INTRODUCTION

The teaching literature describes many applications of games of chance to the teaching of genetic concepts. Playing cards (1, 2) and real or electronic dice (3–5) have been employed to illustrate the principles of inheritance, natural selection, and mutation. The classic experimental work which first quantified spontaneous mutation in bacterial cell populations, the Luria-Delbrück fluctuation test (6), has been adapted to wet lab (7–9) and computer-based (10) teaching activities. Recent pedagogical work has shown that student instruction in fluctuation-test theory helps dispel the incorrect notion that the environment causes directed mutations (11). Clearly, a proper understanding of mutation and mutation rate is conceptually valuable for all students of biology. The dice-roll exercise described here was developed to illustrate the use of the Drake mutation rate equation (12, 13) and to provide practice with mutation rate calculation for a semester-long, undergraduate, medical microbiology laboratory course investigating bacterial antibiotic resistance. This activity’s simplicity and one-hour time allotment make it amenable to any biology classroom or laboratory exploring the frequency of mutation.

In 1970, J. W. Drake developed a mutation rate equation conceptually distinct from that of Luria and Delbrück (6), who assumed random mutation and applied the Poisson probability distribution. Drake’s equation is based on the rate of change of the mutant fraction in a population (12). Drake very simply posited that the rate of increase in the number of mutants, \( M \), in a culture of \( N \) cells is equal to the total mutant fraction of the population at any time during population increase. The mutant fraction is the sum of the fraction which experiences mutation (\( \mu \), the mutation rate) and the fraction produced by multiplication of previously generated mutants:

\[
dM/dN = \mu + M/N \quad \text{Eq. 1}
\]

Here, \( N \) is very large and essentially equal to the number of cell division events required to produce the population from a single cell. This equation assumes that mutant cells multiply at the same rate as wild-type cells and do not revert.

In order to derive a useful equation for mutation rate measurement, the mutant fraction \( f \) is defined as \( f = M/N \), which is also expressed \( M = fN \). Since \( f \), \( M \), and \( N \) increase as the population grows, the product rule for differentiation is employed:

\[
dM = f(dN) + N(df)
\]

Substituting this equation for the \( dM \) term of Eq. 1 and multiplying both sides by \( dN \) produces

\[
f(dN) + fN(df) = \mu(dN) + M(dN/df)
\]

Dividing the left side by \( f \) and the right side by the equivalent expression \( M/N \) gives

\[
dN + N(df)/f = \mu(dN)(N/M) + dN
\]

or, in factored form,

\[
dN + N(df)/f = [\mu(N/M) + 1](dN)
\]

Dividing both sides by \( N \) gives

\[
(df)/f = [\mu(dN)] / M
\]

And substituting \( fN \) for \( M \) produces

\[
df = \mu(1/N)dN
\]

Since the mutation rate, \( \mu \), is a constant, albeit one which is accurately measured only when \( N \) is large, both sides of this equation may be easily integrated to produce Drake’s first mutation rate equation:

\[
\mu = (f - f_0) / [\ln(N/N_0)] \quad \text{Eq. 2}
\]

The “zero” subscripts indicate the initial condition before population growth; that is, the culture was begun from a low number of non-mutant cells.

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In 1991, Drake adapted his equation to multiple-culture fluctuation tests (13). Beginning with Equation 2, he redefined \( N_0 \) as the large cell number at which the first mutant cell appears. At this point \( N_0 \) equals \((1/\mu)\). Substituting \( \mu \) for \( 1/N_0 \), neglecting \( f_0 \), and replacing the \( f \) of Equation 2 with \( M/N \) produces the final mutation rate equation:
\[
\frac{M}{N} = \mu \times \ln(N\mu) \quad \text{Eq. 3}
\]

Experimentally, the median number of mutants per culture, \( M \), should be determined from at least 40 cultures, which is a standard for mutation rate calculation by fluctuation test (14). \( N \) is the same very large number of cells from all cultures.

**PROCEDURE**

The only materials needed for this exercise are one 20-sided die per student and access to a spreadsheet program such as MICROSOFT EXCEL for facile data analysis. Dice may be obtained from a variety of educational and gaming suppliers, such as EAI Education of Oakland, New Jersey.

To perform this exercise, each student rolls his or her die a total of 100 times as five sets of 20 rolls each. “Non-12” results are recorded as a minus sign (−), and “12” results are noted as a plus sign (+) on the data sheet shown in Appendix 1. Each student’s results collectively represent both a single population and a single fluctuation test culture. In order to simulate the appearance of mutant organisms as a population of cells expands, a single roll of a 20-sided die is considered a cell division event, \( N \). Furthermore, a “12” result arbitrarily models the appearance of a mutant cell. In this context, the theoretical mutation rate is 1/20 or 5.00%. A spreadsheet is then used to calculate the Drake mutation rate as described in Appendix 2.

The format for recording data used in Appendix 1 is useful, since many columns of 20 rolls will produce no “12” results. Therefore, if desired, student data may be further analyzed using the Luria-Delbrück mutation rate equation:
\[
\mu = -\ln(P_0) / N \quad \text{Eq. 4}
\]

To apply Equation 4, \( P_0 \) is the class-wide fraction of 20-roll data sets which yield no “12” results, and \( N \) equals 20 (see reference 15). Note that this application of Equation 4 utilizes a factor of five simulated cultures (5 × 20 rolls per student) in place of the single culture in Equation 3 (1 × 100 rolls per student) and is therefore more accurate.

**CONCLUSION**

Student data from two medical microbiology classes are shown in Table 1. A five-minute timed quiz was administered to the 2015 class following measurement of the mutation rate of *Serratia marcescens* bacteria to nalidixic acid resistance at 25 µg/mL. Four students correctly determined both the Drake and Luria-Delbrück rates; no students correctly determined the Drake rate only; five students correctly determined the Luria-Delbrück rate only; and five students determined neither rate correctly. Taken together, these results show that both equations model a simulated fluctuation test reasonably well and provide students with conceptual practice regarding calculation of mutation rates.

**SUPPLEMENTAL MATERIALS**

Appendix 1: Student instructions
Appendix 2: Instructor preparation

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**TABLE 1.**

|                  | Spring 2015 | Spring 2017 |
|------------------|-------------|-------------|
| Number of students | 19          | 9           |
| Theoretical mutation rate | 5.00%      | 5.00%      |
| Class-wide Measured mutation rate | 4.37%      | 6.67%      |
| “12” rolls / total rolls | 4.37%     | 6.67%      |
| Drake mutation rate (\(\mu_{Drake}\)) | 3.33%      | 4.19%      |
| Luria-Delbrück mutation rate (\(\mu_{L-D}\)) | 4.45%     | 5.84%      |
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