

## CHAPTER 19

### LURIA/DELBRÜCK: MUTATIONS OCCUR AT RANDOM—THE FLUCTUATION TEST

*In 1943, Salvador Luria and Max Delbrück performed a classic experiment that conclusively demonstrated that favorable mutations such as antibiotic resistance in bacteria were “happy accidents,” preexisting mutations, and not the consequence of some sort of environmental influence causing the specific mutation to occur.*

### DARWIN'S THEORY OF SELECTION

Much of our view of mutation has been structured by the original viewpoints of Charles Darwin and Hugo de Vries. Darwin's view has had particular importance because of the central role mutation plays in his theory of biological evolution. Mutation provides the variation (raw material) upon which natural selection acts. Implicit in this view is a very important assumption. In Darwin's model, selection acts by choosing from among variants that are already there. The environment does not direct the genetic system to produce particular variants that would be advantageous, but rather passively selects from whatever happens to be available. In Darwin's view there is no connection between the production of variation and its utilization in evolution. Indeed, this is the heart of Darwin's theory: evolution is a passive process. Adaptation is constrained by the environment, not produced by it.

### ACQUIRED CHARACTERISTICS ARE NOT INHERITED

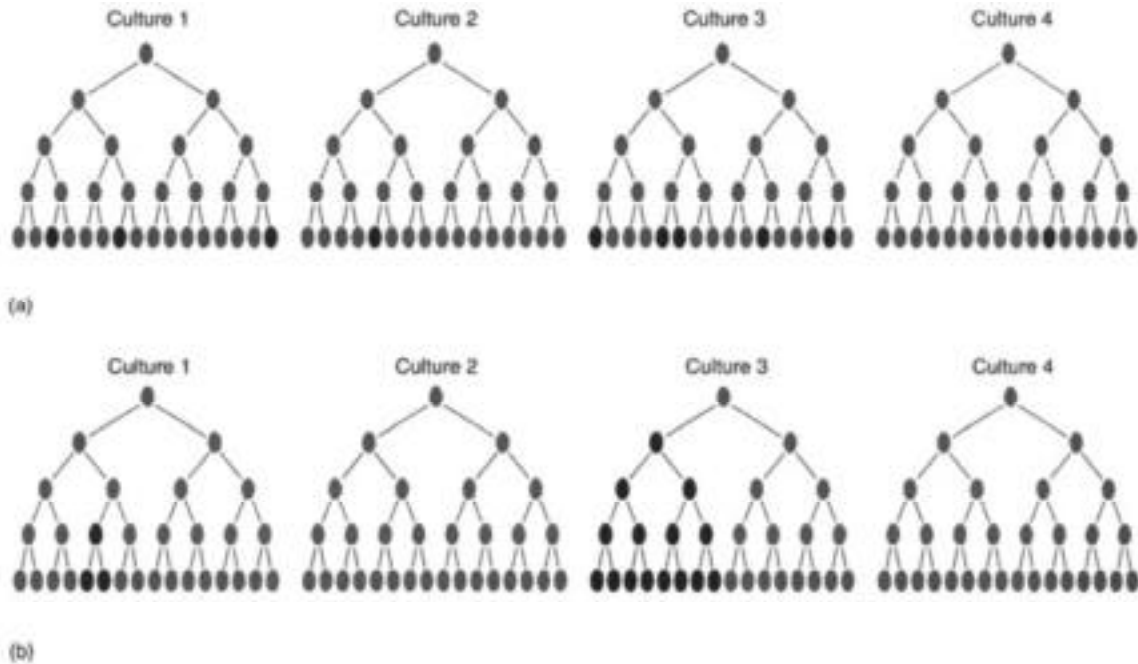
This was just a hypothesis at this point. There have been alternative viewpoints, also strongly held by first-class biologists. Jean Baptiste Pierre de Lamarck, one of the greatest biologists of the last century, argued that the environment directs the production of those particular mutations that will be favorable. The classic example is that of the giraffe that stretches (“grows”) its neck to reach higher branches and then passes this newly-acquired long neck trait to its offspring. This is a reasonable alternative hypothesis. Indeed, it was just this view of directed mutation that was espoused by Trofim Denisovich Lysenko in Russia during his tenure as “Soviet Lord of Biology” from 1936 to 1963. Until he was deposed, this forced exclusion of other theories led to the functional suppression of genetics education in Russia with serious agricultural consequences. For we now know this “acquisition of inherited characteristics” hypothesis to be incorrect. Darwin was right. Selection chooses from among mutations that already exist. A population is *preadapted*, if you will, in that it contains members potentially suited for a variety of unanticipated future situations.

