

The Luria-Delbrück Fluctuation Test as a Classroom Investigation in Darwinian Evolution

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Abstract

Microbial cultures swiftly adapt to lethal agents such as antibiotics or viruses by acquiring resistance mutations. Does this remarkable adaptability require a Lamarckian explanation, whereby the agent specifically directs resistance mutations? Soon after the question arose, Luria and Delbrück devised a clever experiment, the *fluctuation test*, that answered this question in the negative: microbial adaptation, they showed, is entirely consistent with a Darwinian explanation. Their 1943 article is a classic of biology

literature, with practical and theoretical implications that continue to expand today. Implementing an updated fluctuation test in a college teaching lab provides a simple experimental setting in which beginning students learn to apply basic principles of evolutionary biology and scientific reasoning, while gaining hands-on experience in core technical advances of contemporary life science.

Keywords: Darwin, Lamarck, mutation, yeast, likelihood function, nonsense suppression, stress, cancer

Introduction

Breast cancer patients whose tumors are hormone-responsive are typically treated with anti-estrogen drugs. In the course of therapy tumors often become treatment-resistant. Were resistant mutations lurking in a small subpopulation of cancer cells before chemotherapy, or did they arise during treatment? Next generation sequencing reveals that both can be true (Robinson et al., 2013); the ongoing clinical relevance of this question is part of the 70-year legacy of the Luria-Delbrück experiment.

Luria and Delbrück (Luria and Delbrück, 1943) sought to explain a striking characteristic of microbial cultures, which seemed to challenge Darwinian orthodoxy: when such cultures are exposed to a lethal agent—e.g., an antibiotic or virus—variant cells that are resistant to the agent emerge with remarkable speed and regularity. The cultures appear to “adapt” rapidly to the lethal challenge. The survivors’ resistance is heritable, in that their progeny remain resistant after the agent is withdrawn. In the language of modern molecular genetics, the resistant variants have DNA mutations that confer heritable resistance.

Luria and Delbrück considered two opposing explanations for the origin of microbial resistance mutations, which we will call the *post-exposure* versus *pre-exposure* hypotheses. Post-exposure mutations arise only *after* the microorganisms encounter the agent. Such mutations were envisaged by Luria and

Delbrück's contemporaries as "Lamarckian," in that heritable resistance to the agent would be directed by the microorganisms' struggle to survive the agent. Pre-exposure mutations, in contrast, arise *before* contact with the agent, and thus independently of the selective advantage they confer. This hypothesis can be said to be "Darwinian," in that resistance to a particular agent emerges because that agent *selects* for resistant mutants, not because it specifically directs mutations that confer resistance to itself.

To distinguish these two hypotheses, Luria and Delbrück inoculated several dozen small *non-selective* medium cultures with a few non-resistant bacteria each; the non-selective medium lacked the lethal agent. After incubating the cultures to saturation, they spread each culture on *selective* agar medium in a Petri dish. The selective medium contained the lethal agent, so that only resistant mutants could grow; the dishes were incubated to allow resistant colonies to form. The results were striking: colony counts varied widely from dish to dish. This *fluctuation* can be expressed in statistical language: the *variance* in colony counts was vastly greater than the *mean* (average), a result that overwhelmingly favors the pre-exposure over the post-exposure hypothesis (see Performance and Results of the Fluctuation Test).

The fluctuation test has since been replicated countless times with a great variety of microbes and lethal agents. In each case, variance is vastly larger than the mean, corroborating the pre-exposure hypothesis. Luria and Delbrück's work showed that fluctuation must be taken to account in estimating microbial mutation rates, which today are almost always measured by some variant of their method (see Estimating Mutation Rates). Meanwhile, it has become clear that post-exposure mutations do occur under some conditions of stress, and that stress-induced mutability is an important aspect of microbial physiology and cancer biology. Nevertheless, there is no definitive evidence that these post-exposure mutations are Lamarckian in the sense of being specifically directed at genes that can relieve the stress (see Post-Exposure Mutations in Microbes and Cancer).

