

climate change. The “4 per 1000” Initiative (which aims to increase carbon storage in soil by 0.4% annually), launched at the 21st Conference of the Parties in Paris (<http://4p1000.org>), and the latest report of the Intergovernmental Panel on Climate Change indicate that soils have the potential to store annually a substantial amount of carbon – on a scale relevant in comparison to the emission of carbon dioxide through human activities¹⁰. Achieving this high storage goal, and, in particular, deciding where and how to focus our efforts (see go.nature.com/3chahft), requires an understanding of underlying biological processes in the soil^{11,12}.

The fate of carbon that enters the soil, that is, whether it is emitted or stored, depends on the diversity and function of the soil microbial community. Bacteria produce compounds that ‘glue’ soil particles together, thereby forming aggregates in which carbon is stored. Fungi incorporate more carbon in their biomass than the amount they release through respiration, and this carbon is trapped in soil aggregates as recalcitrant ‘necromass’ (dead biomass)¹³. Guerra and colleagues’ map shows, in a spatially explicit way, carbon storage together with the diversity of the soil organisms involved. This information will surely help efforts to discover and understand the soil microbial and environmental conditions under which carbon storage takes place, and how that might be optimized.

A next step in developing future versions of this map should be to gather data representing other locations, so as to capture further soil types, forms of land use and climates. The Global Soil Biodiversity Observatory, an initiative that aims to monitor and forecast the status of soil biodiversity and soil health (go.nature.com/3ea7tgc), might contribute to future efforts to monitor soil biodiversity and to the further development of this highly useful soil biodiversity map of the world.

Peter C. de Ruiter is at Biometris, Wageningen University, 6708 PB Wageningen, the Netherlands. **Elly Morriën** and **P.C.d.R.** are at the Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 1090 GE Amsterdam, the Netherlands. e-mails: peter.deruiter@wur.nl; w.e.morrien@uva.nl

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Microbiology

Mystery find of microbial DNA elements called Borgs

Christian Rinke

Microorganisms store some genetic information on non-chromosomal elements. The emergence of a surprising version of these elements shifts our understanding of their diversity and potential roles. **See p.731**

Most microorganisms have a single chromosome that harbours almost all of their genetic information^{1,2}. Extra genomic information is frequently encoded in non-chromosomal DNA segments called extrachromosomal elements (ECEs), which exist in the form of structures such as plasmids that consist of DNA in a linear or circular form. Al-Shayeb *et al.*³ report on page 731 the discovery of unusually large ECEs containing numerous and diverse genes that encode proteins involved in metabolic activity. The authors hypothesize that these ECEs increase the capacity of their microbial hosts to consume the greenhouse gas methane.

The authors discovered 19 types of large ECE when analysing samples, such as of wetland soil, underground (sediment-associated) water and discharges from mines, taken from sites in the United States. The cells that are thought to host the ECEs are from the genus *Methanoperedens*, a group of archaea – unicellular microorganisms that are evolutionarily distinct from bacteria (Fig. 1). These ECEs are inferred to replicate in *Methanoperedens* cells, and have, like most ECEs, a tendency to acquire genetic information from other organisms and their environment. To highlight this ability to assimilate genes, especially from their *Methanoperedens* host, Al-Shayeb and colleagues named these ECEs Borgs, after a group of aliens in the science-fiction franchise Star Trek – creatures that assimilated thousands of species from across the Galaxy.

Most of the recovered Borg sequences are partial representations of these ECEs, but there are four complete sequences, which share a common genome organization. The sequences were inferred to be linear and to range in size from 662 to 918 kilobase pairs of DNA.

Most of the Borg-encoded proteins corresponded to unknown hypothetical proteins,

but approximately 21% matched archaeal proteins, most of which (on the basis of gene- and protein-sequence similarity and other characteristics) were assigned to *Methanoperedens*. Al-Shayeb and colleagues ruled out the idea that these sequences represent previously unknown archaeal genomes, because of their near-complete lack of characteristic archaeal genes – marker genes or genes encoding components (ribosomal proteins) of the machinery that makes proteins. The authors conclude that these novel sequences cannot exist independently in microbes, and must be bona fide ECEs. The abundance ratios between Borgs and their potential hosts ranged from 2:1 to 8:1, suggesting that *Methanoperedens* cells can harbour multiple copies of the same Borg. Interestingly, several samples with relatively high abundances of *Methanoperedens* lacked Borgs, supporting the idea that these ECEs are associated only with specific types of *Methanoperedens* host.

