

Some Stochastic Models for Plasmid Copy Number

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Received July 22, 1982

Some stochastic models for the copy number of plasmids in a cell line are studied. When considering the behavior of copy number in the *whole* cell line, the theory of multitype branching processes is appropriate. Attention is paid to the cure rate in the cell line, and the asymptotic fractions of cells containing a given number of plasmids. These quantities are used to compare the models numerically.

1. INTRODUCTION

In this paper, we study the behavior of some stochastic models of plasmid reproduction, and the maintenance of plasmid copy number. The motivation for this study arose from work of Gardner *et al.* (1977), Novick and Hoppensteadt (1978), and Emerson and Hoppensteadt (1981).

We consider a cell line initiated by a single cell at time $n = 0$, and growing by binary splitting at times $n = 1, 2, \dots$. The initial cell contains a number of plasmids, which replicate in the cell, and are then distributed to the two daughter cells at cell division. We shall analyze two ingredients of this process. The first is the *replication* mechanism, the second the *partition* mechanism. The replication process determines how plasmids within a given cell replicate before cell division, whereas the partition mechanism describes how the plasmids, after replication in a cell, are divided among the two daughter cells.

Several models for the replication process have been considered. Random replication is taken to mean that plasmid replication occurs sequentially, so that a given plasmid may replicate several times before partition (Rownd, 1969). Models of this type, which are related to the classical Polya urn schemes, have been discussed in the context of plasmid incompatibility by Novick and Hoppensteadt (1978) and by Ishii *et al.* (1978).

In a recent report, Gardner *et al.* (1977) described another collection of stochastic replication schemes. In essence, they modeled the behavior of the

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number of plasmids contained in a cell in any specific generation of a randomly chosen line of descent through the cell population. The aim of their study was to quantify the differences between their replication schemes with respect to the following properties:

(a) *Stability of plasmid copy number.* This includes the mean and variance of the number of plasmids in the line of descent at particular times

(b) *The cure rate.* Along a random line of descent, the cells must eventually be *cured* (i.e., contain no plasmids; see Section 2). How fast does curing occur?

Emerson and Hoppensteadt (1981) also consider aspects of these problems using computational methods.

The focus of the present paper is the simultaneous study of plasmid copy number and cure rate *in the whole cell line* rather than along a random line of descent, using the models of Gardner *et al.* We concentrate on two measures to assess quantitatively the differences between the replication mechanisms: the asymptotic cure rate (Section 3.1) and the asymptotic proportions of cells containing a given number of plasmids (Section 3.2). In Section 4, numerical comparisons are made. The basic tool we use is the (finite and countable) multitype branching process which describes the evolution of plasmid numbers through the cell line. Some of the more technical derivations are relegated to the Mathematical Appendix.

We conclude this section with a description of the replication and partition mechanisms that we will analyze.

1.1. *The Replication Processes*

There are three such mechanisms that we shall describe.

(a) *The Multiplicative (or Doubling) Model (M).* Suppose that at cell contains i (>0) plasmids just before replication. We assume that each plasmid replicates once independently of all others, giving rise to either one offspring plasmid (with probability $1-p$) or two offspring (with probability p), where $0 < p \leq 1$. If we define

$$R_{ii} = \Pr\{l \text{ plasmids in cell before partition} \\ i \text{ before replication}\}, \quad (1.1)$$

then in the present case we have

$$R_{ii} = \binom{i}{l-i} p^{l-i} (1-p)^{2i-l}, \quad l = i, i+1, \dots, 2i. \quad (1.2)$$

When $p=1$, the model reduces to one in which each plasmid produces exactly two offspring; hence the term "doubling." The parameter p takes account of random variation in this reproduction scheme.

(b) *The Additive Model (A)*. We again consider a situation in which a cell contains i (>0) plasmids. In this case, there is a fixed number N of replication events, each of which is successful with probability p , independently for each such event. The number of plasmids in the cell prior to partition and after replication is then equal to $i + Y$, where Y is a binomial random variable with parameters N and p . This model can be ascribed to Kasamatsu and Rownd (1970) (in the case $p = 1$), who wanted to model a regulation mechanism to control the replication process.

It is readily seen that for $i > 0$,

$$R_{ii} = \binom{N}{l-i} p^{l-i} (1-p)^{N-l+i}, \quad l = i, i+1, \dots, N+i. \quad (1.3)$$

(c) *The Equilibrium Model (E)*. This process also seeks to describe a replication control mechanism. Given i (>0) plasmids in a cell, and a replication event success probability of $p = 1$, the number of plasmids in the cell before partition is $2N$, regardless of the value of i . This model reflects the inability of a given cell to contain more than a given number of plasmids. When $p < 1$, we view the process as leaving $i + Y$ offspring plasmids, where Y is a binomial random variable with parameters $2N - i$ and p . It follows that

$$R_{ii} = \binom{2N-i}{l-i} p^{l-i} (1-p)^{2N-l}, \quad l = i, \dots, 2N. \quad (1.4)$$

In all three models, it is assumed that p , and the form of the replication probabilities R_{ii} , remain constant through time. From a standpoint of replication control mechanisms, it might be argued that p should depend on the plasmid copy number i ; the larger i , the smaller should be p . We shall not attempt an analysis of this process. Finally, since we are supposing that cured cell (one containing no plasmids) leaves cured daughter cells, we must have $R_{00} = 1$.