A Classroom Yeast Fluctuation Test

We have developed a yeast fluctuation test that has been successfully performed by 20 freshmen over the last 4 years. It is the flagship module in a lab that substitutes for the regular lab part of our beginning biology majors course, and that replaces multiple stand-alone laboratory “exercises” with a small number of extended investigations. Each investigational module is intended to engage students actively in learning, and to connect multiple threads of biology within a coherent intellectual framework. The fluctuation test module affords students with no prior lab experience an opportunity to test a key prediction of evolutionary biology by simple means. Their investigation integrates scientific reasoning and simple mathematics with basic microbiology and molecular genetics. It counters common student misconceptions that are shared by some high school biology teachers (Mead and Scott, 2010; Yates and Marek, 2014): that evolution can’t be tested; that microbes evolve resistance “on purpose.”

The entire module, summarized in Table 1, consists of 11 interconnected lab experiments, computer labs, demonstrations, lectures and discussions; session numbers in this article refer to the table. In the final section, we describe flexible options for partial implement requiring as few as 3 sessions. A detailed instructors’ manual and teaching materials are available at mls@missouri.edu ~~mathinlifesciences@missouri.edu/lab_modules/yeast_fluctuation_test~~. Here we review the logic of the yeast fluctuation test, emphasizing the learning opportunities it affords the beginning biology student.

Table 1. Elements of the yeast fluctuation test module. Each session lasts 100 minutes.

Session	Timing	Activity	Type
1		Start yeast cultures in non-selective liquid medium	Lab experiment
2	3–5 days after Session 1	Spread yeast cultures on selective agar Petri dishes	Lab experiment
3	Before Session 8	Random sampling and the Poisson distribution	Computer lab
4	At least 7 days after Session 2	Count Petri dishes; extract colony DNA; polymerase chain reaction (PCR)	Lab experiment
5	After Session 4	Purify PCR product; sequencing reactions; gel electrophoresis	Lab experiment
6	Enough time after Session 5 for sequence data to be returned	Edit sequence files	Computer lab
7	After Session 6	Align sequences; identify the “red gene” bioinformatically	Demonstration
8	After Sessions 4 & 7	Discuss results of yeast fluctuation test	Discussion
9–10		How genes specify proteins via the genetic code; genetic design of the starting yeast strain (section on Genetic Design)	Lecture and discussion
11	After Sessions 3 & 4	Estimating mutation rates from fluctuation test data	Computer lab

The fluctuation test uses a parent yeast strain constructed by Sue Jinks-Robertson and colleagues, and is based on a genetics lab she taught at Emory University. The lethal agent is the antibiotic canavanine. Two types of spontaneous mutation result in canavanine-resistant colonies: mutations in the

“red” gene (as we call it) give rise to red colonies, while mutations in the “white” gene give rise to white colonies. The identities of the genes don’t emerge until late in the students’ investigation (Sessions 7, 9 and 10; section on Genetic Design).

Performance and Results of the Fluctuation Test

Just before Session 1, 22.5-ml non-selective liquid medium cultures (the “bulk cultures”) are inoculated with parent yeast cells at a density of 10^5 cells/ml. The non-selective medium contains no canavanine, and therefore allows cells to grow equally whether or not they harbor a canavanine resistance mutation. Very few if any of the cells at the outset are canavanine-resistant mutants. The students dispense 60- μ l portions from the bulk cultures into 70 13-ml culture tubes. Those tubes, and the remainders of the bulk cultures, are shaken until Session 2 3–5 days later, by which time cell density has increased 1000-fold to 10^8 cells/ml.

In Session 2, students first dispense replicate 60- μ l samples from one of the bulk cultures into 50 additional 13-ml culture tubes. Then they spread the contents of all 120 culture tubes—the 70 individual 60- μ l cultures and the 50 60- μ l samples of the bulk culture—onto Petri dishes with selective agar medium, each Petri dish receiving 6 million cells. The selective medium contains canavanine, so that only canavanine-resistant mutants can grow into colonies. The Petri dishes are incubated at least a week until Session 4, allowing canavanine-resistant colonies to develop fully.

In a computer lab (Session 3), students use a spreadsheet program such as Microsoft Excel to calculate the mean and variance of a series of numbers generated randomly and by experiment (counting yeast cells under the microscope). They learn how the Poisson distribution describes random sampling error, predicting that mean and variance will approximate each other. No prior mathematical background beyond high school algebra is assumed. While this session isn’t essential, it enhances students’

understanding of the logic of the fluctuation test. The Poisson distribution reappears in Session 11 (see Estimating Mutation Rates); it also appears in other modules.

