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

[Presented by: Steve Carr (scarr@mun.ca), 14 January 2014]

Background

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
Bacteria have no nuclei: do they have "*genes*"?
Bacterial "phenotypes" are the manifestations of 10^6s of bacteria simultaneously
Bacteria don't have 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] ⇒ *no* bacterial colonies grow: bacteria are **Ton**^{s} ("T-one sensitive")
[phage] ~ [bacteria] ⇒ *some* bacterial colonies grow: bacteria are **Ton**^{r} ("T-one resistant")
Ton^{r} phenotype is **stable**
all descendant bacteria are **Ton**^{r}
phenotype *persists* in the absence of **T1**

Two Hypotheses (d'Herelle 1926 *vs* Brunet 1929)

1. **Ton**^{r} phenotype is ***induced*** *by exposure* of bacteria to phage
   Each bacterium has a (small, finite) chance of survival (~ 1 / 10^7);
   Survivors have altered *metabolic phenotype*, which is *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 ~ 1 / 10^7) are already **Ton**^{r}
   These bacteria have undergone **genetic mutation** to a stable *genotype*
   [*phenotype* persists in absence of phage]
   a ***Darwinian*** hypothesis: **Ton**^{r} bacteria are ***selected***

Materials & Methods

Hypotheses make different predictions as to
numerical distribution of **Ton**^{r} phenotypes among bacterial cultures.

Induction (Adaptation) Hypothesis predicts: $\mathbf{n / N = a}$
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: $\mathbf{n / N = ga2^g / 2^g = ga}$
where **a** = **mutation rate** (# mutations / cell / generation)
g = # **generations** to go from **1** → **N** bacteria, so that
N = $\mathbf{2^g}$ doublings occur, of which
n = $\mathbf{ga2^g}$ produce mutant **Ton**^{r} bacteria

[because a mutation in the ***i* th** generation contributes $a2^{i}2^{g-i} = a2^{g}$ 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

Statistical foundations of **Luria - Delbruck experiment**

Thought experiment:

Consider four cultures each 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}

Suppose **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%

observe = **3, 1, 5,** & **1 Ton**^{r} colonies

mean = **10 / 4** = **2.5 Ton**^{r} per culture
variance = **2.75**
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 = **(2 + 0 + 8 + 0) / 4** = **2.5 Ton**^{r} as before

After 4 generation, earlier **Ton**^{r} **mutations** leave more offspring (as in **Culture 3**)
variance = **10.75**

after 5 generations, when the number of **Ton**^{r} cells has *doubled* in each culture:
variance = **48.00**

Mutation Hypothesis predicts variance >> mean, as **g** increases

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Experimental procedure:

"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

	Bulk Cultures	Individual Cultures
Experiment ##	**1, 10, 11, 15**	**16,17, 21a**
Mean	**16.7**	**11.3**
Variance	**15**	**694**

Bulk cultures:

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

variance ~ mean ⇒ random distribution

Expected result if changes are either induced or spontaneous

[essentially a **control** experiment]

Individual cultures:

mean ~ mean in bulk

variance >> variance in bulk:

Experiment supports prediction of Mutation Hypothesis !

Calculation of **Mutation rate** (**a**)

mean # mutations / culture = **aN**

Poisson distribution predicts $p_0 = e^{(-a / N)}$

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

Rewrite as $a = -\ln (p_0 / N)$

p_0 **= 11 / 20 = 0.55** from data in Experiment 16

N = 0.2 ml **x** 10^8 bacteria / ml

Then **a = -*ln* 0.55 / (0.2 x 10^8) = 3 x 10^{-8}** mutations / cell / generation

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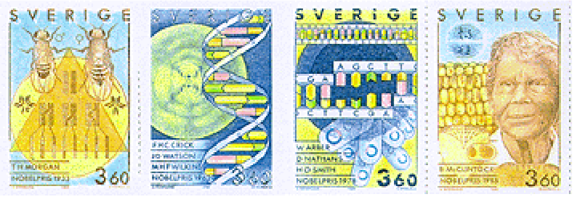
Conclusions

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



January 24, 1943

From: Max

To: Salvador

***"You are right about the difference in fluctuations of resistants, when plating samples from one or from several cultures. In the latter case, the number of clones has a Poisson distribution**. I think what this problem needs is a worked out and written down theory, and I have begun doing so."*

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**