### “PREADAPTIVE” VS. “POSTADAPTIVE” VARIATIONS

The prior existence of selected mutations is most easily seen in the study of bacteria (figure 19.1). The issue arose anew in such studies because of the marked ability of bacterial cultures to adapt to selective pressures imposed by the investigator. If sensitive bacteria are exposed to penicillin, sooner or later the culture becomes resistant to the drug. It is as if the antibiotic called up the necessary resistance. The issue in this case is clear-cut:

1. The variation is “postadaptive.” Directed mutations occur in the bacteria when placed on the selective medium. The selective medium dictates which mutations occur.

- The variation is “preadaptive.” A random collection of mutations exists prior to exposure of bacteria to the selective medium. Selection chooses the new types, but does not produce them.



**Figure 19.1**

**Are specific mutations induced? The fluctuation test.** To test whether bacterial viruses induce resistance mutations in hosts exposed to them, researchers examined the distribution of resistant cells in parallel cultures of infected bacteria. (a) If the  $T_1$  virus is inducing the mutation, the distribution of resistant colonies should be much the same in all four cultures. (b) If resistance arises spontaneously, it can arise in any generation, and some cultures show far greater numbers of resistant colonies than others.

## **LURIA AND DELBRÜCK'S FLUCTUATION TEST**

The issue was settled in 1943 with the development of the fluctuation test. Mutations are indeed present *before* selection. The production of mutations is random with respect to their effects on the phenotype. They act like a “random number generator” within the genetic system, constantly churning out “mistakes” that may prove to be beneficial under other circumstances. Salvador Luria and Max Delbrück set out to prove that bacteria were *not* being directed in some manner to produce the required mutations.

Luria and Delbrück noticed that while infection of a bacterial population with the bacteriophage T1 killed most cells, a few resistant cells always survived to found resistant populations. Did the T1 cause specific mutation to T1 resistance in the bacterial population? To test this, they devised this simple experiment:

- They inoculated a single culture with very few cells, which were permitted to grow and divide, eventually forming a population of millions of cells. From this, a small sample was spread on each of several culture plates containing the T1 virus, and the number of resistant colonies noted. The number of resistant colonies they observed was similar on all plates (the *variance* in colony number was low).
- They then repeated the procedure for several different cultures, testing cultures from each for resistance. The variance in resistant colony number was very much greater between cultures than within them!

