Pick Your Poisson: An Educational Primer for Luria and Delbrück's Classic Paper

Philip M. Meneely¹

Department of Biology, Haverford College, Haverford, Pennsylvania 19041

ABSTRACT The origin of beneficial mutations is fundamentally important in understanding the processes by which natural selection works. Using phage-resistant mutants in *Escherichia coli* as their model for identifying the origin of beneficial mutations, Luria and Delbrück distinguished between two different hypotheses. Under the first hypothesis, which they termed “acquired immunity,” the phages induced bacteria to mutate to immunity; this predicts that none of the resistant mutants were present before infection by the phages. Under the second hypothesis, termed “mutation to immunity,” resistant bacteria arose from random mutations independent of the presence of the phages; this predicts that resistant bacteria were present in the population before infection by the phages. These two hypotheses could be distinguished by calculating the frequencies at which resistant mutants arose in separate cultures infected at the same time and comparing these frequencies to the theoretical results under each model. The data clearly show that mutations arise at a frequency that is independent of the presence of the phages. By inference, natural selection reveals the genetic variation that is present in a population rather than inducing or causing this variation.

KEYWORDS primer; education; *Escherichia coli*; acquired immunity; mutation to immunity

Background

One of the seminal experiments in the history of genetics owes part of its inspiration to the presence of slot machines at a faculty mixer at the University of Indiana in 1942 (Luria and Delbrück 1943). Salvador Luria himself described this experience in an essay in *Phage and the Origins of Molecular Biology*, a volume of essays dedicated to Luria's co-author, Max Delbrück, for Delbrück's sixtieth birthday (Luria 1966), and used the experience for the title of his autobiography as well (Luria 1984). Luria's essay provides an informative and personal background to this experiment and to the thought processes of the authors, as well as to the nature of scientific collaboration 75 years ago (Luria 1966).

A slot machine has a very low probability of a payout during any single spin, but when the payout occurs, it is a jackpot. While not many people would have recognized the statistical underpinnings of slot machines and applied them to the origins of mutations in bacteria, Luria did. Luria had been working with Delbrück on several different biological questions related to viral or phage infections in bacteria spo-

radically over the preceding two years, but they had also been discussing experiments that could measure the rate at which new mutations arose in bacteria. In a letter to Delbrück in January 1943, Luria outlined the subsequent experiments almost precisely, and Delbrück responded immediately, also recognizing the implications. While waiting for the summer to conduct the experiments together at Cold Spring Harbor Laboratories, Delbrück worked out the theoretical aspects of what they could expect under different hypotheses. The result of that collaboration is “[Mutations of Bacteria from Virus Sensitivity to Virus Resistance](#),” which appeared in *Genetics* in November of 1943 (Luria and Delbrück 1943).

While bacteriophages (*i.e.*, viruses that infect bacteria) were instrumental in the early days of molecular genetics, few modern students are likely to have much experience in working with them, so a brief experimental background is in order. A bacterial culture growing in nutrient broth is cloudy. When an aliquot of phages in excess of the number of bacteria is added to the culture, the culture clears because the phages are infecting and then lysing the bacterial cells as the phages reproduce. After a time of continued incubation, the culture becomes cloudy again because phage-resistant bacterial cells proliferate. This growth of virus-resistant bacteria after infection was referred to as *secondary growth*. The resistant cells could be isolated and grown as pure cultures and continued

to exhibit phage resistance over subsequent generations. The resistance after the initial infection also did not depend on the continued presence of the phages, so change to resistance was heritable and stable. The same result occurs when an excess of phages is spread onto an agar plate before the bacteria are plated; most bacteria lyse, but after a time, a few resistant bacterial colonies appear.

Two models were proposed for the origins of these resistant cells in the phage-infected cultures. (While the paper clearly lays out the two models and their predictions, it does not always list them in the same order.) The fundamental question was this: does the phage infection directly induce the bacteria to mutate to resistance, or does the phage infection reveal the presence of preexisting resistant mutants in the culture? The first of these hypotheses is called *acquired immunity*, while the second is called *mutation to immunity*.

While there had been a few previous experiments to distinguish between these ideas, Luria and Delbrück wrote, “Neither of these views seems to have been rigorously proved in any single instance.” Interpretation of the results depended in large part on the rates at which spontaneous mutations arose in bacteria. This rate was unknown. Previous attempts to measure the mutation rate had been frustrated by highly variable results, and the lack of reproducible results in these experiments was seen as a significant shortcoming. Part of the genius of Luria and Delbrück was to realize that the fluctuation in previous results was inherent in the underlying question of bacterial variation; rather than being a problem for understanding the origins of mutations, fluctuations in the rates at which resistant mutants arise could help to provide the solution. Their experiment is often referred to as the *fluctuation test* because of this insight.

