TOWARDS A BETTER UNDERSTANDING OF THE EVOLUTION OF SENESCENCE, APOPTOSIS AND TUMOUR GROWTH

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A thesis submitted in partial fulfilment of the requirements for the degree of
PhD in Computing

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Abstract

Senescence (ageing) and apoptosis (programmed cell death) are phenomena that have troubled theoreticians and experimentalists. Previous research showed that the mortality curve of the yeast population followed the Gompertz-Makeham equation. We develop a generalised theoretical model which shows that the mortality of the organism can be expressed as a function of Ageing Factors such as ERCs. We use this idea to explain why senescence leads to apoptosis.

Antagonistic pleiotropy and disposable soma theory suggest that senescence (and accordingly apoptosis) is a ‘side effect’. Although the altruistic benefits of apoptosis have been suggested before, we are attempting to show that in a resource-restricted environment, apoptosis can be a strategic choice. We show that the interactions between apoptotic and non-apoptotic organisms can be modelled using game theory and differential equations. We find that switching to apoptotic mode gives the organism an advantage over the non-apoptotic organisms in a resource-restricted environment. Mathematical analysis indicates that apoptosis is a stable strategy provided the conditions remain the same. We also find that one apoptotic organism can invade a population of non-apoptotic organisms. This begs the question - why do tumours (which are non-apoptotic) occur if apoptosis is the best strategy? We show that apoptosis and angiogenesis play a significant role in the development of tumours. We studied the effects of these two parameters on the dynamics of tumour and apoptotic populations. We find that the mixed strategy of avoidance of apoptosis and angiogenesis gives neoplasms an advantage over apoptotic organisms in certain conditions. Accordingly, the tumour organisms can invade apoptotic tissues. We also find that this strategy is not beneficial in the long-term.
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Statement of Originality

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Acknowledgements

A number of people have assisted in the production of this work. Firstly, I would like to thank my supervisor Dr. André Grüning, as well as my co-supervisors Dr. Matthew Casey and Prof. Paul Krause. Dr. Grüning has provided me with much useful advice and support throughout my PhD research and helped me in refining this thesis. Discussions with Dr. Matthew Casey and Dr. Uli Steiner have been greatly appreciated. I thank Dr. Colin Gillespie and Dr. David Sinclair for providing the necessary data on yeast ageing. Further people who have provided comments, useful information, or support, include (amongst many others) Dr. Lilian Tang (who was the examiner for the confirmation of my PhD studies and provided valuable feedback), Prof. Bob Malcolm, Dr. Matthew Karlsen and Kendi Muchungi.

I thank the Department of Computing as a whole for granting me the Ph.D. studentship that made this research possible.

I am grateful to my partner Deepali for proof reading this document and for putting up with me whilst I was writing this thesis.

Finally, I would like to thank my parents Prabhulla Chandran and Geetha as well as the rest of my family for their patience and support.
List of Acronyms

**ABM**: Agent Based Modelling

**AF**: Ageing Factor

**CR**: Calorie Restriction

**DISC**: Death-Inducing Signalling Complex

**DNA**: Deoxyribonucleic acid

**DR**: Death Receptors

**EGT**: Evolutionary Game Theory

**ERCs**: Extrachromosomal rDNA circles

**ESS**: Evolutionary Stable Strategy

**GT**: Game Theory

**ODE**: Ordinary Differential Equation

**PAPI**: Players, Actions, Payoffs, and Information

**PCD**: Programmed cell death

**PDE**: Partial Differential Equation

**rDNA**: Ribosomal DNA

**RNA**: Ribonucleic acid

**ROS**: Reactive oxygen species

**SSE**: Sum of Squared Errors of Prediction

**SSR**: Sum of Squared Residuals

**UDS**: Unscheduled DNA Synthesis

**UV**: Ultraviolet
To himself everyone is immortal; he may know that he is going to die, but he can never know that he is dead.

SAMUEL BUTLER
1

Introduction

"The idea is to die young as late as possible." - Ashley Montagu

Ageing is the increase in the susceptibility of organisms as they grow to factors that may cause death. It is a problem that has troubled evolutionary biologists for decades. Though one could simply look at ageing as the accumulation of damage (referred to as Ageing Factors in this work), the causes of ageing are in fact much more complex. The ageing process at both the cellular and molecular levels is now much clearer - thanks to the past 60 years of research. One biological phenomenon that goes with ageing is death. Amongst the different types of death apoptosis is the most perplexing one. It is a morphologically distinct form of cell death and is programmed. Apoptosis itself raises a number of questions; the most important one being: why would an organism be programmed to die? Explaining this from an evolutionary theorist’s point of view is extremely complicated.

Interestingly these questions can also be addressed from a theoretical point of view using synthetic biology, which is the approach that we have chosen to adopt in this study. We show how apoptosis might be selected as an Evolutionary Stable Strategy (ESS) (see Chapters 5 and 6). We use simulations and theoretical analyses to support our hypotheses. Since it is difficult to model apoptosis without dealing with the problem of ageing, we attempt to combine these issues and form a generalised model. We also discuss exceptions
to the rules of ageing and apoptosis, such as in the case of tumour populations where cells evade apoptosis and thrive over the apoptotic population (which displays apoptosis).

This thesis is divided into 8 chapters including this introductory chapter. In the second chapter, we look at the background information required to address the problem. We discuss various existing models of population dynamics and different theories of ageing. Concepts such as the Gompertz-Makeham law are also studied in this chapter. We also present a case (using previous studies) for the use of yeast as the ideal sample for studying ageing and apoptosis. In addition, we describe how ageing and apoptosis occur in yeast. The chapter ends with a discussion on apoptosis and tumour growth.

The next chapter is an analysis of the literature we discussed in Chapter 2. We also use Chapter 3 to make a case for our work by outlining what is missing in the current body of research. Here we look at ageing as an evolutionary process and study various approaches adopted by researchers. Furthermore, we critically analyse the research in the fields of ageing, apoptosis and tumour growth. We also explain where the gaps and pitfalls are in the current research and how they can be used to carry out further research in this area. Since tumour growth is an important exception to the rule of apoptosis, we look to determine whether the process of apoptosis itself is reversible. Chapter 3 ends with a discussion on the gaps in the research on apoptosis and tumours.

Our work is discussed from Chapter 4 onwards. In Chapter 4, we explain the importance of quantifying the progression of ageing in an organism. A heuristic approach is adopted to see how difficult it is to find a relationship between mortality and the amount of damage present in the body of an organism. We find that in fact it is possible to formulate a relationship albeit a very crude one. Deriving inspiration from this conclusion, we proceed to develop a model from first principles and show that the ageing process in yeast can be modelled using the model we developed from first principles. We also show that this can be done without increasing the complexity of the model and also by using different models such as Leslie matrix based models. An existing model of ageing in yeast is critically analysed and we show how it can be improved. This is followed by a review of all the models developed so far and the goodness of the fitness graphs obtained in each
case. The chapter ends with concluding remarks.

Having developed a model of ageing, we attempt to simulate apoptosis. In Chapter 5, we describe a game theory-based model and the assumptions associated with it. Then the model is simulated and the results are analysed. We vary the values of the parameters over a wide range of values and see how the model behaves. The model is also scaled up to see if it makes any difference to the results obtained previously. This is followed by a mathematical analysis of the equilibrium of the populations used in the simulations. The chapter ends with a discussion and conclusion section.

In Chapter 6, we show that similar results can be obtained by using a simpler model involving the use of differential equations. Though the interactions between the organisms are not demonstrated explicitly, we find that this model can also be used to simulate the population dynamics of an apoptotic and a non-apoptotic population. The results are then analysed and the parameter values are systematically altered to search for any changes in the outcome. Results and their interpretations are then discussed in the next section of this chapter. The stability and steady state of the populations are mathematically analysed. This is followed by a discussion and conclusion section.

In Chapter 7 tumour populations are analysed. Since tumour populations are a specific type of non-apoptotic population, we investigate why their behaviour gives them the upper hand over apoptotic populations, given the right conditions. The model describes how the characteristics of tumour populations are implemented. This is followed by simulations and a discussion of the results. We also show that similar results can be obtained using both the game theory-based model and the differential equation-based model we developed in Chapters 5 and 6. Most of the simulations are done using the differential equation-based model as it is easier to simulate. We also vary the parameters systematically and look for notable results. This is followed by a discussion section and stability analysis as carried out previously in Chapter 6. The chapter ends with a discussion and conclusion section.

In the concluding chapter (Chapter 8), we critically analyse the models we have developed. Since we have already discussed and analysed the results from all the models in the
previous chapters, in this chapter we look at the shortcomings of each model and the original contributions we have made. We start by making a case for ageing (which is essentially an extension of the existing body of research) and then we make a good argument for apoptosis using our models. We also explore the question of why tumour cells have an advantage in some situations and explain how oncologists could potentially use our model to look for ways to treat tumour populations. The Evolutionary Stable Strategy (ESS) of tumour populations is then discussed. The chapter ends with the section titled ‘Final Conclusion’.

Some of the special cases and exceptions are discussed in the Appendices which are provided towards the end of the thesis (after Bibliography). In the Appendices, we have discussed ways to improve some of our models. We have also provided some explanations for some the equations we have used in the main text.
Background

I Introduction

In this chapter, we review the recent developments in the study of senescence, apoptosis and tumour growth. We introduce some of the modelling approaches that we use in our own research. Experimental results from the fields of molecular biology and gerontology are discussed in detail.

We start this chapter by introducing some of the oldest methods in modelling population dynamics. We show that these tools are powerful enough to model various complex scenarios. Some the definitions used in the field of population dynamics are then discussed. This is followed by a brief discussion on the different theories of ageing. Furthermore, we show why yeast is a good example for studying both ageing and apoptosis. Previous modelling approaches using yeast are then discussed in detail. A discussion on ageing as an evolutionary process as we understand it now is also included. Since tumour growth is an exception (tumours evade apoptosis), we have included a section dedicated to reviewing research in this field.

Ageing is considered to be a long-standing problem in the field of evolutionary biology. Apoptosis is an associated problem which is equally perplexing. Although the physical reasons for ageing and apoptosis are now better known, these are still important funda-
mental questions which evolutionary biology still cannot fully explain. We use modelling to understand the ageing process and apoptosis from an evolutionary standpoint. We utilise concepts from synthetic biology to simulate the growth of populations. Accordingly, the actual biomolecular aspects of senescence and apoptosis are discussed only in this chapter. We derive inspiration from the biological world and formulate simple rules for the synthetic world we use.

I.I Population Dynamics: Models

In order to simulate the growth of the population we need an effective model for predicting the dynamics of the population. We start by briefly reviewing some of the oldest methods and then we discuss the matrix model in more detail (both of these are used in our models).

It is important to mention that often simple models are quite powerful. In some of our models, we use differential equations to model the growth of populations. In order to comprehend the effectiveness of differential equations-based models, we need to review some earlier modelling approaches.

**Lotka-Volterra model:** This is one of the simplest models of predator-prey interactions (Murray, 2003)

\[
\frac{dx}{dt} = rx - axy \\
\frac{dy}{dt} = bxy - my
\]  

(2.1)

(2.2)

where \(x\) is the density of prey, \(y\) is the density of predators, \(r\) is the rate of change in prey population, \(a\) is predation rate, \(b\) is reproduction rate of predators per one prey eaten and \(m\) is the predator mortality rate. This simple model can simulate the growth of both predator and prey populations effectively.

**General Predator-Prey Model:** This is a more generic approach (Huang, 2002)
\[
\frac{dx}{dt} = F(x) - G(x)y
\] (2.3)
\[
\frac{dy}{dt} = cG(x)y - my
\] (2.4)

where \( x \) is the biomass of prey, \( y \) is the biomass of predators, \( F(x) \) is the growth rate of prey, \( c \) is the interaction parameter, and \( G(x) \) is the predation per predator.

**Age Structured Dynamics:** Here we group a population into different classes based on their age. This is a powerful method as their physiological features change with age (see the section *Leslie Matrices* of this chapter). We use this method to study the age based population dynamics of yeast populations (see Chapter 4).

**Evolutionary Dynamics:** Changes in the behaviour and genetic patterns of the organisms are accounted for by using evolutionary methods. Researchers commonly use evolutionary game theory to incorporate the elements of evolution (Allen, 1976). This concept is used when we study the evolutionary stable strategies of populations (see Chapters 6 and 7).

**Agent Based Modelling:** An agent is used to represent an individual of a population and local rules are used to quantify the actions and interactions of the agent. Globally, the result will represent the dynamics of the population as a whole. It was found that this model can incorporate many features (local rules) as observed in nature (Kreft et al., 1998). We use this powerful tool when we study the dynamics of apoptotic and non-apoptotic populations (see Chapter 5).

**Leslie matrices:** In the case of organisms that take different physiological forms during their life cycle, it is necessary to classify a population into different groups based on their age.

This can be done by using the approach followed by Leslie (1945):
where \( n_i \) is the number of individuals in class \( i \), \( F_i \) is the rate of reproduction and \( P_i \) is the survival rate. Due to the simplicity and powerfulness of Leslie’s matrix, many researchers tried to extend the model by using different mathematical tools (see Fig. 2.1). This inspired us to use a matrix based model whilst modelling population dynamics of yeast population. This is explained further in Section III of this chapter.
I.II Goodness of Fit

We used the sum of squared residuals (SSR) or the sum of squared errors of prediction (SSE) for determining the goodness of fit where we have compared our data against data from experiments (as shown in Chapter 4). The chi-square factor is defined as:

\[ SSE = \sum_{i=1}^{n} (y_i - f_{x_i})^2 \]  
(2.6)

where \( y_i \) is the observed value and \( f_{x_i} \) is the predicted value.

II Definitions

In this section we define some of the words that are frequently used when discussing the population dynamics:

**Ageing:** It is the process of becoming older. In literature the words senescence and ageing are used as synonyms. Though very few researchers reserve the word *senescence* only to refer to the gradual deterioration of bodily functions associated with ageing, we consider both words as synonyms to be consistent with the large amount of literature already existing and to avoid any confusion.

**Apoptosis:** It is the process of Programmed Cell Death (PCD) (see the section ‘Apoptosis’ in this chapter for more details).

**Non-apoptotic organisms:** Organisms with no apoptotic machinery present. They die predominantly due to starvation or environmental causes.

**Tumour:** A tumour is an abnormal growth of tissue. These cells tend to be non-apoptotic.

**Fertility \( (F_i) \):** The number of offspring produced by an individual of age \( i \) in unit time (i.e. while survives from age \( i \) to \( i + 1 \))

**Survival \( (P) \):** The probability that an individual at time \( t \) will live to time \( (t + 1) \) i.e. the individual should move from age class \( i \) to \( (i + 1) \)
**Mortality rate**: It is the rate of deaths in a given population per unit of time.

**Survivorship function** \((l(i))\) : The probability that an individual will survive from birth to age \(i\).

Using the *survivorship function*, we can quantify the survival probability of an individual as:

\[ P_i = \frac{l(i+1)}{l(i)} \]  

**Fertility function** \((m(i))\) : The expected number of offspring in unit time per member of age \(i\)

### III Matrix Model

Whelpton (1936) introduced the concept of matrix models in population dynamics. Bernardelli (1941) and Lewis (1942) improved his ideas and Leslie (1945) formalised the model which we use currently. Due to the success of the model, all matrix models are now commonly known as Leslie matrix population models.

The original model (Leslie, 1945) is discrete in nature and dependent upon the age of the population. For the purposes of simplicity, individuals in a population are grouped into different age classes. The matrix representation helps us to determine:

- the growth of the population itself; and
- the age distribution of individuals after a period of time.

Leslie (1948) demonstrated that the basic model was similar to the exponential growth equation

\[ N_t = N_0e^{rt} \]  

\[ (2.8) \]
where $t$ is the time, $N_0$ and $N_t$ are the numbers of individuals at time 0 and $t$ respectively and $r$ is the rate of growth.

It can also be shown that when the population size approaches the equilibrium ($K$), the growth of the population itself may be approximated as:

$$N_t = \frac{N_{eq}}{1 + Ke^{rt}} \quad (2.9)$$

where $N_{eq}$ is the population at equilibrium. We employ these tools when we discuss the age structured dynamics of yeast populations in Chapter 4.

IV Analysing Matrix Models

Let’s call the matrix which contains the reproductive and survival probabilities in equation (2.5) ‘A’. We know that the entries of the first row of the matrix $A$ are given by the fertility ($F_i$) and the sub-diagonal (the elements just below the top-left to bottom right diagonal) is given by the survival probability ($P_i$). One can introduce variable parameters in order to model complex scenarios. This variability could be either internal (e.g. a density dependent feature) or external (e.g. using a deterministic or stochastic process). In very complex cases, the parameters can be both internally and externally generated.

Leslie matrices may be employed to analyse how the percentage of the population classes changes over time and see if they converge to a stable age distribution.

It is interesting to note that, since the fertility of an age class is dependent upon the distribution of the number of individuals born and dead, we can easily introduce density-dependent parameters. Since the Leslie matrix is a square matrix, we can find $n$ possible eigenvalues and eigenvectors (provided the matrix is $n \times n$) which satisfy an equation of the form:

$$A\nu = \lambda\nu \quad (2.10)$$
where \( \nu \) is the eigenvector corresponding to eigenvalue \( \lambda \)

These vectors and values further give us insight into the dynamics of the population that we are dealing with. By examining these values one can predict whether the size of the population is increasing, constant or decreasing. The biological interpretations of these values make the analysis particularly interesting from the point of view of synthetic biology as it gives us a good indication about the dynamics of the population. Amongst the eigenvalues, one of the values will be greater than the others in terms of magnitude. This essentially determines the growth of the population. This higher value is called *dominating eigenvalue* (or latent root).

### V Applying Reliability Theory to Ageing

Deriving inspiration from Gavrilov and Gavrilova (2001b), we can say that the mortality and the reliability of an organism are interlinked. Furthermore, reliability (of a system or organism) can be measured using different methods (the biological equivalents of the processes are provided in brackets):

- Average time to failure (lifespan of the organism);
- Number of failures per unit time (number of death in each age class per unit time);
- and
- Survival Probability (mortality rate of the organism).

This gave us inspiration to study how the survival probabilities changes when the fitness associated with the organism changes. The heuristic studies that we have done using this are briefly mentioned in Chapter 4.
VI Theories of Ageing

Despite the fact that the process of ageing is not as obscure as it was about 50 years ago, the causes of ageing are still debatable (Kirkwood, 2008). There are a number of different theories of ageing and in this section, we review some of the most important ones.

