

Parasites go the full monty

Patrick J. Keeling

Energy metabolism is an essential function of life. Yet a resourceful parasite with a minimalist genome has discarded much of its metabolism, developing a unique alternative in the process.

Parasites are well known for stripping themselves down to the bare essentials, but how far will they go? Microsporidia are one group of single-celled parasites that have taken it to extremes and gone the 'full monty'. On page 450 of this issue¹, Katinka and colleagues expose the shocking extent of this striptease in the complete genome sequence of the microsporidian *Encephalitozoon cuniculi*, which parasitizes a range of mammals, including humans. This genome is a mere shadow of those found in other eukaryotes (organisms with nucleated cells), as it consists of only 2.9 million base pairs — less than 0.1% the size of the human genome. This is even smaller than the genomes of many bacteria. Nevertheless, the genome is a treasure trove of information on the powerful reductive forces that shape these unusual parasites.

By any criterion, microsporidia are odd — they can survive only by living inside other cells (Fig. 1), and their method of invading a host cell is truly startling. Microsporidian spores contain a long coiled tube that can be blasted from the spore by a process akin to turning a garden hose inside-out. If this projectile hits a nearby cell, the contents of the spore are forced through the tube and into the cell². This combination of harpoon and hypodermic needle, powered by incredible physical force and requiring seemingly impossible cellular gymnastics, is one of the most sophisticated infection mechanisms in biology.

Yet apart from the structures needed to drive this remarkable machine, and a nucleus to house their DNA, microsporidian spores are pretty spartan. It seems that while they were honing their ability to parasitize other cells, they also abandoned many of the common eukaryotic cell features that biologists take for granted. The *E. cuniculi* genome sequence¹ reflects this reduction at many levels. Missing are genes for a variety of cellular processes and for many metabolic pathways. Moreover, the remaining genes are tightly packed, with little 'junk' DNA between them. Most surprisingly, though, the genes themselves are noticeably shorter than their counterparts in other organisms.

The severe reductionism of microsporidia, together with their amazing specialization, has nearly erased the genomic record of their evolutionary history, making it difficult to determine how they originated. Their simplicity, especially the lack of mitochon-

dria (the powerhouses that convert sugar to energy in cells), gave rise to the compelling idea that microsporidia might be a primitive eukaryotic lineage that evolved before the acquisition of mitochondria³. Early evolutionary trees lent support to the notion, but recent molecular data suggest that this view needs revising. First, genes derived from mitochondria have been found in microsporidia, showing that their ancestors were in fact endowed with mitochondria^{4–6}. Second, most of the evidence from evolutionary trees now points to microsporidia being highly evolved fungi, rather than ancient eukaryotes⁷. The genome should end this debate, by providing an enormous number of new genes with which to examine microsporidian origins. Indeed, Katinka and colleagues¹ have already identified several genes that strongly support a fungal origin for these parasites. So, characteristics previously considered primitive could now be seen as recent adaptations.

Nevertheless, the apparent lack of mitochondria in microsporidia is still of great interest. Mitochondria are the focal point of energy metabolism in most nucleated cells, so how do microsporidia generate energy without them? Until now, we had very little idea. But Katinka and colleagues use the genome sequence to reconstruct the parasite's core metabolism, with surprising results.

From other organisms, we know that core energy metabolism can be cobbled together in slightly different ways as mitochondria degenerate or are lost, but all these variations share similar adaptations⁸. Microsporidia, however, have invented a unique system of core energy metabolism. They use parts of typical mitochondrial metabolic pathways, mixed with pathways found in other organisms that lack mitochondria, resulting in a system that is fundamentally different from either^{1,9}. In particular, microsporidia lack pyruvate:ferredoxin oxidoreductase — a key enzyme in other organisms that lack mitochondria, and a common target for anti-parasitic drugs — and use mitochondrial pyruvate dehydrogenase in its place^{1,9}. Once again, we see François Jacob's image of evolution as a tinkerer¹⁰, using bits and pieces of existing machines to build a completely new one — in this case, something as central to life as a new form of energy metabolism.

