J. Math. Biol. 42, 145–174 (2001)
Digital Object Identifier (DOI):
10.1007/s002850000053

Mathematical Biology

Wolfgang P. Angerer

An explicit representation of the Luria–Delbrück distribution

Received: 10 April 1999 / Revised version: 29 February 2000 /
Published online: 26 January 2001 –

Abstract. The probability distribution of the number of mutant cells in a growing single-cell population is presented in explicit form. We use a discrete model for mutation and population growth which in the limit of large cell numbers and small mutation rates reduces to certain classical models of the Luria–Delbrück distribution. Our results hold for arbitrarily large values of the mutation rate and for cell populations of arbitrary size. We discuss the influence of cell death on fluctuation experiments and investigate a version of our model that accounts for the possibility that both daughter cells of a non-mutant cell might be mutants. An algorithm is presented for the quick calculation of the distribution. Then, we focus on the derivation of two essentially different limit laws, the first of which applies if the population size tends to infinity while the mutation rate tends to zero such that the product of mutation rate times population size converges. The second limit law emerges after a suitable rescaling of the distribution of non-mutant cells in the population and applies if the product of mutation rate times population size tends to infinity. We discuss the distribution of mutation events for arbitrary values of the mutation rate and cell populations of arbitrary size, and, finally, consider limit laws for this distribution with respect to the behavior of the product of mutation rate times population size. Thus, the present paper substantially extends results due to Lea and Coulson (1949), Bartlett (1955), Stewart et al. (1990), and others.

1. Introduction

Since the introduction of fluctuation analysis by Luria and Delbrück in 1943, there has been good reason to believe that the production of mutant varieties by a growing population of bacteria is a random process. Luria and Delbrück argued that the large fluctuations observed in successive attempts to determine the proportion of phage-resistant bacteria in growing cultures of *Escherichia coli* would not support the hypothesis that it is the presence of the phage that induces resistance in the cells (Luria and Delbrück 1943). To illustrate this, consider a collection of bacterial cultures and suppose that in some of these cultures, one or several bacteria accidentally gain resistance to the phage at a very early stage of culture growth. As proliferation continues, these bacteria will eventually divide into a large number of

W.P. Angerer: Department of Molecular Genetics, Institute of Cancer Research, Borschkegasse 8a, A-1090 Vienna, Austria. e-mail: a8505892@unet.univie.ac.at

Present address: Department of Mathematics, University of Vienna, Strudelhofgasse 4, 1090 Vienna, Austria

Key words: Bacterial mutation – Fluctuation analysis – Birth-and-mutation process – Mutation event

mutant clones, so that after being plated with the phage on solid medium, a substantial proportion of cultures will produce a much larger number of colonies than would be consistent with the hypothesis that resistant cells arose only after the bacteria had been exposed to the phage. Clearly, this argument applies to more general settings than the development of phage-resistance in sensitive bacteria, and the experimental approach devised by Luria and Delbrück has been employed repeatedly to study the emergence of mutants in single-cell populations. With this approach, it is presupposed that it is primarily the distribution of mutants in a sample of cell cultures that carries the relevant biological information and not, for instance, the time it takes for mutant cells to develop into visible colonies. Hence, the need arises for a model from which this distribution can reasonably be calculated.

It would be desirable to refer to this distribution simply as 'the' Luria–Delbrück distribution. However, any attempt to make 'the' Luria–Delbrück distribution a well-defined object of mathematical investigation inevitably relies on explicit assumptions about the mutational process and about how cell proliferation proceeds in the population. To convey an idea to the reader of how these assumptions look like, we shall briefly review several existing models for the calculation of the Luria–Delbrück distribution (an excellent account of a lot of important work that has been devoted to the study of the Luria–Delbrück distribution during the last fifty years has recently been given by Zheng (1999)). The most general approach so far appears to be the one by Stewart et al. (1990), who require that (i) the probability that a mutation takes place in the (small) time interval between t and $t+dt$ equals the length of the interval, dt, times a certain function $\Phi(t)$ of time only (but does not depend on how the population is composed of mutant and non-mutant cells), and (ii), the probability[1] $p(k;t)$ that a mutation occurring at time t will be represented by a (mutant) clone of size k by the time T when the cells are plated on solid medium is a function of the parameters t, k, and T, and in particular does not depend on what may happen at other times (this assumption will not hold if, for instance, mutants have to compete for resources). It was demonstrated by Stewart et al. that under these two assumptions, the probability generating function (PGF) $P(z)$ of the number of mutants present in the population at time T is given by

$$P(z) = \mathrm{e}^{-\Lambda} \exp\left(\sum_{k=1}^{\infty} \lambda_k z^k\right) \quad , \qquad (*)$$

where $\lambda_k = \int_0^T p(k;t)\Phi(t)dt$, and $\Lambda = \sum_{k=1}^{\infty} \lambda_k$. Under the assumption that[2] $p(k;t) = \mathrm{e}^{-\lambda(T-t)}(1-\mathrm{e}^{-\lambda(T-t)})^{k-1}$ (λ denotes the growth rate of cells) and the mutation rate is constant, the model of Stewart et al. model reduces to the one of Lea and Coulson (Lea and Coulson 1949; Stewart et al. 1990). It is especially this latter model that has become popular among geneticists, which is mostly due to its comparative simplicity and Lea and Coulson's thorough discussion of how to employ this model

[1] In this section, we adhere to the notation of the respective authors.

[2] This assumption is justified if cell proliferation proceeds in an asynchronous manner as explained in Section 2.1. See also the discussion following Equation (67) below.

for the evaluation of experimental data. It does, however, lead to a distribution of mutant cells with infinite moments. Although this is not so much a drawback from a practical point of view, it is clear that the expected number of mutant cells (for instance) diverges because Lea and Coulson's model predicts too large a probability for an arbitrary culture of bacteria to contain a very large number of mutants. Since there are simply no bacterial populations that contain an infinite number of mutants, it follows that the tailing of the distribution towards large numbers of mutants, which is a vital point in Luria and Delbrück's original argument, is not described correctly in Lea and Coulson's model. Notwithstanding that, Lea and Coulson's model has attracted considerable recent interest (Ma et al. 1992; Pakes 1993; Kemp 1994; Goldie 1995; Prodinger 1996) and justifiably has become the most widely used model underlying the evaluation of fluctuation experiments.

Another very general model is the one by Tan (1982). Tan derives the following expression for the PGF $\zeta_n(s)$ of the number of mutants that are present in a cell population that is grown from an initially very large number of cells after these cells have gone through n successive rounds of replication (this is the Lemma that appears on p. 721 of Tan (1982)):

$$\zeta_n(s) = \{h_n(s)\}^{x_0} \exp\left\{\lambda_1 \sum_{j=1}^{n} \mu_1^j [h_{n-j}(s) - 1]\right\} \quad , \qquad (**)$$

where $h(s)$ is the PGF of the progeny size of a mutant cell, and $h_n(s) = h(h_{n-1}(s))$. Furthermore, x_0 is the number of mutants initially present in the population, μ_1 is the expected number of cells in the progeny of a non-mutant cell, and λ_1 is a measure for the intensity of the mutational process, in the sense that $p_1 = \lambda_1 M_0^{-1} + o(M_0^{-1})$, where p_1 denotes the probability of mutation of a non-mutant cell. $M_0 \gg \lambda_1$ is the initial size of the population. Clearly, the fact that essentially only the PGF $h(s)$ enters the calculation of $\zeta_n(s)$ allows Equation (**) to be tailored to cover a wide variety of different scenarios. In particular, Tan can allow for both forward and backward mutation (the latter, however, turns out to be negligible if the rate p_2 of backward mutation is of the order $p_2 = \lambda_2 M_0^{-1} + o(M_0^{-1})$, where λ_2 is small compared with M_0), and for the death of both mutant and non-mutant cells. Moreover, Tan also showed how to incorporate the phenotypic delay of new mutants into his model (this is actually straightforward if one considers the fraction of 'newborn' mutants that manage to express the mutant phenotype in a certain time interval; see Tan (1982) for details).

To get an intuitive grasp of Equation (**), consider a sufficiently large culture of bacteria such that the probability that M_0 non-mutant cells will produce a certain number k of mutants during the first round of replication is $\frac{p_1^k \mu_1^k M_0^k}{k!} \mathrm{e}^{-p_1 \mu_1 M_0} \sim \frac{\lambda_1^k \mu_1^k}{k!} \mathrm{e}^{-\lambda_1 \mu_1}$. By the time of the n^{th} round of replication, the probability distribution of the number of cells in the progeny of these k mutant cells will have the generating function $[h_{n-1}(s)]^k$. Therefore, the PGF of the number of mutant cells in the population at the time of the n^{th} round of replication that result from the mutation of non-mutant cells during the first round of replication will by given by

$\sum_{k=0}^{\infty} \frac{\lambda_1^k \mu_1^k}{k!} [h_{n-1}(s)]^k e^{-\lambda_1 \mu_1} = \exp\{\lambda_1 \mu_1 [h_{n-1}(s) - 1]\}$. The other factors in Equation (**) arise in a similar manner [3] (for a generalization of this model to multivariate branching processes, see Tan (1989)).

Both Tan's model as well as that of Stewart et al. (1990) require that the mutation rate be small, i.e. that the number of mutant cells in the population be negligible in comparison with the number of non-mutant cells. This is a somewhat unfortunate limitation. We will follow a different approach here, which allows the mutation rate to assume any value between (and including) 0 and 1, and which allows the number of mutant cells in a population to be large. The crucial point is to forget about the conventional modelling of population growth as a continuous process over time, and to formulate our model in the language of partial difference equations. We then arrive at an explicit representation of a probability distribution which is related to the models of Lea and Coulson (1949) and Bartlett (1955) in much the same way as is the binomial distribution to the Poisson distribution. This point will be made clear in Section 5.

For simplicity, we shall only deal with bacterial populations. However, it is clear that our results apply whenever the assumptions (i)–(v) of Section 2.1 or the slightly more general assumptions of Section 3 are fulfilled, and in particular to what might be called a Luria–Delbrück theory outside the field of fluctuation analysis, namely the mapping of certain disease-related genes through linkage disequilibrium (Hästbacka et al. 1992; Lehesjoki et al. 1993; de la Chapelle 1993). Here, the idea is as follows: When a disease-related gene is first introduced into a (human) population, it will be in complete linkage disequilibrium with the marker alleles on the same chromosome. As parents inherit the gene to their progeny, this linkage disequilibrium will tend to dissipate because of recombinations that occur between the gene and the surrounding markers. Now, from the point of view of fluctuation analysis, the one gene introduced into the population together with its haplotype of markers may be thought of as an ancestral bacterial cell, and any recombination which occurs between a gene and a marker allele can be seen as a mutation. Since the mutation rate for this kind of mutation clearly increases with the distance between the gene of interest and the marker, the fraction of disease-bearing chromosomes that do not anymore display the ancestral haplotype may serve as a measure for the genetic distance between the disease gene and the marker. A key difference, however, to a fluctuation experiment is that one uses essentially a single observation (namely the fraction of disease-bearing chromosomes that are derived from the ancestral chromosome and have not undergone recombination yet) to infer on the recombination rate, which to do is at least slightly opposed to Luria and Delbrück's original line of argument. Still, it is even in this very simplified setting that the fine-scale mapping of genes can be remarkably successful. Indeed, the diastrophic dysplasia gene that applying Luria and Delbrück's method of 'likely' averages (Luria and Delbrück 1943) had been predicted to lie within 64 kb from

[3] Rather than proceeding with the analysis of (**), Tan makes use of the assumption that M_0 is very large to pass from (**) to a continuous-time model that avoids the cumbersome calculation of the functional iterates $h_n(s)$.

a polymorphism in the colony-stimulating-factor-1 receptor gene later was found to lie at 70 kb proximal to this region (Hästbacka et al. 1994; Jorde 1995). However, it is clear that questions such as the reliability of linkage information from Luria–Delbrück-like models or even for the best measure of linkage disequilibrium for fine-scale mapping purposes call for a more sophisticated treatment (see, for instance, Kaplan et al. 1995; Kaplan and Weir 1995; Xiong and Guo 1997; also Devlin and Risch (1995), and Guo (1997), which contain a very useful list of references).