In Session 4, students record the red and white colony counts on all 120 Petri dishes (they also begin the sequence analysis described in the next section). The red colony counts from the most recent year are graphed in Figure 1. They are typical not only of 4 years' results in the lab course, but also of fluctuation tests in general, including the original Luria-Delbrück experiment. Bulk culture sample counts cluster as predicted for random sampling by the Poisson distribution, with a variance (10.75) close to the mean (9.18). Individual culture counts, in contrast, fluctuate widely, with a variance (611) 95 times greater than the mean (6.40); 43 of the 70 dishes have no red colonies at all, while two “jackpot” dishes have 99 and 175. White colony counts (not shown) had the same striking pattern: bulk culture samples: variance (10.09) close to the mean (9.70); individual cultures: variance (6179) 250 times greater than the mean (24.7).

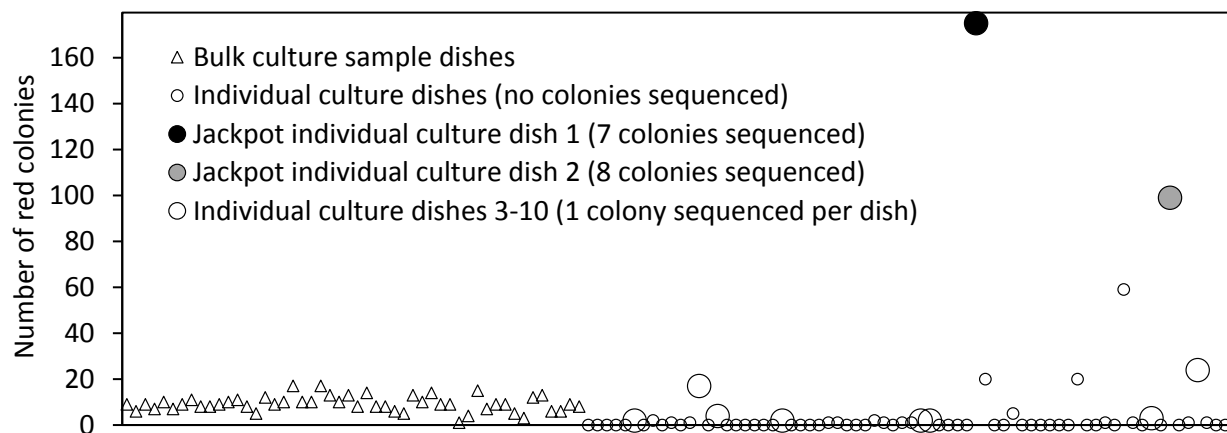


Figure 1. Scattergram of red colony counts in Fall 2013. Triangles correspond to the 50 60- μ l bulk culture samples; circles correspond to the 70 individual 60- μ l cultures. The large black, gray and open circles correspond to the 10 individual culture dishes whose colonies were sequenced (next section).

These results differ sharply from the expectations of the post-exposure hypothesis, according to which resistance mutations don't arise until *after* cells first encounter canavanine on the selective agar medium. In that scenario, each dish, whether from an individual culture or a bulk culture sample, receives 6 million cells, none of which is already resistant. Once they land on the selective medium, all cells have the same low probability of successfully mutating to resistance and thus founding a colony. Each cell mutates independently of all other cells, regardless of its clonal history. Under the post-exposure hypothesis, therefore, colony counts should be Poisson-distributed on *both* bulk culture sample and individual culture dishes, in stark contrast to the actual results in Figure 1.

On the other hand, the results are fully consistent with the pre-exposure hypothesis, according to which resistance mutations occur randomly during growth in non-selective medium. In this scenario, resistant mutants are already present in the cultures before spreading on selective medium; each mutant gives rise to a colony, while unmutated cells—the overwhelming majority—are killed by canavanine. The number of resistance *mutation events* during non-selective growth in individual cultures will be randomly distributed according to the Poisson distribution. In contrast, the number of resistant *mutants* following non-selective growth (and thus the number of colonies on the selective Petri dishes) will fluctuate widely from culture to culture. That's because mutation events during non-selective growth are randomly distributed not only with regard to *number*, but also with regard to *time*. Mutation events occurring early during non-selective growth give rise to exponentially larger clones of resistant mutants than do mutation events occurring late, as shown in Figure 2 (*upper half*). The extreme fluctuation (high variance) evident in the individual culture colony counts in Figure 1, with many dishes having zero colonies and a few having jackpots, is therefore entirely expected. Unlike the individual culture dishes, the bulk culture sample dishes represent random samples from a single population of fully-grown cells; the only source of variance is therefore random sampling error, as described by the Poisson distribution (Figure 2, *lower*