The sheer number of genes for mitochondrial metabolic enzymes retained in the

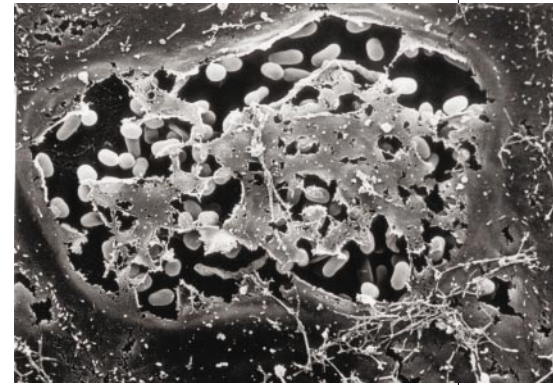


Figure 1 Infection by parasites. A scanning electron micrograph of *Encephalitozoon cuniculi* spores inside a human host cell.

genome is interesting in itself as it raises the question: have microsporidia really shed their mitochondrion, or have we simply failed to recognize it? This debate deepens with the sequencing of the genome, as it is not absolutely clear from the genes alone whether these enzymes operate in a mitochondrion, or whether they have been relocated to the cytoplasm. There is credible evidence either way^{1,9}, but in this case, the gene sequences only raise the questions. The answers await the localization of the enzymes within the cell.

Although it may seem perverse, parasites are probably the most common and diverse form of life on Earth today. Yet we know little about where they come from, how they end up the way they are, or in some cases even what they are. The genome sequence of *E. cuniculi* is an important step towards answering these questions for microsporidia. Before long, genome sequences from *Plasmodium*, *Cryptosporidium*, *Toxoplasma*, *Entamoeba*, *Trypanosoma*, *Giardia* and others will usher in an age of comparative parasite genomics. This is an exciting time for parasitology, and if *E. cuniculi* is an honest herald, many 'rules' are about to be broken. ■

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Cosmology

The matter with density

Trevor Ponman

Theories of how the Universe developed structure assume a similar distribution of matter in small and large structures. But observations of gas densities in galaxy clusters suggest that this is not the case.

Most galaxies, like our own Milky Way, are part of a small group or larger cluster of galaxies. Ordinary baryonic matter accounts for only 10–20% of the mass of such clusters, the rest being made up of mysterious, and as yet unidentified, 'dark matter'. Most of the baryonic matter is intergalactic gas at a temperature of millions of degrees kelvin, with the galaxies themselves accounting for only around 5% of the total mass. Cosmologists predict that the distribution of matter within large clusters should look very similar to that in small ones — the two being related by a simple spatial scaling. But it has been clear for some time that baryonic matter, at least, does not behave like this: the gas density in the inner regions of small clusters is systematically lower than that in large ones. On page 425 of this issue, Voit and Bryan¹ offer an explanation for these observations, which follows with a certain inevitability from the relationship between the entropy of gas and the time it takes to cool.

Modern cosmology says that structure develops hierarchically. Observations of the cosmic microwave background^{2,3} — the relic radiation left over from the Big Bang — reveal the imprint of small fluctuations in matter density from when the Universe was young. According to theorists, these small fluctuations grow steadily with time, as gravity pulls more mass into regions that are already denser than average. Eventually, the densest regions collapse to form stable structures known as haloes, which are dominated by dark matter, and, as the gas within them cools, star formation results, establishing a galaxy at the centre of each dark-matter halo. When small dark-matter haloes coalesce into larger ones, the galaxies they contain may either merge to form larger galaxies or retain their identity, producing a group of galaxies within a single dark halo. Further mergers of these groups create rich galaxy clusters containing many hundreds of galaxies — these are the largest stable structures found in the Universe today.