In Section 2 we study the role of the parameters p and N on the evolution of plasmid copy number in a random line of descent.

2. RANDOM LINES OF DESCENT

A random line of descent through the cell line is defined as follows. We choose with probability $\frac{1}{2}$ one of the two daughter cells at time $n = 1$, with probability $\frac{1}{2}$ one of the daughter cells of that chosen at time $n = 1$, and so on. Now let X_n be the number of plasmids in the cell (before replication) at time n , $n = 0, 1, 2, \dots$. For the three models described earlier, it is clear that

$\{X_n, n \geq 0\}$ is a Markov chain, and its one-step transition probabilities (p_{ij}) are given by

$$p_{ij} = \Pr\{X_{n+1} = j | X_n = i\} = \sum_l R_{il} P_{lj}, \quad (2.1)$$

where, as in (1.1),

$$R_{il} = \Pr\{l \text{ plasmids in cell before partition} | i \text{ before replication}\},$$

and

$$P_{lj} = \Pr\{\text{randomly chosen daughter cell inherits } j \text{ plasmids} | \text{mother cell had } l \text{ before partition}\}.$$

So as not to obscure the effects of the replication process, we shall in all cases assume a *random* partition process. This means that any plasmid in a cell before partition is assigned independently and at random to either of the two daughter cells. In particular,

$$P_{lj} = \binom{l}{j} 2^{-l}, \quad j = 0, 1, \dots, l. \quad (2.2)$$

The type of Markov chain represented by the transition probabilities (2.1) is most easily ascertained by looking at the probability generating functions (pgf) $f_i(s)$, $i > 0$, given by

$$f_i(s) = \sum_j p_{ij} s^j, \quad 0 \leq s \leq 1. \quad (2.3)$$

Using (2.2) and the replication probabilities (1.2)–(1.4), these pgf's are readily evaluated. The results are given in Table I. From this table, it is

TABLE I

Model	pgf $f_i(s)$, $i > 0$
Multiplicative	$\left(\frac{1}{2} + \frac{s}{2}\right)^i \left(1 - \frac{p}{2} + \frac{ps}{2}\right)^i$
Additive	$\left(\frac{1}{2} + \frac{s}{2}\right)^i \left(1 - \frac{p}{2} + \frac{ps}{2}\right)^N$
Equilibrium	$\left(\frac{1}{2} + \frac{s}{2}\right)^i \left(1 - \frac{p}{2} + \frac{ps}{2}\right)^{2N-i}$

immediate that for the multiplicative model, X_n is a Galton–Watson branching process with offspring pgf $f(s)$ given by

$$f(s) = \left(\frac{1}{2} + \frac{1}{2}s\right) \left(1 - \frac{p}{2} + \frac{ps}{2}\right). \tag{2.4}$$

Similarly, the additive model may be viewed as a branching process with immigration, stopped whenever 0 is reached. The offspring pgf $f(s)$ and immigration pgf $b(s)$ are given by

$$f(s) = \frac{1}{2} + \frac{1}{2}s, \quad b(s) = \left(1 - \frac{p}{2} + \frac{ps}{2}\right)^N. \tag{2.5}$$

The equilibrium model has no such convenient representation.

All three processes have an absorbing state at 0, which corresponds to curing of the line of descent. We shall write the transition matrix $P = (p_{ij})$ in the form

$$P = \begin{pmatrix} 1 & \mathbf{0}' \\ \mathbf{p} & Q \end{pmatrix}, \tag{2.6}$$

where Q corresponds to transitions among the transient states $\{1, 2, \dots\}$. Of fundamental importance in such models is the Perron–Frobenius eigenvalue, ρ (say), of the matrix Q . For the multiplicative model, the representation of $\{X_n\}$ as a branching process allows us to obtain from Seneta and Vere-Jones (1966) and (2.4) that

$$\rho_M = f'(1) = (1 + p)/2, \quad p < 1. \tag{2.7}$$

The equilibrium model has a finite Q matrix, and if $p > 0$, this matrix is primitive. We have been unable to compute ρ_E explicitly, but we can establish the bounds, good for $p \uparrow 1$, from the maximum and minimum row sums of Q (Seneta, 1981, p. 8),

$$1 - (2 - p)^{-1}(1 - p/2)^{2N} \leq \rho_E \leq 1 - \left(\frac{1}{2}\right)^{2N}. \tag{2.8}$$

