Mutation and Selection

It is clear that evolution shapes the phenotypes of all organisms - there is variation in a population, and natural selection allows the propagation of the variants that are most fit. Heritable variations are caused by mutation, a change in the genome sequence. One of the fundamental questions about evolution is **where do mutations come from?** This question was elegantly addressed by Salvador Luria and Max Delbruck in 1943. They posed 2 competing hypotheses: 1) **mutations are induced by challenges in the environment**, and 2) **mutations arise randomly**. The answer to this question has important implications for how evolution works. If hypothesis 1 is correct, a population of organisms adapts in response to the environment - when things are bad, make mutations! If hypothesis 2 is correct, mutations are always being made and the environment selects among the existing pool of phenotypes.

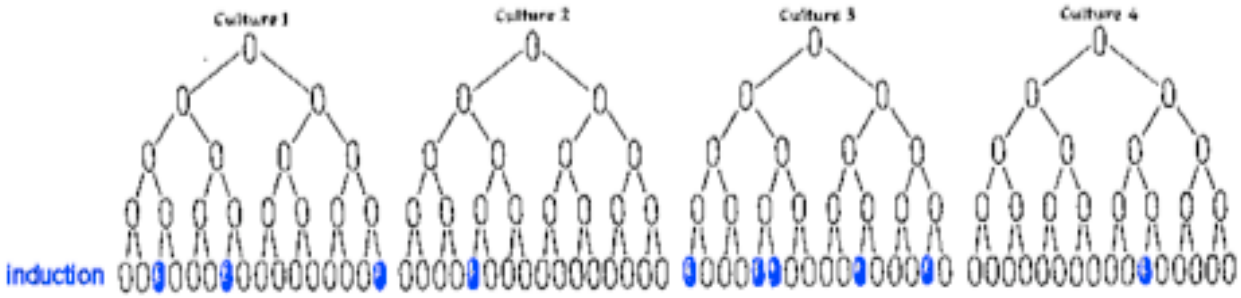
The Luria-Delbruck Fluctuation Test

An easy system to look at mutation and selection is the interaction of bacteria and phage. Luria and Delbruck used *E. coli* and phage T1 (we will be using phage T4). Wild-type *E. coli* will be killed by T1, but if you grow a large culture of *E. coli* and plate it on T1 some cells will survive and make colonies. According to hypothesis 1, the mutations arise when the *E. coli* are challenged by T1. According to hypothesis 2, mutations that make *E. coli* resistant arise spontaneously during growth, but you can only see that they are there when the cells are challenged by T1. The explanation each hypothesis provides is different, but the predicted result of the experiment is the same - most cells die, but resistant mutants survive. So how can these hypotheses be tested?

Luria and Delbruck realized that even though looking at the average number of *E. coli* that survive on T1 would not discriminate between the hypotheses, the two hypotheses made different predictions about the **variance** that would be observed in the number of E. coli that survive on T1 between different cultures. Variance is a statistical measure of how data distribute or are spread out around the mean of a dataset.

According to hypothesis 1, all cultures of *E. coli* are the same when they are plated on T1 - they are wild-type until they are challenged by the phage, and the challenge induces mutations. In this case, the difference in the number of survivors in different cultures should be small and should cluster around the mean. The data should show a “normal” or “Gaussian” distribution, like the grade distribution in organic chemistry.

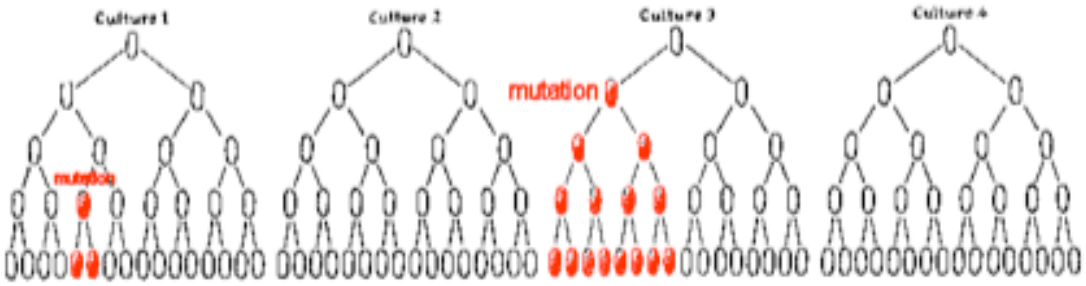
Here is a picture:



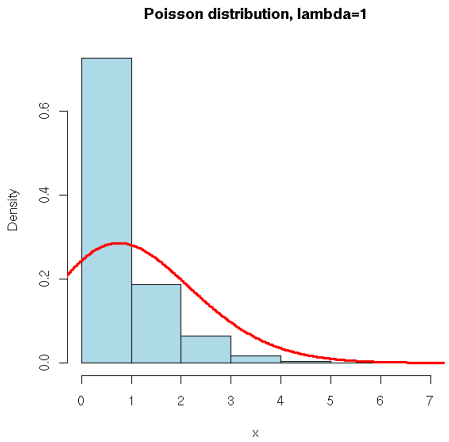
In generation 1 there are no mutants in any of the cultures. Likewise, in generations 2, 3, and 4 there are no mutants. In the last generation the cells are plated on T1 and mutant (blue cells) arise. There is some variation in the number of mutants in the different cultures, but the **variance** is small.