It is possible to group the theories into two groups: stochastic and genetic. There are dealt with separately below:

VI.I Stochastic Theories of Ageing

The major theories in this group are summarised as follows:

1. Somatic Mutation and DNA Repair: This idea was proposed by Failla (1958), who stated that the ageing process itself is a result of the decline in cellular functions (particularly maintenance). This theory is also known as the somatic mutation theory of ageing. The core idea behind this theory is that when vital proteins and amino acids are produced in reduced quantities, this will affect the functioning of the cell. If the repair rate of the cell is not equal to the error rate then it can lead to ageing. There are random mutations occurring in the cell that may also have hazardous effects. It was hypothesised that these random errors will grow with age (due to the obvious cascading effect) (Jaime, 1991). This also observed in nature. We can extend this idea and say that when the repair rate falls below a threshold value the integrity of the genome itself is lost (Jaime, 1991). In certain studies, DNA repair was measured after subjecting the cell to have Unscheduled DNA Synthesis (UDS) by exposing it to ultraviolet (UV) radiation (Zorn et al., 1979).

2. Error-Catastrophe: This theory considers the excessive RNA mutations to be the cause of ageing. This is of particular interest when it comes to organisms such as viruses where there is only RNA present. It is based on the idea that RNA repair is not as good as the DNA repair mechanism. This theory can also explain the
extinction of a population to an extent in the case of viruses (Lewis and Tarrant, 1972).

3. **Protein Modification**: This theory introduced the idea that the accumulation of oxidatively damaged proteins, nucleic acids and lipids might be responsible for the ageing process itself (Stadtman, 2001). It was found that the number of modified proteins (oxidatively damaged proteins) increases with age (Stadtman, 2001). It is also observed that the amount of protein carbonyl content appears to go up with age.

4. **Free Radical Theory**: This is one of the more widely accepted theories. According to this theory, it is the free radicals (atoms with unpaired electrons) that are responsible for ageing, as they cause damage to the cell structure of the organism. It is interesting to note that mitochondrion (which is known as the powerhouse of the cell) produces a large number of free radicals since it is actively engaged in the process of producing chemical energy. As a result, the cell is damaged heavily by the production of free radicals (Harman, 1956). It was also discovered that if one could limit the amount of free radicals by dietary restriction (or even using genetic methods), it would extend the lifespan of the individual (Chung et al., 1992).

### VI.II Genetic Theories of Ageing

Here we review the widely accepted theories that consider ageing as a genetic process (i.e. the process of ageing is somehow triggered by gene expression).

1. **Longevity Genes**: According to this theory, there are genes designed specifically for longevity (hence their ability to function affects the ageing process). However, an explanation of this from an evolutionary point of view seems difficult (as it is hard to explain why such genes got picked up in the first place). Nonetheless, the theory could help to explain ageing in a number of organisms including filamentous fungi, yeast, mice and nematodes. In many cases, researchers could clone these genes that
are responsible for the lifespan of the organism (suggesting the viability of such genes) (D’mello et al., 1994). The main problem with this theory is that these genes are also responsible for various cellular functions (hence they cannot be considered just as Longevity Genes). Furthermore, there could be multiple mechanisms of the ageing process (which make finding the relationships between the genes and the functions difficult).

2. **Accelerated Ageing Syndromes:** This is considered more as an assisted cause rather than the main cause of ageing. According to this theory there are various conditions (commonly referred to as ‘segmental progeroid syndromes’) that are responsible for the acceleration of the ageing process. These include Hutchinson-Gilford progeria, Werner’s syndrome and Cockayne’s syndrome. The theory also suggests that the group could be large and may include Down’s syndrome, Ataxia telangietasia and Bloom’s syndrome. It is postulated that these syndromes are activated slowly as time progresses and are responsible for ageing (based on the cytochemical analysis) (Dyer and Sinclair, 1998).

3. **Neuro-endocrine:** Another popular idea is that the ageing process is continuous and is caused by the loss of sensitivity in the hypothalamic region of the brain. This causes that part of the brain to be less sensitive to the negative feedback inhibitions of neurotransmitters and hormones (Weinert and Timiras, 2003).

4. **Immunologic:** It has been found that with the increase in age of a vertebrate organism, there is an increase in the immunogenetic diversification of its dividing cells. This diversification process causes loss of recognition patterns between different cells in the body (Walford, 1964), which in turn triggers various autoimmune-like reactions. Hence according to this view, the ageing process itself is a mild but extended auto-immune phenomenon

5. **Cellular Senescence:** This is one of the most well researched ideas in ageing theory. After each cell division chromosomal telomeres are shortened and it is postulated that this places an upper limit on the number of cell divisions, thereby
causing ageing (Levy et al., 1992). Our work also derives inspiration from this. It should be noted that cellular senescence is seen in many organisms. We are particularly interested in cellular senescence in unicellular organisms (such as yeast). The role of telomeres in cellular senescence (since the size of telomeres reduces after each cellular division) is crucial. Accordingly, the cell stops diving after a particular limit called Hayflick limit (Hayflick, 1961). This is a widely researched area but our work does not cover this in detail since we are restricting ourselves to a synthetic biology-based generalised case.

6. Antagonistic pleiotropy: This idea was proposed by Williams (1957a) and is a currently accepted theory (Kirkwood and Rose, 1991b). Pleiotropy is a term used when one gene controls more than one phenotypic trait. Antagonistic pleiotropy predicts that one of these qualities could be a beneficial one to the organism and another might be harmful for the organism (such as the typical characteristics of ageing). In addition, there is a possibility that natural selection favoured this gene because the positive effects are seen during the early part of an organism’s life span and the harmful effects are seen during the latter part (when the effect of natural selection is feeble i.e. after the reproductive stage). In this report, we try to show that this is not the only possibility.

VII Ageing as an Evolutionary Process

Having discussed the theories of ageing, we need to see how ageing occurs from an evolutionary point of view. Our work attempts to formalise the ageing process (and subsequent death) as an evolutionary process. We consider the biological organism as a complex machine whose reliability decreases over time.

It has been observed that one could increase the average time span of a population of organisms by providing proper care i.e. by investing more on maintenance (Kirkwood and Rose, 1991a). However, the maximum life span remains unchanged. This can be made clear with the help of Fig. 2.2 which shows that the average life span increases
tremendously from around 15 years to around 70 years due to advancements in medicine and health care. But the maximum life span reams almost constant.

![Figure 2.2: Changes in human mortality rates over years. Figure adapted from Jazwinski (1989).](image)

This forces us to doubt the veracity of theories such as Somatic Mutation and DNA Repair since the data from Fig. 2.2 shows the characteristics of a genetic process rather than a random process. Further, it may seem that ageing process itself is a programmed one (as organisms mostly follow a survival curve). However at the same time, the simple deterioration (wear and tear) theory provides a good explanation for the human ageing process.

Until the 1950s, senescence was considered to be an ‘unsolved problem of biology’ (Medawar, 1952). Even though there are various unknown factors, the picture is much clearer now (Kirkwood, 2005b). The biochemical deteriorative process is widely studied and there are models for ageing of different species. Unfortunately, a common theory is yet to emerge.

It is interesting to note that the life span of different organisms varies widely. Even within the mammalian domain it varies over a 1:100 range. A typical example is the Argentine desert mouse which lives for about 10 months whilst a human being lives for about 75 years.

The main debate is whether the ageing process itself is programmed (i.e. a direct consequence of a gene dedicated for ageing) or not. In non-programmed ageing, there is no genetic component that favours ageing. There are few studies that support the idea of non-programmed ageing (Kirkwood, 2008). Those studies also provide a good explana-
tion for lifespan observations. On the other hand programmed ageing is active in nature and genetic components play an active role in accelerating the ageing process. Common apoptosis machineries are also seen in organisms (Gourlay et al., 2006a). It is interesting to note that some new discoveries are increasingly in favour of a programmed process (Aravind et al., 2001).

Medawar (1952) suggested that age calculated ‘relative to the age of first reproductive capability’ is a primary factor in the evolutionary selection process. Later Williams (1957a) and Kirkwood and Rose (1991b) suggested that the process itself could be a side effect (i.e. ideas similar to antagonistic pleiotropy).

In the literature review (see the Section VI of this chapter), it was found that the number of studies that consider ageing as an altruistic act are very low in number. In this context it is important to mention the cell suicide mechanism that allows a cell to destroy itself. This mechanism is also seen in large organisms such as octopus, marsupial mouse and salmon which all commit biological suicide. Some of these individuals die shortly after reproduction in order to allow the next generation to grow easily (Goldsmith, 2010).

Kirkwood (2008) suggested that an organism will try to make reproduction as easy as possible. However this is not the case in general since various organisms have different mating rituals (some of which are very complex). Excessive reproductive maturity age is another issue that contradicts this point of view.

Different approaches were proposed during last fifty years in order to find a theory that is more appealing from an evolutionary point of view. This includes group selection theory (Wynne-Edwards, 1986), kin selection theory (Michod, 1982) and selfish gene theory (Williams, 1982).

The idea of senescence and the consequent death as an altruistic behaviour is not new. It is suggested by Weismann (1882) that by limiting the life span of the individual organism, one might be able to increase the resources available to younger organisms (who are the more evolved members). Skulachev (2001) further suggested that it might even challenge older individuals which in turn can also influence the evolution process (see Longo et al.

Accordingly, non-programmed theories of ageing are now widely accepted. However, one of the main difficulties for non-programmed theories is explaining the benefits of *calorie restriction (CR)*. When rats were fed a ‘CR but nutritious diet’, it was found that their average life span increased by about 50 percent. This could help the population to survive a famine. According to the non-programmed theory the ageing process should have been quickened as the energy available for maintenance and repair is decreased. Another important issue is the discovery of genes that apparently cause ageing. It was reported that by disabling these genes in nematodes, their lifespan increased by a factor of 10 (Apfeld and Kenyon, 1998).

## VII.I Common Deductions

The existing evolutionary theories present the following conclusions (Kirkwood, 2005b):

- Genes that are designed to promote ageing are unlikely to exist as they may not be of interest to the benefit of the organism. However the chances of having genes that favour reproduction to maintenance are likely to be present as they give the population an evolutionary advantage.

- Ageing may not be programmed. It could simply be due to the accumulation of somatic damage. Since there is always a trade-off between investments in maintenance and reproduction, the organism may not spend much energy on avoiding the process of senescence.

- Death may be programmed. There is a chance that apoptosis was selected due to its altruistic benefits. It is interesting to note that we have seen apoptotic machinery in a number of organisms (this is explained in the next chapter).

- There may be pleiotropic genes that could benefit the organism in the earlier part
of its lifetime and which may become hazardous during the latter part of the organism’s lifetime.

In this report we show that it is possible to quantify the altruistic benefits associated with apoptosis (see Chapters 5 and 6).

VIII Gompertz-Makeham Law

In the previous section we saw how ageing might look programmed. In this context, it is necessary to discuss the Gompertz-Makeham law. It is an empirical law that governs the increase in mortality rate with increase in age.

The mortality rate is given by (Witten and Satzer, 1992)

\[
\lambda(t) = \lambda_0 e^{\beta t}
\]  

(2.11)

where, \( \lambda_0 \) is an age independent factor, \( t \) is the time period, \( \beta \) is the age dependent factor.

And the survival probability \( (S(t)) \) can be expressed as

\[
S(t) = e^{\frac{\lambda_0}{\beta}(1-e^{\beta t})}
\]  

(2.12)

It is worth noting that the values of these parameters varies from population to population (depending on the environmental factors and rate of ageing).

It is also interesting that most living organisms obey this empirical law. Research has shown (Witten and Satzer, 1992) that in most populations the lifespan of the organism and the survival probability can be predicted using Gompertz-Makeham law. This inspired us to investigate more into the survival probability and the age of the organisms in a population and identify the cause of this relation using synthetic biology.
IX  Yeast: A Case Study

In order to study the ageing process we need a good biological specimen. Budding yeast, *Saccharomyces cerevisiae*, is a good example to study the phenomenon of senescence (Jazwinski, 1990; Sinclair et al., 1998; Defossez et al., 1998). It also exhibit the characteristics associated with apoptosis (Gourlay et al., 2006a). The mother cells of *S. cerevisiae* can only undergo a limited number of cell divisions before death. The mother cell divides asymmetrically and it produces a daughter cell that is relatively smaller in size. Therefore, we can mark one of final cells as the original mother cell. The age of the mother cell can easily be calculated by counting the number of ‘bud scars’ on its surface (Sinclair et al., 1998).

However, the age of the daughter cells produced (especially in the early phase) in the process of division is not normally influenced by the age of the mother cell (unless they are produced in the very late stage of the life of mother cell). The main cause of this ‘ageing’ is the accumulation of extrachromosomal ribosomal DNA circles (ERCs) in the cell and during the early stages of the life of mother cell hardly any ERCs are allowed to migrate to daughter cells. It is worth mentioning that various methods such as elutriation and a biotin–streptavidin magnetic sorting procedure are used by experimentalists (Emilgez and Jazwinski, 1989; Smeal et al., 1996) for isolating the old cells.

In this work we re-evaluate the experimental data and try to formulate a general model from the first principle with less artificial (implicit) assumptions than Gillespie et al. (2004a). Sinclair et al. (1998) showed that the mortality curve of *S. cerevisiae* is in agreement with the Gompertz-Makeham equation. It was also shown that this equation is a direct consequence of reliability theory (Gavrilov and Gavrilova, 2001a). This allows us to investigate the entire process (and stages) of senescence in *S. cerevisiae*. A single cell based numerical evaluation is done using the model in order to check the accuracy of the predictions of the model against experimental data.

Gillespie et al. (2004a) used heuristic calculations to find the values of ERC replications and excisions. Gillespie et al. (2004a) also assumed that a cell will die only if it has
1000 ERCS. No explanation for the logic behind this assumption was provided and it is considered to be an empirical observation from Sinclair and Guarente (1997b). In this report we show that the survival probability curve can be derived without making that assumption (see Chapter 4).

Gillespie et al. (2004a) started with a single cell and used a time-dependent probability for ERC excision, a constant probability for ERC replication and a binomial probability for migration. A cell was allowed to die once it reached 1000 ERCS. Furthermore Gillespie et al. (2004a) used cells from new generations (i.e. daughter and granddaughter cells along with the original set of mother cells ) to get a better fit for the curve. However, in the original experiment by Sinclair and Guarente (1997a), they used only the initial generation of mother cells which were free of ERCS when they started the experiment.

Gillespie et al. (2004a) considered two schemas for allowing migration of ERCS from the mother cell to daughter cells. They defined a critical value ($N_{max}$) up to which the mother cell keeps almost all the ERCs produced. After reaching this level, the mother cell starts diffusing more ERCS towards the daughter cell.

### IX.I ERC Replication and Migration

It has been found that the presence of ERCS is correlated with explanation of the ageing process in yeast (Sinclair and Guarente, 1997a). Although it is possible to have other factors as well that contribute to the process of senescence (Laun et al., 2001; Defossez et al., 1998; Kaeberlein et al., 2007; Jazwinski, 2000), in this report, we assume the effects of other parameters to be negligible. Therefore, according to this assumption, ERCS are considered as abnormalities present in the cell as they negatively influence the longevity of the cell and adversely affect its overall reproductive capability.

The ERC accumulation process starts with the excision of an ERC from the rDNA locus (Murray and Szostak, 1983). During the initial stages of life the mother cell keeps all the newly formed ERCS and hence the age of the newly formed daughter cell is set to zero (Kaeberlein et al., 2007). The ERCS also grow by replication. Both of these effects
contribute to the exponential amplification (Sinclair, 1998) of the ERC number. After a particular stage, the daughter cells also receive a portion of the ERCs present and in this case their life span is shortened accordingly (Kennedy et al., 1994). The overall process can be represented as shown in Fig. 2.3.

![Figure 2.3: Migration of ERCs from mother to daughter cells (where n, m, o, and p represents the generations with n < m < o < p). The figure shows a single mother cell at 6 different stages of its lifespan and four daughter cells produced.](image)

Gillespie et al. (2004a) hypothesised that the probability of the formation of ERC can be quantified as

\[
P_{for} \simeq \min(\kappa t^n, 1)
\]  

where \( \kappa \) is a constant, \( t \) is the number of times the mother has produced daughter cells and \( n \) is any real number.

In their simulation, Gillespie et al. (2004a) also assumed that the mother cell usually keeps the newly formed ERCs and the probability with which the ERCs may migrate to daughter cells increases with the increase in the number of ERCs present in the mother cell. The resultant data curve was found to be in agreement with the experimental data from Sinclair et al. (1998) as shown in Fig. 2.4.
Chapter 2 Section IX

IX.II Apoptosis in Yeast

In the previous section, we saw that mother cells ultimately cease to reproduce. The question now is what happens to those old cells. Researchers have discovered that they undergo apoptosis (programmed cell death) (Aravind et al., 2001). It has been found that programmed cell death (PCD) is an important feature that assists in the development and maintenance of the population amongst metazoans (Gourlay et al., 2006a). In this context it is worth mentioning that there are different types of PCDs: apoptosis (either caspase dependent or independent), apoptosis-like PCD, necrosis, necrosis-like PCD, accidental necrosis, autophagic cell death and mitotic catastrophe (Gourlay et al., 2006a). However, we are interested only in apoptosis as the mechanism behind this appears to be programmed (Koonin and Aravind, 2002).

It was suggested that apoptosis (especially those involving caspases) is developed further along with multi-cellularity (Aravind et al., 2001). It is found that the apoptotic pathways seen in prokaryotic and eukaryotic organisms have common ancient origins as we can find common pathways for both of them (Koonin and Aravind, 2002). This suggests that the
apoptotic process itself is programmed. In our research we are particularly interested in finding out how the apoptosis process serves an altruistic purpose. Since we are not examining the molecular biology associated with this, a review of these mechanisms is beyond the scope of this work. For a detailed review of the processes involved please refer Gourlay et al. (2006a); Madeo et al. (1997); Longo (2004).

These concepts inspired us to investigate the possibility of using ERCs as the wear and tear component and apoptosis is a means to save the population from exhausting resources.

X Apoptosis

Apoptosis is essentially the regulated destruction of a living cell. It has been observed in both single celled organisms and multicellular organisms. It has also been reported that there are a number of different genes that affect the cell’s likelihood of activating the apoptotic machinery. Once it has been activated, the process itself requires a well harmonised activation together with the execution of various other sub-programmes.

With regard to unicellular organisms, the activation of the apoptotic machinery essentially results in the destruction of the entire organism. Apoptosis works slightly differently in multicellular organisms (due to their complexity). Apoptosis has been established as a very efficient way to manage cell or tissue growth. It can efficiently get rid of all excess cells that have accumulated. It has already been established (by studying tumour populations - See Chapter 7) that if the excess cells are not managed effectively, then this can be detrimental to the organism itself (since the excess cells can critically affect the homeostasis of the organism).

