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 2016]

Background

Max **Delbruck** (1906 -1981) & Salvador **Luria** (1912 - 1991)
1969 Nobel Prize in Physiology or Medicine

Bacteriology <1940 not influenced by genetic thinking
No nuclei: do they have "*genes*"?
No individual "*phenotypes*": colonies of 10^6s of bacteria simultaneously
No sex: genetic 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, heritable**
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 ***induced*** *by exposure* of bacteria to phage
Each bacterium has small, finite prob.of survival (~ 1 / 10^7);
Survivors have altered *metabolic phenotype*, *transmitted to offspring:*
changed *phenotype* persists in *genotype*
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 (~ 1 / 10^7) are already **Ton^r**
have undergone **genetic mutation** to a stable *genotype*
changed *genotype* regenerates *phenotype*
a ***Darwinian*** hypothesis: **Ton^r** bacteria are ***selected***

Materials & Methods

Hypotheses predict *different* distributions of **Ton^r** phenotypes among cultures

Induction Hypothesis predicts: **n / N = a**
where **n** = number of **Ton^r** bacteria observed out of
N = number of **Ton^s** bacteria plated, where
a = **probability of conversion** from **Ton^s** to **Ton^r**
Then, **n** should be *constant* wrt **N**

Mutation Hypothesis predicts: **$n / N = ga2^g / 2^g = ga$**
where **a** = **mutation rate** (# mutations / cell / generation)
g = **# generations** to go from **1** → **N** bacteria, so that
N = **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?

Thought experiment:
Consider **four** cultures each started from a **single** bacterium
after **g = 4** generations, expect **64 cells** from **60 divisions**
plate each culture separately w/ **T1**, count total # **Ton^r**
Suppose **10** **Ton^r** colonies observed: what distribution *(***variance***, "fluctuation")* expected?

Induction Hypothesis:

Ton^r *induction* occurs in *last* 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**
Expect **Poisson Distribution** for rare, random events: **variance = mean**
***Homework*: Evaluate 16 plants / 64 quadrats distribution** by **Chi-Square**

Mutation Hypothesis

Ton^r *mutations* occur 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 generations, early **mutations** leave more offspring
variance = **10.75**
after 5 generations, # of **Ton^r** cells *doubles* 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 ##	**10a**	**16**
Mean	**16.7**	**11.3**
Variance	**15**	**694**

Single bulk culture (*e.g.*, **Experiment 10**a):
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 for either induced *or* spontaneous hypotheses: a **control**

Multiple individual cultures (*e.g.*, **Experiment 16**):
mean ~ mean in bulk
variance >> variance in bulk:
Experiment supports Mutation Hypothesis !

Calculation of **Mutation rate** (**a**)
mean # mutations / culture = **aN**
Poisson predicts ***null class*** = $p_0 = e^{(-\ a/N)}$
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 **Experiment 16**
N = 0.2 ml **x** 10^8 bacteria / ml
Then **$a = -ln\ 0.55 / (0.2 \times 10^8) = 3 \times 10^{-8}$** mutations / cell / generation

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Conclusion

January 24, 1943

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."*

Max

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 Molecular Genetics"***
bacteria can be used to measure **extremely low gene mutation rates**

***Homework*: repeat all statistical calculations for Experiments 3 & 21a**