half). The low variance in colony counts observed for these control dishes in Figure 1 is thus anticipated in both post-exposure and pre-exposure scenarios.

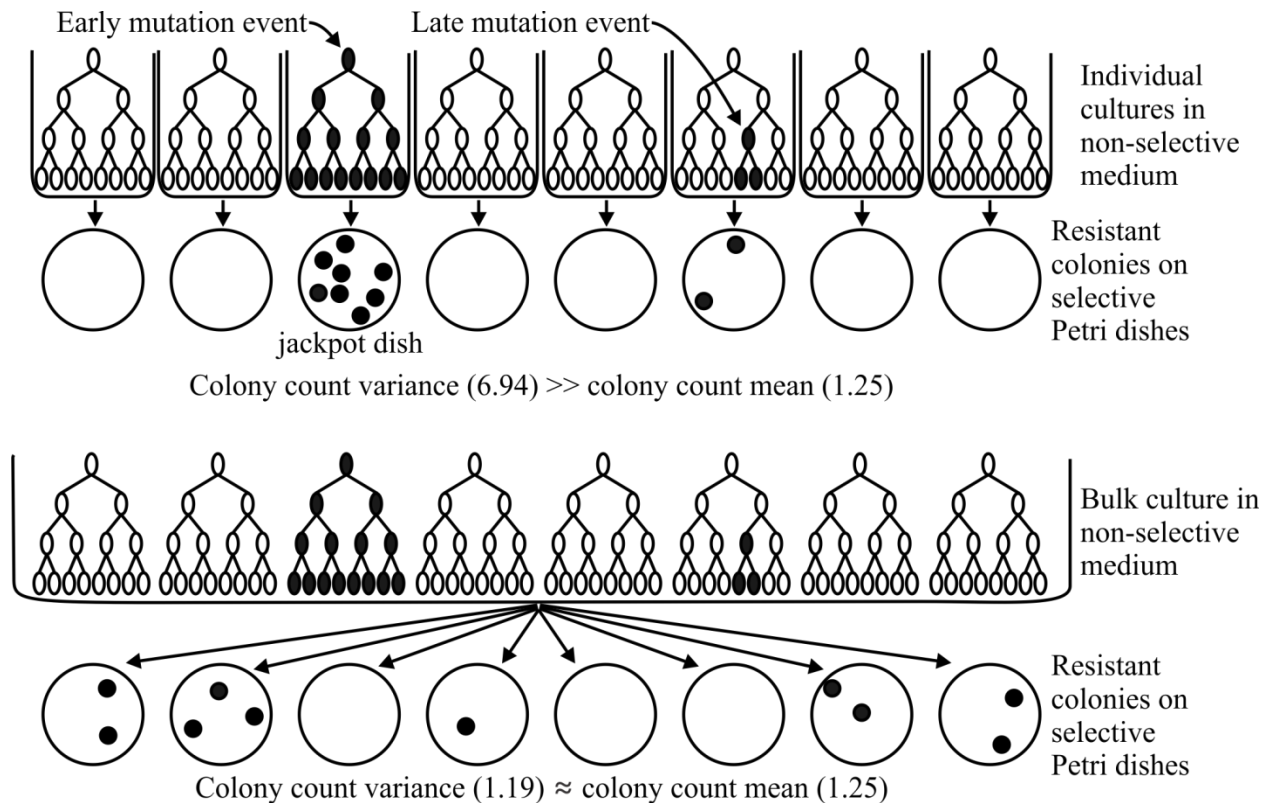


Figure 2. Expectations of the pre-exposure hypothesis. *Upper half:* individual 60- μ l cultures and corresponding individual culture dishes. *Lower half:* control bulk culture and corresponding 60- μ l bulk-culture sample dishes; a bulk culture is equivalent to hundreds of individual cultures mixed together in a single culture vessel. Bifurcating lineages represent exponential growth in non-selective medium; black ovals in these lineages represent canavanine-resistant mutant cells. Black circles in the Petri dishes represent canavanine-resistant colonies. Early mutation events in individual cultures give rise to exponentially larger mutant clones than late mutation events. The variance in colony counts on the corresponding individual culture dishes is therefore much higher relative to the mean than predicted by the Poisson distribution for random sampling error. The same large disparity in mutant clone size occurs in the bulk culture, but the mutant and parent cells from all lineages are mixed together in a single