X.I Apoptosis as a Type of Programmed Cell Death

Apoptosis is essentially a type of programmed cell death. It plays an important role in the growth, development and homeostasis of both multicellular and unicellular organisms
(Henson and Hume, 2006). Reportedly, failings in the apoptotic machinery can cause autoimmune diseases, neurodegenerative diseases and cancer in multicellular organisms (Steller, 1995). In the case of mammals, apoptotic mechanisms can be triggered by either extracellular or intracellular stimuli, thereby triggering the extrinsic or intrinsic pathways depending on the stimulus in question. The extrinsic pathway is triggered by the stimulation of Death Receptors (DR) on the plasma membrane of the cell (Ashkenazi and Dixit, 1999). This further causes the formation of the Death-Inducing Signalling Complex (DISC) and caspase-8 protein activation. Researchers have identified the link between caspase-8 and apoptosis (Ashkenazi and Dixit, 1998).

Gillespie et al. (2004b) have shown that the accumulation of ERCs can impact on the mortality of cells. Herker et al. (2004) have shown that the chronological ageing of yeast can lead to apoptosis. In this data, only those cells that die because of apoptosis are accounted for and those die due to external factors are eliminated. It is interesting to note that this survival curve is, roughly, a Gompertz curve. This is consistent with our hypothesis.

In our model, we represent the change from non-apoptotic to apoptotic by switching on the apoptotic pathway (a binary system is adapted and is explained under the section on modelling) once the effect of the AFs have crossed a minimum threshold value.

Even though the protein CD95 is known as “death receptor”, it also plays an important role in the activation of non-apoptotic machinery (Steller et al., 2011). It has been demonstrated through research that CD95 plays a vital role in both tumour growth and invasion. It also plays a significant role in proliferation and necroptosis (Fuchs and Steller, 2011; Tang et al., 2011). From a modelling perspective this is interesting as one can investigate a number of different scenarios simply by altering key parameters. This is discussed in detail in Chapter 6.
X.II Modelling Apoptosis

Systems biology, which is a biology focused inter-disciplinary field that deals primarily with complex interactions in biological systems, has been widely used to understand the cellular and subcellular processes in the areas of electrophysiology and metabolic pathway control. This allowed researchers to identify the complex bio-chemical pathways which are essential in many phenomena such as apoptotis. This method of study even pre-dates developments in molecular biology. However, using systems biology in predictive systems modelling is relatively new (Rehm and Prehn, 2013). Usually in systems biology data from experiments are widely used to comprehend and predict the behaviour of biological systems. We follow this approach in the chapter on ageing. However, in the chapter on apoptosis modelling we used empirical observations to formulate a hypothetical environment rather than using pure data from experiments. Since we are interested in formulating a generalised model, we restrict ourselves to synthetic biology.

One can argue that experimental data and mathematical modelling in systems biology are connected through a cyclical workflow (Kitano, 2002). The experimental data is fed into the mathematical model and this, in turn, predicts biological phenomena. In our model of apoptosis, we use generic observations obtained from biological experiments and produce generic predictions.

In the systems biology of apoptosis, methods such as the quantitative Western Blot, single cell analysis, mass spectrometry and single death assays are used to study the apoptotic machinery. When it comes to modelling formalisms, the Boolean modelling of protein-protein interactions has been very successful where the protein-protein interactions are simulated just by looking at which genes are activated and in turn which proteins they activate. The actual interaction itself is not considered and the modeller simply examines whether the protein or gene has been active or not. This allows the modeller to predict the chain of events. However, this modelling approach cannot simulate the temporal dynamics of protein concentrations. In order to describe the quantitative temporal dynamics of the population, ordinary differential equations (ODE) are used in biology. It has been
reported that stochastic effects and diffusion can be neglected without impacting upon the viability of the model (Schleich and Lavrik, 2013). ODE modelling assumes that the protein molecules are in abundance and are well-mixed. These interactions can be described using simple mass-action kinetics. It is also interesting to note that the dynamic model includes a high number of kinetic parameters that cannot be measured through biological experimentation. We have studied apoptosis using ODE and this is discussed in Chapter 6). It is also worth noting that ODE’s assume a homogenous distribution and neglect spatial information. To account for this, partial differential equations (PDEs) are used. We derive inspiration from these approaches and use them to develop our synthetic model (see Chapters 6 and 7).

XI  Basic Concepts from Game Theory

In Chapter 5, we use game theory to study the dynamics of apoptotic and non-apoptotic populations. Using the basic ideas from game theory we are trying to show (with the help of Agent Based Modelling) that the processes of ageing and apoptosis can be represented as evolutionary co-operative games played between individuals of two different species. In this report we will demonstrate that apoptosis is a strategic decision chosen by the individuals in order to allow the population to grow at a much faster rate (see Chapter 5). A brief review of game theory-based approaches in modelling biological systems is also given in Chapter 5.

XI.I  Apoptosis in Literature and Previous Modelling Approaches

It was Weismann (1892) who originally theorised that ageing (senescence) could be a way of removing the old from the population. However, that approach failed to answer why those individuals that had genes responsible for ageing were selected over other individuals that had no such genes (Hamilton, 1966). The first successful theory (to a great extent) of ageing was formulated by Pedawar (1952). He predicted that ageing is
caused by *mutation accumulation*. Williams (1957b) extended this idea and formulated the theory of antagonistic pleiotropy. In antagonistic pleiotropy one of the two effects of a gene is beneficial and the other is detrimental. It predicts that the genes that offer benefits early in life are selected even if they may prove detrimental towards the end of the life. One of the main drawbacks of this theory is that, from an evolutionary perspective, one would expect the linkage between the beneficial and detrimental effects to be too rigid so that evolution cannot ward off the deleterious function. This is not observed in nature. A strong contender in the field is the *disposable soma theory* proposed by Kirkwood et al. (1977). According to this, there is a trade-off between the energy spent on maintenance and the energy required for reproduction. Therefore, the organism will not be able to spend too much energy on maintenance. However, experiments involving *Calorie Restriction (CR)* (low calorie intake without malnutrition) found that animals live longer when fed substantially less than controls (Masoro, 1990; Weindruch, 1996). This is not easily reconcilable with the *disposable soma theory* because a decrease in the supply of calories should have been detrimental to the organism. In fact, organisms subjected to CR tend to live longer.

It is interesting to note that these theories mostly deal with senescence rather than apoptosis (Kuan and Passaro Jr, 1998). Death is considered only as a consequence of senescence. Apoptosis certainly has evolutionary benefits (Kuan and Passaro Jr, 1998), but the reason why apoptosis is activated late in life still remains unknown to this day. It has been found that when yeast colonies are subjected to stress, this activates apoptosis and forces the organisms to commit ‘altruistic suicide’ (Gourlay et al., 2006b). This suggests that apoptosis might not be just a side-effect. More importantly it has been found that the apoptotic machinery has evolved over time (Aravind et al., 1999).

**XII Tumour Growth**

Since tumour tissues evade apoptosis, it is an important problem for us to consider whilst dealing with apoptosis. Neoplasms (tumour) are essentially an abnormal growth of cells
(Vakkila and Lotze, 2004). Prior to this abnormal growth (collectively known as neoplasia), cells normally undergo metaplasia (the reversible replacement of a differentiated cell type) or dysplasia (a change in the cell phenotype) (Younes et al., 1993). The cells may also exhibit hyperplasia (proliferation of cells) (Berry et al., 1984). Neoplasms themselves can be benign (carcinoma in situ) or malignant (England et al., 1989). For the purposes of our work, we will be focusing on malignant neoplasms as the characteristics of these are well-defined.

At the microscopic level, we can observe many pathological changes. They include anisocytosis (unequal cell size), poikilocytosis (abnormal cell shape), hyperchromatism (increase in the amount of pigmentation present) and high mitotic activity (unusual cell growth) (Oluwole et al., 2009; Nigg, 2002). It is also worth noting that tumour growth normally relies on a single population of neoplasms. It has been established that tumours are mainly caused by DNA damage\(^1\) (Kastan et al., 1991). The reason why neoplasms are able to colonise is due to the disruption of apoptosis pathways in the cells, which in turn allows the neoplasms to undergo replication at a faster rate (Ghobrial et al., 2005). Apoptosis was initially identified by its morphological characteristics (which include shrinkage of the cell, membrane blebbing\(^2\), nuclear fragmentation and so on). It is now clear that oncogenic mutations can also disrupt apoptosis (Lowe and Lin, 2000), which can lead to tumour initiation, progression or metastasis.

Researchers have identified the presence of apoptotic pathways in a number of species (see the section ‘Apoptosis’ of this chapter). Since it is a genetically programmed process, apoptosis can be influenced by genetic mutations. Apoptosis indicates that programmed cell death is just like any other metabolic or developmental process. In the early 1970s, researchers first identified the importance of apoptosis in tumour populations (Lowe and Lin, 2000). Experiments indicated that changes in apoptosis can significantly influence the malignant phenotype of the tumour.

Many oncogenes (those genes that have the potential to cause tumour growth) were

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1 This is an example of an ageing factor (AF) we would be using in our models.
2 Protrusion of the membrane
identified later in the 1980s and 1990s. Amongst these, a prominent oncogene is \textit{bcl-2} (Kroemer, 1997). The cloning and expression of \textit{bcl-2} oncogene confirmed the influence of apoptosis in tumour growth (Adams and Cory, 1998). Interestingly, the behaviour of \textit{bcl-2} gene is different from that of a typical oncogene. A typical oncogene simply disrupts the proliferation controls; whereas \textit{bcl-2} blocks apoptotic pathways and increases the survival probability of the cell. It has been observed in transgenic mice that an over-expression of \textit{bcl-2} increases \textit{lymphoproliferation} (lymphocytes are produced in excessive quantities) and stimulates \textit{c-Myc-induced lymphomagenesis} - both can in turn increase tumour growth (Xiao et al., 2008; Lowe and Lin, 2000). To date, a small number of \textit{bcl-2} type proteins have been found in mammalian tissue (Abrams, 1991; Adams and Cory, 1998).

In the treatment of tumours it is essential to identify the factors that can promote apoptosis. It has been reported that many factors such as growth factor depletion, problems in cell-matrix interactions, hypoxia and strong radiation can induce apoptosis (Lowe and Lin, 2000). For example, \textit{IGF-1} improves the survival rate of a cell (through the \textit{PI-3 kinase pathway}) and a reduction in the volume of \textit{IGF-1} or any other survival factors can cause ‘death by default’. The best known apoptotic pathways involve those triggered by various ‘death receptors’. These include \textit{Fas/CD95}, \textit{TNFR1}, \textit{DR3}, \textit{DR4} and \textit{DR5} (Schneider et al., 1997; Aggarwal, 2003).

It has also been found that once the tumours that have exceeded the capacity to acquire more nutrients, this can lead to hypoxia and in turn can activate \textit{p53} to promote apoptosis. It should be noted here that those cells that have \textit{p53} mutations can survive hypoxic stress, which in turn can cause vigorous tumour growth (Sansome et al., 2001). However, once the tumour cell crosses the threshold value, the survival probability of the cell will be affected dramatically. Researchers have also found that some oncogenic changes promote apoptosis rather than suppress it (Lowe and Lin, 2000). Experiments done on the \textit{c-myc} oncogene show the importance of oncogene-induced apoptosis in tumours especially at the latter stages of the tumour growth (Evan et al., 1992).

One of the most established ways to treat tumour growth is to induce apoptosis. In the
1970s researchers found that radiation and chemotherapy could induce apoptosis (Debatin et al., 2002). Though it is hard to verify that the process (cell death) itself is apoptotic, the morphological features exhibited are similar to that of apoptosis.

The second focus of our research is on pathological angiogenesis (see Chapter 7), which is another hallmark of cancer. It was established more than 100 years ago that angiogenesis occurs commonly around tumours. This allows the tumour cells to have better access to nutrients. The hypothesis that neoplasms release a diffusible ‘angiogenic’ substance was suggested in 1968 (Carmeliet and Jain, 2000). Folkman (1990) suggested that the progression of tumour and metastasis are dependent heavily on angiogenesis. Accordingly, the restriction of angiogenesis could be used a way to prevent the spread of neoplasms.

In 1976 Gimbrone and Gullino (1976) showed that without the ability to release chemicals that induce angiogenesis, a tissue cannot easily become cancerous. The effect of pro-angiogenic molecules can be countered using anti-angiogenic molecules. These could be from the immune system or externally supplied. When the concentration of these two molecules are equal to one another, the ‘angiogenic switch’ is said to be off. There are many signals that can turn the switch on. These include low pO2, low pH, stress caused by the growing neoplasm, immune response, activation of oncogenes or deactivation of tumour-suppressor genes (Rofstad, 2000).

The tumour vessels form by sprouting or intussusception from an existing nearby vessel. They can also develop around a pre-existing vessel to form a perivascular cuff. Some tumours can develop using bone marrow-derived endothelial precursors. Growth factors such as the Vascular Endothelial Growth Factor and angiopoietin play a significant role in promoting angiogenesis (Saaristo et al., 2000).

In our research both of these factors, namely apoptosis and angiogenesis, are considered (see Chapter 7). We studied the effect played by both factors in the dynamics of the Neoplasm population.
Analysis of literature: Making a Case for Our Research

“It is remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed”

– Williams (1957b)

In the previous chapter (Chapter 2) we reviewed the progress made in this field of research. In this chapter, we analyse the implications of the research discussed previously and show how it motivated us to conduct our own research. Here we discuss the pitfalls and shortcomings of previous research attempts and show how these gaps could be used as a pathway to pursue our research. We primarily focus on previous modelling attempts of ageing and apoptosis. Since tumour growth is an important exception to the rule of apoptosis, we also look to determine whether the process of apoptosis itself is reversible. Furthermore, deductions from biological experiments are used to make a case for our own work.
I Ageing as an Evolutionary Process

Although we cannot be completely certain, it is widely accepted that ageing\(^1\) occurs because of limitations in somatic maintenance (thereby, accumulating damage) and not by active gene programming (Kirkwood, 2005a). There are many factors that affect the rate of ageing in an organism and its life span. They include a number of ecological factors such as the availability of food in the environment and hazard rates (which measures the force of mortality). It has been proved that the trade-off between investing in growth and reproduction plays a key role in determining the ageing pattern of a species. It is worth noting here that changes in dietary patterns can also affect the ageing of an organism.

The role of senescence in the evolutionary development of an organism is difficult to explain. Even after half a century of research, this explanation is not complete. At the very outset ageing might appear as though it contradicts what is expected during the process of evolution, since evolution favours organisms programmed for survival. As described the previous chapter, ageing is now explained by considering the following two factors:

1. The force of natural selection declines with reproductive age; and
2. There is a trade-off between maintenance (repair and growth) and reproduction

For the purposes of our work, we are not going to consider the former as what it essentially says is that ageing is a random factor. Antagonistic pleiotropy suggests that ageing is just the downside of a gene that helped the organism in the earlier part of its life cycle (Rose, 1982). As the evolutionary pressure decreases towards the end of the life cycle of an organism, despite the fact that ageing causes negative effects the gene is selected because it contributes many positives in the earlier part of the lifespan. In other words, ageing is a random side-effect that just got picked up. One could argue that if this is the case, why didn’t some organisms randomly mutate to avoid this side effect? Even though

\(^1\)We use the same definitions we discussed in the previous chapter for ageing, senescence, death, apoptosis and mortality
the evolutionary pressure is low towards the end of the life cycle it should still have an impact. Hence this theory can be challenged from a theoretical point of view.

Thus we focus on the latter (2). If the trade-off is significant, then the organism should show ageing. One can also assume that it would develop a mechanism using which the older individuals can be removed from the population so that the rest of the population can thrive at a faster rate. Kirkwood and Rose (1991a) have already shown that there exists an optimal amount of nutrients that can be spent on maintenance (and the rest on reproduction) if the resource (nutrient) available is limited. However, a simple and direct correlation between the damages accumulated in the body of a biological organism and the survival rate has not been proved theoretically. If one could do this, then the modelling of ageing and apoptosis would become much easier.

In the previous chapter we also discussed how the evolutionary theory predicts some of the underlying mechanisms that change the complex cellular and molecular behaviour that in turn causes senescence. Accordingly, once we have a theoretical model that accounts for the relationship between the amount of damage (which we already defined as Ageing Factors (AFs)) and the mortality rate, then in theory, one can model the population of dynamics of ageing populations using simple rules. In the coming chapters, we show that a simplified approach is still valid in the biological world and can act as a generalised model. We also restrict our model to a hypothetical environment which is free from any other influences. This is interesting when we consider the fact that, although apoptosis is an intrinsic biological process, it is seen only in protected environments. A good example of this is when you take the case of a human being living in the developed world, where 85% of new-born human babies live up to the age of 65 whilst nine out of ten of a population of new-born wild mice will die before they reach the age of 10 months (Austad, 1997). If we keep wild mice in a protected environment, most of them will survive for more than 2 years. This makes the theoretical study of apoptosis in the biological world fairly difficult (as in most cases the data available is for uncontrolled environments).

We have also seen some organisms showing no senescence (Martinez, 1998) and some can even exhibit negative senescence (Vaupel et al., 2004). Various models have been
developed to account for this including the dependence on the quantity of nutrients available in the environment (Martinez, 1998). Some organisms are present only in very resource rich environments. For the purposes of our research, we restrict ourselves to resource-restricted environments.

II  Research in Ageing

Ageing is arguably the most familiar biological phenomenon; but it is also one that is extremely complex. Cell biologists and microbiologists have unravelled the mysteries behind the process to a great extent. Even though the idea of programmed ageing has been suggested before, it should be noted that there is hardly any evidence that supports this notion. In fact, the evolutionary theory suggests that the process is very unlikely to be programmed. Since it is a complex process, there tend to be multiple theories that explain the process of ageing as shown by Medvedev (1990). However, later research has shown that this approach of having multiple theories is flawed since in order to comprehend the problem, a generalised theory is required (Kirkwood, 2005a) and this development helped other researchers to simplify the theoretical model.

One aspect that needs to be addressed is the discovery of “genes that promote programmed ageing”. For example, in nematode Caenorhabditis elegans experimentalists have discovered the presence of such genes. This might look like evidence for programmed ageing. However, it has been shown that this need not be the case by studying the differences in the ageing process in mono-zygotic human twins (Finch and Kirkwood, 2000). If programmed, the programming is hardly visible. Hence it cannot be considered adequate evidence for programmed ageing.

Experimental work on ageing has primarily been concerned with how ageing occurs. Only an evolutionary theory can explain why ageing occurs. The concepts of mutation accumulation and antagonistic pleiotropy provide the basis for the development of many of the current theories on ageing and death. The idea (Disposable Soma Theory of Ageing) suggested by Kirkwood and Rose (1991a) is the most important in this case. Their
deduction is compatible with the data and is argued from an evolutionary point of view. The organisms that show senescence tend to live in a resource-restricted environment. The trade-off between investment in maintenance and growth is the root cause of ageing. In our models we also share this view. This allows us to investigate what happens to the old individuals in the population that continue to consume nutrients. This also allows us to hypothesise that perhaps programmed death can be of assistance here. In other words apoptosis could serve an altruistic purpose. It should be noted that as this aspect of apoptosis has been suggested before (as discussed in the previous chapter), our focus will be primarily on the accumulation of AFs and how this impacts upon the population. It is necessary to make the distinction between the possibility of programmed death as we are suggesting (i.e. apoptosis) and not programmed ageing. Apoptotic machinery can kick-in once the senescence level has crossed a particular level.

