

Frame by frame

Bacteria provide a way of watching thousands of generations evolve in a few years, says s ananthanarayanan

CHARLES Darwin's theory of the evolution of the species provided the device of natural selection by which specific random variations in generations were favoured for survival. Gregor Mendel's work was on the mechanics of inheritance and he showed that traits were passed on in units called genes. There has been discussion about whether the great specialisation that has been seen in evolution, like development of flight, or of organs like the eye, could come about through random processes. Should there not be some "direction" to the variations that were selected?

Darwin himself had said, "... Natural selection acts only by taking advantage of slight successive variations; she can never take a great and sudden leap, but must advance by short and sure, though slow steps. ... If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down." But evolution takes so long in happening, with evidence only in prehistoric fossil records, that there is no way of seeing what the intermediate steps actually were.

Zachary D Blount, Jeffrey E Barrick, Carla J Davidson and Richard E Lenski of Michigan, Texas and Calgary, Canada, report in the journal *Nature* that they played the course of evolution in quick-time by observing bacteria rapidly multiply through 55,000 generations in just 25 years.

The bacterium *E. coli* is an organism just two microns in size, found in the intestines of warm-blooded animals. The species arose about 100 million years ago, along with warm-blooded animals and has remained unchanged for over 20 million years. The organisms were discovered by Theodor Escherich, a German paediatrician, in 1885, who called them *Bacterium coli*, as they were found in the colon.

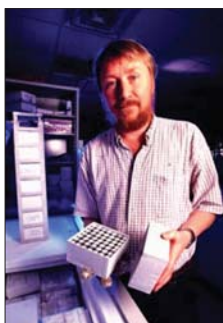
E. coli, as a simple and easily grown laboratory bacterium, has been a very useful base of research in biotechnology and microbiology. Its special importance is that it is a fully equipped living organism and, more important, that it reproduces every few minutes. This pace of reproduction is to create 75 generations in a day and over 2000 generations in a year.

Lenski, co-author of the paper, had started this long running project in experimental evolution in 1988. A group of fast-growing *E. coli*, descended from the same parent cell, was used to found 12 independent populations in 12 different culturing flasks containing the same simple culture medium, which had the components essential for



Jeffrey E Barrick.

growth and glucose as the sole source of carbon. Every day, for nearly 25 years, a sample from each flask has been transferred to a fresh flask to start a new culture. What we have now is thus 12 lines of some 9,000 flasks with about 55,000 generations of *E. coli*. In addition to fresh cultures, every few days samples from the flasks were also stored at -80° Celsius. We thus have with us the time-frozen samples of *E. coli* as they had evolved at various times along these 25 years. Unlike fossil records,



Richard E Lenski and Carla J Davidson.

that one of the 12 lines of *E. coli* began to use citrate as a nutrient, as if it were in oxygen-free conditions!

This development was different from the usual,

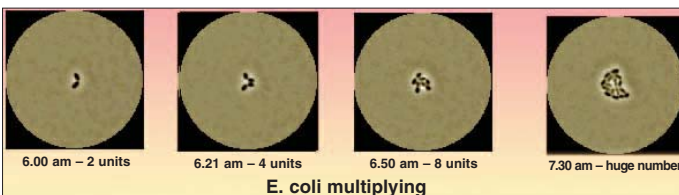
But where and what changes had taken place were to be identified.

Action reply

The researchers went "back in time" with the help of the frozen samples and resuscitated ancestors of the current *cit⁺* population. The resuscitated ancestors were then cultured to see which ones had the ability to evolve into *cit⁺*. The cut-off would then identify the single change that provided the ability for this evolution. Investigation showed that the *cit⁺* evolution potential existed only in a few, most recent non-*cit⁺* samples. What was the change at these stages, which were the *potentialised* stages, was difficult to trace but when *cit⁺* did appear, ie, the *actualisation* step, was easier to pin down. The action of citrate entering the cell, which is what *cit⁺* cells permitted, takes place because of a protein that provides the molecular handle for this action. The researchers, therefore, analysed the different *E. coli* DNA at the region that carries the gene *citT*, which enables generation of that protein. It was found that in the original *E. coli* cells, *citT* was located downstream of *citG*, another gene related to citrate use and also to *rimK*, an unrelated gene that affects energy metabolism. But in *cit⁺* cells, the genes were rearranged, with *rimK* and *citG* being fused, and



Zachary Blount.



