

their support for these competing hypotheses. Trees built using single genes from many species tend to support L–E–D<sup>8</sup>, but analyses using many genes from a few complete genomes support A–P–C<sup>5,6</sup>. The number of species represented in a phylogenetic study can have two effects on tree reconstruction. First, without genomes to represent most animal phyla, genome-based trees provide no information on the placement of the missing taxonomic groups. Current genome studies do not include any members of the Lophotrochozoa. More notably, if a species' genome is evolving rapidly, tree reconstruction programs can be misled by a phenomenon known as long-branch attraction<sup>9</sup>.

In long-branch attraction, independent but convergent changes (homoplasies) on long branches are misconstrued as 'shared derived' changes, causing artefactual clustering of species with long branches. Because these artefacts are systematic, confidence in them grows as more data are included, and thus genome-scale analyses are especially sensitive to long-branch attraction. Long branches can arise in two ways. One is when a distantly related organism is used as an 'outgroup' to root the tree of the organisms of interest. The other is when one organism of interest has a very different, accelerated pattern of evolution compared with the rest. Unfortunately for whole-genome studies, the usual outgroup, yeast, is very distantly related to animals, and *C. elegans* is a long-branch species<sup>5</sup>. Long-branch attraction will therefore tend to result in nematodes moving to the base of the tree, generating erroneous support for A–P–C. Not all whole-genome studies are tainted: analysis of rare insertions and deletions of genomic features (introns) in some animal genomes, characters that may be immune to the insidious charms of long-branch attraction, does not support A–P–C<sup>10</sup>.

Philippe *et al.*<sup>7</sup> have overcome these problems by using data from 'expressed sequence tags' (ESTs) in addition to complete genome sequences. Sequencing ESTs efficiently samples just the genes in any genome, avoiding the non-coding parts. The vastly lower cost of an EST project compared with sequencing a complete genome means that large numbers of ESTs have been generated for a much wider range of organisms, and we and others have been decorating the animal tree with EST data, including data from the neglected Lophotrochozoa<sup>11</sup>.

Using this expanded data set, Philippe *et al.*<sup>7</sup> find convincingly in favour of L–E–D (Fig. 1). They include many more data than previous non-genomic studies (35,371 amino acids from 146 genes) and more species than genome studies (35 species representing 12 animal phyla and 14 outgroups including choanoflagellates, thought to be the protozoan phylum most closely related to animals).

When only a distant outgroup (yeast) was

used<sup>7</sup>, nematodes emerge at the base of the tree. But with closer outgroups (protozoans related to animals, and jellyfish), nematodes cluster with arthropods, as predicted by the L–E–D hypothesis. In the complete data set, however, lophotrochozoan flatworms cluster with the ecdysozoan nematodes, and not with their supposed lophotrochozoan relatives (the molluscs and annelid worms). Suspecting that this was another long-branch artefact, Philippe *et al.* selectively eliminated genes expected to contribute most to long-branch attraction — those with a greater evolutionary rate in some species (such as nematodes) compared with others (such as deuterostomes). Indeed, as the most biased genes were removed, support for Ecdysozoa and Lophotrochozoa increased.

Will this be the last, defining statement in the controversy? There remain some unresolved problems with Philippe and colleagues' analysis, such as the position of the phylum Tardigrada (water bears). Tardigrades are unquestionably close to arthropods (they have eight stumpy legs), but appear as the sister phylum to the Nematoda. Have the nematodes lost the legs they once had, or are tardigrades misplaced?

Additionally, only 12 of the 35 animal phyla are currently represented: will addition of more phyla — particularly lophotrochozoan phyla — change the tree significantly? Have coeloms in protostome and deuterostome animals very different developmental

origins: have they arisen independently? Some really obscure bilaterally symmetrical animal phyla, such as acoel flatworms, are thought to have separated from the main animal lineage before the divergence of protostomes and deuterostomes. Will these illuminate the evolution of our own complex bodies?