Borgs are probably not chromosomes. Neither do they qualify as viruses, because of their lack of sequences encoding recognizable proteins needed for the construction of a protective viral coat, which is essential to encase the viral genetic material and to help viruses to infect cells⁴. Thus, these large ECEs probably represent plasmids. Plasmids in the form of bacterial or archaeal DNA molecules are, in most cases, smaller than their host’s chromosome(s) and generally dispensable, and they typically encode proteins that provide functions which give their host an advantage under specific conditions^{5,6}. All of these criteria might apply to Borgs, and, therefore, on the basis of their large size, Borgs can be classified as ECEs called megaplasmids.

Numerous bacteria and archaea harbour megaplasmids. These large ECEs have greater

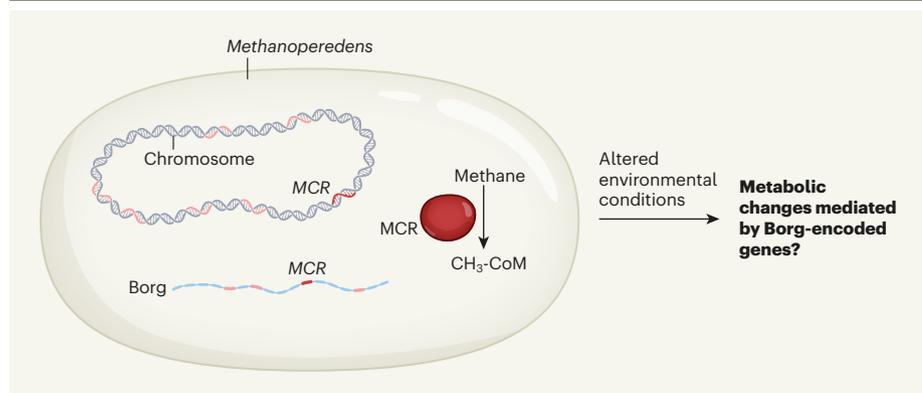


Figure 1 | *Methanoperedens* species contain unusually large extrachromosomal elements (ECEs).

Genetic information in *Methanoperedens* species – microorganisms in a group called archaea – is stored on a circular chromosome. However, Al-Shayeb *et al.*³ discovered that some of these species also encode genetic information on ECEs, which the authors called Borgs. It seems that, like most ECEs, Borgs tend to acquire genes, especially from their *Methanoperedens* hosts (red DNA). *Methanoperedens* and some Borgs encode enzymes that could contribute to the oxidation of the greenhouse gas methane to produce the molecule CH₃-CoM (although whether these enzymes are expressed from Borgs remains to be determined). The key enzyme required for this oxidation reaction is MCR, and all subunits of this enzyme are encoded in the host and in two Borgs identified by the authors. Al-Shayeb *et al.* suggest that the Borg-encoded genes might expand the gene repertoire and boost the host's ability to modulate its metabolic capacity under various environmental conditions. For example, MCR might be preferentially produced from the host or from the Borg depending on substrate availability, if a system exists similar to the one used by some microbes to regulate production of different versions of MCR depending on hydrogen concentration²⁰.

genomic flexibility than do chromosomes, which enables ECEs to acquire numerous genes from other microbes through a process called horizontal gene transfer⁷.

One of the largest linear plasmids recovered so far was found more than a decade ago, in bacteria of the genus *Streptomyces*. These megaplasmids are up to 1.8 megabase pairs in size and are densely packed with genes that encode enzymes for antibiotic production^{6,8}. Another bacterial lineage known for megaplasmids of up to 1.2 megabase pairs in length is the genus *Sphingomonas*. These megaplasmids encode enzymes involved in the degradation of molecules such as hydrocarbons and organochlorides, and they contribute to the metabolic flexibility of this genus⁹.

