Stochastic Modeling of *Chlamydia*

Ph.D. Thesis

Máté Szalai

Supervisor:

Péter Kevei, associate professor, Ph.D.

Doctoral School in Mathematics
University of Szeged, Bolyai Institute

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Introduction

Stochastic modeling of biological systems holds significant importance in the natural sciences, as highlighted in [9]. The interest in mathematical modeling of natural systems has significantly increased over the past century. A large number of researchers have endeavored to comprehend the operation of biological systems through mathematical techniques. The basic approach is to treat a biological phenomenon as a physical state wherein there is an interaction between energy and matter. However, this type of physical state is highly organized, intricate, and complex, akin to a machine where each part is more intricate. Due to this complexity, it is preferable to analyze biological systems via probabilistic models. One of the best tools for modeling natural systems is the branching processes.

1.1 Branching processes

The theory of branching processes is an area of mathematics that describes situations in which an individual exists for a time and then may be replaced by one, two, or more individuals of a similar or different type, see in [15]. This field is thoroughly explored and dynamic, attracting both theoretical inquiry and practical implementation.

Since Francis Galton's examination of the decline of surnames within the British peerage during the 19th century, the theory of branching processes has significantly advanced and made noteworthy contributions to the fields of biology and medicine. In recent times, branching processes have proven to be effective tools for shedding light on various challenges across molecular biology, cell biology, developmental biology, immunology, evolution, ecology, medicine, and related domains. Branching processes have been instrumental for experimentalists and clinicians in comprehending seemingly counterintuitive observations, devising novel experiments and clinical protocols, and offering testable predictions that have been validated in real-world scenarios. For mathematicians, the task of grasping novel biological and clinical findings has served as a ca-

talyst for pioneering advancements in the realm of branching processes, spurring the development of new mathematical frameworks.

A central question in the theory of branching processes is the probability of extinction, wherein no individuals exist after some finite number of generations. If we begin with a single individual in the initial generation, the excepted population size in generation n is m^n, where m represents the expected number of offspring. If $m < 1$ (subcritical process), then the excepted number of individuals decreases swiftly towards zero, indicating extinction with a probability one according to Markov's inequality. Alternatively, if $m > 1$ (supercritical process), then the probability of extinction is less than 1 (though not necessarily zero). If $m = 1$ (critical process), then extinction transpires with a probability one, unless each individual produces exactly one offspring. One of the most frequently used types of branching processes is represented by Galton–Watson processes.

1.1.1 The Galton–Watson process

The Galton–Watson process stands as the earliest, most straightforward, and widely recognized branching process [15]. It can be described as follows.

A single individual exists for precisely one unit of time. Upon its demise, it generates a stochastic number of offspring according to a predetermined probability distribution. Each of the offspring in the first generation behaves autonomously, just as the initial individual did, operating independently of one another. It exists for a single unit of time and generates a random number of offspring. Each of the offspring in the second generation behaves similarly, and this pattern continues for subsequent generations. Because all particles have identical lifespans of one unit, the process can be mathematically represented using a discrete-time index, which corresponds to the number of successive generations. The particle counts Z_n in the successive generations $n \geq 0$ (where generation 0 consists of the single initial individual) constitute a sequence of random variables possessing numerous intriguing properties, such as the Markov property. The properties of the Galton–Watson process offer insights into more intricate branching phenomena.

The simplicity of the Galton–Watson process renders it a suitable and commonly utilized tool for the initial exploration of proliferation processes in biology. It is applicable whenever the assumption of discrete, non-overlapping generations holds true.

1.2 Markov control process

In Chapter 3, and 4 we give a model that leads to a stochastic optimization problem.

In an optimal control problem, we are presented with a dynamical system whose behavior can be influenced or regulated by appropriately selecting certain variables of the system, known as control, action, or decision variables, see in [11]. The controls that can be applied at any given time are selected based on ,,rules" known as control policies. In addition, we are provided with a function known as a performance criterion (or performance index), defined over the set of control policies, which evaluates the system's response to the control policies being employed in some manner. The optimal control problem then entails identifying a control policy that optimizes (i.e. either minimizes or maximizes) the performance criterion.

Optimal control problems are initially classified based on the mathematical model of the system under investigation as (i) deterministic or stochastic, and (ii) continuous-time or discrete-time models. A second classification is into finite or infinite horizon problems, depending on whether the system is to be operated over a finite or an infinite time interval, respectively. A third classification pertains to the form of the performance criterion. Indeed, there are numerous other possible classifications. For instance, one may distinguish problems with full or incomplete state information, with a finite, countable, or uncountable number of states, with constraints or without them, adaptive or not, and so on.

Here, our focus lies on a class of discrete-time, stochastic control systems referred to as Markov control processes (MCPs). These systems are encountered in various fields, including engineering, economics, population control, and the management of renewable and nonrenewable resources.

A discrete-time Markov control model is represented by a five-tuple

$$(X, A, \{A(x), x \in X\}, Q, c), \tag{1.1}$$

where X and A are given sets, referred to as the state space and the control (or action) set, respectively. $\{A(x) : x \in X\}$ is a family of nonempty subsets $A(x)$ of A, where $A(x)$ represents the set of feasible controls (or actions) in the state $x \in X$. Finally, Q represents a transition law, and c denotes a cost-per-stage (or one-stage cost) function. (In some problems, it may be more convenient to consider a reward function r instead of the cost c.)

The control model (1.1) represents a controlled stochastic system observed at times $t = 0, 1, \ldots$. Denoting by x_t and a_t the state of the system and the control (or action) applied at time t, respectively, the evolution of the system may be described as follows. If the system is in

state $x_t = x \in X$ at time t, and the control $a_t = a \in A(x)$ is applied, then two events occur: (i) a cost $c(x, a)$ is incurred, and (ii) the system transitions to the next state x_{t+1}, which is an X-valued random variable with distribution $Q(\cdot \,|\, x, a)$, i.e.,

$$Q(B \,|\, x, a) := \mathbf{P}(x_{t+1} \in B \,|\, x_t = x, a_t = a), \quad B \subset X. \tag{1.2}$$

Once the transition to the new state has occurred, a new control is selected, and the process is repeated. (i) and (ii) are the key characteristics of an MCP; that is, at any given time, the cost (or reward) and the transition law depend solely on the current state of the system and the current action.

For now, let us interpret a control policy as a sequence $\pi = \{a_t\}$ of control actions which are feasible in the sense that $a_t \in A(x_t)$ for all $t = 0, 1, \ldots$, and let Π be the set of all policies. A policy π and an initial state $x_0 = x$ determine a "Markov-like" stochastic process called a Markov control process (MCP)-also known as a Markov decision process (MDP).

In many applications, the evolution of an MCP is specified by a discrete time (or difference) equation of the form

$$x_{t+1} = F(x_t, a_t, \xi_t), \quad t = 0, 1, \ldots; \quad x_0 \text{ given}, \tag{1.3}$$

where $\{\xi_t\}$ is a sequence of independent and identically distributed (iid) random variables with values in some space S and common distribution μ, and independent of the initial state x_0. In this case, the transition law Q in (1.2) is given by

$$\begin{aligned} Q(B|x, a) &= \mu(\{s \in S | F(x, a, s) \in B\}) \\ &= \int_S \mathbb{I}_B[F(x, a, s)]\mu(\mathrm{d}s) \\ &= \mathbf{E}\mathbb{I}_B[F(x, a, , \xi)], \end{aligned} \tag{1.4}$$

where $\mathbb{I}_B(\cdot)$ stands for the indicator function of the set B, and ξ stands for a generic random variable with distribution μ. To specify an optimal control problem, in addition to a dynamic system and a set of policies, we require a performance criterion, also known as a performance index or objective function. In our case, a typical performance criterion is the expected total cost up to a certain time N, denoted as:

$$J_N(\pi, x) := \mathbf{E}_x^\pi \left[\sum_{t=0}^{N} c(x_t, a_t) \right], \tag{1.5}$$

where $\mathbf{E}_x^\pi$ stands for the expected value when using the policy $\pi = \{a_t\}$, given the initial state $x_0 = x$. Then the optimal control problem is to

minimize the function $\pi \to J_N(\pi, x)$ over Π, for all x. A policy π^* such that

$$J_N(\pi^*, x) = \inf_{\Pi} J_N(\pi, x), \quad \text{for all } x \in X \tag{1.6}$$

is said to be an optimal policy, and the minimum cost (1.6), i.e.,

$$J_N^*(x) := \inf_{\Pi} J_N(\pi, x), \quad x \in X,$$

is referred to as the control problem's value function or optimal cost.

Finally, if the one-stage cost $c(x, a)$ is replaced by a one-stage reward (or revenue or income) function $r(x, a)$, then the resulting optimal control problem is to maximize the given performance criterion.

Example 1. *Portfolio selection.* This example concerns the problem faced by a "small investor" (i.e., an economic agent whose actions cannot influence the market prices) who has to decide on the best consumption-investment strategy, given that he/she wishes to allocate the total investment among various assets with different rates of return. We consider two assets: one of them is a risk-free or safe asset (e.g., a bond) with a fixed interest rate i, and the other one is a risky asset (stock) with a stochastic rate of return ξ_t of investment at time t. A consumption-investment policy is a sequence $\pi = \{(p_t, c_t), t = 0, 1, \ldots\}$ consisting of a *portfolio process* $\{p_t\}$ and a *consumption process* $\{c_t\}$. That is, at each time t, p_t (resp. $1 - p_t$) is the fraction of wealth invested in the stock (resp. the safe asset), and c_t is the amount of wealth consumed; they must satisfy the constraints

$$0 \le p_t \le 1, \quad 0 \le c_t \le x_t, \tag{1.7}$$

where x_t denotes the investor's wealth at time t. Thus, the state or wealth process $\{x_t\}$ evolves according to the equation

$$x_{t+1} = [(1 - p_t)(1 + i) + p_t \xi_t](x_t - c_t), \quad t = 0, 1, \ldots, \tag{1.8}$$

with a given initial wealth $x_0 = x > 0$.

In this example, we may take the state space $X = \mathbb{R}_+ := [0, \infty)$ and the control set $A = [0, 1] \times \mathbb{R}_+$. From (1.7), the set of feasible controls $a = (p, c)$ is $A(x) = [0, 1] \times [0, x]$ whenever the state or wealth is x. Assuming that $\{\xi_t\}$ is a sequence of iid random variables with distribution μ, the transition law Q is determined from (1.8), as in (1.3)-(1.4). Finally, to complete the specification of a Markov control model in the form (1.1) we introduce a one-stage reward function $r(x, a)$ (instead of a cost c). A typical choice of r in financial economics is a "utility from consumption" i.e., with $a = (p, c) \in A(x)$,

$$r(x, a) := u(c),$$

where u is a given "utility" function. The corresponding optimal control problem is of course to maximize this criterion over the set of all consumption-investment policies that satisfy (1.7).

1.3 Biological outlook

This thesis predominantly incorporates real measurement results for modeling, with the tested organism being predominantly the bacterial species *Chlamydia trachomatis*. In Figure 2.3 and 2.4, we can observe measurement results obtained from the Department of Medical Microbiology and Immunobiology, University of Szeged. Figure 3.1 shows temporal analysis of chlamydial developmental forms using a three-dimensional electron microscopy approach, [16].

Chlamydia trachomatis infections, which are sexually transmitted, pose a significant global public health challenge. These infections affect millions of individuals worldwide, including men, women, and children, often leading to severe medical complications. *Chlamydiae* are obligate intracellular bacteria that primarily infect epithelial cells of the conjunctiva, respiratory tract and urogenital tract.

Chlamydiae have an unique developmental cycle, with two phenotypic bacterial forms, the elementary body (EB) and the reticulate body (RB), see in Figure 1.1. The EB is the infectious form, the RB multiplies in the host cell by binary fission in the inclusion, which is a specific area of the infected host cell.

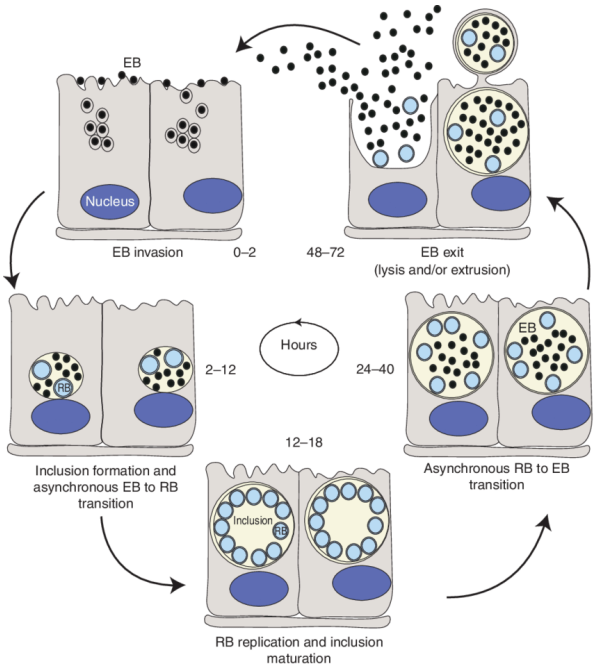

Figure 1.1: Life cycle of *Chlamydia* [2].

Within the initial 2 hours following internalization into cells, EBs merge to establish a nascent inclusion. Between 2 and 6 hours post-internalization, EBs initiate differentiation into RBs. By 12 hours post-infection (hpi), RBs are observed dividing through binary fission, and their numbers peak by 18 to 24 hpi. Subsequently, an increasing number of RBs revert back to EBs around 24 hpi and continue this differentiation process until lysis or release occurs between 48 and 72 hpi, depending on the species of *Chlamydia* involved. The EBs are ready to infect new host cells.

This unique life-cycle triggered a lot of mathematical work to model the growth of the population. Wilson [25] worked out a deterministic model taking into account the infected and uninfected host cells and the extracellular *Chlamydia* concentration. Wan and Enciso [24] formulated a deterministic model for the quantities of RB's and EB's, and solved an optimal control problem to maximize the quantity of EB'swhen the host cell dies. The same problem in a stochastic framework was investigated by Enciso et al. [6] and Lee et al. [16]. Bornali Das (from the University of Szeged) devotes her entire thesis to deterministic modeling of *Chlamydia trachomatis* [4]. They have provided, among others, an optimal control for *Chlamydia* treatment.

There is a third form of the bacterium, the aberrant body or persistent body. This form of the bacterium is induced by various adverse environmental stimuli, such as the lack of nutrients and the presence of antibiotics, see Panzetta et al. [19]. The persistent body is not capable to multiply. After elimination of the stress stimuli, the persistent body may reenter the normal developmental cycle, differentiates to RB, multiplies and redifferentiates to EB. If there is an excess of antibiotics reaching the so-called bactericide concentration, the bacterium is killed, and no multiplication can be observed. A lower antibiotic concentration does not kill all of the bacterium, but leads to the formation of non-multiplying aberrant bodies. Further lowering the antibiotic concentration more RB can be observed, while the formation of aberrant body decreases. At very low antibiotic concentration, the antibiotic has no effect on the bacterial growth and all the bacteria enter the normal developmental cycle.

This thesis is structured as follows. In Chapter 2, we provide a Galton–Watson model for the growth of a bacterial population in the presence of antibiotics. Chapter 2 is based on [3]. We assume that bacterial cells either die or duplicate, and the corresponding probabilities depend on the concentration of the antibiotic. Assuming that the mean offspring number is given by $m(c) = 2/(1 + \alpha c^{\beta})$ for some α, β, where c stands for the antibiotic concentration we obtain weakly consistent, asymptotically normal estimator both for (α, β), and for the minimal inhibitory concentration (MIC), a relevant parameter in pharmacology. For the measurements of *Chlamydia* growth quantitative polymerase chain reac-

tion (qPCR) technique was used. The 2-parameter model fits remarkably well to the biological data.

Section 2.6 is a part of Chapter 2, based on [13]. The model assumption is entirely similar, as in Chapter 2; however, the measurement method differs. We assume that bacterial cells either die or duplicate, with probabilities $p_0(c)$, and $p_2(c)$, where $p_2(c) = 1/(1 + \alpha c^\beta)$ for some positive real numbers α, β. Using measurements based on colony counting method we obtain weakly consistent, asymptotically normal estimator for the parameters.

In Chapter 3, we explore the unique life cycle of *Chlamydia*. Chapter 3 is based on [14]. We model the population growth by a 2-type discrete-time branching process, where the probability of duplication depends on the state. Maximizing the EB production leads to a stochastic optimization problem. Simulation study shows that our novel model is able to reproduce the main features of the development of the population, something deterministic models had not been able to achieve until now.

In Chapter 4, we establish a connection with our previous findings. Specifically, at a given antibiotic concentration, we determine the optimal transition of *Chlamydia* from the RB form to the EB form. First and foremost, we assume that the antibiotic solely affects the RB body, but not the EB body. This assumption is biologically plausible since EB bodies have the capability to form inclusions, aiding their survival under adverse conditions. In this scenario, we can numerically determine the optimal strategy, see in Section 4.1. In the alternate case, we assume that the antibiotic affects both the RB and EB bodies, see in Section 4.2. It is important to emphasize that, as far as our knowledge extends, there is no real-world measurement data available for these models.

2

Stochastic Modeling of In Vitro Bactericidal Potency

Since the discovery of penicillin, antibiotics have been used increasingly worldwide to treat bacterial infections. However, due to the easy access to, and the general misuse of antibiotics some bacteria became resistant to them. These *superbugs*, the extensively drug resistant bacterias, are one of the main threats of the future. Resistance appears in bacterial population naturally due to random mutation. By killing the vulnerable bacteria the use of antibiotics increase the portion of resistant bacteria in the population. Therefore, for the safe and proper use of antibiotics the determination of bactericidal potency is of the utmost importance.

In this Chapter the bacterial population is modeled by a special two-type Galton–Watson branching process, where the types represent the alive and dead bacterias, respectively. We assume that the offspring distribution, in particular the offspring mean $m(c)$ depends on the antibiotic concentration $c > 0$ as

$$m(c) = m_{\alpha,\beta}(c) = \frac{2}{1 + \alpha c^{\beta}}, \tag{2.1}$$

where $\alpha > 0$, $\beta > 0$ are unknown parameters. This flexible 2-parameter model captures the basic features of antibiotic dependence: (1) $m(0) = 2$, that is each bacterial cell duplicates in an antibiotic-free environment; (2) m is monotone decreasing and continuous, that is increasing the antibiotic concentration decreases the chance to duplicate; (3) $\lim_{c\to\infty} m(c) = 0$ that is sufficiently large antibiotic concentration kills the bacteria. Under this model the minimal inhibitory concentration (MIC), the smallest antibiotic concentration preventing bacterial growth, is the smallest c for which $m(c) = 1$, that is $\alpha^{-1/\beta}$. MIC is a very important parameter in pharmacology. Its estimation is rather troublesome, since due to the usual two-fold dilution technique one can observe only the bacterial growth under antibiotic concentration $c_0, 2c_0, \ldots, 2^k c_0$. Therefore one can claim only that the MIC belongs to some interval $[c, 2c]$, or give an

upper bound for it. Based on measurements at different concentrations we obtain weakly consistent asymptotically normal estimator both for (α, β), and for the MIC. Although the mathematical model has only 2 parameters, it fits remarkable well to real data.

We assume that the bacterial population is homogeneous, all the cells behave similarly. In particular, there is no resistant type. As mutation is rare under normal conditions and in short time, this is a natural assumption for our data set. Long-term evolution of bacterial populations with both *resistant* and *susceptible* types was investigated in several papers using deterministic models, see Svara and Rankin [22] and Paterson et al. [21], and the references therein. Closest to our model is the deterministic model given by Liu et al. [17]. In Liu et al. [17] a deterministic expression for the number of colony forming units is obtained in terms of the antibiotic concentration.

Branching processes are classical tools to model cell proliferation, see the monographs by Haccou et al. [10], Kimmel and Axelrod [15]. However, to the best of our knowledge for estimation of bactericidal potency of antibiotics only deterministic models are used.

In the experiments growth of *Chlamydia trachomatis* bacterial population was analyzed by quantitative PCR (qPCR) method with 12 different antibiotic concentrations and 2 different antibiotics.

Azithromycin and doxycycline are the most commonly used antibiotics in *Chlamydia* infections (Miller [18]), but *Chlamydiae* are also sensitive to quinolone type antibiotics (Vu et al. [23]). Now *Chlamydia trachomatis* infected cells were treated with azithromycin and the quinolone ciprofloxacin. The dose response curves, the concentration dependent impacts of these antibiotics on chlamydial growth were measured 48 hours post infection. A major challenge is the accurate measurement of chlamydial growth. The golden standard is the immunofluorescent labeling and manual counting of the chlamydial inclusions. This very tedious but precise method was used recently in Lee et al. [16]. Instead of counting the bacterial cells, the quantity of bacterial genomes (which is a constant times the number of bacteria) can also be measured. Chlamydial genome concentration in the infected host cells can be measured by qPCR technique. This method is accurate and theoretically measures the genome of all individual bacteria. Eszik et al. [7] developed a version of the qPCR, the so-called direct qPCR method for chlamydial growth monitoring. Direct qPCR is capable to perform qPCR measurements without the labor-intensive deoxyribonucleic acid (DNA) purification.

The qPCR method gives a so-called cycle threshold (Ct) value to each bacterial sample. If the effectivity of the qPCR is 100%, then in the exponential phase of the PCR, when there are enough reagents the amount of PCR product doubles in each cycle. In a qPCR experiment the amount of the PCR product is monitored continuously after each

qPCR cycle. The less is the original amount of qPCR template (here *Chlamydia trachomatis* DNA) the higher number of cycles are needed to reach a certain level of PCR product (in fact fluorescence intensity). Fixing a threshold level the needed cycle number is the Ct value, see e.g.Yuan et al. [26]. As an example, if sample A has a Ct value of 22 and sample B has a Ct value of 24, then sample A contains 4 times as much chlamydial DNA than sample B. Therefore, the theoretical Ct value equals $a - \log_2 Z^{(i)}_{n;c,x_0}$, where $a \in \mathbb{R}$ is an unknown constant, which depends on the choice of the threshold level, and can be estimated as described in (2.30) below, and $Z^{(i)}_{n;c,x_0}$ stands for the total number of dead and alive bacterial cells at antibiotic concentration $c > 0$, after n generations starting with x_0 bacterias, in experiment i. Adding a measurement error, the measurements have the form

$$C_i(c, x_0) = a - \log_2 Z^{(i)}_{n;c,x_0} + \varepsilon_{i;c}, \qquad i = 1, \dots, N, \tag{2.2}$$

where measurement error $\varepsilon_{i;c}$ is assumed to be Gaussian with mean zero, and variance σ^2_ε. This simple linear model is suggested by Yuan et al. [26]. Due to the measurement method lower the Ct value means higher genome concentration. The dose response curves measured by a direct qPCR method were used in this paper, see Figures 2.3 and 2.4.