A few other points are important to recognize as we begin to discuss their results. Phage reproduction and lysis of the cell occur by a burst rather than slowly over time. Thus, mutation to resistance is a binary event that could be treated by binomial statistics. Delbrück trained as a physicist, and although Luria trained as a physician and microbiologist, he had spent a year studying physics at the University of Rome. This training allowed them to recognize that the occurrence of mutations follows a Poisson distribution, a probability distribution familiar to physicists but not so familiar to most biologists. Because a Poisson distribution is the occurrence of an event during a particular time interval, Luria and Delbrück also realized that for bacteria growing in culture, an appropriate measure of time intervals is the number of cell divisions. In addition to their quantitative backgrounds, Luria had the necessary background in microbiology to work with phages. Rather than work with an isolated mixture of different phages, Luria had pure cultures of the phage and had developed reliable methods for working with them. In the paper, the phage is referred to as α ; it was subsequently renamed T1. (In his essay, Luria says that he named it α because his typewriter keyboard had such a key, so it was easy to type.)

The key experiment and its results

The basic procedure for the experiment was as follows: a broth culture of *Escherichia coli* B was grown from a small number of cells. Meanwhile, the phages were plated and spread over the surface of an agar plate with complete medium in numbers in excess of the number of bacteria to be plated. A suspension of the bacteria was then seeded onto the plates. Most of the bacteria lysed, but after a day or two, colonies of resistant bacteria were found on the plate and could be counted.

In a control experiment, 10 samples were taken from the same bacterial broth culture, with the results from three separate experiments shown in their Table 1. Each sample was shown as a row in the table, while the columns showed different cultures. The reproducibility of their plating methods, which is essential to interpreting the fluctuation in results with the experiment cases, can be seen from a comparison of the results from the 10 samples in one experiment. The three cultures had different average numbers of resistant colonies, but the key parameters can be found by comparing the results for different samples from the same culture. We will discuss the implications of these results later.

The experimental cases to determine the origin of the resistant bacteria followed essentially the same protocol for growing the bacteria and plating them on an excess of phages. The key difference was that rather than taking samples from the same culture to plate, 9–20 different cultures were grown separately and plated at the same time. Thus, in their Table 2, the columns are a series of individual cultures grown at the same time, while the rows are each a separate culture.

Much more of the paper is devoted to calculating the mutation rate and comparing it to expected values. While I will briefly discuss the importance of this result, it is probably sufficient to look at their Table 4. The estimated mutation rate to resistance was 2.45×10^{-8} per bacterium per division cycle. Separate experiments and slightly different methods and assumptions give essentially the same rate. The most important conclusion here is that their method to measure the mutation rate, for example, by calculating based on the number of divisions, did not show the variation that had been found by others, supporting the validity of their theoretical treatment.

Unpacking the Work

The Poisson distribution and Tables 1 and 2

For students with experience in statistics and probability, the importance of the results in Tables 1 and 2 may not be so difficult to understand. For students with less background in statistics and probability, these results need to be unpacked and analyzed. After all, they constitute strong evidence that mutations arise at random without regard to their selective advantage or disadvantage, which is a key tenet of evolutionary theory. Let’s look at these data more closely.

Many events we encounter in genetics have two discrete outcomes: peas are wrinkled or round, flies have red or white eyes, and a mutation has occurred or it has not, for example. The frequencies of these two outcomes will follow some binomial probability distribution. (Many introductory statistics classes focus on events that have a continuous set of outcomes, with a probability distribution that follows a curve such as a Gaussian distribution rather than a binomial distribution.) A familiar nongenetics example of a binomial probability distribution is a coin flip: heads and tails are two discrete outcomes, and the probability of each is 0.5 on any single flip. One type of binomial distribution is the Poisson distribution, which expresses the probability of a given number of occurrences of an event that occurs during a fixed time period. Perhaps it is simpler to express this as a question: if we know that some event occurs at a particular rate, what is the probability that we will find x occurrences in the next hour (or some other time interval)? One of the most famous applications of the Poisson distribution involved the number of soldiers in the Prussian army killed per year by horse kicks, but a more familiar example is radioactive decay in a given time period. The Poisson distribution is particularly appropriate when one of the two outcomes is rare compared to the other, which is true in the case of resistance mutations. Most of the bacterial cells do not mutate to resistance in the next cell division, just as most radioactive atoms do not undergo radioactive decay and most Prussian soldiers did not die from a horse kick during a year.

