

## In Retrospect

# A spotlight on bacterial mutations for 75 years

**In the debate about how bacterial mutations arise, an experiment in 1943 showed that they can occur spontaneously and independently of a selection pressure. This study also popularized the use of maths-driven analysis of biological data.**

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Do bacteria acquire mutations randomly, or do mutations arise adaptively as a direct response to environmental pressures? This question has wide implications in areas ranging from evolution to the treatment of bacterial infections. In 1943, writing in *Genetics*, Luria and Delbrück<sup>1</sup> revealed, by a combination of experimental analysis and profound mathematical insight, that bacteria evolve through random mutations that arise independently of an environmental stress, and that occur even before bacteria encounter such selective conditions. Their study was a milestone in a debate about the nature and causes of bacterial evolution that is still ongoing. Moreover, this work has inspired the fields of microbial evolution and quantitative biology.

Luria and Delbrück worked at a time when scientists disagreed on the fundamental nature of bacterial evolution<sup>2</sup>, despite tremendous advances in molecular biology and microbiology. For plants and animals, there was a general consensus that, consistent with Charles Darwin's theory of evolution, natural selection acted on mutations that arose randomly, regardless of their benefit to the organism. However, the unusual nature of bacterial genetics — such as the absence of sexual reproduction — sparked a vigorous debate about whether the principles that drive animal evolution also apply to bacteria (see [go.nature.com/2brojqp](http://go.nature.com/2brojqp)). The main alternative hypothesis was Lamarckian evolution, named after the French biologist Jean-Baptiste Lamarck. In this model, the specific mutations that provide an advantage to an organism are acquired directly in response to the organism's environment<sup>3</sup>.

For present-day microbiologists, this debate might seem strangely contrived — after all, if other organisms evolve in a manner consistent with the Darwinian principles of randomly occurring organismal variation that selection can act on, why should bacteria be an exception? Yet, it's worth having sympathy for our scientific predecessors. Even though we now accept that bacteria evolve through Darwinian mechanisms, 'quasi-Lamarckian' processes of bacterial evolution are still being discovered and debated<sup>4–6</sup>.

Luria and Delbrück themselves encountered

some difficulties when they entered the debate about how bacterial evolution occurs. To establish an approach to study mutations in bacteria, they allowed individual *Escherichia coli* cells to grow into large populations in individual test tubes, and added the cells from each of these tubes to Petri dishes containing agar coated with viruses known to kill the bacteria. Luria and Delbrück monitored the number of visible bacterial colonies on each of the plates. Each of these virus-resistant colonies arises from a cell and its descendants that had a mutation enabling the cells to survive the viral attack. Yet, for a simple experiment, their results were initially confusing: the number of colonies was highly variable between the different plates, a result that the authors initially attributed to an experimental error (see [go.nature.com/2brojqp](http://go.nature.com/2brojqp)). But in a moment of clarity, Luria realized<sup>7</sup> that the high variability in the number of bacterial colonies might be an important clue, not an error.

Let's consider the experimental variance in the number of virus-resistant colonies per Petri dish expected under the process of either adaptive or random mutation. If mutations arise by an adaptive process, each bacterial cell would have a chance of acquiring a resistance mutation only on encountering the virus. Assuming each cell's chance of becoming resistant is small, the prediction would be that the number of virus-resistant colonies per Petri dish would vary according to a Poisson distribution (a standard probability distribution for random events, in which the standard deviation of the data equals the square root of the mean).

But, if evolution is driven by random mutations, mutations that confer viral resistance would arise during the growth of the bacterial population before viral exposure. In this case, the experimental variance in the number of virus-resistant bacterial colonies between different Petri dishes would be much higher than in the adaptive-mutation scenario, because the number of virus-resistant bacteria in a given test tube would depend on the random timing of when mutations occurred. A single virus-resistance mutation that occurred early in the growth of the bacterial population would result in a large number of virus-resistant bacterial descendants of the original mutated cell, whereas mutations that arose much later during the growth of the bacterial culture, just



## 50 Years Ago

After the wreck of the Torrey Canyon in March 1967, some 8,000 seabirds were taken to cleansing stations in Britain — but well under ten per cent ... of these birds were rehabilitated and returned to the sea. Even this figure gives too optimistic a picture of the cleansing operation, for a large proportion of the so-called rehabilitated birds were recovered dead within a few days. Although exact figures are hard to come by, the Torrey Canyon episode revealed the complete inadequacy of the current methods of rehabilitating oiled birds ... Legislation can never totally eliminate accidental pollution and it is estimated that even the much vaunted "load on top" system of washing tankers, although a great improvement on previous practice, produces pollution at a rate of 400,000 tons a year.

From *Nature* 30 November 1968

## 100 Years Ago

[U]ndoubtedly the war has been responsible for an enormous amount of destruction of capital; but when estimates are given ... of the percentage of loss in Belgium, France, Italy, Serbia and other countries, it is not usually borne in mind that capital does not merely consist of gold and silver, of bricks and mortar ... or even of railways, steamships and machinery ... but of scientific knowledge ... When, therefore, we compile estimates of the losses due to the war, let us not forget that our greatest asset, the vast store of knowledge that Science has gathered together for us ... is still intact. It is a store that has slowly been accumulating ever since the beginning of the world — a store which enables man more and more to triumph over Nature, and one that for ever remains practically indestructible as the real permanent capital of the race, and by far its most precious heritage.

From *Nature* 28 November 1918

before viral encounter, would produce many fewer virus-resistant bacteria.

