

**S. E. Luria and M. Delbruck (1943). Mutations of bacteria from virus sensitivity to virus resistance.
Genetics 28:491**

[Presented by: **Steve Carr** (scarr@mun.ca), 07 January 2012]

Background & Introduction

[Max Delbruck](#) (1906 -1981) & [Salvador Luria](#) (1912 -1991)

Shared [1969 Nobel Prize](#) in Physiology or Medicine

Bacteriology in 1940s not heavily influenced by genetic thinking

No nuclei, do they have "genes"?

Bacterial "*phenotypes*": manifestations of 10^6 s of bacteria simultaneously

No sex: crosses not possible

[Discovery of bacterial sex led to [1958 Nobel Prize](#)]

bacteriophages ("**phages**") - "*subcellular parasites that infect, multiply within, & kill bacteria.*"

T1 phages are active on [E. coli](#)

[phage] >> [bacteria] \Rightarrow no bacterial colonies grow: bacteria are **Ton^S** ("T-one sensitive")

[phage] ~ [bacteria] \Rightarrow some bacterial colonies grow: bacteria are **Ton^R** ("T-one resistant")

Ton^R phenotype stable

all descendant bacteria **Ton^R**

phenotype *persists* absent T1

Two Hypotheses (d'Herelle 1926 vs Brunet 1929)

1. **Ton^R phenotype** *induced* by exposure of bacteria to phage

Each bacterium has (small, finite) chance of survival (say $\sim 1 / 10^7$);

Survivors have **altered metabolic phenotype**, transmitted to offspring

[distinction between *phenotype* & *genotype* not clear]

Bacteria *adapt* to their environment :

a **Lamarckian** hypothesis: **inheritance of acquired characteristic**

2. **Ton^R phenotype** occurs **spontaneously**, prior to exposure of bacteria to phage

Some rare bacteria (say $\sim 1 / 10^7$) *already* **Ton^R**

genetic mutation to a stable *genotype*

[*phenotype* persists absent phage]

a **Darwinian** hypothesis: **Ton^R bacteria selected**

Theory:

Hypotheses make different predictions as to

statistical distribution of **Ton^R** phenotypes among bacterial cultures.

Induction (Adaptation) Hypothesis predicts: **$n / N = a$**

where **n** = number of **Ton^R** bacteria observed out of

N = number of **Ton^S** bacteria plated, and

a = **probability of conversion** from **Ton^S** to **Ton^R**

Then, **n** should be a *constant* fraction of **N**

Mutation Hypothesis predicts: **$n / N = ga2^g / 2^g = ga$**

where **a** = **mutation rate** (# mutations / cell / generation)

g = # **generations** to go from **1** \rightarrow **N** bacteria, so that

N = **2^g** doublings occur, of which

n = **ga2^g** produce mutant **Ton^R** bacteria

[because a mutation in the i th generation contributes $a2^i2^{g-i} = a2^g$ mutants]

Then, n should increase wrt N , as g increases

How can differences in n be evaluated?

Suppose c cultures are started from a single Ton^S mutant each
after g generations there are $N = 2^g$ bacteria in each culture

mean number of Ton^r bacteria is $\bar{x} = \sum(x_i) / c$

variance is $\sigma^2 = \sum [\bar{x} - x_i]^2 / c$

Thought experiment:

Consider four cultures started from a single bacterium

after $g = 4$ generations, expect 16 cells from 15 divisions @,
total 64 cells from 60 divisions

plate each culture separately w/ T1, count total # Ton^r

10 Ton^r colonies observed: what distribution ("fluctuation") expected?

Induction Hypothesis:

Ton^r induction occurred only in fourth generation upon exposure to T1

probability of induction (a) is uniform / bacterium

$a = 10$ inductions / 64 cells = 15%

mean occurrence = $10 / 4 = 2.5 \text{Ton}^r$ per culture

variance = $[(2.5 - 3)^2 + (2.5 - 1)^2 + (2.5 - 5)^2 + (2.5 - 1)^2] / 4 = 2.75$ [alternative calculation]

