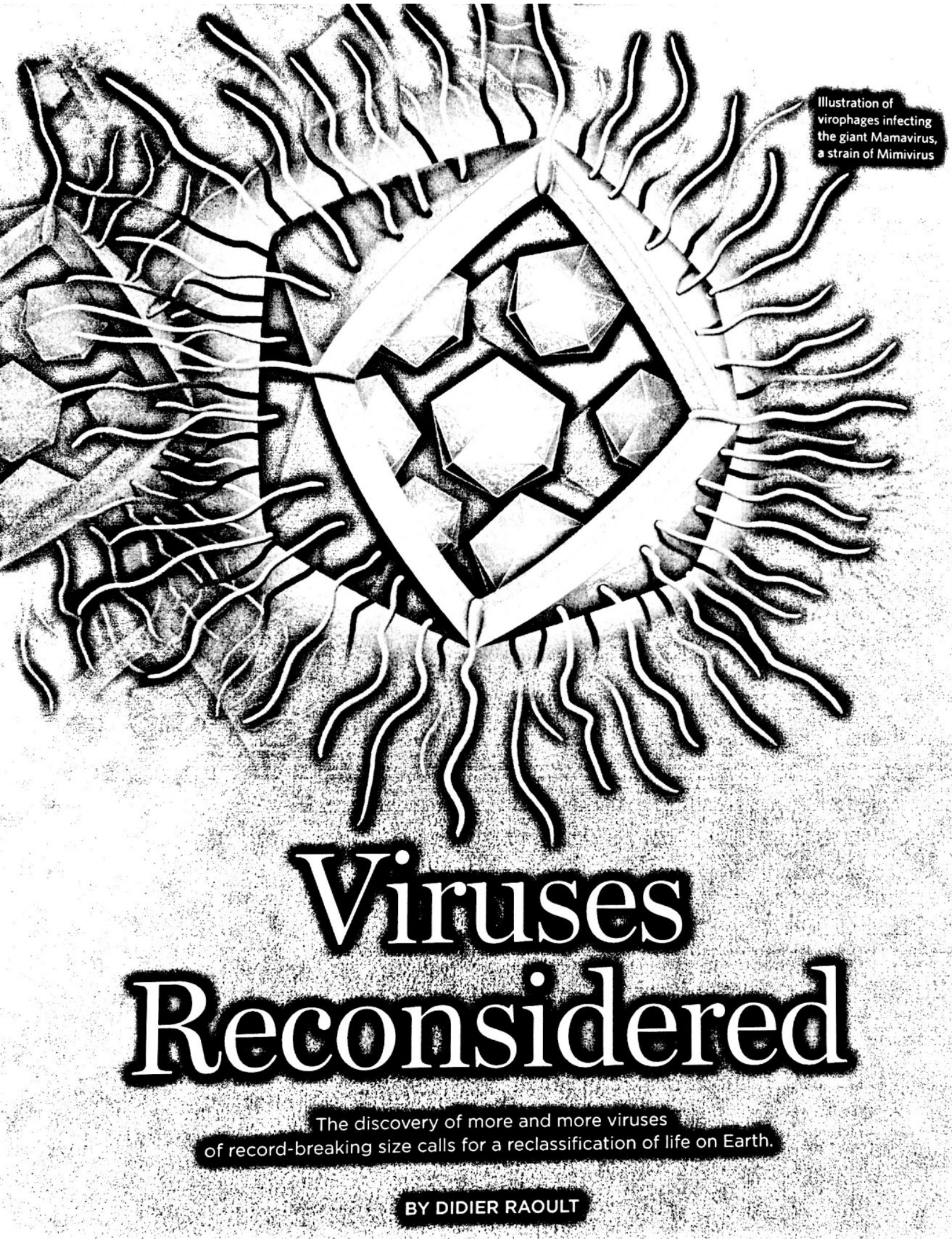

Illustration of virophages infecting the giant Mamavirus, a strain of Mimivirus

Viruses Reconsidered

The discovery of more and more viruses of record-breaking size calls for a reclassification of life on Earth.