population, from which random samples are spread on selective dishes. The only source of variance in colony counts is therefore random sampling error, as suggested in the lower half of the figure.

Sequence Analysis of Resistant Mutants

Many distinct mutations in what we're calling the "red" gene result in red canavanine-resistant colonies (Giroux et al., 1988; Mudrak et al., 2009). Only by rare coincidence will two independently-arising mutant colonies harbor identical mutations. Conversely, if multiple red canavanine-resistant colonies from a single Petri dish harbor identical red gene mutations, it's likely that they're identical by descent rather than by coincidence: i.e., that they're members of a single mutant clone, arising from a single mutation event in an ancestral cell. That's an expected occurrence on the pre-exposure hypothesis, according to which jackpot dishes represent early resistance mutation events that found large clones of identical resistant mutants. The post-exposure hypothesis, in contrast, predicts that no two mutants in different colonies, even on the same Petri dish, should be identical by descent. That's because post-exposure mutation events occur in each cell independently after it has landed on the selective agar medium. Sequencing the red gene from multiple colonies on a single jackpot dish thus provides an additional critical test of the post-exposure versus pre-exposure hypotheses. The test is cheap and easy using polymerase chain reaction (PCR) and DNA sequencing technology. Students sequence the red gene from multiple red colonies on 2 jackpot dishes, and from single red colonies on 8 additional dishes as controls (half of Session 4; Sessions 5–7).

The results for 2013 are shown in Figure 3. All clones from a single jackpot dish have identical mutations, while clones from different dishes have different mutations. This fully corroborates the pre-exposure hypothesis, which readily explains jackpot mutants on a single dish as identical by descent; but

strongly argues against the post-exposure hypothesis, which can only explain the outcome as multiple improbable coincidences.

Parental red gene	CTCTCGGTAGCCAAGTTGGTTTAAGGCGCAAGACTTTAATTTATCACTACGAAATCTTGAGATCGGGCGTTCGACTCGCCCCGGGAGA
Dish 1 Colony 1	A
Dish 1 Colony 2	A
Dish 1 Colony 3	A
Dish 1 Colony 4	A
Dish 1 Colony 5	A
Dish 1 Colony 6	A
Dish 1 Colony 7	A
Dish 2 Colony 1 T
Dish 2 Colony 2 T
Dish 2 Colony 3 T
Dish 2 Colony 4 T
Dish 2 Colony 5 T
Dish 2 Colony 6 T
Dish 2 Colony 7 T
Dish 2 Colony 8 T
Dish 3 Colony 1 T
Dish 4 Colony 1 T
Dish 5 Colony 1 G
Dish 6 Colony 1 A
Dish 7 Colony 1 G
Dish 8 Colony 1 G
Dish 9 Colony 1 G
Dish 10 Colony 1	G

Figure 3. Sequence alignment of the parental red gene with the mutant red gene observed in 23 red canavanine-resistant colonies. Bold letters in the colony sequences indicate nucleotides that differ from the corresponding nucleotides in the parental gene; dots indicate positions where the colony sequence and the parental red gene are identical.

Colony sequencing acquaints students with two foundational technologies of modern biology, PCR and DNA sequence analysis. Learning how they work reinforces an understanding of the biology of chromosome replication. Editing sequence files and aligning the resulting sequences in order to identify canavanine resistance mutations (Sessions 5 and 7) highlights the indispensable role of bioinformatics in life sciences.

