



Figure 2 Negative refraction confirmed. These data from Parazzoli *et al.*³ compare refraction in their negative-index material (red points) with the response of a normal, positive-index material, Teflon (blue points). Moreover, the experimental data are in fair agreement with simulations (solid lines).

held in place by fibreglass sheets, and current circulating in them produces a negative magnetic response; in addition, thin straight copper strips respond to electric fields, also in a negative manner (Fig. 1). The new ingredients, different in each of the two experiments, are in the manner the data are collected. Parazzoli *et al.* made their measurements in free space, up to 66 cm from their material sample. The data show a clear, well-defined, negatively refracted signal (Fig. 2). Houck *et al.* work within the confines of a planar waveguide, but sample the electromagnetic field at many points. They too observe unambiguous negative refraction. In most people's minds this will settle the long-running argument about the issue of negative refraction, which has also now been lent more theoretical support by extended computations⁹.

Houck *et al.*⁴ go on to tackle another, even more controversial issue. Veselago¹ observed that a slab of negative-index material would focus light, like a lens. Because they measured the transmitted light at many points around the sample, Houck *et al.* were able to verify some limited focusing capabilities of their material. This is an area in which further work can be expected.

It has been said that tackling a new scientific problem is like going into a darkened room. First you fall over the furniture, then you collide with other people in the room; arguments might develop. With time things settle down, as you learn where most of the furniture is and don't fall over so often. Eventually someone finds the light switch and everything becomes obvious.

I think I just heard the click of a switch. ■

John Pendry is in the Condensed-Matter Theory Group, The Blackett Laboratory, Imperial College, London SW7 2AZ, UK.

e-mail: j.pendry@imperial.ac.uk

1. Veselago, V. G. *Sov. Phys. Usp.* **10**, 509–514 (1968).
2. Shelby, R. A., Smith, D. R. & Schultz, S. *Science* **292**, 77–79 (2001).
3. Parazzoli, C. G., Gregor, R. B., Li, K., Kolteneh, B. E. C. & Tanielian, M. *Phys. Rev. Lett.* **90**, 107401 (2003).
4. Houck, A. A., Brock, J. B. & Chuang, I. L. *Phys. Rev. Lett.* **90**, 137401 (2003).
5. Pendry, J. B., Holden, A. J., Stewart, W. J. & Youngs, I. *Phys. Rev. Lett.* **76**, 4773–4776 (1996).
6. Pendry, J. B., Holden, A. J., Robbins, D. J. & Stewart, W. J. *IEEE Trans. Microw. Theory Techniques* **47**, 2075–2084 (1999).
7. Smith, D. R., Padilla, W. J., Vier, D. C., Nemat-Nasser, S. C. & Schultz, S. *Phys. Rev. Lett.* **84**, 4184–4187 (2000).
8. Valanju, P. M., Walsler, R. M. & Valanju, A. P. *Phys. Rev. Lett.* **88**, 187401 (2002).
9. Foteinopoulou, S., Economou, E. N. & Soukoulis, C. M. *Phys. Rev. Lett.* **90**, 107402 (2003).

Genomics

Relative pathogenic values

Julian Parkhill and Colin Berry

The bacterium that causes anthrax has several close relatives. Comparison of their genome sequences should provide insight into the biology of these organisms as agents of disease — and of terrorism.

Particular notoriety has been accorded to *Bacillus anthracis* of late. As the causative agent of anthrax, this bacterium was used in the 2001 postal attacks in the United States¹, and it has reportedly been 'weaponized' as a warfare agent on at least one occasion². On pages 81 and 87 of this issue, Read *et al.*³ and Ivanova *et al.*⁴

bring the power of genomics to bear on efforts to understand this pathogen and its close relatives.

Bacillus anthracis is a member of a group of closely related organisms that includes *B. cereus*, an opportunistic pathogen of humans, and *B. thuringiensis*, an insect pathogen that has been used worldwide as



100 YEARS AGO

The discovery by Monsieur and Madame Curie that a sample of radium gives out sufficient energy to melt half its weight of ice per hour has attracted attention to the question of the source from which the radium derives the energy necessary to maintain the radiation; this problem has been before us ever since the original discovery by Becquerel of the radiation from uranium. It has been suggested that the radium derives its energy from the air surrounding it, that the atoms of radium possess the faculty of abstracting the kinetic energy from the more rapidly moving air-molecules while they are able to retain their own energy when in collision with the slowly moving molecules of air. I cannot see, however, that even the possession of this property would explain the behaviour of radium... I think that the absence of change in the radium has been assumed without sufficient justification; all that the experiments justify us in concluding is that the rate of change is not sufficiently rapid to be appreciable in a few months. There is, on the other hand, very strong evidence that the substances actually engaged in emitting these radiations can only keep up the process for a short time; then they die out... I have recently found that water from deep wells in Cambridge contains a radio-active gas, and I am anxious to see whether water from other sources possesses the same property. I should be greatly obliged if any of your readers who have access to deep level water would fill a clean two-gallon can with it and forward it to the Cavendish Laboratory. I should, of course, pay the carriage and return the can. J. J. Thomson
From *Nature* 30 April 1903.