BY DIDIER RAOULT

The theory of evolution was first proposed based on visual observations of animals and plants. Then, in the latter half of the 19th century, the invention of the modern optical microscope helped scientists begin to systematically explore the vast world of previously invisible organisms, dubbed "microbes" by the late, great Louis Pasteur, and led to a rethinking of the classification of living things.

In the mid-1970s, based on the analysis of the ribosomal genes of these organisms, Carl Woese and others proposed a classification that divided living organisms into three domains: eukaryotes, bacteria, and archaea. (See "Discovering Archaea, 1977" on page 80.) Even though viruses were by that time visible using electron microscopes, they were left off the tree of life because they did not possess the ribosomal genes typically used in phylogenetic analyses. And viruses are still largely considered to be nonliving biomolecules—a characterization spurred, in part, by the work of 1946 Nobel laureate Wendell Meredith Stanley, who in 1935 succeeded in crystallizing the tobacco mosaic virus. Even after crystallization, the virus maintained its biological properties, such as its ability to infect cells, suggesting to Stanley that the virus could not be truly alive.

Recently, however, the discovery of numerous giant virus species—with dimensions and genome sizes that rival those of many microbes—has challenged these views. In 2003, my colleagues and I announced the discovery of Mimivirus, a parasite of amoebae that researchers had for years considered a bacterium.[1] With a diameter of 0.4 micrometers (μm) and a 1.2-megabase-pair DNA genome, the virus defied the predominant notion that viruses could never exceed 0.2 μm. Since then, a number of other startlingly large viruses have been discovered, most recently two Pandoraviruses in July 2013, also inside amoebas. Those viruses harbor genomes of 1.9 million and 2.5 million bases, and for more than 15 years had been considered parasitic eukaryotes that infected amoebas.[2]

Now, with the advent of whole-genome sequencing, researchers are beginning to realize that most organisms are in fact chimeras containing genes from many different sources—eukaryotic, prokaryotic, and viral alike—leading us to rethink evolution, especially the extent of gene flow between the visible and microscopic worlds. Genomic analysis has, for example, suggested that eukaryotes are the result of ancient interactions between bacteria and archaea. In this context, viruses are becoming more widely recognized as shuttles of genetic material, with metagenomic studies suggesting that the billions of viruses on Earth harbor more genetic information than the rest of the living world combined. (See "Going Viral," *The Scientist*, September 2013.) These studies point to viruses being at least as critical in the evolution of life as all the other organisms on Earth.

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A giant discovery

Despite the fact that viruses use the same genetic code as verifiably living things, science long classified them as mere collections of biomolecules. And because scientists assumed that viruses had both an upper size limit of just 0.2 μm and a parasitic nature, they classified them in a not-quite-biological world of their own.

That thinking started to change in the early 2000s, when my colleagues and I identified an unknown virus living inside an amoeba. It was as big as some bacteria and archaea and was visible under an optical microscope—qualifying it as a microbe under Pasteur's original definition. I named it Mimivirus as a personal joke about the stories that my father, a biomedical scientist, told me when I was a child to explain evolution; the stories were based on the life of "Mimi the amoeba." I initially disguised the true source of this name, however, pretending that Mimivirus came from "MiMicking microbe."

Researchers had first noticed Mimivirus in 1992, but based on its appearance under light microscopy it had been considered an intracellular bacterium for several years. Transmission electron microscopy images depicting its ultrastructure, along with the determination of its genome sequence in 2004,[3] however, confirmed that it was, in fact, part of the viral world. Mimivirus has no ribosomal genes, but its genome contains more than 1,200 genes—three times more than any virus known at the time. Its genome is larger than that of many bacteria and archaea and comparable to some eukaryotic genomes. Mimivirus was no ordinary virus.