For the additive model, the situation is more complicated. We define

$$\begin{aligned} Q_r(s) &= 1 & (r = 0) \\ &= \prod_{i=0}^{r-1} b(f_i(s)) & (r \geq 1), \end{aligned}$$

where

$$f_0(s) = s, \quad f_i(s) = f(f_{i-1}(s)), \quad i \geq 1, \tag{2.9}$$

and $f(s)$ and $b(s)$ are given in (2.5). Then if $a_r = Q_{r-1}(0) - Q_r(0)$, $r \geq 1$, the equation

$$\sum_{l=1}^{\infty} a_l s^l = 1 \quad (2.10)$$

has a unique solution s^* , and $\rho_A = s^{*-1}$, and $\frac{1}{2} < \rho_A < 1$. The reader is referred to Seneta and Tavaré (1983) for details.

This done, it is in principle straightforward to analyze the behavior of the cure time $T_0 = \inf\{n > 0: X_n = 0\}$. It is elementary to show that curing must eventually occur, the random line of descent eventually containing no plasmids. Some numerical computations of the distribution of T_0 have been given (Emerson and Hoppensteadt, 1981; Gardner *et al.*, 1977) in the case $p = 1$. We shall content ourselves by considering the (asymptotic) cure rate θ , say, defined by

$$\begin{aligned} \theta &= \lim_{n \rightarrow \infty} \Pr\{X_{n+1} = 0 | X_n > 0\} \\ &= 1 - \lim_{n \rightarrow \infty} \frac{\Pr\{T_0 > n+1\}}{\Pr\{T_0 > n\}}. \end{aligned} \quad (2.11)$$

If we assume that $X_0 = i > 0$ is fixed, then it is readily established that

$$\theta = 1 - \rho. \quad (2.12)$$

For the doubling model, this is contained in Kolmogorov's result (cf. Harris, 1963, p. 18) when $p < 1$, and for the additive model this follows from Seneta and Tavaré (1983). For the equilibrium model, it is a straightforward consequence of nonnegative matrix theory (see Seneta (1981, Theorems 1.2 and 4.6)).

3. PLASMID COPY NUMBER IN THE WHOLE CELL LINE

The behavior of copy number in a random line of descent gives a poor picture of the distribution of copy number in the whole cell line. We give a simultaneous study of copy number in all cells in this section. Our method is based on considerations of Harris (1963, p. 47). See also Novick and Hoppensteadt (1978, p. 426).

For $j \geq 0$, let $X_n^{(j)}$ be the number of cells in generation n containing j plasmids before replication. Clearly, $\sum_{j \geq 0} X_n^{(j)} = 2^n$, so we may disregard $X_n^{(0)}$ (which counts the number of cured cells in generation n), and focus on the vector process $\mathbf{X}'_n = (X_n^{(1)}, X_n^{(2)}, \dots)$. Now consider a cell containing i

(>0) plasmids before replication. We compute the mean number m_{ij} of cells containing j plasmids produced by a cell containing i . Given that the parent cell produces k plasmids before partition, the mean number of j -type cells produced is, from (2.2),

$$1 \times \left\{ \binom{k}{j} 2^{-k} + \binom{k}{k-j} 2^{-k} \right\} = 2 \binom{k}{j} 2^{-k}, \quad j \neq k/2, \quad (3.1)$$

or

$$2 \binom{k}{k/2} 2^{-k} \quad \text{if } j = k/2.$$

In either case, the (conditional) mean number is $2P_{kj}$. Averaging over the distribution of k gives

$$m_{ij} = \sum_k R_{ik} 2P_{kj} = 2p_{ij}, \quad i, j \geq 1. \quad (3.2)$$

Writing $M = (m_{ij})$, and using (2.6), we find

$$M = 2Q. \quad (3.3)$$

Clearly, cells behave independently of one another, and their offspring distributions do not change with time. The pgf of the distribution of the number of different types produced by an i -type cell is then

$$f^{(i)}(s_1, s_2, \dots) = \sum_{k>i} R_{ik} \sum_{j=0}^k P_{kj} s_j s_{k-j}, \quad i \geq 1, \quad (3.4)$$

where $s_0 = 1$. It follows that

$$\left. \frac{\partial f^{(i)}}{\partial s_j} \right|_{s=1} = 2p_{ij} = m_{ij},$$

in agreement with (3.2). The process \mathbf{X}'_n is now identified as a multitype Galton–Watson branching process (cf. Harris (1963, p. 36)) with mean matrix M and offspring pgf's $f^{(i)}$. We note that extinction ($\mathbf{X}' \equiv \mathbf{0}'$) is impossible in these models, since a cell with $i > 0$ plasmids necessarily produces at least one cell with positive plasmid copy number.