50 YEARS AGO

Royal Society: Foreign Members. The following have been elected foreign members of the Royal Society: Louis Victor Pierre Raymond, Prince de Broglie (Paris), distinguished for his contributions to quantum theory; Marie Jules Constant Robert Courrier (Paris), distinguished for his contributions to endocrinology; Hermann Joseph Muller (Bloomington, Indiana), distinguished for his contributions to the chromosome theory of heredity; Wolfgang Pauli (Zurich), distinguished for his contributions to theoretical physics, in particular the formulation of the exclusion principle.
From *Nature* 2 May 1953.

a pesticide for over 40 years. Read *et al.*³ describe the genome sequence of a strain of *B. anthracis* that was isolated from a Texan cow and subsequently used as a standard laboratory strain around the world. The same group has previously shown that this strain is virtually identical to that used in the US postal attacks⁵ and is, therefore, still representative of a fully virulent strain. Read *et al.*³ also generated an incomplete sequence of a strain of *B. cereus* for comparative purposes, and manufactured a microarray to extend this comparison to further strains of *B. cereus*. Ivanova *et al.*⁴ have completed the sequence of a different strain of *B. cereus*, and compared it to the unfinished sequence of the postal bioterror strain generated by Read *et al.* in their earlier work.

To understand the biology of this group of organisms, one must first realize that bacteria often carry more than one form of genetic material. In addition to the chromosome that bears the bulk of the genes, bacteria often carry extra DNA molecules called plasmids — small, self-replicating and often mobile elements that carry genes for accessory functions. Plasmid functions are extremely diverse, ranging from the undetectable to the essential — the genes concerned often encode ‘virulence factors’; proteins that confer resistance to antibiotics; and, crucially in this context, toxins.

It has been known for some time that plasmids confer distinct capabilities on the various members of the *B. cereus* group — which include *B. cereus* itself, *B. anthracis*, *B. thuringiensis* and related bacteria. For example, the plasmids of *B. anthracis*, pXO1 and pXO2, encode toxin and other proteins

essential for full virulence in animals, and a range of *B. thuringiensis* plasmids carry insect toxins that allow the bacterium to attack its favoured hosts. In addition, the size of the *B. cereus* genome is very variable between isolates, ranging from about 2.4 megabases to 6.4 megabases⁶. In contrast, *B. anthracis* isolates represent a group that is closely related genetically, probably indicating a recent evolutionary origin⁷. The unanswered questions around these certainties concern exactly how closely these organisms are related. Are they really separate species or, as has been suggested, the same species carrying different plasmids⁸?

The new work^{3,4} goes a long way towards answering these questions. It is clear from the results that the genomes sequenced are highly conserved, and that even many of the *B. anthracis* plasmid genes are present in other strains of the *B. cereus* group. The only significant and consistent differences between *B. anthracis* and *B. cereus* are the genes encoding toxin components, which are carried on an apparently recent insert (a pathogenicity island) within the *B. anthracis* pXO1 plasmid, and a loss by mutation in the *B. anthracis* genome of a regulator (PlcR) of the expression of a wide range of genes, many associated with virulence⁹. It is also clear from these analyses that factors involved in virulence are not restricted to the plasmid. Instead, the chromosome itself, which is often considered to be a relatively inert background for the virulence-determining plasmids, carries many genes that seem to be involved in virulence.

Read *et al.*³ and Ivanova *et al.*⁴ found that genes involved in pathogenicity for animal

and insect hosts were present on the chromosomes of both *B. cereus* and *B. anthracis*, and in many other representatives of the species. These include genes similar to those known to be involved in the pathogenicity of *Listeria* (a common food-poisoning organism in humans), and genes similar to those used by insect pathogens to attack the gut walls of their hosts. Far from being a repository of the staid metabolic genes, the chromosome is replete with its own arsenal of pathogenicity determinants. This suggests that the individual plasmids are not the only controls of virulence, but are instead more like accessory factors that can drive the overall virulence capabilities of the organism towards individual specific hosts.