The rest of this Chapter is organized as follows. The model and some basic properties are given in Section 2.1. The estimator of $m(c)$ for c fixed is provided in Section 2.2, while in Section 2.3 we consider different antibiotic concentrations together. Section 2.4 contains a small simulation study, and real data is analyzed in Section 2.5.

2.1 The theoretical model

We consider a simple Galton–Watson branching process where the offspring distribution depends on the antibiotic concentration $c \geq 0$. Each bacteria either dies (leaves no offspring), survives (leaves 1 offspring), or divides (leaves 2 offsprings) with respective probabilities $p_0 = p_0(c)$, $p_1 = p_1(c)$, and $p_2 = p_2(c)$. Let $f(s) = f_c(s)$ denote the offspring generating function and $m = m(c)$ the offspring mean if the antibiotic concentration is c, i.e.

$$\begin{aligned} f(s) = f_c(s) = \mathbf{E}s^{\xi_c} = \sum_{i=0}^{2} p_i(c) s^i, \quad s \in [0,1], \\ m = m(c) = f_c'(1) = \mathbf{E}\xi_c, \end{aligned} \tag{2.3}$$

where ξ_c is the number of offsprings. The process starts with $X_0 = x_0$ initial individuals, and

$$X_{n+1;c} = \sum_{i=1}^{X_{n;c}} \xi^{(n)}_{i;c}, \tag{2.4}$$

where $\{\xi_c, \xi_{i;c}^{(n)} : i \geq 1, n \geq 1\}$ are iid random variables with generating function f_c. Note that the offspring distribution does depend on the antibiotic concentration c, but here and in the next section we suppress this dependence from the notation.

Using the qPCR method the observed quantity is the genom of all individual bacteria, which is a constant times the *total* number of bacteria, that is live and dead cells together. Therefore, we have to keep track of the dead bacterias too. In order to do this we consider a two-type Galton–Watson branching process $\mathbf{X}_n = (X_n, Y_n)$, $n \geq 0$, where X_n, Y_n stands for the number of alive, dead bacterias respectively, in generation n. Then the total number of bacteria at generation n is $Z_n = X_n + Y_n$. We also write Z_{n,x_0} to emphasize that $X_0 = x_0$. The process evolves as

$$\begin{aligned} X_{n+1} &= \sum_{i=1}^{X_n} \xi_i^{(n)} \\ Y_{n+1} &= Y_n + \sum_{i=1}^{X_n} \eta_i^{(n)}, \quad n \geq 0, \end{aligned} \tag{2.5}$$

$(X_0, Y_0) = (x_0, 0)$, where $(\xi, \eta), (\xi_i^{(n)}, \eta_i^{(n)})$, $n = 1, 2, \ldots$, $i = 1, 2, \ldots$ are iid random vectors such that $\mathbf{P}((\xi, \eta) = (0, 1)) = p_0$, $\mathbf{P}((\xi, \eta) = (1, 0)) = p_1$, $\mathbf{P}((\xi, \eta) = (2, 0)) = p_2$. The offspring mean matrix $\mathbf{M}$ has the form

$$\mathbf{M} = \begin{pmatrix} \mathbf{E}\xi & \mathbf{E}\eta \\ 0 & 1 \end{pmatrix} = \begin{pmatrix} m & p_0 \\ 0 & 1 \end{pmatrix}.$$

In this $\mathbf{M}$ matrix, the first row and the first column represent the living individuals, while the second row and the second column represent the dead individuals. Next we determine the mean vector of $\mathbf{X}_n$.

Lemma 1. *If $x_0 = 1$ then for the mean we have $\mathbf{E}X_n = m^n$, and $\mathbf{E}Y_n = p_0(1 + m + \ldots + m^{n-1})$, thus*

$$\mu_n := \mathbf{E}Z_{n,1} = \begin{cases} m^n \left(1 + \frac{p_0}{m-1}\right) - \frac{p_0}{m-1}, & m \neq 1, \\ 1 + p_0 n, & m = 1. \end{cases}$$

Furthermore

$$\mathbf{Var}X_n = \begin{cases} \frac{\sigma^2 m^{n-1}(m^n - 1)}{m-1}, & m \neq 1, \\ n\sigma^2, & m = 1, \end{cases} \tag{2.6}$$

where $\sigma^2 = \mathbf{Var}\xi$. For $m \neq 1$

$$\begin{aligned} \mathbf{Var}(Y_n) = {} & m^{2n} \frac{p_0^2 \sigma^2}{m(m-1)^3} - n m^n \frac{2p_0^2 \nu}{m(m-1)^2} \\ & + m^n \left(\frac{p_0(p_0\nu + m(m-1))}{m(m-1)^2} + \frac{2p_0^2}{(m-1)^2} \right) \\ & - \frac{p_0(p_0\nu + (m-1)^2)}{(m-1)^3} - \frac{p_0^2}{(m-1)^2}, \end{aligned}$$

with $\nu = \sigma^2 + m(m-1)$, *and*

$$\mathbf{Cov}(X_n, Y_n) = m^{2n}\frac{p_0\sigma^2}{m(m-1)^2} - m^n\frac{p_0}{m(m-1)}\left(n\nu + \frac{\sigma^2}{m-1}\right),$$

while for $m = 1$

$$\mathbf{Var}(Y_n) = p_0^2\sigma^2\frac{(n-1)n(2n-1)}{6} + p_0 n - p_0^2 n^2,$$

and

$$\mathbf{Cov}(X_n, Y_n) = p_0\sigma^2\frac{n(n-1)}{2} - p_0 n.$$

Proof of Lemma 1.

$$\mathbf{E}\left[\mathbf{X}_{n+1}|\mathbf{X}_n\right] = \begin{pmatrix} mX_n \\ p_0X_n + Y_n \end{pmatrix} = \mathbf{X}_n\mathbf{M},$$

thus

$$\mathbf{E}\mathbf{X}_n = \mathbf{X}_0\mathbf{M}^n.$$

We have, by induction on n that

$$\mathbf{M}^n = \begin{pmatrix} m^n & p_0(1 + \ldots + m^{n-1}) \\ 0 & 1 \end{pmatrix},$$

thus

$$\begin{aligned} \mathbf{E}Z_n &= m^n + p_0(1 + m + \ldots + m^{n-1}) \\ &= \begin{cases} m^n\left(1 + \frac{p_0}{m-1}\right) - \frac{p_0}{m-1}, & \text{if } m \neq 1, \\ 1 + np_0, & \text{if } m = 1, \end{cases} \end{aligned} \tag{2.7}$$

as claimed.

To ease notation put

$$p_0 \circ X_n = \sum_{i=1}^{X_n} \eta_i^{(n)}.$$

Then

$$Y_n = Y_{n-1} + p_0 \circ X_{n-1}$$

thus

$$Y_n^2 = Y_{n-1}^2 + 2Y_{n-1}(p_0 \circ X_{n-1}) + (p_0 \circ X_{n-1})^2.$$

Conditioned on X_{n-1} the variable $p_0 \circ X_{n-1}$ has binomial distribution, therefore

$$\mathbf{E}(p_0 \circ X_{n-1})^2 = \mathbf{E}\left[X_{n-1}p_0(1-p_0) + X_{n-1}^2p_0^2\right].$$

Since (X_n) is a single type GW process, (2.6) holds. Furthermore,

$$\mathbf{E}[Y_{n-1}\,(p_0 \circ X_{n-1})] = p_0 \mathbf{E} X_{n-1} Y_{n-1}.$$

Summarizing

$$\mathbf{E}Y_n^2 = \mathbf{E}Y_{n-1}^2 + 2p_0\mathbf{E}X_{n-1}Y_{n-1} + p_0(1-p_0)\mathbf{E}X_{n-1} + p_0^2\mathbf{E}X_{n-1}^2. \quad (2.8)$$

Next we obtain a recursion of $\mathbf{E}X_nY_n$. Using the definition

$$\begin{aligned}\mathbf{E}X_nY_n &= \mathbf{E}\sum_{i=1}^{X_{n-1}} \xi_i^{(n-1)} \left(Y_{n-1} + \sum_{i=1}^{X_{n-1}} \eta_i^{(n-1)} \right) \\ &= m\mathbf{E}X_{n-1}Y_{n-1} + \mathbf{E}\sum_{i=1}^{X_{n-1}} \xi_i^{(n-1)} \sum_{i=1}^{X_{n-1}} \eta_i^{(n-1)}.\end{aligned}$$

For fixed k, using that (ξ_i, η_i) are iid

$$\mathbf{E}\sum_{i=1}^{k}\xi_i \sum_{i=1}^{k}\eta_i = \sum_{i=1}^{k}\mathbf{E}\eta_1\xi_1 + k(k-1)\mathbf{E}\xi\mathbf{E}\eta = k(k-1)mp_0.$$

Substituting back

$$\mathbf{E}X_nY_n = m\mathbf{E}X_{n-1}Y_{n-1} + mp_0\mathbf{E}X_{n-1}(X_{n-1}-1).$$

Using induction and (2.6) we obtain the closed formula

$$\begin{aligned}\mathbf{E}X_nY_n &= \sum_{i=1}^{n-1} p_0 m^i \mathbf{E}X_{n-i}(X_{n-i}-1) \\ &= \begin{cases} m^n \frac{p_0\nu}{(m-1)m}\left(\frac{m^n}{m-1} - \left(n + \frac{1}{m-1}\right)\right), & m \neq 1, \\ p_0\sigma^2\frac{n(n-1)}{2}, & m = 1, \end{cases}\end{aligned}$$

where, to ease notation we put $\nu = \sigma^2 + m(m-1)$. From (2.8)

$$\mathbf{E}Y_n^2 = 2p_0\sum_{i=0}^{n-1}\mathbf{E}X_iY_i + p_0(1-p_0)\sum_{i=0}^{n-1}\mathbf{E}X_i + p_0^2\sum_{i=0}^{n-1}\mathbf{E}X_i^2, \quad (2.9)$$

where we have a closed formula for all the ingredients.

First assume that $m \neq 1$. For the last two terms simply

$$\begin{aligned}\sum_{i=0}^{n-1}\mathbf{E}X_i &= \frac{m^n-1}{m-1}, \\ \sum_{i=0}^{n-1}\mathbf{E}X_i^2 &= \frac{\nu}{m(m-1)}\frac{m^{2n}-1}{m^2-1} - \frac{\sigma^2}{m(m-1)}\frac{m^n-1}{m-1}.\end{aligned}$$

For the first term, using that

$$\sum_{i=1}^{n-1} i\, m^i = \frac{1}{(m-1)^2}\left[nm^n(m-1) - m^{n+1} + m\right],$$

we obtain

$$\begin{aligned}\sum_{i=0}^{n-1} \mathbf{E}X_iY_i &= \frac{p_0\nu}{m(m-1)^2}\frac{m^{2n}-1}{m^2-1} - \frac{p_0\nu}{m(m-1)^2}\frac{m^n-1}{m-1} \\ &\quad - \frac{p_0\nu}{m(m-1)}\frac{1}{(m-1)^2}\left[nm^n(m-1) - m^{n+1} + m\right] \\ &= m^{2n}\frac{p_0\nu}{m(m-1)^3(m+1)} - (n-1)m^n\frac{p_0\nu}{m(m-1)^2} - \frac{p_0\nu m}{(m-1)^3(m+1)}.\end{aligned}$$

Substituting back into (2.9)

$$\begin{aligned}\mathbf{E}Y_n^2 &= m^{2n}\frac{p_0^2\nu}{m(m-1)^3} - nm^n\frac{2p_0^2\nu}{m(m-1)^2} + m^n\frac{p_0(p_0\nu + m(m-1))}{m(m-1)^2} \\ &\quad - \frac{p_0(p_0\nu + (m-1)^2)}{(m-1)^3}.\end{aligned}$$

While for $m = 1$ substituting back into (2.9) after some calculation we obtain

$$\mathbf{E}Y_n^2 = p_0^2\sigma^2\frac{(n-1)n(2n-1)}{6} + p_0 n.$$

Substituting into the definition of variance and covariance, the result follows. □

The strong law of large numbers and the central limit theorem imply that for each fixed n as $x_0 \to \infty$

$$\frac{Z_{n,x_0}}{x_0} \to \mu_n \quad \text{a.s.} \tag{2.10}$$

and

$$\frac{Z_{n,x_0} - x_0\mu_n}{\sqrt{x_0}} \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_n^2), \tag{2.11}$$

where $\xrightarrow{\mathcal{D}}$ stands for convergence in distribution, and

$$\sigma_n^2 = \mathbf{Var}(Z_n) = \mathbf{Var}(X_n) + \mathbf{Var}(Y_n) + 2\mathbf{Cov}(X_n, Y_n).$$

It is clear that the geometric growth rate of $\mathbf{E}Z_n$ is the offspring mean m, while the precise distribution determines only the constant factor. Simple analysis shows that if $m = p_1 + 2p_2 > 1$ then

$$m^n \le \mu_n = \frac{p_2 m^n - p_0}{m-1} \le \frac{m(m^n-1)}{2(m-1)} + 1, \tag{2.12}$$

if $m = 1$ then

$$1 \le \mu_n = 1 + p_0 n \le 1 + \frac{n}{2}, \tag{2.13}$$

while for $m < 1$

$$1 \le \mu_n = \frac{p_0 - p_2 m^n}{1 - m} \le \frac{m(1 - m^n)}{2(1 - m)} + 1. \tag{2.14}$$

The upper bound is attained at $(p_0, p_1, p_2) = (1 - m/2, 0, m/2)$, while the lower bound is attained at $(p_0, p_1, p_2) = (0, 2 - m, m - 1)$ for $m \ge 1$, and at $(p_0, p_1, p_2) = (1 - m, m, 0)$ for $m \le 1$.

The process (X_n) is a single type Galton–Watson process with offspring mean $m = p_1 + 2p_2$. If $m \le 1$ then the process dies out almost surely, that is $X_n = 0$ for some n (if $m = 1$ we exclude the degenerate case $p_1 = 1$) while if the process is supercritical, i.e. $m > 1$ then the probability of extinction is the smaller root of $f(q) = q$, which is $q = p_0/p_2$; see e.g. Theorem 5.2 in Haccou et al. [10]. By the martingale convergence theorem

$$\frac{X_n}{m^n} \to W \quad \text{a.s.}, \tag{2.15}$$

where W is a nonnegative random variable. For $m \le 1$ clearly $W \equiv 0$, while if $m > 1$ then $\mathbf{P}(W = 0) = q$, and the distribution of W is absolutely continuous on $(0, \infty)$.

The process $\mathbf{X}_n = (X_n, Y_n)$ is decomposable, which, in the 2-type case only means that type-2 individual cannot have type-1 offspring. Limit theorems for supercritical decomposable processes were obtained by Kesten and Stigum [12]. The eigenvalues of $\mathbf{M}$ are m and 1, therefore the process is supercritical if and only if $m > 1$. The left eigenvector corresponding to m is

$$u = \left(u_1, \frac{p_0}{m - 1} u_1 \right).$$

Applying Theorem 2.1 by Kesten and Stigum [12] we obtain for $m > 1$ that

$$\lim_{n \to \infty} \frac{1}{m^n} (X_n, Y_n) = W \left(1, \frac{p_0}{m - 1} \right),$$

where W is the nonnegative random variable from (2.15).

2.2 Estimation of the offspring mean

Recall that the measurements are given in the form (2.2), where $Z^{(i)}_{n;c,x_0}$ stands for the total number of dead and alive bacteria at generation n

starting with x_0 bacteria under antibiotic concentration c at experiment i, $i = 1, \ldots, N$. We assume that the sequence $\{\varepsilon_{i;c} : i \geq 1, c \geq 0\}$ is iid, independent of the process $\mathbf{X}_n$, and Gaussian with mean 0 and variance σ_ε^2.

By (2.11), an application of the delta method (see e.g. Agresti [1] Section 14.1) implies as $x_0 \to \infty$, for any $i = 1, 2, \ldots, N$

$$\sqrt{x_0} \log_2 \left(1 + \frac{Z_{n;c,x_0}^{(i)} - x_0 \mu_n}{x_0 \mu_n} \right) \xrightarrow{\mathcal{D}} \mathcal{N}\left(0, \sigma_n^2 (\mu_n \log 2)^{-2}\right), \tag{2.16}$$

in particular, as $x_0 \to \infty$

$$\log_2 Z_{n;c,x_0}^{(i)} - \log_2(x_0 \mu_n) \xrightarrow{\mathbf{P}} 0, \tag{2.17}$$

where $\xrightarrow{\mathbf{P}}$ stands for convergence in probability. In the following, we frequently use the delta method. Put

$$\log_2 \widehat{\mu}_n = a - \log_2 x_0 - \frac{\sum_{i=1}^N C_i(c, x_0)}{N}.$$

In the next results both x_0 and N tend to infinity. Taking iterated limits are always understood as first $x_0 \to \infty$ and then $N \to \infty$. The next statement is a simple consequence of (2.16), (2.17), the law of large numbers, and the central limit theorem.

Proposition 1. *As first $x_0 \to \infty$ and then $N \to \infty$*

$$\log_2 \widehat{\mu}_n \xrightarrow{\mathbf{P}} \log_2 \mu_n,$$

which implies that $\widehat{\mu}_n$ is weakly consistent estimatior of μ_n. Furthermore, as first $x_0 \to \infty$ and then $N \to \infty$

$$\frac{1}{\sigma_\varepsilon} \sqrt{N} \left[\log_2 \widehat{\mu}_n - \log_2 \mu_n \right] \xrightarrow{\mathcal{D}} \mathcal{N}(0, 1),$$

which implies that

$$\frac{1}{\sigma_\varepsilon \mu_n \log 2} \sqrt{N} \left(\widehat{\mu}_n - \mu_n \right) \xrightarrow{\mathcal{D}} \mathcal{N}(0, 1),$$

Thus we can estimate μ_n. The problem is that μ_n does not determine uniquely m, only gives a possible range for it. This range can be deduced from the sharp bounds in (2.12), (2.13), (2.14). In Figure 2.1 we see the corresponding upper and lower bounds for $\log_2 \mu_n$ for $n = 10$. If $\log_2 \mu_{10} = 8$ we can deduce that m has to be in the range $(1.709, 1.741)$, while if $\log_2 \mu_{10} = 1$, than $m \in (0.671, 1.072)$. The larger values of $\log_2 \mu_n$ imply more precise bouns for m. Furthermore, larger n also implies more

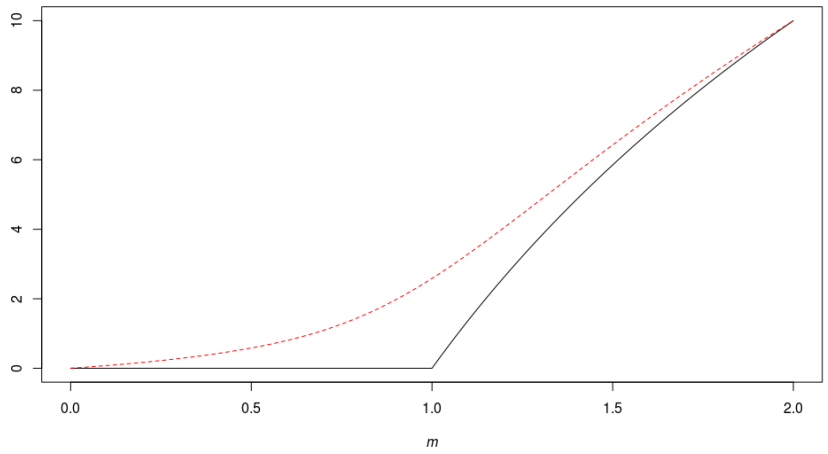

Figure 2.1: Upper and lower bound for $\log_2 \mu_n$ for $n = 10$.

precise bounds. However, for $m \leq 1$ one cannot determine the value m from Z_i. This is reasonable, since for both $p_0 = 1$ and $p_1 = 1$ we have $\mu_n = 1$, whereas $m = 0$ in the former and $m = 1$ in the latter case.

To overcome this difficulty, we assume that $p_1 \equiv 0$. This is clearly reasonable for *bactericide* antibiotics, which either kill the bacteria, or let it duplicate. While, if a *bacteriostatic* antibiotic blocks the duplication of a single bacteria then it keeps blocking in the later generations as well. Therefore, we can equally count a 'blocked' bacteria as a dead one.

Assume now that $p_1 \equiv 0$. Then μ_n is Lemma 1 simplifies to

$$\mu_n(m) = \frac{m}{2}\left(m^{n-1} + \ldots + 1\right) + 1 = \begin{cases} \frac{m(m^n-1)}{2(m-1)} + 1, & m \neq 1, \\ \frac{n}{2} + 1, & m = 1. \end{cases} \tag{2.18}$$

Then μ_n is a strictly increasing convex function, $\mu_n(0) = 1$, $\mu_n(2) = 2^n$. Its inverse function $\psi_n : [1, 2^n] \to [0, 2]$ is continuous strictly increasing. Define the estimate

$$\widehat{m} = \psi_n(\widehat{\mu}_n). \tag{2.19}$$

From Proposition 1 it follows that $\widehat{m}$ is a weakly consistent estimator of m, and by the delta method

$$\begin{aligned} &\frac{\sqrt{N}}{\psi_n'(\mu_n(m))\sigma_\varepsilon \mu_n \log 2}\left(\psi_n(\widehat{\mu}_n) - \psi_n(\mu_n(m))\right) \\ &= \frac{\sqrt{N}}{\psi_n'(\mu_n(m))\sigma_\varepsilon \mu_n \log 2}\left(\widehat{m} - m\right) \xrightarrow{\mathcal{D}} \mathcal{N}(0,1). \end{aligned}$$

Noting that $\psi_n'(\mu_n(m)) = 1/\mu_n'(m)$ we obtain the following.