It has also been observed that many organisms such as yeast (*Saccharomyces cerevisiae*), nematode worms (*Caenorhabditis elegans*) and fruitflies (*Drosophila melanogaster*) all have genes that influence the ageing process. However a gene that helps the organism to completely avoid ageing is not observed in any species. It is also often argued that if 90% of the population of a species dies due to environmental causes, then any mechanism that assists in body maintenance will only help 10% of the population. Hence the evolutionary pressure will be lower in this scenario. However, this argument is counter-intuitive. It depends on whether the newly mutated organisms can give rise to a species that reduces the mortality rate itself. It should be noted that the trade-off argument is still valid. This explicit focus on the evolution of the optimal levels of investment of resources (also known as the disposable soma theory) in cell maintenance is now widely accepted (Kirkwood et al., 1977; Kirkwood and Holliday, 1979). The theory predicts that the amount of energy spent on cellular maintenance and repair at molecular level affects the longevity of the organism. This has been found to be true in many species such as *Peromyscus leucopus* which is a long-lived rodent species that has a very low generation of reactive oxygen species (ROS - a by-product of the metabolism of oxygen and may damage cell
structures (Sohal et al., 1993).

It is also widely seen that organisms that live for a long time reproduce at a slower rate and exhibit greater ability to repair molecular damage (Martin et al., 1996). They also respond well to stress (Kapahi et al., 1999). Although their lifespan is relatively longer, they also follow the same Gompertz survival curve. We are therefore interested primarily in the relationship between the AFs present in the organism and the survival curve.

Although a number of species have been studied for ageing, for the purposes of our research we restrict ourselves to yeast populations since in yeast both ageing and apoptotic behaviours are present. It is also worth mentioning that these apoptotic machineries are also found in higher-level organisms including humans.

We have data from Sinclair and Guarente (1997b) that shows the chronological ageing of yeast cells. One could study the relationship between the AFs (ERCs in this case) and survival probability using this data as we know how the ERCs replicate (see Chapter 4). Once this is clear, one can then analyse how apoptosis affects the old individuals in a population and how this determines the population dynamics (see Chapters 5 and 6).

III Research in Apoptosis

The theoretical modelling of apoptosis is one of the more actively researched areas in evolutionary biology. Yet, the number of research works on the modelling of the population dynamics of generic apoptotic and non-apoptotic populations is small. Most works focus on the interactions between tumour cells (which tend to be non-apoptotic) and normal somatic cells (apoptotic). However, this would not provide us with a generalised view of the bistable dynamics of the system (where both populations can co-exist). In our modelling approach, we use the hypothesis that the apoptotic machinery can be triggered

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2 On the other hand, *Mus musculus* shows higher generation of ROS and has a shorter lifespan than *Peromyscus leucopus*. 

3 Researchers have also seen evidence of cell damage (mutation) accumulation and trade-offs between longevity and fertility in organisms like Fruitflies.

4 We need to distinguish apoptosis from necrosis here. Even though necrosis occurs through the activation of molecular pathways like apoptosis, it is in fact a premature death of cells by autolysis. We are only interested in the programmed death of a cell because of senescence.
once the AFs cross a threshold value. This is true in many species including yeast as we have seen in the previous chapter.

Ageing is commonly described as a progressive, generalised impairment of the fitness (ability to perform a task) of an organism which in turn decreases the probability of survival. Therefore, another way to look at apoptotic machinery activation is as a function of the AFs present. A simplified approach would suggest that modelling can be done using two sets of ageing populations but only in one of those is the apoptotic machinery activated, once the AFs cross a threshold value.

We discussed in the previous chapter that Weismann (1892) was probably the first to suggest that ageing might be programmed. However, his definition of ageing is quite obscure. There were many other theories that emerged after him which also supported his view. The important aspect of these theories was that these ideas were pivoted around programmed ageing and not programmed death. One of the key arguments used is the idea that ageing evolved to facilitate the turnover of generations. This idea has many flaws and is discussed in detail in Kirkwood and Cremer (1982). The main issue is the focus on ageing (senescence) and not on apoptosis. It is a fact that since in the wild most animals die young, natural selection hardly plays a role in the process of senescence. However, some of the older members do survive and the pressure from them on the resources available could be significant. We explore this idea in our modelling of apoptosis.

The main argument used by Kirkwood and Cremer (1982) is that since the effects of ageing on an organism are minacious it is likely that an organism that has randomly mutated to avoid the gene that causes programmed ageing would have a clear advantage over the organisms with this gene. However, this is true only if we look at ageing as a programmed process. If we consider ageing as a natural result of opting for the optimal strategy (or even the idea that ageing is a side-effect), then apoptosis (programmed death) can potentially play a significant role in governing the dynamics of the populations.

We could study this in two ways - using game theory-based model and differential equations. Both are valid approaches as described in Chapter 2. Basanta et al. (2008) have
already shown that game theory is a good tool to use to simulate the population dynamics of apoptotic and non-apoptotic populations (even though his model uses tumour cells). Apoptosis is generally characterized by specific morphological characteristics in the cell. In our modelling approach instead of focusing on cell cycle machinery and signalling pathways, we look at the process on a more abstract level. Our approach is essentially a binary approach. We allow the non-apoptotic cells to randomly mutate into apoptotic cells to check if the strategy is evolutionarily stable\(^5\). Once the AFs levels have crossed the threshold we assume that the apoptotic machinery has been activated. For the non-apoptotic cells, there would not be any such activation and the cells will continue to live till they starve to death. This simplifies the problem to a considerable extent and allows us to focus just on the evolutionary advantages of the phenomenon.

There are advantages to both (game theory-based model and differential equations-based model) modelling approaches. Even though the game theory-based model is complex and computationally intensive, it allows us to formulate local rules and allows cells to operate under these local rules. The end result will be the product of the behaviour of the cells acting under these local rules. In this way one can easily capture complex biological processes and be very close to the biological environment. The differential equations approach is easier to compute and is simple, but some intricate cell behaviours are difficult to encode using this modelling approach. Here we focus on the ‘big picture’ and the model allows us to study the effect of different parameters (which have biological values) on the population dynamics of the system. Even though we can have parameters in the game theory-based model that are biologically relevant, it often has less meaning on a macroscopic level (as the parameters are more difficult to measure) as we demonstrate later in Chapter 5.

The strategic interactions between cells can be represented using simple game theory models. In the context of these organisms, the strategic interaction is essentially an intelligent choice. This does not mean that the cells are actively making the choice. It

\(^5\)This is done by allowing a single apoptotic organism to compete against a population of non-apoptotic organisms.
is widely accepted that many organisms have the ability to locate the presence of nearby nutrients, predators or other individuals using changes in chemical gradients. It simply implies that natural selection has favoured such behaviours (strategies) as a response to a given situation. As a result of evolutionary selection, those individuals who made the wrong strategies have been wiped out. This way each set of organisms will be able to opt for the best strategy (e.g. compete and acquire nutrients or migrate and locate new nutrients) in our model and in turn can lead to the indirect choosing of a phenotype (this can be either apoptotic or non-apoptotic).

IV Is Apoptosis Reversible?

Many genes associated with apoptosis have been identified. The molecular biology behind the process is also clearer now (Kirkwood and Rose, 1991a). Until 2000, apoptosis was widely considered as an irreversible process with caspase activation, which in turn triggers the process that causes the death of the cell. Hoeppner et al. (2001) found that the cell survival probability in Caenorhabditis elegans (a nematode) increases when the engulfment genes are blocked. This is particularly evident when the cells are subjected to weak pro-apoptotic signals. It was shown that any mutation that induces the partial loss of function of engulfment genes causes the cell to avoid apoptosis. The survival probability of the cell depends on the mutations in these engulfment genes. It was also shown by Reddien and Horvitz (2000) and Lundquist et al. (2001) that these mutations alone can improve the survival rate of the population and even cause differentiation of some cells which otherwise would have died due to apoptosis.

So this begs the question: what happens when a rogue apoptotic cell becomes non-apoptotic like a tumour cell? One would assume that the result is the same as the results we get when we model apoptotic and non-apoptotic populations. However, in actual fact it is quite different. Tumour cells are no ordinary non-apoptotic cells. They also come with a specific set of strategies (which are discussed in detail in Chapter 7) that are not available to apoptotic cells. A good example is angiogenesis. We have seen in the
previous chapter that this causes the cells to have better access to nutrients. As a result, the distribution of nutrients will be different (i.e. biased towards tumour cells). A model that studies the growth of apoptotic and non-apoptotic populations where nutrient is a key parameter can also be used to study the new scenario.

V Apoptosis and Tumour Growth

The main problem with studying non-apoptotic cells using tumour cell modelling is that tumour cells are essentially special types of non-apoptotic cells. Hence there will be a bias. It is also worth mentioning here that the characteristics of tumour cells vary from colony to colony. Accordingly we will restrict ourselves to the general features of tumour cells. Since the phenotypical behaviour of these cells varies from that of normal apoptotic cells, it is easier to model the population dynamics of the system based on these behavioural changes. It has also been reported (but the evidence itself is not strong) that tumour cells can gain an advantage over apoptotic cells by actively harming them. Interestingly, some of these tumour cells also thrive by harming neighbouring cells (Smith, 1982). This allows us to test for cases where cooperation is evident and those where there is no cooperation.

In most game theory-based modelling (discussed in Chapter 2), the players (organisms) are allowed to opt for fixed or variable strategies that are genetically determined. This could be played against the environment and against each other. In our models the organisms are allowed to compete with each other. In the game theory models we allow organisms to cooperate with one another provided they both belong to the same phenotype. This allows us to test the model for different scenarios. Researchers tend to consider a number of biologically-plausible situations and simulate the population dynamics. We explore the dynamics of the population by looking at mixed strategies and allow the individual cells to choose a strategy depending on the circumstances.

It has been reported that the models that suggest successful strategies also suggest that they could lead to genetic polymorphisms (Tomlinson, 1997). This is particularly interesting since we could argue that if the strategy adopted is evolutionarily stable it could
possibly lead to the change in the behaviour of the organism (i.e. an apoptotic cell can become non-apoptotic). In the case of tumour growth, one could also investigate what happens when an increasing number of cells become cancerous. The evolutionary stability of the system on the long run will be of particular interest to oncologists. It should be noted here that we would be restricting ourselves to simple cells that reproduce asexually. This makes it easy to model the dynamics and apply game theory without much complexity.

In the next chapter (Chapter 4) we look at the effects of AFs on the mortality rate of the organisms. Once we have a good understanding of this relationship, we can build a model that would allow us to study both apoptosis and tumour growth.
Modelling of Ageing

Ageing was considered as a long-standing problem in biology (Medawar, 1952). Though the microbiology behind ageing is better known now, it is still an important question which evolutionary biology cannot fully explain.

‘Budding yeast’, *Saccharomyces cerevisiae*, is a good example to study the phenomenon of senescence (Jazwinski, 1990; Sinclair et al., 1998; Defossez et al., 1998) (as discussed in Chapter 2). It exhibits the characteristics associated with apoptosis (Gourlay et al., 2006a). The mother cells of *S. cerevisiae* can only undergo a limited number of cell divisions before death. The mother cell divides asymmetrically and it produces a daughter cell that is relatively smaller in size. Hence we can mark one of final cells as the original mother cell. And the age of the mother cell (in terms of divisions) can easily be calculated by looking at the number of ‘bud scars’ on its surface. (Sinclair et al., 1998)

However, the daughter cells produced in the process of division are not normally influenced by the age of the mother cell unless they are produced in the very late stages of the life of mother cell. The main cause of this ‘ageing’ is the accumulation of extrachromosomal ribosomal DNA circles (ERCs) in the cell, and during the early stages of the life of the mother cell hardly any ERCs migrate to the daughter cells.

In this chapter we re-evaluate the experimental data and try to formulate a general model from the first principle with less artificial (implicit) assumptions than Gillespie
et al. (2004a). Sinclair et al. (1998) showed that the mortality curve of *S. cerevisiae* is in agreement with the Gompertz-Makeham equation. It was also shown that this equation is a direct consequence of reliability theory (Gavrilov and Gavrilova, 2001a). This allows us to investigate the entire process (and stages) of senescence in *S. cerevisiae*. A single-cell based numerical evaluation is done using the model in order to check the correctness of the predictions of the model against experimental data.

Gillespie et al. (2004a) used heuristic methods to find the values of ERC replication and excision. Gillespie et al. (2004a) also assumed that the cell will die only when it has acquired 1000 ERCs. No explanation for the logic behind this assumption was provided and it is considered as an empirical observation based on data from Sinclair et al. (1998). In this chapter we attempt to derive the survival probability curve without making that assumption.

Gillespie et al. (2004a) started with a single cell and used a time dependent probability for ERC excision (which is an arbitrary assumption), a constant probability for ERC replication and a binomial probability for migration. A cell was allowed to die once it reached 1000 ERCs. Furthermore Gillespie et al. (2004a) used cells from new generations (i.e. daughter and granddaughter cells along with the original set of mother cells) to obtain a better fit for the curve. However in the original experiment by Sinclair and Guarente (1997a), they used only the initial generation of mother cells, which were free of ERCs, when they started their experiment.

Gillespie et al. (2004a) considered two phases for allowing migration of ERCs from mother to daughter cell. They defined a critical value (*N*<sub>max</sub>) up to which the mother cell keeps almost all the ERCs produced. In the second phase, the mother cell starts diffusing ERCs more aggressively towards the daughter cell.

### I ERCC Replication and Migration

It has been found that the presence of ERCs is associated with the ageing process in yeast (Sinclair and Guarente, 1997a). Although it is possible for there to be other factors...
as well that contribute to the process of senescence (Laun et al., 2001; Defossez et al., 1998; Kaeberlein et al., 2007; Jazwinski, 2000), in our work (similar to Gillespie et al. (2004a)), we assume the effects of other parameters to be negligible. Thus, according to this assumption, ERCs are considered as abnormalities present in the cell as they negatively influence the longevity and the overall reproductive capability of the cell.

The ERC accumulation process starts with the excision of an ERC from the rDNA locus (Murray and Szostak, 1983). During the initial stages of life the mother cell keeps all the newly formed ERCs and hence the age of the newly formed daughter cell is set to zero (Kaeberlein et al., 2007). The ERCs also grow by replication. Both of these growths contribute to the exponential amplification (Sinclair, 1998) of the ERC number. After a particular stage, the daughter cells also receive a portion of the ERCs present and in this case their life span is shortened accordingly (Kennedy et al., 1994).

Gillespie et al. (2004a) assumed that the probability of the formation (at the time of each replication) of ERCs \( P_{for} \) can be quantified as

\[
P_{for} \simeq \min(\kappa t^n, 1)
\]

(4.1)

where \( \kappa \) is a constant, \( t \) is the number of times the mother has produced daughter cells and \( n \) is any real positive number.

In their simulation, Gillespie et al. (2004a) also assumed that the mother cell usually keeps the newly formed ERCs and the probability of ERCs diffusing to daughter cells increases with the increase in the number of ERCs present in the mother cell. The resultant data curve was found to be in agreement with the experimental data from Sinclair et al. (1998) as shown in Fig. 4.1.

Gillespie et al. (2004a) showed that there is a link between the number of ERCs present and the mortality of the cells. In our work, we extend this idea to formalise this relationship.
Figure 4.1: Comparison of Gillespie et al. (2004a) with data from Sinclair et al. (1998). The error (SSE) in this case is 0.0018

II Hypothesis

Inspired by the experimental observations described previously, we hypothesise the following:

*Ageing factors (such as ERCs in yeast) can be considered as the products of ‘wear and tear’ in a system and they can be used to predict the survival probability. Gavrilov and Gavrilova (2001a) showed that system reliability theory can be applied to biological systems. We hypothesise that the amount of ageing factors (e.g. ERCs in yeast) present in a cell at a given time is related to the probability of the survival of the individual as ageing factors affect their reliability (fitness).*
III Heuristic approach: Using Reliability Theory-Based Model

This section is merely a chronological description of how we started experimenting to see the way ERCs and the mortality rates are linked. Our contributions to the existing body of research begin from the section ‘Model From First Principles’. We use the ideas developed here for formulating a model from first principles.

In order to study the effects of ERCs we used a reliability based model. The methodology adopted here is essentially a heuristic approach.

Reliability theory has been applied to the problem of ageing and longevity (see Gavrilov and Gavrilova (2001a) for details). According to this theory, the failure rate ($\lambda(t)$), can be represented as

$$\lambda(t) = -\frac{dP(t)}{P(t)dt}$$

(4.2)

where $P(t)$ is the probability of survival of the system up to time $t$.

In the case of the senescence in $S. \textit{cerevisiae}$ caused by the ERCs present, the failure rate can be expressed as a function of ERCs ($E$) (since we have seen that it can used as an age-dependent parameter in Gompertz equation).

Now if we assume:

$$\lambda(t) = f(E(t))$$

(4.3)

This will allow us to simulate a whole range of scenarios.

After experimenting, we found that it is indeed possible to derive a relationship between the mortality and age of the organism in a population. However, considering the fact the approach was very speculative (it was in fact a ‘trial and error’ method) the details of the results are not included in this report as they cannot be used as proofs.
Chapter 4 Section IV

IV Model from First Principles

We have found that (using the heuristic method) it is possible to derive a relationship between the AFs and the survival probability of the cells. Deriving inspiration from that, we formulate the general form for the probability of survival \( P(t) \) from first principles.

Inspired by the Gompertz law, we define the death rate \( \lambda(t) \) as:

\[
\lambda(t) = \lambda_0 + \lambda_{ERC}(E(t))
\]  

(4.4)

where \( \lambda_0 \) represents a constant factor independent of the number of ERCs present.

Probability \( P(t) \) of survival until time \( t \) then has the following relationship to \( \lambda \):

\[
P(t)\lambda(t) = -\frac{dP}{dt}, \quad t \geq 0
\]  

(4.5)

If \( \lambda(t) \) is known, solving the above for \( P(t) \) yields (with initial condition \( P(t) = 1 \)):

\[
P(t) = e^{-\int_0^t \lambda(\tau)d\tau}
\]  

(4.6)

This completely independent of all the choices of \( \lambda \) and results from the theory of ordinary differential equations. \( P(t) \) is non-increasing with \( t \), as \( \lambda \) can only be non-negative.

\( P(t) \) has two interpretations:

1. Individualistic: \( P(t) \) relates to a single individual and hence stands for the probability of survival until \( t \).