On the basis of this insight, Luria and Delbrück generated a statistical distribution (the Luria–Delbrück distribution) to describe the prevalence of virus-resistant bacterial mutants that would be expected if mutations arose randomly before the bacterial population came under selective pressure from the virus. Compared with a Poisson distribution expected for adaptive mutations, this Luria–Delbrück distribution has a long ‘tail’ at the end of the distribution pattern. In the context of the authors’ experiments, this tail would correspond to Petri dishes that have a high number of bacterial colonies, corresponding to early mutational events that lead to a large number of mutant descendants.

The 1943 paper reported the results of the authors’ experiments, termed fluctuation tests, that took this mathematical approach to analyse the number of virus-resistant colonies in *E. coli* populations. The authors’ findings were consistent with mutations following a Luria–Delbrück distribution rather than a Poisson distribution, demonstrating that bacterial mutations arose randomly, and independently of an encounter with a virus.

Luria and Delbrück’s work shaped subsequent studies of biology and evolution in many ways. Luria himself was reported as saying that their fluctuation test removed bacteria from “the last stronghold of Lamarckism” (see [go.nature.com/2fbxujf](http://go.nature.com/2fbxujf)). The fluctuation test is still a standard procedure for accurately measuring mutation rates in diverse systems, from bacteria<sup>8</sup> and yeast<sup>9</sup> to cancer cells<sup>10</sup>. Their study also popularized the use of *E. coli* and the viruses that attack it as a simple experimental model system for biology<sup>11</sup>. Beyond its direct impact in laboratories, the experiment became a textbook example of how mathematical thinking combined with simple experimentation can lead to profound biological insights<sup>12</sup>. For their contributions to bacterial and viral genetics, Luria and Delbrück won the Nobel Prize in Physiology or Medicine in 1969 (which they shared with the biologist Alfred Hershey).

Their insight into mutational processes also has implications in settings such as the clinic. In analogy to the original experiment, imagine a population of patients who have the same type of bacterial infection and who are being treated with the same antibiotic (the antibiotic replaces the virus as the selection pressure here). According to the random-mutation model, even if all else is equal among the patients, the number of antibiotic-resistant bacterial mutants initially present will vary highly between the patients, which could lead to markedly variable treatment outcomes. Because such high inherent variability in treatment efficiency reflects resistance mutations arising in a population before treatment, using DNA sequencing or other types of analysis to identify the presence and number

of antibiotic-resistant bacterial mutants before treatment could improve our ability to predict treatment outcome.

Did the Luria and Delbrück study really close the door on Lamarckism? As far as bacteria are concerned, the answer is much more complicated than the duo could probably ever have anticipated.

It is undeniable today that randomly occurring mutations and natural selection are central tenets of how bacterial evolution occurs<sup>5</sup>. However, scientists are uncovering and debating an increasing array of other evolutionary processes at work in bacteria, some of which are suspiciously Lamarckian in character<sup>4–6</sup>. For example, we now know that the genome-wide mutation rate, and even the mutation rates of specific genes, can be shaped by evolution and affected by the environment<sup>13–15</sup>. An even more striking example is bacterial adaptation through the CRISPR–Cas viral-defence system, in which bacteria can incorporate viral genetic material into their own genomes and use it, as an adaptive mechanism, to protect themselves and their descendants against current and subsequent viral attacks<sup>16,17</sup>. These quasi-Lamarckian mechanisms presumably evolved by random mutations and natural selection. They do not necessarily undermine the lessons learnt from Luria and Delbrück’s work, but rather, show the power of evolution to sculpt living organisms in endlessly interesting ways.

It is intriguing to imagine an alternative scientific history that might have occurred if Luria and Delbrück had stumbled upon one of these quasi-Lamarckian mechanisms. The CRISPR–Cas defence mechanism is mainly repressed in the *E. coli* that they studied, but it is active in other bacterial species, such as *Streptococcus thermophilus*.

A fun challenge would be to repeat the Luria–Delbrück experiment under conditions that might favour the evolution of resistance by such adaptive mechanisms, for example by replacing *E. coli* with *S. thermophilus*. Would the distribution of the number of resistant mutants indicate random or adaptive mutations? What would Luria and Delbrück have concluded had they used a species that had the CRISPR–Cas system? The contingency of this historic choice underscores the fact that, like evolution, science perhaps also progresses both adaptively and randomly. ■

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#### PALAEANTHROPOLOGY

## The not-so-dangerous lives of Neanderthals

**Have Neanderthals gained an unfair reputation for having led highly violent lives? A comparison of skulls of Neanderthals and prehistoric humans in Eurasia reveals no evidence of higher levels of trauma in these hominins. [SEE LETTER P.686](#)**

MARTA MIRAZÓN LAHR

Injuries are part of everyday life, from a scratch on the skin to a broken bone to a fatal trauma. Although many injuries are accidental, others can arise as a consequence of an individual’s or a group’s behaviour, activity or social norms — characteristics that tell us about societies and the inherent tensions and risks within and between different groups.

On page 686, Beier *et al.*<sup>1</sup> provide evidence that challenges the long-standing view<sup>2</sup> that Neanderthal populations experienced a level of traumatic injuries that was significantly higher than that of humans. The result calls into question claims<sup>2,3</sup> that the behaviour and technologies of Neanderthals exposed them to particularly high levels of risk and danger.

Reports of injuries and deaths are constantly in the news. As well as being drawn to read the