Genome sequences from many other animals are now being gathered, and EST projects are under way or planned for many more. No doubt the tree will sprout new shoots — and new controversies — very soon. ■

Martin Jones and Mark Blaxter are at the Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Ashworth Laboratories, King's Buildings, Edinburgh EH9 3JT, UK.  
e-mail: mark.blaxter@ed.ac.uk

1. Aguinaldo, A. M. A. *et al.* *Nature* **387**, 489–493 (1997).
2. Winnepenninckx, B. *et al.* *Mol. Biol. Evol.* **12**, 1132–1137 (1995).
3. Adoutte, A., Balavoine, G., Lartillot, N. & de Rosa, R. *Trends Genet.* **15**, 104–108 (1999).
4. Mushegian, A. R., Garey, J. R., Martin, J. & Liu, L. X. *Genome Res.* **8**, 590–598 (1998).
5. Blair, J. E., Ikeo, K., Gojobori, T. & Hedges, S. B. *BMC Evol. Biol.* **2**, 7 (2002).
6. Wolf, Y. I., Rogozin, I. B. & Koonin, E. V. *Genome Res.* **14**, 29–36 (2004).
7. Philippe, H., Lartillot, N. & Brinkmann, H. *Mol. Biol. Evol.* **22**, 1246–1253 (2005).
8. Peterson, K. J. & Eernisse, D. J. *Evol. Dev.* **3**, 170–205 (2001).
9. Felsenstein, J. *Syst. Zool.* **27**, 401–410 (1978).
10. Roy, S. W. & Gilbert, W. *Proc. Natl Acad. Sci. USA* **102**, 4403–4408 (2005).
11. Davison, A. & Blaxter, M. L. *Parasitology* **130**, 1–14 (2004).

## Technology

# Warm fusion

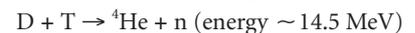
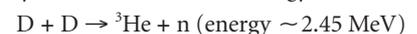
Michael J. Saltmarsh

A device that could fit in your lab-coat pocket uses nuclear fusion, and just a little heat, to produce neutrons. The advantages in simplicity and portability over conventional neutron generators could be considerable.

On page 1115 of this issue, Naranjo, Gimzewski and Putterman<sup>1</sup> report the successful demonstration of an intriguingly simple neutron generator that produces neutrons possessing an energy of 2.5 mega-electronvolts (MeV) from reactions involving the fusion of two nuclei of deuterium. This device, it must be stressed, will not generate net energy, and is not related to past controversies about 'cold fusion'.

Neutrons can penetrate significant quantities of matter, and interact primarily with the nucleus rather than the electronic structure of an atom. As a result, portable neutron generators have found a wide range of applications, including well-logging for oil exploration, and the screening of baggage for airline security. Several commercial devices are available that use fusion reactions of deuterium (D) and tritium (T), whose nuclei contain one and two

neutrons respectively (ordinary hydrogen nuclei have none). The reactions generate helium and a single neutron that carries away most of the reaction energy:



These neutron generators rely either on an ion beam from a miniature accelerator producing reactions in a solid target loaded with deuterium and/or tritium, or on the electrostatic confinement of a D–D or D–T plasma. In both cases high-voltage power is required, and the apparatus is fairly complex.

The device reported by Naranjo *et al.*<sup>1</sup> falls into the solid-target category, only without much of the complexity. Indeed, in some ways it is remarkably low-tech — the only input is a few tens of volts, to bias an electron-suppression grid, and some gentle



## 100 YEARS AGO

The water-supply for the occupants of our huge prehistoric “camps” has always been somewhat of a mystery, and it has been suggested that they were only temporary refuges... But the watering of men and animals on the scale indicated by the areas enclosed would be a formidable task even for a day, and other explanations must be sought. The late General Pitt-Rivers, for example, held that the water-level of the combs was higher then than now, and streams would have been plentiful on the slopes; but, feeling the inadequacy of this view, he also had recourse to the dew-pond theory... An exposed position innocent of springs was selected, and straw or some other non-conductor of heat spread over the hollowed surface. This was next covered with a thick layer of well puddled clay, which was closely strewn with stones. The pond would gradually fill, and provide a constant supply of pure water, due to condensation during the night of the warm, moist air from the ground on the surface of the cold clay... Some ponds of this kind, no doubt of very early and perhaps of Neolithic date, may still be seen in working order.

From *Nature* 27 April 1905.