The paper is organized as follows. In Section 2, we explain the assumptions which are basic to our model and derive the Luria–Delbrück distribution in explicit form. Section 3 is concerned with the influence of cell death on the distribution and an alternative model of mutation. In Section 4, we present an algorithm to calculate efficiently the distribution. Sections 5 and 6 are devoted to the study of limiting distributions for large populations. Section 7 introduces the distribution of the number of mutation events. In Section 8, we shall be concerned with the limiting distribution of mutation events.

2. The Luria–Delbrück distribution

2.1. Model assumptions and notation

We begin with specifying a number of assumptions which are sufficient to formulate our model in its simplest form.

(i) We are concerned with bacterial populations of arbitrary size. In particular, we do not require that the size of the population is large. We will, however, assume that the population has been grown from a single non-mutant cell.

(ii) The number of mutant cells in a population of given size n is a non-negative, integer-valued random variable ρ_n, which takes on values between 0 and $n-1$. Similarly, the number of non-mutant cells in this population is a random variable ω_n such that $\rho_n + \omega_n = n$.

(iii) Whenever a cell divides, it is replaced by two cells which are capable of further proliferation. Mutant cells divide only into cells with mutant properties, whereas the division of a non-mutant (i.e. 'wild-type') cell may result in the formation of a mutant cell as well. We will always assume that mutation occurs only at the time of cell division.

(iv) The probability for any cell to be the next to divide is the same for all cells in the population. To be explicit, we assume that in a population of n cells, r of which are mutants, the probability that a mutant cell is the next to divide is r/n, the probability that it is a non-mutant cell, is $(n-r)/n$. Clearly, this assumption can only be an approximation, even if the cultures are grown under conditions which equally support the proliferation of mutant and wild-type cells.

(v) We define the *mutation rate* α_i as the probability that the division of a wild-type cell in a population of i cells ends up with the formation of one mutant and one non-mutant cell. If the mutation rate is constant, i.e. if $\alpha_i = \alpha_j$ for any pair of indices $i, j \geq 1$, we shall simply denote it by α. The fact that we allow the mutation rate to vary with population size should be considered as

mainly technical. We shall only draw on it as a convenient tool to allow for the possibility that a culture is grown from $n_0 > 1$ cells, r_0 of which are mutants.

Whenever the distribution of the number of mutants in a bacterial population is calculated from the above assumptions, we shall refer to it as an 'ordinary' Luria–Delbrück distribution.

We will now briefly introduce the most frequently used symbols. We shall in general denote the probability that a culture of n cells contains r mutants by $p_\rho(n, r; \vec{\alpha}_{n-1})$, where $\vec{\alpha}_{n-1}$ is short for $(\alpha_1, \alpha_2, \ldots, \alpha_{n-1})$. Similarly, we shall denote the probability that this population contains k wild-type cells by $p_\omega(n, k; \vec{\alpha}_{n-1})$. Clearly, $p_\rho(n, r; \vec{\alpha}_{n-1}) = p_\omega(n, n-r; \vec{\alpha}_{n-1})$. If the mutation rate is such that $\alpha_i = 0$ for $1 \le i < n_0 - r_0, \alpha_i = 1$ for $n_0 - r_0 \le i < n_0$, and $\alpha_i = \alpha$ for $i \ge n_0$, this is clearly equivalent to a population that is grown from r_0 mutants and $k_0 = n_0 - r_0$ non-mutant cells which mutate with constant probability, and we shall write $p_\rho(n, r|n_0, r_0; \alpha)$ and $p_\omega(n, k|n_0, k_0; \alpha)$ instead of $p_\rho(n, r; \vec{\alpha}_{n-1})$ and $p_\omega(n, k; \vec{\alpha}_{n-1})$. If $n_0 = k_0 = 1$, we shall simply write $p_\rho(n, r; \alpha)$ and $p_\omega(n, k; \alpha)$. In the same spirit, we shall generally denote the expected value of the number of wild-type cells in a population of size n by $E_\omega(n; \vec{\alpha}_{n-1})$ or, if the mutation rate is constant and the population is grown from a single wild-type cell, simply by $E_\omega(n; \alpha)$. We shall also use the probability generating functions $g_\rho(n, s; \alpha) = \sum_{r=0}^{n-1} p_\rho(n, r; \alpha) s^r$ and $g_\omega(n, s; \alpha) = \sum_{k=1}^{n} p_\omega(n, k; \alpha) s^k$. Clearly, $g_\rho(n, s; \alpha) = s^n g_\omega(n, 1/s; \alpha)$. An expression like $p_\omega(1, k; \vec{\alpha}_0)$ also has a meaning: It denotes the probability that one picks k wild-type ($k = 0$ or 1) cells to start the culture. Finally, we shall denote the limit of a sequence $\{X_n\}$ that exists with probability 1 as $n \to \infty$ by $p - \lim_{n\to\infty} X_n$.

2.2. The Luria–Delbrück distribution

Suppose that a culture of n cells contains r mutants. This can only be the case if either the culture of $n-1$ cells has already contained r mutants and the last cell doubling that took place did not produce a new mutant, or if the culture of $n-1$ cells has contained $r-1$ mutants and the last cell doubling *did* produce a new mutant. Now, a cell division in a culture of $n-1$ cells, r of which are mutants, will *not* lead to the formation of another mutant cell if one of $n-1-r$ wild-type cells divides (the probability for this event is $(n-r-1)/(n-1)$), and if its division does *not* lead to the formation of a mutant cell (this probability is $1-\alpha_{n-1}$). Hence, with probability

$$(1-\alpha_{n-1})\frac{n-r-1}{n-1} p_\rho(n-1, r; \vec{\alpha}_{n-2}) \ ,$$

a culture of n cells containing r mutants derives from a culture of $n-1$ cells that has already contained r mutant cells. A similar argument shows that the probability that a culture of n cells containing r mutants derives from a culture of $n-1$ cells with $r-1$ mutants is

$$\frac{r-1}{n-1} p_\rho(n-1, r-1; \vec{\alpha}_{n-2}) + \alpha_{n-1}\frac{n-r}{n-1} p_\rho(n-1, r-1; \vec{\alpha}_{n-2}) \ ,$$

so that in total, we obtain

$$p_\rho(n,r;\vec{\alpha}_{n-1}) = (1-\alpha_{n-1})\left[1-\frac{r}{n-1}\right]p_\rho(n-1,r;\vec{\alpha}_{n-2}) + \left[\alpha_{n-1}+(1-\alpha_{n-1})\frac{r-1}{n-1}\right]p_\rho(n-1,r-1;\vec{\alpha}_{n-2}) \quad . \qquad (1)$$

It is generally more convenient to concentrate on the probability distribution $p_\omega(n,k;\vec{\alpha}_{n-1})$ of wild-type cells in the population. Since $k := n-r$, we find from Equation (1)

$$p_\omega(n,k;\vec{\alpha}_{n-1}) = (1-\alpha_{n-1})\frac{k-1}{n-1}p_\omega(n-1,k-1;\vec{\alpha}_{n-2}) + \left[1-(1-\alpha_{n-1})\frac{k}{n-1}\right]p_\omega(n-1,k;\vec{\alpha}_{n-2}) \quad . \qquad (2)$$

Clearly, $p_\omega(n,k;\vec{\alpha}_{n-1}) \geq 0$ for any n and k, and $\sum_{k=1}^{n} p_\omega(n,k;\vec{\alpha}_{n-1}) = 1$ for all n. It is then a straightforward calculation to verify

Theorem 2.1. *The probability $p_\omega(n,k;\vec{\alpha}_{n-1})$ that a bacterial culture of n cells contains k wild-type cells is*

$$p_\omega(n,k;\vec{\alpha}_{n-1}) = \frac{1}{(n-1)!}\sum_{i=1}^{k}(-1)^{i-1}\binom{k-1}{i-1}\prod_{j=1}^{n-1}[j-i(1-\alpha_j)] \quad . \qquad (3)$$

The probability $p_\rho(n,r;\vec{\alpha}_{n-1})$ that it contains exactly r mutants is simply given by $p_\rho(n,r;\vec{\alpha}_{n-1}) = p_\omega(n,n-r;\vec{\alpha}_{n-1})$.

One easily obtains the following corollary to Theorem 2.1:

Corollary 2.2. *For constant α, the probability $p_\omega(n,k;\alpha)$ that a culture of size n contains exactly k wild-type cells if it has been grown from a single wild-type cell is*

$$p_\omega(n,k;\alpha) = \sum_{i=1}^{k}(-1)^{n-i}\binom{k-1}{i-1}\binom{i(1-\alpha)-1}{n-1} \quad . \qquad (4)$$

Alternatively, the probability $P_\omega(n,k;\alpha)$ that it contains at most k wild-type cells is given by

$$P_\omega(n,k;\alpha) = \sum_{i=1}^{k}(-1)^{n-i}\binom{k}{i}\binom{i(1-\alpha)-1}{n-1} \quad . \qquad (5)$$

As already mentioned in Section 2.1, the fact that we allow the mutation rate to be variable allows us to derive immediately the probability distribution $p_\omega(n,k|n_0,k_0;\alpha)$ of the number of wild-type cells in a population which is grown from n_0 cells, k_0 of which are wild-types that mutate with constant probability. In fact, the distribution of wild-types in such a population evolves in the same manner

as if the culture were grown from a single wild-type cell with the mutation probability set equal to zero for the first $k_0 - 1$ cell divisions, then switched to 1 for another $n_0 - k_0$ cell divisions, and finally adjusted to its constant value $\alpha_i = \alpha$ for $n \geq n_0$. Then Theorem 2.1 immediately yields the following

Corollary 2.3. *For constant α, the probability $p_\omega(n, k|n_0, k_0; \alpha)$ that a culture of size n contains exactly k wild-type cells if it has been grown from a culture of $n_0 > 1$ cells with k_0 wild-type cells is*

$$p_\omega(n, k|n_0, k_0; \alpha) = \frac{(n-n_0)!(n_0-1)!}{(n-1)!(k_0-1)!} \sum_{i=k_0}^{k} (-1)^{n-n_0+k_0-i} \times \frac{(k-1)!}{(k-i)!(i-k_0)!} \binom{i(1-\alpha)-n_0}{n-n_0} \quad . \tag{6}$$

Equation (6), however, is of limited use in practice; the main reason is that in general it is difficult to determine the population size with an accuracy of order n_0. Moreover, the number of wild-type cells in the inoculum cannot reasonably be adjusted unless the pre-culture is grown under conditions which guarantee that only wild-type cells proliferate or mutant cells are sorted out, in which case $k_0 = n_0$. In any other case, $n_0 \gg 1$ implies that the random sampling of mutants from a large pre-culture into the inoculum must be taken into account. Equation (6) does not serve this purpose. We will employ (6) only to discuss the relation between our model and the ones formulated by Lea and Coulson (1949) and Bartlett (1955) in Section 5.

