

Out on a limb

From giant viruses to unexplained marine DNA, there may be more to the tree of life than the three domains currently defined.



The e-mail arrived two weeks after Jonathan Eisen's paper was published. Tongue-in-cheek, it read: "Welcome to the 'Fourth Domain' club." Eisen, an evolutionary biologist at the University of California, Davis, chuckled. His paper¹,

which came out in March, hinted at bizarre new forms of microscopic life in the ocean. The e-mail was from Didier Raoult, a biologist at the University of the Mediterranean in Marseilles, France, who for years has argued that the colossal viruses he has identified belong to a unique, hitherto unknown, branch of life: the fourth domain.

Raoult believes that this domain branched off from the very base of life's evolutionary tree — a declaration that has been met with strong scepticism. Researchers disagree with the way he interprets sequence data. The debate highlights how hard it is to decipher the original relationships between microorganisms that have swapped, tinkered with and generally scrambled their DNA over billions of years.

Whether or not the fourth domain exists, the hunt for evidence has turned up several surprises. Raoult's microbes blur the boundaries between viruses — generally considered non-living things because they cannot function on their own — and the cellular organisms that host them, reigniting a debate about what constitutes life. Raoult says that some viruses are sophisticated enough to warrant special consideration. Eisen's approach, which sifted DNA from thousands of seawater samples, picked out sequences from — well, nobody quite knows what. "I would call it the dark matter of the biological universe," says Eisen of the countless unidentified DNA sequences lurking in the environment. "There is potentially enormous diversity out there, and this is the way to look for it."

CELLULAR DIVISION

Before the advent of DNA sequencing, biologists divided life into two domains based on cellular structures. Eukaryotes, which encompass everything from amoebas to trees to humans, have large cells with internal structures such as nuclei and mitochondria. Prokaryotes are smaller and for the most part lack these structures. But in 1977, Carl Woese, a microbiologist at the University of Illinois at Urbana-Champaign, turned taxonomy upside down when he used RNA sequences to show

BY GWYNETH DICKEY ZAKAIB that prokaryotes comprise two very different groups: bacteria, which are distantly related to the eukaryotes, and archaea, which sit much closer to them on the evolutionary tree². Woese faced

considerable backlash from the scientific community. It wasn't until the mid-1980s that his theory was accepted, bolstered by mounting genetic and molecular evidence. The textbooks were duly rewritten to feature three domains: eukaryotes, bacteria and archaea.

Raoult's claim for a fourth domain stems from his study of giant viruses. He and his colleagues identified the first of these³, Mimivirus, in 2003. The microbe had been found years before, infecting amoebas in the water of a cooling tower in Bradford, UK, but it was so big that it was mistaken for a bacterium. When Raoult and his colleagues couldn't identify it from its DNA, they looked at it under a microscope — and saw a virus.

The discovery stunned microbiologists. At 750 nanometres, Mimivirus was the first viral particle to be visible under a light microscope. "It was an exciting moment," says Jean-Michel Claverie, an evolutionary microbiologist at the University of the Mediterranean, who collaborated with Raoult on the discovery and analysis of Mimivirus. "People realized we didn't know anything about microbial biodiversity."

In 2004, Raoult and his group published the genome of Mimivirus⁴. At 1.2 million base pairs, its genome was at least twice as large as the largest known viral genome. And some of its more than 1,000 genes were involved in translating genetic information into proteins, something never before seen in viruses because they usually must rely on host translation machinery. The group drew up phylogenetic trees by comparing sequence information for seven proteins from Mimivirus with counterparts from organisms in the three domains. The giant virus, they wrote "appears to define a new branch", suggesting that it arose from an ancestor that could have predated the eukaryotes. According to Raoult, some viruses might deserve this special status on the tree because of their complexity and genetic make-up.

Objections ensued. David Moreira and Purificación López-García of the University of Paris-Sud in Orsay, France, included theirs in a list of ten reasons why viruses should be excluded from the tree of life⁵. For one thing, viruses can steal genetic material from their hosts in a

The outer capsid of Mimivirus: this giant was originally mistaken for a bacterium.

C. XIAO ET AL. PLOS BIOL. 7, E1000092 (2009)

process called horizontal gene transfer, which could be the source of the protein-translation genes.

Raoult, meanwhile, had found more giant viruses, including one in a Parisian cooling tower. Last year, he published a paper⁶ that incorporated genetic data from the newly discovered giant viruses and from some other known large DNA viruses into phylogenetic trees. Because the sequences from these viruses seem to cluster together on their own branch of the tree, he argued, their genes must have been inherited together through evolution, and the group, called nucleocytoplasmic large DNA viruses (NCLDVs), shared a common ancestor (see 'Family trees').

But resolving lineage using genetic sequences is not straightforward, particularly over great evolutionary distances. Over time, genes can mutate one way and later revert back to their former sequences, erasing the evolutionary trail of breadcrumbs that scientists try to follow. Two species can also independently evolve highly similar gene sequences, even though they aren't related, in a process called convergence. So organisms such as the NCLDVs could appear to belong together on close branches when really they are farther apart.

