

Luria-Delbrück, revisited: The classic experiment does not rule out Lamarckian evolution

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Abstract. We re-examined data from the classic Luria-Delbrück fluctuation experiment, which is often credited with establishing a Darwinian basis for evolution. We argue that, for the Lamarckian model of evolution to be ruled out by the experiment, the experiment must favor pure Darwinian evolution over both the Lamarckian model and a model that allows both Darwinian and Lamarckian mechanisms. Analysis of the combined model was not performed in the original 1943 paper. The Luria-Delbrück paper also did not consider the possibility of neither model fitting the experiment. Using Bayesian model selection, we find that the Luria-Delbrück experiment, indeed, favors the Darwinian evolution over purely Lamarckian. However, our analysis does not rule out the combined model, and hence cannot rule out Lamarckian contributions to the evolutionary dynamics.

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1. Introduction

From the dawn of evolutionary biology, two general mechanisms, Darwinian and Lamarckian, have been routinely considered as alternative models of evolutionary processes. The *Darwinian hypothesis* posits that adaptive traits arise continuously over time through spontaneous mutation, and that evolution proceeds through natural selection on this already existing variation. In contrast, the *Lamarckian hypothesis* proposes that adaptive mutations arise in response to environmental pressures. The Nobel Prize winning fluctuation test by Salvador Luria and Max Delbrück [1] is credited with settling this debate, at least in the context of evolution of phage-resistant bacterial cells.

Luria and Delbrück realized that the two hypotheses would lead to different variances (even with the same means) of the number of bacteria with any single adaptive mutation. Specific to the case of bacteria exposed to a bacteriophage, this would result in different distributions of the number of surviving bacteria, cf. Fig. 1. In the Darwinian scenario, there is a possibility of a phage-resistance mutation arising in generations prior to that subjected to the phage. If this mutation happens many generations earlier, there will be a large number of resistant progeny who will survive (a “jackpot” event). However, there will be no survivors if the mutation does not exist in the population at the moment the phage is introduced. If the same experiment were repeated many times, the variance of the number of survivors would be large. In contrast, in the Lamarckian scenario, the distribution of the number of survivors is Poisson. Indeed, each occurring mutation (and hence each survivor) happens with a small probability, independent of the others. This would result in the usual square-root scaling of the standard deviation of the number of survivors, a much smaller spread than in the Darwinian case.

To test this experimentally, Luria and Delbrück let the cells grow for a few generations, exposed them to a phage, plated the culture, and then counted the number of emergent colonies, each started by a single resistant, surviving bacterium. They found that the distribution of the number of survivors, as measured by the number of colonies grown after plating, was too heavy-tailed to be consistent with the Poisson distribution. They concluded then that the bacteria must evolve using the Darwinian mechanism. They could not derive an analytical form of the distribution of survivors in the Darwinian model, so that their data analysis was semi-quantitative at best. In particular, they could only establish that the Darwinian model fits the data better than the Lamarckian/Poissonian one, but they could not quantify *how good* the fit is.

Potentially even more importantly, the original paper contrasted only two scenarios: pure Lamarckian and pure Darwinian ones. However, it is possible that both processes have a role in bacterial evolution, as is abundantly clear now in the epoch of CRISPR and epigenetics [2, 3, 4, 5]. Ruling out a significant Lamarckian contribution to evolution would require us to show not only that the Darwinian model explains the data better than the Lamarckian one, but also that the Darwinian model is more likely than the *Combination model*, which allows for both types of evolutionary processes. Evolution

could also proceed through an entirely different mechanism, so that neither of the proposed models explain the data. Distinguishing between these possibilities requires evaluating whether a specific model fits the data well, rather than which of the models fits the data better.

Unlike Luria and Delbrück in 1943, we have powerful computers and new statistical methods at our disposal. Distributions that cannot be derived analytically can be estimated numerically, and model comparisons can be done for models with different numbers of parameters. In this paper, we perform the quantitative analysis missing in the Luria-Delbrück paper and use their original data to evaluate and compare the performance of three models: Darwinian (D), Lamarckian (L), and Combination (C) models. The comparison is somewhat complicated by the fact that both the L and D models are special cases of the C model, so that C is guaranteed to fit not worse than either L or D. We use Bayesian Model Selection [6, 7], which automatically penalizes for more complex models (such as C) to solve the problem. We conclude that, while the L model is certainly inconsistent with the data, D and C explain the data about equally well when this penalty for complexity is accounted for. Thus the Lamarckian contribution to evolution cannot be ruled out by the 1943 Luria and Delbrück data. Further, while D and C fit equally well, neither provide a quantitatively good fit to one of the two primary experimental data sets of Luria and Delbrück’s paper, suggesting that the classic experiment may have been influenced by factors or processes not considered.