Proposition 2. *Assume that $p_1 = 0$. As first $x_0 \to \infty$ and then $N \to \infty$, the estimate $\widehat{m}$ is a weakly consistent estimator of m, and*

$$\frac{\mu_n'(m)}{\sigma_\varepsilon \mu_n(m) \log 2} \sqrt{N}(\widehat{m} - m) \xrightarrow{\mathcal{D}} \mathcal{N}(0,1).$$

2.3 The dependence of m on the antibiotic concentration

Assuming $p_1 \equiv 0$ we can estimate the mean for $c > 0$ fixed as described in Proposition 2. Next we combine our estimator for different concentrations.

We assume that the offspring mean as a function of c satisfies (2.1) for some unknown parameters $\alpha > 0$, $\beta > 0$. This is a quite flexible model, and we show that empirical data fits very well to this model. Rewriting (2.1)

$$\log \alpha + \beta \log c = \log \left(\frac{2}{m(c)} - 1 \right). \tag{2.20}$$

Assume that we have measurements for $K \geq 2$ different concentrations $c_1 < c_2 < \ldots < c_K$, and we obtain the estimator for the offspring mean $\widehat{m}(c_i)$, $i = 1, 2, \ldots, K$. Standard least square theory implies that the expression

$$\sum_{i=1}^{K} \left(\log \left(\frac{2}{\widehat{m}(c_i) - 1} \right) - \beta \log c_i - \log \alpha \right)^2$$

attains it minimum at $(\alpha, \beta) = (\widehat{\alpha}, \widehat{\beta})$, with

$$\begin{aligned} \widehat{\beta} &= \frac{K \sum_{i=1}^{K} h_i \ell_i - \sum_{i=1}^{K} h_i L_1}{K L_2 - L_1^2} \\ \widehat{\alpha} &= \exp \left\{ \frac{\sum_{i=1}^{K} h_i - \widehat{\beta} L_1}{K} \right\}, \end{aligned} \tag{2.21}$$

where to ease notation we write

$$h_i = \log \left(\frac{2}{\widehat{m}(c_i)} - 1 \right), \quad \ell_i = \log c_i, \tag{2.22}$$

and

$$L_1 = \sum_{i=1}^{K} \ell_i, \quad L_2 = \sum_{i=1}^{K} \ell_i^2. \tag{2.23}$$

Note that by the Cauchy–Schwarz inequality the denominator of $\widehat{\beta}$ is strictly positive for $K \geq 2$.

The *minimal inhibitory concentration* (MIC) is the smallest antibiotic concentration that stops bacteria growth. In mathematical terms

$$\vartheta := \mathrm{MIC} = \min\{c : m(c) \leq 1\},$$

which, under the assumption (2.1)

$$\vartheta = \mathrm{MIC} = \alpha^{-1/\beta}.$$

Define the estimator

$$\widehat{\vartheta} = \widehat{\alpha}^{-1/\widehat{\beta}}. \tag{2.24}$$

In the following statement we summarize the main properties of these estimators. Introduce the notation

$$k_i = \frac{2}{m(c_i)(2 - m(c_i))} \frac{\sigma_\varepsilon \mu_n(m(c_i)) \log 2}{\mu_n'(m(c_i))}, \quad i = 1, 2, \ldots, K. \tag{2.25}$$

Proposition 3. *Assume that first $x_0 \to \infty$ and then $N \to \infty$. Then the estimates $\widehat{\alpha}, \widehat{\beta}$, and $\widehat{\vartheta}$ are weakly consistent estimators of the corresponding quantities. Furthermore, as $x_0 \to \infty$ and then $N \to \infty$*

$$\sqrt{N}(\widehat{\alpha} - \alpha, \widehat{\beta} - \beta) \xrightarrow{\mathcal{D}} (U, V),$$

where (U, V) is a two-dimensional normal random vector with mean 0 and covariance matrix

$$\begin{pmatrix} \sigma_\alpha^2 & \sigma_{\alpha\beta} \\ \sigma_{\alpha\beta} & \sigma_\beta^2 \end{pmatrix},$$

where

$$\begin{aligned}
\sigma_\alpha^2 &= \frac{\alpha^2}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 (L_2 - L_1 \ell_i)^2 \\
\sigma_{\alpha\beta} &= \frac{\alpha}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 (K\ell_i - L_1)(L_2 - L_1 \ell_i) \\
\sigma_\beta^2 &= \frac{1}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 \left(K\ell_i - L_1\right)^2,
\end{aligned}$$

and

$$\sqrt{N}(\widehat{\vartheta} - \vartheta) \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_\vartheta^2),$$

with

$$\sigma_\vartheta^2 = \frac{\vartheta^2 (\log \alpha)^2}{\beta^2 (KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 \left(\frac{L_2 - L_1 \ell_i}{\log \alpha} - \frac{K\ell_i - L_1}{\beta} \right)^2 .$$

Proof of Proposition 3. In what follows all the iterated limits aremeant as first $x_0 \to \infty$ and then then $N \to \infty$. By Proposition 2 and the delta method

$$\begin{aligned} &\frac{\sqrt{N} m(c_i)(2 - m(c_i))\mu_n'(m(c_i))}{2\sigma_\varepsilon \mu_n(m(c_i)) \log 2} \left(h_i - \log \left(\frac{2}{m(c_i)} - 1 \right) \right) \\ &= \frac{\sqrt{N}}{k_i} \left(h_i - \log \left(\frac{2}{m(c_i)} - 1 \right) \right) \xrightarrow{\mathcal{D}} \mathcal{N}(0, 1), \end{aligned}$$

for $i = 1, 2, \ldots, K$. Recall the notation in 2.23. Then using the independence of $h_i's$

$$\sqrt{N} \sum_{i=1}^{K} \left(h_i - \log \left(\frac{2}{m(c_i)} - 1 \right) \right) (K\ell_i - L_1) \xrightarrow{\mathcal{D}} \mathcal{N}(0, s_n^2),$$

with

$$s_n^2 = \sum_{i=1}^{K} k_i^2 (K\ell_i - L_1).$$

Substituting back into 2.21

$$\sqrt{N}(\widehat{\beta} - \beta) \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_\beta^2). \tag{2.26}$$

Similarly

$$\sqrt{N}(\widehat{\alpha} - \alpha) \xrightarrow{\mathcal{D}} \mathcal{N}(0, z_n^2), \tag{2.27}$$

with

$$z_n^2 = \sum_{i=1}^{K} \frac{k_i^2 (L_2 - L_1 \ell_i)^2}{(KL_2 - L_1^2)^2},$$

which implies

$$\sqrt{N}(\widehat{\alpha} - \alpha) \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_\alpha^2).$$

The statement for the covariance follows the same way. From (2.26) and (2.27) we obtain

$$\sqrt{N}(\widehat{\vartheta} - \vartheta) \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_\vartheta^2),$$

as claimed.

□

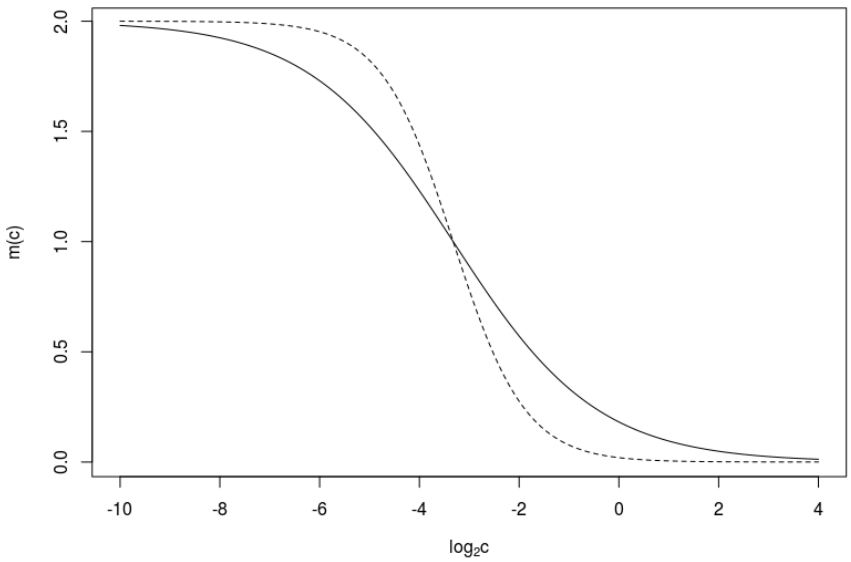

Figure 2.2: $m(c)$ in a logarithmic scale (solid $(\alpha, \beta) = (10, 1)$, dashed $(\alpha, \beta) = (100, 2)$).

2.4 Simulation study

Regardless of the fixed values $\mathbf{c} = (c_1, \ldots, c_K)$ the estimator $(\widehat{\alpha}, \widehat{\beta})$ is weakly consistent and asymptotically normal as $x_0 \to \infty$ and $N \to \infty$. However, the asymptotic variances in Proposition 3 do depend on the specific choice of $K \geq 2$ and the values $c_1 < \ldots < c_K$. If the antibiotic concentration is too low we essentially see a freely growing bacterial population, while for too large concentration the antibiotic already kills all the bacteria, and we only see the initial population. Therefore, intuitively it is clear that we should choose values for the concentration c_i such that $m(c_i)$ is not close to 0, nor to 2. Otherwise we cannot tell at which concentration the antibiotic starts to work.

Consider the following example. Assume that

$$\alpha = 10, \quad \beta = 1, \quad n = 10, \quad x_0 = 10^4, \quad \sigma_\varepsilon = 0.2. \tag{2.28}$$

It turns out that this is a reasonable choice, since roughly we obtain these estimates for the azithromycin data, see the next section. The mean offspring function $m(c)$ is given on Figure 2.2.

Choose $K = 3$ different concentrations such that $\mathbf{c}_1 = (2^{-6}, 2^{-4}, 2^{-2})$. Then for the asymptotic covariances we obtain

$$\sigma_\alpha^2 = 8.63, \quad \sigma_{\alpha,\beta} = 0.25, \quad \sigma_\beta^2 = 0.00767, \quad \sigma_\vartheta^2 = 0.00012. \tag{2.29}$$

concentrations	σ_α^2	$\sigma_{\alpha,\beta}$	σ_β^2	σ_ϑ^2
$\mathbf{c}_1 = (2^{-6}, 2^{-4}, 2^{-2})$	8.63	0.25	0.00767	$1.2 \cdot 10^{-4}$
$\mathbf{c}_2 = (2^{-2}, 2^{-1}, 1)$	112	9.41	0.833	0.012
$\mathbf{c}_3 = (2^{-9}, 2^{-8}, 2^{-7})$	967	18.7	0.364	0.0298
$\mathbf{c}_4 = (2^{-8}, 2^{-7}, 2^{-1}, 1)$	58	1.17	0.0257	0.00179
$\mathbf{c}_5 = (2^{-9}, 2^{-8}, \dots, 1)$	23	0.568	0.0157	$5.1 \cdot 10^{-4}$

Table 2.1: Asymptotic variances for different choices of $\mathbf{c}$ for $(\alpha, \beta) = (10, 1)$.

However, as we see in Table 2.1 wrong choice of the concentrations might results much larger variances. For $\mathbf{c}_2$ we only observe the process at large concentrations, killing all the bacteria, while in case $\mathbf{c}_3$ the concentration is small, the antibiotic does not have any effect. The combination of large and small values as in $\mathbf{c}_4$ does not help either. Less obvious is the fact that choosing too many points is contraproductive too. This is the case for $\mathbf{c}_5$.

Choosing the values as in (2.28), $K = 3$ and $\mathbf{c}_1 = (2^{-6}, 2^{-4}, 2^{-2})$ we simulated the process as follows. For a given concentration c_k, $k = 1, \dots, K$, we calculate $m(c_k)$ from (2.1), and choose the offspring distribution

$$p_{0;k} = 1 - \frac{m(c_k)}{2}, \quad p_{1;k} = 0, \quad p_{2;k} = \frac{m(c_k)}{2}.$$

With this offspring distribution we simulate $n = 10$ generations of the two-type Galton–Watson process (X_n, Y_n) described in Section 2.1. Therefore we obtain $Z_{10;c_k,x_0}$. Independently, we repeat the simulation N times for each concentration c_k. Independent of the Z's take an iid sequence of Gaussian random variables $\{\varepsilon_{i;c_k} : i = 1, \dots, N; k = 1, \dots, K\}$ with mean zero and variance σ_ε^2. Take $a = 0$ in (2.2). The resulting sequence $\{C_i(c_k, x_0) : i = 1, \dots, N; k = 1, \dots, K\}$ is one simulated measurement. From each measurement we calculate the estimation $(\widehat{\alpha}, \widehat{\beta})$ as described in (2.21). We simulated the measurement this way 1000 times. The resulting means and empirical variances of $\sqrt{N}(\widehat{\alpha}-\alpha, \widehat{\beta}-\beta)$ and $\sqrt{N}(\widehat{\vartheta}-\vartheta)$ are given in Table 2.2. We see that the empirical values are very close to the theoretical ones in (2.29) even for $N = 3, 10$. It is somewhat surprising that the estimates work even for $N = 3$, which is the suggested number of measurements at each concentration in microbiology (see e.g. [26, 7]).

Next we investigate our estimator with a steeper killing curve. Let $\alpha = 100$ and $\beta = 2$, and the other values as in (2.28). This is also a possible choice, see the estimates for the ciprofloxacin data in the next section. In Figure 2.2 wee see the mean offspring function $m(c)$ for $(\alpha, \beta) = (10, 1)$ and for $(\alpha, \beta) = (100, 2)$. Note that the MIC value is 0.1 in both cases.

N	$\overline{\alpha}$	$\overline{\beta}$	$\overline{\vartheta}$	$\widehat{\sigma}^2_\alpha$	$\widehat{\sigma}_{\alpha,\beta}$	$\widehat{\sigma}^2_\beta$	$\widehat{\sigma}^2_\vartheta$
3	10.359	1.004	0.0998	12.95	0.325	0.00891	0.000121
10	10.106	1.002	0.1	9.27	0.262	0.00789	0.000116
50	10.03	1.0005	0.1	9.3	0.265	0.008	0.000124
100	9.999	0.9999	0.1	8.83	0.258	0.008	0.000117
∞	10	1	0.1	8.63	0.25	0.00767	0.00012

Table 2.2: Empirical mean and variances for $(\alpha, \beta) = (10, 1)$.

concentrations	σ^2_α	$\sigma_{\alpha,\beta}$	σ^2_β	σ^2_ϑ
$\mathbf{c}_1 = (2^{-6}, 2^{-4}, 2^{-2})$	11298	35.6	0.0124	$3.64 \cdot 10^{-4}$
$\mathbf{c}_6 = (2^{-5}, 2^{-4}, 2^{-3})$	1431	5.49	0.0216	$1.26 \cdot 10^{-5}$
$\mathbf{c}_7 = (2^{-7}, 2^{-6}, \ldots, 2^{-1})$	42490	129.3	0.429	0.00142

Table 2.3: Asymptotics variances for different choices of $\mathbf{c}$ for $(\alpha, \beta) = (100, 2)$.

Therefore, we can compare two rather different and practically relevant scenarios. In the latter case the curve is much steeper, therefore there are less relevant concentrations, so we expect larger variances. In Table 2.3 we see that this is partly true, however the estimate of ϑ is good.

2.5 The experiment

In the experiment $50,000$ mother cells were infected by *Chlamydia trachomatis*. The multiplicity of infection (MOI) value, the ratio of the initial number of bacteria and number of mother cells is 0.2. That is $x_0 = 10,000$. The measurements correspond to 12 different antibiotic concentrations using twofold dilution technique, meaning that $c_i = 2^i c_0$, $i = 0, 1, \ldots, 11$. For each concentration 3 measurements were taken. For the technical details of the experiment we refer to Eszik et al. [7].

We analyze two antibiotics: azithromycin and ciprofloxacin. These antibiotics have different antimicrobial effects: azithromycin is a bacteriostatic antibiotic, meaning that it does not necessarily kill the bacteria, only prevents growth, while ciprofloxacin is a bactericide antibiotic, which usually kills bacteria. In Figures 2.3 and 2.4 we see the qPCR measurements as a function of $\log_2 c$.

If c is large enough, i.e. at very high antibiotic concentration $m(c)$ is close to 0, that is $Z_{n;x_0,c} \approx x_0$, since all the bacteria dies without offspring. Therefore, for c large enough we can estimate the constant a in (2.2) as

$$\widehat{a}_N = \frac{1}{N} \sum_{i=1}^{N} C_i(c, x_0) + \log_2 x_0. \tag{2.30}$$

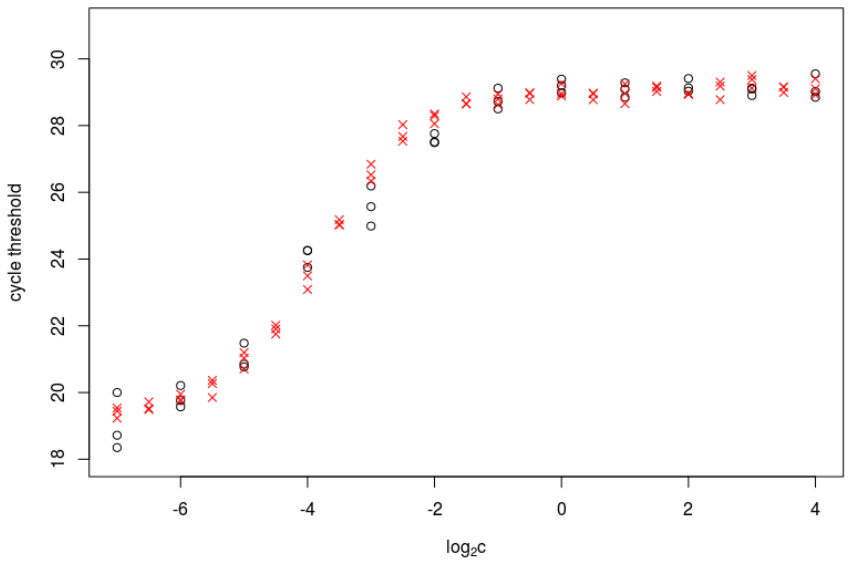

Figure 2.3: Measured (∘) and simulated (×) Ct values for azithromycin.

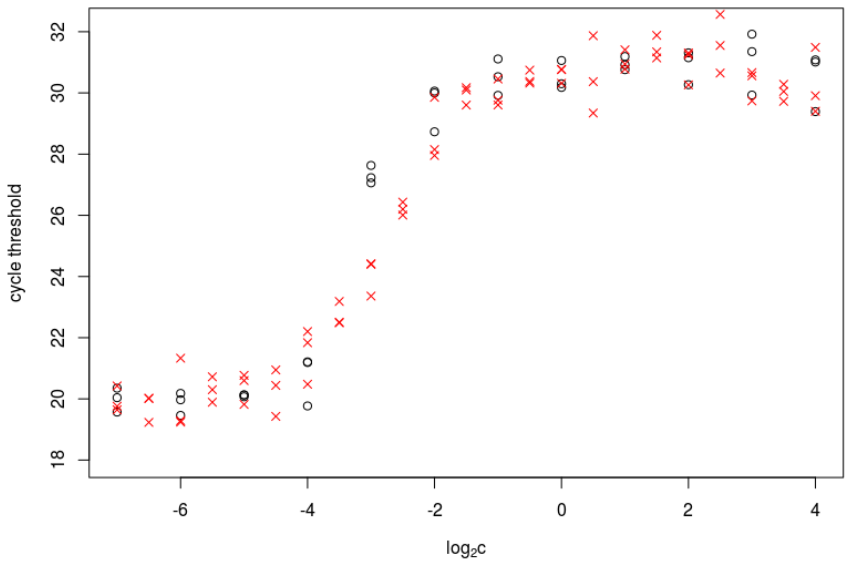

Figure 2.4: Measured (∘) and simulated (×) Ct values for ciprofloxacin.

Then $\widehat{a}_N$ is normally distributed with mean a and variance σ^2/N. Furthermore, σ_ε can also be estimated from these data. For azithromycin we used those measurements where $c \geq 2^{-1}$, while for ciprofloxacin those for which $c \geq 1$.

Chlamydiae cannot replicate indefinitely, because they propagate in a closed system, where the available nutrients are finite. A special feature of *Chlamydia* is that it has an infectious elementary form that is not capable to grow. After the infection of the host cell, it differentiates to a reticulate body which is capable to propagate by binary division, but the number of its divisions is limited. Then the reticulate body redifferentiates into the elementary body, exits the original host cell, infects a new one and its developmental cycle starts again in another host cell. Our wet-laboratory experiment followed one round of the developmental cycle, which is approximately 48 hours. Therefore, the number of generations n is typically a fixed small number, in our experiments around 10. If c is small then there is no antibiotical effect so the bacterial population grows freely, that is $Z_{n,x_0,0} \approx 2^n x_0$. We can estimate n as

$$\widehat{n}_N = \widehat{a}_N - \log_2 x_0 - \frac{1}{N}\sum_{i=1}^{N} C_i(c, x_0).$$

Then $\widehat{n}_N$ is normally distributed with mean n and variance $2\sigma_\varepsilon^2/N$. To estimate $\widehat{n}_N$ we used the smallest possible concentration, $c = 2^{-7}$.

Using Proposition 2 we estimate $m(c)$. In Figures 2.5 and 2.6 we see the estimated means and the corresponding fitted curve $m(c)$, where the parameters α, β are estimated as described in (2.21). In the previous section we showed that the best strategy is to choose few concentration where the mean offspring is not close to 0, nor to 2. For the azithromycin we chose $\mathbf{c} = (2^{-5}, 2^{-4}, 2^{-2}, 2^{-1})$ and obtained $\widehat{\alpha} = 9.1$, $\widehat{\beta} = 1.12$, and $\widehat{\vartheta} = 0.139$. (We obtain similar estimates for various reasonable choices.) For ciprofloxacin in Figure 2.4 we see a rapid drastic change; for $c \geq 2^{-2}$ the population dies out, while for $c \leq 2^{-4}$ the population freely grows. We chose $\mathbf{c} = (2^{-4}, 2^{-3}, 2^{-2})$ and obtained $\widehat{\alpha} = 71.8$, $\widehat{\beta} = 2.46$, $\widehat{\vartheta} = 0.175$. (These values are less stable to the change in $\mathbf{c}$.) Simulated measurements with the estimated values are given in Figures 2.3 and 2.4, where the circles are the real measurements and the crosses denote the values of the simulated ones. In both cases we obtain a remarkably good fit.

