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COMPLEXITY OF MUTATIONS IN BACTERIAL POPULATIONS

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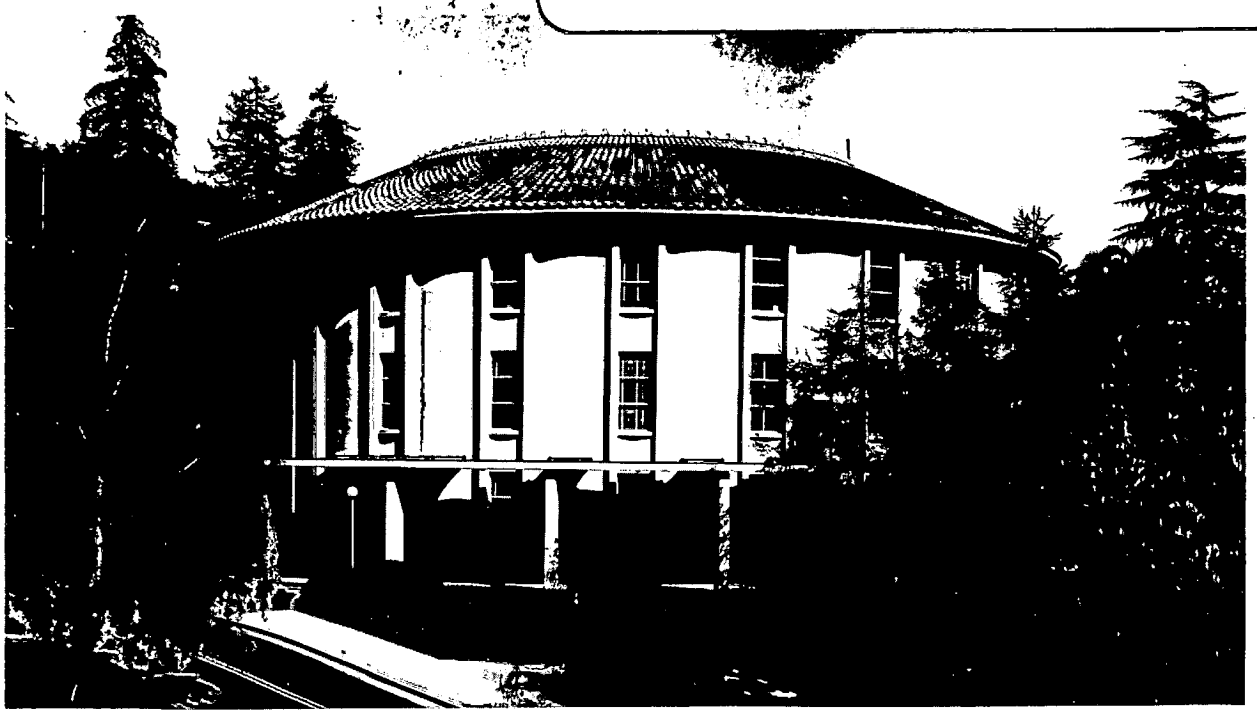

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COMPLEXITY OF MUTATIONS IN BACTERIAL POPULATIONS

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SUMMARY

Spontaneous mutation has as its most basic effect increasing the fraction of mutants in a population in direct proportion to the number of generations of continuous culture (Luria & Delbruck, 1943; Ryan & Wainwright, 1954; Stent & Calendar, 1978). What is not at all obvious without further consideration is that the fraction of independently arising mutants actually decreases relative to the total number of mutants. This is an important limitation when mutants are picked from a single mutagenesis experiment. An approach is suggested for the most efficient isolation of a library of unique mutants in experiments where there is a constant and low rate of mutagenesis.

1. Introduction

In a population of dividing bacteria a mutation gives rise to identical mutants belonging to the same clone. I define the complexity of the bacterial mutations in the population as the total number of clones. This neglects the loss of identical clones by coincident mutation or the loss of mutants by reversion. In the following mathematical analysis equations are derived for complexity. This is further reflected in the fraction of unique or independently arising mutants as a function of the number of generations of continuous culture.