2. Methods

2.1. Models and Notational preliminaries

There have been many theoretical attempts, with varying degrees of success, to find closed-form analytical expressions of the distribution of mutants under the Darwinian scenario (the Luria-Delbrück distribution) following different modeling assumptions [8, 9, 10, 11]. For example, models with constant and synchronous division times [10, 11], exponentially distributed division times [12, 13], and with different growth rates for the wild-type and the mutant populations [14, 15, 16] have been proposed to find the distribution of survivors (see Ref. [17] for a recent review). Here we follow Haldane’s modeling hypotheses [10] and assume that (i) normal cells and mutants have the same fitness until the phage is introduced, (ii) all cells undergo synchronous divisions, (iii) no cell dies before the introduction of the phage, (iv) mutations occur only during divisions, with each daughter becoming a mutant independently (D case), or only when the phage is introduced (L case), and (v) there are no backwards mutations.

With these assumptions, the D and the C models are able to produce very good fits to the experimental data (see below), which suggests that relaxation of these assumptions and design of more biologically realistic models is unnecessary in the context of these experiments.

For the subsequent analyses, let N_0 be the initial number of wild-type, phage-

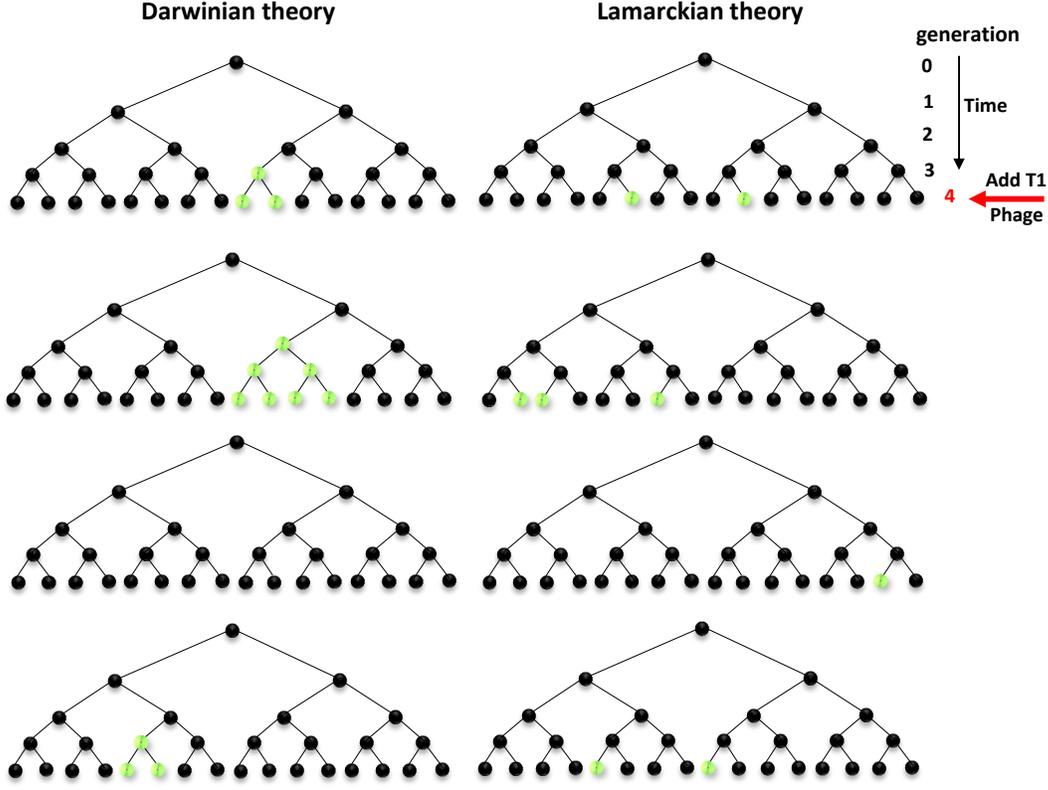


Figure 1. Schematics of the two theories tested by the Luria-Delbrück experiment. Here black dots denote bacteria susceptible to the bacteriophage, and green dots resistant bacteria. Each tree represents one realization of the experiment, which starts with a single bacterium (top). The bacterium then divides for several generations, and the phage is introduced into the culture at the last generation (bottom row of each tree). Darwinian theory (left column) of evolution predicts that mutations happen spontaneously throughout the experiment. Thus different repeats of the experiment (different trees) will produce very broadly distributed numbers of survivors (from 0 to 4 in this example). In contrast, in the Lamarckian case (right column), mutations only occur when the phage is introduced, so that the standard deviation of the number of survivors in different repeats (from 1 to 3 in this example) scales as the square root of their mean, which is much smaller than in the Darwinian case.