2. Ensemble: \( P(t) \) does not relate to an individual, but to a large population of such, then \( P(t) \) is the fraction of initial population that survives until \( t \).

For mathematical convenience we focus on the individualistic approach. We define \( E(t) \) as the number of ERCs, \( r(t) \) as the growth rate of existing ERCs and \( p(t) \) as the production rate of ERCs.
This yields:

\[ E(0) = 0 \] (4.7)
\[ E(t) = rE(t - 1) + p(t) \] (4.8)

or in continuous time:

\[ \frac{dE}{dt} = rE(t) + p(t) \] (4.9)

But according to Gillespie et al. (2004a) \( r(t) = r \) and we have also seen that:

\[ p(t) \ll rE(t) \] (4.10)

when \( t \) is high. Hence we can assume \( p(t) = p \) and using (See Appendix - equation (A.4)):

\[
E(t) = \int_0^t pe^{r(t-\tau)}d\tau + c \\
= p \int_0^t e^{r(t-\tau)}d\tau + c \\
= pe^rt \int_0^t e^{-r\tau}d\tau + c \\
= pe^rt \left[-\frac{1}{r}e^{-r\tau}\right]_0^t + c \\
= \frac{p}{r} e^rt \left[1 - e^{-rt}\right] + c \\
= \frac{p}{r} \left[e^rt - 1\right] + c
\] (4.11)

with \( c \) an arbitrary constant for which \( E(0) = c \).

Now for mathematical convenience we assume that, \( \lambda_{ERC}(E) \) is linear in \( E \), ie:

\[ \lambda_{ERC}(E(t)) = cE(t) \] (4.12)

and inserting this into equation (4.4) gives:
\[ \lambda(t) = \lambda_0 + cE(t) \]
\[ = \lambda_0 + c \frac{P}{P} \left( e^{rt} - 1 \right) \]
\[ = \lambda_0 + c \left( e^{rt} - 1 \right) \] (4.13)

where integration constants have been collected and simplified without limiting the generality using Reeh’s universal constant (see Appendix Section I). Since \( p \) is a constant it could be merged with one of the integrating constants.

\[ P(t) = e^{-\int_0^t \lambda(\tau) d\tau} \]
\[ = e^{-t\lambda_0 + c \int_0^t (e^{r\tau} - 1) d\tau} \]
\[ = e^{-t\lambda_0 - c \left( \left[ \frac{e^{r\tau} - 1}{r} \right]_0^t + t \right)} \]
\[ = e^{-t\lambda_0 - c \left( \frac{e^{rt} - 1}{r} + t \right)} \]
\[ = e^{-t\lambda_0 - c \left( e^{rt} - 1 + \frac{t}{r} \right)} \] (4.14)

It is found (using the curving tool from Matlab) that for values \( r=0.6 \) (which is the same value used by Gillespie et al. (2004a)), \( c=0.000004 \) and \( \lambda = 0.01005 \) we obtain a curve that is similar\(^1\) to case 6 in the previous section. This is shown in Fig. 4.2.

The biological meaning of the parameter \( c \) could be that it represents the impact of the ERC number on the mortality of the cell.

**IV.I With ERC migration**

ERC migration can be incorporated by altering the value of \( r \) by an ‘effective net growth rate’ of ERCs:

\[ r(t) = r - r_d(E(t)) \] (4.15)

\(^1\)Goodness of fit using SSE is 0.08744
where $r$ is the gross production rate of ERCs, $r(t)$ is the effective net rate, and $r_d$ is the rate of sharing with the daughter:

Biologically it makes sense to assume that sharing sets in gradually around a certain threshold $E_0$ so in principle $r_d$ is a sigmoidal function, for example the exponential sigmoidal (but which could also be the square root sigmoid):

$$r_d(E) = r_D \frac{1}{1+e^{-a(E-E_0)}}$$  \hfill (4.16)

where $r_D$ is the asymptote for $E$, $E_0$ is the value of maximal change of $r_d$, i.e. the ‘threshold’, which to be chosen such that rate with which ERCs migrate from the mother cell to daughter cells is negligible for $E \approx 0$ and $a$ is the steepness of the threshold.

A simplification (for mathematical convenience) of the above sigmoidal behaviours is to assume that the threshold is hard at $E_0$, i.e.: replacing the sigmoid with a Heaviside function:

$$r_d(E) = r_d H(E - E_0)$$  \hfill (4.17)

This means that the equation (4.11) breaks into two parts with different but constant rates $r$ for $E < E_0$ and $r - r_d$ for $E \geq E_0$. 

Figure 4.2: Model from first principles : Survival probability Vs Time (reproductive life time).
With the initial condition $E(0) = 0$ we get the specific solution:

$$E(t) = \frac{p}{r} \left[ e^{rt} - 1 \right]$$

(4.18)

ie $c = 0$. Now we estimate the value $E_0$ (using curve fitting) and want to know at what time $t_0$ this is reached:

$$E(t_0) = E_0$$

(4.19)

$$r \frac{E_0}{p} = (e^{rt_0} - 1)$$

(4.20)

$$r \frac{E_0}{p} + 1 = e^{rt_0}$$

(4.21)

$$\log \left( \frac{r}{p} E_0 + 1 \right) = rt_0$$

(4.22)

$$\log \left( \frac{r}{p} E_0 + 1 \right) = \frac{t_0}{r}$$

(4.23)

(4.24)

One can calculate $E_0 = E(t_0)$ easier from $t_0$ then $t_0$ as $t_0(E_0)$ from the inverse function.

$$E(t) = \begin{cases} 
\frac{p}{r} \left[ e^{rt} - 1 \right], & t < t_0 \\
\frac{p}{r_D} \left[ e^{r_D(t-t_0)} - 1 \right] + E_0(t_0) & 
\end{cases}$$

(4.25)

We assume that the Heaviside function starts operating around generation 15. It is at this point that the model with no ERC migration starts deviating (as shown in Fig. 4.2). This is a biologically valid assumption as well.

The survival probability for the organism after $t_0$ can be written as:
Due to the nature of the equation, the fitting is done for generations versus log of the survival probability. So the equation becomes:

\[
\ln(P(t)) = k_a e^t + k_b t + k_c \tag{4.27}
\]

The results are shown in Fig. 4.3 and Fig. 4.4.

The values estimated (with 95% confidence bounds) for the parameters are:

\( k_a = -3.425 \times 10^{-16} \)

\( k_b = -0.1533 \) and

\( k_c = 2.881. \)

Goodness of fit is found to be (using SSE) 1.493. It should be noted that the parameters are chosen so as to get the best result.
Figure 4.3: General model fitted with transformed data (exponential of survival probabilities) from Sinclair et al. (1998). Here the whole lifespan is considered. Survival probability versus Time (reproductive life time) graph.

Figure 4.4: General model fitted with transformed data (exponential of survival probabilities) from Sinclair et al. (1998). Survival probability versus Time (reproductive life time) graph.

V Matrix Model of Survival Probability

In all of the models discussed previously in this chapter we were concerned only about the survival probabilities of the cells. Now, let us use the matrix population model to
determine how the size of the population itself is changing. Even though we would expect it to have a similar output to that of the previous models, there could be a slight deviation (in terms of fitness - See Table 4.1) due to the fact that this model is overly simplified\(^2\).

Inspired by various matrix population models (discussed in Chapter 2), we also propose a matrix model\(^3\) for understanding ageing in yeast. We divided the cells into three different classes:

1. Cells with no ERCs present \((n_1)\);
2. Cells with few ERCs present such that the mother cell keeps almost all of them \((n_2)\); and
3. Cells with large number of ERCs present such that mother cell will try to diffuse them towards the daughter during reproduction \((n_3)\).

Such a collection of yeast cells may be loosely represented as:

\[
\begin{bmatrix}
  n_1 \\
  n_2 \\
  n_3 \\
\end{bmatrix}
(t + 1) =
\begin{bmatrix}
  p_1 & 1 & 0 \\
  1 - p_1 & p_2 & 1 \\
  0 & 1 - p_2 & p_3 \\
\end{bmatrix}
\begin{bmatrix}
  n_1 \\
  n_2 \\
  n_3 \\
\end{bmatrix}(t)
\]

\[\text{(4.28)}\]

Please note that this is not a perfect representation as few factors are neglected (such as the element corresponding to the production of very few daughter cells with no ERCs present by a mother belong to class 3).

As we are only interested in the original set of virgin mother cells (i.e. daughters, grand-daughter and so on are neglected), we need only\(^4\)

\(^2\)Reasons for using this simplified model are explained later in this section.
\(^3\)Derivation of a generic model is shown in the appendix. Please see Matrix model section in the appendix for details
\(^4\)The following equation is obtained by ignoring the components that represent reproduction in the matrix
\[
\begin{bmatrix}
n_1 \\
n_2 \\
n_3 \\
\end{bmatrix}
(t + 1) =
\begin{bmatrix}
p_1 & 0 & 0 \\
1 - p_1 & p_2 & 0 \\
0 & 1 - p_2 & p_3 \\
\end{bmatrix}
\begin{bmatrix}
n_1 \\
n_2 \\
n_3 \\
\end{bmatrix}(t)
\]

(4.29)

Equation (4.29) can also be represented as:

\[ n_1 = p_1 n_1 \] (4.30)

\[ n_2 = (1 - p_1) n_1 + p_2 n_2 \] (4.31)

\[ n_3 = (1 - p_2) n_2 + p_3 n_3 \] (4.32)

where \((1-p_1)\) is the excision probability, \(p_2\) is the chance of a cell in class 2 producing a class 3 cell, \(p_3\) is the chance of a cell in class 3 surviving from \(t\) to \((t + 1)\). It should be emphasised that this representation is a very simplistic approach and for a better ‘fit’ we may have to include more elements. Nonetheless, this should produce a curve that is roughly in agreement with the data from Sinclair and Guarente (1997a).

If we consider the biological (Gillespie et al., 2004a) and statistical aspects of the parameters we can say that:

\[ 0 \leq p_1 \leq 1 \] (4.33)

\[ 0 \leq p_2 \leq 1 \] (4.34)

\[ 0 \leq p_3 \leq 1 \] (4.35)

\[ p_1 \gg p_2 \] (4.36)

\[ p_2 > p_3 \] (4.37)
We found that (using curve fitting) for $p_1 = 0.8$, $p_2 = 0.65$ and $p_3 = 0.35$ we can obtain a good curve (since we are interested only in ‘ageing’ and survival probability, this curve will suffice) as shown in Fig. 4.5 and Fig. 4.6. It also shows that the initial number of individuals is not a determining factor in governing the dynamics. From this curve we can predict the maximum survival time\(^5\) (which was used as a direct empirical observation in Gillespie et al. (2004a)). The growth of the population (only the initial generation of 50 members\(^6\) is considered) is shown in Fig. 4.7.

From equation (4.29) we can have\(^7\)

$$(1 - p_1 - \lambda)(p_2 - \lambda)(p_3 - \lambda) = 0$$  \hspace{1cm} (4.38)

\(^5\)Shown later in this section  
\(^6\)This initial number does not determine the nature of the curves and can be varied.  
\(^7\)The following equation may be used for further analysis - including the stability of the population.
Figure 4.6: Survival curve obtained from matrix model starting with 50 individuals in class 1 (with no ERCs present). The error (SSE) in this case is also found to be 0.2869.

Figure 4.7: Growth of the three classes of yeast cells according to the matrix model.
VI  Modified Gillespie’s Model

Since Gillespie et al. (2004a)’s model is inconsistent with the experimental conditions followed by Sinclair et al. (1998), we decided to redo the simulations to check if their model is consistent with the experimental data available. We found that, for the following values of parameters, we obtain a curve (see Fig. 4.8) which is almost in agreement (goodness value of 0.3911) with the data from Sinclair et al. (1998) to a considerable extent (see Section VII.II of this chapter for fitness values):

- $N_{max}$ (which is a critical value up to which the mother cell tries to keep all the ERCs formed) =800
- Production probability=0.6
- Replication probability=0.6
- ERC migration probability before $N_{max}$=0.98
- ERC migration probability after $N_{max}$= 0.75

These are the same values used by Gillespie et al. (2004a). The only difference is that we have included only the original set of mother cells while calculating the resultant population.

We also tested the model by varying $N_{max}$ to 175 and 900.

The resultant curves are shown in Figs. 4.9 and 4.10. We have also used a low constant death rate (due to environmental effects) to account for the initial decline in the survival curve.

We also simulated the model by changing the value of the number of ERCs at which the cell fails to replicate to check whether we could obtain a better curve. We found that for the values:

---

8Gillespie et al. (2004a) included multiple generation (daughter and granddaughter cells) in their simulations even though Sinclair et al. (1998) considered only the original population of mother cells. We also done experiments by altering our model to include multiple generations and found that there is a slight change in the survival probability values of the initial time period ($t=1-10$). And the rest of the curve is unaffected.
Figure 4.8: Modified Gillespie's model with cells dying when they reach 1000 ERC limit with $N_{\text{max}}=175$. The error (SSE) in this case is found to be 0.0534.

Figure 4.9: Modified Gillespie's model with cells dying when they reach 1000 ERC limit with $N_{\text{max}}=800$. The error (SSE) in this case is found to be 0.0075.
Figure 4.10: Modified Gillespie’s model with cells dying when they reach 1000 ERC limit with \( N_{\text{max}} = 900 \). The error (SSE) in this case is found to be 0.0077.

- \( N_{\text{max}} = 175 \)
- production probability = 0.6
- replication probability = 0.6
- ERC migration probability before \( N_{\text{max}} = 0.95 \)
- ERC migration probability after \( N_{\text{max}} = 0.77 \)

we can obtain a better curve if we set the number of ERCs required for senescence to occur as 300. The curve is shown in Fig. 4.11.
Figure 4.11: Modified Gillespie’s model with cells dying when they reach 300 ERC limit. The error (SSE) in this case is found to be 0.0188.

**VII Discussion**

We used biologically inspired arguments to explain the nature of the curve and then employed empirical equations, based on these arguments, to construct the new model. Accordingly, it provides a better understanding of the actual processes involved in the ageing of *S. cerevisiae*. We have already seen that the yeast model is consistent with the Gompertz-Makeham equation (Sinclair et al., 1998).

We can also explore, mathematically, a special case where we have a hypothetical mechanism that keeps removing ERCs from the system. This will enable the system to maintain itself without senescence. Therefore, it can also avoid apoptosis. However, such a mechanism is not observed in nature. The reason for this could be better understood if we analyse the system with the aid of a fitness function that is defined in terms of the Malthusian parameter (which essentially is growth rate), \( r \), that is obtained from solving the Lotka equation (Kirkwood and Rose, 1991a):

\[
\int_0^T e^{-rt} l(t; s)m(t; s)dt = 1
\]  

\[(4.39)\]
where $t$ is the age, $s$ is the maintenance, $T$ is the lifespan, $l(t; s)$ and $m(t; s)$ represent the survivorship and fecundity of the cell respectively. It can be shown that the optimum value of $s$ is less than the minimum value required for avoiding senescence (Kirkwood and Rose, 1991b). In other words, the system finds it to be optimal to age rather than spending energy on resources to remove ERCs more aggressively. This essentially answers the question of why the system enters into a mode of an aggressive ERC eviction (diffusion) phase and still does not try to remove all the ERCs present.

**VII.I Summary of Models**

A summary of the models we have developed so far and some of the features of those models are described here:-

i) **Model derived from first principles**: We further showed that the above model can be generalised and can be derived from first principles:

$$P(t) = e^{k_a e^t + k_b t + k_c} \quad (4.40)$$

ii) **Modified Gillespie’s model**: We also modified the model used by Gillespie et al. (2004a) since the simulation was inconsistent with experiment by Sinclair (1998). We found that the modified model also provides a good fit but the parameter values are different from that of Gillespie et al. (2004a).

iii) **Matrix Model**: For understanding the population dynamics of the cells simulated we derived a matrix-based model. In the case of yeast, we found that we could have a simplified model that could capture the big picture accordingly:

$$\begin{bmatrix} n_1 \\ n_2 \\ n_3 \end{bmatrix}(t + 1) = \begin{bmatrix} p_1 & 0 & 0 \\ 1 - p_1 & p_2 & 0 \\ 0 & 1 - p_2 & p_3 \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ n_3 \end{bmatrix}(t) \quad (4.41)$$
VII.II Goodness of Fit

The goodness of the fits are shown in Table 4.1.

Table 4.1: Goodness of the fitted curves obtained in three cases (compared against experimental data from Sinclair et al. (1998). Parameter values are not listed here since the type of parameters used in each model varies.

<table>
<thead>
<tr>
<th>No</th>
<th>Method</th>
<th>Error (SSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gillespie et al. (2004a)</td>
<td>0.0018</td>
</tr>
<tr>
<td>2</td>
<td>Model from first principles (with no migration)</td>
<td>0.08744</td>
</tr>
<tr>
<td>3</td>
<td>Modified Gillespie’s model (best case with Nmax=800)</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

Even though when the model based on first principles was used the error was worse than Gillespie et al. (2004a), the approach itself is elegant as it is devoid of many implicit assumptions. More importantly, we can also say that in most of our models we used less implicit assumptions than Gillespie et al. (2004a).

VIII Concluding Remarks

The concept of senescence is an unresolved problem in evolutionary biology (Kirkwood, 2005b). Sinclair (1998) defined it mathematically as the “likelihood of death of individuals within a population increases exponentially with age”.

Although different attempts have been made to explain the process of ageing, no successful attempts explaining the evolutionary case have been made (Gavrilov and Gavrilova, 2003; Partridge and Gems, 2006). The new model proposed here has the ability to accommodate multiple parameters along with main cause, (i.e. the presence of ERCs) as suggested by some of the recent studies (Kaeberlein et al., 2007; Lamming et al., 2004) (by modifying what is included as AFs).

Studying the process of ageing in yeast is a very effective and efficient way to comprehend the problem in general as it has already been shown by Sinclair (1998) that the ageing curves are very similar for both unicellular and multicellular organisms.
This would also help us to investigate if a mutation is triggered by such an event in a unicellular organism and is later picked up by other organisms as well. There is ample evidence to suggest that a distress in a cell could cause such a mutation (Ishii et al., 1998; Lin and Beal, 2006; Finkel and Holbrook, 2000). This idea is used to develop our idea further and is discussed in Chapters 5 and 6.

In our model, we saw that the mother cell diffuses more ERCs to daughter cell towards the end of its lifespan and this could lead to such type of a stochastic mutation. We also found that (in section VII of this chapter) the optimal maintenance is lower than the value required to avoid senescence. This may result in the natural selection of a mutation that causes a cell to start producing a chemical that will kill itself (a typical example could be the release of enzyme lyase) towards the end of its life span (programmed death).