The following result is interesting in its own right. It is most conveniently proved by insertion into Equation (1):

Corollary 2.4. *For $\alpha = 1/2$, the probability that a culture of size n contains r mutants if it is grown from a single wild-type cells is*

$$p_\rho(n, r; 1/2) = \binom{n+r-1}{r} \frac{1}{2^{n+r-1}} \quad . \tag{7}$$

To conclude this section, we will calculate the moments of the probability distribution in Theorem 2.1. For any integer $z \geq 1$, we define the factorial moments of the distribution of the number of wild-type cells in a population of size n as follows:

$$E_\omega^z(n; \vec{\alpha}_{n-1}) = \sum_{k=1}^{n} k(k+1)\cdots(k+z-1)\, p_\omega(n, k; \vec{\alpha}_{n-1}) \quad . \tag{8}$$

Note that this definition is non-standard in comparison with the usual one, which involves a descending factorial. It is also easy to check that if we define $E_\omega^0(n; \vec{\alpha}_{n-1}) = 1$, we obtain for the generating function $\Xi_\omega(n, s; \vec{\alpha}_{n-1})$ of the factorial moments $E_\omega^z(n; \vec{\alpha}_{n-1})$,

$$\Xi_\omega(n, -s; \vec{\alpha}_{n-1}) = \sum_{z=0}^{\infty} \frac{E_\omega^z(n; \vec{\alpha}_{n-1})}{z!} (-s)^z = g_\omega\left(n, \frac{1}{1+s}; \vec{\alpha}_{n-1}\right) \quad , \tag{9}$$

where $g_\omega(n, s; \vec{\alpha}_{n-1})$ is the generating function of the probability distribution (3). We have

Theorem 2.6.

$$E_\omega^z(n;\vec{\alpha}_{n-1}) = z!\prod_{j=1}^{n-1}\frac{j+z(1-\alpha_j)}{j} \quad . \tag{10}$$

Proof. From Equation (2), it follows immediately that

$$\begin{aligned}
&(n-1)E_\omega^z(n;\vec{\alpha}_{n-1})\\
&\quad=(n-1)\sum_{k=1}^{n}k(k+1)\cdots(k+z-1)\,p_\omega(n,k;\vec{\alpha}_{n-1})\\
&\quad=(n-1)\sum_{k=1}^{n}k(k+1)\cdots(k+z-1)\,p_\omega(n-1,k;\vec{\alpha}_{n-2})\\
&\qquad-(1-\alpha_{n-1})\sum_{k=1}^{n}k^2(k+1)\cdots(k+z-1)\,p_\omega(n-1,k;\vec{\alpha}_{n-2})\\
&\qquad+(1-\alpha_{n-1})\sum_{k=1}^{n}(k-1)k\cdots(k+z-1)\,p_\omega(n-1,k-1;\vec{\alpha}_{n-2})\\
&\quad=[n-1+z(1-\alpha_{n-1})]E_\omega^z(n-1;\vec{\alpha}_{n-2}) \quad ,
\end{aligned}$$

and Theorem 2.6 is confirmed by induction. □

With reference to elementary properties of the gamma function (see, for instance, Lebedev 1972), one immediately obtains the following important

Corollary 2.7. *For constant* α,

$$\begin{aligned}
E_\omega^z(n;\alpha) &= \frac{\Gamma(z+1)}{\Gamma(z(1-\alpha)+1)}\frac{\Gamma(n+z(1-\alpha))}{\Gamma(n)}\\
&=\frac{\Gamma(z+1)}{\Gamma(z(1-\alpha)+1)}n^{z(1-\alpha)}\left[1+z(1-\alpha)\frac{z(1-\alpha)-1}{2n}+O(n^{-2})\right] \quad .
\end{aligned} \tag{11}$$

Note that in particular, from Corollary 2.7,

$$E_\omega(n;\alpha) := E_\omega^1(n;\alpha) \sim \frac{n^{1-\alpha}}{\Gamma(2-\alpha)} \quad , \tag{12}$$

so that for $\alpha > 0$, $E_\omega(n;\alpha)/n \to 0$ as n tends to infinity.

3. Two extensions of the model

The assumptions which underlie the derivation of Equation (1) are somewhat simplifying. One might, for instance, wish to consider the possibility of back mutation, or the possibility that it might take more than one step of mutation for the mutant phenotype to become manifest. Although it is easy to introduce the appropriate corrections into Equation (1), the entrance of additional terms into this equation complicates the computation of an explicit solution. We will treat here two extensions of our model where such a solution can be found.

3.1. Cell death

The rôle of cell death in fluctuation analysis has not been discussed extensively so far (see, however, Tan 1982; also Kimmel and Axelrod 1994). The reason for this is probably that a fluctuation experiment requires that cells be able to grow on solid medium. Cells that are genotypically mutants but fail to grow on the selection medium remain invisible during the experiment. Thus, one might dispense with the problem of cell death simply by redefining the mutation rate as the probability that the division of a non-mutant cell results in the formation of a cell whose progeny is able to survive until the culture is plated on solid selection medium and to form colonies there. We will, however, adhere to our less restrictive concept of mutation rate introduced in Section 2.1 as far as possible.

We keep only the assumptions (i) and (ii) of Section 2.1. Furthermore, we make the following assumptions:

(i) The population is composed of colony-forming cells (CFCs) and dead cells. A colony-forming cell is a mutant or non-mutant cell that is capable of further proliferation such that there is always at least one CFC in its progeny. The number of CFCs in a population of n cells is a random variable c_n which may take on any integer value between 1 and n.

(ii) Whenever a cell divides, it is replaced by two cells *at least one of which* is capable of further proliferation. A mutant cell may divide into two mutants, or into one mutant and one dead daughter cell. A wild-type cell may divide into two wild-type cells, or into one wild-type and one mutant daughter cell, or into one wild-type and one dead daughter cell, but not into one mutant and one dead daughter cell. We will assume that whether a cell is mutant or dead can be decided immediately after the division of its mother cell.

(iii) We will assume that if a population contains n_c CFCs, r of which are mutants, the probability that a mutant cell is the next to divide is r/n_c, and similarly for the non-mutant fraction of CFCs. This is reasonable because from the point of view of fluctuation analysis, a cell is ascribed a 'mutant' or 'non-mutant' phenotype according to its capacities to develop into a colony under certain selective circumstances. Therefore, if any bacterial culture produces mutant colonies after being plated on solid medium, these colonies must have originated from colony-forming cells.

(iv) We define the *mutation rate* α_i as the probability that the division of a wild-type cell in a population *which contains i CFCs* results in the formation of one mutant and one non-mutant daughter cell. Similarly, we introduce the probability δ_i that the division of any cell in a population which contains i CFCs ends up with the formation of one dead and one colony-forming cell. For simplicity, we shall assume that δ_i is not too large, such that $\frac{\alpha_i}{1-\delta_i} \leq 1$. Note that the actual value of δ_i must be the same for both mutant and non-mutant cells, since otherwise the growth conditions would preferentially support the proliferation of one or the other cell type, which contradicts assumption (iii) above.

We denote the probability that a culture of n cells contains n_c CFCs and r mutants by $p_{c,\rho}(n, n_c, r; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1})$, where $\vec{\alpha}_{n_c-1}$ is as above and $\vec{\delta}_{n_c-1}$ is of course short

for $(\delta_1, \delta_2, \ldots, \delta_{n_c-1})$. Since only colony-forming cells divide, it is clear from a brief inspection of Equation (1) that we must have

$$\begin{aligned} & p_{c,\rho}(n, n_c, r; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1}) \\ & \quad = (1 - \alpha_{n_c-1} - \delta_{n_c-1}) \frac{n_c - r - 1}{n_c - 1} p_{c,\rho}(n-1, n_c - 1, r; \vec{\delta}_{n_c-2}, \vec{\alpha}_{n_c-2}) \\ & \qquad + \alpha_{n_c-1} \frac{n_c - r}{n_c - 1} p_{c,\rho}(n-1, n_c - 1, r-1; \vec{\delta}_{n_c-2}, \vec{\alpha}_{n_c-2}) \\ & \qquad + (1 - \delta_{n_c-1}) \frac{r-1}{n_c - 1} p_{c,\rho}(n-1, n_c - 1, r-1; \vec{\delta}_{n_c-2}, \vec{\alpha}_{n_c-2}) \\ & \qquad + \delta_{n_c} p_{c,\rho}(n-1, n_c, r; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1}) \ . \end{aligned} \tag{13}$$

Because of assumption (iii), we might try to express the probability $p_{c,\rho}(n, r, n_c; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1})$ as the product of the probability $p_c(n, n_c; \vec{\delta}_{n_c-1})$ that it contains n_c colony-forming cells times the probability that there are r mutants among these cells. The problem is whether this latter probability can be taken to be $p_\rho(n_c, r; \vec{\alpha}_{n_c-1})$ (the answer is no), since, by assumption (ii) of this section, the possibility to undergo mutation increases the chances for a non-mutant cell to survive. In other words, cells that are still alive are more likely to have mutated. This point is clarified by the following

Theorem 3.1. *Let $p_c(n, n_c; \vec{\delta}_{n_c-1})$ denote the probability that a culture of n cells contains n_c colony-forming cells, and let $p_\rho(n, r; \vec{\Delta}_{n_c-1})$ be an ordinary Luria–Delbrück distribution with*

$$\vec{\Delta}_{n_c-1} := \left(\frac{\alpha_1}{1 - \delta_1}, \frac{\alpha_2}{1 - \delta_2}, \ldots, \frac{\alpha_{n_c-1}}{1 - \delta_{n_c-1}} \right) \ .$$

Then the probability $p_{c,\rho}(n, n_c, r; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1})$ that a culture of size n contains exactly n_c colony-forming cells and r mutants among them is given by

$$p_{c,\rho}(n, n_c, r; \vec{\delta}_{n_c-1}, \vec{\alpha}_{n_c-1}) = p_c(n, n_c; \vec{\delta}_{n_c-1}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1}) \ . \tag{14}$$

Proof. If we set $p_c(1, 1; \vec{\delta}_0) = p_\rho(1, 0; \vec{\Delta}_0) = 1$ and $p_c(1, n_c; \vec{\delta}_0) = p_\rho(1, r; \vec{\Delta}_0) = 0$ otherwise, the theorem is correct for $n = 1$ and any value of n_c and r. Therefore, it only remains to prove that the product $p_c(n, n_c; \vec{\delta}_{n_c-1}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1})$ fulfils the recursion relation (13), i.e., to check that

$$\begin{aligned} & p_c(n, n_c; \vec{\delta}_{n_c-1}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1}) \\ & \quad = (1 - \alpha_{n_c-1} - \delta_{n_c-1}) \frac{n_c - r - 1}{n_c - 1} p_c(n-1, n_c - 1; \vec{\delta}_{n_c-2}) p_\rho(n_c - 1, r; \vec{\Delta}_{n_c-2}) \\ & \qquad + \alpha_{n_c-1} \frac{n_c - r}{n_c - 1} p_c(n-1, n_c - 1; \vec{\delta}_{n_c-2}) p_\rho(n_c - 1, r-1; \vec{\Delta}_{n_c-2}) \\ & \qquad + (1 - \delta_{n_c-1}) \frac{r-1}{n_c - 1} p_c(n-1, n_c - 1; \vec{\delta}_{n_c-2}) p_\rho(n_c - 1, r-1; \vec{\Delta}_{n_c-2}) \\ & \qquad + \delta_{n_c} p_c(n-1, n_c; \vec{\delta}_{n_c-1}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1}) \ . \end{aligned} \tag{15}$$