Further analysis depends on the behavior of powers of the matrix M , which in view of (3.3) is essentially covered in Section 2. Let ρ be the general symbol for the eigenvalue in the discussion (2.7)–(2.10), and let \mathbf{v}' , \mathbf{w} denote the corresponding left and right eigenvectors of Q , respectively. Clearly, 2ρ is the Perron–Frobenius eigenvalue of M , with associated vectors

\mathbf{v}' , \mathbf{w} . In all cases $2\rho > 1$ (except when $\rho = 1$ in the multiplicative model, a case we shall not consider further). Using results of Seneta and Tavaré (1983) and Seneta and Vere-Jones (1966) for the two cases of countably infinite Q , we may take \mathbf{v}' , \mathbf{w} to be normalized so that

$$\sum_{i>1} v_i = 1, \quad \sum_{i>1} v_i w_i = 1. \quad (3.5)$$

3.1. Cure Rates

Conditional on \mathbf{X}_n , the mean number of uncured cells at time $n + 1$ is $\mathbf{X}'_n M \mathbf{1}$, where $\mathbf{1}' = (1, 1, \dots)$, and so the mean proportion remaining uncured is $(\mathbf{X}'_n M \mathbf{1}) / (2\mathbf{X}'_n \mathbf{1})$. Hence the unconditional proportion cured from the n th to the $(n + 1)$ st generation is given by

$$1 - E \left(\frac{\mathbf{X}'_n M \mathbf{1}}{2\mathbf{X}'_n \mathbf{1}} \right). \quad (3.6)$$

Since extinction is impossible, we see that

$$0 < \frac{\mathbf{X}'_n M \mathbf{1}}{2\mathbf{X}'_n \mathbf{1}} = \frac{2\mathbf{X}'_n Q \mathbf{1}}{2\mathbf{X}'_n \mathbf{1}} \leq \frac{X'_n \mathbf{1}}{X'_n \mathbf{1}} = 1. \quad (3.7)$$

If we now concentrate on the equilibrium model (which has a finite number of types), then we have from a theorem of Kesten and Stigum (1966) that there is a r.v. W , with $\Pr[W > 0] = 1$ such that

$$(2\rho)^{-n} \mathbf{X}'_n \rightarrow W \mathbf{v}' \quad \text{a.s. as } n \rightarrow \infty. \quad (3.8)$$

It follows that

$$\mathbf{X}'_n M \mathbf{1} / 2\mathbf{X}'_n \mathbf{1} \rightarrow \rho \quad \text{a.s.} \quad (3.9)$$

Thus from (3.9), the dominated convergence theorem, and (3.7), we obtain

$$\lim_{n \rightarrow \infty} \left\{ 1 - E \left(\frac{\mathbf{X}'_n M \mathbf{1}}{2\mathbf{X}'_n \mathbf{1}} \right) \right\} = 1 - \rho. \quad (3.10)$$

For the other two models, the presence of a countable number of types makes the analysis less simple. Assuming, by analogy with the equilibrium model that $\Pr[W > 0] = 1$, we can establish, using a theorem of Moy (1967) that (3.8) and (3.9) hold with convergence a.s. replaced by convergence in probability. Details of this, and a discussion of the assumption $\Pr[W > 0] = 1$, are given in the Mathematical Appendix. This is sufficient to ensure that (3.10) holds also. Note that (3.10) and (2.12) give the same expression for the asymptotic cure rate. It is worth noting that in the

equilibrium model the fraction $2^{-n}X'_n/1$ of uncured cells converges a.s. to 0 as $n \rightarrow \infty$ (and to 0 in probability in the other cases). Thus even though the total number of uncured cells grows to infinity, they become sparser and sparser.

3.2. Copy Number Density

There are several ways in which the evolution of copy number could be measured. The simplest for numerical comparisons (see Section 4) seems to be the asymptotic fraction of cells containing a given number of plasmids. For the equilibrium model, we know from (3.8) that

$$\frac{X'_n}{X'_n/1} \rightarrow v' \quad \text{a.s. as } n \rightarrow \infty, \tag{3.11}$$

so that v_i can be viewed as the asymptotic fraction of cells containing i plasmids; (3.11) holds for the other cases in the sense of convergence in distribution on the set $\{W > 0\}$ (see Mathematical Appendix). In any case, we use v as a simple means of comparing the processes. In particular, the mean μ and variance σ^2 of v have useful interpretations in this context.

