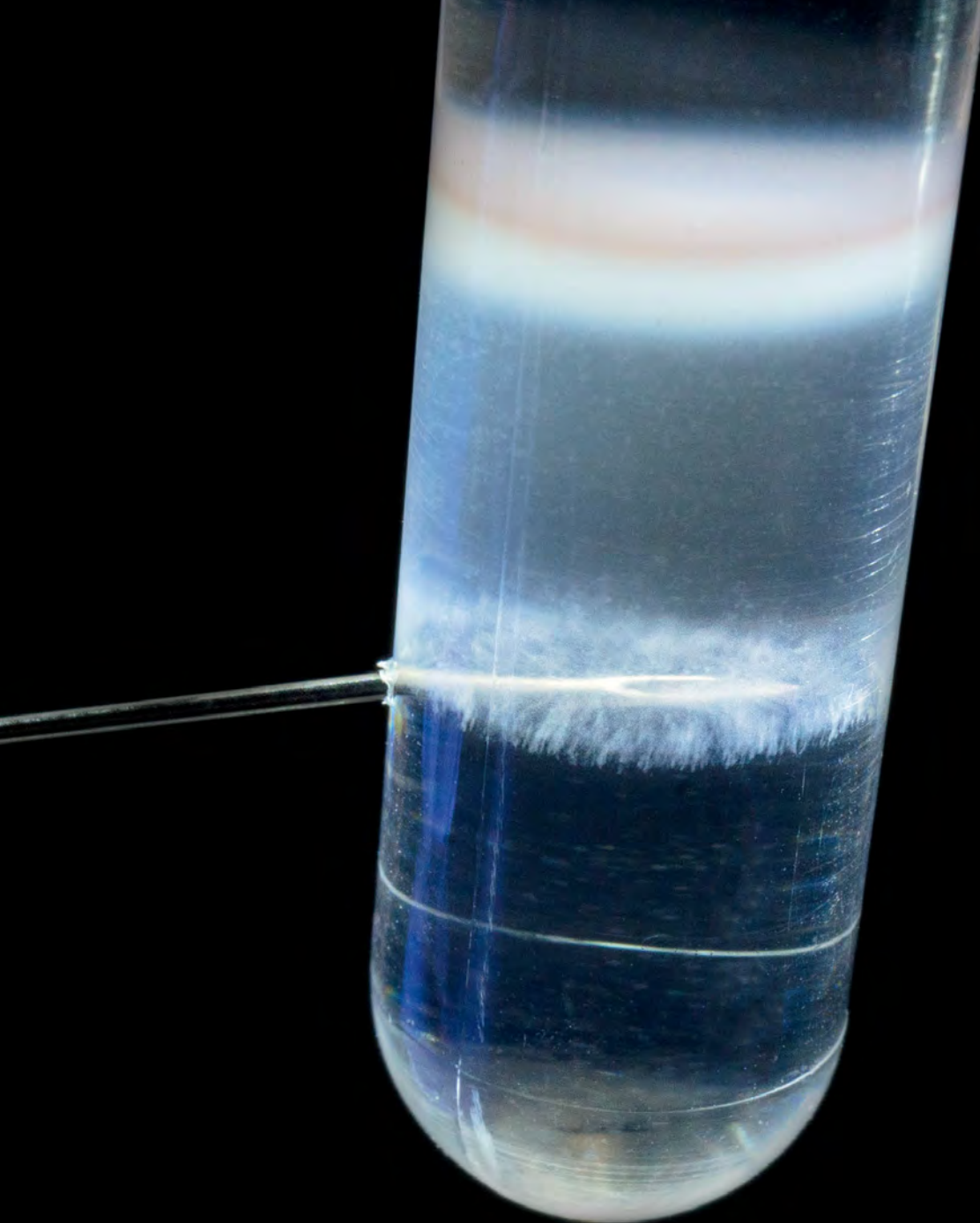




# Tiny giants

Viruses are usually incredibly small, but some deviate from the norm and reach sizes greater than that of a bacterial cell. **Matthias Fischer** from the **Max Planck Institute for Medical Research** in Heidelberg is one of a small number of scientists working on giant viruses of this kind.