sensitive bacteria, and g be the number of generations before the phage is introduced, so that the total number of bacteria after the final round of divisions is $N = 2^g N_0$, and the total that have ever lived is $2N - N_0$. We use θ_D to denote the probability of an adaptive (Darwinian) mutation during a division, and θ_L to denote the probability of an adaptive Lamarckian mutation when the phage is introduced. With this, and discounting the probability of another mutation in the already resistant progeny, the mean number of adaptive Darwinian mutations at generation g is $m_D = \theta_D(2N - N_0)$, and the mean number of adaptive Lamarckian mutations is $m_L = \theta_L N$. The number of survivors in the L model is Poisson-distributed with the parameter m_L :

$$P_L(k|\theta_L, N_0) = \frac{e^{-m_L} m_L^k}{k!} \quad (1)$$

For the D model, there are multiple ways to have a certain number of resistant bacteria, k , in the population of size N before introducing the phage. For instance, there are four ways to have 5 resistant bacteria (i. e., $k = 5$): (i) One mutation occurs 2 generations before the phage introduction (where the total living population is $N/4$ at that generation), resulting in 4 resistant progenies in the last generation, and one more mutation at the last generation, making a total of 5 resistant cells before the phage introduction. This is the most likely scenario with probability $P_5^{(i)} = (1 - \theta_D)^{(2N-N_0)-8} \theta_D^2 \binom{N/4}{1} \binom{N-4}{1}$, where $(2N - N_0)$ is the total number of cells that have ever lived in the entire experiment, so that $(2N - N_0) - 8$ is the total number that have ever lived without mutating. The θ_D^2 factor indicates that a total of two mutations have occurred in the population. The first choose factor denotes the number of independent mutational opportunities 2 generations before the phage introduction, and the second one denotes the number of mutational opportunities in the last generation. (ii) Two mutations occur 1 generation before the phage introduction and one more mutation in the last generation. This is less likely than (i) with probability $P_5^{(ii)} = (1 - \theta_D)^{(2N-N_0)-7} \theta_D^3 \binom{N/2}{2} \binom{N-4}{1}$, where, there are a total of 7 mutant that have ever lived in the history of the experiment and 3 mutational events before the introduction of the phage. (iii) One mutation occurs 1 generation before the phage introduction and 3 more mutations in the last generation. This is less likely than (ii) with probability $P_5^{(iii)} = (1 - \theta_D)^{(2N-N_0)-6} \theta_D^4 \binom{N/2}{1} \binom{N-2}{3}$. (iv) Five mutations occur in the last generation before introducing the phage. This is the least likely scenario with probability $P_5^{(iv)} = (1 - \theta_D)^{(2N-N_0)-5} \theta_D^5 \binom{N}{5}$. In general, for an arbitrary number of resistant cells, k , let Π_k denote the set of sequences (a_0, a_1, \dots) that satisfy $k = \sum_0^\infty a_s 2^s$, such that $a_s \in \mathbb{Z}_{\geq 0}$. This condition captures all the possible sequences of $\{a_s\} \in \Pi_K$ that produce k number of resistant cells. For instance, in case (i) the corresponding sequence is $\{a_2 = 1, a_1 = 0, a_0 = 1\}$, and in case (ii) it is $\{a_1 = 2, a_0 = 1\}$. Then, following Haldane's approach [10], we can write $P_D(k)$, the probability of finding k resistant cells given the Darwinian model of evolution, as

$$P_D(k|\theta_D, N_0) = \sum_{\{a_s\} \in \Pi_K} (1 - \theta_D)^{(2N-N_0) - \sum_{s=0}^\infty a_s (2^{s+1}-1)} \theta_D^x \prod_{s=0}^\infty \binom{N}{2^s} - \sum_{n=s+1}^\infty a_s (2^{n-s}) \Bigg), \quad (2)$$

where $x \equiv \sum_{\{a_s\}} a_s$ and the probability $P_D(k|\theta_D, N_0)$ is summed over all the possible sequences $\{a_s\} \in \Pi_K$ that produce the number k ; in the case of $P_{k=5}$ mentioned earlier, $P_D(5|\theta_D, N_0) = P_5^{(i)} + P_5^{(ii)} + P_5^{(iii)} + P_5^{(iv)}$.