According to hypothesis 2, different cultures are not the same when they are plated on T1. The chance of a mutation arising that makes the cells resistant to T1 is small, so most of the cultures will have no mutants and no survivors. In some cultures a mutation will have occurred and there will be survivors, but the number of mutants that can survive will depend on when the mutation occurred - if it occurred early in the culture growth there will be lots of mutants, but if it occurred late there will be few.

Here is a picture:



In this case, the average number of survivors may be the same as for hypothesis 1, but the **variance** will be huge - most of the cultures will have no survivors, some will have a small number, and a few will have many survivors. A distribution like this is similar to a “Poisson distribution”, and for rare events, like mutations, the curve will look like this:



Here is how the experiment works. A series of samples from (1) the same bacterial culture and (2) from lots of different bacterial cultures are plated on agar plates coated in T1. If Hypothesis 1 is correct and all of the resistant colonies arose after the bacteria were spread on the plate, then the variance will be similar for both series of samples. If Hypothesis 2 is correct, then the resistant bacteria arose before exposure to T1 and the number of resistant mutants in each culture will depend on when the mutation arose during culture growth. As a result, the variance for the trials with different cultures (scenario 2 above) will be much higher that the variance for trails from the same culture (scenario 1 above).

Challenge

> For this experiment you will use Luria and Delbruck's fluctuation test to determine which hypothesis, random or adaptive mutation, best explains where mutations that make *E. coli* resistant to phage T4 come from. We will give you bacteriophage T4, a strain of *E. coli* B that is susceptible to T4, and a protocol for the fluctuation test. Make sure to design appropriate controls for this experiment.

Key Questions:

An enormous problem in public health is the increase in the number of pathogenic bacteria that are resistant to antibiotics. Resistance can come from mutations that make bacteria impervious to the antibiotic. Given your results from this experiment, explain how antibiotic treatment influences the selection of resistant mutants.

Some bacterial strains have much higher mutation rates than other strains. What would be the benefits of a high mutation rate? What would be the downside to a high mutation rate?

In 1996, a new set of drugs to treat HIV were introduced. The new drugs stopped the replication of HIV. However, within a few months some strains of HIV were found that were completely resistant to the new drugs, and today many HIV strains exist that are resistant to these drugs. How can you explain the emergence of HIV resistance to the drugs?

Fluctuation Test Protocol

We will use bacteriophage T4 and a strain of *E. coli* B that is susceptible to T4 for this experiment. To do the fluctuation test, you will grow and plate aliquots from lots of different cultures and plate multiple aliquots from a single culture as described below.

Preparation of Cultures- Lab Class Day 1:
Each group will set up 10 independent cultures as follows.

1) Add 100 µl of the *E. coli* B culture to a tube with 5 ml of nutrient broth.

2) Repeat for a total of 10 tubes per group of 2.

3) The instructor will prepare one additional culture. It will be the used by everyone in the class to measure variance from a single culture.

4) All the cultures will be grown with shaking overnight at 37 °C, then stored at 4 °C.

Plating cultures- Lab Class Day 2: Make sure to use appropriate controls!
Each group will plate an aliquot from the 10 cultures they grew on Day 1 and six aliquots from the single culture that will be shared by the whole class.

5) To select for T4-resistant *E. coli*, the phage must first be spread on the LB plates. Spread 200 µl of the T4 phage stock onto 16 LB plates. Make sure that the liquid is completely absorbed onto the plate before continuing.

6) Spread 50 µl from one of your cultures from Day 1 onto a plate with T4 phage. Repeat for the other cultures.

7) Everyone will be given a sample from the culture started by the instructor. Spread 50 µl of this culture onto a plate with T4 phage. Repeat for the remaining 5 plates.

8) The plates will be incubated at 37 °C for ~18 h then placed at 4 °C.

Counting colonies: Lab Class Day 3:

9) Count the number of colonies on each of your plates and calculate the average and variance for each data set.

Variance = $\Sigma(X-\mu)^2/N$ where X=measurement, m=average of all measurements, N= total number of measurements

or

1) calculate the mean
2) for each number, subtract the mean and square the result
3) average the numbers from step 2 - this is the variance

Questions to consider:

How do your results compare to those from the rest of the class?

On Day 1 we will also perform a plaque assay to determine the number of phage in the T4 sample that you will use for this experiment. How do the number of phage used compare to the number of bacteria plated?

Can you calculate the mutation frequency?