TEXT **STEFANIE REINBERGER**



In the laboratory of Matthias Fischer at the Max Planck Institute in Heidelberg, vials containing water samples are lined up against one another, each containing a whole world of aquatic single-celled organisms and viruses. The labels reveal the origins of the samples: Guenzburg, Kiel, but also more exotic locations such as Tallinn or the British Virgin Islands. “The collection is the result of many years of work,” the microbiologist explains.

Although they look like nothing more than vials of water to the naked eye, the samples are actually teeming with life, which only becomes visible when viewed through a microscope: countless tiny dots are scurrying back and forth. “The smaller ones are bacteria, which are devoured by larger cells that have a nucleus. These so-called protists are the reason we created the collection in the first place,” Fischer explains. Indeed, these protists are susceptible to attack by giant viruses, which are Fischer’s

As giant viruses are about the same size as bacteria, it is almost impossible to purify them by filtration only. However, as viruses and bacteria have different densities, they form layers when spun in an ultracentrifuge. Scientists can then extract the viral band using a syringe and needle.



Global microcosms: each vial teems with single-celled organisms and viruses isolated from different environments. Wheat grains in the vials stimulate the growth of bacteria, which in turn serve as a food source for protists.

main interest. Together with his colleagues, he therefore screens the waters of the Danube, the Baltic Sea or the Indian Ocean for single-celled organisms that can act as hosts for giant viruses.

### A VIRUS DISGUISED AS A BACTERIUM

These viruses may be tiny by human standards, but they are the giants of the virus world. Indeed, they are so much bigger than other viruses that scientists initially mistook them for bacteria. The first example of these viral giants was discovered by researchers in 1992 in an amoeba found in the water circuit of an industrial cooling tower in Bradford (UK). Assuming the virus to be a bacterium, the researchers initially named it *Bradfordcoccus*.

It was not until 2003 that researchers at Aix-Marseille University realized that they were actually dealing with a virus – one that was even bigger than some bacteria, with a diameter of 750 nanometers. Moreover, for a virus, it also had a huge genome: with 1.2 million base pairs, it was twice as big as the largest known virus genome to date and encoded some 1,000 genes. By comparison, influenza viruses and HIV carry about a dozen genes.

The French researchers gave the giant a new, more suitable name: mimi-virus, from mimicking microbe – a virus that pretends to be a bacterium. Many other giant viruses have since been discovered in oceans, salt lakes, sewage treatment plants, tree barks, forest soils and permafrost. Although the virus particles differ widely in terms of their genomes, structures and shapes, they have one thing in common: they

blur the previously firmly established boundary between cells and viruses.

The unwritten rule among virus researchers was that viruses had a maximum diameter of 200 to 300 nanometers. Virus experiments were therefore carried out using small-pore filters that only allowed representatives meeting the common size definition to pass. As a result, giant viruses were simply filtered out of the sample material along with bacteria and other single-celled organisms – and therefore quite simply overlooked. “Ultimately, the fact that they remained undiscovered for so long is caused by our own preconceptions. One of the lessons giant viruses teach us is therefore to always question our assumptions if we genuinely want to discover something new,” says Matthias Fischer.

### A GROWING ARMY OF GIANT VIRUSES

Although giant viruses have received greater attention in recent years, there are still only a few scientists worldwide who study them. This may be due to the exotic nature not only of the viruses themselves, but also of their host organisms. Protists play an important role in the world’s food webs, but only few species are of medical or economic significance, such as the pathogens responsible for malaria, sleeping sickness and toxoplasmosis.

So far, hardly any research has been conducted into the majority of protists, many of which are also hosts of giant viruses. The circle of colleagues with whom Matthias Fischer can discuss ideas and exchange knowledge and methods is thus rather small. Often, the