Unlike most other viruses, Mimivirus carries genes that encode translational machinery for replication. Its genes encode what is known as the "viral factory," a ribosome-less transcription-and-translation system that copies the Mimivirus genome and assembles viral offspring. The system gathers nucleotides from the virus's host, draining the amoeba's nucleus of its own DNA and digesting it into individual nucleotides that are reassembled into Mimivirus sequences. Within about 16 hours, the amoeba is dead, and a single virus has replicated into 10,000 particles in an incredibly rapid and dynamic process.[4]

Since this initial discovery, scientists have isolated a number of other giant viruses from hosts such as algae, flagellates, and other amoebae. Genes identified in metagenomic studies hint at the presence of such giant viruses all over the world. Researchers are now searching in earnest for new giant viruses, and numerous groups are scouring myriad environments for more clues about this new viral form. In our laboratory alone, we have found more than 50 different types of giant virus belonging to two new viral families: Mimiviridae and Marseilleviridae.

Analyzing the genomes of these viruses, we realized that they are chimeras—in other words, these giant viruses exchange genetic material with their

Computer-generated image of a Sputnik virophage particle

VIRAL PARASITES

Given the size of newly discovered giant viruses, perhaps it should come as no surprise that we have even identified parasitic viruses that infect giant viruses.[1] We found the first one—dubbed Sputnik by my colleague Bernard La Scola of Centre National de la Recherche Scientifique (CNRS) in France—serendipitously in 2006 when examining a new strain of Mimivirus, named Mamavirus, using transmission electron microscopy. In those images, we saw a small virus infecting the giant virus. I named these new virus-infecting viruses virophages, echoing the term bacteriophages, which is used to describe viruses that infect bacteria.

Sputnik replicates using the viral factory that Mamavirus creates within its amoeba host. An analysis of Sputnik's 18-kilobase genome showed that, like its viral host, it also takes genes from different hosts. Other researchers have since found virophages in the giant virus known as the *Cafeteria roenbergensis* virus (CroV) and the alga-infecting large DNA viruses called phycodnaviruses. The presence of virophages in these viruses appears to inhibit the growth of the giant virus, which would otherwise kill the host cell. Parallel examples are seen in the bacteriophages, with those viruses often mediating the effects of bacterial parasites on eukaryotic hosts. Thus, like other viruses, virophages may play a critical role in interspecies dynamics and entire ecosystems.

And virophages aren't the giant viruses' only parasites. We also discovered a parasitic element that I named the transpoviron.[2] Equivalent to transposons—the selfish jumping genes observed in bacteria—the transpoviron also multiplies into millions of copies in the viral factory created by giant viruses. This transpoviron can jump into the genome of the virophage or of the giant virus to be reproduced within the amoeba host. These gene-shuffling parasites make giant viruses a hotbed of diversity. Virophages, the transpoviron, and previously identified self-splicing introns have appropriately been named the "mobilome" of giant viruses and are critical for transferring genes among viruses.

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2. C. Desnues et al., "Provirophages and transpovirons as the diverse mobilome of giant viruses," *PNAS*, 109:18078-83, 2012.

hosts and with other parasites that share the same hosts. The viruses also share several genes with large DNA viruses, a class of viruses too small to be viewed under an optical microscope. Large DNA virus genomes range from 50 to 200 kilobases and have similar architecture: a variable region comprises genes from other organisms, and a more stable region is unique to the viruses. This genome architecture is largely shared by the giant viruses known to science, prompting the field to classify them as a new order, Megavirales, and place them as a sister group to large DNA viruses in the viral tree.[5] Extreme diversity in the genomic variable regions favors the hypothesis that these virus lineages are as old as those of other microbes on Earth.

A fourth branch of life?