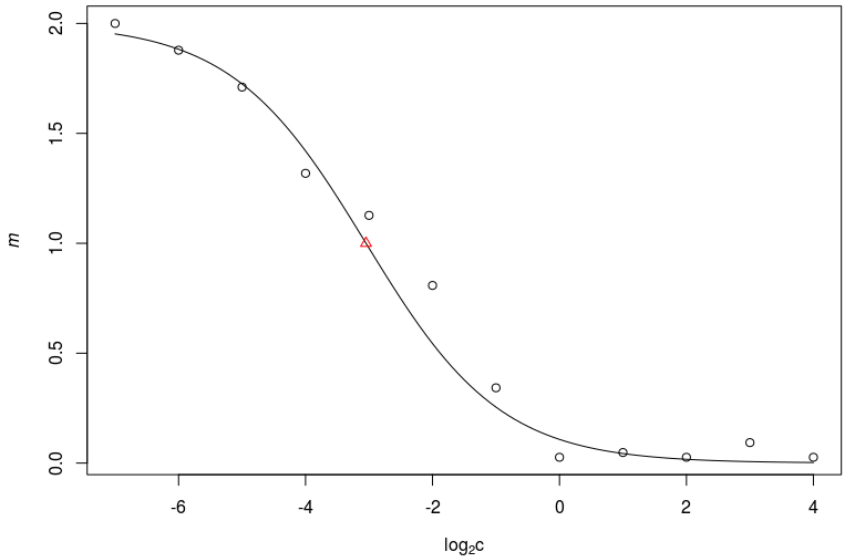

Figure 2.5: Estimated means and the fitted curve for azithromycin.

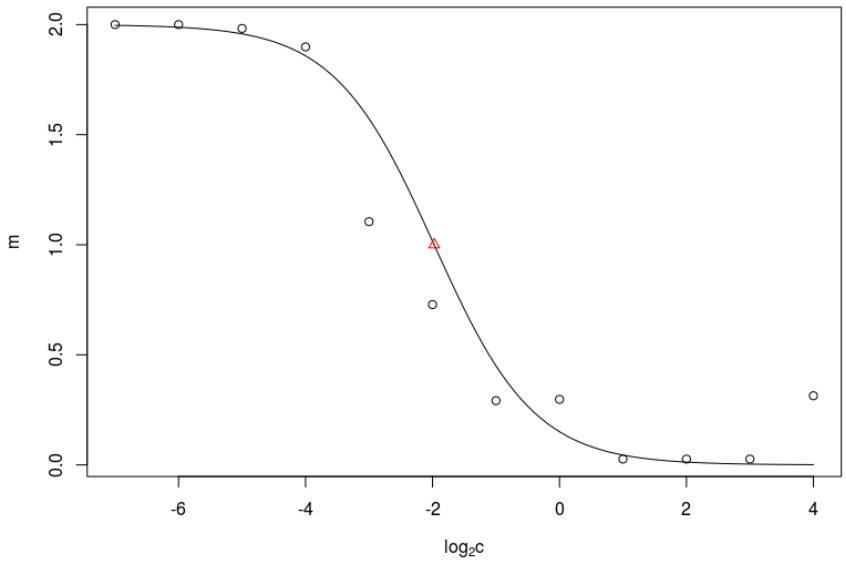

Figure 2.6: Estimated means and the fitted curve for ciprofloxacin.

2.6 Estimation of in vitro bactericidal potency based on colony counting method

Accurately estimating the bactericidal potency is a crucial concern for ensuring the safe and appropriate utilization of antibiotics. In Bogdanov et al. [3] we worked out a Bienaymé–Galton–Watson branching model for the growth of the bacterial population, and we obtained weakly consistent asymptotically normal estimators for the relevant parameters when for the biological measurements quantitative PCR (qPCR) method is used. In [3] we found that the 2-parameter model fits very well to real biological data. In this section we provide an estimator under the same model assumptions but for different biological data: we assume that the experimental data was obtained using colony counting method. The qPCR method measures the total bacterial genom, which is the total number of *dead and alive* bacterial cells multiplied by a constant. On the other hand, colony counting gives an estimator for the extinction probability. The basic experiment is the following. Originally, x_0 bacterial cells (e.g. *Escherichia coli*) are inoculated onto agar plates containing a series of antibiotic concentration, and after the incubation period all the viable colonies are enumerated, see e.g. Liu et al. [17].

As in [3] we assume that the bacterial population is homogeneous, in particular, there is no resistant type. Long-term evolution of bacterial populations with both *resistant* and *susceptible* types was investigated in several papers using deterministic models, see Svara and Rankin [22], Paterson et al. [20], and the references therein. Closest to our model is the deterministic model given by Liu et al. [17], where the biological measurements were obtained by colony counting. In [17] a deterministic expression for the number of colony forming units was obtained in terms of the antibiotic concentration.

The rest of this Section is organized as follows. The model and some basic properties are given in Subsection 2.6.1. The estimation of the parameters for fixed antibiotic concentration is provided in Subsection 2.6.2. Subsection 2.6.3 contains a small simulation study.

2.6.1 The theoretical model

Consider a simple Galton–Watson branching process where each bacteria either dies (leaves no offspring) or divides (leaves 2 offsprings) with respective concentration dependent probabilities

$$p_0 = p_0(c), \quad p_2 = p_2(c) = 1 - p_0(c).$$

Let $f(s) = f_c(s) = p_0 + p_2 s^2$ denote the offspring generating function and $m = m(c) = 2p_2(c)$ the offspring mean if the antibiotic concentration is c. The process starts with a single ancestor $X_{0;c} = 1$, and

$$X_{n+1;c} = \sum_{i=1}^{X_{n;c}} \xi_{i;c}^{(n)},$$

where $\{\xi_c, \xi_{i;c}^{(n)} : i \geq 1, n \geq 1\}$ are iid random variables with generating function f_c. We further assume that the offspring distribution is given by

$$p_2(c) = \frac{1}{1 + \alpha c^\beta}, \tag{2.31}$$

where $\alpha > 0$, $\beta > 0$ are unknown parameters. Note that as $m = 2p_2$ this is the same assumption as in [3]. Under this model the MIC, the smallest antibiotic concentration preventing bacterial growth, is the smallest c for which $m(c) = 1$, that is $\alpha^{-1/\beta}$.

If $m \leq 1$ then the process dies out almost surely, while if the process is supercritical, i.e. $m > 1$ then the probability of extinction is the smaller root of $f_c(q) = q$, which is in our setup

$$q(c) = \begin{cases} \frac{1-p_2(c)}{p_2(c)}, & \text{if } p_2(c) > 1/2, \\ 1, & \text{if } p_2(c) \leq 1/2. \end{cases} \tag{2.32}$$

2.6.2 Estimation of the parameters

Assume that the initial number of bacterial cells is x_0, that is we observe x_0 independent copies of the Galton–Watson process $(X_{n;c})$. Then the number Y_c of living colonies has binomial distribution with parameters x_0 and $1 - q(c)$. Therefore, the natural estimator for $q(c)$ is

$$\widehat{q}(c) = 1 - \frac{Y_c}{x_0}.$$

The law of large numbers and the central limit theorem implies that $\widehat{q}(c)$ is a weakly consistent estimator, and as $x_0 \to \infty$

$$\frac{\sqrt{x_0}}{\sqrt{q(c)(1-q(c))}} \left(\widehat{q}(c) - q(c)\right) \xrightarrow{\mathcal{D}} \mathcal{N}(0,1). \tag{2.33}$$

From (2.32) we see that we can estimate $p_2(c)$ only if $q(c) < 1$, or equivalently $m(c) > 1$, in which case

$$\widehat{p_2}(c) = \frac{1}{1 + \widehat{q}(c)}. \tag{2.34}$$

We assume that the offspring mean as a function of c satisfies (2.31) for some unknown parameters $\alpha > 0$, $\beta > 0$. Rewriting (2.31)

$$\log \alpha + \beta \log c = \log \left(\frac{1}{p_2(c)} - 1 \right).$$

Assume that we have measurements for $K \geq 2$ different concentrations $c_1 < c_2 < \ldots < c_K$, such that $m(c_K) > 1$. As in (2.34), we obtain the estimator $\widehat{p}_2(c_i)$ at different concentrations, from which, using simple least squares estimator we obtain the estimator

$$\widehat{\beta} = \frac{K \sum_{i=1}^K f_i \ell_i - \sum_{i=1}^K f_i L_1}{K L_2 - L_1^2},$$
$$\widehat{\alpha} = \exp\left\{ \frac{\sum_{i=1}^K f_i - \widehat{\beta} L_1}{K} \right\},$$

where to ease notation we write

$$f_i = \log\left(\frac{1}{\widehat{p}_2(c_i)} - 1 \right), \quad \ell_i = \log c_i,$$

and

$$L_1 = \sum_{i=1}^K \ell_i, \quad L_2 = \sum_{i=1}^K \ell_i^2.$$

By the Cauchy–Schwarz inequality the denominator of $\widehat{\beta}$ is strictly positive for $K \geq 2$.

Under the assumption (2.31) the MIC equals $\vartheta = \alpha^{-1/\beta}$, therefore its natural estimator is

$$\widehat{\vartheta} = \widehat{\alpha}^{-1/\widehat{\beta}}.$$

Using (2.33), as in [3] we can prove that these estimators are asymptotically normal. Introduce the notation

$$k_i = \frac{p_2(c_i)}{1 - p_2(c_i)} \sqrt{q(c_i)(1 - q(c_i))}, \quad i = 1, 2, \ldots, K.$$

Proposition 4. *Assume that $c_1 < \ldots < c_K$ are given concentrations such that $m(c_K) > 1$. Then as $x_0 \to \infty$, $\widehat{\alpha}, \widehat{\beta}$, and $\widehat{\vartheta}$ are weakly consistent estimators of the corresponding quantities. Furthermore, as $x_0 \to \infty$*

$$\sqrt{x_0}(\widehat{\alpha} - \alpha, \widehat{\beta} - \beta) \xrightarrow{\mathcal{D}} (U, V),$$

where (U, V) is a two-dimensional normal random vector with mean 0

and covariance matrix $\begin{pmatrix} \sigma_\alpha^2 & \sigma_{\alpha\beta} \\ \sigma_{\alpha\beta} & \sigma_\beta^2 \end{pmatrix}$, *where*

$$\sigma_\alpha^2 = \frac{\alpha^2}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 (L_2 - L_1\ell_i)^2,$$

$$\sigma_{\alpha\beta} = \frac{\alpha}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 (K\ell_i - L_1)(L_2 - L_1\ell_i),$$

$$\sigma_\beta^2 = \frac{1}{(KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 \left(K\ell_i - L_1\right)^2,$$

and

$$\sqrt{x_0}(\widehat{\vartheta} - \vartheta) \xrightarrow{\mathcal{D}} \mathcal{N}(0, \sigma_\vartheta^2),$$

as $x_0 \to \infty$, *with*

$$\sigma_\vartheta^2 = \frac{\vartheta^2 (\log \alpha)^2}{\beta^2 (KL_2 - L_1^2)^2} \sum_{i=1}^{K} k_i^2 \left(\frac{L_2 - L_1\ell_i}{\log \alpha} - \frac{K\ell_i - L_1}{\beta} \right)^2.$$

2.6.3 Simulation study

If $m(c_K) > 1$, then regardless of the fixed values $\mathbf{c} = (c_1, \ldots, c_K)$ the estimate $(\widehat{\alpha}, \widehat{\beta})$ is weakly consistent and asymptotically normal as $x_0 \to \infty$. However, the asymptotic variances in Proposition 4 do depend on the specific choice of $K \geq 2$ and the values $c_1 < \ldots < c_K$. Intuitively, it is clear that we should choose values for the concentrations where the derivative of m is large, that is m is close to 1, see Figure 2.2.

As in [3] we compare two rather different biologically relevant scenarios: $(\alpha, \beta) = (10, 1)$ and $(\alpha, \beta) = (100, 2)$. In Figure 2.2 we see the mean function for these two cases. Table 2.4 contains the theoretical variances given in Proposition 4 for different choices of the concentrations. For the steeper function ($(\alpha, \beta) = (100, 2)$) the variances of α and β are significantly larger, however the variance of the MIC is of the same order. We also see that a wrong choice of the concentrations might result much larger variations. For $\mathbf{c}_3$ all the concentrations are small, the antibiotic does not have any effect, so we cannot make a good estimate from observations at these concentrations.

concentrations	σ_{10}^2	σ_1^2	$\sigma_{0.1}^2$	σ_{100}^2	σ_2^2	$\sigma_{0.1}^2$
$\mathbf{c}_1 = (2^{-7}, 2^{-4})$	2424	2.87	0.015	$2.98 \cdot 10^6$	38	0.0027
$\mathbf{c}_2 = (2^{-5}, 2^{-4.5}, 2^{-3.4})$	875	1.36	0.0016	$3.54 \cdot 10^5$	5.5	0.0014
$\mathbf{c}_3 = (2^{-9}, 2^{-8}, 2^{-7})$	$8.99 \cdot 10^4$	32	2.89	$3.84 \cdot 10^8$	1448	29

Table 2.4: Asymptotic variances for $(\alpha, \beta) = (10, 1)$ and $(\alpha, \beta) = (100, 2)$.

x_0	$\overline{\alpha}$	$\overline{\beta}$	$\overline{\vartheta}$	$\widehat{\sigma}^2_\alpha$	$\widehat{\sigma}_{\alpha,\beta}$	$\widehat{\sigma}^2_\beta$	$\widehat{\sigma}^2_\vartheta$
50	11.25	1.01	0.101	1464	41	1.3	0.002
100	10.79	1.01	0.1004	1349	43	1.48	0.0019
300	10.23	1.003	0.1	981	36.2	1.44	0.0018
500	10.17	1.003	0.1	931	34.9	1.34	0.0016
∞	10	1	0.1	875	34	1.36	0.0017

Table 2.5: Empirical mean and variances for $(\alpha, \beta) = (10, 1)$.

Choosing the right antibiotic concentration is important to get a good estimate. The larger variances above are not surprising, because in the present setup the estimator for the mean $m(c)$ works only for supercritical processes, that is for those c, for which $m(c) > 1$. That is we can sample only from the upper part of the mean function $m(c)$ in Figure 2.2. This is in sharp contrast to the situation treated in [3], where the total number of dead and alive bacteria was counted, and the estimator for the mean works for any c.

With $\alpha = 10$, $\beta = 1$ and concentration vector $\mathbf{c}_2$ we simulate the process as follows. For a given concentration c_k, $k = 1, \ldots, K$, we calculate $p_2(c_k)$ from (2.31). From each measurement we calculate the estimation $(\widehat{\alpha}, \widehat{\beta})$ as described in [3]. We simulated the measurements 1000 times. The resulting means and empirical variances of $\sqrt{x_0}(\widehat{\alpha} - \alpha, \widehat{\beta} - \beta)$ and $\sqrt{x_0}(\widehat{\vartheta} - \vartheta)$ are given in Table 2.5. We see that even for small initial number of bacteria the empirical variances are close to the theoretical counterparts.

3

Branching model with state dependent offspring distribution for *Chlamydia* spread

Chlamydiae are obligate intracellular bacteria which have a unique two-stage developmental cycle, with two forms, the elementary body (EB) and the reticulate body (RB). The EB is the infectious form and it is not capable to multiply. After infecting the host cell, the EB differentiates to RB. The RB multiplies in the host cell by binary fission. After some time RBs redifferentiate to EBs. The EBs are then released from the host cell ready to infect new host cells. It was shown recently by Lee et al. [16] using 3D electron microscopy method and manual counting that this conversion occurs asynchronously, so that some RBs are converting into EBs, while others continue to divide, see in Figure 3.1.

Figure 3.1 shows entire chlamydial inclusions from representative infected cells at 16, 28, and 36 hpi (hours post-infection), scale bar: 1000 nm. Pie charts showing mean numbers of each chlamydial form per inclusion are grouped into three developmental phases: RB replication only (no IBs or EBs), onset of RB-to-EB conversion (IBs + EBs $\leq$50% of *Chlamydiae*), and EB accumulation (IBs + EBs >50% of *Chlamydiae*). All four chlamydial forms inside each inclusion were identified and counted: 12 h.p.i. ($n = 50$), 16 h.p.i. ($n = 31$), 20 h.p.i. ($n = 22$), 24 h.p.i. ($n = 10$), 28 h.p.i. ($n = 13$), 32 h.p.i. ($n = 10$), 36 h.p.i. ($n = 9$), 40 h.p.i. ($n = 10$), now n specifies the number of inclusions. The inclusion is the distinct region within the host cell, where the doubling of RB bodies, and conversion from RB to EB, occurs. Note that these inclusions are copies of each other. It's also crucial to note that if such a process is halted to count the RB and EB bodies, then the process stops and cannot continue. The intermediate body (IB) serves as the transitional stage between the RB and the EB forms, although it can be effectively regarded as an infectious form.

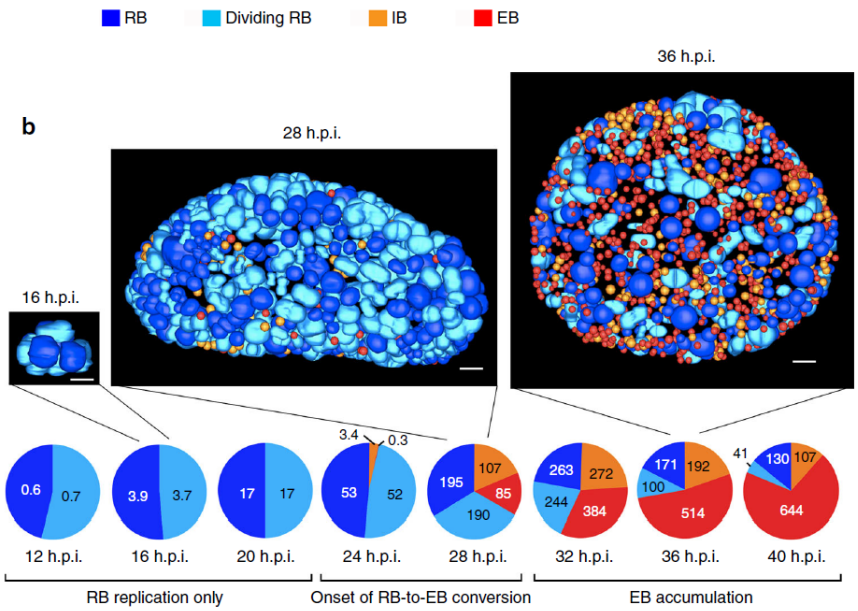

Figure 3.1: Temporal analysis of chlamydial developmental forms using a three-dimensional electron microscopy approach, [16].

Mathematical models suggested up to now are unable to reproduce this asynchronous conversion, since both in the deterministic differential equation model in [24] and in the stochastic model in [6] the optimal conversion strategy is the so-called 'bang-bang' strategy, that is, up to some time the population duplicates, then converts to EBs with the maximal possible rate.

Branching processes are well-known tools to model cell proliferation, see the monographs by Haccou et al. [10], Kimmel and Axelrod [15]. In [6], a continuous time Markov chain model was introduced with time-dependent transition rates, and the cell death was assumed to be independent of the population process. However, as heavily infected cells are more likely to die, the latter independence assumption, while mathematically convenient, is not realistic. In this Chapter we use a discrete-time branching process model, where the probability of duplication and the time of the cell death depends on the state of the process. Finding the optimal conversion strategy leads to a stochastic optimization problem, a so-called *discrete-time Markov control process*, see e.g. Hernández-Lerma and Lasserre [11]. The only input of the process is a *death-probability function* $d(x, y)$, which determines the probability that the host cell dies if there are x RBs and y EBs. Simulation study shows that with a simple death-probability function our model is able to capture the real behavior described recently in [16].

This Chapter is organized as follows. In Section 3.1 we describe our theoretical model. In Sections 3.2 and 3.3 we analyze the cases when the

host cell's death time is independent of, or depends on the process. The latter case is biologically more relevant. Section 3.4 contains a simulation study.

3.1 The theoretical model

Consider a two-type discrete-time Galton–Watson branching process $\mathbf{X}^\pi = (\mathbf{X}_n^\pi)_n = (X_n^\pi, Y_n^\pi)_n$, $n \geq 0$, together with a sequence of probabilities $\pi = (p_n)_n$. We assume that π is adapted to the natural filtration $(\mathcal{F}_n)_n$ generated by $\mathbf{X}$, i.e. $\mathcal{F}_n = \sigma(\mathbf{X}_k^\pi, k \leq n)$. Initially $\mathbf{X}_0^\pi = (1, 0)$, and the process evolves as

$$\begin{aligned} X_{n+1}^\pi &= \sum_{i=1}^{X_n^\pi} \xi_{n,i}, \\ Y_{n+1}^\pi &= Y_n^\pi + \sum_{i=1}^{X_n^\pi} \left(1 - \frac{\xi_{n,i}}{2}\right), \quad n \geq 0, \end{aligned} \tag{3.1}$$

where $(\xi_n, \xi_{n,i}), n = 1, 2, \ldots, i = 1, 2, \ldots$ are conditionally independent random variables given $(p_n)_n$, for fix n the variables $(\xi_n, \xi_{n,i}), i = 1, 2, \ldots$ are identically distributed, such that $\mathbf{P}(\xi_n = 2|p_n) = p_n$, $\mathbf{P}(\xi_n = 0|p_n) = 1 - p_n$.

Here X_n^π stands for the number of RBs and Y_n^π for the number of EBs in generation n. In generation n each RB duplicates with probability p_n and converts into EB with probability $1-p_n$. If $\xi_{n,i} = 2$, then the ith RB in generation n duplicates, while if $\xi_{n,i} = 0$ then it converts to EB. The process π, the sequence of duplication probabilities, is adapted to $(\mathcal{F}_n)_n$, which intuitively means that based on the whole past of the process the population determines its duplication probabilities. In what follows, we call the random process π a *strategy*.

For the conditional expectations we obtain

$$\begin{aligned} \mathbf{E}[(X_{n+1}^\pi, Y_{n+1}^\pi)|\mathcal{F}_n] &= (2p_n X_n^\pi, Y_n^\pi + (1 - p_n)X_n^\pi) \\ &= \mathbf{X}_n^\pi \begin{pmatrix} 2p_n & 1 - p_n \\ 0 & 1 \end{pmatrix}. \end{aligned} \tag{3.2}$$

If p_n depends only on the actual state (X_n, Y_n), then the process is Markovian.