For the Combination model, both the L and the D processes contribute to generating survivors. Thus we write the distribution of the number of survivors in this case as a convolution

$$P_C(k|\theta_L, \theta_D, N_0) = \sum_{k'=0}^k P_D(k'|\theta_D, N_0) P_L(k - k'|\theta_L, N_0). \quad (3)$$

Further analytical progress on the problem is hindered by additional complications. First, in actual experiments, the initial number of bacteria in the culture N_0 is random

and unknown. We view it as Poisson-distributed around the mean that one expects to have, denoted as $\Pi(N_0|\bar{N}_0)$. This gives:

$$P_{D/L/C}(k|\theta_D, \theta_L, \bar{N}_0) = \sum_{N_0=0}^{\infty} P_{D/L/C}(k|N_0)\Pi(N_0|\bar{N}_0). \quad (4)$$

Finally, in some of the Luria-Delbrück experiments, they plated only a fraction r the entire culture subjected to the phage. This introduced additional randomness in counting the number of survivors after the plating, k_p , which we again model as a Poisson distribution with the mean rk , $\Pi(k_p|rk)$ [18, 19, 20], resulting in the overall distribution of survivors:

$$P_{D/L/C}(k_p|\theta_D, \theta_L, \bar{N}_0) = \sum_k \Pi(k_p|rk)P_{D/L/C}(k). \quad (5)$$

2.2. Computational models

The expressions in the previous section appear sufficiently simple. However, evaluating $P_C(k_p)$ in Eq. (5) involves two nested sums in the expression for $P_D(k)$, Eq. (2), a convolution to combine L and D processes, and two more convolutions to account for randomness in N_0 and during plating. These series of nested (infinite) summations make it inefficient to use the analytical expression in Eq. (5) for data analysis. Instead, we resort to numerical simulations to evaluate $P_{D,C}(k_p)$ (expressions for the pure L model remain analytically tractable).

Our simulations assume that each culture begins with a Poisson-distributed number of bacteria, with a mean number of 135, as in the original paper. The bacteria were modeled as dividing in discrete generations for a total of $g = 21$ generations. Both of the numbers are easily inferable from the original paper using the known growth rate and the final cell density numbers. Cells divide synchronously, and each of the daughters can gain a resistance mutation at division with the probability θ_D , which is nonzero in C and D models. Daughters of resistant bacteria are themselves resistant. Non-resistant cells in the final generation are subjected to a bacteriophage, which induces Lamarckian mutations with probability θ_L , nonzero in C and L models. We note again that this total number of Lamarckian-mutated cells is Poisson-distributed with the mean θ_L times the number of the remaining wild type bacteria.

To speed up simulations of the Darwinian process, we note that the total number of cells that have ever lived is $N_t = 2N_02^g - N_0$. Thus the total number of Darwinian mutation attempts is Poisson distributed, with mean $N_t\theta_D$. We generate the number of these mutations with a single Poisson draw and then distribute them randomly over the multi-generational tree of cells, marking every offspring of a mutated cell as mutated. We then correct for overestimating the probability of mutations due to the fact that the number of mutation attempts in each generation decreases if there are already mutated cells there. For this, we remove original mutations (and unmark their progenies) at random with the probability equal to the ratio of mutated cells in the generation

when the mutation appeared to the total number of cells in this generation. Note that since mutations are rare, such unmarking is not very common in practice, making this approach substantially faster than simulating mutations one generation at a time.

To estimate $P_C(k_p|\theta_D, \theta_L)$, we estimate this probability on a 41x41 grid of values of θ_D and θ_L . For each pair of values of these parameters, we perform $n = 30,000,000$ simulation runs (see below for the explanation of this choice) starting with a Poisson-distributed number of initial bacteria, then perform simulations as described above, and finally perform a simulated Poisson plating of a fraction of the culture if the actual experiment we analyze had such plating. We measure the number of surviving bacterial cultures k_p in each simulation run and estimate $P_C(k_p|\theta_D, \theta_L)$ as a normalized frequency of occurrence of this k_p across runs, $f_C(k_p|\theta_D, \theta_L)$. The Darwinian case is evaluated as $P_C(k_p|\theta_D, \theta_L = 0)$, and the Lamarckian case as $P_C(k_p|\theta_D = 0, \theta_L)$.