But this is almost obvious, since the probability $p_c(n, n_c; \vec{\delta}_{n_c-1})$ that a bacterial population contains n_c CFCs regardless of the number of mutants it contains clearly satisfies

$$p_c(n, n_c; \vec{\delta}_{n_c-1}) = (1-\delta_{n_c-1})\, p_c(n-1, n_c-1; \vec{\delta}_{n_c-2}) + \delta_{n_c} p_c(n-1, n_c; \vec{\delta}_{n_c-1}) \ , \quad (16)$$

because of the very definition of δ_i. Then, because of Equation (1), the first three terms on the right-hand side of Equation (15) can be collected to give $(1-\delta_{n_c-1})$ $p_c(n-1, n_c-1; \vec{\delta}_{n_c-2}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1})$, and we finally obtain

$$p_c(n, n_c; \vec{\delta}_{n_c-1}) p_\rho(n_c, r; \vec{\Delta}_{n_c-1}) = [(1-\delta_{n_c-1}) p_c(n-1, n_c-1; \vec{\delta}_{n_c-2}) + \delta_{n_c} p_c(n-1, n_c; \vec{\delta}_{n_c-1})] p_\rho(n_c, r; \vec{\Delta}_{n_c-1}) \ ,$$

which obviously completes the proof of the theorem. □

Note that in practice there is no need to calculate the distribution $p_c(n, n_c; \vec{\delta}_{n_c-1})$, since both the total number of cells as well as the number of CFCs in a bacterial population are experimentally accessible quantities. In fact, Theorem 3.1 tells us that, conditional on the fact that a bacterial culture of given size n contains n_c CFCs (which can be decided in the experiment), the probability that it contains exactly r mutants is simply $p_\rho(n_c, r; \vec{\Delta}_{n_c-1})$.

3.2. An alternative model of mutation

In the present section, we shall be interested in what happens if the mutation of a wild-type cell may also result in the production of two mutant daughter cells. We keep the assumptions of Section 2.1, with the exception of (v), which we replace by the following:

(i) We denote by $\alpha^\bullet$ the probability that the division of a wild-type cell in a population of arbitrary size ends up with the formation of one mutant and one non-mutant cell. Similarly, we denote by $\alpha^{\bullet\bullet}$ the probability that a wild-type cell divides into two mutant daughter cells. We will assume that both $\alpha^\bullet$ and $\alpha^{\bullet\bullet}$ are constant during the whole period of population growth, and that both $\alpha^\bullet$ and $\alpha^{\bullet\bullet}$ are not too large. Specifically, we assume that $\alpha^\bullet + 2\alpha^{\bullet\bullet} < 1$ (the reason for this somewhat strange condition will become clear later).

Since we are dealing with a different model of mutation here, we introduce some new notation. We now denote the probability that a culture of n cells contains r mutants by $\sigma_\rho(n, r; \alpha^\bullet, \alpha^{\bullet\bullet})$, that it contains k wild-type cells, by $\sigma_\omega(n, k; \alpha^\bullet, \alpha^{\bullet\bullet})$. It is clear that

$$\begin{aligned}\sigma_\rho(n, r; \alpha^\bullet, \alpha^{\bullet\bullet}) = \ & (1-\alpha^\bullet-\alpha^{\bullet\bullet}) \frac{n-r-1}{n-1} \sigma_\rho(n-1, r; \alpha^\bullet, \alpha^{\bullet\bullet}) \\ & + \frac{r-1}{n-1} \sigma_\rho(n-1, r-1; \alpha^\bullet, \alpha^{\bullet\bullet}) \\ & + \alpha^\bullet \frac{n-r}{n-1} \sigma_\rho(n-1, r-1; \alpha^\bullet, \alpha^{\bullet\bullet}) \\ & + \alpha^{\bullet\bullet} \frac{n-r+1}{n-1} \sigma_\rho(n-1, r-2; \alpha^\bullet, \alpha^{\bullet\bullet}) \ . \end{aligned} \quad (17)$$

We are looking for a solution of equation (17) in terms of known quantities. It is provided by

Theorem 3.2. *Define*

$$\Theta := \alpha^{\bullet} + 2\alpha^{\bullet\bullet}, \quad \theta := \frac{\alpha^{\bullet\bullet}}{1-\Theta} \ . \tag{18}$$

Furthermore, let $\hat{p}_\rho(n, r; \Theta)$ *denote an 'ordinary' Luria–Delbrück distribution such that the quantities* $\hat{p}_\rho(n, r; \Theta)$ *fulfil the recursion relation* (1) *with* Θ *in the role of* α*, but*

$$\hat{p}_\rho(1, 0; \Theta) = \frac{1}{1+\theta}, \quad \hat{p}_\rho(1, 1; \Theta) = \frac{\theta}{1+\theta} \ , \tag{19}$$

and $\hat{p}_\rho(1, r; \Theta) = 0$ *otherwise. In other words, suppose that a population of bacteria has been grown from a single cell that is wild-type with probability* $\frac{1}{1+\theta}$ *and mutant with probability* $\frac{\theta}{1+\theta}$*, and let the mutation rate in the population be equal to* Θ*. Then*

$$\sigma_\rho(n, r; \alpha^{\bullet}, \alpha^{\bullet\bullet}) = (1+\theta)^{n-r} \sum_{i=0}^{r} \binom{n-i}{r-i} (-\theta)^{r-i} \hat{p}_\rho(n, i; \Theta) \ . \tag{20}$$

Sketch of proof. The fact that $\sigma_\rho(1, 0; \alpha^{\bullet}, \alpha^{\bullet\bullet}) = 1$ and $\sigma_\rho(1, r; \alpha^{\bullet}, \alpha^{\bullet\bullet}) = 0$ for any other r is straightforward. Next, one uses Equation (1) to express each single $\hat{p}_\rho(n, i; \Theta)$ in terms of $\hat{p}_\rho(n-1, i; \Theta)$ and $\hat{p}_\rho(n-1, i-1; \Theta)$, and thus checks that the sum on the right-hand side of Equation (20) does fulfil the recursion relation (17). Since any two quantities that fulfil the same partial difference equation and coincide on a sufficiently large set of initial data are identical, this will complete the proof of Theorem 3.2. The details of the calculation, however, are somewhat messy, and not particularly interesting in themselves. The reader is invited to obtain them from the author. □

Corollary 3.3. *The probability distribution* $\sigma_\omega(n, k; \alpha^{\bullet}, \alpha^{\bullet\bullet})$ *of the number of wild-type cells in a population of size n has the generating function*

$$\sum_{k=0}^{n} \sigma_\omega(n, k; \alpha^{\bullet}, \alpha^{\bullet\bullet}) s^k = \frac{\theta}{1+\theta} + \frac{1}{1+\theta} g_\omega(n, s-\theta(1-s); \Theta) \ , \tag{21}$$

where

$$g_\omega(n, s; \Theta) := \sum_{k=1}^{n} p_\omega(n, k; \Theta) s^k \ . \tag{22}$$

is the generating function of the probability distribution (4), with Θ *in the rôle of* α*.*

Proof. Because of Theorem 3.2 and a straightforward application of the binomial theorem, we have

$$\begin{aligned} \sum_{k=0}^{n} \sigma_\omega(n, k; \alpha^{\bullet}, \alpha^{\bullet\bullet}) s^k &= \sum_{i=0}^{n} \hat{p}_\omega(n, n-i; \Theta) \sum_{k=0}^{n-i} \binom{n-i}{k} (-\theta)^{n-k-i} (1+\theta)^k s^k \\ &= \hat{g}_\omega(n, s-\theta(1-s); \Theta) \ , \end{aligned}$$

where we have denoted by $\hat{g}_\omega(n, s; \Theta)$ the generating function of the probability distribution $\hat{p}_\omega(n, k; \Theta)$. Because of the definition of the quantities $\hat{p}_\omega(n, k; \Theta)$, this generating function is equal to $g_\omega(n, s; \Theta)$ with probability $1/(1+\theta)$ (cf. Equation (19)), whereas with probability $\theta/(1+\theta)$, it is equal to 1. Since $\hat{g}_\omega(n, s; \Theta) = E(E(s^{\omega_n}|\omega_1))$ by the very definition of a generating function, this already completes the proof of Corollary 3.3. □

4. A note on computation

In many applications of fluctuation analysis, the cell numbers involved may build up to $\sim 10^6 - 10^8$. It is then clear that for the calculation of the distribution neither Equation (1) nor Theorem 2.1 will be suitable. To efficiently calculate the distribution in such a case, it is natural to try and develop an algorithm similar to the one described by Ma et al. (1992) (also Sarkar et al. 1992). This is possible if the wild-type cells mutate with constant probability. The procedure is as follows. Consider the generating function

$$\gamma_k(x; \alpha) := \sum_{n=k}^{\infty} p_\omega(n, k; \alpha) x^{n-1} \quad , \tag{23}$$

where the summation could begin with any $n \leq k$, since we expect that $p_\omega(n, k; \alpha)$ is zero for any $n < k$. Then, because of Corollary 2.2 and a routine application of the binomial theorem, (23) yields

$$\begin{aligned}
\gamma_k(x; \alpha) &= \sum_{n=1}^{\infty} (-1)^{n-1} \sum_{i=1}^{k} (-1)^{i-1} \binom{k-1}{i-1} \binom{i(1-\alpha)-1}{n-1} x^{n-1} \\
&= \sum_{i=1}^{k} (-1)^{i-1} \binom{k-1}{i-1} \sum_{n=1}^{\infty} \binom{i(1-\alpha)-1}{n-1} (-x)^{n-1} \\
&= (1-x)^{-\alpha} [1 - (1-x)^{(1-\alpha)}]^{k-1} \quad ,
\end{aligned} \tag{24}$$

so that after integrating and multiplying by $\frac{k}{(1-\alpha)^{k-1}} x^{-k}$, we obtain

$$\begin{aligned}
\frac{k}{(1-\alpha)^{k-1}} \sum_{n=k}^{\infty} \frac{1}{n} \, p_\omega(n, k; \alpha) x^{n-k} &= \left(\frac{1 - (1-x)^{1-\alpha}}{(1-\alpha)x} \right)^k \\
&= \left(\sum_{i=0}^{\infty} \frac{\Gamma(\alpha + i)}{\Gamma(\alpha)\Gamma(i+2)} x^i \right)^k \quad .
\end{aligned} \tag{25}$$

The problem of calculating the distribution thus reduces to determining the coefficients in the power series expansion of $\left(\frac{1-(1-x)^{1-\alpha}}{(1-\alpha)x} \right)^k$; but this is immediate from a well-known theorem about power series raised to powers (e.g. Gradshteyn and Ryzhik 1980). In our instance, it reads

$$\frac{k}{(1-\alpha)^{k-1}} \frac{1}{n} \, p_\omega(n, k; \alpha)$$

$$= \frac{1}{n-k}\sum_{i=1}^{n-k}\frac{\Gamma(\alpha+i)}{\Gamma(\alpha)\Gamma(i+2)}(ik-n+k+i)\frac{k}{(1-\alpha)^{k-1}}\frac{1}{n-i}p_\omega(n-i,k;\alpha) \ ,$$

so that finally, we obtain

$$p_\rho(n,r;\alpha) = \frac{n}{r}\sum_{i=1}^{r}\frac{\Gamma(\alpha+i)}{\Gamma(\alpha)\Gamma(i+2)}\frac{(n+1)i-r(i+1)}{n-i}p_\rho(n-i,r-i;\alpha) \ . \quad (26)$$

with $p_\rho(n-r,0;\alpha) = (1-\alpha)^{n-r-1}$.