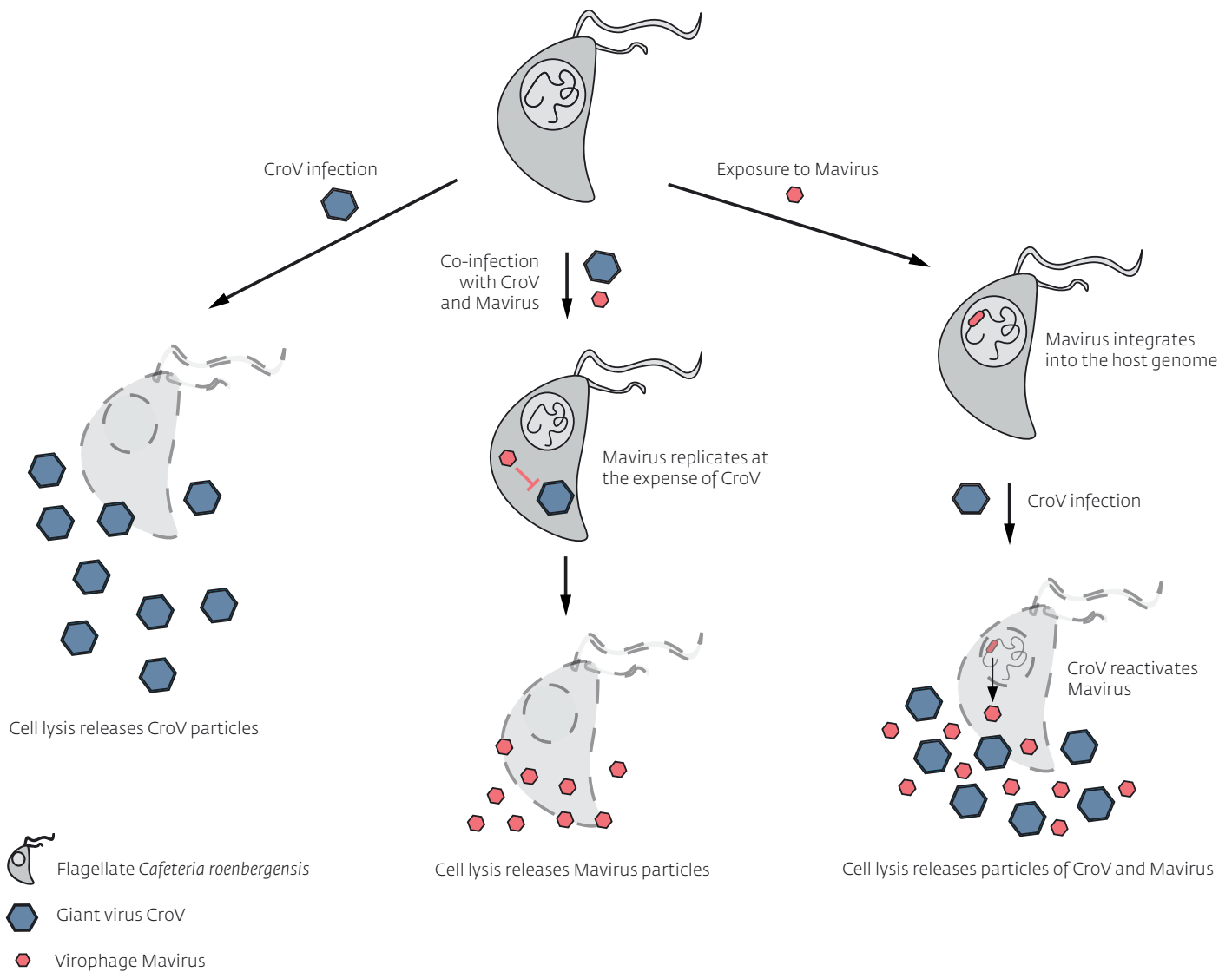
Matthias Fischer uses pulsed-field gel electrophoresis to separate the genomes of giant viruses for further analysis.

standard laboratory techniques don't work for giant viruses or their hosts. In that case, Fischer has to spend a great deal of time finding alternatives – or new techniques altogether. “On the upside, however, there's a good chance we'll stumble across something new and unexpected in each of our projects,” he says.

You could describe the 42-year-old microbiologist as a modern-day discoverer. He has a sparkle in his eye as he talks about the unsolved mysteries of giant viruses, and you can sense his eagerness to crack as many of them as possible. And there is no shortage of riddles surrounding these giants. One question is how many different types of giant viruses remain to be discovered, another one is their unresolved evolutionary origin and why they are so big in the first place.