2.3. Quality of fit

In the original Luria and Delbrück publication [1], no definitive quantitative tests were done to determine the quality of fit of either of the model to the data well. We can use the estimated values of $P_C(k_p|\theta_D, \theta_L)$ for this task. Namely, Luria and Delbrück have provided us not with frequencies of individual values of k_p , but with frequencies of occurrence of k_p within bins of $x \in (0, 1, 2, 3, 4, 5, 6 - 10, 11 - 20, 21 - 50, 51 - 100, 101 - 200, 201 - 500, 501 - 1000)$. By summing $f_C(k_p|\theta_D, \theta_L)$ over $k_p \in x$, we evaluate $f_C(x|\theta_D, \theta_L)$, which allows us to write the probability that each experimental set of measurements, $\{n_x\}$, came from the model:

$$P(\{n_x\}|\theta_D, \theta_L) = \mathcal{C} \prod_x \left(\frac{n_x}{\sum_x n_x} \right)^{f_C(x|\theta_D, \theta_L)}, \quad (6)$$

where \mathcal{C} is the usual multinomial normalization coefficient. This probability can also be viewed as the likelihood of each parameter combination, and the peak of the probability gives the usual Maximum Likelihood estimation of the parameters [21]. To guarantee that the estimated value of the likelihood has small statistical errors, we ensured that each of the (θ_D, θ_L) combinations has 30,000,000 simulated cultures. Then, at parameter combinations close to the maximum likelihood, each bin x has at least 10,000 samples. Correspondingly, at these parameter values, the sampling error in each bin is smaller than 1%.

Finally, to evaluate the quality of fit of a model, rather than to find the maximum likelihood parameter values, we calculate empirically the values of $\log_{10} P(\{n_x^*\}|\theta_D, \theta_L)$ for each parameter combination, where $\{n_x^*\}$ are synthetic data generated from the model with θ_D, θ_L . Mean and variance of $\log_{10} P$ gives us the expected range of the likelihood if the model in question fits the data *perfectly*.

2.4. Comparing models

In comparing the L, the D, and the C models, we run into the problem that C is guaranteed to have at least as good of a fit as either D or L since it includes both of them as special cases. Thus in order to compare the models quantitatively, we need to penalize C for the larger number of parameters (two mutation rates) compared to the two simpler models. To perform this comparison, we use Bayesian model selection [6, 7], which automatically penalizes for such model complexity.

Specifically, Bayesian model selection involves calculation of probability of an entire model family $M = \{L, D, C\}$ rather than of its maximum likelihood parameter values:

$$P(M|\{n_x\}) \propto \int d\vec{\theta}_M P(\vec{\theta}_M|\{n_x\}, M) P(M) \quad (7)$$

where the posterior distribution of $\vec{\theta}$ is given by the Bayes formula,

$$P(\vec{\theta}_M|\{n_x\}, M) \propto P(\{n_x\}|\vec{\theta}_M, M) P(\vec{\theta}_M|M), \quad (8)$$

and $P(\{n_x\}|\vec{\theta}_M, M)$ comes from Eq. (6). Finally, $P(M)$ and $P(\vec{\theta}_M|M)$ are the *a priori* probabilities of the model and the parameter values within the model, which we specify below.

The integral in Eq. (7) is over as many dimensions as there are parameters in a given model. Thus while more complex models may fit the data better at the maximum likelihood parameter values, a smaller fraction of the volume of the parameter space would provide a good fit to the data, resulting in an overall penalty on the posterior probability of the model. Thus posterior probabilities $P(M|\{n_x\})$ can be compared on equal footing for models with different number of parameters to say which specific model is *a posteriori* more likely given the observed data. Often the integral in Eq. (7) is hard to compute, requiring analytical or numerical approximations. However, here we already have evaluated the likelihood of combinations of (θ_L, θ_D) over a large grid, so that the integral can be computed by direct summation of the integrand at different grid points.

To finalize computation of posterior likelihoods, we must now define the *a priori* distributions $P(M)$ and $P(\vec{\theta}_M|M)$. We choose $P(C) = P(M) = P(L) = 1/3$, indicating our ignorance about the actual process underlying biological evolution. The choice of $P(\vec{\theta}_M|M)$ is tricky, as is often the case in applications of Bayesian statistics. We point out that the experiment was designed so that the number of surviving mutants is almost always 1 or less, for a population with $\approx 0.25 \times 10^8$ individuals, which indicated that *a priori* both θ_L and θ_D are less than 4×10^{-9} . Further, we assume that, for the combined model, $P(\vec{\theta}_M) = P(\theta_L)P(\theta_D)$. Beyond this, we do not choose one specific form of $P(\vec{\theta}_M)$, but explore multiple possibilities to ensure that our conclusions are largely independent of the choice of the prior.