This conclusion has led both groups^{3,4} to reassess the potential evolutionary niche of the ancestral organism of the *B. cereus* group. Using the genome to probe the potential metabolism of the organisms, they show that, unlike many of their relatives found in the wider environment, these bacteria seem to shun simple sugars and other carbohydrates of plant origin as a source of nutrients. Rather, they favour a more carnivorous diet of proteins, amino acids and the complex carbohydrates used by insects to build their bodies — a dietary foible taken to further extremes by a more distantly related insect pathogen, *B. sphaericus*, which is completely unable to metabolize any carbohydrates. All of this suggests that the immediate ancestor of the *B. cereus* group species was not simply a harmless soil-dwelling organism, but that it may have been saprophytic or even parasitic — that is, have preyed on the dead, or living, bodies of

Earth science

Subduction the hard way

Diamonds are a geologist’s best friend. Trapped inside the hardest natural substance is a relatively pristine record of the history of the continents — a record that can be exploited to investigate whether material that once formed the ocean floor of the early Earth was subducted into the mantle and preserved beneath the continental landmasses.

Earlier support for this view came from mantle rocks known as eclogite xenoliths. These are fragments of green, basalt-like material that were transported to the surface in volcanic magma. The ratio of the oxygen isotopes ¹⁸O and ¹⁶O in the eclogites suggested that they had originated as altered basalt on the ocean floor.

But the hot, turbulent past of the xenoliths means that there is no guarantee that this geological record is pure.

In this issue (*Nature* 423, 68–70; 2003), Daniel Schulze and colleagues report their analysis of diamonds mined in Guaniamo, Venezuela. This image, captured through cathodoluminescence, shows one of their samples, 2 mm across and known as ‘Picasso’s diamond’ for its resemblance to the cubist masterpieces of the Spanish painter.

Trapped inside this diamond (and others like it) is coesite, a form of silicon dioxide. The oxygen-isotope ratio in the coesite matches that of altered ocean-floor



basalt — “compelling evidence”, say Schulze *et al.*, in favour of subduction of oceanic plates

being instrumental in the formation of the early continents.

Alison Wright

NICOLA CANZIER, ALISON DIAS

insects and other animals. Indeed, organisms that seem to be members of the *B. cereus* group have been isolated from insect digestive tracts and have been proposed to be gut-attached stages of the bacterium¹⁰. This predisposition of the *B. cereus* group may have been built upon and fine-tuned by the ready exchange of plasmids encoding accessory factors that allow the bacteria to exploit other animals or insects more directly as hosts and sources of nutrients.

All of this serves to underline the point that, whatever crude attempts are made by human beings, the true biowarfare experts are the bacteria themselves — they are constantly ready and exquisitely able to adapt to, and exploit, any environmental or pathogenic niche that presents itself. We can only hope that the availability of these sequences

will assist those who are working to protect us against these agents of disease. ■

Julian Parkhill is at the Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

e-mail: parkhill@sanger.ac.uk

Colin Berry is at the Cardiff School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK.

e-mail: berry@cf.ac.uk

1. Jernigan, D. B. *et al.* *Emerg. Infect. Dis.* **8**, 1019–1028 (2002).
2. Seelos, C. *Nature* **398**, 187–188 (1999).
3. Read, T. D. *et al.* *Nature* **423**, 81–86 (2003).
4. Ivanova, N. *et al.* *Nature* **423**, 87–91 (2003).
5. Read, T. D. *et al.* *Science* **296**, 2028–2033 (2002).
6. Carlson, C. R. & Kolsto, A.-B. *Mol. Microbiol.* **13**, 161–169 (1994).
7. Keim, P. *et al.* *J. Bacteriol.* **179**, 818–824 (1997).
8. Helgason, E. *et al.* *J. Bacteriol.* **66**, 2627–2630 (2000).
9. Mignot, T. *et al.* *Mol. Microbiol.* **42**, 1189–1198 (2001).
10. Margulis, L. *et al.* *Proc. Natl Acad. Sci. USA* **95**, 1236–1241 (1998).

Chemical physics

How to keep dry in water

Philip Ball

What does water look like close to biological surfaces? The question has provoked heated debate for decades. Experiments suggest that this 'vicinal' water may be markedly different from the bulk liquid.

Water is notorious for behaving strangely, but it is possible that we don't yet know the half of it. Amid the crush of biological macromolecules and membranes in a cell, the water of the cytoplasm rarely achieves thicknesses of more than a few molecular layers. Might this confinement between macromolecular surfaces induce behaviour very different from that of the bulk liquid? A flurry of recent papers^{1–3}, in *Langmuir* and *Physical Review Letters*, lends support to the idea — but there is, as yet, no consensus in sight⁴.