For the multiplicative model (with $p < 1$) standard theory (see Harris 1963, p. 17) shows that

$$\mu_M = \lim_{n \rightarrow \infty} \frac{\rho_M^n}{1 - f_n(0)}, \tag{3.12}$$

where $f_n(s)$ is given in (2.9) and $f(s)$ in (2.4). The variance is

$$\sigma_M^2 = \mu_M \left\{ \frac{2p}{1 - p^2} + 1 - \mu_M \right\}. \tag{3.13}$$

For the additive model, one obtains (Seneta and Tavaré, 1983)

$$\mu_A = \frac{Nps^*}{2 - s^*} = \frac{Np}{2\rho_A - 1} \tag{3.14}$$

with variance

$$\sigma_A^2 = (\rho_A - \frac{1}{4})^{-1} \frac{Np}{2} \left(\frac{(N-1)p}{2} + \mu_A \right) + \mu_A - \mu_A^2. \tag{3.15}$$

For the equilibrium model, the mean and variance are most easily calculated on a computer. However, when $p = 1$ a complete analysis is possible. Indeed $\rho_E = 1 - 2^{-2N}$,

$$v_j = \rho_E^{-1} \binom{2N}{j} 2^{-2N}, \quad j = 1, \dots, 2N, \tag{3.16}$$

and

$$\mu_E = N\rho_E^{-1}, \quad \sigma_E^2 = N\rho_E^{-1}(N + \frac{1}{2} - N\rho_E^{-1}). \quad (3.17)$$

4. NUMERICAL RESULTS

With these theoretical results established, we can compute ρ , \mathbf{v} , and compare models. For the additive and multiplicative processes, where Q is countable, we employed the truncation algorithms of Seneta (1981, Section 6.4). Let ${}_{(n)}Q$ be the n -square top left-hand corner of Q , and set ${}_{(n)}\mathbf{x}'_0 = (1, \dots, 1)$. The simplest algorithm to find ρ , \mathbf{v} proceeds via a series of iterations:

For $k \geq 0$, set ${}_{(n)}\mathbf{y}'_k = {}_{(n)}\mathbf{x}'_k \cdot {}_{(n)}Q$, and define ${}_{(n)}\rho_k$ as the first element of ${}_{(n)}\mathbf{y}'_k$. Then

$${}_{(n)}\mathbf{x}'_{k+1} = {}_{(n)}\rho_k^{-1} {}_{(n)}\mathbf{y}'_k, \quad k \geq 0. \quad (4.1)$$

The scheme is iterated until $|{}_{(n)}\rho_{k+1} - {}_{(n)}\rho_k| < \varepsilon$, for some tolerance ε ; we chose $\varepsilon = 10^{-5}$. The sequence ${}_{(n)}\rho$ increases to ρ as n increases, and ${}_{(n)}\mathbf{x}'$ converges to \mathbf{v}' , normalized so that $v_1 = 1$. Typically, $n = 30$ was sufficient to produce no change in ${}_{(n)}\rho$ to four or five decimal places, and the first 20 elements of ${}_{(n)}\mathbf{x}'$ differed little also. For the equilibrium model, the same technique was used for the whole finite matrix of transient states Q . The mean in (3.12) was computed via an algorithm of Pollak (1969), and the values of $s^* = \rho_A^{-1}$ found by algorithm (4.1) were checked for consistency with the solution of (2.10).

In Tables II–IV, we compare the cure rates and asymptotic copy

TABLE II
Asymptotic Cure Rates (3.10)

p	Multi- multiplicative	Additive			Equilibrium ^a		
		$N = 5$	10	15	$N = 5$	10	15
0.1	0.45	0.3194	0.2098	0.1377	0.2373	0.1056	0.0457
0.3	0.35	0.1327	0.0337	0.0078	0.0611	0.0051	0.0004
0.5	0.25	0.0494	0.0039	0.0003	0.0165	0.0003	
0.7	0.15	0.0157	0.0003		0.0049	3×10^{-5}	
0.9	0.05	0.0041			0.0016		
					(.00098)	9.5×10^{-7}	9×10^{-10})

^a Parenthetical values for $p = 1$ from (3.16).

TABLE III
Means of Copy Number Distribution (3.11)

p	Multi- plicative ^a	Additive ^b			Equilibrium ^c		
		$N = 5$	10	15	$N = 5$	10	15
0.1	1.104	1.384	1.723	2.070	1.599	2.250	2.974
0.3	1.353	2.042	3.217	4.571	2.547	4.652	6.927
0.5	1.729	2.774	5.039	7.505	3.409	6.669	10.000
0.7	2.504	3.613	7.004		4.141	8.236	
0.9	6.020	4.537			4.745	9.474	
					(4.995)	≈10.0	≈15.0)

^a From (3.12).

^b From (3.14).

^c Parenthetical results for $p = 1$ from (3.17).

distribution for a range of values of p and N . Our choice of values of N was predicated on the use of reasonable amounts of computer time, although there is some evidence (Gardner *et al.*, 1977) that these values may not be unreasonable.

To provide some detail about the effect of p on the copy number distribution (3.11), we plot in Fig. 1 the distributions $\{v_j\}$ for the additive model with $N = 5$. Figure 2 gives the analogous distributions for the equilibrium model with $N = 5$.