3. Results

Luria and Delbrück’s paper provided data from multiple experiments, where in each experiment they grew a number of cultures, subjected them to the phage, and counted survivors. Most of the experiments have $O(10)$ cultures, which means that their statistical power for distinguishing different models is very low. We exclude these experiments from our analysis and focus only on experiments No. 22 and 23, which have $n = 100$ and $n = 87$ cultures, respectively. The experimental protocols differ in that Experiment 23 plated the entire culture subjected to the phage, while Experiment 22 plated only 1/4 of the culture. We analyze these experiments separately from each other.

3.1. Experiment 22

We evaluated the posterior probability of different parameter combinations, $P(\vec{\theta}_M | \{n_x\}, M)$, numerically, as described in *Methods*. The likelihood (posterior probability without the prior term) is illustrated in Fig. 2. Note that the peak of the likelihood is at $\theta_L^{22} \approx 4.0 \times 10^{-10} \neq 0$, $\theta_D^{22} \approx 1.8 \times 10^{-9}$, illustrating that the data suggests that the Combination model is better than either of the pure models in explaining the data, though the pure Darwinian model comes close. The fit of the maximum likelihood Combination model is shown in Fig. 3. The quality of the best fit $\log_{10} P(\{n_x\} | \theta_D^{22}, \theta_L^{22}) \approx 63.7$. This matches surprisingly well with the likelihood expected if the data was indeed generated by the model, $\log(P(\{n_x\} | \theta_D^{22}, \theta_L^{22})) = 62.1 \pm 5.1$. Thus the model fits the data perfectly despite numerous simplifying assumptions, suggesting no need to explore more complex physiological scenarios, such as asynchronous divisions, or different growth rates for mutated and non-mutated bacteria.

Next we evaluate the posterior probabilities of all three models by performing the Bayesian integral, Eq. (7). We use two different priors for θ_L and θ_D to verify if our conclusions are prior-independent: uniform between 0 and 4×10^{-9} and uniform in the logarithmic space between 1×10^{-10} and 4×10^{-9} . For the uniform prior,

$$\frac{P(D)}{P(C)} \approx \frac{2.8}{1}, \quad \frac{P(L)}{P(C)} \approx 10^{-10^6}. \quad (9)$$

In other words, the purely Lamarckian model is ruled out by an enormous margin, as suggested in the original publication. However, the ratio of posterior probabilities of the Darwinian and the Combination models is only 2.8, and this ratio is 2.0 for the logarithmic prior, which is way over the usual 5% significance threshold for ruling out a hypothesis. In other words,

The Darwinian and the Combination models of evolution have nearly the same posterior probabilities after controlling for different number of parameters in the models. Thus contribution of Lamarckian mechanisms to evolution in the Luria-Delbrück Experiment 22 cannot be ruled out.

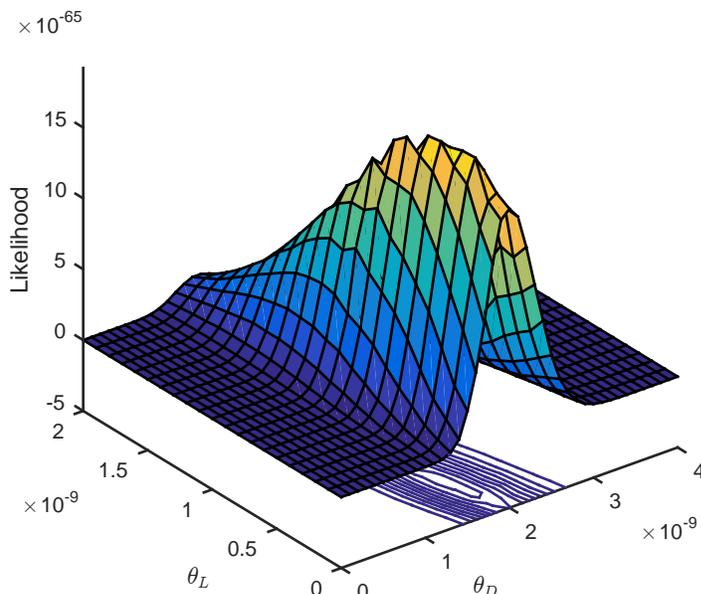


Figure 2. Posterior likelihood of the Darwinian, θ_D , and the Lamarckian, θ_L , mutation parameters evaluated for the Luria-Delbrück Experiment 22. Notice that the likelihood peaks away from $\theta_L = 0$.