For the sake of completeness, and because the result will be needed below, we will also treat the more general case when the inoculum used to seed the experiment contains mutants and wild-type cells. To this end, we compute the $(n_0-1)^{\text{th}}$ derivative of the corresponding generating function,

$$\gamma_{k,n_0,k_0}(x;\alpha) := \sum_{n=n_0+k-k_0}^{\infty} p_\omega(n,k|n_0,k_0;\alpha)x^{n-1} \ . \quad (27)$$

Here, the summation could of course begin with any $n \le n_0+k-k_0$. Then, because of Equation (6),

$$\begin{aligned}
&\gamma_{k,n_0,k_0}^{(n_0-1)}(x;\alpha)\\
&\quad= \frac{(n_0-1)!}{(k_0-1)!}\sum_{i=k_0}^{k}(-1)^{k_0-i}\frac{(k-1)!}{(k-i)!(i-k_0)!}\sum_{n=n_0}^{\infty}\binom{i(1-\alpha)-n_0}{n-n_0}(-x)^{n-n_0}\\
&\quad= \frac{(n_0-1)!}{(k_0-1)!}\sum_{i=k_0}^{k}(-1)^{k_0-i}\frac{(k-1)!}{(k-i)!(i-k_0)!}(1-x)^{i(1-\alpha)-n_0}\\
&\quad= (n_0-1)!\binom{k-1}{k_0-1}(1-x)^{k_0-n_0-\alpha k_0}[1-(1-x)^{(1-\alpha)}]^{k-k_0} \ , \quad (28)
\end{aligned}$$

which in comparison with (24) is less useful.

5. The limiting distribution (first case)

The purpose of this section is to derive a limit law for the Luria–Delbrück distribution that applies when the population size is large and the mutation rate is small, and thereby to establish a connection between our model and the ones of Lea and Coulson (1949) and Bartlett (1955). We express this connection as the following

Theorem 5.1. *Consider a bacterial culture of n_0 cells that contains r_0 mutants, and denote by $p_\rho(n,r|n_0,r_0;\alpha)$ the probability that this culture will contain r mutants when it has grown to a size of n cells (Corollary 2.2). Furthermore, suppose that $\nu := \lim_{n\to\infty}\frac{n_0}{n}$ exists and that r_0 remains finite as $n\to\infty$. Then, for any nonnegative $\varphi < \infty$, $\lim_{n\to\infty} p_\rho\left(n,r|n_0,r_0;\frac{\varphi}{n}\right)$ exists and is in fact the probability distribution of a nonnegative, integer-valued random variable with generating function*

$$\sum_{r=r_0}^{\infty} \lim_{n\to\infty} p_\rho\left(n, r|n_0, r_0; \frac{\varphi}{n}\right) s^r = \left(\frac{\nu s}{1-(1-\nu)s}\right)^{r_0} (1-s+\nu s)^{\varphi(1-s)/s} \quad . \quad (29)$$

Proof. We will employ the functions $\gamma_{k,n_0,k_0}(x;\alpha)$ (27) from Section 4, or rather their $(n_0-1)^{\text{th}}$ derivative. Thus,

$$\begin{aligned} x^{n_0-k}\gamma_{k,n_0,k_0}^{(n_0-1)}\left(x;\frac{\varphi}{k}\right) &= \sum_{n=k+n_0-k_0}^{\infty} \frac{(n-1)!}{(n-n_0)!} p_\omega\left(n, k|n_0, k_0; \frac{\varphi}{k}\right) x^{n-k} \\ &= \sum_{r=r_0}^{\infty} \frac{(k+r-1)!}{(k+r-n_0)!} p_\rho\left(k+r, r|n_0, r_0; \frac{\varphi}{k}\right) x^r \quad , \end{aligned}$$

so that, by Equation (28), and if we simply write n instead of k,

$$\begin{aligned} &\frac{(k_0-1)!}{(n_0-1)!}\frac{(n-k_0)!}{(n-1)!}\sum_{r=r_0}^{\infty}\frac{(n+r-1)!}{(n+r-n_0)!} p_\rho\left(n+r, r|n_0, r_0; \frac{\varphi}{n}\right) x^r \\ &\quad = x^{n_0-k_0}(1-x)^{k_0-n_0-\varphi k_0/n}\left[\frac{1-(1-x)^{1-\varphi/n}}{x}\right]^{n-k_0} \quad . \end{aligned} \quad (30)$$

Furthermore,

$$\lim_{n\to\infty}\left[\frac{1-(1-x)^{1-\varphi/n}}{x}\right]^{n-k_0} = (1-x)^{\varphi(1-\nu)(1-x)/x} \quad ,$$

according to the conditions of the theorem, and

$$\lim_{n\to\infty}\frac{(k_0-1)!}{(n_0-1)!}\frac{(n-k_0)!}{(n-1)!}\frac{(n+r-1)!}{(n+r-n_0)!} = \nu^{-r_0}(1-\nu)^{-r+r_0} \quad ,$$

if we recall that $n_0-k_0=r_0$. Therefore, we obtain

$$\begin{aligned} &\sum_{r=r_0}^{\infty}\lim_{n\to\infty} p_\rho\left(n+r, r|n_0, r_0; \frac{\varphi}{n}\right)\nu^{-r_0}(1-\nu)^{-r+r_0}x^r \\ &\quad = x^{r_0}(1-x)^{-r_0-\varphi\nu}(1-x)^{\varphi(1-\nu)(1-x)/x} \quad , \end{aligned} \quad (31)$$

at least for $x<1$, which, as an identity between power series, proves that $\lim_{n\to\infty} p_\rho$ $(n+r, r|n_0, r_0; \frac{\varphi}{n})$ exists. Since the convergence of $p_\rho(n+r, r|n_0, r_0; \frac{\varphi}{n})$ clearly entails that of $p_\rho(n, r|n_0, r_0; \frac{\varphi}{n})$, we may substitute one for the other in Equation (31). It is then easy to check that a change of variables $x =: (1-\nu)s$ transforms the resulting equation into (29), thereby completing the proof of Theorem 5.1. □

The expression $(1-s+\nu s)^{\varphi(1-s)/s}$ that appears on the right-hand side of Equation (29) has often been referred to as Bartlett's generating function (or, for $\nu=0$, as Lea and Coulson's generating function). Zheng (1999) calls it the exact PGF for the Lea-Coulson formulation of the Luria–Delbrück model. In view of the fact that there exists another model of Bartlett's to study growth-and-mutation processes in bacterial populations (see Discussion), this is probably advisable. A very readable

account of the fortunes of the generating function $(1-s+\nu s)^{\varphi(1-s)/s}$ during the 1950s can be found in Zheng (1999).

As far as the alternative model of mutation introduced in Section 3.2 is concerned, we have the following

Theorem 5.2. *Let $\psi_\rho(n,s;\alpha^\bullet,\alpha^{\bullet\bullet})$ denote the probability generating function of the distribution* (20)*. Then, for any two nonnegative numbers φ, $\phi<\infty$,*

$$\lim_{n\to\infty}\psi_\rho\left(n,s;\frac{\varphi}{n},\frac{\phi}{n}\right)=e^{\phi(1-s)}(1-s)^{(\varphi+2\phi)(1-s)/s}=e^{\phi(1-s)}g_{LC}(s)\ , \quad (32)$$

where $g_{LC}(s)$ denotes Lea and Coulson's (1949) generating function.

Proof. Because of Corollary 3.3, we have

$$\begin{aligned}\psi_\rho(n,s;\alpha^\bullet,\alpha^{\bullet\bullet})&=s^n\frac{1}{1+\theta}g_\omega\left(n,\frac{1+\theta(1-s)}{s};\Theta\right)+s^n\frac{\theta}{1+\theta}\\&=\frac{(1+\theta-\theta s)^n}{1+\theta}\sum_{r=0}^{n-1}\left(\frac{s}{1+\theta(1-s)}\right)^r p_\rho(n,r;\Theta)+s^n\frac{\theta}{1+\theta}\ ,\end{aligned} \quad (33)$$

where Θ and θ are as specified by Equation (18). With $\theta=\alpha^{\bullet\bullet}/(1-\alpha^\bullet-2\alpha^{\bullet\bullet})=\phi/(n-\varphi-2\phi)$, it is clear that the first factor in (33) tends to $e^{\phi(1-s)}$ as $n\to\infty$, whereas the last term simply disappears. If now we fix for the moment an arbitrary (small) value for θ, it follows from Theorem 5.1 that

$$\begin{aligned}&\lim_{n\to\infty}\sum_{r=0}^{n-1}\left(\frac{s}{1+\theta(1-s)}\right)^r p_\rho\left(n,r;\frac{\varphi+2\phi}{n}\right)\\&\quad=\left(\frac{(1+\theta)(1-s)}{1+\theta-\theta s}\right)^{(\varphi+2\phi)(1+\theta)(1-s)/s}\ ,\end{aligned}$$

since we have derived the probability distribution (20) under the assumption that $n_0=1$, which implies that $\nu=n_0/n\to 0$ as $n\to\infty$. If now we let $\theta=\phi/(n-\varphi-2\phi)\to 0$ as well, we obtain the desired result. □

6. The limiting distribution (second case)

The crucial point about the derivation of Theorem 5.1 is that the product αn of mutation rate and population size converges as the population size tends to infinity. In general, however, one would expect that the number of mutations which occur in a bacterial population as it grows to infinite size also increases without bound, unless the mutation rate is zero for most of the time. If we exclude the possibility of backward mutation, the number of mutants in a bacterial population is at least as large as the number of times that non-mutant cells have mutated in this population, and one may ask, say, for the probability that the ratio of the number of mutants to

the total size of the population exceeds 1/2. The division by the number of all cells in a population may obviously be too crude a normalization to yield interesting results, and we will therefore investigate the probability that the number of mutant cells (or, which is basically the same thing, the number of non-mutant cells) deviates significantly from its expected value. We keep all the assumptions of Section 2.1, but assume the mutation rate as constant.