For some time, the question as to their origin has divided the small community of researchers into two camps. Some believe the giant viruses emerged from cells that gradually lost most of their genetic material. With the remaining genes, the diminished cells were no longer able to multiply on their own and became dependent on the machinery of other cells. Giant viruses may therefore be the remnants of an otherwise extinct domain of life, alongside bacteria, archaea and eukaryotes (cells with a nucleus). “However, our research findings – and those of colleagues – suggest that giant viruses rather evolved from smaller viruses,





One host, two viruses: when the giant virus CroV infects its host, the flagellate *Cafeteria roenbergensis*, it multiplies inside the cell and kills it at the end of the infection cycle (left). If the flagellate is simultaneously infected with the virophage Mavirus, the giant virus is unable to produce new infectious particles and instead, Mavirus replicates. Although the infected cell ultimately dies, other host cells are protected from CroV infection by the action of Mavirus (center). Mavirus alone cannot multiply in the flagellate, but it incorporates itself into the host's genetic material, where it can persist for many generations until CroV infection reactivates the virophage (right).

continually incorporating new genes from other organisms while also duplicating their own," says Fischer.

### NEW TERRITORY FOR RESEARCHERS

Fischer's personal interest in viruses was sparked towards the end of his degree in biochemistry in Bayreuth, when he came across a scientific article on the diversity of viruses in the ocean. "I was absolutely fascinated because, until

then, I had no idea that every liter of seawater contains several billion virus particles," Fischer recalls. He then contacted the author of the article, Curtis Suttle, in Vancouver. "I applied for a doctoral position, booked a flight and stayed for six years."

This work set the course for Fischer's further scientific career. The first years in Vancouver demanded a great deal of perseverance. The young doctoral student from Germany set out to study a new virus, *Cafeteria roenbergensis* virus

»» The small virus operates like a defense system, protecting the flagellates from the giant virus. Not all viruses are therefore parasites of their host organisms; rather, some viruses exist in a mutually beneficial symbiosis with their hosts.

(CroV), which later turned out to belong to the same family as the mimiviruses. CroV infects a single-celled organism by the name of *Cafeteria roenbergensis*, a widespread species of marine zooplankton that feeds on bacteria and is one of the most numerous predators on the planet.

### TOXIC DNA

At the time, researchers had known about the giant virus for a number of years but hadn't investigated it further. It was Fischer's job to change that. Initially, however, luck didn't appear to be on his side: he planned to decode the viral genome by incorporating pieces of it into bacteria in order to multiply and decipher them piece by piece – but this experiment failed repeatedly. The viral DNA was apparently toxic for the bacteria. A few years later the arrival of new sequencing technologies would allow him to finally read more than 600,000 letters of the virus genome.

In the meantime, Fischer had other problems to deal with: the virus yield from his infection experiments was often so low that he couldn't use it for further studies. But this circumstance ultimately led to an important discovery, which continues to occupy the scientist to this day. Fischer realized that CroV itself is infected by a much smaller virus – a so-called virophage by the name of Mavirus. As Mavirus only multiplies within a host cell that is simultaneously infected with CroV, it does not harm the host. On the contrary, because Mavirus acts as a parasite of CroV,

it prevents the production of new giant viruses and thereby helps its host *C. roenbergensis* to survive albeit indirectly. This single-celled organism typically dies upon infection with CroV, which is a lytic virus: once it has multiplied in sufficient quantities, the host cell bursts and the viral offspring are released. However, when CroV is parasitized by the smaller Mavirus, the cell releases new Maviruses particles instead of CroV particles. Although this doesn't make a difference for the infected cell, it matters for its not yet infected neighbors: they are spared from infection with CroV and the population survives.

### PROTECT YOUR KIN

"The small virus therefore acts like an altruistic defense system for the host cell in order to protect members of the same species against the giant virus," says Fischer. "Among microorganisms, it seems to be a widespread principle that single individuals are sacrificed to ensure the survival of the population as a whole." Not all viruses are therefore parasites of their hosts; rather, a mutually beneficial symbiosis exists between *C. roenbergensis* and Mavirus.