Liquid water is held together by a random, fluctuating, three-dimensional network of hydrogen bonds. This unique liquid-state structure is responsible for many of water's anomalies, and it has long been thought to play a role in the hydrophobic interaction. This attraction between hydrophobic surfaces in water is one of the key stabilizing forces of protein folding and of multi-subunit protein assemblies.

Close to a hydrophobic surface the hydrogen-bonding pattern of water is disrupted, without the compensatory interactions that might operate at hydrophilic surfaces. How does water cope with this loss of stabilization? The classic model of hydrophobic interactions⁵ proposes that, around hydrophobes, water molecules lock into a more rigidly defined cage that preserves hydrogen bonding in an 'ice-like' solvation shell. The attraction then results from the gain in entropy as some of this solid-like water is liberated by the overlapping of solvation shells when the

two hydrophobes come together. It is an appealing picture, but turns out not to be supported by any evidence of increased ordering of water around small hydrophobic species⁶.

Modern theories of inhomogeneous fluids show that in fact one needn't invoke water's unusual structure to anticipate that strange things will happen at interfaces. Capillary condensation in narrow pores shows how confinement shifts the phase diagram of a fluid. Conversely, when the interactions between a liquid and a surface are relatively unfavourable (as with water and hydrophobes), there may be a lower fluid density at the interface, leading in the extreme case to the formation of a gas-like layer (a process known as 'drying').

Lum *et al.*⁷ predicted that this is just what should happen between hydrophobic objects of various shapes (plates and cylinders, for example, approximating protein surfaces): if the surfaces are large enough and close enough together, capillary evaporation creates a bubble between them. This theoretical model included none of the geometrical nuances of a hydrogen-bonded network; it was generic to any confined fluid.

Yet water will not give up its special status so readily. There is, for example, the mystery of the long-range hydrophobic attraction. Distinct from the shorter-range force that binds proteins, this interaction between hydrophobic surfaces seems to extend over distances of up to 100 nm — equivalent to many hundreds of molecular diameters. It

has compelled some researchers to seek explanations, seemingly fantastic at face value, in terms of some kind of extended, collective ordering of the intervening water molecules.

The long-range hydrophobic interaction was potentially unified with the issue of drying at a single interface by the suggestion of Attard and co-workers⁸ that the force might arise from the growth and bridging of sub-microscopic bubbles between the surfaces. The bridging meniscus would pull the surfaces together. Tyrrell and Attard reported⁹ that, using an atomic force microscope (AFM), they had observed pancake-shaped 'nanobubbles' on hydrophobic surfaces.

Might the formation of these bubbles be promoted by partial drying at the interface — that is, by depletion of water density in this region? At least two of the new studies support this notion. Noting that in the earlier work the AFM itself might have nucleated the bubbles, Steitz *et al.*¹ have used a less invasive technique — neutron reflectivity — to look at the interface. Neutrons are strongly scattered by deuterium (D), and the researchers look at 'heavy' water, D₂O, in contact with deuterated polystyrene, a hydrophobic polymer. The scattering densities of these two substances are nearly equal, so any inhomogeneities at the interface should show up in an otherwise 'uniform' sample.

Steitz *et al.* see a surface layer 2–5 nm thick with a density about 6–12% lower than that of bulk D₂O, which they interpret as evidence of partial drying. (It is hard, however, to rule out the possibility of an apparent density deficit arising from surface migration of protonated impurities, which scatter neutrons more weakly.) They also use an AFM to reveal a surface covered with flat bubbles, each 50–120 nm wide and up to 18 nm high. They propose that the thin depletion zone acts as a precursor to the nanobubbles (which might still be nucleated by the AFM tip itself).

Jensen *et al.*² find a similar depletion zone in X-ray reflectivity measurements of the interface of a heavy-alkane monolayer floating on the surface of water. Again, the density depletion is around 10% of the bulk water density, corresponding to about one H₂O molecule for every 0.25–0.30 nm² of surface. But the depletion layer appears to be thin — less than 1.5 nm, perhaps reduced by 'capillary waves' that ruffle the surface of the free monolayer.

Another neutron-reflectivity study has been conducted by Schwendel *et al.*³, who confess that their studies on contrast-matched mixtures of D₂O–H₂O against alkylated surfaces show a density deficit in the first 2 nm or so that is so large as to be unphysical. They suspect that nanobubbles or other air inclusions might be distorting the results. In a personal communication, Thomas and co-workers indicate that they