Previously identified archaeal megaplasmids, mainly known from salt-loving archaea, are up to 698 kilobase pairs in size^{10,11}. Some archaeal megaplasmids carry genes deemed essential for cell viability, and have been termed minichromosomes, blurring the terminology boundary between plasmids and chromosomes¹². Overall, the limited number of archaeal megaplasmids reported so far makes it difficult to discern general features of these ECEs. However, compared with currently known archaeal megaplasmids, Borgs stand out because of their distinctive characteristics. They represent the largest-known archaeal plasmids and the first examples of linear megaplasmids that are currently associated with the archaeal domain of life. In addition, they are characterized by a range of repeating DNA sequences of unknown function.

Borgs encode enzymes that have a broad

spectrum of metabolic functions, including enabling reactions such as the oxidation of methane. *Methanoperedens*, like many other archaea, have an important role in the global carbon cycle – a key process regulating Earth's climate, in which methane is a major compound¹³. Methane is produced and consumed by archaea in large quantities. Indeed, archaea account for more than 50% of the methane produced globally per year¹⁴. However, methane release into the atmosphere is also combated by methane-consuming microbes, and in oxygen-deprived environments, this role is carried out by microbes such as *Methanoperedens*. These microbes break down methane using a pathway¹⁵ that depends on an enzyme called MCR. Two of the newly discovered Borgs have sequences that encode all of the subunits of MCR.

This discovery of MCR-encoding genes in ECEs is remarkable. Several Borgs also encode enzymes involved in the biosynthesis of a non-protein 'helper' molecule (a cofactor) needed for the catalytic function of MCR, and for enzymes involved in the biosynthesis of tetrahydromethanopterin, which is a component, called a coenzyme, that is required for methane production and consumption in oxygen-lacking (anaerobic) habitats¹⁶. These genes are also encoded in chromosomes of *Methanoperedens* species.

Al-Shayeb and colleagues suggest that ECE-encoded genes expand the host's generic repertoire and might enable the host to extend its metabolic functions under different conditions. One ECE, named the Lilac Borg, encodes 16 multi-haem cytochrome molecules. These

cytochromes might enable *Methanoperedens* species to transfer electrons during methane consumption. The authors speculate that the high number of copies of these genes increases the capacity of *Methanoperedens* species to metabolize methane. Experimental validations are needed to prove that this is the case and to show that the Borg-encoded genes are transcribed as a response to changing environmental conditions.

A major roadblock to validating Borg function in *Methanoperedens* species or any other archaeal lineage that can metabolize methane in the absence of oxygen is the lack of a way to culture these cells *in vitro*. Although several systems to enrich this type of microbe have been established, some of which have archaeal communities dominated by methane-metabolizing microbes¹⁷, none has been reported to contain large linear ECEs. A preprint study describes large plasmids associated with *Methanoperedens* in bioreactors¹⁸. However, these plasmids are described as being circular, up to only 192 kilobase pairs in length, and occurring in *Methanoperedens* species that do not host Borgs. Therefore, the great hunt for Borg-containing microbes grown using conditions *in vitro* begins.

Until such cultures are established, researchers will probably depend on culture-independent methods to examine *Methanoperedens* and their ECEs in environmental samples. In doing so, methods such as long-read DNA sequencing should be used to improve the assemblies of the partial sequences of Borgs. This approach would help to reveal the composition of the many repeated DNA sequences, and to confirm or disprove Borg linearity.

The physical location of Borgs inside *Methanoperedens* cells could be verified using techniques such as cell-sorting-based single-cell genomic approaches. This would enable the physical separation and DNA sequencing of single microbial cells and the determination of their entire genome, including chromosome(s) and ECEs¹⁹. In addition, methods such as metatranscriptomics to analyse gene expression under a range of conditions could help to decipher whether and, if so, when, Borg-associated genes are expressed in their hosts. Such evidence might support the hypothesis that Borg-encoded enzymes extend the conditions under which *Methanoperedens* can metabolize methane.

Borgs are an exciting discovery based on the interpretation of genome sequences. Their large size, linear nature, repeat-rich features and numerous metabolic genes expand the repertoire of known megaplasmids. It will be exciting to see whether Borgs affect the carbon cycle in their native habitats, in particular regarding the consumption of methane. It may be that, in contrast to those in Star Trek, the Borgs in our Universe use assimilation to save rather than to conquer a world.

