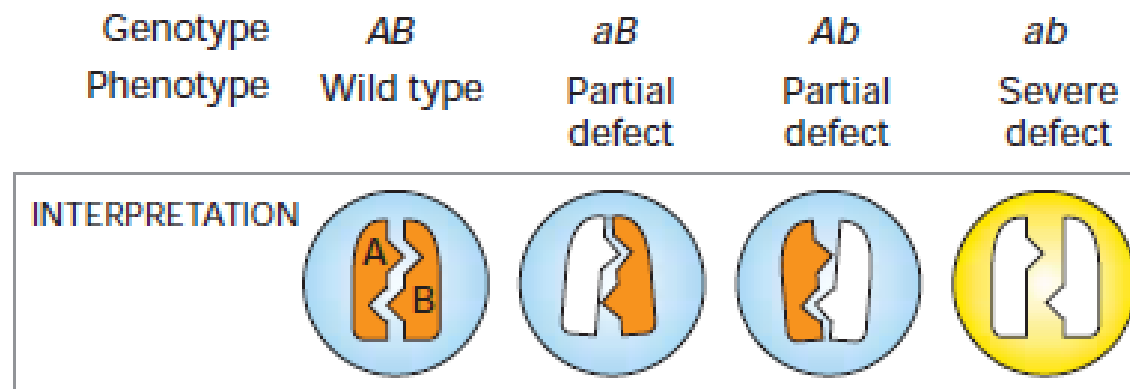


- *synthetic* is used here for its ancient Greek meaning: the combination of two entities to form something new.

(b) Synthetic lethality 1



Spontaneous mutation

One of the first questions asked by geneticists was whether spontaneous mutations are induced in response to external stimuli, or whether variants are present at a low frequency in most populations.

An ideal experimental system to address this important question was the analysis of mutations in bacteria that confer resistance to specific environmental agents not normally tolerated by wild types.

Luria and Delbrück fluctuation test

It was known at the time that if *E. coli* bacteria are spread on a plate of nutrient medium in the presence of phage T1, the phage soon infect and kill the bacteria.

However, rarely but regularly, colonies were seen that were resistant to phage attack; these colonies were stable and so appeared to be genuine mutants.

However, it was not known whether these mutants were produced spontaneously but randomly in time or whether the presence of the phage induced a physiological change that caused resistance.

Luria reasoned that if mutations occurred spontaneously, then the mutations might be expected to occur at different times in different cultures; so the resulting numbers of resistant colonies per culture should show high variation.

Luria and Delbrück designed their “**fluctuation test**” as follows:

They inoculated 20 small cultures, each with a few cells, and incubated them until there were 10^8 cells per milliliter.

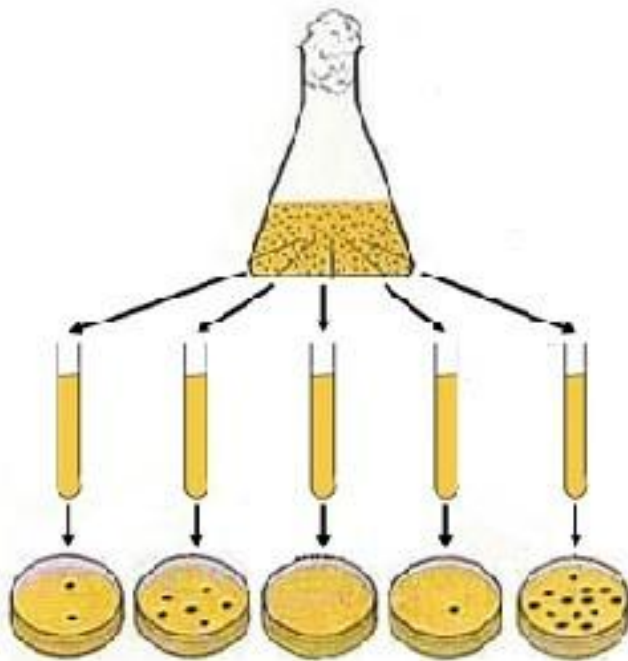
At the same time, a much larger culture also was inoculated and incubated until there were 10^8 cells per milliliter.

The 20 individual cultures and 20 samples of the same size from the large culture were plated in the presence of phage.

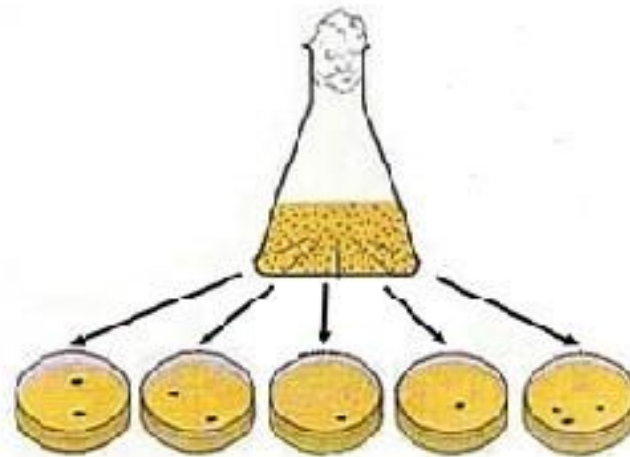
The 20 individual cultures showed high variation in the number of resistant colonies: 11 plates had 0 resistant colonies, and the remainder had 1, 1, 3, 5, 5, 6, 35, 64, and 107 per plate.