The process ends at a random time $T \in \{1, 2, \ldots\}$ when the infected host cell dies. The aim of the bacterial population is to produce as many EBs as possible, that is to maximize $\mathbf{E}(Y_T^\pi)$ over all possible strategies (p_n). Denoting by $\mathcal{P}$ the set of all strategies, a strategy $\mathbf{q}$ is *optimal*, if

$$\sup_{\pi \in \mathcal{P}} \mathbf{E}(Y_T^\pi) = \mathbf{E}(Y_T^{\mathbf{q}}).$$

Note that we do not claim neither existence nor uniqueness, see the remark after Theorem 1.

The cause of the host cell's death and the distribution of its time is not yet well-understood. Experiments indicate that the lysis times of different host cells vary between 48 and 72 hpi, see Elwell et al. [5]. Here we consider two models. If T is independent of the process, than we can calculate explicitly an optimal strategy, which turns out to be deterministic 'bang-bang' strategy. Depending on the distribution of T, the population doubles up to some deterministic time ($p_n = 1$), and then all the RBs convert to EBs immediately ($p_n = 0$). This phenomena is analogous to the findings in the continuous time setup in [6], where independence of T and $\mathbf{X}^\pi$ was tacitly assumed. Therefore, this model cannot explain the asynchronous conversion. In our second model we assume that the host cell dies at time n with a probability depending on $\mathbf{X}_n^\pi$, such that more bacteria imply higher death probability. In this more complex and more realistic model we can determine an optimal strategy only numerically. We found that asynchronous conversion happens naturally. In simulations we obtained similar behavior as in real experiments in [16].

3.2 Death time T is independent of X

Assume that the host cell's death time T is independent of the process $\mathbf{X}^\pi$. Introduce the notation $\pi_\ell = (1, 1, \ldots, 1, 0, 0, \ldots)$, where the first $\ell \geq 0$ components are 1.

Theorem 1. *Assume that $T \geq 1$ is bounded and it is independent of $\mathbf{X}^\pi$. Let ℓ be such that*

$$2^\ell \mathbf{P}(T > \ell) = \sup_{k \geq 0} 2^k \mathbf{P}(T > k). \tag{3.3}$$

Then π_ℓ is an optimal strategy, with optimal value

$$\sup_{\pi \in \mathcal{P}} \mathbf{E}(Y_T^\pi) = \sup_{k \geq 0} 2^k \mathbf{P}(T > k).$$

Note that if T is unbounded, then it is possible that

$$\lim_{k \to \infty} 2^k \mathbf{P}(T > k) = \infty,$$

in which case it is easy to see that there is no optimal strategy.

Furthermore, one can construct distributions for which ℓ in (3.3) is not unique, showing that the optimal strategy is not necessarily unique. Indeed, the simplest example is a two-valued T for which $\mathbf{P}(T = 1) = \mathbf{P}(T = 2) = \frac{1}{2}$. Then both π_0 and π_1 are optimal strategies with optimal value 1. Under π_0 the gain is constant 1, i.e. deterministically $Y_T^{\pi_0} \equiv 1$, while under π_1 the gain is 2 or 0 with probability $\frac{1}{2} - \frac{1}{2}$.

Proof. To ease notation we suppress π. Since $T \leq N$, for some N, in an optimal strategy $p_{N-1} = 0$. Next, using the independence of T and X

$$\begin{aligned}&\mathbf{E}\left[Y_T | T > N-2, \mathcal{F}_{N-2}\right]\\ &= Y_{N-2} + 2p_{N-2}X_{N-2}\mathbf{P}(T = N | T > N-2) + X_{N-2}(1-p_{N-2})\\ &= Y_{N-2} + X_{N-2}\left(1 + p_{N-2}(2\mathbf{P}(T = N | T > N-2) - 1)\right).\end{aligned}$$

Thus, the resulting expression is linear in p_{N-2}, therefore choosing $p_{N-2} = 0$ or 1, maximizes the expectation. (We emphasize that there is no uniqueness in general, since if $\mathbf{P}(T = N | T > N-2) = \frac{1}{2}$, than any $p_{N-2} \in [0,1]$ maximizes the expression.) Since p_{N-2} only depends on the distribution of T, and not on (X_{N-2}, Y_{N-2}), we have

$$\begin{aligned}&\mathbf{E}[Y_T | T > N-3, \mathcal{F}_{N-3}]\\ &= Y_{N-3} + X_{N-3}(1-p_{N-3}) +\\ &+ X_{N-3}2p_{N-3}(1-p_{N-2})\mathbf{P}(T > N-2 | T > N-3) +\\ &+ X_{N-3}4p_{N-3}p_{N-2}\mathbf{P}(T > N-1 | T > N-3)\\ &= Y_{N-3} + X_{N-3}(1 + p_{N-3}(2(1-p_{N-2})\mathbf{P}(T > N-2 | T > N-3) +\\ &+ 4p_{N-2}\mathbf{P}(T > N-1 | T > N-3) - 1)).\end{aligned}$$

Again, the resulting expression is linear in p_{N-3}, therefore choosing $p_{N-3} = 0$ or 1, maximizes the expectation. Iteration gives that there is an optimal strategy for which each p_i is either 0 or 1.

This means that there exists an optimal strategy of the form π_k, for some k. Under π_k the population doubles up to generation k, then all the RBs convert to EBs. These strategies are easy to compare. Under π_k simply $Y_T = \mathbb{I}(T \geq k+1)2^k$,

$$\mathbf{E}(Y_T) = \mathbf{P}(T > k)2^k.$$

Taking the maximum in k, we obtain that π_ℓ is indeed an optimal strategy. □

One can consider a more general model, in which each bacterium cell is allowed to wait, that is, neither divides, nor converts. Then instead of a single p_n, we have a vector (p_n, q_n), such that $\mathbf{P}(\xi_n = 2 | (p_n, q_n)) = p_n$, $\mathbf{P}(\xi_n = 1 | (p_n, q_n)) = q_n$, and $\mathbf{P}(\xi_n = 0 | (p_n, q_n)) = 1 - p_n - q_n$. In this more general setting Theorem 1 remains true with the identical proof. Intuitively it is clear that the population loses nothing by adding RBs, as T is independent of $\mathbf{X}$. Therefore, in an optimal strategy $q_n \equiv 0$. However, this more general setup might allow us to model stress factors for the bacteria, such as antibiotic treatment. Then, depending on the antibiotic concentration, there is an minimal probability $q_{\min} > 0$, such that a single bacterium waits, that is $q_n \geq q_{\min}$. This extended model will be the subject of further research.

3.3 Death time T depends on X

Here we assume that T, the death time depends on the process $\mathbf{X}^\pi$. Given that the host cell is alive in generation $n-1$, the probability that it dies in the next step is $d(X_n^\pi, Y_n^\pi)$, that is

$$\mathbf{P}(T = n | T > n-1, \mathcal{F}_n) = d(X_n^\pi, Y_n^\pi).$$

The deterministic *death-probaility* function d describes the effect of RBs and EBs to cell's death. It is not clear which type is more harmful to the host cell, since RB particles are larger, while EB particles secrete chemicals poisoning the host cell, see e.g. [5]. Assume that

$$\exists\, C > 0 \;\text{ such that }\; d(x,y) = 1 \;\text{ whenever }\; x + y \geq C. \tag{3.4}$$

That is, if the total number of bacteria exceeds C the host cell necessarily dies. This is biologically a natural assumption.

In this scenario the process is a special *discrete-time Markov control process* (or Markov decision process). For theory and properties of these processes we refer to the monograph by Hernández-Lerma and Lasserre [11]. To see that our model fits in the theory we slightly modify our process. Recall that $X_n = X_n^\pi$ depends on the strategy π, however for notational ease we suppress the upper index. Let $\widetilde{X}_n = X_n \mathbb{I}(T > n)$, $\widetilde{Y}_n = Y_n \mathbb{I}(T \geq n)$. Note that $\widetilde{X}_T = 0$, $\widetilde{Y}_T = Y_T$, and $\widetilde{Y}_{T+1} = 0$, which is convenient at the definition of the reward function in (3.6). Define a Markov chain $(\widetilde{X}_n, \widetilde{Y}_n)_n$ on the state space $\{0, 1, \ldots\}^2$, where the possible controls are given by the duplication probabilities $p_n \in [0,1]$. The transition probabilities are, for $x > 0, y \geq 0$,

$$\begin{aligned}
&\mathbf{P}\left(\widetilde{X}_{n+1} = 2j, \widetilde{Y}_{n+1} = y + x - j | \widetilde{X}_n = x, \widetilde{Y}_n = y, p_n = p\right) \\
&= \binom{x}{j} p^j (1-p)^{x-j} (1 - d(2j, y + x - j)), \quad j = 1, \ldots, x, \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y + x - j | \widetilde{X}_n = x, \widetilde{Y}_n = y, p_n = p\right) \\
&= \binom{x}{j} p^j (1-p)^{x-j} d(2j, y + x - j), \quad j = 1, \ldots, x, \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y + x | \widetilde{X}_n = x, \widetilde{Y}_n = y, p_n = p\right) \\
&= (1-p)^x,
\end{aligned} \tag{3.5}$$

while if $x = 0$

$$\mathbf{P}(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = 0 | \widetilde{X}_n = 0, \widetilde{Y}_n = y, p_n = p) = 1.$$

The first two formulae correspond to the possibility that $j \geq 1$ bacteria duplicate (with probability $\binom{x}{j} p^j (1-p)^{x-j}$) and the host cell remains

alive, or die, while the third formula corresponds to the possibility that all the RBs convert to EBs, and in this case it does not matter whether the host cell dies or not. The fourth equation states that $(0,0)$ is the unique absorbing state, which is a convenient condition for the form of the reward function.

The *reward function* (-1 times the cost function in [11]) gives the number of EBs upon cell's death, that is

$$c(x,y) = \begin{cases} y, & x = 0, \\ 0, & \text{otherwise} \end{cases} \tag{3.6}$$

Define the *value function*

$$h(x,y) = \begin{cases} \sup_{\pi\in\mathcal{P}} \mathbf{E}\left[\sum_{n=0}^{\infty} c(\widetilde{X}_n, \widetilde{Y}_n) | (\widetilde{X}_0, \widetilde{Y}_0) = (x,y)\right], & d(x,y) < 1, \\ y, & d(x,y) = 1. \end{cases} \tag{3.7}$$

which is the optimal number of expected EBs upon host cell's death, given that the host cell is alive and $(\widetilde{X}_0, \widetilde{Y}_0) = (x,y)$, if $d(x,y) < 1$. If $d(x,y) = 1$ then the cell cannot be alive at state (x,y), thus the reward is y. Clearly $h(0,y) = y$. Due to the fact that $(0,0)$ is the only absorbing state in the infinite sum in (3.7) there is only one non-zero term.

We are looking for the value $h(x,y)$ and an optimal strategy π. This stochastic optimization problem is in fact a finite-horizon problem, see [11, Chapter 3]. Indeed, from any state $(\widetilde{X}_n, \widetilde{Y}_n) = (x,y)$ either the total number of bacteria increases ($j \geq 1$ and the host cell survives in (3.5)), or $\widetilde{X}_{n+1} = 0$, meaning that the cell dies. Therefore, by condition (3.4) from any initial state (x,y) the process reaches the absorbing state $(0,0)$ in at most $C+1$ steps. So in (3.7) in the summation the upper limit can be changed to C. Using Theorem 3.2.1 in [11] both the value function h and the optimal strategy can be determined by backward induction on time. In our setup, backward induction on the total number of bacteria is more natural, and this goes as follows.

Theorem 2. *Assume that* (3.4) *holds. Then* $h(x,y) = y$ *if* $x + y \geq C$, *and* $h(0,y) = y$ *for any* y. *Assume that* $h(x,y)$ *is determined whenever* $x + y \geq m$ *for some* $m \leq C$, *and let* $x + y = m - 1$. *Then*

$$h(x,y) = \max_{p\in[0,1]} \sum_{j=0}^{x} \binom{x}{j} p^j (1-p)^{x-j} \\ \times \left[d(2j, y+x-j)(y+x-j) + (1 - d(2j, y+x-j))h(2j, y+x-j)\right], \tag{3.8}$$

where all the values of h *on the right-hand side are determined. The maximum in* p *of the continuous function on the right-hand side of* (3.8) *is attained at* $p(x,y)$, *which gives the optimal strategy.*

Proof. From definition (3.7) we see that $h(x, y) = y$ if $x + y \geq C$ or $x = 0$. Formula (3.8) follows from the Markovian structure and from the transition probabilities in (3.5).

Indeed, from (x, y), $x + y = m - 1$, the process can jump to states $(0, y+x-j), j = 0, 1 \ldots, x$, and $(2j, y+x-j), j = 1, 2, \ldots, x$, depending on whether the host cell dies or not. In the first case $h(0, y + x - j) = y + x - j$, while in the second case the total number of bacteria equals $y + x + j \geq m$, therefore $h(2j, y + x - j)$ is determined by the induction assumption. Thus all the quantities in (3.8) are known, so $h(x, y)$ can be calculated. □

One could consider again the more general model mentioned at the end of Section 3.2, where each bacterium is allowed to wait. Then instead of a single p_n, the possible controls are given by a vector (p_n, q_n), with $p_n + q_n \leq 1$. A version of Theorem 2 remains true, except in (3.8) the maximum is taken in (p, q). We found that for reasonable death-probability functions (i.e. d_2 in (3.12)), the optimal values of q are small, or even 0. Here we will present an example, where $C = 200$, and the death probility function see 3.12, where now $c_0 = 0.003$, and $(\alpha, \beta) = (2, 2)$. We see in Section 3.4 that these are sensible parameter choices. These numerical computations even with a computer take a very long time, so we cannot choose the important auxiliary parameter (C) to be large. However, we encountered a problem here, as illustrated by the following simple example

$$\begin{aligned} h(1,2) = \max_{p+q\leq 1} \{ & q[d(1,2)\cdot 2 + (1-d(1,2))\cdot h(1,2)] \\ & + (1-p-q)[d(0,3)\cdot 3 + (1-d(0,3))\cdot h(0,3)] \\ & + p[d(2,2)\cdot 2 + (1-d(2,2))\cdot h(2,2)]\} \end{aligned} \tag{3.9}$$

In this case, both $h(0, 3)$ and $h(2, 2)$ values are known. However, we encountered a problem that did not occur in Theorem 2, namely the possibility of remaining in the same state, which is now $(1, 2)$ in example (3.9). However, the value of $h(1, 2)$ is unknown, and we aim to calculate it presently. In order to address this issue, we adjust our model as follows. We make the assumption that transitioning to the (x, y) state from the same (x, y) state is not possible, and that the host cell cannot perish while in the (x, y) state. Therefore, we need to divide the formula by $(1 - q)(1 - d(x, y))$. What we are calculating is the probability that the process do not transition to the same state for which h is computed, and simultaneously, the host cell does not perish in that state.

Theorem 3. *Assume that* (3.4) *holds. Then* $h(x, y) = y$ *if* $x + y \geq C$, *and* $h(0, y) = y$ *for any* y. *Assume that* $h(x, y)$ *is determined whenever*

$x + y \geq m$ for some $m \leq C$, and let $x + y = m - 1$. Then

$$h(x,y) = \max_{p+q\leq 1} \sum_{j=0}^{x} \sum_{\ell=0}^{x-j} \binom{x}{j} \binom{x-j}{\ell} p^j q^\ell (1-p-q)^{x-j-\ell} \times [d(2j+\ell, y+x-j-\ell)(y+x-j-\ell) + (1-d(2j+\ell, y+x-j-\ell))h(2j+\ell, y+x-j-\ell)] / A(x,y) \tag{3.10}$$

where $A(x,y) = (1-q)(1-d(x,y))$, and all the values of h on the right-hand side are determined. The maximum of the continuous function is attained at $p(x,y) + q(x,y) \leq 1$, which gives an optimal strategy.

The proof of the Theorem 3 remains identical to that of Theorem 2.

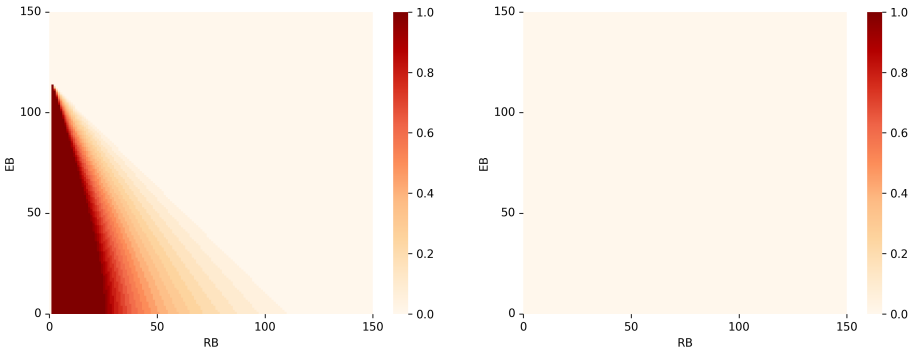

Figure 3.2: The numerical p (left) and q values (right) corresponding to the death-probability function (3.12) with $C = 200$, $(\alpha, \beta) = (2, 2)$, and $c_0 = 0.003$.

In Figure 3.2, we observe the optimal values for p and q when $C = 200$, $(\alpha, \beta) = (2, 2)$, and $c_0 = 0.003$. It can be observed that the q values are zero in all instances. It's worth reiterating that even with the aid of software, determining these optimal values can be time-consuming. The question may arise whether $C = 200$ suffices as a threshold choice. Our conjecture is that if the value c_0 is appropriately selected, then as $C \to \infty$, we have $h(x,y) \to a_{x,y}$, for all (x,y). In Section 3.4, it is demonstrated that the aforementioned parameter selection is indeed correct.

3.4 Simulation studies

For a given death-probability function d, we can determine numerically the value function and an optimal strategy using Theorem 2. Then,

the process is a simple Galton–Watson branching process with state-dependent offspring distribution, which can be simulated easily. In each examples below the empirical mean of RBs and EBs are calculated from 1000 simulations.

First we consider a simple threshold death-probability function, that is for some $C > 0$

$$d(x, y) = d_1(x + y) = \begin{cases} 1, & \text{if } x + y \geq C, \\ 0, & \text{otherwise.} \end{cases} \tag{3.11}$$

This is a simple, but biologically very unnatural death-probability function. In this case, typically some bacteria convert to EBs at an early stage, while others still divide. As long as the total number of bacteria is below C, the population is safe, in the sense that the host cell cannot die. Consequently, the population does not rush to expand, rather tries to find a state, from which the optimal value $C - 1$ can be obtained deterministically. Indeed, the population exhibits a slow growth, taking numerous generations to eventually reach the optimal value $C - 1$. This unnatural behavior is apparent in Figure 3.4, where we plotted six trajectories of the process, with $C = 300$.

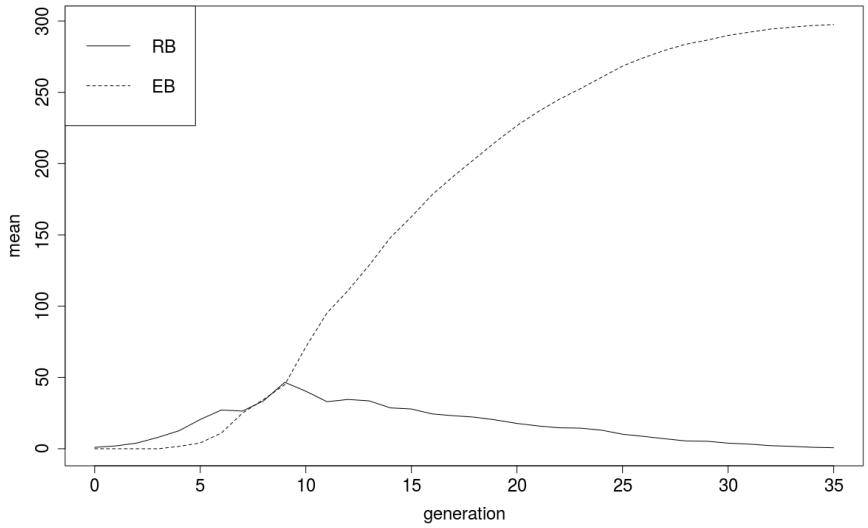

Figure 3.3: The mean number of EBs and RBs for the death-probability (3.11) with $C = 300$.

For simulations we choose $C = 300$. The value function is almost constant 299 with $h(1, 0) = 298.7$. In Figure 3.3 we see that the number of RBs is typically small, while the number of EBs starts to increase at an early stage. In Figure 3.4 there are six trajectories of the process. On Figure 3.11 (top left) we see the numerical p values. The structure of

the death-probability function causes the discontinuity of the p function. Note e.g. that $p(x, y) \equiv 1$ on the line $\{(x, y) : 2x + y = 299\}$, since after one duplication the population reaches the maximum possible value 299.

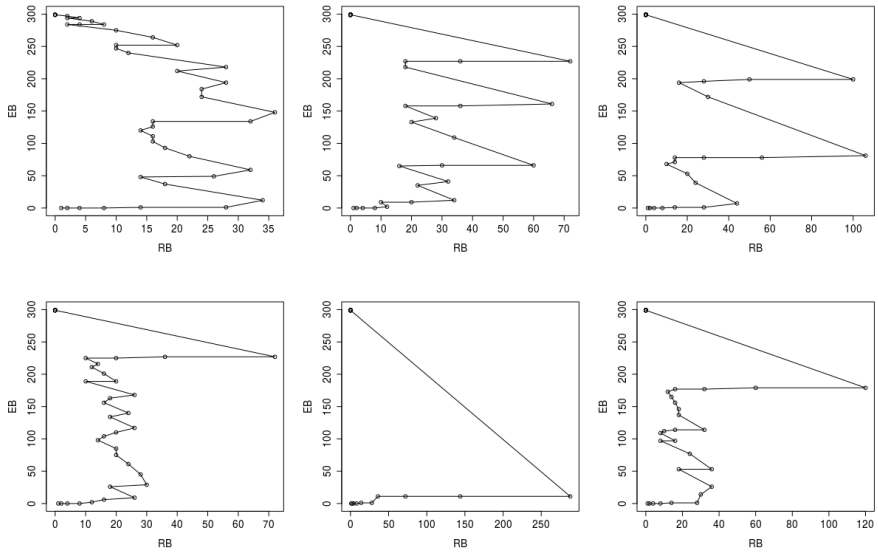

Figure 3.4: Simulations of the process with death-probability (3.11) and $C = 300$.