2. Formulation of Complexity Equations

For purposes of simplifying the mathematics, we start with an inoculum which on the average has only one mutant:

$$a N_o = 1$$

where a is the mutation rate and N_o is the number of bacteria in the inoculum. The inoculum is the 0th generation. This initial condition simply means that the counting of generations begins when the population reaches a size which generates one mutation, on the average. From this point, there will be C_g new clones, on the average, generated in each of the following gth generations:

$$C_g = 2^g$$

After a total of G generations there are C clones (complexity):

$$C = \sum_{g=0}^{G} 2^g$$

By considering partial sums this becomes:

$$C = 2^{G+1} - 1 \qquad \text{Eq. 1}$$

The total number of mutants, n, belonging to all clones:

$$n = (G+1)2^G$$

The fraction of unique mutants, F:

$$F = C/n \qquad \text{Eq. 2}$$

Substituting,

$$F \doteq \frac{2^{G+1}-1}{(G+1)2^{G}}$$

$$F \simeq \frac{2}{G+1} \qquad \text{Eq. 3}$$

The fraction of unique mutants in a bacterial population is inversely proportional to the number of generations of continuous culture past the point where, on the average, one mutation occurs. The equation is otherwise independent of the mutation rate and the size of the initial inoculum. However, the mutation rate determines the number of generations which are necessary to acquire a certain number of clones. Low mutation rates necessitate more generations, so the fraction of unique mutants is lower.

3. Limitations on Complexity in Mutagenesis

Equation 3 limits mutagenesis experiments when there is a constant and low rate of mutation, as in the case of spontaneous mutation or insertional mutagenesis by some transposons under steady-state conditions (a is a constant during the mutagenesis). Equation 3 also limits chemical mutagenesis experiments when the mutagen is administered to the culture over many generations. Equation 3 does not have a significant effect in experiments where a mutagen is applied briefly and the culture is plated before extensive replication of the independent mutants occurs. The latter case includes mutagenesis induced by chemicals or ultra-violet light where the exposure is limited to less than a few generations, and insertional mutagenesis by zygotically induced transposons (Biek & Roth, 1980) where the rate of transposition is very high for only one generation.

As an example of an experiment where Equation 3 is limiting, consider a mutagenesis experiment with a mutation rate equal to 10^{-7} per bacterium per generation. Start with an inoculum of 10^7 bacteria which grows exponentially for ten generations to a total of 1.024×10^{10} bacteria. On the average, there will be 11,264 total mutants belonging to 2,047 clones such that only 18% of the mutants are unique (arose independently).

The limitations of Eq. 3 become severe when it is necessary to isolate a large number of unique mutants from a single mutagenesis experiment. An example is in the construction of a library of transposon mutagenized plasmids when there is a low frequency of transposition. As shown in the example above, an average experiment may yield only 18% unique inserts. This does not consider the preference of transposons for certain target sequences which has the effect of reducing the number of unique inserts still further. For a review on transposable elements, see Calos and Miller, 1978.

4. Resolution of the Problem of Complexity in Mutagenesis

The limitations of Eq. 3 may be avoided by the following procedure. Any mutagenesis procedure will yield at least one mutant which is necessarily unique. One mutagenesis experiment should be done for each unique mutant. By doing N experiments in parallel, N unique mutants are obtained by picking only one mutant from each experiment. In most cases, it should be more expedient to increase the number of mutagenesis cultures than to screen redundant mutants. For the insertional mutagenesis of a plasmid this translates into more matings and less plasmid preparations and restriction digests. Care must be taken that the experiments are indeed independent of each other. In the case of matings where a plasmid rescues a

transposon by conjugation (Youvan et. al., 1982), this means that the donors should begin from inocula of single bacteria (single colonies) rather than liquid stocks which may already be limited in complexity.

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