Consider the sequence $\{W_n\}$ of random variables

$$W_n := \frac{\omega_n(\alpha)}{E_\omega(n;\alpha)} \quad , \tag{34}$$

where $E_\omega(n;\alpha)$ is the expected value of the number of wild-type cells in a population of size n (Equations (11) and (12)). Then we shall prove

Theorem 6.1. *Let $0 < \alpha < 1$. As $n \to \infty$, the sequence $\{W_n\}$ converges with probability 1. Furthermore, the probability $P_W(x;\alpha)$ that $W := p - \lim_{n\to\infty} W_n$ assumes a value not exceeding x is given by*

$$P_W(x;\alpha) = \sum_{i=1}^{\infty} (-1)^{i-1} \frac{\sin(\pi i(1-\alpha))}{\pi} \frac{\Gamma(i(1-\alpha))}{i!} \left(\frac{x}{\Gamma(2-\alpha)}\right)^i \quad , \tag{35}$$

for $x > 0$, and $P_W(x;\alpha) = 0$ otherwise.

Proof. We first observe that a culture of size $n-1$ that contains k wild-type cells will on average contain

$$(1-\alpha)\frac{k}{n-1}(k+1) + \alpha\frac{k}{n-1}k + \frac{n-k-1}{n-1}k = \frac{n-\alpha}{n-1}k$$

wild-type cells when it has reached size n. Therefore we have for the expectation of W_n conditional on W_{n-1},

$$E(W_n|W_{n-1}) = \frac{n-\alpha}{n-1}\frac{k}{E_\omega(n;\alpha)} = \frac{k}{E_\omega(n-1;\alpha)} = W_{n-1} \quad . \tag{36}$$

This proves that the sequence $\{W_n\}$ of random variables (34) is a martingale. Furthermore, $E(W_n) = 1$ and $W_n > 0$ for all finite n, so that $W := p - \lim_{n\to\infty} W_n$ exists because of Doob's theorem.

Since convergence in probability implies convergence in distribution, the calculation of the distribution function $P_W(x;\alpha)$ is now standard. We fix a value for $x > 0$ and then pick an integer $k_n := k_n(x)$ such that $k_n < xE_\omega(n;\alpha) \le k_n + 1$. Then, because of Corollary 2.2,

$$\begin{aligned} P_W(x;\alpha) &= \lim_{n\to\infty} P_\omega(n,k_n;\alpha) \\ &= \lim_{n\to\infty} \sum_{i=1}^{k_n} (-1)^{n-i} \frac{[E_\omega(n;\alpha)]^i}{i!} x^i [1 - O(E_\omega(n;\alpha)^{-1})] \binom{i(1-\alpha)-1}{n-1} \quad , \end{aligned}$$

where $O(\,)$ is Landau's order symbol. The proof of Theorem 6.1 is now completed by noting that, because of the asymptotic expression for the gamma function already quoted in Corollary 2.7,

$$\begin{aligned}
&\lim_{n\to\infty}(-1)^{n-1}[E_\omega(n;\alpha)]^i\binom{i(1-\alpha)-1}{n-1}\\
&\quad=\left(\frac{\Gamma(n+1-\alpha)}{\Gamma(2-\alpha)\Gamma(n)}\right)^i\frac{\Gamma(n-i(1-\alpha))}{\Gamma(n)\Gamma(1-i(1-\alpha))}\\
&\quad=\frac{1}{\Gamma(1-i(1-\alpha))}\frac{1}{[\Gamma(2-\alpha)]^i}\\
&\quad=\frac{\sin(\pi i(1-\alpha))}{\pi}\frac{\Gamma(i(1-\alpha))}{[\Gamma(2-\alpha)]^i}\ ,
\end{aligned}$$

and that (as is easy to check) the series in Equation (35) has infinite radius of convergence. The fact that $P_W(x;\alpha)=0$ for $x\le 0$ is obvious. □

Theorem 6.1 tells us that the probability that a very large culture of bacteria contains a number of mutants larger than $n - xE_\omega(n;\alpha)$ can be sufficiently well approximated by $P_W(x;\alpha)$. Thus, we find that for any η, $0\le\eta<1$, the probability that a large culture of (say) n bacteria contains more than ηn mutant cells is given by $P_W\left(\frac{(1-\eta)n}{E_\omega(n;\alpha)};\alpha\right)$. This tends to unity as n increases.

For completeness, we quote an interesting corollary to Theorem 6.1.

Corollary 6.2. *For $\alpha=1/2$, the probability density $p_W(x;\alpha)$ of $W=p-\lim_{n\to\infty}W_n$ is*

$$p_W(x;1/2)=\frac{2}{\pi}e^{-\frac{x^2}{\pi}}\ , \tag{37}$$

if $x>0$, while $p_W(x;1/2)=0$ otherwise.

We now return to a discussion of the probability distribution (20) which emerges in the context of the alternative model of mutation introduced in Section 3.2. Here, we consider a sequence $\{\Omega_n\}$ of random variables

$$\Omega_n:=\frac{1}{1+\theta}\frac{\omega_n}{E_\omega(n;\Theta)}\ . \tag{38}$$

Then we have

Theorem 6.3. *Let $0<\Theta<1$. The sequence Ω_n converges in distribution, and the limiting distribution function is*

$$P_\Omega(x;\Theta,\theta)=\frac{\theta}{1+\theta}+\frac{1}{1+\theta}P_W(x;\Theta)\ , \tag{39}$$

where $P_W(x;\Theta)$ is the distribution function (35) with Θ in the rôle of α.

Proof. We will make use of the theory of Laplace-Stieltjes (LS) transforms. By definition, the LS-transform $\lambda_{W_n}(y;\alpha)$ of the distribution function of the random variable W_n (34) is the expected value of e^{-yW_n}, i.e.

$$\lambda_{W_n}(y;\alpha) = \sum_{k=1}^{n} \mathrm{e}^{-ky/E_\omega(n;\alpha)}\, p_\omega(n,k;\alpha) = g_\omega(n, \mathrm{e}^{-y/E_\omega(n;\alpha)};\alpha) \ . \tag{40}$$

We denote the LS-transform of the probability distribution $P_W(x;\alpha)$ (Theorem 6.1) by $\lambda_W(y;\alpha)$ (this function may easily be given in explicit form, but we do not need it at this point; see, however, Equation (65) below). Finally, the LS-transform $\lambda_{\Omega_n}(y;\Theta,\theta)$ of the distribution function of the random variables (38) is

$$\lambda_{\Omega_n}(y;\Theta,\theta) = \frac{\theta}{1+\theta} + \frac{1}{1+\theta} g_\omega(n, (1+\theta)\mathrm{e}^{-y/(1+\theta)E_\omega(n;\Theta)} - \theta;\Theta) \ , \tag{41}$$

because of Corollary 3.3. Our aim is to prove that

$$\lim_{n\to\infty} \lambda_{\Omega_n}(y;\Theta,\theta) = \frac{\theta}{1+\theta} + \frac{1}{1+\theta}\lambda_W(y;\Theta) \tag{42}$$

uniformly in y on any interval $[0,z)$, $z<\infty$. Since, for any such y,

$$\begin{aligned} 0 &\le \mathrm{e}^{-ky/E_\omega(n;\Theta)} - [(1+\theta)\mathrm{e}^{-y/(1+\theta)E_\omega(n;\Theta)} - \theta]^k \\ &\le k[\mathrm{e}^{-y/E_\omega(n;\Theta)} - (1+\theta)\mathrm{e}^{-y/(1+\theta)E_\omega(n;\Theta)} + \theta] \\ &\le \frac{1}{2}\frac{\theta}{1+\theta}\frac{ky^2}{[E_\omega(n;\Theta)]^2} \ , \end{aligned} \tag{43}$$

it follows immediately from (41) and (43) that

$$0 \le \lambda_{W_n}(y;\Theta) - (1+\theta)\lambda_{\Omega_n}(y;\Theta,\theta) + \theta \le \frac{\theta}{1+\theta}\frac{1}{E_\omega(n;\Theta)}\frac{z^2}{2} \ ,$$

which implies Equation (42) as well as Theorem 6.3. □

7. The distribution of mutation events

For the purpose of this and the next section, we will again make use of the assumptions listed in Section 2.1, with the exception that we will assume the mutation rate is constant throughout. Furthermore, we adopt the following convention:

(i) Whenever a wild-type cell divides into one mutant and one non-mutant cell, we call it a *mutation event*. The number of mutation events that have occurred in a population of given size n is a non-negative, integer-valued random variable, which we denote by μ_n.

Practically speaking, the distribution of mutation events should be of greater importance than the distribution of mutants, since the number of mutations that have occurred in a population (which might be in practice a population of tumor cells) is a more accurate measure of its mutability than the number of mutants it contains. Because of the fact that the mutant cells themselves multiply, it might

seem that the distribution of mutation events is easier to access than the distribution of mutant cells. However, even if we know that a population contains r mutant cells (which is significantly more information than to know that it contains n mutant and non-mutant cells), these r cells may still be the result of any number of mutation events from 1 to r. Furthermore, the probability that a mutation occurs in a population of given size does not only depend on the number of mutations that have already occurred, but also on *when* they did. For example, if the first mutation already occurs at the time of division of the first cell in the population, there will be only $\sim n/2$ cells available for mutation later on. Therefore, the precise form of the distribution of mutation events for arbitrary values of α should be of significant interest also from the theoretical point of view.

If we denote by $p_{\rho,\mu}(n, r, m; \alpha)$ the probability that a population of size n in which m mutation events have occurred contains exactly r mutants, we immediately find from Equation (1)

$$\begin{aligned} p_{\rho,\mu}(n, r, m; \alpha) = (1-\alpha)\left[1 - \frac{r}{n-1}\right] p_{\rho,\mu}(n-1, r, m; \alpha) \\ + \frac{r-1}{n-1} p_{\rho,\mu}(n-1, r-1, m; \alpha) \\ + \alpha \frac{n-r}{n-1} p_{\rho,\mu}(n-1, r-1, m-1; \alpha) \quad . \end{aligned} \tag{44}$$

The probability $p_\mu(n, m; \alpha)$ that m mutation events have occurred in a culture of size n which contains an arbitrary number of mutants would then be given by

$$p_\mu(n, m; \alpha) = \sum_{r=0}^{n-1} p_{\rho,\mu}(n, r, m; \alpha) \quad . \tag{45}$$

Unfortunately, the recursion relation (44) is much less amenable for an explicit solution than the corresponding recursion (1). However, with some effort it is possible to prove the following

Theorem 7.1. *Let the distribution of the number of wild-type cells be given as in Theorem 2.1, and denote by $g_\omega(n, s; \alpha)$ its generating function for arbitrary but fixed population size (22). Furthermore, let*

$$g_\mu(n, s; \alpha) = \sum_{m=0}^{n-1} p_\mu(n, m; \alpha) s^m \tag{46}$$

denote the probability generating function of the distribution of the number of mutation events, where $p_\mu(n, m; \alpha)$ is the probability that exactly m mutations have occurred in a population of n cells. Then

$$g_\mu(n, s; \alpha) = \frac{1-\alpha s}{1-\alpha} g_\omega\left(n, \frac{1-\alpha}{1-\alpha s}; \alpha s\right) \quad . \tag{47}$$

Sketch of proof. The idea of proof is to introduce first a new notation for the probability that no mutation occurs, say $1 - \alpha =: \beta$, and to realize that the probability $p_\mu(n, m; \alpha)$ must in some way be proportional to α^m times some possibly complicated polynomial in β. On the other hand, we have

$$p_\rho(n, r; \alpha) = \sum_{m=0}^{n-1} p_{\rho,\mu}(n, r, m; \alpha) \quad . \tag{48}$$