TABLE IV
Variance of Copy Number Distribution (3.11)

p	Multi- plicative ^a	Additive ^b			Equilibrium ^c		
		$N = 5$	10	15	$N = 5$	10	15
0.1	0.108	0.328	0.766	1.178	0.600	1.343	2.186
0.3	0.414	1.082	2.432	3.900	1.489	3.447	5.308
0.5	1.045	1.812	4.071	6.227	2.059	4.432	6.668
0.7	3.108	2.506	5.352		2.346	4.844	
0.9	26.811	3.068			2.460	4.980	
					(2.522)	≈5.0	≈7.5)

^a From (3.13).

^b From (3.15).

^c Parenthetical results for $p = 1$ from (3.17).

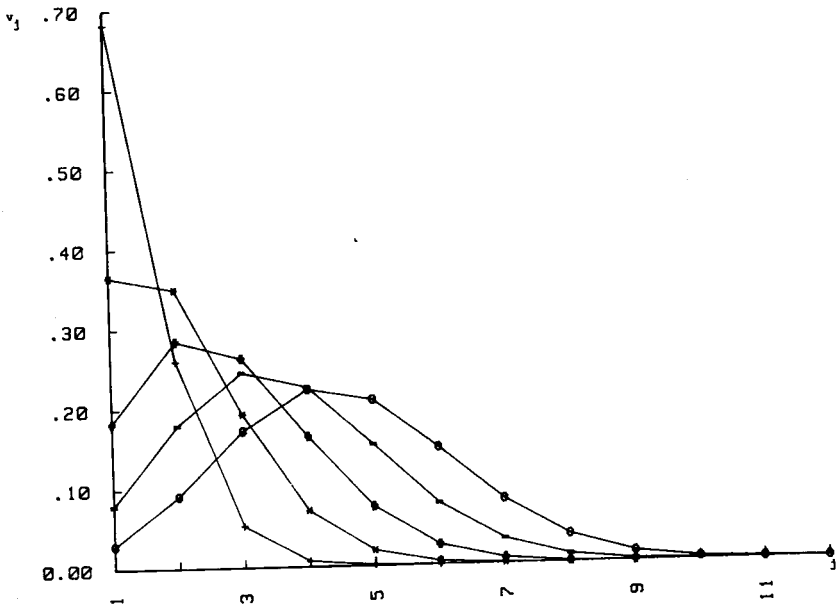


FIG. 1. Copy number distributions (3.11) for additive model with $N=5$. +, $p=0.1$; *, $p=0.3$; #, $p=0.5$; -, $p=0.7$; O, $p=0.9$.

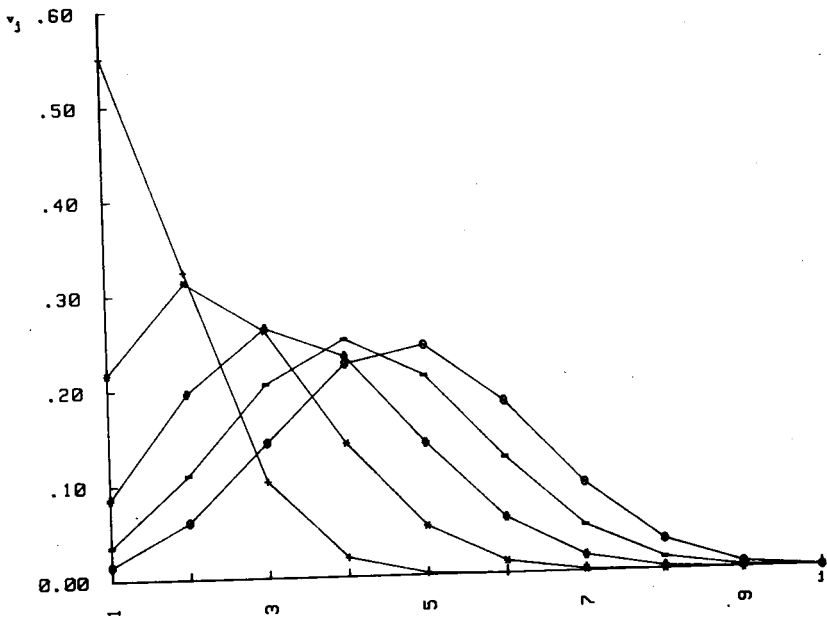


FIG. 2. Copy number distribution (3.11) for equilibrium model with $N=5$. +, $p=0.1$; *, $p=0.3$; #, $p=0.5$; -, $p=0.7$; O, $p=0.9$.