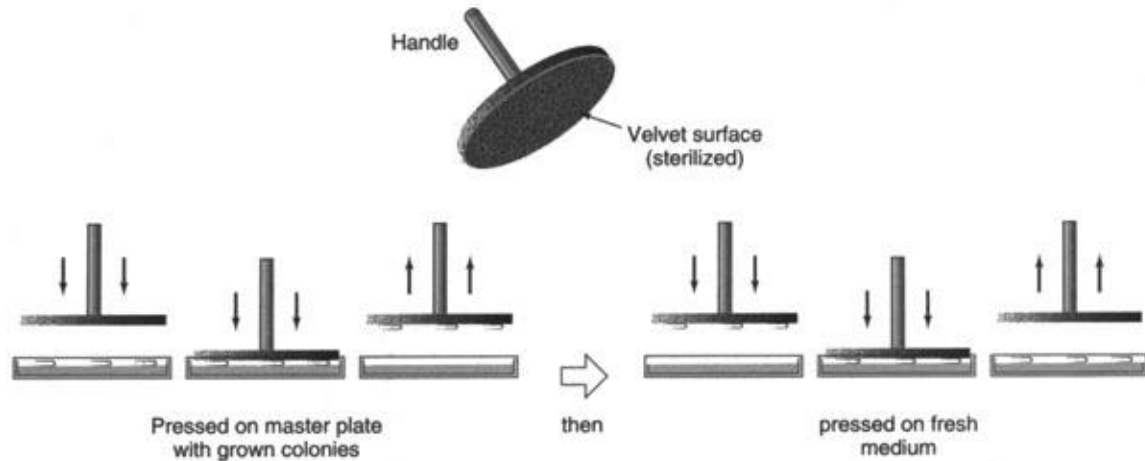
If bacteriophage T1 caused T1 resistance, then the variance in the two procedures should be the same. If, however, the event leading to T1 resistance was random, then it might occur at different times in different cultures, leading to different final proportions of T1-resistant cells in different cultures, and thus to a high variance in mean number of resistant cells per culture.

The conclusion of this experiment is inescapable: the mutation to T1 resistance arises in a random manner within bacterial populations (table 19.1). This is a general result that seems true of most organisms. Most mutation is not directed at specific genes by selective forces, but rather is random with respect to genotype. Rare exceptions have been documented in corn and wasps, but as a rule, mutation is blind to genotype.

**TABLE 19.1** The Fluctuation Test of the Spontaneous Origin of T1 Phage-Resistant *E. coli* Mutants

Individual Cultures		Samples from Bulk Culture	
<i>Culture Number</i>	<i>Ton'</i> <i>Colonies Found</i>	<i>Sample Number</i>	<i>Ton'</i> <i>Colonies Found</i>
1	1	1	14
2	0	2	15
3	3	3	13
4	0	4	21
5	0	5	15
6	5	6	14
7	0	7	26
8	5	8	16
9	0	9	20
10	6	10	13
11	107		
12	0		
13	0		
14	0		
15	1		
16	0		
17	0		
18	64		
19	0		
20	35		
Mean ( $\bar{n}$ )	11.3		16.7

From S. E. Luria and M. Delbrück, *Genetics* **28**, 491 (1943), Genetics Society of America, Bethesda, MD. Reprinted by permission.