There are two reasons why natural selection might favour such a mutation:

1. The cell undergoing such type of mutation will subsequently die due to an increase in the presence of the new hazardous chemical (Aravind et al., 1999). Therefore, this reduces the number of ‘older’ daughter cells produced (with ERCs present). Accordingly, such a group might thrive as a result.

2. Since an affected cell is eventually killed, the net resources available for the rest of the group of cells increases.

These are analogous to the arguments used to support altruism. This could be a possible explanation for apoptosis from an evolutionary point of view. This is investigated in the next chapter (Chapter 5) along with the concept of cooperation amongst cells.
Modelling of Apoptosis

We know from Kirkwood and Rose (1991b) that ageing occurs in most organisms because it is the optimal strategy in a resource-restricted environment. In this chapter we investigate the population dynamics when you allow one set of populations to thrive without any programmed death machinery and another with this machinery built-in.

We explore a theoretical model and study the population dynamics of a set of non-apoptotic and apoptotic populations. The change in population dynamics when an individual selects apoptosis as a strategic choice is studied. We also demonstrate how our model could potentially solve the problems associated with the disposable soma theory. The dependency of each variable used in the model is carefully studied by varying the values over significant ranges. We start by developing a very simple system and test it for a short period of time. This is later extended to reflect a biological scenario.

This chapter is divided into five main sections. We start by introducing the problem we are addressing. In the second section we show how we use concepts developed in the previous chapter are employed in the model described in this chapter. Since a review of the phenomenon (apoptosis) itself has been detailed in the review chapter (see Chapter 2), we discuss elements that are significant only from a modelling perspective here. The reasons for opting for Synthetic Ecology model are also discussed. In the following section we set out details of our modelling approach where the rules and algorithms used in the model are described. We end this chapter with a conclusion section (the final section).
I Introduction

Both antagonistic pleiotropy and disposable soma theory have predicted senescence (and in turn apoptosis) to be a ‘side effect’ of ‘growing old’ (Kirkwood, 2005a). It has also been shown that senescence causes apoptosis (Campisi and di Fagagna, 2007). In the previous chapter we have shown how senescence impacts on the mortality of an organism. Although the altruistic benefits of apoptosis have been suggested before (Alison and Sarraf, 1992), we are attempting to show that in a resource-restricted environment, apoptosis can be a strategic choice. In order to demonstrate this we will employ a model based on game theory and computer simulations using Agent Based Modelling (ABM). Evolutionary Game Theory (EGT) is widely used to design simple mathematical models of populations where the interactions between individuals determine their dynamics (Smith, 1982). It has also been discovered that environmental and genetic mutations can transform the cells in a healthy tissue into a population of individualistic tumour cells (Nowell et al., 1976). From a theoretical point of view, the reverse is also feasible (i.e. non-apoptotic organisms can also become apoptotic through mutation). We use the above mentioned concepts (Game theory (GT) and the idea that non-apoptotic organisms can become apoptotic by undergoing mutation) to develop the model. Our approach is similar to that of Basanta et al. (2008) and we use the model to predict the stable strategy of the population set. In the model, the rate of growth of ageing factors is governed by the Gompertz function (which is consistent with the deductions we made in the previous chapter) and the threshold for apoptosis to activate is set to an arbitrary value.

The individuals are allowed to compete for food, which is allocated according to the strategies adopted by the individual and the pay-off matrix. Once an individual secures enough nutrients, it is allowed to replicate and the newborn individual becomes part of the population. After each time-step the nutrients are replenished.

This process is repeated over many time-steps (see Section IV.I for more details). We find that switching to the apoptotic mode gives the individual cell an advantage over non-apoptotic organism in a resource-restricted environment. Using EGT, the stability
of the new strategy is explored. The analysis indicates that apoptosis is an Evolutionary Stable Strategy (ESS) as long as the conditions of the model (i.e. parameters) remain the same. The model is tested extensively by varying the values of all the parameters used and the impact of each parameter is studied. We find that once angiogenesis is activated, apoptosis is no longer the best strategy. This is consistent with the behaviour of tumour cells (Ruf and Mueller, 1996). This model is further explored in Chapter 7.

II From Ageing to Apoptosis

In the previous chapter (see Chapter 4) we showed that ageing plays a significant role in the population dynamics of yeast populations. Kirkwood and Rose (1991a) have shown that it is ideal for an organism to spend only an optimal amount of energy on maintenance. If the organism manages to avoid apoptosis, then the resulting population will accumulate a number of old cells over time. This idea inspired us to hypothesise the following 3 ideas:

1. Apoptosis is likely to play a positive role in removing the old organisms from the population;

2. Apoptosis is an altruistic act by an organism to facilitate the faster growth of the population; and

3. Organisms with apoptotic machinery have an evolutionary advantage over those with no such mechanism.

Interestingly, modelling using yeast populations has also been successfully established as a method for studying apoptotic regulation as they have shown markers of apoptotic machinery and it is quite easy to grow yeast colonies (MacLean et al., 2001). Even today, the advantages of apoptosis for a unicellular organisms such as yeast are unknown. Our work attempts to shed more light on this subject by studying the dynamics of apoptotic and non-apoptotic populations in a resource-restricted environment. Research has shown that yeast cultures that have chronologically aged exhibit markers associated
with apoptosis (Herker et al., 2004). They also show elevated levels of oxygen radicals and exhibit caspase activation.

Herker et al. (2004) has also shown that the apoptotic pathway can be delayed by the over-expression of YAP1. Even though a reduction in yeast caspase YCA1 leads to a higher probability of survival (i.e. low apoptosis), the cells that do survive show a reduction in their ability to re-grow. This clearly indicates that pre-damaged cells amass in the absence of proper apoptotic machinery. This fact inspired us to hypothesise that apoptosis could play a positive role in the population dynamics of various organisms.

**Game Theory (GT)**

Game Theory (GT) is widely used to design simple mathematical models of populations where interactions between individuals determine their dynamics. We use this tool to model our problem. GT was formalised by Von Neumann and Morgenstern and used primarily as a tool for analysing economic scenarios (Von Neumann and Morgenstern, 2007). Since then it has been successfully applied to various problems in evolutionary biology (Smith, 1982; Hofbauer, 2001; Hofbauer and Sigmund, 2003). Since cancer has long been recognised as an evolutionary disease (Nowell et al., 1976), EGT is widely used to study tumour cell interactions (Bach et al., 2001). Since tumour growth is essentially an evolutionary and ecological process, EGT is an appropriate modelling tool to analyse the population dynamics under various micro-environmental circumstances (Gatenby and Maini, 2003). In this scenario different phenotypes of tumour cells compete for space and resources (Merlo et al., 2006; Levitis, 2011). In our model, we use GT to study the behaviour of the organisms and then check that the strategies adopted by the most successful players are evolutionary stable. Tomlinson (1997); Tomlinson and Bodmer (1997) have already shown that one can achieve a realistic model even if simple rules are used to reduce the complexity of the model. In their models (Angiogenesis and PCD), they used simple kinetic equations to predict the large scale picture of the population dynamics.
II.I Agent Based Modelling

ABM (or multi-agent simulation), which simulates the actions and interactions of autonomous agents to replicate their effects on the system as a whole, allows us to implement our model in the best possible way, since we are dealing with an ecosystem of individuals competing for space and resources. Mansury et al. (2006) proposed a model to explore the ‘genotype-phenotype’ link using ABM. Similarly in our model, we assume that when the non-apoptotic individual becomes apoptotic through random mutation the phenotype (presence of apoptosis) also changes. ABM has widely been used for simulating biological scenarios (Walker et al., 2004). ABM has also been used in multi-scale agent-based cancer modelling (Zhang et al., 2009). In our model we are simulating the actions and interactions of the population and these are in turn dependent on the GT model. A determining element of the analysis is to check that the strategy chosen by the successful population (i.e. the one that is able to produce a considerably greater number of progenies than the other) is in fact an ESS. A strategy is ESS if, when adopted by a population, it cannot be successfully invaded by an alternative strategy during the process of evolution (Taylor and Jonker, 1978).

Bellomo and Delitala (2008) have shown that GT can be used to study the modelling of mutations. They also showed that the model could be used to study the onset, progression and immune competition of cancer cells. Our modelling approach is inspired by Basanta et al. (2008) and we use our model to study the evolutionary advantages of apoptosis. We start by defining a GT model and simulate the interactions using ABM. Unlike in Basanta et al. (2008), in which they used GT only to estimate the equilibrium point, we use the GT modelling rules even whilst simulating the population dynamics.

III Hypothesis

We hypothesis the following:

*Apoptosis is likely to improve the population growth of a species as it would allow the*
population to eliminate older organisms that are consuming resources without producing any healthy offspring. If the effects of the Ageing Factors are significant, apoptosis can be a strategic choice (a mutually beneficial act) made by the species to increase population growth. Accordingly, apoptotic organisms are likely to thrive over non-apoptotic organisms in the long run (at least in certain circumstances) - in other words, apoptosis leads to overall larger population and higher inclusive fitness.

IV Modelling

IV.I Game Theory Model

We start by defining a simplified model and then we alter the assumptions (see Section IV.III) to show that this model works in a more realistic environment as well. The simulation environment is characterised by a 100 x 100 lattice space (in 2D) in which each lattice space can host up to two individuals. The individuals are allowed to move around inside the lattice boundary of these 100 x 100 lattice cells. The carrying capacity of a lattice cell corresponds to the area of influence of one organism over another (i.e.: organisms within the same lattice cell only can compete with one other). An initial amount of nutrients, $N_0$, is randomly distributed (to ensure that there is no bias in the allocation of nutrients) in the lattice space (for the specific details see Section IV.II of this chapter). A single lattice cell is also not allowed to have more than 2 units of nutrients. The amount of nutrients available determines the growth of the population. We start with a fixed number of individuals ($P_0$) belonging to two phenotypes - apoptotic and non-apoptotic. Individuals belonging to both population sets are allowed to undergo senescence. The strategy set consists of two choices - compete or migrate.

We also assume that cells can mutate between apoptotic and non-apoptotic states; thereby changing their phenotypes. This is consistent with what is observed in experiments (Knudson and Strons, 1972; Knudson, 1971). In our model we assume that a set of

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1Since we are interested in the population dynamics and migration, it is better to set this parameter to a lower value as it will effectively show which population is good at migration and doing it efficiently
cells have mutated to become apoptotic and we simulate the population dynamics when equal numbers of each population are present (i.e. $N_0/2$ of each). We then check what happens when the numbers of each population are not equal.

In each time-step 50 pairs of organisms (which is an arbitrary value and this is later changed in the extended model where all the individuals are allowed to compete) are randomly chosen (provided they are in the same cell, if not an organism from a nearby cell is allowed to migrate into the cell as described below) and are allowed to compete for nutrients. Cooperation has been observed in various populations and it has been established that similar organisms are likely to compete together against others (Axelrod et al., 2006). In our model, we introduce cooperation in such a way so that the competition will always be between two individuals belonging to two different populations (i.e. apoptotic and non-apoptotic). Even though there is experimental evidence to support this as shown by Axelrod et al. (2006), we later modify this assumption to allow every organism in a cell to participate in competition (see Section IV.III of this chapter). Nutrient amounts are expressed in terms of biomass. Since the individual organisms can interact only with other organisms in the same lattice cell, these two randomly selected cells should belong to the same cell. If there is only one organism in a lattice cell, a nearby organism is picked and is allowed to migrate to the lattice cell by paying the cost of migration. This is because in the biological world, a nearby organism is attracted to the nutrient through bio-chemical change in the environment. If the organism does not migrate, the other individual will get the full amount of nutrients available. This assumption is consistent with approaches adopted in similar experiments (Basanta et al., 2008). If the other cell finds nutrient on its way or has access to nutrients in the original host cell, then it devours those nutrients (i.e.: there won’t be any competition). The randomly selected individuals decide whether to compete or migrate to another cell. The decision to migrate to the nearest cell is influenced by the population density of those cells (i.e. a lower population density favours migration to the lattice cell)$^2$.  

$^2$If there is only one nearby lattice cell and it is empty, there is a 50% chance of migrating to that cell. This chance goes down when the density of the nearby lattice cell is increased. The probability of migration of a cell with $j$ organisms to a cell with $n$ organisms is set as $p = 0.5 - \frac{n}{n^2}$. 

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Each new organism will have a ‘nutrient reserve’ of 1 unit. When an individual gains nutrients it is added to this reserve. If this level falls to 0 or below 0, the individual dies. In every time-step two individuals compete (This is later altered in Section IV.III of this chapter). Whilst competing an individual organism uses resources from its ‘nutrient reserve’. The aim of the competition is to obtain the maximum nutrients available. Nutrients worth at least \( n_f \) are required to reproduce. Nutrients worth \( n_r \) are used for the process of reproduction so that after the reproduction the daughter cell will have a ‘nutrient reserve’ of 1 and the mother cell will have a ‘nutrient reserve’ of \((n_f - n_r)\). The new offspring will be created in the same lattice cell provided the carrying capacity of the lattice cell has not been exceeded. If the carrying capacity has been exceeded, the offspring is allowed to migrate to the nearest low density lattice cell by paying the cost of migration.

The AF level of the organism will have a negative effect every time the individual tries to obtain nutrients or migrate \((f(t))\) to another lattice cell. This is accounted by deducting some amount (depending on the AFs present) from the ‘nutrient reserve’ and is used for ‘maintenance’. AFs are allowed to increase after each time step provided the individual did partake in the competition. The effect of AFs is expressed as \(f(t)\) and is set to be equal to the mortality rate of the individual. From the Gompertz-Makeham law (Makeham, 1867), the mortality \((\mu(t))\) is given by

\[
\mu(t) = \alpha e^{\beta t} + \gamma
\]

(5.1)

where \(\alpha\), \(\beta\) and \(\gamma\) represent ‘basal vulnerability’, ‘actuarial ageing rate’ and age independent ‘extrinsic mortality’ respectively. It should be noted that this selection is completely arbitrary. This can be replaced even using a simple linear function and we would obtain a similar result. However, we have used this function as we found the nature of the function to be consistent with similar biological phenomena.

Accordingly,
\[ f(t) = \alpha e^{\beta t} + \gamma \]  

(5.2)

where \( t \) is the age of the individual. In our model we assume \( f(t) \) to be a linear function of \( \mu(t) \) with the value of \( \alpha \) equals 1. It should be noted that we are only assuming the rate of production of AF here and not the death rate of the population itself.

If a cell does not partake in competition it is assumed to be in a ‘hibernation mode’ (The effect of this assumption is further tested in Section IV.III of this chapter) and its AFs will not increment. In the case of apoptotic cells, a cell is allowed to die once \( f(t) \) crosses an arbitrary level, \( f_+(0) \). The process is repeated over many time-steps.

Figure 5.1: Migration of individuals from one lattice cell to another.

The update of the lattice space is as follows:

1. 50 pairs of individuals are randomly selected (the effect of the number of pairs chosen is tested in Section IV.III of this chapter by allowing all the individuals in the population are allowed to enter into competition ) and allowed to compete.

2. Whilst competing, the individual organisms simultaneously and randomly decide whether they want to continue competing for nutrients or migrate to neighbouring lattice cells. This probability of migration is implemented as simple binomial distribution (Bernoulli distribution) based on the number of individuals already present in a lattice cell, the amount of nutrients available and the number of neighbouring individuals present.

3. Based on their strategy a reward is given according to the following pay-off matrix:
where $f(t)$ is the effect of ageing factors, $c$ is the cost of competing and $a$ is the cost of migration. This matrix is specific to each of the individuals participating. For the biological significance of these parameters please see Section IV.II.

4. As a result, a cell may obtain all available nutrients in entirety, obtain half of the nutrients or may leave the competition and migrate.

5. If a cell’s ‘nutrient reserve’ is more than $n_f$ it is allowed to replicate and its reserve goes down to $n_f-n_r$.

6. The new cell is formed in the lattice cell or migrates to a nearby lattice cell depending on the carrying capacity of the lattice cell.

7. If the nutrient reserve of an individual falls below 0, it dies.

8. If the individual organism can undergo apoptosis and its $f(t)$ is greater than $f_+(0)$, apoptotic machinery is activated and the cell is allowed to die.

9. Every time-step $n_0$ units of nutrients is replenished and is randomly allocated (using Random() function in Java) to different lattice cells (the effect of nutrients is further tested by varying the parameter value. See Section IV.III of this chapter). The probability of each lattice cell acquiring nutrients depends upon whether it has reached the maximum allowed value 2 (nutrient units) and the number of nutrient units already present.

10. If a lattice cell and neighbouring cells have reached the carrying capacity, the individual will not be able to reproduce.

This is repeated over $t$ time steps. A simplified illustration of the migration process is shown in Fig. 5.1.
IV.II Simulation and Discussion

The GT-based model was used to produce several simulations each lasting 6000 time-steps ($t$). In the simulation, each lattice cell can accommodate up to 2 individual organisms. The amount of nutrients supplied whilst initialising the simulation ($N_0$) was 1000 units. 10 individuals of each population ($P_0$) were randomly placed in the lattice grid. The value of $n_r$ was set to 1 unit and $n_f$ to 2 units. We have tested the model across a range of parameter values and found that this indeed lies within the appropriate range that makes it biologically plausible (see Section IV.III of this chapter).

Even though we have used a random function for this experiment, the error bar associated with the simulation data is so low compared to the number of individuals present (hence not shown).

The result of the simulation is shown in Fig. 5.2. It shows that the apoptotic population has a significant advantage over the non-apoptotic population. Up to time-step 500, the difference is hardly noticeable. After that the increase in the number of apoptotic cells is much more noticeable. The reason why the apoptotic cells are thriving is because the old cells are constantly being removed from the system. In the case of the non-apoptotic population the old organisms live a lot longer and they can hardly co-operate with other non-apoptotic cells as the old organisms are very week. The old non-apoptotic cells can
hardly reproduce since $f(t)$ takes a huge toll on the nutrient reserve. This provides an opportunity for the relatively young apoptotic population to thrive over non-apoptotic population. Towards the end of the simulation, one can see that the populations are reaching their respective saturation values. The final states of the populations are studied later by extending this model.

Figure 5.3: Change in the population over 6000 generations when an average amount of 5 nutrient units was added per time-step.

The simulation has used over 30,000 nutrient units over time. In order to check if the randomness of the nutrient allocation had played any role in governing the dynamics, we allocated 5 nutrient units per time-step (which is the average from the previous simulations) and the results are shown in Fig. 5.3. Although there are minor variations, overall the shape of the graph remains the same. This allows us to conclude that the randomness of the nutrient allocated does not play a huge role in governing the population dynamics. We kept the randomness only to emulate a biologically similar environment as explained in the previous section (see the section Modelling of this chapter).