Consider a smoother death-probabilityfunction

$$d_2(x, y) = \begin{cases} 1 - e^{-c_0(\alpha x + \beta y)}, & x + y \leq C - 1, \\ 1, & x + y \geq C. \end{cases} \tag{3.12}$$

When the total number of bacteria is small, then it is unlikely that the host cell dies. The parameters α, β allows us to tune the relative effect of EBs and RBs on the host cell's death. On the one hand RBs are much larger than EBs suggesting $\alpha > \beta$, on the other hand EBs secrete chemicals enhancing cell death. Note that biological experiments suggests that *chlamydia* controls host cell survival, see [5, p. 392]. We explored three scenarios, with $c_0 = 0.0003$ in each cases and $(\alpha, \beta) = (1, 3)$, $(2, 2)$, and $(3, 1)$, with $C = 2500, 1500, 3000$, respectively. We chose C large enough, so that an optimal strategy does not depend on its specific value. The rationale of choice of the different threshold values C can be seen from Figure 3.11. For the empirical mean of 1000 simulations and some typical trajectories see Figures 3.5 – 3.10.

The population dynamics of RB and EB cells depend strongly on the value (α, β). For $(\alpha, \beta) = (1, 3)$ the relative effect of EBs on cell-death is much larger. Therefore, the process prefer to have only RBs up to some point (generation 11), and then all RBs convert to EBs immediately, resulting an 'bang-bang' strategy. The exponential increase of RBs and

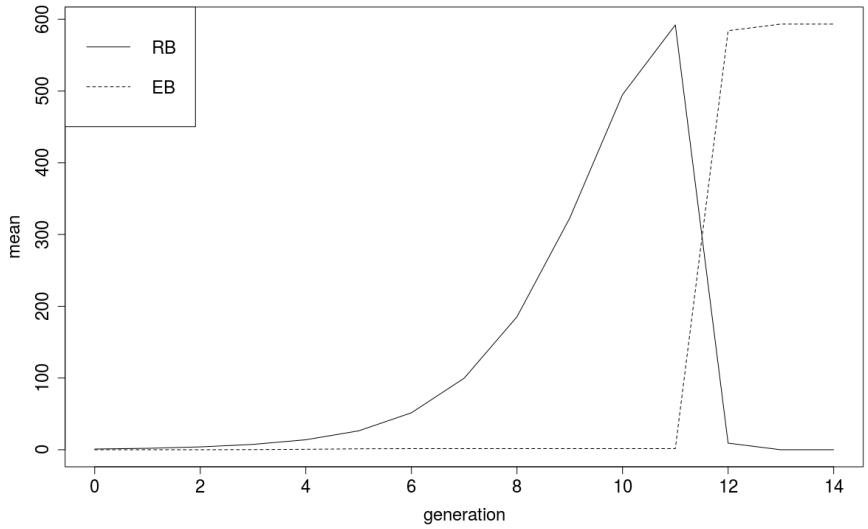

Figure 3.5: The mean number of EBs and RBs for the death-probability (3.12), with $(\alpha, \beta) = (1, 3)$.

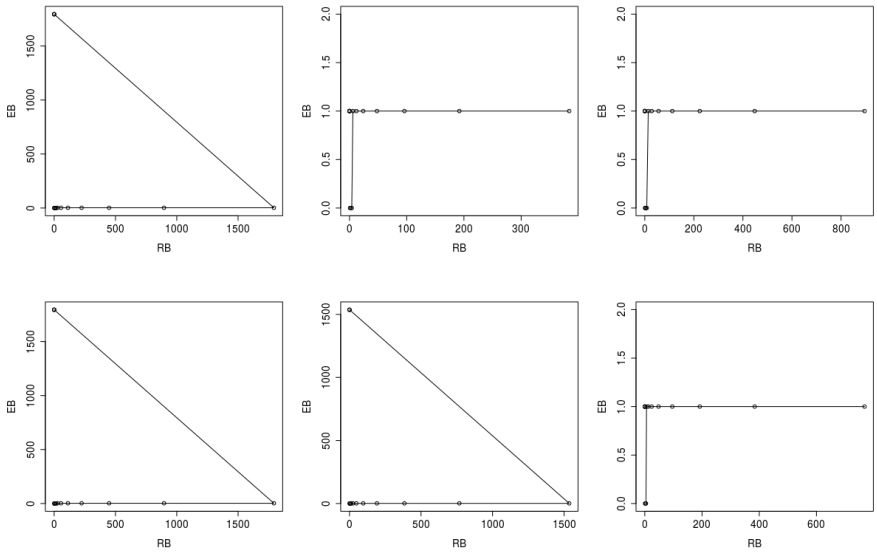

Figure 3.6: Simulations of the process with death-probability (3.12), with $(\alpha, \beta) = (1, 3)$.

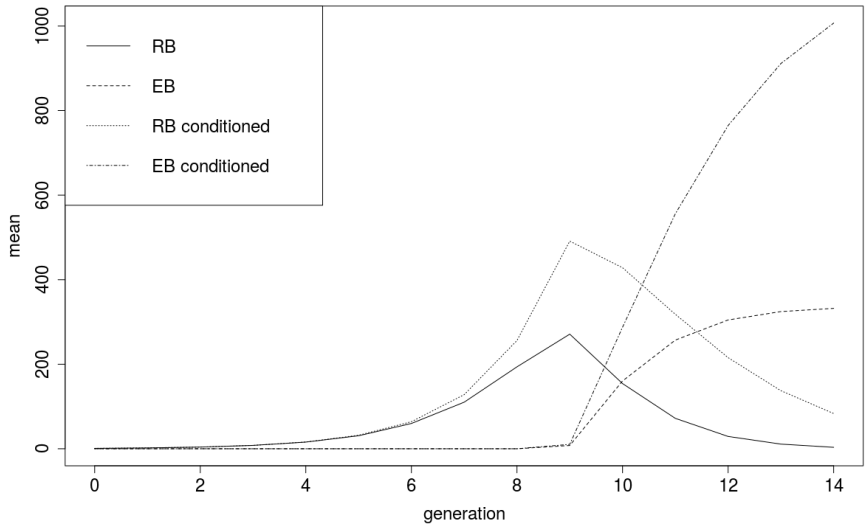

Figure 3.7: The mean and conditional mean of EBs and RBs for the death-probability (3.12), with $(\alpha, \beta) = (2, 2)$.

the sudden change is clearly visible both on the means (Figure 3.5), and on the trajectories (Figure 3.6). Here $h(1, 0) = 605$. The optimal p values on Figure 3.11 (top right) show the same pattern: in each state either all cells duplicate ($p = 1$), or all cells convert ($p = 0$).

For $(\alpha, \beta) = (2, 2)$ the relative effect of RBs and EBs is the same. The RBs duplicate and increase exponentially fast up to generation 9, then they start to convert to EBs. In Figures 3.7 and 3.8 we see that in generations 9–12 the EBs and RBs simultaneously appear, showing the asynchronous conversion obtained in real experiments in [16]. Here $h(1, 0) = 324$.

In Figure 3.7 we also plotted the empirical means of the EBs and RBs conditioned on that the host cell is alive. In the real experiment in Lee et al. [16] only those inclusions are counted where the host cell is alive. This clearly causes a bias. We can transform the generation time to real time, hours-post-infection (hpi). After the EB enters the host cell, it takes approximately 12 hours to convert to RB and to start to duplicate. Between 12 and 24 hpi the doubling time is about 1.8 hours, and around 28 hpi RB-EB conversion starts, see [16, p. 2]. In Table 3.1 we copied the measurements from [16] together with the simulation results corresponding to different values of (α, β) around $(2, 2)$, to see that our model captures remarkably well the experimental data.

For the optimal p values in Figure 3.11 (bottom left) we do see values other than 0 and 1. There are no big jumps in the p values, which makes it biologically relevant.

Finally, for $(\alpha, \beta) = (3, 1)$ the relative effect of RBs is much larger,

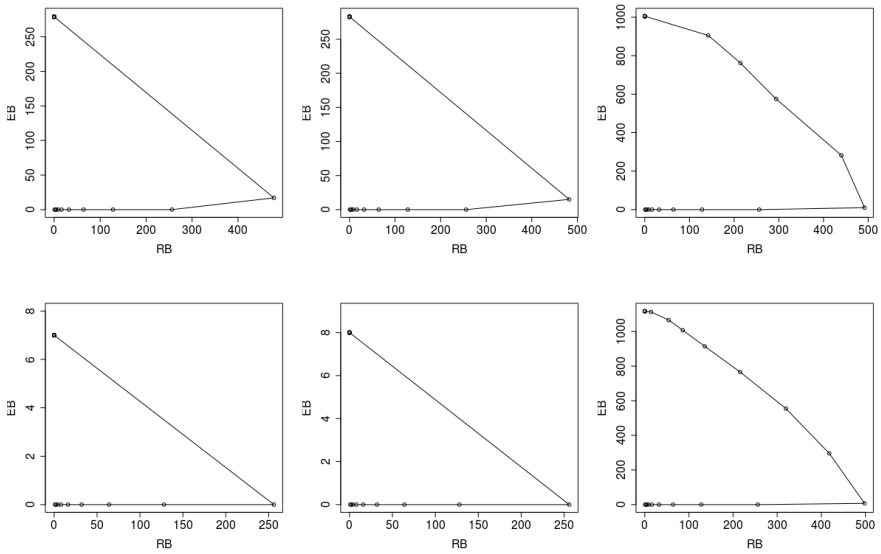

Figure 3.8: Simulations of the process with death-probability (3.12), with $(\alpha, \beta) = (2, 2)$.

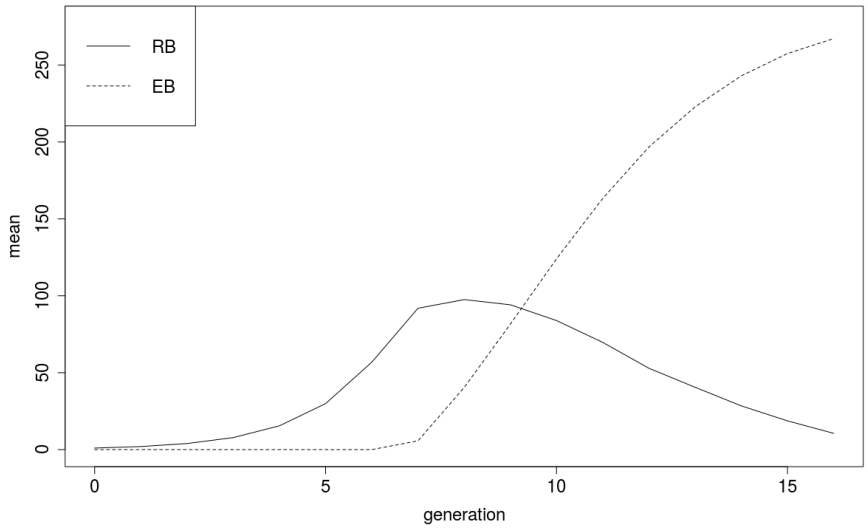

Figure 3.9: The mean number of EBs and RBs for the death-probability (3.12), with $(\alpha, \beta) = (3, 1)$.

(α, β)	gen	0	3	5	7	10	11	12	13
	hpi	12	16	20	24	28	32	36	40
$(1.8, 2.2)$	RB	1	8	32	128	634	286	286	87
	EB	0	0	0	0	194	687	687	940
$(1.9, 2.1)$	RB	1	8	32	128	500	314	177	104
	EB	0	0	0	0	262	603	825	945
$(2, 2)$	RB	1	8	32	128	429	309	211	136
	EB	0	0	0	0	287	563	768	910
$(2.1, 1.9)$	RB	1	8	32	128	390	323	244	177
	EB	0	0	0	0	268	497	697	858
$(2.2, 1.8)$	RB	1	8	32	128	356	314	256	195
	EB	0	0	0	0	261	460	648	804
measured	RB	1.3	7.6	34	105	385	507	271	171
	EB	0	0	0	3.7	192	656	706	751

Table 3.1: Conditional means for different values of (α, β) in our simulations, and real data from Lee et al. [16] (last two rows)

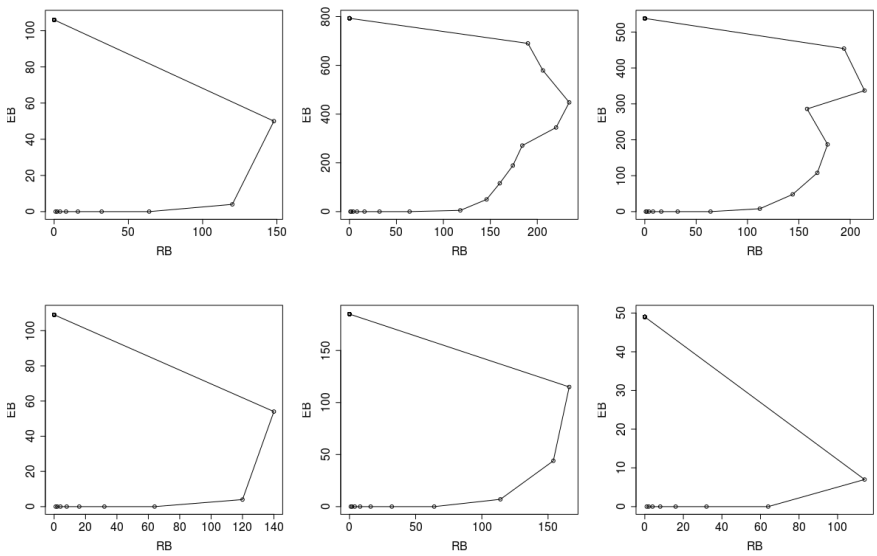

Figure 3.10: Simulations of the process with death-probability (3.12), with $(\alpha, \beta) = (3, 1)$.

which implies a shorter period of exponential increase in the RB population, and a longer coexistence of RB and EB population, see Figures 3.9 and 3.10. These results suggest that the effect of RBs on host cell's death is larger, or at least as large as the effect of EBs. Here $h(1, 0) = 285.7$. The p values in Figure 3.11 are even smoother than in the previous case, and the population prefers to have not too many RBs.

We note again that calculating the optimal probabilities is slow. From

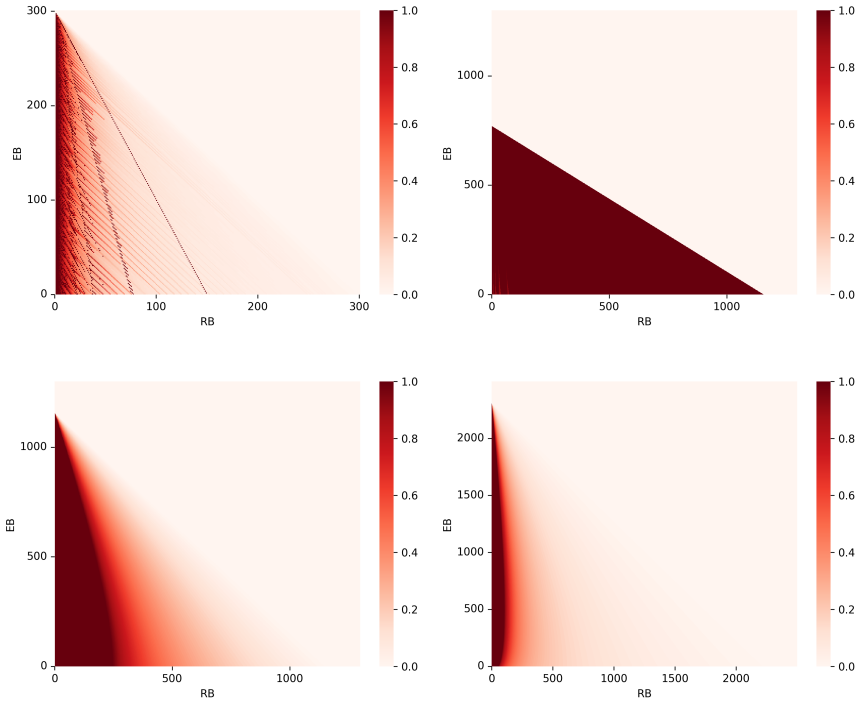

Figure 3.11: The numerical p values corresponding to the death-probability function (3.11) with $C = 300$ (top left), and to (3.12) with $(\alpha, \beta) = (1, 3)$ (top right), $(\alpha, \beta) = (2, 2)$ (bottom left), $(\alpha, \beta) = (3, 1)$ (bottom right).

(3.8) we see that the runtime is $O(C^3)$. For the death-probability function d_2 in (3.12) with $C = 3000$ it takes about 12 hours on a 5-year-old normal PC. Once we have the optimal probabilities simulations are fast. We calculated the optimal probabilities with the given $c_0 = 0.0003$, in the neighborhood of $(\alpha, \beta) = (2, 2)$. Simulations show that the optimal fit to the measurements is obtained around $(2, 2), (2.1, 1.9)$, see Table 3.1.

4

Branching model for the spread of *Chlamydia* under the influence of antibiotics

In Chapter 2 we formulated a model depicting the rise in bacterial count in relation to the concentration of antibiotics. We defined the expected value of the offspring as $m(c)$ and proceeded to fit a two-parameter function to it. Through our analysis, we observed that the parameters exhibit weakly consistency and asymptotic normality. As for real data, we analyzed the measurement results of the *Chlamydia trachomatis* bacterium.

In Chapter 3, we developed a model to characterize the optimal spread of *Chlamydia*. We were able to explicitly define the optimal strategy in the case where the time of host cell death was independent of the process, irrespective of the number of RBs and EBs within the host cell. As far as we are aware, the pending case has not been dealt with before. In this instance, we were able to numerically determine the optimal strategy, and we gave the conditional means for different values of (α, β) in our simulations, see in Table 3.1. We can conclude that there exists a high degree of similarity between the simulated data and the measured data. It's worth noting that antibiotics were not considered in that Chapter.

In this Chapter, we investigate the combined behavior of the two models, specifically determining the optimal spread of *Chlamydia* in the presence of antibiotic concentration. It's essential to emphasize that, as far as our knowledge extends, this does not involve actual measured data; however, it addresses a biologically relevant question entirely. So the novelty lies in the fact that an RB can either duplicate, transform into an EB, or undergo cell death. Initially, we neglect the scenario in which EBs can not perish, see in Section 4.1. Consequently, we require a three-type Galton–Watson process, wherein RBs, EBs, and deceased entities are treated distinctly. Following that, in Section 4.2 we also explore the scenario where the antibiotic affects both the RB and the EB.

4.1 The theoretical model (the antibiotic has no effect on EB)

Consider a three-type discrete-time Galton–Watson branching process $\mathbf{X}^\pi = (\mathbf{X}_n^\pi)_n = (X_n^\pi, Y_n^\pi, Z_n^\pi), n \geq 0$, together with a sequence of probabilities $\pi = (p_n)_n$. We assume that π is adapted to the natural filtration $(\mathcal{F}_n)_n$ generated by $\mathbf{X}^\pi$, i.e. $\mathcal{F}_n = \sigma(\mathbf{X}_k^\pi, k \leq n)$. Initially $\mathbf{X}_0^\pi = (1, 0, 0)$, and the process evolves as

$$\begin{aligned} X_{n+1}^\pi &= \sum_{i=1}^{X_n^\pi} \xi_{n,i}, \\ Y_{n+1}^\pi &= Y_n^\pi + \sum_{i=1}^{X_n^\pi} \eta_{n,i}, \\ Z_{n+1}^\pi &= Z_n^\pi + \sum_{i=1}^{X_n^\pi} \zeta_{n,i}, \quad n \geq 0, \end{aligned}$$

where (ξ_n, η_n, ζ_n), $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i})$ $n = 1, 2, \ldots$, $i = 1, 2, \ldots$ are conditionally independent random variables given $(p_n)_n$, for fix n the variables are identically distributed, such that

$$\begin{aligned} &\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (2, 0, 0) | p_n\right) = p_n, \\ &\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (0, 1, 0) | p_n\right) = 1 - p_n - p_c, \\ &\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (0, 0, 1) | p_n\right) = p_c. \end{aligned}$$

X_n^π and Y_n^π again stands for the number of RBs and number of EBs in generation n, while Z_n^π denotes the number of dead in generation n. In generation n each RB duplicates with probability p_n, or die, with a predetermined probability p_c, or converts into EB with probability $1 - p_n - p_c$. It's worth mentioning that p_c remains independent of the current generation, and it represents the probability of bacterial demise, contingent upon the antibiotic concentration. If $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (2, 0, 0)$, then the ith RB in generation n duplicates, if $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (0, 1, 0)$ then the ith RB converts to EB in generation n, while if $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (0, 0, 1)$ then the ith RB died in generation n. Similarly, we call π the strategy, the sequence of duplication probabilities is adapted to $(\mathcal{F}_n)_n$.

For the conditional expectations we obtain

$$\begin{aligned} \mathbf{E}[(X_{n+1}^\pi, Y_{n+1}^\pi, Z_{n+1}^\pi) | \mathcal{F}_n] &= (2p_n X_n^\pi, Y_n^\pi + (1 - p_n - p_c) X_n^\pi, Z_n^\pi + p_c X_n^\pi) \\ &= \mathbf{X}_n^\pi \begin{pmatrix} 2p_n & 1 - p_n - p_c & p_c \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}. \end{aligned}$$

The process also ends at a random time $T \in \{1, 2, \ldots\}$ and, if denoting by $\mathcal{P}$ the set of all strategies, a strategy $\mathbf{q}$ is optimal, if

$$\sup_{\pi \in \mathcal{P}} \mathbf{E}(Y_T^{\pi}) = \mathbf{E}(Y_T^{\mathbf{q}}).$$

As observed in Section 3.2, the independent case cannot exhibit asymmetric behavior. Hence, we will solely focus on the dependent case.