Hence, one will try to find a suitable expansion of the probabilities $p_\rho(n, r; \alpha)$ into powers of α and β and then collect terms of the same order in α. Specifically, one introduces auxiliary variables

$$p_{\rho,\mu}(n, r, m; \alpha) =: \alpha^m \beta^{n-r-1} \frac{(n - r - 1)!}{(n - 1)!} q_{\rho,\mu}(n, r, m) \quad , \tag{49}$$

and then proves that

$$q_{\rho,\mu}(n, r, m) = \sum_{j=0}^{m} D_{j,r,m} \binom{n + j - 1}{r + j} \quad , \tag{50}$$

where the coefficients $D_{j,r,m}$ are defined recursively such that

$$D_{j,r+1,m} = r D_{j,r,m} - (r + j) D_{j,r,m-1} + (r + j) D_{j-1,r,m-1} \tag{51}$$

and certain boundary conditions are met (e.g., $D_{j,r,m} = 0$ for $r < m$). In a similar fashion, one proves that

$$p_\rho(n, r; \alpha) = \beta^{n-r-1} \frac{(n - r - 1)!}{(n - 1)!} \sum_{j=0}^{r} C_{j,r;\alpha} \binom{n + j - 1}{r + j} \quad , \tag{52}$$

where

$$C_{j,r;\alpha} = \sum_{m=j}^{r} \alpha^m D_{j,r,m} \quad . \tag{53}$$

Together with Equations (22) and (46), this leads to Theorem 7.1. The actual calculations, though elementary, seem to be more difficult to be followed than to be done by oneself. The reader is therefore encouraged to do so, or to obtain details of the proof from the author. □

8. The limiting distribution of mutation events

This final section is devoted to the derivation of limit laws for the distribution of the number of mutation events similar to those expressed by Theorems 5.1 and 6.1. If, as in Section 5, the mutation rate is small and the cell number is large such that the product αn converges as n tends to infinity, the calculations are rather simple.

Theorem 8.1. *Let $p_\mu(n, m, \alpha)$ denote the probability distribution of the number of mutation events in a population of size n, and let $g_\mu(n, s; \alpha)$ denote its generating function as specified by Theorem 7.1, Equation (47). Then, for any nonnegative number $\varphi < \infty$, both $\lim_{n\to\infty} p_\mu\left(n, m; \frac{\varphi}{n}\right)$ and $\lim_{n\to\infty} g_\mu\left(n, s; \frac{\varphi}{n}\right)$ exist, and*

$$\lim_{n\to\infty} g_\mu\left(n, s; \frac{\varphi}{n}\right) = e^{\varphi(s-1)} \quad . \tag{54}$$

Proof. For the proof, we fix a value for s between zero and one, and rewrite Equation (47) as

$$g_\mu\left(n, s; \frac{\varphi}{n}\right) = \sum_{r=0}^{n-1} \left(\frac{1-\varphi/n}{1-\varphi s/n}\right)^{n-r-1} p_\rho\left(n, r; \frac{\varphi}{n}s\right) \quad .$$

Since $\frac{1-\varphi/n}{1-\varphi s/n} \leq 1$, it follows at once that

$$g_\mu\left(n, s; \frac{\varphi}{n}\right) \geq \left(\frac{1-\varphi/n}{1-\varphi s/n}\right)^{n-1} \quad . \tag{55}$$

On the other hand, Theorem 5.1 implies that the family of probability distributions $p_\rho\left(n, r; \frac{\varphi}{n}s\right)$ is tight, which in our instance means that for any positive number ε there exists an index r_ε such that $\sum_{r=0}^{r_\varepsilon} p_\rho\left(n, r; \frac{\varphi}{n}s\right) > 1-\varepsilon$ for all n. Thus

$$\begin{aligned} g_\mu\left(n, s; \frac{\varphi}{n}\right) &\leq \left(\frac{1-\varphi/n}{1-\varphi s/n}\right)^{n-r_\varepsilon-1} \sum_{r=0}^{r_\varepsilon} \left(\frac{1-\varphi/n}{1-\varphi s/n}\right)^{r_\varepsilon-r} p_\rho\left(n, r; \frac{\varphi}{n}s\right) + \varepsilon \\ &\leq \left(\frac{1-\varphi/n}{1-\varphi s/n}\right)^{n-r_\varepsilon-1} + \varepsilon \quad , \end{aligned} \tag{56}$$

which together with Equation (55) already implies Theorem 8.1, since ε is arbitrary, and r_ε is finite.

If, on the other hand, $\alpha n \to \infty$ as $n \to \infty$, we consider a sequence $\{M_n\}$ of random variables

$$M_n := \frac{1-\alpha}{\alpha} \frac{\mu_n}{E_\omega(n; \alpha)} \quad . \tag{57}$$

The proof of the following theorem owes much to the advice of Anthony Pakes, whose idea it was to bound the difference (63) of LS-transforms by means of moment-generating functions.

Theorem 8.2. *Let $0 < \alpha < 1$. The sequence M_n converges in distribution, and the limiting distribution function is*

$$P_M(x; \alpha) = P_W(x; \alpha) \quad , \tag{58}$$

with $P_W(x; \alpha)$ as given by Theorem 6.1.

Proof. Because of Theorem 7.1, Equation (47), the LS-transform $\lambda_{M_n}(y;\alpha)$ of the distribution function of the random variables M_n is

$$\lambda_{M_n}(y;\alpha) = \frac{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}{1-\alpha} \times g_\omega\left(n, \frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}; \alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}\right) . \quad (59)$$

The first factor on the right-hand side of Equation (59) tends to unity as $n \to \infty$. We may therefore concentrate on the term which involves the generating function. Write, for simplicity,

$$\zeta_n := \alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)} . \quad (60)$$

Then we shall prove that

$$\lim_{n\to\infty} g_\omega\left(n, \frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}; \zeta_n\right) = \lim_{n\to\infty} g_\omega(n, e^{-y/E_\omega(n;\alpha)}; \zeta_n) = \lambda_W(y;\alpha) . \quad (61)$$

As compared with the proof of Theorem 6.3, the argument requires a little more care, since we only know that $\lim_{n\to\infty} g_\omega(n, e^{-y/E_\omega(n;\alpha)};\alpha)$ exists and is equal to $\lambda_W(y;\alpha)$, but not whether the same is true for $\lim_{n\to\infty} g_\omega(n, e^{-y/E_\omega(n;\alpha)};\zeta_n)$. To prove that this is indeed the case, consider the sequence $\{Z_n\}$ of random variables

$$Z_n := \frac{\omega_n(\zeta_n)}{E_\omega(n;\alpha)} . \quad (62)$$

Now, because $\frac{1-\alpha}{1-\alpha e^{-(1-\alpha)y/\alpha}} > \frac{1}{1+y} > e^{-y}$ for any $y > 0$, it is straightforward to derive the inequalities

$$0 \le \frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}} - e^{-y/E_\omega(n;\alpha)} < \frac{1}{2\alpha(1-\alpha)} \frac{y^2}{[E_\omega(n;\alpha)]^2} ,$$

and

$$\left(\frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}\right)^k - e^{-yk/E_\omega(n;\alpha)} \le k\left(\frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}\right)^{k-1}\left(\frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}} - e^{-y/E_\omega(n;\alpha)}\right) .$$

It follows that

$$\left| g_\omega\left(n, \frac{1-\alpha}{1-\alpha e^{-y(1-\alpha)/\alpha E_\omega(n;\alpha)}}; \zeta_n\right) - g_\omega(n, e^{-y/E_\omega(n;\alpha)}; \zeta_n)\right| \le \frac{E_\omega(n;\zeta_n)}{[E_\omega(n;\alpha)]^2} \frac{y^2}{2\alpha(1-\alpha)} ,$$

which together with Corollary 2.7 implies that the random variables Z_n and M_n have the same limiting distribution. It remains to show that Z_n is distributed like W_n in the limit $n \to \infty$. To prove this, write $y = E_\omega(n;\alpha)\log(1+\eta/E_\omega(n;\alpha))$ (log denotes natural logarithm) and then check that, because of Equation (9) and Corollary 2.7,

$$|\lambda_{Z_n}(y;\zeta_n) - \lambda_{W_n}(y;\alpha)| = \left| \sum_{z=0}^{\infty} \frac{E_\omega^z(n;\zeta_n)}{[E_\omega(n;\alpha)]^z} \frac{(-\eta)^z}{z!} - \sum_{z=0}^{\infty} \frac{E_\omega^z(n;\alpha)}{[E_\omega(n;\alpha)]^z} \frac{(-\eta)^z}{z!} \right|$$

$$< \sum_{z=0}^{\infty} C_{n,z} \frac{E_\omega^z(n;\alpha)}{[E_\omega(n;\alpha)]^z} \frac{(\eta n^{\alpha-\zeta_n})^z}{z!} \quad , \tag{63}$$

where

$$C_{n,z} = z\eta \frac{\alpha \log n}{E_\omega(n;\alpha)} + z^2\eta \frac{\alpha^2}{nE_\omega(n;\alpha)} + O(n^{-2}) \quad . \tag{64}$$

If we ignore for the moment the coefficients $C_{n,z}$ in (63), it follows from Corollary 2.7 that the remaining series $\sum_{z=0}^{\infty} \frac{E_\omega^z(n;\alpha)}{\left[E_\omega(n;\alpha)\right]^z} \frac{\eta^z}{z!}$ still has an infinite radius of convergence. Therefore, the right-hand side of (63) tends to zero essentially like $\log n/E_\omega(n;\alpha)$. This concludes the proof of Theorem 8.2. □

Incidentally, we may employ (63) to calculate finally the LS-transform of the random variable W. It is sufficient to set $\lambda_{Z_n}(y;\zeta_n) = 0$ and to forget about the absolute value in (63). Invoking Corollary 2.7 once more, we obtain

$$\lambda_W(y;\alpha) = \sum_{z=0}^{\infty} \frac{[\Gamma(2-\alpha)]^z}{\Gamma(z(1-\alpha)+1)}(-y)^z \quad , \tag{65}$$

provided that $\alpha < 1$, since it is only then that η as defined by $y = E_\omega(n;\alpha)\log(1+\eta/E_\omega(n;\alpha))$ tends to y as n tends to infinity. For $\alpha = 1$, we have in fact $\lambda_W(y;1) = \frac{1}{1+\eta} = \mathrm{e}^{-y}$, as expected.

9. Discussion

This paper provides an explicit representation of a Luria–Delbrück distribution that has remained unnoticed for about fifty years. However, the investigation of the model formulated here is not only justified because it allows for such a representation, but also because of its practical and theoretical implications. These concern in particular the derivation of the limit laws (35), (39), and (58). As far as the practical side of the problem is concerned, the evaluation of a fluctuation experiment is a subject well worth of study on its own. It is, however, not necessarily connected with the problem of calculating the Luria–Delbrück distribution. Still, this investigation does contribute to that issue. Theorem 3.1, for instance, tells us that in general one need not worry about cell death during the evaluation of a fluctuation experiment. At first, one may proceed as if the cultures were composed of colony forming cells only. The difference is just that the

mutation rate measured in such an experiment will be larger by a factor $1/(1-\delta)$ in comparison with the true mutation rate α. Now, either the probability that any cell divides into a colony-forming and a dead cell is small, in which case the influence of cell death can be ignored, or it is not, in which case this probability can readily be determined (at least as long as it is constant). In fact, it is clear that for large populations, the number n_c of CFCs in the population will be very close to its expected value $(1-\delta)n$. As mentioned in Section 3.1, both the total number of cells in a population as well as the number of CFCs it contains are readily accessible quantities. The easiest way to determine them is to take aliquots of the culture to be counted under the microscope (possibly after suitable dilution) or to be plated on solid medium. In either case, the results coincide with the true value of n (n_c) only up to an accuracy of at most $\sqrt{n}$ ($\sqrt{n_c}$). If, therefore, one wishes to get an idea about the magnitude of δ, it is necessary that the experiment be conducted such that $n - n_c \cong \delta n \gg \sqrt{n} + \sqrt{n_c}$, i.e. $\delta \gg 2\sqrt{n}/n$, which poses no problem if δ is really so large that cell death must be taken into account.