Living particles that could be seen under the optic microscope were first lumped into one category, the microbes; then split into two categories, when Édouard Chatton distinguished between eukaryotes and prokaryotes in the first half of the 20th century; and next grouped into three categories, when Carl Woese added archaea to the tree of life in 1977. Now, I propose that there are four.[6]

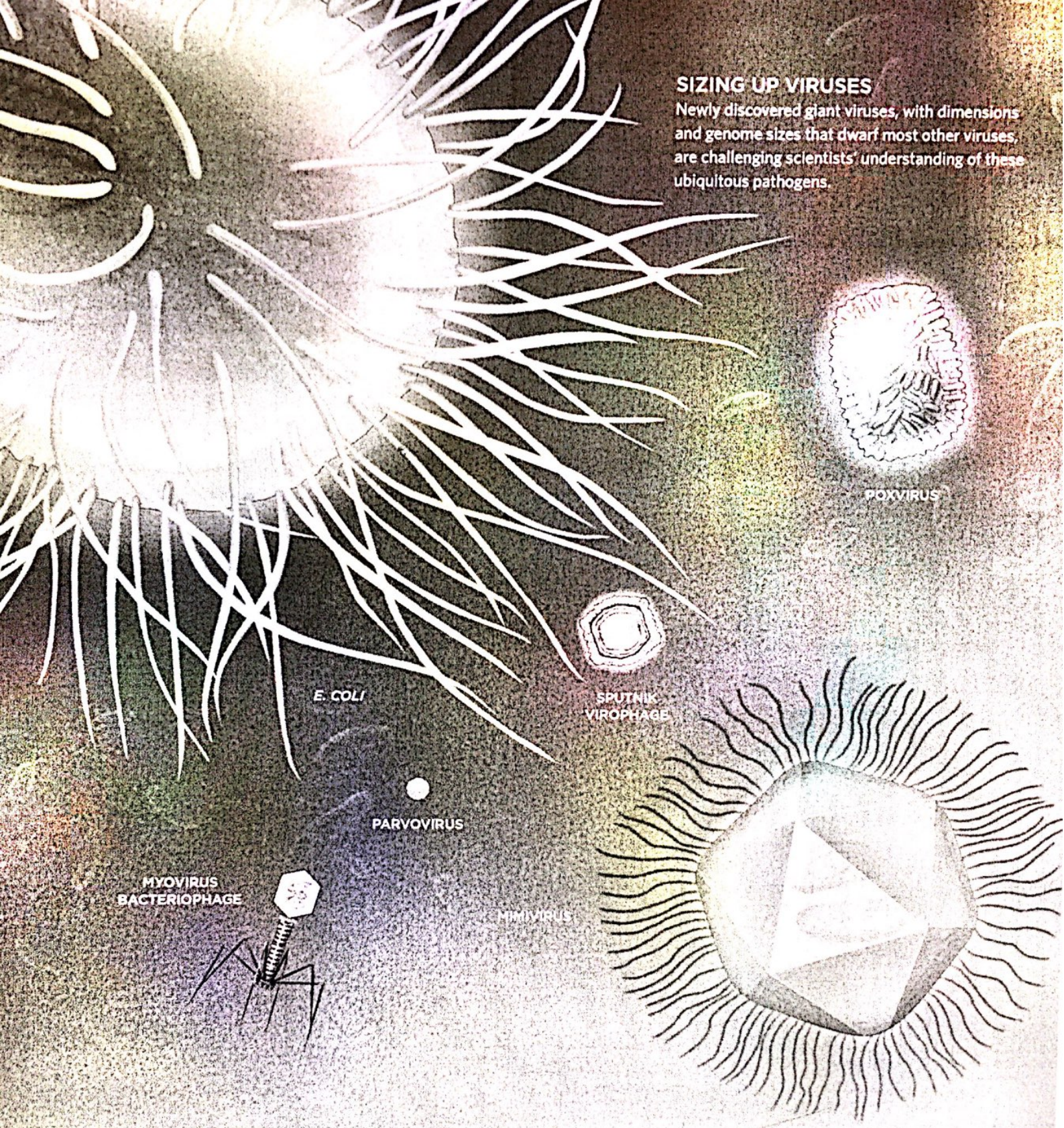
The available data show that some well-conserved genes of giant viruses could help reconstruct the evolutionary history of viruses. Of course, phylogenetic trees are typically constructed using ribosomal genes, which are totally absent in viruses. Viruses do, however, carry genes for transfer RNA and RNA polymerase. Trees constructed using these genes show that viruses are at least as old as the three traditional domains proposed by Woese. By clustering genes with a known function we found that four groups with different genetic repertoires emerged, corresponding to giant viruses, archaea, bacteria, and eukaryotes. In this way, giant viruses should take their place among microbes—and, more importantly, among the living—as a fourth branch of life. Whether viruses should be classified as a new domain, a superkingdom, or, as I suggested recently, TRUC (Things Resisting Uncomplete Classification), which means "stuff" in French slang, is still a matter of debate.[7]