Follows a **Poisson Distribution**: variance = mean

Mutation Hypothesis

Ton^r mutation has occurred spontaneously, prior to exposure to T1

mutation rate (a) = 2 events / 60 cell divisions = 0.033 mutations / cell / generation

mean rate of occurrence = $(2 + 0 + 8 + 0) / 4 = 2.5 \text{Ton}^r$ as before

earlier Ton^r mutations leave more offspring (as in Culture 3)

variance = $[(2.5 - 2)^2 + (2.5 - 0)^2 + (2.5 - 8)^2 + (2.5 - 0)^2] / 4 = 10.75$

after 5 generations, when the number of Ton^r cells has doubled in each culture:

variance = $[(5.0 - 4)^2 + (5.0 - 0)^2 + (5.0 - 16)^2 + (5.0 - 0)^2] / 4 = 48.00$

Mutation Hypothesis predicts variance >> mean, as g increases

Methods:

"The first experiment was done on the following Sunday morning.

(In a letter dated January 21 [1943], Delbruck exhorted me to go to church)"

Twenty x 200 ul "individual cultures"

One x 10 ml "bulk culture"

Inoculate with ~ 10^3 bacteria @

Grow for $g = 17$ generations

⇒ ~ 10^8 bacteria / ml

Plate entire "individual cultures"

& 200 ul aliquots of "bulk culture" on petri dish w/ T1

Results

	<u>Bulk</u>	<u>Individual</u>
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	Cultures	Cultures
Experiment ##	1, 10, 11, 15	16, 17, 21a
Mean	16.7	11.3
Variance	15	694

In **bulk** cultures,

$$a = n / N = (16.7 / (0.2 \text{ ml} \times 10^8 \text{ bacteria / ml})) = 8 \times 10^{-7} \text{ variants / cell}$$

variance ~ **mean** \Rightarrow **random** distribution

Expected result if changes are induced (also compatible with mutation)
[essentially a **control** experiment]

In **individual** cultures,

mean ~ **mean** in bulk

variance \gg **variance** in bulk:

Experiment supports prediction of Mutation Hypothesis !

Mutation rate (a) can be calculated

mean # mutations / culture = **aN**

Poisson distribution predicts $p_0 = \exp(-a / N)$

where p_0 = fraction of cultures with *no* **Ton^r** mutants

Rewrite as **a = -ln p₀ / N**

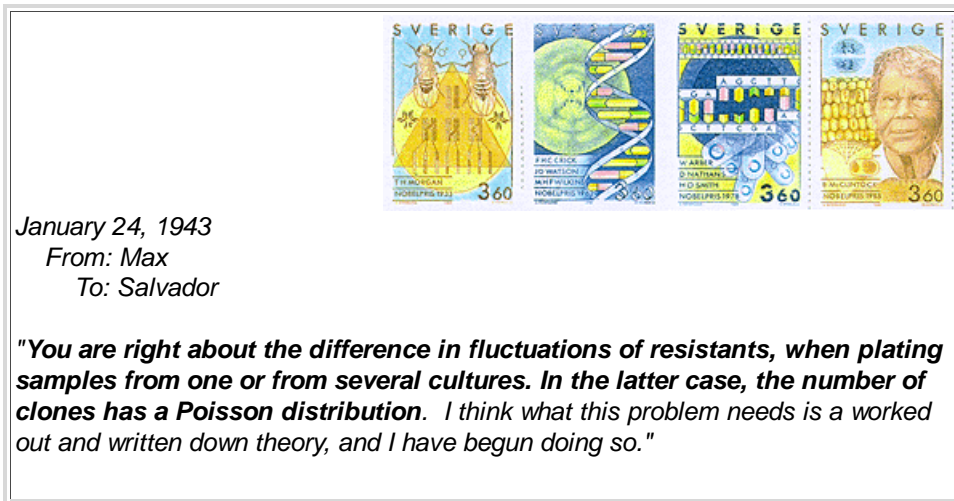
p₀ = 11 / 20 = 0.55 from data in [Experiment 16](#)

N = 0.200 ul x 10⁸ bacteria / ml

Then **a = -ln 0.55 / (0.2 x 10⁸) = 3 x 10⁻⁸ mutations / cell / generation**

Conclusions

"On a postcard dated **January 24**, Delbruck replied:



The MS of the theory arrived on February 3rd"

Luria on the significance of these experiments:

- (1) "**Adequate**" evidence of **spontaneous mutation** as source of genetic variation
- (2) Provided **method** for measuring **mutation rates**, and therefore is
- (3) "**The Birth of Bacterial Genetics**"
bacteria can be used to measure **extremely low mutation rates**