As with other probability distributions, a Poisson distribution is defined by two parameters—the mean and the variance. The mean is the “average” number of colonies appearing on a plate, while the variance is calculated from the differences between each observation and the mean. A key defining feature for events that follow a Poisson distribution is that the mean and the variance are equal. Thus, we need to look at the variances in [Tables 1 and 2](#) and compare them to the mean.

Notice in [Table 1](#) that the mean for each experiment is nearly the same as the variance. The parameter P gives the probability that the amount of variation observed could arise by chance given this mean and variance, and the P -values are quite high for all three of the experiments. In other words, the results in [Table 1](#) agree with expectations when a large population is sampled repeatedly for an event that occurs rarely.

We can now turn to [Table 2](#), in which the results are quite different. Look, for example, at the 20 cultures in experiment 16. While the average (mean) per sample is 11.35 resistant colonies, none of the individual cultures had close to this number of resistant colonies. In fact, 11 of the 20 cultures had no resistant colonies, and six more cultures had six or fewer colonies. However, three cultures had many more resistant colonies—from 35 to 107. These are Luria’s “jackpots.” A simple visual inspection of the data suggests that the 10 cultures in this experiment must not be separate

samples from the same large population. While a visual inspection is helpful, calculation of the variance is more meaningful for drawing conclusions. Note that the variance is much larger than the mean in every experiment, so these results cannot be explained by assuming a Poisson distribution. Clearly, these cultures do not represent different samples from the same large population but instead show that mutations to resistance arise (or not) independently in every sample.

Let’s think about this result and the two hypotheses about the origin of resistant mutations. If the acquired-immunity hypothesis were correct, separate samples should have approximately the same mean number of resistant colonies, and the variance would be equal to the mean. Although the 20 samples in experiment 16 were grown separately, the phages were introduced at the same time in each sample, so if the phages are inducing mutation to resistance, the results should resemble those in [Table 1](#). This hypothesis does not account for the results in [Table 2](#). However, under the mutation-to-immunity hypothesis, each separate culture mutates to immunity independently of when the phages are applied. By chance, some cultures will have no cells that mutate to immunity, even though the phages have been applied. Other cultures will have cells that mutate to immunity a few cell divisions before being plated and will have only a few resistant colonies. However, a few cultures will have cells that mutated to immunity early in the growth phase, so these cultures will have many resistant colonies.

Mutation rates

While [Tables 1 and 2](#) present the most important results in the paper for understanding the origin of beneficial resistant mutations, it is worth looking at the data more fully to illustrate some other features of the experiment. The general equation for a Poisson distribution is

$$P_x = \left(h^x e^{-h} \right) / x!$$

where x is any given number, and h is the number of mutation hits. From this, the probability that a culture will have no hits (that is, $x = 0$) is

$$P_0 = h^0 e^{-h} / 0! = e^{-h}$$

By taking the natural log and multiplying by -1 , $h = -\ln P_0$. The number of mutations can be calculated directly from the number of cultures that have no mutations.

In experiment 16 cited earlier, 11 of 20 (0.55) cultures had no hits, so $h = -\ln(0.55) = 0.60$ mutations per culture. Because the number of cell divisions in each culture was known and the number of bacteria was known (5.6×10^8 in this experiment), the mutation rate per cell division can be easily computed by dividing the number of mutations per culture by the number of cells in the culture. Similar rates were found for every culture.

The genetic basis for resistance was not known at the time of these experiments. Subsequent analysis indicates that the mutation to resistance probably occurred in the *fhuA* gene of *E. coli*, which acts as the receptor for the T1 phage. It is not straightforward to compare these rates to more modern methods to estimate mutation rates, which arrive at estimates of about 10^{-8} per base pair per generation.

Connections to Genetic Concepts

The most important connection that this makes to a key genetic concept—in fact, the key genetic concept that this paper demonstrated—is that mutations occur at random with respect to selective pressure. How does variation arise in a population, and what happens to it over time?

Mutations do not arise because they are beneficial to the organism. Mutations arise at random. Some will prove to be beneficial when a selective agent is applied, but selection does not produce the beneficial mutation. This is such a key concept that it may be taken for granted, but we take it for granted only because Luria and Delbrück demonstrated it to be true.