## 50 YEARS AGO

In 1949 Burnet and Fenner postulated that antibody production against a particular antigen can be specifically suppressed by exposure to the same antigen during embryonic life... The decisive step which brought the principle of immunological tolerance from the sphere of Nature's eccentricity into the domain of an experimental method of possibly very wide applicability was the artificial production of a similar type of tolerance... The present investigations were carried out on birds by the method devised by Billingham, Brent and Medawar, and had a threefold purpose: (1) investigation of whether tolerance could be acquired to cells of foreign species; (2) if so, whether the tolerance would also extend to a virus from the donor animal to which the recipient was not normally susceptible; (3) whether a sexual cross between species which is not normally possible could be made so by means of acquired tolerance. Positive answers to the first two questions have been obtained; the answer to the third awaits the sexual maturation of the treated birds.

Morten Simonsen

From *Nature* 30 April 1955.

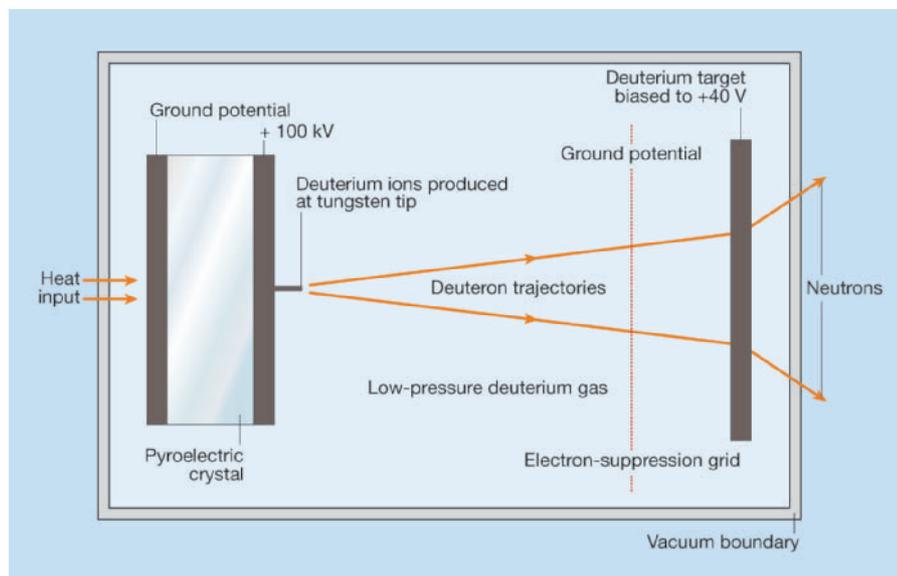


Figure 1 Naranjo and colleagues' apparatus for neutron generation<sup>1</sup>. The chamber is filled with deuterium gas at low pressure (0.7 pascals). As the crystal is heated, the potential builds across the crystal. Deuterium ions (deuterons) are generated at the tungsten tip, and accelerated towards the target; the electrons fall back to the crystal electrode. The ions strike the deuterium target ( $\text{ErD}_3$ ), and some generate 2.5-MeV neutrons. Electrons knocked from the target surface are repelled by the suppression grid and fall back on to the target rather than being accelerated back to the crystal.

heat (around 2 watts). A minute or two after the heat is applied, neutron emission starts, reaching a peak of about 1,000 per second; once the heat source is removed, the device gradually switches itself off. The key to the device's simplicity lies in the replacement of the miniature ion-source and accelerator in existing generators by a system based on a combination of two well-known phenomena — the pyroelectric effect and field ionization.

The pyroelectric effect — the fact that some materials become charged when heated — was probably first recorded in 314 BC by Theophrastus<sup>2</sup>, Aristotle's student and successor, from his studies of the gemstone tourmaline. More recently, various man-made materials have been investigated, and potentials of around 100,000 volts reported for crystals such as lithium tantalate ( $\text{LiTaO}_3$ ), with the emission of energetic electrons under suitable conditions. This effect was used by Brownridge<sup>3,4</sup> to produce a small pyroelectric X-ray generator, of which a commercial version, powered by a 9-volt battery, is now available<sup>5</sup>.