5. DISCUSSION

We have examined each of three models of plasmid replication proposed in Gardner *et al.* (1977) and Novick and Hoppensteadt (1978) under the assumption of random partition from the standpoints of a random line of descent and of a whole cell line. The concepts of asymptotic cure rate, defined for each approach, coincided. The second standpoint allowed consideration also of the asymptotic fractions of cells containing a given number of plasmids, and seems useful in that the study of copy number by random line of descent (Gardner *et al.*, 1977; Emerson and Hoppensteadt, 1981) is likely to be generally misleading. The study of the evolution of the whole cell line was achieved by the use, in a simple way, of the extensive theory of multitype branching processes.

The multiplicative model allows for "unrestricted" replication, and is unlikely to be accepted as plausible. Numerical results indicate that a decrease in cure rate, with increasing p for a given type of model, or for fixed moderate p in proceeding from multiplicative to additive to equilibrium is accompanied by an increase in the mean of the corresponding copy number distribution (as might have been anticipated). The variance of this distribution is somewhat higher for the equilibrium model than for the additive one, for the same values of the parameters N and p . For p close to unity, the multiplicative model has both high mean and variance in this distribution, which highlights its unsuitability.

MATHEMATICAL APPENDIX

The deduction that (3.8) and (3.9) hold with convergence in probability and that (3.10) continues to hold, for the multiplicative and additive models, may be made by first checking that the conditions of (Moy, 1967, Theorem 1) are satisfied. We give sufficient information for the interested reader to reconstruct the full argument. The underlying R -classification theory of nonnegative matrices may be found in Seneta (1981).

1. Since we are assuming $p \neq 0$, in view of (2.4) for the multiplicative model, and (2.5) for the additive model, the matrix Q of (2.10) is irreducible and aperiodic. In the multiplicative model (where we assume further $p < 1$), Q is R -positive with convergence radius $R = 2/(1+p)$, with R -invariant measure $\mathbf{v}' = \{v_i\} > \mathbf{0}'$ normed according to (3.5) given by the Yaglom conditioned-limit distribution, where the corresponding R -invariant vector $\mathbf{w} = \{w_i\}$ is given by $w_i = i/\mu_M$, $i \geq 1$ (with μ_M given by (3.12)); see Seneta and Vere-Jones (1966). In the additive model, Q is R -positive with $R = s^*$, $1 < s^* < 2$, and the left and right R -invariant vectors $\mathbf{v}' (> \mathbf{0}')$ and $\mathbf{w} (> \mathbf{0})$ can be taken to satisfy (3.5) (Seneta and Tavaré, 1983). Since $M = 2Q$, M

has analogous “Perron–Frobenius” structure, with convergence radius $R/2 = (2\rho)^{-1} (<1)$, and the same \mathbf{v}' and \mathbf{w} as Q . Thus the preliminary conditions of (Moy, 1967, Section 2), on the Galton–Watson process with a countable number of types, are satisfied.

2. Write $C(i) = E((\mathbf{X}'_1 \mathbf{w})^2 | \mathbf{X}_0 = \mathbf{e}_i)$, $i \geq 1$, where \mathbf{e}_i is the vector with unity in the i th position and zeros elsewhere. If

$$\sum_{i=1}^{\infty} C(i) v_i < \infty. \tag{A1}$$

Theorem 1 of May (1967) implies that for a fixed starting vector of form \mathbf{e}_i , there is a proper random variable W satisfying $0 < E(W^2) < \infty$ and such that for any vector $\mathbf{f} = \{f_i\}$, $i \geq 1$, satisfying

$$|f_i/w_i| \leq \text{const}, \quad i \geq 1, \tag{A2}$$

we can conclude

$$(2\rho)^{-n} (\mathbf{X}'_n \mathbf{f}) \rightarrow (\mathbf{v}' \mathbf{f}) W,$$

convergence being in mean square. We show below that in both multiplicative and additive multitype models, (A1) is satisfied and (A2) is satisfied with $\mathbf{f} = \mathbf{1}$. Since $M\mathbf{1} = 2Q\mathbf{1} \leq 2\mathbf{1}$, it follows (A2) is also satisfied with $\mathbf{f} = M\mathbf{1}$. Hence $(2\rho)^{-n} \mathbf{X}'_n \mathbf{1} \rightarrow W$, $(2\rho)^{-n} \mathbf{X}'_n M\mathbf{1} \rightarrow (\mathbf{v}' M\mathbf{1}) W = 2\rho W$, convergence being taken in probability. If it is true in our specific framework, in analogy to the case of a finite number of types, that $\text{Pr}[W = 0] = 0$ (see below), (3.9) holds with convergence in probability. In view of (3.7), $\mathbf{X}'_n M\mathbf{1} / (2\mathbf{X}'_n \mathbf{1})$ is uniformly integrable, and since convergence in probability implies convergence in distribution, (3.10) follows.

Equation (3.11) can be established in the sense of convergence of the finite-dimensional distributions of the vector $\mathbf{X}'_n / (\mathbf{X}'_n \mathbf{1})$ using the previous discussion and the Cramér–Wold device.