The stability of the model was tested by varying the initial populations. As shown in Fig. 5.4 and Fig. 5.5 the initial population of the apoptotic population was kept constant and the population of non-apoptotic population was varied. In Fig. 5.4 the initial population
of the apoptotic and non-apoptotic populations was 20 and 1600 receptively. It was found that for these conditions, the apoptotic population is still the dominant one as it thrives over the non-apoptotic population at a faster rate. In Fig. 5.5 the stable states are different. Here the initial populations were set at 20 and 3800 for apoptotic and non-apoptotic populations respectively. It is clear from the graph that in this case the non-apoptotic population dominates over the apoptotic population. Logically, the only reason why this happens is because there are large numbers of non-apoptotic individuals and they can easily cooperate and kill the apoptotic population simply by using up all the nutrients. This inspires one to investigate the bistable dynamics of the system. This is done using the model developed using differential equations.

Figure 5.4: Change in the population over 6000 generations with initial population set to 20 and 1600 for apoptotic and non-apoptotic populations respectively.
For finding the value of $f(t)$ in (5.2), we put

- $\gamma$ is equal to 1;
- $\alpha$ is equal to -1; and
- $\beta$ is equal to -0.01.

Simplicity was the main guiding factor that led us to choose the above values since we will obtain a simple exponential curve to represent $f(t)$. We have also found that this relation could be varied, thereby allowing us to model the impact of the rate of growth of AFs on apoptosis (see Section IV.III of this chapter). Since the nutrients are expressed in terms of biomass and an individual dies when its ‘nutrient reserve’ falls below 0, it is reasonable to assume that the value of $f(t)$ starts with a low value and progresses according to the modified form of Gompertz-Makeham equation. This will give us:

$$f(t) = 1 - e^{-0.01t}$$  \hspace{1cm} (5.4)

The value of $f_+(0)$, at which the apoptotic machinery is activated, is set at 0.1. In the simulation we set the value of $c$ as 0.1 and $a$ as 0.05. Since the aim of the organism
is to acquire nutrients and during many competitions it may receive only nutrients less than 0.5 units (i.e. when there is only 1 unit of nutrient available and it has to compete with another individual), it is logical to assume that $c$ should be less than 0.5. In view of the fact that migration is not competitive, $a$ should be less than $c$. The value of $n_0$ is randomly selected based on the properties of individual lattice cells. Many of these simplifications are necessary and similar to the approaches adopted by Tomlinson (1997) and Tomlinson and Bodmer (1997).

From the simulation (see Fig. 5.2) it is found that individuals that can undergo apoptosis have got a significant advantage over non-apoptotic cells in a resource restricted environment. This may not be the case if the nutrient supply is unlimited. From Fig. 5.2 it is clear that during the first 700 time-steps, both apoptotic and non-apoptotic populations were growing at almost the same rate as each other (at 700, the ratio of the average number of younger individuals belonging to apoptotic population is 1.5 times the average number of younger individuals in non-apoptotic population). This is due to the fact that most of the individuals in the population were young and they had access to a sufficient amount of nutrients and space. However, when the shape of the graph changes, apoptotic populations continue to grow and the non-apoptotic population is almost in a stagnant state. Towards the end of the simulation, we see that the non-apoptotic population is decreasing in number. This is because many of the non-apoptotic individuals carry high amounts of AFs that depletes them of the nutrients required for reproduction and many of them die when the ‘nutrient reserve’ hits 0 or less. Even though there are two factors why organism would die (i.e.: running out of nutrients and apoptosis), the effect of ‘nutrient reserve’ hitting 0 or less is same for both populations. Accordingly, the change in the dynamics should be due to apoptosis. From the values obtained from the simulations we find that the number of individuals that died due to apoptosis was higher than the ones died by running out of nutrient reserves. This essentially helped the apoptotic population to clean up the old members of the population, and further gave the population an evolutionary advantage.
Biological validity of the parameters

We have used four parameters \((f(t), c, a \text{ and initial number of individuals and nutrients })\) in the model. Every single one of the parameters has been chosen to reflect the biological world. Even though the implementation of some of the parameters is complex, we show that the complexity is required in order to be faithful to the physical world. The actual values of the parameters themselves do not have any biological significance even though the way they are related to each other does matter and is highly relevant from a biological point of view.

i) \(AFs\) (and the associated \(f(t)\)): This parameter is the primary one that distinguishes an apoptotic individual from a non-apoptotic one. In many organisms (both uni-cellular and multicellular organisms) there are various AFs present that cause similar effects. Examples include free radical accumulation and Extrachromosomal rDNA Circles in yeast (Discussed in the previous chapter). The way this parameter increases in value in the model is also in accordance with what is observed in nature (Breitenbach et al., 2004). Though the actual values of this parameter are arbitrary provided, it is set to a comparable value for both populations. On the other hand, the rate of increase of the parameter does matter.

ii) \(c\) (cost of competing): It is well established in literature that cost of competition plays a key role whilst individual cells attempt to acquire nutrients. Menéndez et al. (2010) and Moreno (2008) have both reported that it is particularly important when the nutrient is a key factor (whilst modelling apoptotic and non-apoptotic cells). Our model is particularly interesting when dealing with a resource restricted environment.

iii) \(a\) (cost of migration): This is another important parameter when modelling competition. The one aspect one needs considering is that the value has to be lower than that of cost of competition since competing requires more energy. It should also be higher than that of the energy spent by an individual organism in hibernate state.

It is worth mentioning here that the way nutrients are added to the system is consistent with the way nutrients appear in biological world. There is a maximum limit on the
amount of nutrients that can appear in a particular area and the way they appear depends upon a range of environmental factors. In order to simulate this, we set a maximum carrying capacity for the nutrient and the way nutrient appears depends upon the existing number of nutrients, other individuals present (since a very high density of any population can hinder the growth of other populations) and a random factor (which will account for the environmental factors in our model). Similar to previous experiments, the probability was dependent upon a binomial distribution of the factors.

It is also interesting to note that in the physical world an organism does not die if it cannot secure nutrition for a small period of time. This is simulated in the model by allowing the individual cells to use a small amount of energy from the nutrient reserve every time they do not secure nutrient units. In the simulation, if the individual organism decides to hibernate 0.001, units are deducted from its nutrient reserve.

We have also simulated the model to see how the initial number of individuals affect the population dynamics. This important is some environments such as tumour populations, since the number of non-apoptotic cells are lower than that of the apoptotic cells. This further discussed in the next two chapters.

**Shape of the curve:** We can see from Fig. 5.2 that both populations reach almost a stable state (we verify the stability further when we extend the model). It is clear from the graph that population is likely to stabilise because towards the upper end (where the number of individuals produced is equal to number of individuals that died and the nutrient added at each time-step is used for this).

**Is it an ESS?:** If we had started with a single population and allowed some of the individuals to mutate into apoptotic populations, they would also have the same advantage exhibited by the apoptotic population in our experiment. As long as the nutrient access is restricted, the apoptotic population will grow at a much faster rate and outnumber the non-apoptotic population. From Fig.5.2, it is clear that the apoptotic population grows and reaches a stable steady state as the total population reaches the carrying capacity of the lattice. On the other hand, the non-apoptotic population starts to decline. However, it is not clear whether apoptosis is an ESS. This is investigated further after extending
the model in the next section (and in the next chapter).

IV.III Towards a More Realistic Model

Fig. 5.2 showed that apoptosis can provide an altruistic benefit in a resource restricted environment. This encouraged us to investigate the scenario more extensively. We made the following changes to the model to have a more realistic representation of the biological system.

1. Extended it to a 1000 × 1000 lattice space;

2. All the individuals participate during each time-step (generation);

3. In the algorithm a single time-step is implemented in two phases;
   - In the first phase the system will look at all the organisms and decide which are organisms that decide to migrate to a new lattice cell to enter into competition
   - In the second phase the competition itself will happen;

4. For simplicity, the carrying capacity of the lattice cell for organisms is restricted to 2 individuals per grid cell;

5. The maximum number of nutrient units in a single cell is set to 2;

6. If an organism decides not to compete and is not in a position to move, a small amount of energy ($h_0$) is deducted from its nutrient reserve; and

7. Since all the cells are participating in a single time-step, the hibernation mode is no longer required

The entire process is summarised in Algorithm 1.

We ran the simulation for 35 generations and we found that apoptotic organisms clearly have an evolutionary advantage over non-apoptotic cells as shown in Fig. 5.6.
Algorithm 1 To simulate the growth of apoptotic and non-apoptotic populations

1: SET initial values : $N_0$, $P_0$(apoptotic), $P_0$(non – apoptotic)
2: foreach nutrient unit
3:   distribute nutrient randomly
4: foreach organism
5:   distribute organism randomly
6: while $t <$ Experiment time
7:   foreach organism
8:     decide to compete or migrate
9:     pay-off based on matrix
10:    if current lattice or nearby by lattice cells can accommodate more organisms
11:       if nutrient reserve $> n_f$
12:          replicate
13:          mother cell is left with nutrient reserve of $n_f - n_r$
14:          daughter cell is with nutrient reserve of 1
15:     if number of organisms in the cell $> carrying capacity of cell$
16:        migrate to nearby cell
17:     if nutrient reserve $< 0$
18:       organism dies
19:     if $f(t) > f_+(0)$ AND organism is apoptotic
20:       organism dies
21:     if organism does not compete or migrate
22:       $h_0$ is deducted from the nutrient reserve
23: $n_0$ nutrients added randomly
Figure 5.6: Simulated a $1000 \times 1000$ lattice with a carrying capacity of 2 individuals per lattice cell. The figure shows the averaged result of 3 different simulations done using the same set of parameters as in the previous simulation.

**Shape of the curve:** We can see from Fig. 5.6 that the apoptotic population reaches a maximum value. This is because towards the upper end, the number of individuals present outnumbers the amount of nutrients available. Once this happens there is chance that some of the individuals will die of starvation and this further causes the quantity of nutrients to increase (according to the model) above the number of individuals present and in turn increases the population itself. This accounts for the wavy nature of the graph towards the end. The average number of nutrients added per cell towards the end is about 0.17 (i.e. about 17,000 units). Approximately, 15,000 individuals die and about the same number of new cells are also born. This means that the system has reached a steady state.

**Impact of competition**

We have seen in Section IV.I of this chapter that competition ($c$) is a key parameter used in the model. We studied the cost of competition by varying the value of $c$ from 0.01 to 0.3 and we find that in both cases apoptotic organisms are greater in number (as shown in Fig. 5.7). However, the effect is more prominent when the cost is low as shown in Fig. 5.8. It is also clear from this graph that, even though both populations thrive equally in the beginning the number of old cells in the non-apoptotic populations takes a huge toll
once the population reaches the peak value.

**Impact of migration cost**

We have also seen in Section IV.I of this chapter that migration cost \(a\) plays an equally important role in the model. By allowing the value of \(a\) to vary from 0.005 to 0.3, we find that a high value of migration is detrimental to both populations but it is worse in the case of the non-apoptotic population because of the large number of old individuals (see Fig. 5.9). This does not mean that if the cost of migration is very high, then apoptosis has no positive impact. It simply shows that the population cannot grow due to the high value of \(a\). However, once the population reaches its peak value, the old individuals in the non-apoptotic population cause the population to shrink to a very low value as shown in Fig. 5.9. The converse is shown in Fig. 5.10.

**Impact of Nutrient concentration**

In the model described previously, we have seen that nutrients play a huge role in governing the population dynamics. It is clear that once the nutrients become more restricted
Figure 5.8: The impact of the cost of competition when the value of $c$ is low (0.01).

Figure 5.9: The impact of the cost of migration when the value of $a$ is high (0.3)
Figure 5.10: The impact of the cost of migration when the value of $a$ is low (0.005)

(i.e.: when the population reaches the saturation level), the apoptotic cells tend to out-perform the non-apoptotic cells by a huge margin. This begs the question as to what happens when the nutrients are not restricted. In order to comprehend this scenario, we set the nutrient carrying capacity to the lattice to double the original number. The results are quite interesting.

From Fig. 5.11 it is clear that once the amount of nutrients has crossed a particular level, apoptosis hardly has a positive impact. Here the number of individuals in each population remain roughly the same. This is because all the individuals have enough nutrients available and even the old individuals can acquire nutrients without much competition. In this scenario, cooperation hardly plays any role as there are sufficient nutrients for everyone. It is clear that the amount of nutrients present in the environment is a key factor determining the impact of apoptosis on population dynamics. This discovery led us to investigate this factor further$^3$ (see Chapter 6).

$^3$In the case of tumour populations, they can facilitate angiogenesis and this can heavily impact the dynamics of the populations if non-tumorous population try to compete with tumour cells
Impact of AF

It is clear from the model that AF is the primary factor that differentially affects the two populations. In the case of an apoptotic population, the organism will die if the AF crosses a threshold level. It is interesting, therefore, to see what happens when AF \( (f(t)) \) is set to a significantly lower value than before. The results corresponding to this are shown in Fig. 5.12.

We find that the lower the value of AF, the lower the benefit of apoptosis. This is due to the fact that AF plays a significant role in the amount of nutrients obtained by the
organism. If the value of AF is set to increase at a very low rate ($\beta$ was set to 0.001), then it will hardly have an impact on the ability to procure nutrients. Therefore, a non-apoptotic population will not suffer much. This result is particularly significant in the case of organisms that have an efficient repair mechanism to ward off AFs as shown in Gómez (2010).

**Parameter testing**

The model has been tested extensively by varying the parameter values. Even though we have discussed the effects of the changes in the values, it is interesting to see how each parameter can affect the population dynamics. Table 5.1 shows the effects of each of the parameter used in the model.
Table 5.1: Relationship between parameters used in game theory-based model for simulating apoptotic and non-apoptotic populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Impact</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of migration (a)</td>
<td>0.01 - 0.3</td>
<td>High</td>
<td>Affects the number of individuals produced and the stable state</td>
</tr>
<tr>
<td>Cost of competition (c)</td>
<td>0.01 - 0.3</td>
<td>High</td>
<td>Affects the number of individuals produced and the stable state</td>
</tr>
<tr>
<td>Initial number of individuals</td>
<td>10-5000</td>
<td>High</td>
<td>Changes the stable state</td>
</tr>
<tr>
<td>Carrying capacity of lattice cells for individuals</td>
<td>2-5</td>
<td>Low</td>
<td>Affects the number of individuals</td>
</tr>
<tr>
<td>Initial amount of nutrients</td>
<td>10-5000</td>
<td>Medium</td>
<td>Affects the numbers individuals produced</td>
</tr>
<tr>
<td>Carrying capacity of lattice cells for nutrients</td>
<td>2-4</td>
<td>High</td>
<td>Affects the number of individuals produced and the stable state</td>
</tr>
<tr>
<td>$\beta$ for Ageing factors ($f(t)$)</td>
<td>0.0001-0.03</td>
<td>High</td>
<td>Affects the number of individuals produced and the stable state</td>
</tr>
</tbody>
</table>
IV.IV Equilibrium Analysis

We have shown that apoptosis is likely to be an ESS. Accordingly, over a significant period of time, we would expect to see only the apoptotic population in the environment. Even though the phenotype is the same for all the members of this population, they have two different strategies in the strategy set. It will be interesting to see how a population of similar individuals (in this case the apoptotic population) behaves when the phenotype employs a mixed strategy, provided there is an equilibrium exists. If \( p \) is the probability of an individual to migrate then using equation (5.3)

\[
P E(m, m) + (1 - p) E(m, n) = p E(n, m) + (1 - p) E(n, n)
\]

where \( E \) is the payoff function and \( n, m \) are the strategies (migrate, compete)

And if we write \(-f(t) - c =: \alpha\) and \(-a - f(t) =: \beta\)

\[
p(\beta) + (1 - p)(\alpha - a) = p(\frac{1}{2} + \alpha) + (1 - p)(1 + \alpha)
\]

\[
p = \frac{1 - \alpha + a}{\beta + \frac{1}{2} - \alpha + a}
\]

Substituting the values back, we get

\[
p = \frac{2(1 + a - f(t) - c)}{1 + 2c}
\]

For simplicity let us replace \( 1 + a - c \) with \( \gamma \) and \( \frac{1}{2} + c \) with \( \theta \)

We can have

\[
p = \frac{\gamma - f(t)}{\beta}
\]
\[ p = \frac{\gamma}{\beta} - \frac{1}{\beta} \times f(t) \]  
(5.10)

Using equation (5.10) we can solve \( p \) as

\[ p = \frac{2(1 + a - c)}{1 + 2c} \]  
(5.11)

where \( a \) and \( c \) should be much less 1 and greater than 0. This will provide the probability of migration amongst young individuals (since \( f(t) \) tends to zero) in a real-world biological set up.

\section*{V Conclusion}

We saw that by switching to apoptotic mode, the individual organism acquires an advantage over non-apoptotic cells in a resource-restricted environment. We have also seen that if the number of non-apoptotic cells are significantly higher, it will alter this stable state. In the biological world, this is unlikely since few individuals can mutate to become apoptotic, it is high unlikely that the non-apoptotic mother cells or their non-mutant daughter cells will quickly evolve to develop cooperation. As cooperation is absent, the apoptotic population can survive and thrive as the non-apoptotic ones cannot compete together against the apoptotic population. Therefore, the best strategy to adopt is to become an apoptotic population. This is likely be an ESS. This is not true in the case where sufficient nutrients are available to the individuals of the population as shown in Section IV.III of this chapter. Also shown in the same section are the major implications in the field of tumour research (we saw that the stable states changed when the amount of nutrients available to the individuals changed). Angiogenesis is a characteristic exhibited by the tumour cells (Hanahan and Weinberg, 2000) and this process does exactly the same. This ability gives tumour cells access to a sufficient amount of nutrients. This changes the dynamics. Using our model, we investigated the impact of angiogenesis on apoptosis. In that case, apoptosis is not the best strategy to adopt as shown in Chapter
7. Vaupel et al. (2004) have made a good case for negative senescence. Although their methodology was analytical, one could potentially investigate it using our model as well (see Chapter 8).

Our model can also be seen as an extension of the disposable soma theory proposed by Kirkwood et al. (1977). This is due to the fact that, according to our model, if the individual does not receive nutrients it will switch to hibernation, thereby increasing its chronological lifespan.
Modelling Apoptosis : Model 2

In this chapter we show that the interactions between apoptotic and non-apoptotic cell colonies can be modelled using differential equations. We also show that apoptotic organisms do possess an advantage over non-apoptotic ones in a resource restricted environment. There have been many attempts to model the dynamics of populations in population ecology. We follow the approach adopted by Scheuring and Yu (2012) to model the interactions between apoptotic and non-apoptotic populations. The equations have been modified to reflect the changes in the organisms involved and the interactions themselves. Simulations are used to imitate the biological interactions. The steady state and stability of the population was then studied theoretically. The results from the experiments were analysed and compared against observations from previous experiments.