Suggestions for Classroom Use

Much of the theoretical background is difficult to understand unless a reader is well versed in differential equations. Fortunately, the most important concepts that the paper demonstrates do not require advanced courses in mathematics and statistics. But other important aspects of the experiment also should not be overlooked.

Detailed questions

The goal of these questions is to stimulate you to think about the design of the experiment, some of the assumptions that were made, and some key insights used in the techniques.

1. A Poisson distribution is used to calculate the number of events that will occur in a given time interval. Luria and Delbrück used cell divisions to define their time intervals and thus calculated the mutation rate per cell division.
 - a. What is the assumption that was made to connect the occurrence of mutations to the number of cell divisions?
 - b. Based on what we now know about the mechanisms by which many mutations arise, why was this such an important assumption? In other words, by using cell division as their measure of time intervals, they were (consciously or unconsciously) connecting the occurrence of mutations to what other cellular process?
2. Luria and Delbrück made the point that the resistant bacteria that arose during secondary growth continued to be resistant even when grown in the absence of phages. Why is this so important?
3. Luria had isolated pure cultures of different types of phages and used one of these for the experiment.
 - a. Why is it important that pure cultures of the phages were used rather than mixed cultures, as some of the previous investigators had done?
 - b. What other very familiar and significant set of experiments in the history of genetics likewise relied on having “pure” cultures rather than a mixture?
4. Why was it important that the number of phages was in excess of the number of bacteria?
5. The various experiments reported in [Tables 1 and 2](#) resulted in different means for the number of resistant colonies. While the significance of the data lies in the comparison between the means and the variance for an experiment, what might be some reasons that different experiments would have different means?
6. Explain how the “jackpots” arose, with some speculation about what connection this made in Luria’s imagination. Or, to ask this another way, how does the fluctuation in the number of different resistant colonies from experiment to experiment, which was considered a significant problem for previous studies, provide insights into the events that occurred here?
7. Luria and Delbrück’s estimate of mutation rate was about 2.45×10^{-8} mutations to resistance per cell division. The best current estimates are that the spontaneous mutation rate is on the order of 10^{-8} per nucleotide per generation.
 - a. What are some of the reasons that these different measures of mutation rate are hard to compare or that they cannot be compared?
 - b. What contemporary methods are used to measure the rate of spontaneous mutation?
 - c. What are some of the reasons that the rates of spontaneous mutation (measured by nucleotide per generation) are so similar in many different organisms?
8. The acquired-immunity hypothesis is essentially that cells acquire heritable characteristics in response to environmental conditions. While this is clearly not generally true, there may be some unusual circumstances in which this occurs. How does the CRISPR response of bacterial cells resemble acquired immunity?
9. Currently, tuberculosis (a bacterial disease) is treated with three antibiotics simultaneously, but resistant strains of bacteria are found regularly.
 - a. Assuming that the probability of resistant “hits” is the same in each case and similar to what was observed by Luria and Delbrück, what would be the chance that resistance to three drugs would appear in a given small culture from your experiment? (This requires using the Poisson distribution.)
 - b. In fact, resistance to all three antibiotics occurs much more frequently than is predicted from your response

to part a. What are some reasons that resistance to three antibiotics simultaneously might occur more often than predicted?

Further Reading

There is an extensive literature that provides more information about this experiment and its background. Two such sources are

- Luria, S. E., 1966 “Mutations of Bacteria and of Bacteriophage” in *Phage and the Origins of Molecular Biology*, ed. by J. Cairns, G. S. Stent, and J. D. Watson. Cold Spring Harbor Press, Cold Spring Harbor, NY
- Luria, S. E., 1984 *A Slot Machine, a Broken Test Tube: An Autobiography*. HarperCollins, New York.

A selection of the collected papers of Luria, including background on this experiment, is available online from the National Library of Medicine Profile Series at <http://profiles.nlm.nih.gov/>.

Literature Cited

- Luria, S. E., 1966 Mutations of bacteria and of bacteriophage, pp. 173–179 in *Phage and the Origins of Molecular Biology*, ed. by J. Cairns, G. S. Stent, and J. D. Watson. Cold Spring Harbor Press, Cold Spring Harbor, NY.
- Luria, S. E., 1984 *A Slot Machine, a Broken Test Tube: An Autobiography*. HarperCollins, New York.
- Luria, S. E., and M. Delbrück, 1943 Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28: 491–511.

Communicating editor: E. A. De Stasio