By incorporating giant viruses into phylogenetic trees, we are now able to begin understanding their role in the evolution of life. Some giant virus genes are highly similar to each other, and to those of other microbe groups, suggesting these genes—and giant viruses—have an ancient origin. Some researchers, such as Patrick Forterre of Paris-Sud University and the Institut Pasteur in France, believe that giant viruses are the origin of the eukaryotic nucleus;[8] the previously established theory of viral eukaryogenesis posits that large DNA viruses played this role.

Forterre and others even speculate that DNA was "invented" by viruses, helping to convert a world of RNA-based organisms to one where DNA became the pervasive unit of heredity. In part due to its catalytic potential, RNA is hypothesized to have been the molecular basis of first life on Earth. (See "RNA World 2.0,"

SIZING UP VIRUSES

Newly discovered giant viruses, with dimensions and genome sizes that dwarf most other viruses, are challenging scientists' understanding of these ubiquitous pathogens.



PARVOVIRUS

- At just 18–26 nm in diameter, these are some of the smallest viruses on Earth.
- Linear, single-stranded DNA; averaging 5,000 base pairs

SPUTNIK VIROPHAGE

- 74 nm in diameter
- Circular double-stranded DNA; 18,343 base pairs

MYOVIRUS BACTERIOPHAGE

- Head diameter of 50-110 nm
- Linear, double-stranded DNA; 33,600 to 170,000 base pairs

POXVIRUS

- Around 200 nm in diameter and 300 nm in length
- Linear, double-stranded DNA; 130,000 to 375,000 base pairs

MIMIVIRUS

- Approximately 400 nm (0.4 μm) in diameter
- Linear, double-stranded DNA; 1.2 million base pairs

E. COLI

- About 2.0 μm long and 0.25–1.0 μm in diameter
- Circular DNA molecule; 4.6 million base pairs

ACANTHAMOEBA POLYPHAGA (NOT PICTURED)

- 15 to 35 μm in length
- Host in which Mimivirus was first discovered

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on page 34.) Forterre argues that early RNA cells and ancient RNA viruses, perhaps derived from these early cells, coexisted at that time and that early RNA cells were likely to have been parasitized by these viruses. Evolving a genome of DNA could have guarded these viruses against attacks from their hosts, which may have begun to evolve RNA-specific defenses to protect themselves against viral infection. Then, as viruses borrowed and returned the genetic material of their hosts, they would have shared DNA genes, which are more stable and would have therefore been favored by natural selection.

