Solutions to Part II of Exam 2

3. Luria and Delbrück[1] devised the following procedure (known as a *fluctuation test*) to estimate the *mutation rate, a*, for certain bacteria:

   Imagine that you start with a single normal bacterium (with no mutations) and allow it to grow to produce several bacteria. Place each of these bacteria in test–tubes each with media conducive to growth. Suppose the bacteria in the test–tubes are allowed to reproduce for n division cycles. After the n^{th} division cycle, the content of each test–tube is placed onto a agar plate containing a virus population which is lethal to the bacteria which have not developed resistance. Those bacteria which have mutated into resistant strains will continue to replicate, while those that are sensitive to the virus will die. After certain time, the resistant bacteria will develop visible colonies on the plates. The number of these colonies will then correspond to the number of resistant cells in each test tube at the time they were exposed to the virus.

   (a) Let Let $M(t)$ denote the number of bacteria that develop a mutation to resistant strains during the time interval $[0, t]$. Make an assumption regarding the distribution of the random variable $M(t)$ and use that assumption to compute
   $$P[M(t) = 0],$$
   the probability that there will be no mutations to resistance in the time interval $[0, t]$.

   ***Solution**:* We may assume that $M(t)$ has a Poisson distribution with parameter $\mu(t)$, where $\mu(t) = E(M(t))$, the expected number of mutations in the time interval $[0, t]$. It then follows that
   $$P[M(t) = 0] = e^{-\mu(t)}.$$
   □

   (b) Estimate the probability, p_o, that at the end of the n division cycles there will be no resistant bacteria. State all assumptions you make and justify your answer.

   ***Solution**:* We can use the fraction of cultures that show no resistant bacteria as an estimate for p_o. □

[1] (1943) *Mutations of bacteria from virus sensitivity to virus resistance. Genetics,* **28**, 491–511

(c) Use the formula for $P[M(t) = 0]$ that you obtained in part (a), together with the estimate for p_o that you obtained in part (b), to estimate, $\mu(t)$, the average number of mutations leading to resistant bacteria in the time interval $[0, t]$, where t corresponds to n division cycles. Explain how you may use the estimate for $\mu(t)$ to estimate the mutation rate, a.

Solution: According to part (b), $P[M(t) = 0]$ can be estimated by p_o, the fraction of cultures that show no resistant bacteria. Thus, using the result from part (a) we have that

$$e^{-\mu(t)} \approx p_o.$$

So, we can estimate $\mu(t)$ by

$$\mu(t) \approx -\ln p_o.$$

To estimate the mutation rate, a, we may use the formula

$$\mu(t) = \frac{a}{k}(N(t) - N_o), \tag{1}$$

where N_o is 1 in this case, $k = \ln 2$, and $t = n$ division cycles. □

(d) Table 1 shows data from an experiment performed by Luria and Delbrück involving 12 similar cultures of about 5×10^8 bacteria each. The first column shows the number–label of the cultures, and the second column shows the number of resistant bacteria in each culture.

Use the data in Table 1 and your results in part (c) to estimate:

i. The average number of mutations, μ, that occurred before the bacteria were exposed to the virus;

Solution: Out of the 12 cultures, 5 showed no resistant bacteria. Thus, according to the result of part (b),

$$p_o = \frac{5}{12} \approx 0.42.$$

Thus, by the results of part (c), the average number of mutations, μ, is approximated by

$$\mu \approx -\ln(0.42) \approx 0.88.$$

□

ii. The mutation rate, a; that is, the probability that a given bacterium will mutate in a division cycle.

Table 1: Luria and Delbrück Experiment No. 17

Culture No. Label	Resistant Bacteria
1	1
2	0
3	0
4	7
5	0
6	303
7	0
8	0
9	3
10	48
11	1
12	4

***Solution**:* From the equation in (1) we get

$$\mu(n) \approx \frac{a}{k} N(n)$$

since $N(n) = 2^n \approx 5 \times 10^8$ is very large compared to $N_o = 1$. We therefore get that

$$a \approx \frac{k}{N(n)}\ \mu(n),$$

so that

$$a \approx \frac{\ln 2}{5 \times 10^8}(0.88) \doteq 1.2 \times 10^{-9}.$$

□