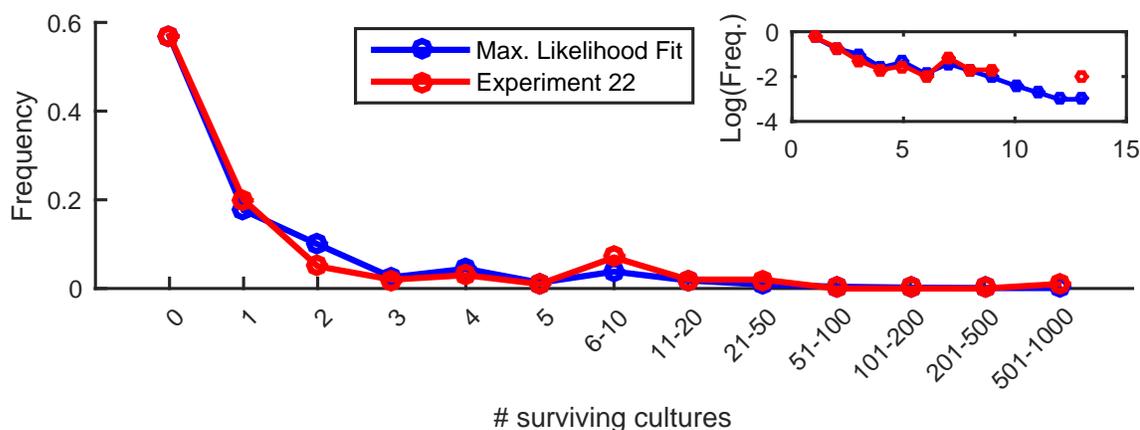


Figure 3. Luria-Delbrück experimental data (red) for Experiment 22 and the maximum likelihood fit of the Combination model with $\theta_L^{22} = 4.0 \times 10^{-10}$ and $\theta_D^{22} = 1.8 \times 10^{-9}$.

3.2. Experiment 23

We performed similar analysis for Experiment 23 and evaluated the posterior likelihood, Fig. 4, for each combination of parameters. Here, however, the posterior is several orders of magnitude smaller than for Experiment 22. This is because the experimental

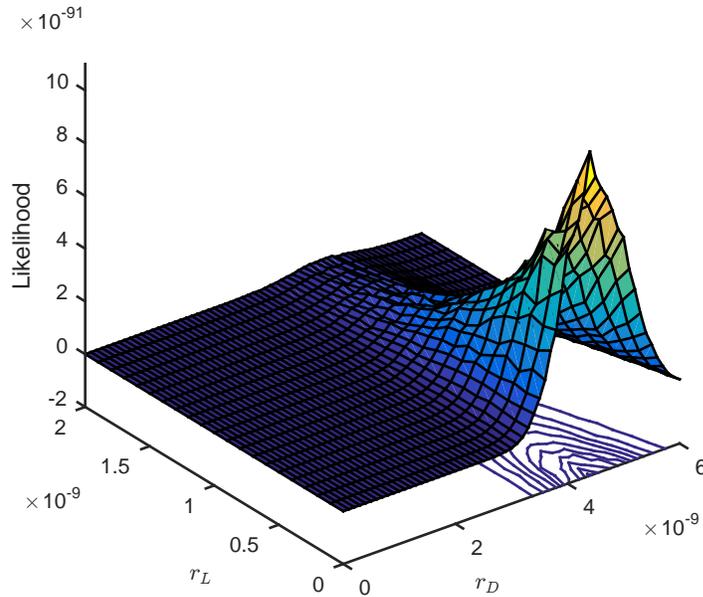


Figure 4. Posterior likelihood of the mutation parameters for Experiment 23. The maximum likelihood is at $\theta_L = 0$. However, neither of the three considered models is capable of fitting the data well (see text).

data has a tail that is *heavier* than typical realizations of even the Darwinian model would predict. Indeed, Luria and Delbrück themselves noted this excessively heavy tail. However, as they were only choosing whether the Darwinian or the Lamarckian model fits better, this led further credence to the claim that the Lamarckian model could not describe the data. Now we are able to quantify this: for Experiment 23, at the maximum likelihood parameters ($\theta_L^{23} = 0, \theta_D^{23} = 4.4 \times 10^{-9}$), the quality of fit is $\log_{10} P(\{n_x\}|\theta_D^{23}, \theta_L^{23}) \approx -90.0$. In contrast, for data generated from the model, we get $\log_{10} P(\text{data}|\theta_D, \theta_L) = -76.9 \pm 3.1$. In fact, by generating 10^5 data sets using these parameter combination, we estimate that the probability of generating data from this model that is as unlikely as the experimental data is $p < 10^{-4}$. Thus the tail of the distribution of the number of mutants in Experiment 23 is so heavy that it cannot be fit well by either of the hypotheses considered. Instead, it is likely that some other dynamics are at play here, such as some form of contamination, or additional non-Darwinian processes. In other words,

Luria-Delbrück Experiment 23 cannot be explained by any of the proposed hypotheses (the Lamarckian, the Darwinian, or the Combination one), and thus cannot be used to rule out one hypothesis over another.