4.1.1 Death time T depends on X, and the antibiotic has no effect on EB

Assume that T, the death time depends on the process $\mathbf{X}^{\pi}$, and the antibiotic has effect on RB, and has no effect on EB. Given that the host cell is alive in generation $n-1$, the probability that it dies in the next step is $d(X_n^{\pi}, Y_n^{\pi}, Z_n^{\pi})$, that is

$$\mathbf{P}(T = n | T > n-1, \mathcal{F}_n) = d(X_n^{\pi}, Y_n^{\pi}, Z_n^{\pi}).$$

The deterministic *death-probability* function d describes the effect of RBs, EBs, and dead to cell's death. Assume that

$$\exists\, C > 0 \;\text{ such that }\; d(x, y, z) = 1 \;\text{ whenever }\; x + y + z \geq C. \tag{4.1}$$

That is, if the total number of bacteria exceeds C the host cell necessarily dies. We again modify our process to fit in [11]. Let $\widetilde{X}_n = X_n \mathbb{I}(T > n)$, $\widetilde{Y}_n = Y_n \mathbb{I}(T \geq n)$, and $\widetilde{Z}_n = Z_n \mathbb{I}(T \geq n)$. Then the state space is $\mathbb{N}^3$, the control set is $[0, 1 - p_c]$, the set of possible duplication probabilities for any state, p_c is the probability of antibiotic concentration-dependent death. Let $A := (\widetilde{X}_n = x, \widetilde{Y}_n = y, \widetilde{Z}_n = z, p_n = p, p_c)$, for $x > 0$, $y \geq 0$,

$z \geq 0$

$$
\begin{aligned}
&\mathbf{P}\left(\widetilde{X}_{n+1} = 2j, \widetilde{Y}_{n+1} = y + x - j - k, \widetilde{Z}_{n+1} = z + k | A\right) \\
&= \binom{x}{j} p^j \binom{x-j}{k} p_c^k (1 - p - p_c)^{x-j-k} \\
&\times (1 - d(2j, y + x - j - k, z + k)), \quad j = 1, \ldots, x, \quad k = 0, \ldots, x - j, \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y + x - j - k, \widetilde{Z}_{n+1} = z + k | A\right) \\
&= \binom{x}{j} p^j \binom{x-j}{k} p_c^k (1 - p - p_c)^{x-j-k} \\
&\times d(2j, y + x - j - k, z + k), \quad j = 1, \ldots, x, \quad k = 0, \ldots, x - j, \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y + x, \widetilde{Z}_{n+1} = z | A\right) \\
&= (1 - p - p_c)^x, \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y, \widetilde{Z}_{n+1} = z + x | A\right) \\
&= p_c^x,
\end{aligned}
\tag{4.2}
$$

while if $x = 0$

$$
\mathbf{P}(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = 0, \widetilde{Z}_{n+1} = 0 | B) = 1,
$$

where $B := (\widetilde{X}_n = 0, \widetilde{Y}_n = y, \widehat{Z}_n = z, p_n = p, p_c)$.

The first two formulae correspond to the possibility that $j \geq 1$ bacteria duplicate, $x - j - k$ bacteria transform, and k bacteria die (with probability $\binom{x}{j} p^j \binom{x-j}{k} p_c^k (1-p-p_c)^{x-j-k}$) and the host cell remains alive, or die, while the third and the fourth formula correspond to the possibility that all the RBs convert to EBs, and all the RBs die, and in this cases it does not matter whether the host cell dies or not. The last formula means that $(0, 0, 0)$ is the unique absorbing state, which is a convenient condition for the form of the reward function.

The *reward function* (-1 times the cost function in [11]) gives the number of EBs upon cell's death, that is

$$
c(x, y, z) = \begin{cases} y, & x = 0, \\ 0, & \text{otherwise.} \end{cases}
$$

Define the *value function*

$$
h(x, y, z) = \begin{cases} \sup_{\pi \in \mathcal{P}} \mathbf{E}\left[\sum_{n=0}^{\infty} c(\widetilde{X}_n, \widetilde{Y}_n, \widetilde{Z}_n) | (\widetilde{X}_0, \widetilde{Y}_0, \widetilde{Z}_0) = (x, y, z)\right], \\ y, \end{cases}
\tag{4.3}
$$

where in (4.3), the first case occurs when $d(x, y, z) < 1$, and the second one occurs when $d(x, y, z) = 1$. The h function in (4.3) is the optimal number of expected EBs upon host cell's death, given that the host cell is alive and $(\widetilde{X}_0, \widetilde{Y}_0, \widetilde{Z}_0) = (x, y, z)$, if $d(x, y, z) < 1$. If $d(x, y, z) = 1$ then the cell cannot be alive at state (x, y, z), thus the reward is y. Clearly $h(0, y, z) = y$. Due to the fact that $(0, 0, 0)$ is the only absorbing state in the infinite sum in (4.3) there is only one non-zero term.

We are looking for the value $h(x, y, z)$ and an optimal strategy π. This stochastic optimization problem is also in fact a finite-horizon problem. Indeed, from any state $(\widetilde{X}_n, \widetilde{Y}_n, \widetilde{Z}_n) = (x, y, z)$ either the total number of bacteria increases ($j \geq 1$ and the host cell survives in (4.2)), or $\widetilde{X}_{n+1} = 0$, meaning that the cell dies. Therefore, by condition (4.1) from any initial state (x, y, z) the process reaches the absorbing state $(0, 0, 0)$ in at most $C+1$ steps. So in (4.3) in the summation the upper limit can be changed to C. Using Theorem 3.2.1 in [11] both the value function h and an optimal strategy can be determined by backward induction on time. In our setup, backward induction on the total number of bacteria is more natural, and this goes as follows.

Theorem 4. *Assume that* (4.1) *holds. Then* $h(x, y, z) = y$ *if* $x+y+z \geq C$, *and* $h(0, y, z) = y$ *for any* y, *and for any* z. *Assume that* $h(x, y, z)$ *is determined whenever* $x+y+z \geq m$ *for some* $m \leq C$, *and let* $x+y+z = m-1$. *Then*

$$\begin{aligned} h(x, y, z) = \max_{p \in [0, 1-p_c]} \sum_{j=0}^{x} \sum_{k=0}^{x-j} \binom{x}{j} p^j \binom{x-j}{k} p_c^k (1-p-p_c)^{x-j-k} \\ \times [d(2j, y+x-j-k, z+k)(y+x-j-k) \\ + (1 - d(2j, y+x-j-k, z+k)) h(2j, y+x-j-k, z+k)], \end{aligned} \tag{4.4}$$

where all the values of h *on the right-hand side are determined. The maximum of the continuous function is attained at* $p(x, y, z)$, *which gives an optimal strategy.*

We note that the proof of Theorem 4 is entirely similar to the proof of Theorem 2.

4.1.2 Simulation study

For a given death-probability function d, we can determine numerically the value function and the optimal strategy using Theorem 4. Then, the process is a simple Galton–Watson branching process with state-dependent offspring distribution, which can be simulated easily. Throughout the numeric calculation, we encountered the issue of sluggish computations. Hence, it is imperative to opt for a probability death function that can be efficiently scaled up, see

$$d_3(x,y,z) = \begin{cases} 1 - e^{-c_0 \frac{\alpha x + \beta y + \gamma z}{2C}}, & x+y+z \leq C-1, \\ 1, & x+y+z \geq C. \end{cases} \tag{4.5}$$

The procedure is as follows. Using Theorem 4 we compute the optimal values for $C = 64$ and $C = 128$, as they execute relatively swiftly. Subsequently, we verify that the values within the p matrices remain unchanged under scaling. The invariance property was consistently upheld, allowing us to scale the values up to $C = 1024$, which represents a roughly significant threshold, as depicted in equation (4.5). We simulate the process as follows. Based on the Theorem 4, we calculate the optimal values for $C = 128$, with a given choice of p_c and (α, β, γ), with probability death function (4.5). After scaling up the values, we can proceed to simulate from the scaled data. It's worth noting that even for $C = 128$, the computations may take up to 5 hours on a typical computer. A straightforward calculation reveals that $p_c < \frac{1}{2}$ must be satisfied when selecting the parameter, otherwise the process will not be supercritical. Throughout the simulations, in all cases $(\alpha, \beta, \gamma) = (2, 2, 1)$, indicating that RB and EB are assigned similar effect, which appears to be a sensible choice, see 3.1, while the dead are assigned a single effect. We conducted simulations for the cases where $p_c \in \{0, 0.2, 0.4\}$. Additionally, we plotted the mean for each simulation, during which we simulated the $\mathbf{X}^\pi$ process 1000 times.

Figure 4.1 displays four trajectories of the case where $p_c = 0$, indicating that the antibiotic has no effect on RB. It is evident that deceased individuals do not appear. It is observable that the RB bodies are roughly divided until the seventh generation, after which the conversion to the EB begins.

In Figure 4.2, we observe the representation of the averages in the case where $p_c = 0$. Naturally, deceased individuals do not appear. Around the seventh generation, EBs begin to emerge.

Figure 4.3 depicts four trajectories of the $\mathbf{X}^\pi$ process, with paramter $p_c = 0.2$, indicating that the antibiotic has a relatively minor impact on the RB body. It is apparent that deceased individuals are already appearing.

In Figure 4.4, the mean are depicted. Interestingly, despite the relatively small value of p_c, the average of the 1000 simulations results in approximately the same number of deceased or EB individuals. So even though the antibiotic doesn't have as much of an effect, we still see about the same number of EBs and dead individuals at the end of the process, which is surprising.

The simulations in Figure 4.5 depict the scenario where the value of $p_c = 0.4$ is already close to the critical value of 0.5. It can be seen that at the end of each trajectory, there are more dead individuals than EB.

As observed in Figure 4.6, it's apparent from the averages that the process struggles to maintain RB and EB. At the end of the process, the

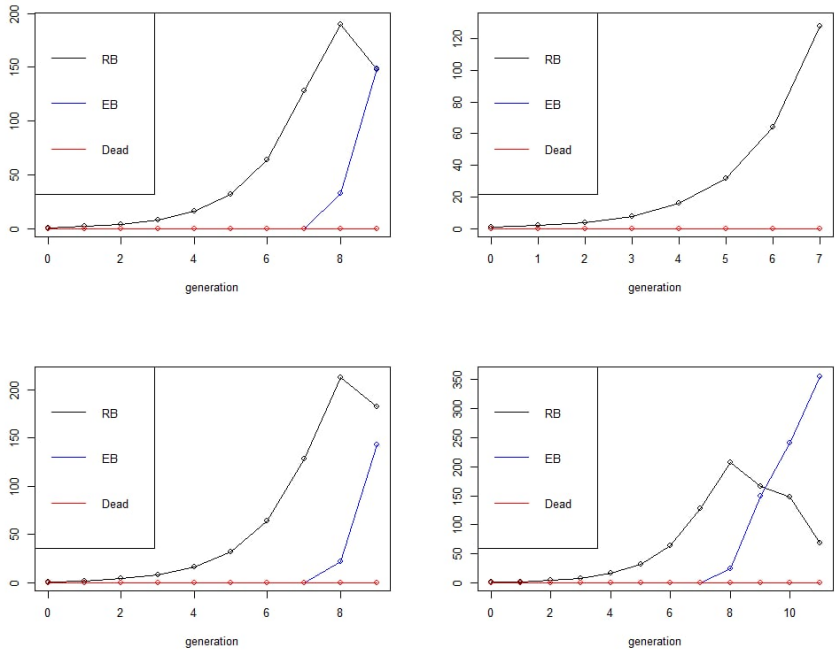

Figure 4.1: Simulations of the process with death-probability function (4.5), with parameters $p_c = 0$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

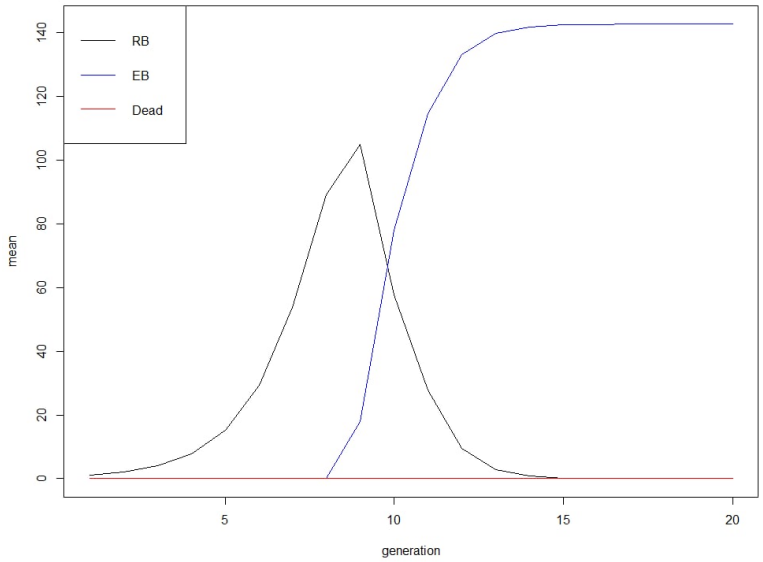

Figure 4.2: The mean of EBs, RBs, and dead with death-probability function (4.5), with parameters $p_c = 0$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

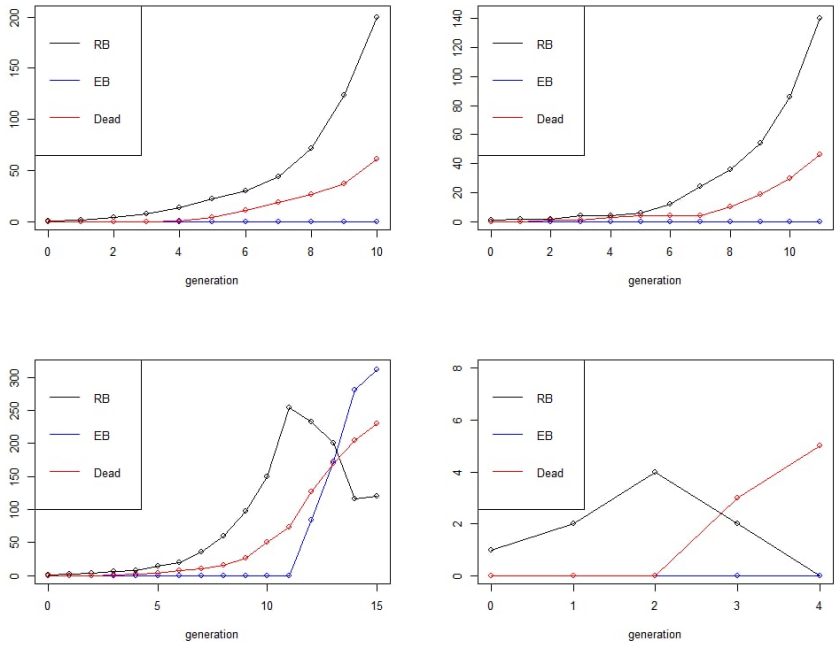

Figure 4.3: Simulations of the process with death-probability function (4.5), with parameters $p_c = 0.2$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

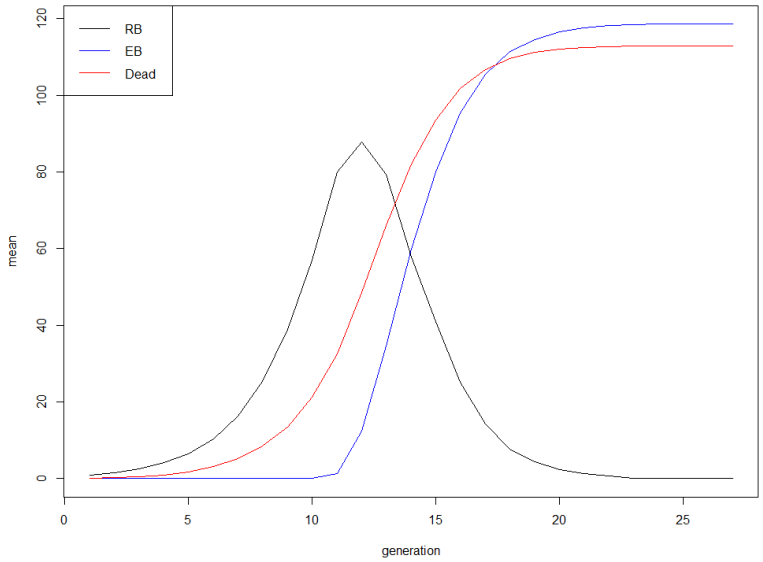

Figure 4.4: The mean of EBs, RBs, and dead with death-probability function (4.5), with parameters $p_c = 0.2$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

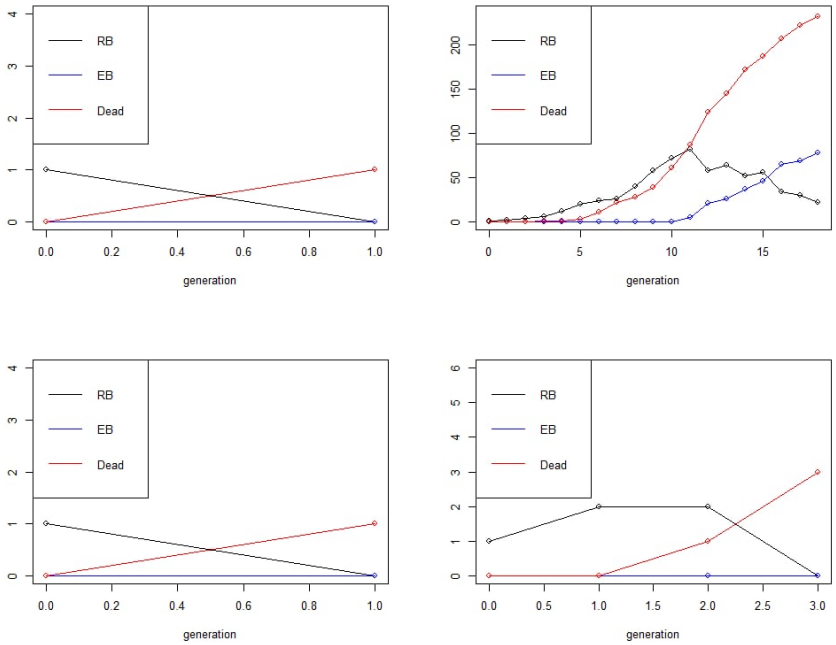

Figure 4.5: Simulations of the process with death-probability function (4.5), with parameters $p_c = 0.4$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

average number of dead individuals will be much higher than the number of EB.

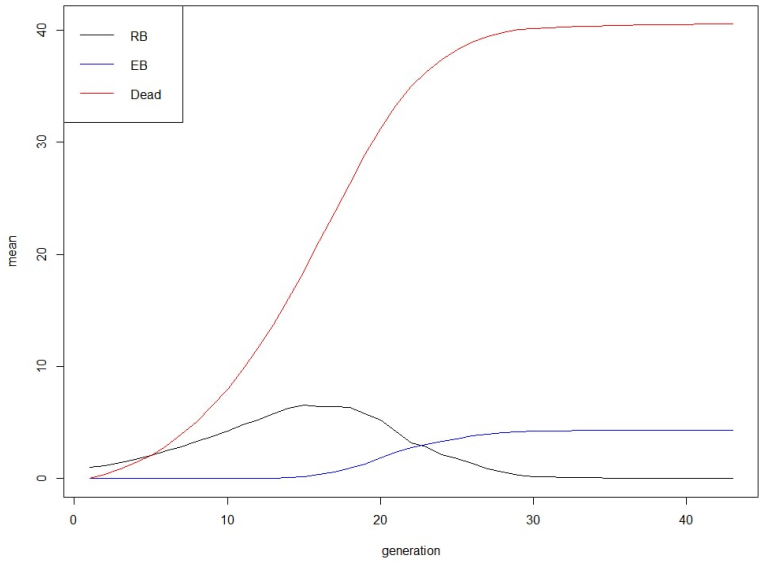

Figure 4.6: The mean of EBs, RBs, and dead with death-probability function (4.5), with parameters $p_c = 0.4$, $(\alpha, \beta, \gamma) = (2, 2, 1)$, and $C = 1024$.

4.2 The theoretical model (antibiotic has effect on both types)

In this Section, we presume that the antibiotic also exerts an influence on the RB body, and EB body as well. This approach is considerably more intricate, and unfortunately, we cannot furnish simulation results in this context due to the extensive computation time required. The main issue that emerged was that, until now, it was assumed that $h(0, y, z) = y$. However, we cannot assert that in this case, as the process does not unequivocally halt in the state $(0, y, z)$, as it has done thus far, since the antibiotic now has the same effect on the EB. Hence, we introduced an finite $N \in \{1, 2, \ldots\}$ value, which aligns with the rationale outlined in Chapter 2. It specifies the time at which the process will end. Note that N was about 10 in the antibiotic experiment, see in Chapter 2.

Again consider a three-type discrete-time Galton–Watson branching process $\mathbf{X}^\pi = (\mathbf{X}_n^\pi)_n = (X_n^\pi, Y_n^\pi, Z_n^\pi), n \geq 0$, together with a sequence of probabilities $\pi = (p_n)_n$. We assume that π is adapted to the natural filtration $(\mathcal{F}_n)_n$ generated by $\mathbf{X}^\pi$, i.e. $\mathcal{F}_n = \sigma(\mathbf{X}_k^\pi, k \leq n)$. Initially $\mathbf{X}_0^\pi = (1, 0, 0)$, and the process evolves as

$$
\begin{aligned}
X_{n+1}^{\pi} &= \sum_{i=1}^{X_n^{\pi}} \xi_{n,i}, \\
Y_{n+1}^{\pi} &= \sum_{i=1}^{X_n^{\pi}} \eta_{n,i} + \sum_{i=1}^{Y_n^{\pi}} (1 - \zeta'_{n,i}), \\
Z_{n+1}^{\pi} &= Z_n^{\pi} + \sum_{i=1}^{X_n^{\pi}} \zeta_{n,i} + \sum_{i=1}^{Y_n^{\pi}} \zeta'_{n,i}, \quad n \geq 0,
\end{aligned}
$$

where $(\xi_n, \eta_n, \zeta_n, \zeta'_n)$, $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i}, \zeta'_{n,i})$ $n = 1, 2, \ldots$, $i = 1, 2, \ldots$ are conditionally independent random variables given $(p_n)_n$, for fix n the variables are identically distributed, such that

$$
\begin{aligned}
&\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (2, 0, 0) | p_n\right) = p_n, \\
&\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (0, 1, 0) | p_n\right) = 1 - p_n - p_c, \\
&\mathbf{P}\left((\xi_n, \eta_n, \zeta_n) = (0, 0, 1) | p_n\right) = p_c,
\end{aligned}
$$

and $\mathbf{P}(\zeta'_n = 0) = 1 - q_c$, $\mathbf{P}(\zeta'_n = 1) = q_c$. X_n^{π} and Y_n^{π} again stands for the number of RBs and number of EBs in generation n, while Z_n^{π} denotes the number of dead in generation n. In generation n each RB duplicates with probability p_n, or die, with a predetermined probability p_c, or convert into EB with probability $1 - p_n - p_c$. In generation n each EB dies with a determined probability q_c. It's worth mentioning that p_c, and q_c remain independent of the current generation, and it represents the probability of bacterial demise, contingent upon the antibiotic concentration, and we assume that $q_c \ll p_c$. If $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (2, 0, 0)$, then the ith RB in generation n duplicates, if $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (0, 1, 0)$ then the ith RB converts to EB in generation n, while if $(\xi_{n,i}, \eta_{n,i}, \zeta_{n,i} = (0, 0, 1)$ then the ith RB died in generation n. If $\zeta'_{n,i} = 1$, then the ith EB died in generation n, while $\zeta'_{n,i} = 0$, the ith EB does not die in the generation n. Similarly, we call π the strategy, the sequence of duplication probabilities is adapted to $(\mathcal{F}_n)_n$.