While all of these theories are just that, and much work is needed to understand the origin and evolution of life on Earth, it is clear that the long-neglected viruses are central to answering these questions. ■

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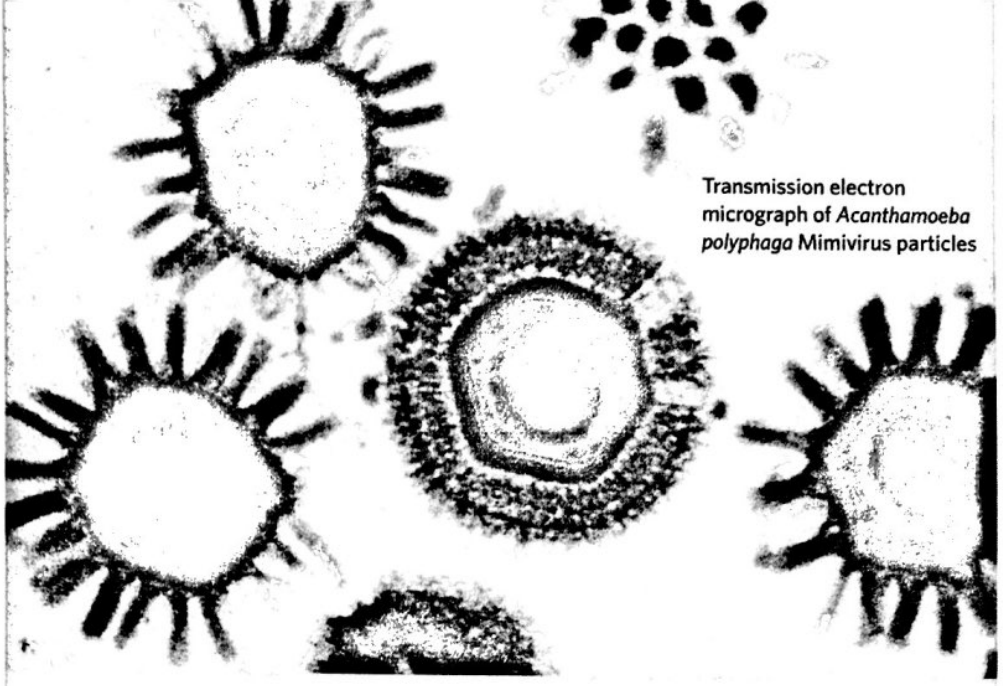

Transmission electron micrograph of *Acanthamoeba polyphaga* Mimivirus particles

A GIANT PROBLEM?

The discovery of giant viruses has also forced a reassessment of the world of experimental virology. Giant viruses have been missed in metagenomic studies of viruses because the first step of these studies is to filter out anything larger than 0.2 micrometers in diameter. For example, when virologists Forest Rohwer of San Diego State University and Jeffrey Gordon of the Washington University School of Medicine in St. Louis sequenced the virome of human stool, they were actually sequencing the "mini"-virome—they missed the giant viruses.[1] Indeed, when my lab investigated the gut microbiota of a young Senegalese man, we found DNA evidence of Mimivirus and the amoeba-infecting giant Marseillevirus, suggesting that these giant viruses may be regular inhabitants of the human microbiome. Furthermore, when we studied the virome of healthy blood donors, sans filtration, we found Marseillevirus.[2]

The role of these viruses in human health and disease is now an important question facing the field of virology. In our lab, a technician working with Mimivirus came down with pneumonia, and after testing his own serum for Mimivirus antibodies, he came into my office and told me he believed he got sick from his contact with the giant virus.[3] Indeed, antibodies against Mimivirus have been reported in other cases of pneumonia.[4,5] Additionally, a strain of Mimivirus was isolated from the lung of a pneumonia patient, though the virus's causative role remains to be confirmed. Meanwhile, however, Marseillevirus has been identified as the culprit in a case of one young child's adenitis, an infection of lymph nodes. Following surgery to remove an 11-month-old boy's enlarged lymph node, it was analyzed to rule out cancer and identify any pathogens. Only Marseillevirus DNA was detected in the node, and the child had antibodies to the giant virus in his blood.

It remains unclear, however, how commonly giant viruses cause acute illness. In addition to being entirely overlooked until recently, the viruses harbor so much gene variability that they can be difficult to detect via PCR. Further investigation is needed to determine the incidence and importance of giant viruses for public health.

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