Estimating Mutation Rates

Mutation rates are a central concern of genetics and evolutionary biology, and because most carcinogens are mutagenic, they are also central to cancer biology and environmental policy. In the 1970's, Bruce Ames and colleagues developed a microbial mutagenicity assay, the "Ames test," in order to rapidly survey environmental agents for their mutagenic, hence carcinogenic, potential (reviewed by Mortelmans and Zeiger, 2000). Luria and Delbrück's work demonstrated that fluctuation (extreme variance) must be taken into account in estimating microbial pre-exposure mutation rates. Contemporary mutagenicity assays use fluctuation tests to assess how mutation rates vary with the dose of the suspected mutagen. In Session 11 students learn how mutation rates can be estimated from colony count data.

We can define the pre-exposure mutation rate μ during non-selective growth as the probability that a cell division gives rise to a mutant daughter cell, assuming the mutation rate is approximately constant during the growth period. It requires almost exactly N cell divisions for the cell population to reach N from a much smaller starting population. Thus when a culture grows in non-selective medium to a population size of N from a much smaller number of cells, the expected number of resistance mutation events \bar{m} is just the product μN (assuming $\mu \ll 1$). In the yeast fluctuation test, $N = 6$ million. The probability that the number of resistance mutation events in a particular individual culture is m is given by the Poisson distribution $\frac{\bar{m}^m e^{-\bar{m}}}{m!}$, where e is the base of the natural logarithms.

In general, we can't count the number of resistance mutation events m that have occurred in a particular culture. Only the number of resulting mutant cells can be observed directly, by counting the number of resistant colonies that result when the culture is spread on selective medium. But there is one special case where the number of resistant colonies on the dish equals the number of resistance mutation

events in the corresponding culture: when both numbers are 0 (this is true only if the *entire culture* is applied to the selective dish, as in our design).

In 2013, 43 of the 70 dishes had no colonies (Figure 1). What does this say about the value of \bar{m} ? We approach this problem by first considering a much simpler one: calculating the probability that an individual dish has 0 colonies, which is the same as the probability that the number of mutation events m in the corresponding culture is 0. We'll call that probability p ; it depends on the value of \bar{m} according to the Poisson distribution above: $p = \frac{\bar{m}^0 e^{-\bar{m}}}{0!} = e^{-\bar{m}}$. Conversely, the probability that a dish has at least 1

colony is $1 - p = 1 - e^{-\bar{m}}$. Mutation events in different cultures occur independently of one another.

Therefore, the joint probability that 43 dishes will have no colonies and 27 at least 1 colony, as was actually observed (Figure 1), is proportional to the product of the individual probabilities:

$p^{43} \times (1 - p)^{27} = (e^{-\bar{m}})^{43} \times (1 - e^{-\bar{m}})^{27}$. This function of \bar{m} is called the *likelihood function*: a function that's proportional to the probability of obtaining the data we actually observed (43 dishes with 0 colonies, 27 dishes with at least one colony) given all the possible values of the parameter \bar{m} in turn. Only *relative* values of the likelihood matter: multiplying the likelihood function by a constant that doesn't depend on the variable parameter \bar{m} yields a new likelihood function with the same meaning as the original one. In the Session 11 computer lab, students use a spreadsheet program such as Microsoft Excel to graph the relative likelihood function, as illustrated in Figure 4.

The likelihood function is a general way of quantifying the strength of evidence for alternative hypotheses that compete for our belief. In this case, the evidence is the colony-count data, and the competing hypotheses are the possible values of the parameter \bar{m} . The value of \bar{m} that maximizes the value of the likelihood function is called the *maximum-likelihood estimate* of \bar{m} ; it's the value to which the data lend maximum support. The students show graphically (and it's easy to prove by the methods of

calculus) that the maximum-likelihood estimate in this case is

$\bar{m} = \ln(70/43) = 0.49$ mutation events/culture, corresponding to a mutation rate

$\mu = \frac{\bar{m}}{N} = \frac{\ln(70/43)}{6 \times 10^6} = 8.1 \times 10^{-8}$. There is obviously uncertainty in this or any other point estimate, but

quantifying uncertainty lies beyond the scope of the module.