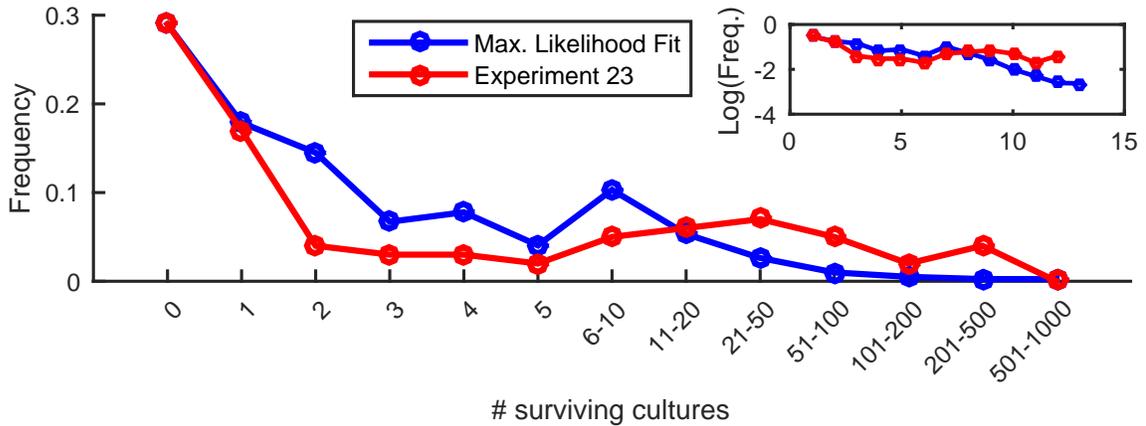


Figure 5. Luria-Delbrück experimental data (red) for Experiment 23 and the maximum likelihood fit of the Darwinian model (also the best fit Combination model) with $\theta_D^{23} = 4.4 \times 10^{-9}$. Here we can see that the tail in the experimental data is too heavy to be reproduced even by the Darwinian model.

4. Conclusion

The classic Luria and Delbrück 1943 experiment [1] is credited with ruling out the Lamarckian model in favor of Darwinism for explaining acquisition of phage resistance in bacteria. However, while heralded as a textbook example of quantitative approaches to biology, the data in the paper was analyzed semi-quantitatively at best. We performed a *quantitative* analysis of the fits of three models of evolution (Lamarckian, Darwinian, and Combination) to these classic data, Experiments 22 and 23. Our analysis was based on a very simplified model of the process: we started each colony with a Poisson-distributed (mean 135) wild-type bacteria and allowed them to replicate synchronously for exactly 21 times, with mutations occurring continuously (Darwinian model) or at the last generation (Lamarckian model). Additionally, we did not consider the possibility that multiple mutations might be needed to acquire resistance, or that growth rates of bacteria may be inhomogeneous. Nonetheless, the simple model fits Experiment 22 data perfectly, suggesting no need for more complex modeling scenarios.

For Experiment 22, by a ratio of $\approx 10^{-106}$, the Lamarckian model is a posteriori less likely than the Darwinian one, agreeing with the original Luria and Delbrück conclusion that the pure Darwinian evolution is a better explanation of the data than the pure Lamarckian evolution. However, the posterior odds of the pure Darwinian model are only 2 – 3 times higher than those for the Combination model (suitably penalized for model complexity), which has nonzero Darwinian *and* Lamarckian mutation rates. Even by liberal standards of modern day hypothesis testing, there is insufficient evidence to rule out the Combination model, and, therefore, contribution of Lamarckian processes to bacterial evolution in this experiment. In contrast, for Experiment 23, neither of the three considered models could quantitatively explain the data, suggesting that additional processes must be in play beyond simple Lamarckian and Darwinian mutations. In

summary, our analysis shows that the classic Luria-Delbrück experiments cannot be used to rule out Lamarckian contributions to bacterial evolution in favor of Darwinism.

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