In June, Eva Heinz, an evolutionary biologist at Newcastle University, UK, and her colleagues published a response to Raoult's redrawn trees⁷. They reanalysed his data and built fresh trees based on models that account for artefacts such as convergence. The new trees showed the NCLDVs sprouting out as 'twigs' from several different branches, rather than being constrained to one branch. Heinz and her co-authors interpreted this to mean that the genes in the NCLDVs could have been stolen from various hosts on those branches through horizontal gene transfer. "With ancient phylogenetics, the model you use really matters," says Tom Williams, one of the co-authors, who is also at Newcastle. "When we used more biologically realistic models, we got a different tree that fitted the data better." And that tree had no fourth domain.

Raoult isn't convinced. It is true that large viruses do not branch together on the new trees, he says, but in his opinion, neither do the eukaryotes. "Maybe their analysis is not able to cluster organisms that have the same origin," he says. Heinz disagrees, saying that the eukaryotes do cluster together in their analysis. So Raoult is currently standing alone. "I'm ready to believe many exotic scenarios," says Claverie, "but my feeling is that at this point we don't have enough data, especially on those large viruses."

GIANT HUNTING

Raoult is still trying to work out how his huge viruses evolved. One idea is that they could have come from a more complex ancestor that was later reduced to a parasitic shell. Raoult continues to search for new giant viruses and wants to use their genome sequences to work out when this group of viruses emerged, based on a molecular clock deduced from the viruses' mutation rates. And he avoids conversing with members of the virology community who are unwilling to challenge the 'dogma' of their field. "I'm more excited by discussions with the people trying to put a little bit of disorder in the way we think about biology," he says.

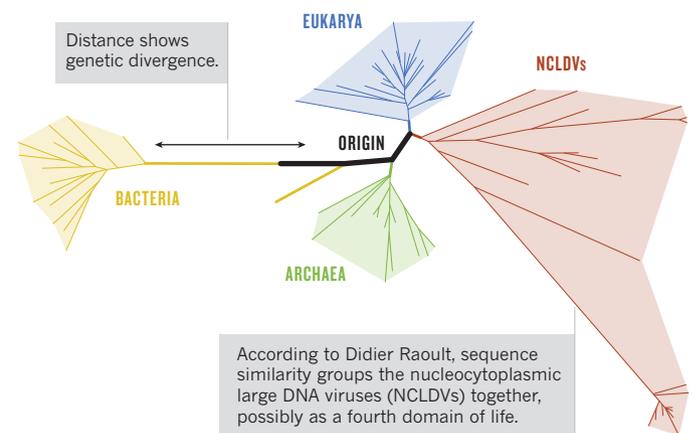
Woese says he can relate to this. "You've got to hang those ideas out there and take what comes," he says. But although Woese's iconoclastic ideas stood up to the criticism back in his day, he says he is less hopeful for Raoult's fourth domain.

So where does this leave Eisen? In his March study, he and his team probed seawater samples from the J. Craig Venter Institute's Global Ocean Sampling Expedition, an around-the-world venture that aims to find novel organisms by sequencing the mix of marine DNA. Most researchers doing environmental genomics search for organisms closely related to those already known, but Eisen cast a wider net. And when he drew up phylogenetic trees based on his haul, several unknowns stood out. A few looked like they might belong in Raoult's fourth branch, whereas others poked out from other branches. But with neither the time nor the funds to identify the

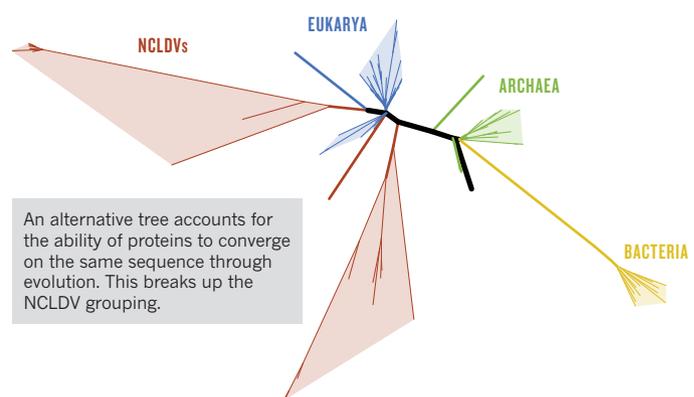
Family trees

Phylogenetic trees try to show how species are related based on similarities in a common gene or protein sequence. In theory, the more similar the sequences, the more closely the organisms are related. But different models of the same sequence, such as the protein RNA polymerase II mapped here, can turn up different results.

BRANCHING OUT



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organisms containing the sequences, Eisen's team published the paper¹ detailing the methods and sequences in order to seek help from others.

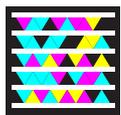
Eugene Koonin, a computational evolutionary biologist at the National Center for Biotechnology Information in Bethesda, Maryland, has done a preliminary analysis of the data that he says turned up nothing too unusual. "Quite a few of these sequences may represent very interesting new branches of bacteria and archaea," he says. But he ultimately sees it as a strong negative result: "They were hunting for the fourth domain but their net came back empty."

Eisen, who has yet to see Koonin's data, is not ready to sign up for Raoult's club. Rather, he says he published his data as a challenge to other researchers to be alert to what is truly unique in environmental samples, rather than discarding it as an artefact, as they often do. And there is plenty left to be discovered within the archaea and bacteria, says Francisco Rodríguez-Valera, a microbiologist at the Miguel Hernández University in Alicante, Spain. "There is a huge amount of microbial diversity that is unknown," he says. "I don't think we need to discover a new domain every ten years to convey to the general public the fact that microbes are important." ■

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