E. coli multiplying

however, these samples of past times can be thawed and revived, for study *in vitro* and for further generations.

Over these 55,000 generations, the samples of *E. coli* did evolve, for greater fitness, generally. But at about 31,000 generations, a remarkable development was noticed. The growth medium in which the *E. coli* were cultured, where glucose was the source of carbon, contained citrate, as the agent that enabled uptake of iron, which is usually insoluble by *E. coli*. While citrate also contains carbon, which *E. coli* can use in oxygen-free conditions, citrate is not a source of carbon in the well-aerated conditions of the experiment. But what was seen, at the 31,000th generation, was

single-step evolution of fitness that was seen generally. To gain the ability to use citrate in the presence of oxygen (a variety of *E. coli* called *cit⁺*) is on par with an evolutionary leap, on the lines of, although not equal to developing flight, for instance. The experiment had thus thrown up an instance of the very kind of evolutionary change whose mechanism was under question. But the design of the experiment allowed investigation up the time-line into the earlier stages to see where exactly the crucial change took place. One thing was clear, that the ability to use citrate was new trait altogether – it was a modification of an existing trait – with refinement of the conditions in which it could be expressed.

rimK being effective in allowing the expression of *citT* and *citG* in the presence of oxygen. In fact, the study has shown that a single copy of the genetic rearrangement was not sufficient to generate *cit⁺*, but an array of two to nine copies were needed. It was cells that inherited the fusion of *rimK* and *citG* and then followed through with

"amplifying" mutations that grew to be *cit⁺*. The discovery, as a result of well-designed, workmanlike investigation into how evolution works, is confirmation that major innovations can be explained by the working of gradual, micro-evolutionary stages, of potentialisation, followed by actualisation and amplification. The work amounts to providing a mechanism of how apparently discontinuous steps in evolution come about through the operation of a series of single-step genetic changes, an answer to anti-evolutionists.

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'Flashfloods are man-made'

anupam chakravarty finds out what triggered the recent landslide in Uttarkashi

ENVIRONMENTAL geologist KS Vaidya lives in Uttarakhand and keeps a tab on the changing processes in the Himalayan region. He was recently conferred with the GM Modi Award for Innovative Science and Technology for his contribution in the fields of Himalayan geology and neotectonics. Formerly a member of the Prime Minister's science advisory council, he recounts what triggered the recent landslide in Uttarkashi. Excerpts:



Flashfloods are becoming recurrent in the Himalayas as well as in other parts of India. What are the geological processes that could trigger them?

Flash floods have little to do with geology. They are related to the rainfall pattern that has changed considerably over the years due to warming up of the atmosphere. There has been evidence that with increasing atmospheric temperature, precipitation during the rainy season is no longer uniform. In summers there is prolonged drought followed by short spells of heavy rainfall. So quick and vast is the rate of precipitation that there is not enough time for water to percolate down the soil. To worsen the situation, there is hardly any tree cover; soil is compacted to the extent that it becomes impermeable. This results in increased river discharge and flash floods.

Could this be the only cause behind the flashflood in Uttarkashi in August that the government termed the worst in 30 years?

It is entirely man-made. A river consists of a deep channel, flanked by floodways on either side, which are extended by floodplains. Historically, people avoided floodways for building houses and only did agriculture there. But decades of human activity have destroyed the



Gangotri bridge in Uttarkashi damaged due to flash flood in August.

geomorphic difference between floodplains and floodways. Now, there is construction work not only on floodways but also close to the river channel. Unlike railway lines and bridges which span the floodways, roads and bridges get easily washed away because developers are least concerned about geological structures. First, they restrict the channel by erecting piers on it. Then they build embankments on both sides of the river to reach the bank. The embankments act as dams, whereas the bridges resemble open sluice gates. In mountainous regions, the developers simply construct culverts or sometimes holes to save cost. In Uttarkashi, all the construction, including roads and bridges, has come up on floodways and terraces. All the new townships coming up on the riverside are on the floodways. So when a river is in spate, what does it do?

Do you think increasing pressure on land in the mountain region is also the reason for devastating flashfloods?

Of course, there is pressure on land. But you will not find village residents building houses on river terraces. They prefer building on the slope. Faulty planning is responsible for such landslides. In the Himalayan region, it is a standard practice to build a road on the debris of previous landslides. This saves the cost of digging or cutting the mountain. So good engineers put pressure on road contractors to build drainage system parallel to the road. But many do not have such a drainage system for the water to flow down. This causes more landslides.