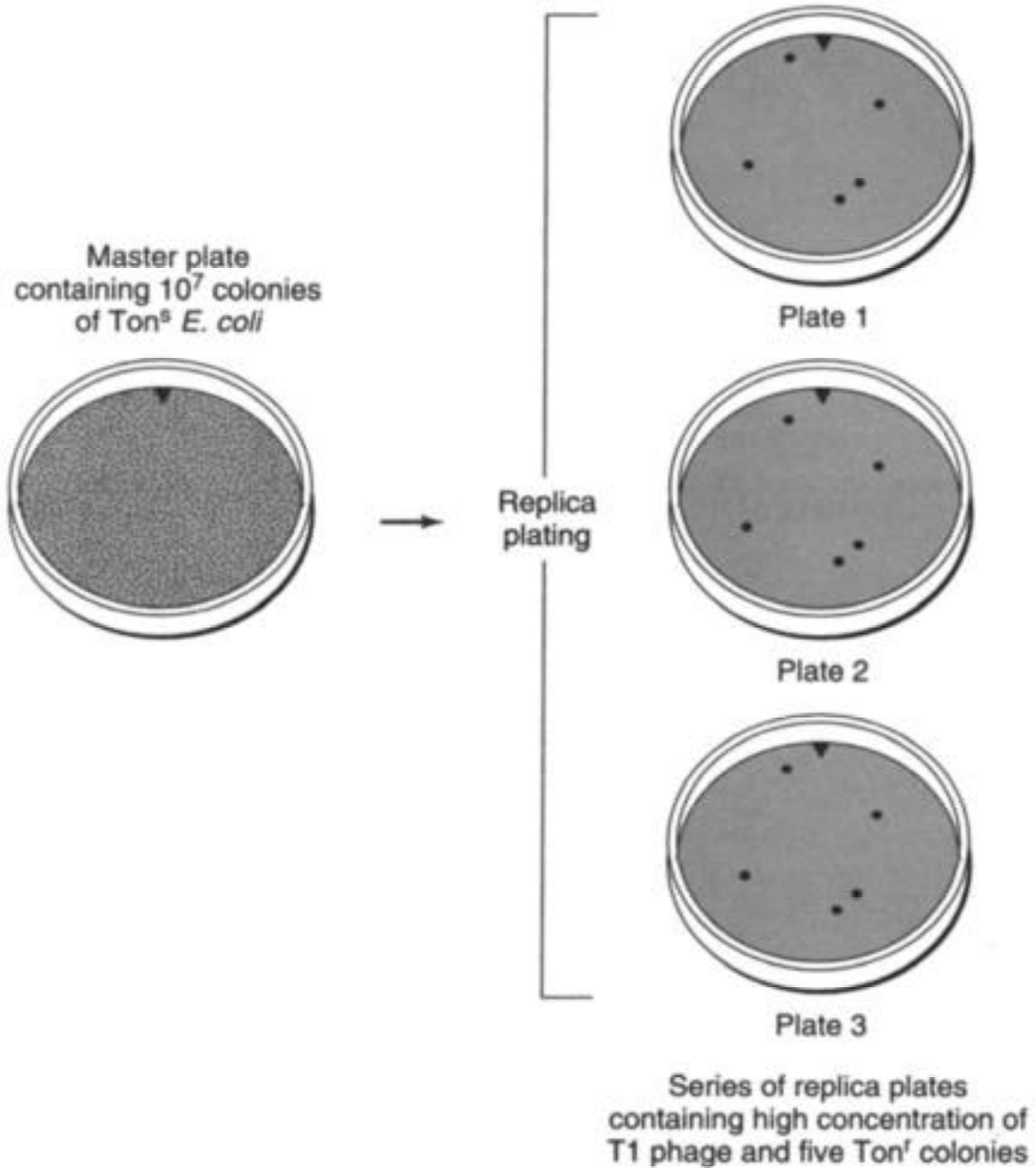
**Figure 19.2**

**The replica plating technique.** A circle of sterile velvet the exact diameter of a petri dish is first touched to the master plate containing the original colonies, and then is immediately touched to a new petri dish with fresh medium. In this way, the exact distribution of bacterial colonies is replicated.

## **ESTHER LEDERBERG'S EXPERIMENT**

In 1953, Esther and Joshua Lederberg developed a similar technique called *replica plating*, in which a Petri dish was inoculated with bacteria and incubated until several colonies were visible, and then the colonies were transferred exactly as they were to other plates that had been inoculated with bacteriophages (figure 19.2). Esther Lederberg used a circular piece of velvet the exact diameter of the Petri dish, pressed it gently onto the colonies, and then pressed the same piece of velvet onto several new Petri dishes that had previously been inoculated with the T1 phage.

This technique ensured an exact transfer, colony by colony, to the phage-infected plates. That made it possible to keep track of each colony and, therefore, each cell line. If resistance to the T1 phage was "preadaptive," then it would be present on the master plate. Replica plating on many T1-containing plates should have given the same T1 colonies each time in the same position, and it did. This proved that T1 resistance must have arisen spontaneously in bacteria and not as an environmentally-dictated mutation (figure 19.3).



**Figure 19.3**

*By replica plating, the spontaneous development of a 4-T1 phage-resistant  $Ton$  colony is shown. The original plate was inoculated only with  $Ton^S$  *E. coli*, so the appearance of 4  $Ton^F$  colonies represents spontaneous mutation.*