The 20 samples from the large culture showed much less variation from plate to plate

Luria-Delbruck Fluctuation Test

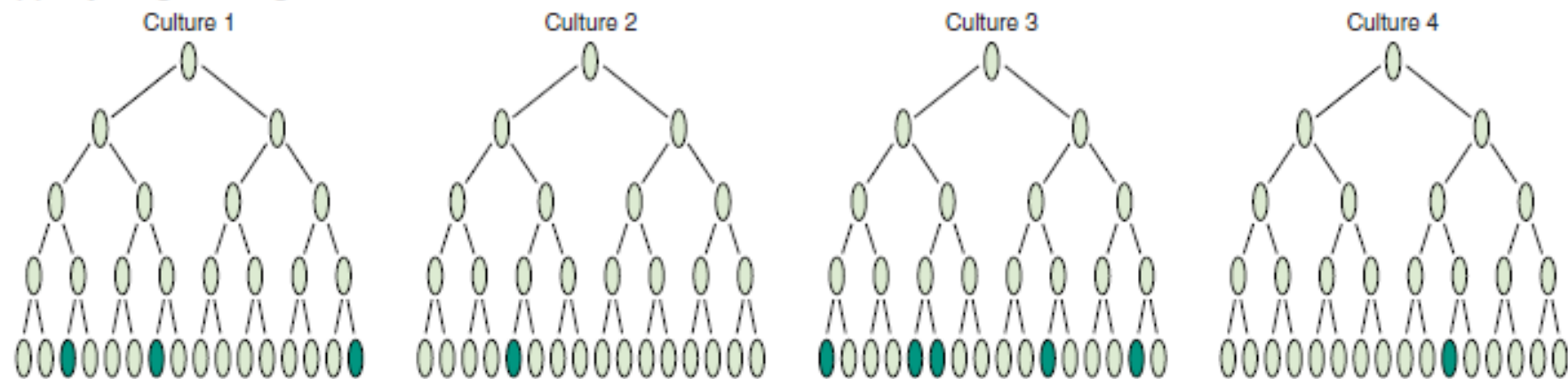


culture separately

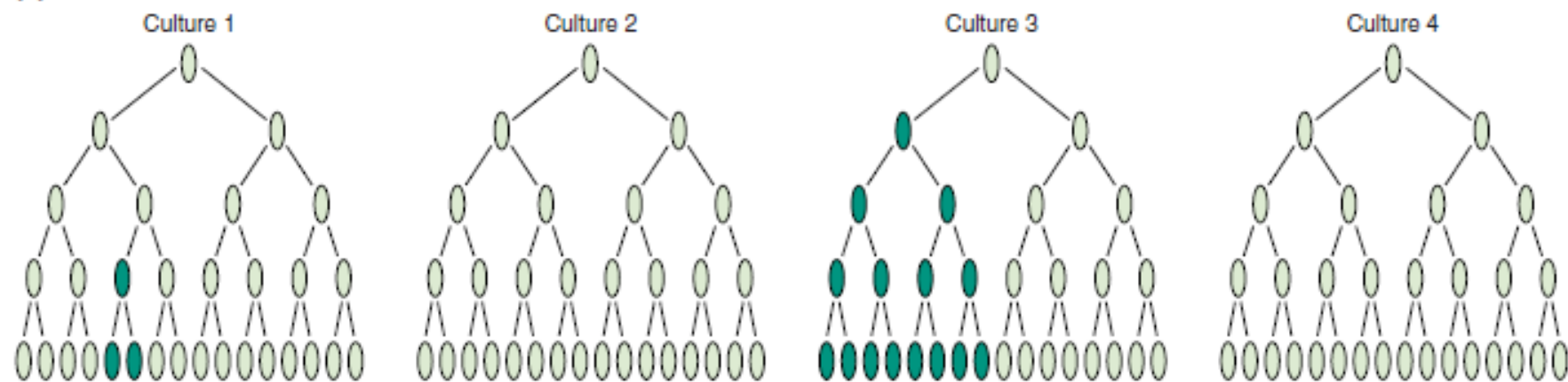


sample repeatedly

(a) Physiological change



(b) Random mutation



If the phage were inducing mutations, there was no reason why fluctuation should be higher on the individual cultures, because all were exposed to phage similarly.

The best explanation was that mutation was occurring **randomly in time**: the early mutations gave the higher numbers of resistant cells because they had time to produce many resistant descendants.

The later mutations produced fewer resistant cells.

This elegant analysis suggests that the resistant cells are selected by the environmental agent (here, phage) rather than produced by it.

Can the existence of mutants in a population before selection be demonstrated directly?

This demonstration was made possible by the use of a technique called **replica plating**, developed by **Joshua and Esther Lederberg** in 1952.

A population of bacteria was plated on nonselective medium—that is, medium containing no phage—and from each cell a colony grew.

This plate was called the ***master plate***.

A sterile piece of velvet was pressed down lightly on the surface of the master plate, and the velvet picked up cells wherever there was a colony.

In this way, the velvet picked up a colony “imprint” from the whole plate.

On touching the velvet to replica plates containing selective medium (that is, containing T1 phage), cells clinging to the velvet are inoculated onto the replica plates in the same relative positions as those of the colonies on the original master plate.

As expected, rare resistant mutant colonies were found on the replica plates, but the multiple replica plates showed identical patterns of resistant colonies.

If the mutations had occurred *after exposure to the selective agents*, the patterns for each plate would have been as random as the mutations themselves.

The mutation events must have occurred before exposure to the selective agent.

