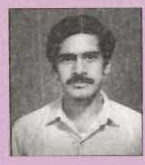
Questioning a Dogma

Do Bacteria Know When and How to Mutate ?

Milind G Watve and Neelima M Deshpande



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For over half a century, all mutations were believed to be 'random', i.e. independent of their effects on the organism in a given environment. This belief was strengthened by experiments by Luria and Delbruck and Lederberg and Lederberg in the middle of the century. Recently, however, many experiments have provided evidence to the contrary. A hot debate therefore ensues at the central stage of evolutionary genetics.

The central dogma of evolutionary biology, widely accepted today, is that of random mutations and natural selection. The genetic information in all living organisms, which is in the form of DNA (sometimes RNA), is conserved and passed on from generation to generation by means of faithful replication of DNA. Errors in replication, however, do take place. These are called mutations. Sometimes mutations are silent, i.e. they do not affect the functioning of the organism in any detectable way. But at other times they affect the characteristics of the organism to a lesser or greater extent. Mutations thus cause variations in the population and natural selection chooses the best of the lot. While the existence and importance of natural selection is seldom questioned, the 'randomness' of mutations has been a case for heated arguments ever since the birth of the concept of 'random mutations'.

A *random* or *spontaneous* mutation means mutation without any purpose or foresight. The organism cannot judge the environment and decide which mutation is likely to be useful under the given circumstances and somehow create it.

Undergraduate students and teachers often conceive of spontaneous mutations as the ones that take place in the absence of a mutagenic agent. This definition is problematic and should be abandoned. If mutations are only errors of replication, they have to be random according to this definition. People have however always found it difficult to believe that the basis of the process that has given rise to systems as complex as the human being is nothing but random errors. Are we humans the result of random errors? Doesn't it sound ridiculous? Neo-Darwinists such as Richard Dawkins have strongly and effectively defended the random mutation - natural selection dogma and argued that such a seemingly simple process can indeed give rise to extremely complex organizations.

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An alternative school, popularly called 'Lamarckian' (after Jean Baptiste de Lamarck 1744-1829) believed that organisms adapt to their environment by changing themselves and these changes are inherited. One can definitely smell a purpose or foresight in the Lamarckian argument. Today there are few takers for this notion. But Lamarckian thought has always played the role of a potential challenger to Darwinian thought. The experiments which turned the opinion strongly in favour of Darwinism were those by Luria and Delbruck, and Lederberg and Lederberg.

The Spontaneous Mutation Strongholds

Luria and Delbruck took cultures of a bacterial species, *Escherichia coli*, which were sensitive to the bacteriophage T1. If one plates a bacterial culture onto a nutrient gel along with a bacteriophage, the phage will kill the sensitive bacteria. Only the resistant ones can grow and form visible colonies. This is a tricky situation. There is no way to know whether a cell is resistant to the given phage unless it is plated in presence of the phage. Therefore there is no simple way to

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know whether a mutation conferring resistance has occurred before or after exposure to the phage. Luria and Delbruck made complex statistical arguments to support the hypothesis that mutations had taken place before exposure to the phage. They divided one culture of *Escherichia coli* (culture A) into many small test tubes and incubated them overnight to allow the cells to multiply for several generations. Another culture (culture B) that was not divided into aliquots was simultaneously incubated. After incubation the contents of each tube were plated out along with the phage. Culture B was also plated a number of times with the phage. The number of colonies on the plates containing the phage, i.e. the number of phage–resistant mutants was counted after incubation.

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The experimenters reasoned that if resistance developed after exposure to the phage, then the number of resistant mutants in each plate should remain more or less the same. On the other hand if mutations occurred before plating out, they may have occurred at any point in time, that is randomly during incubation. Some of the plates may show very few or no mutants. Some of the others, (aptly called 'jackpots') where mutations might have occurred early would have a large number of mutants, as mutants grow exponentially (*Box 1*). Thus after plating, one will observe large variations or 'fluctuations' in the number of colonies in culture A. The undivided set B works as a control in which smaller variations in the number of mutant colonies in different plates are expected since the mutants can be distributed randomly. In statistical jargon, the number of mutants in set B will show Poisson distribution, whereas those in set A will show a highly aggregated frequency distribution.