CSE/Down To Earth Feature Service

Our Web-wired world

Which country has the best Internet access? The worst? And which nation is the most addicted to social networking? A new report reveals all, says peter popham

BROADBAND has been a legal right for citizens of Finland since 2010. But while in most of the developed world access to fast Internet is rapidly becoming a fact of life, like piped water and electricity, many developing countries struggle to attain the vital connective technologies that will make the difference between their people joining the rest of the world or lagging even further behind. In its new report, "The State of Broadband 2012", the Broadband Commission, established by the International Telecommunication Union and Unesco, spells out the momentousness of the revolution we are living through.

"As the price of handsets falls and their functionality increases," the authors write, "soon the vast majority of people on the planet will hold in their hands a device with higher processing power than the most powerful computers from the 1980s."

Since Sir Tim Berners-Lee invented the Internet back in the 1980s, the world's richest and smallest countries have acted fast to anchor what the report calls "the circulation system of the knowledge society" in their infrastructure. Today Switzerland boasts nearly 400 broadband subscriptions per 100 people and Singapore and South Korea have more active mobile broadband subscriptions than

people. Meanwhile, in large areas of Africa, South Asia and Latin America, signals are still spotty and devices are rare as gold dust. In Mali,

Turkmenistan, Eritrea, Bangladesh and 21 other countries, fewer than one per cent have broadband subscriptions or mobile broadband.

And at a time when access to broadband has become critical to economic growth – India, despite having a very patchy network, has created 10 million jobs, thanks to the Internet – failure by governments to take the right measures could be disastrous, the report says.

Economic liberalisation can hinder the efforts of poor countries to build a reliable network. "Liberalisation and competition have created a fragmented market lacking the economies of scale and the regulatory certainty needed," the report argues. One quick fix for reaching "the third billion" is satellite, which avoids problems caused by inadequate infrastructure on the ground. In such countries, cheap pre-paid broadband access

vouchers help spread the Internet habit fast.

Around one-third of the world's population can now access the Internet, but many countries still have a long way to go before their citizens can get online. Here's a pointer to who's online and who isn't:

■ **USA:** Beaten by Canada for the number of fixed broadband subscriptions (Canada has 32 per cent, whereas the USA has 28.7 – the same as the tiny principality of Andorra). However, the USA ranks eighth in the world for mobile broadband subscriptions.

■ **UK:** Ranks 15th in the world in terms of Internet access, with 82 per cent of the population using the Internet in 2011, compared with a global average of 32.5 per cent.

■ **Iceland:** Boasts the highest percentage of Internet users in the world, with 95 per cent of the population going online in 2011. A world leader in Internet usage for years, Iceland has even used online crowdsourcing via social media to help draw up one country's new constitution.

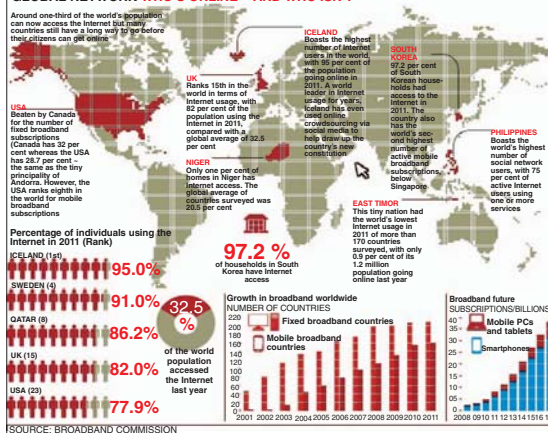
■ **Niger:** Only one per cent of homes in Niger has Internet access. The global average, of countries surveyed, was 20.5 per cent.

■ **South Korea:** 97.2 per cent of South Korean households had access to the Internet in 2011. The country also has the world's second highest number of active mobile broadband subscriptions, below Singapore.

■ **Philippines:** Boasts the world's highest number of social network users, with 73 per cent of active Internet users using one or more services.

■ **East Timor:** This tiny nation had the world's lowest Internet usage in 2011 of more than 170 countries surveyed, with only 0.3 per cent of its 1.2 million population going online last year.

GLOBAL NETWORK WHO'S ONLINE - AND WHO ISN'T



The Independent, London