As far as the alternative model of mutation introduced in Section 3.2 is concerned, we first note that under the assumptions of that section, the probability that the population eventually is composed of mutant cells *only* (which is to say that the population of non-mutant cells becomes extinct) is the same as would be expected for a classical Galton-Watson process. In fact, it is easy to see that under the assumptions of Sections 3.2, the PGF $f(s)$ of the number of non-mutant cells in the progeny of a non-mutant cell is $f(s) = \alpha^{\bullet\bullet} + \alpha^{\bullet}s + (1-\alpha^{\bullet}-\alpha^{\bullet\bullet})s^2$. It is well known that for any Galton-Watson process, the probability q that a population which originates with a single individual finally becomes extinct is the smallest (nonnegative) root of the equation $s = f(s)$. In our case, this implies $q = \frac{\alpha^{\bullet\bullet}}{1-\alpha^{\bullet}-\alpha^{\bullet\bullet}} = \frac{\theta}{1+\theta}$, which is exactly the value predicted by Theorem 6.3. Now, unless the average number of mutant cells per culture in a fluctuation experiment is sufficiently small (such that Theorem 6.3 does not apply), and if none of the N populations in the experiment is composed of mutant cells only (by Theorem 6.3, the probability for this event is approximately $(1+\theta)^{-N}$), one will most likely obtain an ordinary Luria–Delbrück distribution as the outcome of the experiment. Indeed, Theorem 6.3 tells us that the probability that a large culture of bacteria contains fewer than a certain number of wild-type cells, conditional on the fact that it does contain any wild-type cells at all, is just given by $P_W(x;\Theta)$ (one would therefore be tempted to call the parameter $\Theta = \alpha^{\bullet} + 2\alpha^{\bullet\bullet}$ the 'effective' mutation rate).

On the other hand, if the experiment is conducted such that there are only few mutant cells per culture on the average, and if one accepts the fact that under the assumption that wild-type cells *do* occasionally produce two mutants upon division the relevant distribution of mutant cells is given by Theorem 5.2, the picture is a different one. Indeed, if we set $\varphi = 0$ in Equation (32) (such that wild-type cells produce mutants *only* by dividing into two mutant cells), we obtain

$$\lim_{n\to\infty} \psi_\rho\left(n, s; 0, \frac{\phi}{n}\right) = \mathrm{e}^{\phi(1-s)}(1-s)^{2\phi(1-s)/s}$$
$$= \exp\left(-\phi + 2\phi \sum_{i=2}^{\infty} \frac{s^i}{i(i+1)}\right) \quad , \qquad (66)$$

whence it follows that the probability that the culture does not contain any mutant cells is $e^{-\phi}$, the probability that it contains one mutant cell is zero, the probability that it contains two resp. three mutant cells is $e^{-\phi}\frac{\phi}{3}$ resp. $e^{-\phi}\frac{\phi}{6}$, and for four resp. five mutant cells, the respective probabilities are $e^{-\phi}\left(\frac{\phi}{10}+\frac{\phi^2}{18}\right)$ and $e^{-\phi}\left(\frac{\phi}{15}+\frac{\phi^2}{18}\right)$. Thus, for $\phi > 6/5$, the distribution 'zigzags' at least for numbers of mutant cells ≤ 5, and since the distribution is continuous with respect to φ and ϕ, this behavior persists at least for values of φ in some neighborhood of zero. This is of course a reasonable thing to expect (because of the possibility that wild-types divide into two mutant cells, even numbers of mutant cells should be more abundant), and as a similar calculation shows, it is rather different from the behavior of the distribution (32) for $\phi = 0$. However, it is already for $\phi = 3/2$ (and with the additional assumption that $\varphi = 0$) that the probability that the population contains a number of mutants >5 is close to 1/2, and we do not know how pronouncedly the distribution 'zigzags' for these numbers of mutant cells. Furthermore, we may expect that in general $\varphi \gg \phi$, such that for $\phi > 6/5$, the average number of mutants per culture becomes large again. Thus, Theorem 6.3 applies, which predicts that mutant cells should be distributed according to an ordinary Luria–Delbrück distribution (if we ignore the fact that some cultures might not contain any non-mutant cells at all), regardless of the possibility that non-mutant cells might occasionally produce two mutant daughter cells.

Although the assumptions we have imposed on our model may be quite far from being the most general, the picture in this comparatively simple setting appears rather complete. Still, one may object to the model of proliferation underlying the derivation of Equation (1), because cells need to pass through the cell cycle before dividing anew and will not proliferate upon accidentally (say, with probability r/n) being 'invited' to do so. However, the influence of a certain growth model on the distribution of mutant cells does not seem that decisive. Work by Boe et al. (1994) and Tolker-Nielsen and Boe (1994) hints at the possibility that the Haldane distribution (see Sarkar (1991) for the resurrection of the Haldane distribution) might not be substantially different from the one derived from Lea and Coulson's model (1949). Numerical experiments on their own recent model by Lin et al. (1996), which is based on the assumption of cell proliferation proceeding in a completely synchronized manner, have shown that the mutation rate can be rederived from these experiments by applying their own method or the median method of Lea and Coulson (1949) with results differing by at most 30% (although this may seem large, recall that in practice the error in the determination of mutation rates is generally of nearly the same order as the mutation rate itself).

It will be interesting to learn whether this is a consequence of the limit laws in Sections 6 and 8. Indeed, the ratio of the number of mutant (or wild-type) cells to their expected value should be much less responsive to the peculiarities of the mutational process and the underlying model of cell proliferation than the number of mutant cells itself. In fact, we have an example: Theorem 8.2 can be viewed as an instance where a rather peculiar growth model (namely the increase in the number of mutation events in a population) yields exactly the limit law (35). Let us see how this might

come about. Intuitively, one may suspect that the division by $\alpha E_\omega(n;\alpha)$ might be a reasonable way to normalize the number of mutation events, and a rather straightforward calculation confirms intuition inasmuch as the expected number $E_\mu(n;\alpha)$ of mutation events that have occurred in a population of size n turns out to be equal to $\frac{\alpha}{1-\alpha}[E_\omega(n;\alpha)-1]$. Thus, Theorem 8.2 literally claims that the ratio of the number of mutation events to their expected value converges to the probability distribution (35). Incidentally, this also accounts for the appearance of the factor $1-\alpha$ in the definition of the random variables (57). The reason why this factor appears at all is that it is not a fraction α of non-mutant cells that should have produced mutants, but a fraction α of divisions of non-mutant cells. Since any division of a non-mutant cell into one mutant and one non-mutant cell does not alter the number of non-mutant cells in the population, the expected number of wild-type cells tends to underestimate the expected number of divisions of wild-type cells just by the factor $1-\alpha$.

A detailed account of the relation between our model and previous work is beyond the scope of this contribution. However, it would be unduly incomplete if credit were not given to a result of Bartlett's (1955) already alluded to in Section 5. To see what it is about, consider first the generating function in two variables

$$G_\rho(t,s;\alpha) := \sum_{n=1}^{\infty}\sum_{r=0}^{n-1} p_\rho(n,r;\alpha)t^{n-1}s^r \quad .$$

Then, because of Equation (24),

$$\begin{aligned} G_\rho(t,s;\alpha) &= \sum_{k=1}^{\infty}\sum_{n=k}^{\infty} p_\rho(n,n-k;\alpha)(ts)^{n-1}s^{1-k} \\ &= (1-ts)^{-\alpha}\sum_{k=1}^{\infty}[1-(1-ts)^{(1-\alpha)}]^{k-1}s^{1-k} \\ &= \frac{s}{1-ts-(1-s)(1-ts)^\alpha} \quad . \end{aligned} \tag{67}$$

Consider now a single bacterium at time $t=0$, and suppose that the probability that this bacterium or any (mutant or non-mutant) cell in its progeny will divide within the short time interval dt is λdt (this is obviously the continuous-time analogue of the growth model formulated as assumption (iv) in Section 2.1). It is then not too hard to see that the probability that the solitary bacterium will have produced a progeny of size n by the time t is given by $\mathrm{e}^{-\lambda t}(1-\mathrm{e}^{-\lambda t})^{n-1}$ (e.g. Stewart et al. 1990). Therefore, the probability that this bacterium will have produced r mutant cells by the time t is given by the probability that it has produced a progeny of any size times the probability that this progeny contains r mutants, i.e. it is the coefficient of s^r in the generating function

$$\begin{aligned} &\sum_{n=1}^{\infty}\sum_{r=0}^{n-1}\mathrm{e}^{-\lambda t}(1-\mathrm{e}^{-\lambda t})^{n-1}p_\rho(n,r;\alpha)s^r \\ &\qquad = \mathrm{e}^{-\lambda t}G_\rho(1-\mathrm{e}^{-\lambda t},s;\alpha) \end{aligned}$$

$$= \frac{se^{-\lambda t}}{1 - s + s\mathrm{e}^{-\lambda t} - (1-s)(1 - s + s\mathrm{e}^{-\lambda t})^{\alpha}} \quad . \tag{68}$$

Apart from obvious differences in notation, this is exactly the expression derived by Bartlett (1955) (his Equation (12), op. cit., p. 116). It appears strange that this contribution of Bartletts to the theory of bacterial growth and mutation could have been missed even though his book on stochastic processes has been quoted time and again (noteworthy exceptions are Kemp (1994) and, of course, Zheng (1999)). One reason for this may be that, as pointed out by Zheng (1999), the issue of finding an efficient algorithm for the calculation of the probability distribution in (68) is still 'clamoring for solution'. Thus, even if researchers were aware of the generating function (68), they may not have found it useful. Another reason could be an argument of Bartlett's, which, although not erroneous, is at least misleading as it stands. Bartlett argues that the generating function for the probability distribution of the number of mutants in a culture grown from a very large initial number n_0 of bacteria ought to be the n_0^{th} power of the generating function (68), and then sets out to prove that for large n_0 and small α, this expression reduces to Lea and Coulson's generating function (Bartlett 1955). This argument is certainly correct, but it appears to have distracted the attention of researchers from the more exact expression (68).

Acknowledgements. I am indebted to Reinhard Bürger for his continued interest and many useful comments on several previous versions of the manuscript that led to substantial improvement. I owe sincerely thanks to Marek Kimmel, whose review of an earlier version of the paper has encouraged me to investigate the alternative model of mutation introduced in Section 3.2, and who directed my attention to the whole issue of possible applications of fluctuation-analytic methods in population biology. I feel particularly obliged to Anthony Pakes for his tremendously helpful reports on two previous versions of the manuscript and for his support with the proof of Theorem 8.2. The work reproduced in this paper was partially supported by grant P8530-MOB from the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich.

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