The results obtained by Luria and Delbruck supported the spontaneous mutation hypothesis since the distribution in the case of culture A was highly aggregated *(Box 1)*. The kind of distribution generated is often referred to as Luria Delbruck

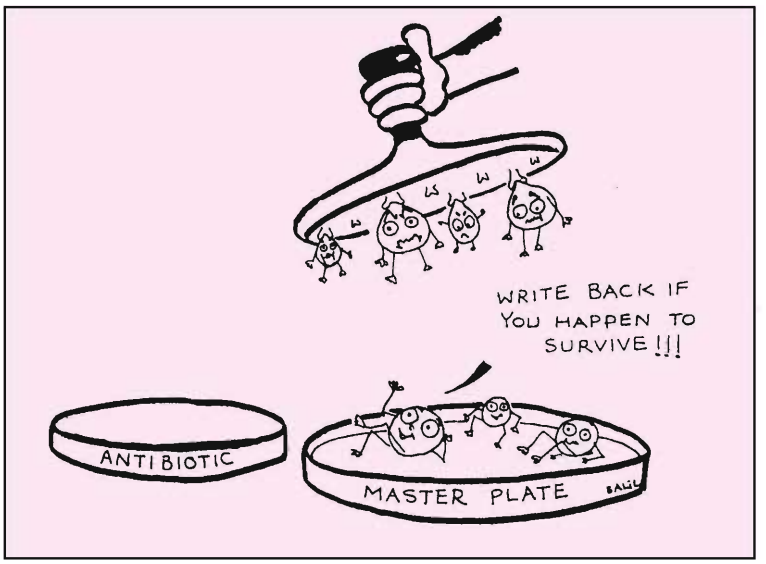
Here is the original experimental data obtained by Luria and Delbruck, which was published in '*Genetics*' in 1943.

Culture A (small individual cultures)	Culture B (samples from large culture)
1	14
0	15
3	13
0	21
0	15
5	14
0	26
5	16
0	20
6	13
107	mean = 16.7
0	variance = 15
0	
0	
1	
16	
17	
18	
19	
20	
0	
0	
64	
0	
35	
mean = 11.4	
variance = 694	

In culture B, as explained in the text, the distribution follows Poisson, an important property of which is that the ratio of variance to mean is approximately equal to 1. In the Luria-Delbruck distribution seen in culture A, on the other hand, variance exceeds the mean several fold. The two jackpots are clearly seen as 107 and 64 respectively.

distribution. Even after half a century of its formulation, it is routinely used for the calculation of mutation rates in bacteria.

These arguments were rather too mathematical for most of the biologists and the most common reactions were either uncritical acceptance or uncritical rejection. Biologists were more convinced by the ingenious 'replica plating' technique developed by Lederberg and Lederberg about ten years later



which made it possible to isolate mutants in pure culture without exposing them or their direct ancestors to a selective agent. Replica plating consists of simultaneously transferring a few cells each from all the colonies on a plate to another plate by means of a velveteen covered stamp pad. This replication of the master plate precisely preserves the geometric location of each colony. And that is the key point. If the master plate is non-selective and the replica plate contains the selective agent, say an antibiotic, only the antibiotic-resistant mutants will grow on the replica plate. This means that the colonies on the master plate, at precisely the same locations, must be antibiotic-resistant mutants. These colonies can be picked up and subcultured. After a couple of repetitions of the procedure, one can be sure that there is an antibiotic-resistant mutant that has not been exposed to the antibiotic anytime. The bacteria we expose to the antibiotic are the siblings of these cells. This in essence is the bacteriological version of 'sib selection'. When one can get antibiotic-resistant mutants without exposure to the antibiotic, it means that mutations are not a response to the selective agent but they arise spontaneously.

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These two celebrated experiments laid the foundations of bacterial genetics and their conclusions remained unchallenged for almost four decades. They became and remained the strongholds of neo-Darwinism. People believed that this was the final proof for all mutations being random. Little wonder therefore that a paper appearing in *Nature* in 1988 which challenged their conclusions stirred a hornet's nest.

A Unicorn Appears

Cairns, Overbaugh and Miller, in their paper, argued that both Luria-Delbruck as well as the Lederbergs were unfair to *E. coli*. They did not allow the organisms to show what they could do if given a decent chance. Luria and Delbruck used a bacteriophage which would kill the cells instantly as a selective agent. We know today that a mutation conferring resistance to phage is expressed only after several generations, a phenomenon called *phenotypic lag*. Therefore even if mutants had appeared after exposure to the phage they wouldn't have survived. The same applies for resistance to many of the antibiotics including streptomycin which the Lederbergs had used. We can of course forgive Luria-Delbruck and the Lederbergs on the grounds that in the early 1940s they did not know the molecular mechanism of phage or antibiotic resistance. In the light of the ever-increasing knowledge of the molecular mechanisms of mutations, someone ought to have reexamined the experimental logic. When Cairns et al did that, they found that what the Luria-Delbruck and the Lederberg experiments proved was only that at least some of the mutations were spontaneous. Their experiments did not prove that all mutations were spontaneous.

