To provide a useful benchmark against which to compare the results of your own actual experiment, we provide two simulations. These *Excel* workbooks ("Acquired Immunity" and "Mutation") illustrate our predictions for outcomes of the Luria-Delbruck experiment under each of the two hypotheses being tested. Each workbook contains user-defined values for relevant parameters, models the experiment under the appropriate hypothesis, calculates the number of bacterial colonies on each plate, and presents these data in the form of numerical, statistical, and graphical output.

Note: Before you make any changes to the values listed in the spreadsheet, you must disable the automatic calculation option, or else the spreadsheet will recalculate before you have entered all the parameter values. The specific procedures for setting up manual calculation vary across different versions of *Excel*. You may therefore need to consult the *Excel* help menu to determine the correct procedure for the version that your computer is running.

Acquired Immunity Hypothesis

Under the acquired immunity hypothesis, resistance arises from exposure to virus, so no bacteria will be resistant prior to plating. Once plating has occurred, each bacterium has a small, fixed probability of acquiring resistance during a single replication cycle. Relevant parameters for this model are therefore the probability of acquiring resistance (p), the initial number of bacteria present on the plate (N_0), and the number of replication cycles (R).

To model this hypothesis, open the "Acquired Immunity" workbook and select the "Replicate Plates" tab in the bottom left-hand corner. Enter the values you wish for p, N_0, and R into the red-lined cells G1, G2, and G3, then recalculate the spreadsheet. The blue-lined cells in column C will display the number of colonies on simulated plates A1-A10, all of which derive from a single tube (Tube A). To see the number of colonies on plates Z1-Z10, which derive from separate tubes (Tubes Z1-Z10), click on the tab for the "Parallel Plates" worksheet in the bottom left-hand corner. Finally, the "Comparison" worksheet tabulates the results of the entire simulated experiment, calculates useful summary statistics (the mean, variance, and coefficient of variation), and plots a histogram of the number of plates with different numbers of colonies. Recalculate the spreadsheet several times without changing the parameter values and see how sensitive the results are to random fluctuations.

Mutation Hypothesis

Under the mutation hypothesis, resistance arises from random mutations during each bacterial replication. We model this process by having each tube initially contain a single (non-resistant) bacterium, which reproduces over 30 replication cycles to a population of 2^{30} (approximately 10^9). During each replication cycle, each bacterium has a small, fixed probability of mutating to become resistant: this resistance will be passed to all of that bacterium's subsequent progeny. Thus, some resistant clones will be exceptionally large, because the mutation occurs during an unusually early replication cycle. After 30 replication cycles, we transfer a certain number of bacteria onto the plate and count the number of resistant bacteria so transferred. Relevant parameters for this model are the frequency of resistance-conferring mutations (μ) and the number of bacteria transferred onto the plate (N_0). Note that our model considers only forward mutations.

To model this hypothesis, open the "Mutation" workbook and select the "Replicate Plates" tab in the bottom left-hand corner. Enter the values you wish for μ and N_0 into the red-lined cells K1 and K2, then recalculate the spreadsheet. Columns C-F will respectively display for each generation the total number of bacteria, the number inheriting resistance from their parent bacterium, the number *not* inheriting such resistance, and the number acquiring resistance through *de novo* mutation. The number of resistant colonies on simulated plates A1-A10, all of which derive from tube A, will be displayed in the blue-lined cells in column H. As before, the number of colonies on plates Z1-Z10 (from separate tubes Z1-Z10) are displayed on the "Parallel Plates" worksheet: you will need to scroll down to view the complete results from this portion of the experiment. Again, the results of the entire simulated experiment are summarized on the "Comparison" worksheet, including the mean, variance, and coefficient of variation for the number of colonies present on each plate. Recalculate the spreadsheet several times, leaving the parameter values constant. How much do random fluctuations affect the results? Compare and contrast these results with your simulated observations from the acquired immunity hypothesis and with your actual data.

1. What are the advantages of using the coefficient of variation rather than the variance when comparing the variation in the number of bacterial colonies between two sets of plates?

2. The mutation model described above ignores back-mutations. How strongly and in what direction do you expect this biologically inaccurate assumption to affect the model results? How could you test your prediction? (Hint: If a random proportion k of the bacterial population undergoes forward mutation, and if we assume that the back-mutation rate equals the forward mutation rate, then what proportion of the resistant population would we expect to undergo back-mutation?)

3. Some model parameters, such as p and μ, would be difficult for an experimenter to manipulate directly. Others, like the aliquot size N_0, are much easier to adjust. Repeat the simulated experiment for a range of N_0 values. What values would make the experiment easiest to conduct? What values yield the greatest statistical power to discriminate between the two hypotheses?

4. How do the acquired immunity and mutation hypotheses relate to Lamarckian vs. Darwinian ideas of inheritance? What are the implications of the Luria-Delbruck experiment for the concept of teleology (i.e., that evolution has a goal or purpose)?