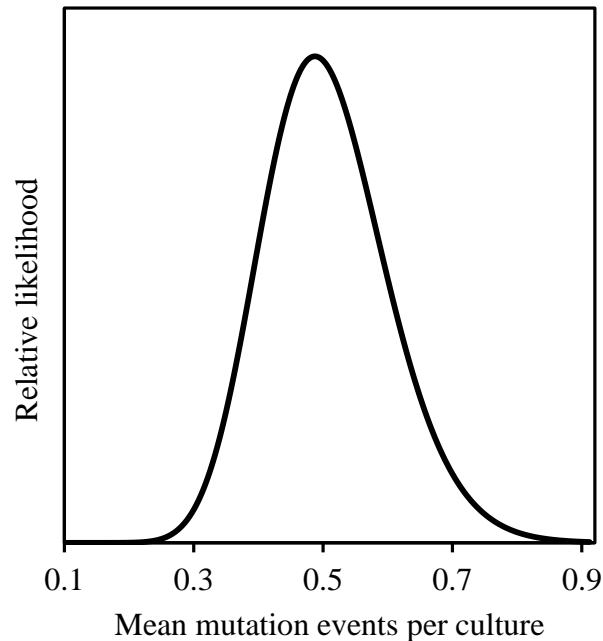


Figure 4. Simple likelihood function for the yeast fluctuation test data in Figure 1. Much more complex likelihood functions take numerical colony counts into account (reviewed in Ycart and Veziris, 2014).

Post-Exposure Mutations in Microbes and Cancer

While fluctuation tests provide definitive evidence for pre-exposure mutations, they don't rule out the possibility of post-exposure mutations. That's because the lethal agent (canavanine in our case) kills parent cells before they have a chance to adapt. Modified fluctuation tests in which the selective medium

allows parent cells to survive, but not to form colonies unless they acquire a new growth-permitting mutation, have in some cases revealed mutations that are post-exposure by stringent criteria (reviewed by Rosenberg, 2001). Post-exposure mutations are generally *induced* by the stress imposed by the selective medium. For instance, bacteria under stress may adopt a hypermutable state by expressing error-prone DNA polymerases and DNA repair systems (Rosenberg, 2001). Breast cancer cells with pre-exposure mutations conferring genetic instability may, under the stress of estrogen deprivation, acquire new mutations facilitating growth in low estrogen (Robinson et al., 2013). Although stress-induced mutations may preferentially target a subset of genes, there is no convincing evidence that they are *directed*: i.e., specifically targeted to a subset of genes that can mutate to permit growth. Stress-induced mutations are an adaptive response to the stress, but as far as we know they are still “Darwinian” rather than “Lamarckian,” in that they are not specifically directed to genes that can relieve the stress.

Genetic Design

Once sequence data are available (Session 7), students can identify the “red” gene bioinformatically as *SUP4-o* (GenBank Accession J01381.1), which encodes an ochre suppressor tRNA. That tRNA differs from the wild-type tyrosine tRNA (GenBank Accession M10266.1) by a G→U substitution at the 5′ position of the anticodon, so that the tRNA recognizes the 5′-UAA-3′ ochre stop codon rather than the 5′-UAC-3′ and 5′-UAU-3′ tyrosine sense codons. By this point in biology instruction, students have learned about the genetic code, preparing them to make the connection between mutations in *SUP4-o* and the red canavanine-resistant phenotype, as explained in this section. While not essential to an understanding of the fluctuation test, this discussion (Sessions 9 and 10) provides a natural context for reinforcing the biology of translation and the genetic code.

The parent yeast strain YFT1 is identical to its direct progenitor SJR1921 (haploid genotype *MATa ura3ΔNco lys2-1 can1-100 ade2-1 leu2-K nearARS306::SUP4-oF*) apart from a few apparently spontaneous mutations. SJR1921 was constructed by Sue Jinks-Robertson and her colleagues based on an earlier design (Pierce et al., 1987). YFT1 and SJR1921 carry three relevant pre-existing mutations:

- The *can1-100* mutation is an AAA to TAA ochre nonsense mutation at codon 47 of the *CAN1* gene (http://wiki.yeastgenome.org/index.php/Commonly_used_auxotrophic_markers); severe loss-of-function mutations in the *CAN1* gene, including the ochre nonsense mutation, result in resistance to canavanine. *CAN1* is the “white” gene.
- The *ade2-1* mutation is a GAA to TAA ochre nonsense mutation at codon 64 of the *ADE2* gene (http://wiki.yeastgenome.org/index.php/Commonly_used_auxotrophic_markers); severe loss-of-function mutations in the *ADE2* gene, including the ochre nonsense mutation, result in accumulation of a red intermediate.
- The *nearARS306::SUP4-oF* mutation (Mudrak et al., 2009) is the *SUP4-o* ochre suppressor tRNA gene inserted artificially in chromosome III; the tRNA is able to suppress both the *can1-100* and *ade2-1* ochre nonsense mutations. As already stated, *SUP4-o* is the “red” gene.