Cairns et al then designed a different set of experiments. The statistical design of the experiment was similar to that of Luria-Delbruck, but instead of using a bacteriophage, they challenged *E. coli* with an environment that did not support the growth of the strain but did not kill it instantly. Now the

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cells had ample time to sense the environment and develop the appropriate mutations, if at all they had the capacity to do so. Here the culture used was a mutant of *E. coli* unable to utilize lactose (called lac^-) and it was plated on a medium containing lactose as the only source of food. Now if mutations that reverted back to lac^+ arose as a result of exposure to lactose, their frequency distribution should have been Poisson. On the other hand if mutations took place before starvation, one would get a Luria-Delbruck distribution. What Cairns et al actually obtained was a composite distribution indicating that both types of mutations might be taking place. The revertant colonies did not appear all at once but accumulated slowly over a period of more than a week. Every day new mutant colonies were observed on the plates suggesting that the starving cells were still mutating after a few days of incubation. Further studies on the rates of specific mutations showed that under the above conditions the rate of lac^- to lac^+ mutation had specifically increased. Rates of other mutations which offered no selective advantage did not so increase. That was simply great. Not only did the organisms know when they needed to mutate, they also knew which gene to mutate.

This came as a shock. Franklin Stahl, in the same volume of *Nature* exclaimed, "What's up? Can bacteria really direct their mutational processes? Did bacteria discover 'directed mutagenesis' before the genetic engineers did?" So unorthodox and unexpected was Cairns' report that Stahl called it "A Unicorn in the Garden".

The First Wave of Reactions

Needless to say the paper created a sensation. A tide of reactions appeared in subsequent issues of *Nature*. A few researchers had noted similar observations before, which had confused them. They became bold and supported Cairns et al.

A host of others were skeptical and found many loopholes in the experimental design and also suggested alternative explanations for the results. Cairns' experiments did have some loopholes and the possibility of alternative explanations. If, for example, the growth rate of mutants was considerably lower than the wild type, an apparently composite distribution like the one obtained by Cairns et al is possible. It was also pointed out that the lac^- cells might be exhibiting slow growth on the lactose medium either because the mutant was leaky or because the lactose was impure. The resultant replication of DNA might allow accumulation of mutants. What might have really bothered the critics were the philosophical implications rather than the experimental design. Did the results support Lamarckism or any modified version of it? Or could the interpretation still be made within the framework of neo-Darwinism? The debate continued. This was but natural. Whenever a team of researchers reports something dramatically different from an existing paradigm it has to pass every scrutiny from all the skeptics of the world.

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But the results were reproducible. Confirmation of the findings followed from several laboratories. Leading among them was Barry Hall. Hall not only confirmed earlier results but tried to improve the experimental design in order to remove all the loopholes pointed out by the critics. Hall also made it clear that the phenomenon, which by now was variously named as 'directed', 'adaptive' or 'Cairnsian' mutation was not confined to the lactose locus but worked as well for a number of different loci and a number of different kinds of mutations many of them involving 'cryptic genes'. Cryptic genes are genes that are normally silent. In order to make these genes active, a specific mutational event is needed. In the case of the cryptic cellobiose utilization operon of *E. coli* it was seen that the frequency of a point mutation that activates the operon was high in an environment in which the gene product was needed.

Although it became increasingly clear that bacteria could really undergo directed mutagenesis, nobody understood the mechanism. Speculations started accumulating from the date of the very first report of directed mutations. But they remained speculations until mid-1995, when a couple of papers appearing in *Science* promised a breakthrough. In order to understand the mechanisms, we will have to peer a little deeper into molecular biology, which we had side-tracked in this article so far. A discussion of these aspects will be taken up in one of the future issues of *Resonance*.

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Suggested Reading

S E Luria and M Delbruck. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28, 491-511. 1943.**

J Lederberg and E M J Lederberg. Replica plating and indirect selection of bacterial mutants. *J. Bacteriology* **63, 399-406. 1952.**

Gunther S Stent. Molecular genetics, An introductory narrative. W H Freeman and Co. 1971.

J Cairns, J Overbaugh and S Miller. The origin of mutants. *Nature* **335, 142-145. 1988.**