Christian Rinke is at the Australian Centre for Ecogenomics, University of Queensland, St Lucia, QLD 4072, Australia.
e-mail: c.rinke@uq.edu.au

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Evolution

Early gills exchanged ions before hosting gas transfer

Dorit Hockman

During evolution, key physiological changes enabled vertebrates to achieve a more active lifestyle. A comparison between living animals challenges current ideas on the timing of one such change in our ancestors. **See p.699**

Gas exchange and ion regulation are vital biological processes. In vertebrates, gas exchange transfers life-supporting oxygen to the blood from the air and removes carbon dioxide. Ion regulation controls the movement of essential ions, such as sodium and calcium, into and out of cells. The relative levels of ions regulate the pH in cells, affecting cellular function. Changes in how these functions were performed were crucial to the evolution of modern vertebrates, but the timing and nature of the changes remain unclear. On page 699, Sackville *et al.*¹ provide insights that adjust some major pieces in this puzzle.

The current view is that, in our pre-vertebrate marine ancestors, ion and gas transfer was initially performed by the skin² (Fig. 1). Gases and ions could easily reach all parts of the body from the skin in these small, relatively inactive creatures. In this scenario, the first gills were used chiefly for filter-feeding – sieving edible particles from the water. Subsequently, early in vertebrate evolution, around 500 million years ago, the gills became the main site for both gas exchange and ion regulation^{2,3}. This shift from the skin to the gills as the site of these processes removed a constraint on animal size, because blood circulating through the gills offered a way to deliver oxygen to the rest of the body.

This enabled vertebrates to become the large, active creatures that exist today.

This theory needs to be tested, but, without travelling back in time to examine the first vertebrates, this is a difficult task. Sackville *et al.* took on the challenge by using the power of comparative biology. Their approach assumes that if an aspect of biology is shared between two living animal species, it was probably also present in their last common ancestor. By comparing where in the body gas exchange and ion regulation probably occur in living vertebrates and their non-vertebrate cousins, the authors built a model of where in the body these key processes were located during vertebrate evolution.

The authors turned first to lampreys (*Entosphenus tridentatus*), which are often referred to as living fossils because they are thought to closely resemble the first vertebrates⁴. Young lampreys, called ammocoetes, are freshwater, burrow-dwelling animals that use their gills for filter-feeding. To measure the levels of gas and ion exchange taking place in different parts of the body, the authors put ammocoetes of varying sizes into a divided chamber, separating their gills from the rest of their bodies. As they tested larger and larger ammocoetes, the authors found that

From the archive

A mysterious plague in frogs, and consideration of the consumption of oxygen after running.

50 years ago

A curious and so far unexplained ailment which killed off millions of frogs in the United States last winter ... may be showing itself again this year ... The symptoms of the ailment seem to indicate that infection of some sort is involved, but it has also been suggested that agricultural chemicals may have done the damage ... [H]eavy rains fell last autumn, and again this year, increasing agricultural runoff just at the time when the frogs entered the waters to hibernate ... [E]vidence to implicate agricultural chemicals comes from [the] finding that frogs caught in September, before they entered the ponds, have hibernated normally and seem to be quite healthy, while those caught in October have died ... So far, however, analyses of lake water samples have failed to turn up evidence of excessive amounts of agricultural chemicals and in any case the bacterial infection itself could have come from the lake waters.

From *Nature* 27 October 1972

100 years ago

In attempting to analyse the factors which underlie muscular efficiency, most observers have been content to concern themselves with a consideration of the oxygen supply. They have devoted themselves to a study of the means by which fuel arrives at the engine rather than to a study of the behaviour of the engine itself ... Prof. A. V. Hill ... was at some pains to point out the errors into which various observers have fallen by neglecting the oxygen consumption which takes place after running stops. ... [I]t is precisely because the oxygen consumption can to a certain extent lag behind the development of energy, it is because the isolated muscle can exert its full strength in the absence of oxygen, that a man can run 100 yards at a much greater speed than he can run 1 mile.

From *Nature* 28 October 1922