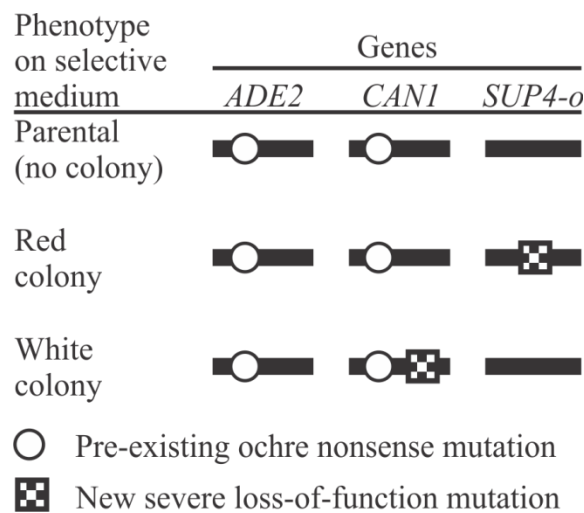


Figure 5. Genotypes and phenotypes of the parent strain and two types of canavanine-resistant mutant.

Figure 5 summarizes genotypes and phenotypes of the parent strain and of the two types of canavanine-resistant mutant that are scored in the fluctuation test. In the parent strain (top line), the suppressor tRNA encoded by the *SUP4-o* gene suppresses the pre-existing *can1-100* ochre nonsense mutation, restoring the function of the Can1 protein and rendering the cell sensitive to canavanine. The parent strain therefore cannot form colonies on selective (canavanine-containing) medium. The *SUP4-o* tRNA also suppresses the *ade2-1* ochre nonsense mutation, restoring Ade2 protein function and preventing accumulation of the red intermediate; the starting strain therefore forms white colonies on non-selective agar medium.

Red canavanine-resistant mutants (Figure 5, *middle*) result from new severe loss-of-function mutations in the *SUP4-o* gene that eliminate functional ochre-suppressing tRNA. As a consequence, no functional Can1 protein is made, so that the cells are resistant to canavanine and thus able to form colonies on selective medium. No functional Ade2 protein is made either, so the canavanine-resistant colonies are red.

White canavanine-resistant mutants (Figure 5, *bottom*) result from new severe loss-of-function mutations in the *CAN1* gene rather than in the *SUP4-o* gene. These cells produce a functional ochre-suppressing tRNA from their non-mutant parental *SUP4-o* gene, thus suppressing the pre-existing *can1-100* and *ade2-1* nonsense mutations. Can1 protein function is nevertheless eliminated or greatly reduced in these mutants as a result of the new loss-of-function mutation, allowing the cells to form colonies on selective medium. These cells produce functional Ade2 protein as a result of suppression, so that the colonies are white.

Options for Implementation

Full implementation of the fluctuation test module uses 11 of our course's 29 100-minute lab sessions. We consider the investment worthwhile because the module brings into play so many fundamental principles of biology and science. Nevertheless, there are flexible options for partial implementation. The yeast fluctuation test as a stand-alone, without the mathematical or genetic emphasis, can easily be accomplished in 3 lab sessions (Sessions 1, 2 and 4). Omitting colony sequencing and associated exercises eliminates half of Session 4 and all of Sessions 5–7, along with most of the expense. Omitting the Poisson distribution eliminates Sessions 3 and 11 and the module's only formal mathematics. Omitting analysis of the genetic design of yeast strains eliminates part of Session 7 and all of Sessions 9 and 10. The module can also easily be adapted for more advanced students by giving them more responsibility for behind-the-scenes preparation, and increasing the depth of explanation and analysis.

In full or partial implementation, the fluctuation test deserves consideration as a standard component of the college biology laboratory, because of its opportunities for student-initiated discovery, its ease of performance, and its ability to shed light on central concepts of biology.

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