Before passing to separate consideration of the multiplicative and additive cases to check (A1) and (A2), we notice that for either

$$C(i) = E(\mathbf{w}' \mathbf{X}_1 \mathbf{X}'_1 \mathbf{w} | \mathbf{X}_0 = \mathbf{e}_i) = \mathbf{w}' E(\mathbf{X}_1 \mathbf{X}'_1 | \mathbf{X}_0 = \mathbf{e}_i) \mathbf{w},$$

and in view of (3.4) the (j, t) entry of $E(\mathbf{X}_1 \mathbf{X}'_1 | \mathbf{X}_0 = \mathbf{e}_i)$ for $i, j, t \geq 1$ is given by

$$\begin{aligned} \left. \frac{\partial^2 f^{(i)}(\mathbf{s})}{\partial s_j \partial s_t} \right|_{\mathbf{s}=\mathbf{1}} &= 2R_{i,j+t} P_{j+t,j}, & j+t \geq \max(2, i) \\ &= 0, & \text{otherwise} \end{aligned}$$

(with the further restriction on the first line that $j + t \leq 2i$ in the multiplicative, $j + t \leq N + i$ in the additive, so

$$C(i) = 2 \sum_{k > \max(2, i)} \sum_{j=1}^{k-1} R_{i,k} P_{k,j} w_{k-j} w_j. \tag{A3}$$

3. *Multiplicative case.* Since $w_i = \text{const } i$, we have from (A3)

$$C(i) \leq \text{const } 2 \sum_{k=i}^{2i} \sum_{j=0}^k \binom{i}{k-i} p^{k-i} (1-p)^{2i-k} \binom{k}{j} 2^{-k} (k-j) j$$

and since $(k-j)j \leq k^2/4$,

$$\leq \text{const} \sum_{r=0}^i \binom{i}{r} p^r (1-p)^{i-r} (r+i)^2$$

which is of the form $\text{const}_1 i + \text{const}_2 i^2$, so (A1) holds since the variance of the Yaglom distribution is finite if and only if the variance of the subcritical offspring distribution is finite. On account of the form of w , (A2) holds with $f = 1$.

4. *Additive case.* In Seneta and Tavaré (1983, Section 2), it is shown that

$$w_i = \text{const} \left(\sum_{i=0}^{\infty} \tilde{c}_i \middle/ \sum_{k=0}^{\infty} k \tilde{a}_k \right),$$

where const and the \tilde{a}_k are positive and independent of i , while $\tilde{c}_0 = 1$, $0 \leq \tilde{c}_i \leq s^{*i} (1 - G_0(f_i(0)))$, where $G_0(s) = s^t$. Since in our case $f(s) = \frac{1}{2} + \frac{1}{2}s$, it follows $f_i(s) = 1 - (\frac{1}{2})^i + (\frac{1}{2})^i s$, so $1 - f_i(0) = (\frac{1}{2})^i$, and in view of the form of G_0 , $\tilde{c}_i \leq i(s^*/2)^i$. Thus, $w_i \leq \text{const } i$. We may now imitate the sequence of inequalities for the multiplicative case to obtain

$$C(i) \leq \text{const}_1 + \text{const}_2 i + \text{const}_3 i^2.$$

Inequality (A1) follows from the fact that, in view of the simple form of $f(s)$ and $b(s)$ given by (2.5) $f''(1-) < \infty$ and $b''(1-) < \infty$; whence we can use dominated convergence along the lines of (13) of Seneta and Tavaré (1983) to show $\sum i^2 v_i < \infty$. That (A2) holds with $f = 1$ follows from the observation that

$$w_i \geq \text{const} \sum_{k=1}^{\infty} k \tilde{a}_k$$

since $\tilde{c}_0 = 1$.

5. We have not been able to establish that $\Pr[W > 0] = 1$ in the multiplicative and additive cases, although, since W is not degenerate at 0, $\Pr[W > 0] > 0$. We are indebted to F. M. Hoppe for pointing out that even though $f(\mathbf{0}) = \mathbf{0}$, simple examples with $\Pr[W = 0] > 0$ can be constructed. In the event that this is so in *our* countable-types cases, the interpretation of $1 - \rho$ as an asymptotic cure rate is still plausible from the fact that $E(\mathbf{X}'_{n+1} \mathbf{1})/E(2\mathbf{X}'_n \mathbf{1}) \rightarrow \rho$ as $n \rightarrow \infty$; while that of the element v_i of \mathbf{v} as the limiting asymptotic fraction of cells containing i plasmids, $i \geq 1$, is still pertinent on the set $\{W > 0\}$.

ACKNOWLEDGMENT

A preliminary version of this paper was presented at the Rocky Mountain Regional Conference on Medical Applications of Statistics, May 1982.

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