Fischer wanted to know more about this unusual alliance. Together with his team, the scientist began to study the infection cycle of Mavirus in greater detail. In the process, the Heidelberg-based researchers made an astonishing discovery: the small virus uses a protein known as an integrase to insert its own genetic material into that of the host cell. So far, this enzyme had only been



Particles of the giant virus CroV (blue) and the Mavirus virophage (red, false color).

known to occur in retroviruses such as HIV and related jumping genes – known as transposons – but not in DNA viruses such as virophages.

For Mavirus, this appears to be a good strategy: if the virus does not rely on CroV for the incorporation of its genetic material, it can be passed from one cell generation to the next even without the giant virus. “This ensures that Mavirus is already present when a suitable giant virus infects the host cell,” says Fischer. For multiplication, however the virophage depends on the giant virus and its replication machinery.

## PARASITES ARE OMNIPRESENT

But does the behavior Fischer observed in the lab really play a role in natural ecosystems? To answer this question, the researcher analyzes *C. roenbergensis* genomes from various water samples. So far, he found Mavirus in all of them. And that’s not all: other virophages are also present in the single-celled organism’s genetic material. Some of the incorporated DNA is very similar to that of Mavirus, while others represent hitherto-unknown virophages. Moreover, some of the virophage genomes are interrupted by transposons. In other words, even virophages – which are parasites of parasites – have their own parasites.

Step by step, Fischer and his team are decoding an interwoven chain of single-celled organisms, viruses and parasitic DNA elements. For him, this is the crucial point. “Parasites that infect other parasites are a widespread phenomenon in nature,” says Fischer. “They are a key driver of evolutionary processes.” The reason for this is that every parasite leaves traces in its host,

either because the host needs to adapt or because it assimilates some of its parasite’s genes and capabilities.

Accordingly, parasites provide their hosts with new genetic information – and therefore new traits. This so-called horizontal gene transfer by viruses plays a central role in the evolution of microorganisms.

It may also have been the driving force for the evolution of giant viruses from smaller viruses. Ultimately, how-

ever, this principle applies to all living things, as the human genome is also littered with parasitic elements, such as transposons or remnants of viruses. In other words, viruses and other parasites played a substantial role in human evolution, too. “This is an incredibly exciting realization,” says Fischer. “Although our research into giant viruses and virophages seems exotic at first glance, it provides us with deep insights into universal processes of evolution.” ◀

### SUMMARY

- Giant viruses can contain over 1,000 genes and presumably evolved from smaller viruses, adopting their hosts’ genetic material as well as duplicating genes of their own.
- Giant viruses are primarily parasites of single-celled eukaryotes (protists). However, they are sometimes infected with parasites themselves: virophages use the giants in order to multiply within the host cell.
- With the ability to incorporate their own genes into foreign genetic material, some viruses modify their hosts’ genome, thus acting as a driving force of evolution.

### GLOSSARY

**Giant viruses:** These unusually complex viruses primarily infect single-celled eukaryotes. The largest specimens are approx. 2 micrometers long and contain over 1,000 genes, whose functions render giant viruses independent of the host cell in many biochemical processes. The particles (capsids) of giant viruses are made up of multiple layers consisting of hundreds of proteins, with special exit portals for the packaged virus genome. Despite their global distribution and diversity, the ecological role of giant viruses is still unknown.

**Transposons:** DNA fragments that can alter their position in the genome. They are also referred to as “jumping genes,” although they often contain multiple genes. Transposons that multiply via an RNA intermediate are known as retrotransposons. These are probably also the origin of retroviruses, which incorporate themselves into the host’s genetic material but can also infect other hosts by forming infectious virus particles.

**Virophages:** So far, only a handful of virophages are known. In addition to Mavirus, examples include Sputnik and Zamilon, which are parasites of mimiviruses. Virophages can only multiply in host cells that are infected with a suitable giant virus at the same time. They replicate at the expense of giant viruses by hijacking some of their enzymes. In other words, virophages are parasites of giant viruses and therefore beneficial to their host cells.

# Setting signs!



Ingo Barth is a senior researcher, and deaf. In daily communication, tech terms such as »tunnel ionization« are giving him hard times as a scientist, because often matching elements are lacking in German sign language. The Foundation supports his project at the Max Planck Institute for Microstructure Physics to develop a German STEM sign language dictionary to enhance equal opportunities and diversity in our research environment.

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