One consequence of this hierarchical build-up of structure is 'self-similarity', which says that large dark-matter haloes

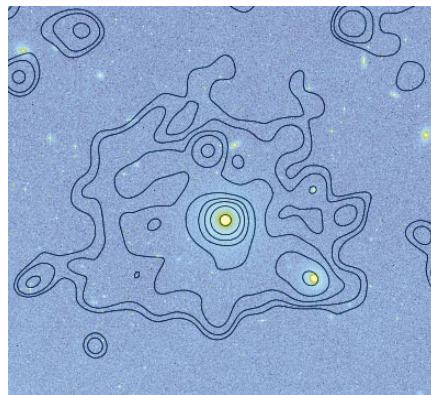


Figure 1 Hot gas in galaxy groups. The contours of X-ray emission recorded by the ROSAT X-ray Observatory are superimposed on an optical image of a galaxy group. Notice the presence of a large central galaxy. The 10-million-degree gas that is responsible for the X-ray emission contains more mass than all the galaxies in the group combined, but its density is lower than expected. Voit and Bryan¹ offer a new explanation of this discrepancy based on the rate at which the gas cools.

are essentially scaled-up versions of small ones. Simple cosmological simulations that include both baryonic and dark matter also predict self-similarity for the distribution of gas within these haloes. Hot, dense gas at 10^6 – 10^8 K emits X-rays, and if self-similarity is true for the gas, then we can expect the X-rays emitted by galaxy clusters (Fig. 1) to have certain scaling properties. For example, larger clusters contain more gas, and have higher characteristic temperatures, and their power output (luminosity, L) in X-rays should scale as the square of the temperature, T . However, it has been clear for many years that this is not the case. The actual relationship is roughly $L \propto T^3$ for large clusters⁴ and is steeper still for small galaxy groups⁵: they are far less luminous than expected. Data from the ROSAT X-ray observatory⁶ revealed the reason for this: the density of gas in the inner regions of small galaxy groups is systematically lower than that in large clusters, contrary to the predictions of self-similarity.

What might explain this discrepancy? Astronomers find it helpful to think in terms of the characteristic entropy of the gas, because this remains unchanged during any adiabatic compression (causing heating) or expansion (causing cooling). Here, entropy is essentially $T/\rho^{2/3}$, where T and ρ are the temperature and density of the gas. The self-similarity model predicts that gas densities should be similar in groups and clusters, so the entropy of the gas should simply scale with T , making it lower in groups, which are cooler. But the entropy of the gas⁶ found in groups is higher than expected, because at a given temperature its density is lower.

These observations support the idea that there is a critical 'floor' value below which the entropy cannot drop. In clusters, most of the gas would naturally lie above this floor value anyway. But in groups, where the entropy is lower as a result of the low temperature, this floor prevents the gas from reaching the high densities required for bright X-ray emission. It has been suggested^{7,8} that this critical value might be set by preheating of the gas — by the energy released in supernova explosions or from quasars — before it was gathered into clusters, but the amount of energy required for this is uncomfortably large.

Voit and Bryan¹ take a different line. They suggest that cooling of the gas, rather than heating, sets the critical value. This is because gas with low entropy cools more quickly than high-entropy gas, so the critical entropy value is set by the gas whose cooling time is equal to the age of the Universe. The authors calculate this critical entropy value, and find that it agrees remarkably well with the observed floor value.

Gas with entropy below the floor value will suffer one of two fates: it either cools and disappears from the hot-gas phase to form stars, or it can be reheated by star formation to above the critical value. Either way, the result is to remove the low-entropy gas that would otherwise dominate the inner regions of clusters and make their X-ray emission more luminous. By simply removing such gas from their models, Voit and Bryan can reproduce the observed luminosity–temperature relation for groups and clusters. To prevent much of the gas in the Universe from cooling within small structures, some form of heating is still required. But the energy needed is likely to be lower than in earlier theories, because the heating is naturally targeted at the low-entropy gas, as this is where star formation, and therefore supernova explosions, occur.

These important insights are not the end of the story. First, we do not yet know the balance between cooling and reheating. In order to establish whether most of the low-entropy gas has been removed by cooling (which removes it from the hot-gas phase altogether) or by reheating (which does not), we need to measure the total gas content of