Field ionization of gases occurs when a potential difference of a few volts exists over atomic distances — equivalent to a field greater than 10 gigavolts ( $1 \times 10^{10}$  volts) per metre. The effect is widely used as the basis of field-ion microscopy. Modest voltages applied to electrodes of very small radius can produce these extremely high fields near the electrode tips, ensuring the ionization of essentially all gas molecules entering the high-field region.

Figure 1 shows how Naranjo *et al.* combined these effects to generate fusion

neutrons. The authors grounded one face of a 1-cm-thick pyroelectric crystal to the inside of a vacuum chamber containing deuterium gas at a pressure of 0.7 pascals (for comparison, Earth's atmospheric pressure is around  $10^5$  pascals). They then attached a tiny tungsten electrode to a plate on the positive face of the crystal. A solid target containing deuterium in the form of erbium deuteride ( $\text{ErD}_3$ ) was placed a few centimetres in front of this electrode.

Raising the temperature of the crystal at a rate of 12.4 °C per minute changed the spontaneous polarization of the crystal, and raised the potential of the positive electrode at a rate of about 50 kilovolts per minute. As the potential rose, the field near the tungsten electrode increased to a value — around 25 gigavolts per metre — sufficient to produce field ionization of the deuterium gas. The positively charged ions (deuterium nuclei, or 'deuterons') produced in this process were accelerated towards the target across essentially the full potential generated by the crystal; the electrons stripped from the deuterium atoms by the ionization experienced a potential drop of only a few volts as they fell back to the crystal. On hitting the target on the opposite wall of the device, the energetic deuterons interacted with the deuterium target to produce 2.5-MeV neutrons via the  $\text{D} + \text{D}$  reaction.

The maximum current obtained in this experiment was about 4 nanoamperes, leading to a maximum neutron production rate of around 1,000 neutrons each second. The accelerating potential can be maintained only while the crystal temperature is changing; thus, the duration of the pulse

at this current level was limited to a few minutes by the attainable temperature rise. Although this output is too small for most applications, the authors outline plans to increase the yield to a million neutrons per second, comparable to that of some commercial portable neutron generators. Nevertheless, even at the level already attained, there are laboratory uses, such as measuring neutron detector response or for student practical demonstrations, for which a simple,

inexpensive, monoenergetic neutron source would be most valuable. ■

Michael J. Saltmarsh is mostly retired from Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, Tennessee 37831-6368, USA. e-mail: msaltmarsh@aol.com

1. Naranjo, B., Gimzewski, J. K. & Putterman, S. *Nature* **434**, 1115–1117 (2005).
2. Donnay, G. *Acta Crystallogr. A* **33**, 927–932 (1977).
3. Brownridge, J. D. *Nature* **358**, 287–288 (1992).
4. Brownridge, J. D. & Raboy, S. *J. Appl. Phys.* **86**, 640–647 (1999).
5. www.amptek.com/coolx.html

HIV

## Viral blitzkrieg

R. Paul Johnson and Amitinder Kaur

It takes years for AIDS to develop from the damage inflicted on the immune system by HIV or its simian counterpart. Surprisingly, as many as half of the body's memory T cells may die at a very early stage of infection.

**H**IV and the related simian immunodeficiency virus (SIV) cause AIDS by infecting the master regulatory cells of the immune system — T helper cells, better known as CD4<sup>+</sup> T cells. It is generally years before enough damage is done to this cellular army for full-blown AIDS to develop. Nevertheless, two reports in this issue<sup>1,2</sup> (pages 1093 and 1148) suggest that the outcome of the battle between SIV and its host may be determined by a dramatic opening salvo, in which the virus eliminates around half of the host's memory CD4<sup>+</sup> T cells within four days — thus setting the stage for a lengthy war of attrition.

CD4<sup>+</sup> T lymphocytes are so called because they express a receptor protein termed CD4; this is necessary for T-cell function, but has also been co-opted by HIV and SIV to gain entry to the cells. Previous results suggested that HIV/SIV replication is restricted

to a relatively small fraction (0.01–1%) of CD4<sup>+</sup> T cells in the chronic stages of infection<sup>3</sup>. This low frequency of infection seemed to reflect the fact that the viruses require the presence of a co-receptor in addition to CD4 to gain entry, and also that they replicate best in activated memory CD4<sup>+</sup> T cells (memory cells being those previously stimulated by foreign antigen). The preferred co-receptor for most HIV and SIV strains is CCR5, which is expressed only in a subset of memory CD4<sup>+</sup> T cells.