For the conditional expectations we obtain

$$
\mathbf{E}[(X_{n+1}^{\pi}, Y_{n+1}^{\pi}, Z_{n+1}^{\pi}) | \mathcal{F}_n] = \mathbf{X}_n^{\pi} \begin{pmatrix} 2p_n & 1 - p_n - p_c & p_c \\ 0 & 1 - q_c & q_c \\ 0 & 0 & 1 \end{pmatrix}.
$$

The process concludes at a predetermined time N, and our objective is to optimize $\mathbf{E}(Y_N)$. It's important to note that this optimization problem differs from the one in Chapter 3 or Section 4.1. In those cases, the process halted at a random time T, whereas here, we are aware that we will terminate the process at a predetermined time N. Certainly, we require the variable T in the same manner.

4.2.1 Death time T depends on X, and the antibiotic has effect on both types (RB and EB)

Here we assume that T, the host cell death time depends on the process $\mathbf{X}^\pi$, and the antibiotic has effect on both type. Furthermore, we assume that the process ends at a predetermined time N. Note that there is no correlation between the times N and T. Given that the host cell is alive in generation $n-1 < N$, the probability that it dies in the next step is $d(X_n^\pi, Y_n^\pi, Z_n^\pi)$, that is

$$\mathbf{P}(T = n | T > n-1, \mathcal{F}_n) = d(X_n^\pi, Y_n^\pi, Z_n^\pi).$$

The deterministic *death-probability* function d describes the effect of RBs, EBs, and dead to cell's death. Assume that

$$\exists\, C > 0 \;\text{ such that }\; d(x,y,z) = 1 \;\text{ whenever }\; x+y+z \geq C. \tag{4.6}$$

We assume that the cells are in vitro, and the process stops after fix N generation time. We must redefine our process once more, taking into account the scenario where if the host cell perishes before time N, the (Y_n, Z_n) process will still persist,

$$\widetilde{X}_n = X_n \mathbb{I}(T > n), \quad \widetilde{Y}_n = Y_n, \quad \widetilde{Z}_n = Z_n.$$

We need to adapt our process in the following manner: if the host cell perishes before reaching time N, then the RB bodies are reset to zero, as they cannot transform into EB bodies outside the host cell, rendering their count irrelevant. However, EB bodies continue to be influenced by the antibiotic in the same manner until time N. Due to these considerations, we must now factor in the number of generations during the optimization calculation. Then the state space is $\mathbb{N}^4$, the control set is $[0, 1-p_c]$, the set of possible duplication probabilities for any state, and the transition probabilities are, for $x \geq 0$, $y \geq 0$, $z \geq 0$, $n \leq N-1$ and

let $A := (\widetilde{X}_n = x, \widetilde{Y}_n = y, \widetilde{Z}_n = z, p_n = p, p_c, q_c)$

$$
\begin{aligned}
&\mathbf{P}\left(\widetilde{X}_{n+1} = 2j, \widetilde{Y}_{n+1} = y - \ell + x - j - k, \widetilde{Z}_{n+1} = z + k + \ell | A\right) \\
&= \binom{x}{j} p^j \binom{x-j}{k} p_c^k (1 - p - p_c)^{x-j-k} \binom{y}{\ell} q_c^\ell (1 - q_c)^{y-\ell} \\
&\times (1 - d(2j, y - \ell + x - j - k, z + k + \ell)), \\
&j = 1, \ldots, x, \quad k = 0, \ldots, x - j, \quad \ell = 0, \ldots, y. \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y - \ell + x - j - k, \widetilde{Z}_{n+1} = z + k + \ell | A\right) \\
&= \binom{x}{j} p^j \binom{x-j}{k} p_c^k (1 - p - p_c)^{x-j-k} \binom{y}{\ell} q_c^\ell (1 - q_c)^{y-\ell} \\
&\times (d(2j, y - \ell + x - j - k, z + k + \ell)), \\
&j = 1, \ldots, x, \quad k = 0, \ldots, x - j, \quad \ell = 0, \ldots, y. \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y + x - \ell, \widetilde{Z}_{n+1} = z + \ell | A\right) \\
&= (1 - p - p_c)^x \binom{y}{\ell} q_c^\ell (1 - q_c)^{y-\ell}, \quad \ell = 0, \ldots, y. \\
&\mathbf{P}\left(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y - \ell, \widetilde{Z}_{n+1} = z + x + \ell | A\right) \\
&= p_c^x \binom{y}{\ell} q_c^\ell (1 - q_c)^{y-\ell}, \quad \ell = 0, \ldots, y,
\end{aligned}
\tag{4.7}
$$

while if $x = 0$

$$
\begin{aligned}
&\mathbf{P}(\widetilde{X}_{n+1} = 0, \widetilde{Y}_{n+1} = y - \ell, \widetilde{Z}_{n+1} = z + \ell | A) \\
&= \binom{y}{\ell} q_c^\ell (1 - q_c)^{y-\ell}, \quad \ell = 0, \ldots, y.
\end{aligned}
$$

The first two formulae correspond to the possibility that $j \geq 1$ RBs duplicate, k RBs bacteria die, $x - j - k$ RBs transform to EBs (with probability $\binom{x}{j} p^j \binom{x-j}{k} p_c^k (1-p-p_c)^{x-j-k}$), and ℓ EBs die, $y-\ell$ EBs persist (with probability $\binom{y}{\ell} q_c^\ell (1-q_c)^{y-\ell}$) and the host cell remains alive, or die. While the third and the fourth formula correspond to the possibility that all the RBs convert to EBs, and all the RBs die, and in this cases it does not matter whether the host cell dies or not (ℓ EBs die, $y - \ell$ EB persist in both cases). The final formula indicates that if $x = 0$, contrary to previous cases, the processes $\widetilde{Y}_n$ and $\widetilde{Z}_n$ will not reset but will continue until time N.
Thus we have that, the reward function

$$
c(x, y, z; n) = \begin{cases} y, & n = N, \\ 0, & \text{otherwise.} \end{cases} \tag{4.8}
$$

This assumption is entirely reasonable, as there is a reward only if $n = N$.

Define the *value function*

$$h(x,y,z;n) = \sup_{\pi\in\mathcal{P}} \mathbf{E}\left[c(\widetilde{X}_N,\widetilde{Y}_N,\widetilde{Z}_N;N)|(\widetilde{X}_0,\widetilde{Y}_0,\widetilde{Z}_0)=(x,y,z)\right]. \quad (4.9)$$

We are looking for the value $h(x,y,z;n)$ and an optimal strategy π. This stochastic optimization problem is also in fact a finite-horizon problem. Indeed, from any state $(\widetilde{X}_n,\widetilde{Y}_n,\widetilde{Z}_n)=(x,y,z)$ either the total number of bacteria increases ($j\geq 1$ and the host cell survives in (4.2)), or $\widetilde{X}_{n+1}=0$, meaning that the host cell dies. It is evident that the process terminates within a finite time, as it will inevitably cease for a fixed N. It's worth mentioning that this value was approximately 10 in the bacterial experiment, as shown in Chapter 2. Another crucial observation is that the process does not halt in the state $(0,y,z;n)$ if $n<N$. In Theorem 5, we can determine the h value of a state using the same backward step recursive method, as in Theorem 2, in Theorem 3, and in Theorem 4.

Theorem 5. *Assume that* (4.6) *holds, and N is given. Then for any $n\leq N$, $h(x,y,z;n)=y(1-q_c)^{N-n}$, if $x+y+z\geq C$, and $h(0,y,z;n)=y(1-q_c)^{N-n}$ for any y, and for any z. Assume that $h(x,y,z;n)$ is determined whenever $x+y+z+n\geq m$ for some $m\leq C+n$, and let $x+y+z+n=m-1$. Then for all $n\leq N-1$*

$$\begin{aligned}
h(x,y,z;n) = &\max_{p\in[0,1-p_c]} \sum_{j=0}^{x}\sum_{k=0}^{x-j}\sum_{\ell=0}^{y}\binom{x}{j}\binom{x-j}{k}\binom{y}{\ell}\\
&\times p^j p_c^k (1-p-p_c)^{x-j-k} q_c^\ell (1-q_c)^{y-\ell}\\
&\times [d(2j,y-\ell+x-j-k,z+k+\ell)(y-\ell+x-j-k)(1-q_c)^{N-(n+1)}\\
&+(1-d(2j,y-\ell+x-j-k,z+k+\ell))\\
&\times (h(2j,y-\ell+x-j-k,z+k+\ell;n+1)(\mathbb{I}(n+1<N)\\
&+(y-\ell+x-j-k)\mathbb{I}(n+1=N)))],
\end{aligned} \quad (4.10)$$

where all the values of h on the right-hand side are determined. The maximum of the continuous function is attained at $p(x,y,z)$, which gives an optimal strategy.

5

Summary

The thesis presents the stochastic modeling of the *Chlamydia* bacterial species.

In Chapter 2, we present a Galton–Watson model describing the growth of a bacterial population in the presence of antibiotic concentration. Our stochastic model is much more natural compared to previous deterministic models, see Liu et al. [17]. We assumed that the expected value of offspring is given by the formula $m(c) = 2/(1 + \alpha c^{\beta})$, where c is the antibiotic concentration, and $\alpha > 0$, $\beta > 0$ are unknown parameters. Considering measurement error in the qPCR technique, we obtained weakly consistent and asymptotically normal estimates for the unknown parameters (α, β) at different antibiotic concentrations.

The *minimal inhibitory concentration* (MIC) is the lowest concentration of an antibiotic that inhibits the growth of bacteria, which is a crucial parameter in pharmacology. Estimating the MIC is quite challenging because with the standard double dilution technique, only bacterial growth can be observed at certain antibiotic concentrations, such as $c_0, 2c_0, \ldots, 2^k c_0$. Therefore, we can only claim that the MIC falls within some interval $[c, 2c]$, or we can provide an upper bound. The majority of the literature does not offer a proper mathematical model for bacterial population growth; it merely defines the MIC as the smallest antibiotic concentration at which no visible bacterial growth occurs. In our work, we provide an explicit mathematical definition of the MIC and a estimation procedure.

From the simulation data, we can observe that the estimates perform well even when the number of simulations varies at different concentrations, set to 3, which is the recommended measurement count in microbiology (see Yuan et al. [26] and Eszik et al. [8]).

We applied the model to real measurement data, where the growth of *Chlamydia trachomatis* bacteria was examined under two different antibiotics. Although our mathematical model has only two parameters, we found an exceptionally good fit to the real data for both bactericidal

and bacteriostatic antibiotics.

In Section 2.6, we retained the model assumption from Chapter 2, but we estimated the probability of extinction using a different measurement procedure. During colony counting, if x_0 individuals (e.g., *Escherichia coli*) are inoculated onto an agar plate containing antibiotics, at the end of the incubation period, all viable colonies are counted. We assumed that the distribution of offspring is given by the formula $p_2(c) = m(c)/2$.

Similarly, we obtained weakly consistent and asymptotically normal estimates for the parameters (α, β) as well as for the MIC.

In Chapter 3, we present a new Galton–Watson branching model to model the evolution of *Chlamydia* populations. In this model, determining the state-dependent offspring distribution is achieved through solving a stochastic optimization problem. The only input information we have about the process is the death-probability function d, which gives the probability of a host cell dying in a given state.

By choosing a natural death function, simulation results show that the process is capable of capturing the asynchronous behavior of bacterial cells, which has recently been supported by experiments in [16]. Furthermore, our simulated data fits extremely well with real measured data, as reported in [16], shown in Table 3.1. To the best of our knowledge, this is the first mathematical model that reproduces this phenomenon.

The exact cause of host cell death is not well understood. Experiments suggest that *Chlamydia* regulates the survival of host cells because early cell death would be disadvantageous for the bacterial population, as seen in [5, p. 394]. However, the quantity of bacteria within the host cell has a pronounced effect. It is not clear which form of the bacterium is more harmful to the host cell, as RBs are physically larger, while EBs release chemical substances. By altering the relative impact of RBs and EBs, our simulation studies indicate that both RBs and EBs have an equal effect on host cell death.

In Chapter 4, we integrated our previous findings. We examined the optimal spread of *Chlamydia* in the presence of antibiotics. It is worth noting that, to the best of our knowledge, there are no real measurement data available, but this assumption is biologically plausible. We assumed that the distribution of offspring follows a Galton–Watson process. In the simplest case, we simplified our model by assuming that the antibiotic affects only the RB form, as shown in Subsection 4.1. This is a biologically reasonable assumption because the infectious EB form is capable of forming inclusions, thus better enduring unfavorable conditions. By choosing a scalable death function, we can simulate the process, where a new parameter p_c represents the antibiotic effect, which remains constant throughout the process. In Subsection 4.2, we assumed that the antibiotic affects both types of *Chlamydia*, introducing another parameter q_c, representing the probability of EB form death. We assumed $q_c \ll p_c$. It

is noteworthy that unlike the models found in Chapter 3 and Subsection 4.1, the process will not stop when the number of RB bodies becomes zero. To address this issue, we introduced a deterministic value N, representing the end of the process in this generation. This value was around 10 based on real measured data, as seen in Chapter 2.

The thesis is based on three articles of the author. These publications are the following:

[**1**] A. Bogdanov, P. Kevei, M. Szalai, D. Virok: Stochastic modeling of in vitro bactericidal potency. *Bulletin of Mathematical Biology* 84 (6), 2022.

[**2**] M. Szalai, P. Kevei: Estimation of in vitro bactericidal potency based on colony counting method. 22nd EYSM 2021 Conference Proceedings.

[**3**] P. Kevei, M. Szalai: Branching model with state dependent offspring distribution for *Chlamydia* spread.

Other publications from the author are as follows:

(i) Máder Attila, Szalai Máté: A kétoldali közelítés, leszámlálás módszere, *Polygon* 27 No. 1 (2024), 8-39.

(ii) Szalai Máté: Szimulációk használata a sztochasztika oktatásában, 2024.

6

Összefoglaló

A disszertáció a *Chlamydia* baktériumfaj sztochasztikus modellezését mutatja be.

A 2. fejezetben megadunk egy Galton–Watson modellt, mely egy baktériumpopuláció növekedését írja le, az antibiotikum koncentráció jelenlétében. A sztochasztikus modellünk sokkal természetesebb a korábbi determinisztikus modellekhez képest, ld. Liu és munkatársai cikkében [17]. Feltettük, hogy az utódok várható értékét az $m(c) = 2/(1+\alpha c^{\beta})$ formula adja meg, ahol c az antibiotikum koncentráció, valamint $\alpha > 0, \beta > 0$ ismeretlen paraméterek. A qPCR technikában figyelembe véve a mérési hibát, különböző antibiotikum koncentráció esetén gyengén konzisztens, valamint aszimptotikusan normális becsléseket kaptunk az ismeretlen (α, β) paraméterekre.

A *minimális gátló koncentráció* (MIC=minimal inhibitory concentration), a legkisebb antibiotikum koncentráció, amely megakadályozza a baktériumok növekedését, mely egy nagyon fontos paraméter a farmakológiában. A MIC-nek a becslése meglehetősen nehézkes, mivel a szokásos kettős hígítási technika miatt $c_0, 2c_0, \ldots, 2^k c_0$, csak a baktériumok növekedése figyelhető meg adott antibiotikum-koncentráció mellett. Emiatt csak azt állíthatjuk, hogy a MIC valamilyen $[c, 2c]$ intervallumba esik, vagy egy felső korlátot tudunk rá adni. A szakirodalmak túlnyomó többsége nem nyújt megfelelő matematikai modellt a baktériumpopuláció növekedésére, csak a MIC értéket határozza meg, mint a legkisebb antibiotikum koncentrációt, látható baktériumnövekedés nélkül. Munkánkban megadjuk a MIC explicit matematikai definícióját, illetve egy becslési eljárását is bemutatjuk.

A szimulációs adatokból láthatjuk, hogy a becslések jól működnek, még akkor is, ha a szimulációk száma különböző koncentrációkban 3, ami a javasolt mérési szám a mikrobiológiában (lásd pl. Yuan és munkatársai [26], illetve Eszik és munkatársai [8] cikkekben).

A modellt valós mérési adatokra alkalmaztuk, ahol a *Chlamydia trachomatis* baktérium növekedését vizsgálták, két különböző antibiotikum

mellett. Noha a matematikai modellünknek csak két paramétere van, mégis rendkívül jó illeszkedést találtunk a valós adatokhoz, mind a baktericid, mind a bakteriosztatikus antibiotikum esetében.

A 2.6. alfejezetben a 2. fejezetben lévő modellfeltevésünket meghagytuk, azonban más mérési eljárást feltételezve a kihalási valószínűséget tudtuk becsülni. A kolóniaszámlálás során, hogy ha x_0 számú egyedet (pl. *Escherichia coli*) oltanak rá egy sor antibiotikumot tartalmazó agarlemezre, akkor az inkubációs időszak végén az összes életképes telepet megszámolják. Feltettük, hogy az utódok eloszlását a $p_2(c) = m(c)/2$ formula adja meg.

Hasonlóan gyengén konzisztens, valamint aszimptotikusan normális becsléseket kaptunk az (α, β) paraméterekre, valamint a MIC-re.

A 3. fejezetben a *Chlamydia* populációk evolúciójának modellezésére egy új Galton–Watson elágazó modellt adunk meg. Ebben a modellben az állapotfüggő utódeloszlás meghatározása sztochasztikus optimalizációs probléma megoldásával történik. A folyamatról az egyetlen bemeneti információnk a d halálozási függvény, mely megadja annak a valószínűségét, hogy a gazdasejt adott állapotban meghal.

Természetes halálozási függvényt választva, a szimulációs eredmények azt mutatják, hogy a folyamat képes megfogni a baktériumsejtek aszinkron viselkedést, amit nem régen kísérletekkel is alátámasztottak [16]. Továbbá, a szimulált adataink rendkívül jól illeszkednek a valós mért adatokhoz, ez látható a 3.1. táblázatban. Legjobb tudomásunk szerint ez az első olyan matematikai modell, amely reprodukálja ezt a jelenséget.

A gazdasejt halálának pontos oka még nem jól ismert. A kísérletek azt sugallják, hogy a *Chlamydia* szabályozza a gazdasejtek túlélését, mivel a korai elhalás hátrányos lenne a baktériumpopulációra vonatkozóan lásd [5, 394.o.]. A gazdasejtben lévő baktériumok mennyisége azonban határozottan erős hatással rendelkezik. Nem világos, hogy a baktérium melyik formája károsabb a gazdasejtre, mivel az RB-k fizikailag nagyobbak, míg az EB-k vegyi anyagokat választanak ki. Az RB-k és EB-k relatív hatását változtatva, a szimulációs vizsgálataink szerint az RB-k és az EB-k ugyanolyan hatással vannak a gazdasejt halálára.

A 4. fejezetben a korábbi eredményeinket kapcsoltuk össze. Megvizsgáltuk a *Chlamydia* optimális terjedését abban az esetben, hogy ha jelen van az antibiotikum. Megjegyzendő, hogy a legjobb ismereteink szerint valós mérési adatok nincsenek, azonban biológiailag egy természetes feltevés. Feltettük, hogy az utódok eloszlása Galton–Watson folyamat szerint alakul. Legelső esetben a modellünket annyiban egyszerűsítettük, hogy az antibiotikum csupán az RB alakra fejti ki hatását, ez látható a 4.1. alfejezetben. Ez biológiailag egy indokolható feltevés, hiszen a fertőző EB forma képes zárványokat létrehozni, ezáltal jobban elviselni a kellemetlen körülményeket. Skálázható halálozási függvényt választva

tudjuk szimulálni a folyamatot, ahol egy új paraméter a p_0, mely megadja az antibiotikum hatását, ami egy konstans érték a folyamat alatt.

A 4.2. alfejezetben feltettük, hogy az antibiotikum mindkét *Chlamydia* típusra hatással van, bevezetve egy újabb q_c paramétert, mely megadja az EB forma halálának valószínűségét. Feltettük, hogy $q_c \ll p_c$. Megjegyzendő, hogy a 3. fejezetben található modell, valamint a 4.1. alfejezetben fellelhető modellekkel ellentétben a folyamat nem fog megállni akkor, amikor az RB testek száma nulla lesz. A probléma áthidalása végett bevezettünk egy N determinisztikus értéket, mely a folyamat végét jelenti ebben a generációban. Ez az érték a valós mért adatoknál olyan 10 körül volt, ez látható a 2. fejezetben.

A disszertáció a szerző három munkáján alapul, ezek a következők:

[1] A. Bogdanov, P. Kevei, M. Szalai, D. Virok: Stochastic modeling of in vitro bactericidal potency. *Bulletin of Mathematical Biology* 84 (6), 2022.

[2] M. Szalai, P. Kevei: Estimation of in vitro bactericidal potency based on colony counting method. 22nd EYSM 2021 Conference Proceedings.

[3] P. Kevei, M. Szalai: Branching model with state dependent offspring distribution for *Chlamydia* spread.

A szerző további munkái:

(i) Máder Attila, Szalai Máté: A kétoldali közelítés, leszámlálás módszere, *Polygon* 27 No. 1 (2024), 8-39.

(ii) Szalai Máté: Szimulációk használata a sztochasztika oktatásában, 2024.

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