequenced seasonal H1N1 viruses[8] from the 1930s. When the authors accounted for the rate difference, trees for all genes had similar branching patterns and showed little evidence of reassortment — instead suggesting that currently circulating seasonal flu viruses descended directly from the strains that caused the 1918 pandemic.

However, Patrono and co-workers outline strong caveats for their conclusions. Bayesian statistical approaches are powerful because they incorporate empirical data and observations from the real world, but sometimes those data are unreliable. The lack of sequences for flu viruses that infected people in the 1920s makes it difficult to pinpoint the timing and cause of rate acceleration. Going forward, understanding the flu pandemic in its full context will require viral sequences from the years that followed — and preceded — 1918.

Sequences of flu viruses from before 1918 could hold the key to understanding why the 1918 pandemic killed so many healthy adults in their twenties. Childhood flu infections can have lasting effects on future immune responses. Currently, researchers must rely on immune signatures retained in human sera (the fluid component of blood) to surmise which flu strains had previously infected the adults killed in the 1918 pandemic. But immunological data can vary between individuals and between laboratories, making it difficult to interpret and reconcile the data with mortality information taken from death certificates[10]. Patrono and co-workers do not tackle serology, or the complex question of whether other H1 flu viruses or different influenza subtypes circulated before 1918 (ref. 9), leaving the door open for future investigations.



NAVENA WIDULIN/BERLIN MEDICAL HISTORY MUSEUM OF THE CHARITÉ

Figure 1 | Century-old lungs from medical archives in Berlin. Patrono *et al*.[1] used samples from these lungs to generate genomic sequences for the influenza A virus that caused the 1918 pandemic.

Lessons from previous pandemics, including their progression in successive waves[11], have guided policy responses to pandemics in the twenty-first century. Patrono and colleagues' work indicates that pandemic viruses evolve dynamically during the early stages, as they optimize replication in people. Many countries did not prioritize genetic sequencing during the early stages of the COVID-19 pandemic, because mutations that reduce vaccine effectiveness were expected to emerge only after several years. Had we known more about the opening months of the 1918 pandemic, we might have been less surprised by the mutations in SARS-CoV-2 that increased viral replication and altered the course of the pandemic during the first year. High-throughput genomic-surveillance pipelines might have been established much earlier in the United States and other countries.

Increases in funding and infrastructure for genomic sequencing during the COVID-19 pandemic have enabled a rapid response to the catastrophe. By contrast, the task of maintaining and cataloguing medical archives receives much less attention, which presents a serious bottleneck to efforts that aim to sequence historical pathogens. The COVID-19 pandemic is a stark reminder that future pandemics are on the horizon — to prepare, we must unlock key lessons buried in the past. Patrono and colleagues' study shows that the trove of archival specimens from previous pandemics is not yet exhausted. But museums low on funding continue to decommission archival collections at an alarming rate. Can genomic advances outpace archival losses? The race is on.

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The authors declare no competing interests.
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This article was published online on 6 July 2022.