Solutions to Assignment #13

Background Information. Luria and Delbrück[1] devised the following procedure (known as the *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.

Problems and Solutions

1. If p_o denotes the fraction of the plates that show no colonies of resistant bacteria, give a formula for estimating the average number of mutations in the n division cycles.

   Solution: We assume that the number of mutations, $M(t)$, in the time interval $[0, t]$ is a random variable that follows a Poisson distribution with parameter $\mu(t)$, the average number of mutations in that interval. It then follows that the probability of no mutations in that time interval is given by

   $$P[M(t) = 0] = e^{-\mu(t)}.$$

   This probability is estimated by the fraction p_o cultures with no resistant bacteria. If t is measured in division cycles, it then follows that

   $$e^{-\mu(n)} \approx p_o,$$

   from which we get that

   $$\mu(n) \approx -\ln p_o. \qquad \square \tag{1}$$

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

2. If p_o denotes the fraction of the plates that show no colonies of resistant bacteria, give a formula for estimating the mutation rate, a, in terms of n and p_o.

   Solution: The average mutation rate, $\mu(t)$, satisfies the differential equation

$$\mu'(t) = aN(t),$$

   where $N(t)$ is the number of bacteria at time t. If t is measured in numbers of division cycles, then

$$\mu'(t) = a2^t.$$

   Integrating the with respect to t yields

$$\mu(t) - \mu(0) = \frac{a}{\ln 2}(2^t - 1),$$

   where $\mu(0) = 0$. If n is very large, then we can make the approximation

$$\mu(n) \approx \frac{a}{\ln 2}2^n.$$

   We then get that

$$a \approx \frac{\ln 2}{2^n}\mu(n),$$

   and so, by (1),

$$a \approx -\frac{\ln 2}{2^n}\ln p_o. \qquad \square \tag{2}$$

Table 1: Number of resistant bacteria in a series of similar cultures

Test–tube #	1	2	3	4	5	6	7	8	9	10	11	12
# of Mutants	1	0	0	7	0	303	0	0	3	48	1	4

3. The data in Table 1 were taken from page 504 of the Luria and Delbrück 1943 paper.

   (a) Estimate the average number of mutations that occured before the bacteria were plated with the virus.

   Solution: The fraction of cultures with no resistant bacteria is $p_o = \dfrac{5}{12}$. Thus, by (1),

$$\mu(n) \approx -\ln\left(\frac{5}{12}\right) \doteq 0.88. \qquad \Box$$

   (b) Given that the number of bacteria in each culture was about 5×10^8, estimate the mutation rate a.

   Solution: Use the estimate for a in (2) with $2^n \approx 5 \times 10^8$ to obtain

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

4. For the data in Table 1:

   (a) Estimate the average number of resistant bacteria right before the plating was made.

   Solution: This is estimated by taking the average of the numbers of resistant bacteria in Table 1; namely, $\overline{r} \approx 30.58$. □

   (b) Use the *sample–variance* formula

   $$s^2 = \frac{\displaystyle\sum_{i=1}^{n}(r_i - \overline{r})^2}{n-1},$$

   where r_i denotes the number of resistant cells in test–tube i and $\overline{r}$ is the average number of resistant bacteria, to estimate the variance of the distribution.

   Solution: $\text{var}(R) \approx s^2 \doteq 6913.74$. □

   (c) Based on your results in the previous part and what you know about the Poisson process, would you say that the number of resistant bacteria follows a Poisson process? Justify your answer.

   Solution: If the number of resistant bacteria truly follows a Poisson distribution, then its mean and variance should be very close. Thus, in a random sample, the sample mean and variance should be very close also. This is not the case for the data in Table 1. Thus, the number of the resistant bacteria does not follow a Poisson distribution. □

5. *[Problem 6.2.5 on page 238 in Allman and Rhodes].* Explain the following results by not referring to the formula $\binom{n}{k} = \dfrac{n!}{k!(n-k)!}$, but in terms of "choosing objects."

   (a) $\binom{n}{1} = n$ and $\binom{n}{n-1} = n$ for any n.

   Solution: $\binom{n}{1}$ is the number of distinct ways of choosing 1 object out of n. This can be done in n ways.

   $\binom{n}{n-1}$ is the number of way of choosing $n-1$ objects out of n. Since in each of these choices we would be missing exactly one of the objects, there are n ways of making the choices. □

   (b) $\binom{n}{0} = 1$ and $\binom{n}{n} = 1$ for any n.

   Solution: There is only one way of choosing no objects at all, and only one way of choosing all the n objects. □