Despite the apparent low frequency of T-cell infection in chronic infection, seminal studies in SIV-infected monkeys<sup>4</sup> — subsequently confirmed in HIV-infected humans<sup>5,6</sup> — revealed a rapid and widespread depletion of CD4<sup>+</sup> T cells in the gut (mucosal T cells) during the first few weeks of infection. T cells in the blood or lymph nodes did not show the same degree of

depletion. This predilection of HIV and SIV for replicating in gut lymphocytes was felt to be a consequence of the relatively large populations of activated CD4<sup>+</sup> T cells at this site that express CCR5. However, the proportion of cells actually infected with SIV was not known; nor was it clear whether T-cell activation was in fact required for infection, or how T cells were killed.

Mattapallil *et al.*<sup>1</sup> have now examined the role of SIV in the depletion of CD4<sup>+</sup> T cells during early (acute) stages of infection. As previously reported<sup>4</sup>, SIV rapidly depleted CD4<sup>+</sup> T cells in the gut. Remarkably, however, 60–80% of memory CD4<sup>+</sup> T cells were concurrently depleted at all sites. Using a technique that can detect a single copy of SIV DNA, the authors determined that 30–60% of all memory CD4<sup>+</sup> T lymphocytes were infected with SIV within 10 days of infection, regardless of their location, and that most of these cells had disappeared 4 days later. These percentages far exceed the number of CD4<sup>+</sup> T cells that express CCR5, but the authors propose that this apparent contradiction may be resolved by their finding of low levels of CCR5-encoding messenger RNA in memory cells in which CCR5 protein could not be detected by flow cytometry. This implies that such cells may in fact express sufficient levels of CCR5 protein to render them permissive for SIV infection. Alternatively, SIV may be entering the cells using other co-receptors.

Li and colleagues' paper<sup>2</sup> provides a complementary perspective on this viral blitzkrieg. By identifying cells expressing SIV RNA in tissue sections, these investigators characterized the activation state of virus-producing cells (viral production requires SIV DNA to be transcribed into RNA). Consistent with their earlier work<sup>7</sup>, they found that most infected cells did not express markers of activation (CD25 or CD69), nor did they express Ki67 — a molecule found in

Behavioural ecology

## Cue for kin

If you yourself can't breed, you can at least help your relatives with their offspring. Such altruistic behaviour occurs in long-tailed tits (*Aegithalos caudatus*, pictured), which Stuart Sharp and colleagues have studied to find out what cues enable a 'helper' to recognize kin. Their report appears elsewhere in this issue (*Nature* **434**, 1127–1130; 2005).

Adult long-tailed tits pair off and attempt to breed each year, but many don't succeed because of high rates of predation on the eggs or nestlings. The childless parents may then turn to assisting kin in feeding their brood — which makes sense in evolutionary terms but requires some

form of recognition system. Long-tailed tits are not the greatest of vocalists. They sing infrequently but do have an individually characteristic contact call, the 'churr', which develops even before fledging and is retained in the adult bird.

The first part of Sharp and colleagues' research involved the playback to individuals of churr calls belonging to a close relative and a non-relative, and a further two trials in which the frequency of these calls had been tweaked. The responses of birds to the untweaked calls of relatives differed significantly from their responses to the other three calls. From this, the authors

conclude that the churr call provides cues involved in kin recognition.

The most innovative part of the study, however, was an investigation into how much churr acquisition owes to nurture (learning) and nature (genetics). This took the form of swapping young birds between nests, so that adult birds were raising foster nestlings along with their true offspring. The churr calls of the fostered birds were the same as those of their nestmates, and unlike those of their biological siblings raised elsewhere — so it seems that the churr is in large part learned.

The pattern of helping observed in long-tailed tits is consistent with



the use of this learned cue in the great majority of cases. But this kin-recognition system evidently isn't flawless: in 6% of cases, an adult helped unrelated nestlings. As the authors point out, recognition systems are rarely perfect. **Tim Lincoln**

ANDREW MACCOLL