I Introduction

Scheuring and Yu (2012) have shown that the behaviour of beneficial microbiomes within the environment can be modelled using the approach developed by Mao-Jones et al. (2010). This inspired us to investigate the possibility of studying the interactions between apoptotic and non-apoptotic cell colonies by employing a similar model. This is because the interactions between the organisms essentially describe the evolution of the populations’ dynamics. Many researchers including Chaston and Goodrich-Blair (2010)
have shown that the interactions in the microbiomes\footnote{Chaston and Goodrich-Blair (2010) have studied the associations between invertebrates and bacteria} can be modelled using game theory. This is also the case with apoptotic and non-apoptotic populations (Bellomo and Delitala, 2008). We have described our own approach using games in the previous chapter. In this chapter we show how the interactions can be described using differential equations.

Prosser et al. (2007) have shown that the biology of microbiomes is essentially a branch of community ecology. It is also worth mentioning here that for a microbiome the environment itself is a host that plays an active role. However, this doesn’t affect our modelling approach as we are only interested in interactions between apoptotic and non-apoptotic cells. In our model we have also introduced a spatial dimension by limiting the amount of nutrients available to the organisms.

It is also interesting to note that gradients in chemical concentrations may have a substantial effect on the qualitative dynamics of the microbial community (Frank, 1994). This has also been proved to a considerable extent by experiments (Chao and Levin, 1981). Our model also uses these principles. For example, when the nutrient gradient in a particular area increases it attracts more organisms towards it. Even though this is not used explicitly, the model implies this.

\section{Model}

The dynamics of substrate ($S$), beneficial microbes ($B$), antibiotics ($A$) and pathogenic microbes ($P$) populations are estimated using the following differential equations (Scheuring and Yu, 2012):

\begin{align*}
\frac{dS}{dt} &= I_s - \frac{r_P f(A)PS}{K+S} - \frac{r_B BS}{K+S} - \delta S \\
\frac{dB}{dt} &= I_B + (1 - \alpha) \frac{r_B BS}{K+S} - \delta B
\end{align*}

(6.1)

(6.2)
\[
\frac{dA}{dt} = \frac{\alpha r_B B S}{K + S} - \delta A 
\]
\[
\frac{dB}{dt} = I_P + \frac{r_P f(A) P S}{K + S} - \delta P 
\]  (6.3)

where \( I_S \) is the substrate production rate, \( I_P \) and \( I_B \) are the Pathogen and Beneficial immigration rates respectively; \( r_P \) and \( r_B \) are the growth rates of Pathogen and Beneficial populations respectively; \( \alpha \) is the uptake rate by the Beneficial population; \( f(A) \) is the effect of the antibiotics on the Pathogen’s net growth rate; \( K \) is the half-saturation constant for the Monod equation and \( \delta \) is the mortality rate.

In our model the interactions are quite different. We have two populations - non-apoptotic \((P_1)\) and apoptotic \((P_2)\). In order to accommodate the effects of ageing, we divide each of these populations into two sets - Adult \((A)\) and Senile \((S)\) such that the total number of organisms in each population will be the sum of these two types in the respective populations.

### II.I Assumptions

We make the following assumptions:

1. Total nutrients consumed are proportional to the population size;

2. The mortality rate of the senile members amongst apoptotic organisms are higher since the rate is affected by both environmental factors and apoptosis (Unlike in non-apoptotic populations where the only factor is environment);

3. The growth rate of senile members amongst apoptotic are higher than senile non-apoptotic organisms; and

4. The period between birth and maturity is defined as adulthood in our model. After this stage, the organism enters into a senile period.
In order to use equations from Scheuring and Yu (2012) we need to alter them to reflect our scenario.

The rate of change in the number of adult individuals \(A\) belonging to the same population is given by:

\[
\frac{dA_i(t)}{dt} = r_{A_i} A_i(t) \frac{N(t)}{(K + N(t))} - \delta_{A_i} A_i(t) + r_{S_i} S_i(t) \frac{N(t)}{(K + N(t))} - r_{C_i} A_i(t) \frac{N(t)}{(K + N(t))}
\]

(6.5)

where \(r_{A_i}\), \(r_{S_i}\)\(^2\) are the growth rates of adults and seniles respectively; \(\delta_{A_i}\) is the mortality rate of adults\(^3\); \(r_{C_i}\) is the rate of transformation from adult to senile; \(A_i(t)\) and \(S_i(t)\) are the number of Adults and Seniles at time \(t\) and \(N(t)\) is the nutrient concentration at time \(t\).

Similarly for the rate of change in the number of seniles:

\[
\frac{dS_i(t)}{dt} = r_{C_i} A_i(t) \frac{N(t)}{(K + N(t))} - \delta_{S_i} S_i(t)
\]

(6.6)

where \(\delta_{S_i}\) is the death rate of senile population.

For the total number of individuals in a population, we have:

\[
P_i(t) = A_i(t) + S_i(t)
\]

(6.7)

The flow of the nutrients is restricted (governed) by:

\[
\frac{dN(t)}{dt} = I_n - \frac{(r_{A_1} A_1(t) + r_{S_1} S_1(t)) N(t)}{(K + N(t))} - \frac{(r_{A_2} A_2(t) + r_{S_2} S_2(t)) N(t)}{(K + N(t))}
\]

(6.8)

This equation (6.8) is defined so that we can add a spatial dimension to the model, which might also help the system to attain a steady state.

\(^2\)Since senile organisms produce adults, this is added to \(A\)

\(^3\)The death could be caused due to environmental factors.
Equations (6.5), (6.6) and (6.8) can be simplified further by having

\[ \frac{N(t)}{K + N(t)} \approx \kappa N(t) \quad (6.9) \]

when \( N(t) \) is low and where \( \kappa \) is a constant. This approximation is valid since we are only interested in the final phase\(^4\).

Equations will change as

\[
\begin{align*}
\frac{dA_i(t)}{dt} & = r^*_a A_i(t) N(t) - \delta A_i A_i(t) + r^*_s S_i(t) N(t) - r^*_c A_i(t) N(t) \quad (6.10) \\
\frac{dS_i(t)}{dt} & = r^*_c A_i(t) N(t) - \delta S_i(t) \quad (6.11)
\end{align*}
\]

where * indicates the new value obtained by multiplying the original with \( \kappa \). As the amount of nutrients are controlled and are simply used to put a constrain on the maximum number of individuals supported by the system, we could also use a simpler version of the equation:

\[
\frac{dN(t)}{dt} = I_n - \alpha (P_1 + P_2) \quad (6.12)
\]

This model assumes that the whole amount of nutrients added to the system is consumed by the organisms present in the system. It also assumes that the rate of intake is same for all organisms present. This could be modified so as to let organisms take up nutrients at different rates. As this will increase the complexity of the situation and will make the analysis of the underlying process difficult, we have not incorporated this in the current model.

Another way to approach this type of synthetic environment, is to consider equation (6.12) as the actual nutrient intake and the nutrient added to the system will be added to the rest of the nutrient already present in the system. We tested for this scenario and the

\(^4\)Please see Appendix II for the other scenario.
results were found to be similar to the one obtained when the organisms are allowed to consume the whole amount of nutrients added (as the only change in this case is the net amount of nutrients available, which will be lower, to both the populations and the case is quite similar to the one where we reduce the nutrient added to the model described above).

II.II Relationship Between Parameters

In the equations (6.10), (6.11) and (6.12) we have used many parameters. Whilst accurate values of these parameters cannot be predicted for a hypothetical environment, we can estimate the range of values that we are interested in. We can say that:

1. $r_A^*$, $r_C^*$ and $r_S^*$ were assumed to be between 0 and 1 as they represent the percentage of organisms that undergo reproduction or transformation in our model;

2. $r_A^*$ should always be higher than $r_S^*$ as the reproductive capacity of adults will always be higher than that of seniles;

3. The sum of $r_A^*$ and $r_C^*$ should be less than or equal to 1 (as they represent percentage of organisms that undergo reproduction or transformation);

4. $\delta_A$ and $\delta_S$ should be less than or equal to 0.1 (lower values than $r_A^*$ - as they represent death rates) since these are mortality rates;

5. $\delta_A < \delta_S$ since the senile organisms die at a faster rate than adults;

6. $\delta_S$ for apoptotic organisms will be higher since they die due to both environmental causes and apoptosis; and

7. $r_S^*$ will be higher for apoptotic organisms since they tend to have, on average, lower ageing factors present (as the older organisms are allowed to die if they are apoptotic and older organisms tend to pass more AFs to younger organisms as observed in yeast populations).
Based on these we simulated the model for a range of parameter values as shown in Table 6.1.

III Simulation

We simulated the model using equations (6.10), (6.11) and (6.12). We started the simulation with 10 units of biomass (where 1 biomass is equivalent to an individual organism) of adult organisms and 1 unit of biomass of senile individual organism from each population and 50 units of nutrients. This number was later varied to ascertain whether the initial number of individuals or nutrient quantity has any impact on the population dynamics of the system. The parameters were selected as follows (These values were later changed to study the impact of each parameter on the system as shown in Table 6.1):

1. For non-apoptotic population ($P_1$):

\[
\frac{dA_1(t)}{dt} = 0.8A_1(t)N(t) - 0.01A_1(t) + 0.2S_1(t)N(t) - 0.4A_1(t)N(t)
\]  
(6.13)

\[
\frac{dS_1(t)}{dt} = 0.4A_1(t)N(t) - 0.05S_1(t)
\]  
(6.14)

2. For apoptotic population ($P_2$):

\[
\frac{dA_2(t)}{dt} = 0.8A_2(t)N(t) - 0.01A_2(t) + 0.6S_2(t)N(t) - 0.4A_2(t)N(t)
\]  
(6.15)

\[
\frac{dS_2(t)}{dt} = 0.4A_2(t)N(t) - 0.1S_2(t)
\]  
(6.16)
Table 6.1: Relationship between parameters used in differential equation-based model for simulating apoptotic and non-apoptotic populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Conditions</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{A_i}$</td>
<td>0.0-1.0</td>
<td>$r_{A_i} &gt; r_{S_i}$</td>
<td>The replicative rate of adults will be higher than that of seniles.</td>
</tr>
<tr>
<td>$r_{S_i}$</td>
<td>0.0-0.8</td>
<td>$r_{S_i} &lt; r_{A_i}$</td>
<td>The replicative rate of adults will be higher than that of seniles.</td>
</tr>
<tr>
<td>$\delta_{A_i}$</td>
<td>0.01-0.5</td>
<td>$\delta_{A_i} &lt; \delta_{S_i}$</td>
<td>The death rate is lower in the case of the adult population.</td>
</tr>
<tr>
<td>$\delta_{S_i}$</td>
<td>0.01-0.5</td>
<td>$\delta_{S_i} &gt; \delta_{A_i}$</td>
<td>The death rate is lower in the case of the adult population.</td>
</tr>
<tr>
<td>$r_{A1}, r_{A2}$</td>
<td>0.0-1.0</td>
<td>$r_{A1} = r_{A2}$</td>
<td>The replicative rate of both adult populations in the case of non-apoptotic and apoptotic populations are the same.</td>
</tr>
<tr>
<td>$r_{S1}, r_{S2}$</td>
<td>0.0-0.8</td>
<td>$r_{S1} &lt; r_{S2}$</td>
<td>The rate of replication of senile cells is higher in the case of the apoptotic population.</td>
</tr>
<tr>
<td>$\delta_{A1}, \delta_{A2}$</td>
<td>0.01-0.05</td>
<td>$\delta_{A1} = \delta_{A2}$</td>
<td>The death rate amongst adults is the same for both non-apoptotic and apoptotic populations.</td>
</tr>
<tr>
<td>$\delta_{S1}, \delta_{S2}$</td>
<td>0.01-0.05</td>
<td>$\delta_{S1} &lt; \delta_{S2}$</td>
<td>The death rate amongst senile cells is higher in the case of the apoptotic population.</td>
</tr>
<tr>
<td>$r_{C_i}$</td>
<td>0.0-0.5</td>
<td>$r_{C1} = r_{C2}$</td>
<td>The number of adults becoming senile is a fraction of the adult population and the value is same for both apoptotic and non-apoptotic populations.</td>
</tr>
</tbody>
</table>
3. And for nutrient \((N)\):

\[
\frac{dN(t)}{dt} = 1 - 0.001(P_1(t) + P_2(t))
\]

(6.17)

However, \(\frac{dN}{dt}\) should always be greater than or equal to 0.

(a) Simulation of the system for up to 1000 generations (impact of nutrients available are reported separately in this chapter).

(b) Simulation of the system for up to 20000 generations.

Figure 6.1: Simulations of the system (using numerical evaluation) for different periods of time.

As discussed in the previous section, the replication rate for the senile adults belong
to the apoptotic population is set to be higher than that of those in the non-apoptotic population (which may have a positive impact on the population growth of the apoptotic population). The effects of this parameter are also studied by altering its value and are reported in this chapter. Since apoptotic senile populations do undergo programmed death, the death rate of the senile apoptotic population is also higher than that of the senile non-apoptotic population (which may have a negative effect on the population growth of the non-apoptotic population). Apart from these two parameters, all other parameter values were kept the same for both sets of populations.

We have simulated the synthetic environment using numeral solutions to the ODEs. From Fig. 6.1(a) it is clear that both of the populations have almost reached a steady state. But it also shows that the apoptotic population is still continuing to go up in number slowly while the number of non-apoptotic ones are going down at very slow pace.

In order to study the steady state of the populations we ran simulations for a considerably longer period of time than in the previous case. When $t = 20000$, it is clear that the populations do reach a near steady state with the apoptotic population at 9779 (up from 11 as we saw in the previous experiment) and the non-apoptotic population at 2 (down from 11) (see Fig. 6.1(b)). Since the growth rate now has become negligible, running simulations for more time is not an efficient way to find the exact steady state. Accordingly, the steady state analysis and stability analysis are carried out theoretically (see the section ‘Analysis’ of this chapter).

It is interesting to see how the sub-populations (Adult and Senile) behave in these conditions. These are shown in Fig. 6.2(a) and Fig. 6.2(b). It is clear that the quantity of adult population changes dramatically in both cases and plays a significant role in determining the shape of the final graph (Fig. 6.1(a)).

Although the value of the parameter that is associated with the replication of adult cells was the same for both populations, the adult cells still play a huge role in governing the dynamics. This is due to the fact that the senile apoptotic cells tend to produce healthier (with low values for AFs) adults than the non-apoptotic ones and this, in turn, is responsible for the higher replication rate amongst them).
(a) Growth of Adult and Senile individuals belonging to non-apoptotic population.

(b) Growth of Adult and Senile individuals belonging to apoptotic population.

Figure 6.2: Growth of Adult and Senile individuals belonging to each population (For the actual values see the section ‘Analysis’).

Since the quantity of the nutrients added to the system is governed by the number of individuals present, the dynamics of the nutrient levels are also of interest to us. This is shown in Fig. 6.3(a). The initial quantity (which is an arbitrary value - in this case it is 50) of nutrient added to the system is not shown. The remaining amount of nutrients available for the individuals from generation 1 onwards is shown in the figure. It is clear from the figure that the nutrient also reaches a steady state. This is investigated further
in the next section (see ‘Analysis’ section of this chapter).

(a) Variation in the quantity of nutrient supplied to the system (as a function of the total population): Data up to 1000 time-steps.

(b) Magnified version of figure (a) up to the 60th time-step.

Figure 6.3: Variation in the quantity of nutrient supplied to the system (as a function of the total population).

However these results beg the question: How vital is the parameter that causes the replication of senile cells \( r_S^* \) when it comes to determining the dynamics of the system? This is done by varying the value of \( r_S^* \) for the non-apoptotic population to 0.4 (instead of 0.2) and keeping all other parameters the same. The result is shown in Fig. 6.4. In this case although the apoptotic cells initially multiply at a faster rate than non-apoptotic
cells, this was later pushed down by the non-apoptotic population. Therefore, this factor is vital when it comes determining the dynamics of the system. This implies that if the apoptotic process does not provide the population with an opportunity to help the older population to replicate at a faster rate, it will severely impact upon the growth of the population.

A similar problem may arise if the apoptotic activity is very high. This can be studied by varying the parameter $\delta_s$ for the apoptotic population. In this experiment we set the value of the parameter to 0.5 (instead of 0.1). The results are quite interesting. Fig. 6.5(a) shows that when we increased this parameter value by a factor of 5, the dynamics of the system also changed. This result implies that if the level of apoptosis is high, it will only hinder the growth of the population and it will not have an altruistic effect. We studied this further by extending our simulation to 20,000 generations (see Fig. 6.6). It clearly shows that in this case the apoptotic population was almost wiped out.

In the previous model (see Chapter 5), we have seen that the amount of nutrients available has an impact on the population dynamics. This idea can be tested using the new model as well. However, unlike in the previous model, there is no bias here (In the previous model, the nutrient concentration was locally increased when the population in the area was optimal). This is done by changing the value of the parameter $I_n$ to 100. From
(a) Studying the effect of $\delta_s$ by setting its value for the apoptotic population to 0.5.

(b) Magnified version of figure (a) up to the 35th time-step.

Figure 6.5: Studying the effect of $\delta_s$ by setting its value for the apoptotic population to 0.5.

Fig. 6.7 it is clear that this has an impact on both populations as shown in the figure (since parts of the figure are not legible, the data from the 500th generation is shown in Fig. 6.8). Here the apoptotic cells do not have any significant advantage over the non-apoptotic population during the early part of the simulation. However, after a while (see Fig. 6.8) the apoptotic population acquires some advantage over the non-apoptotic population.
Figure 6.6: Studying the effect of $\delta_s$ by setting its value for the apoptotic population to 0.5 for up to 20000 generations.

Figure 6.7: The impact of nutrients present.
Figure 6.8: The impact of nutrients present: Data from the 500th generation is shown here. The reasons for the fluctuations in the graph have little to do with the actual dynamics of the system. They are in fact caused by the way we have implemented the system. The equation that governs the allocation of nutrients is set in such a way so that once the value of the population crosses a particular threshold, the nutrients are completely shut off. This affects the population growth and the net population decreases. Since the maximum amount of nutrients supplied to the system is 100 times higher than that of the original value (which was 1), every time a high level of nutrient quantity is added it causes huge fluctuation in the graph.

We repeated the simulation (numerical evaluation) by running it for 20,000 generations to verify that this was in fact what was happening (see Fig. 6.9). It is also interesting to see that this in agreement with results from our previous model (apoptotic populations have an advantage over the non-apoptotic populations). However, the previous model was restricted to a much smaller number of generations due to computational difficulties. Now we can see what happens in the long run. From Fig. 6.9 it is clear that higher nutrient quantity does not offer a good environment for the apoptotic population to thrive over the non-apoptotic population when the populations are small in number (But it should be noted that we have approximated the equations assuming that the amount of nutrients added is relatively low).
Another aspect that needs to be addressed here is the initial number of individuals present. In the previous chapter, we saw that a greater number of non-apoptotic cells than apoptotic ones in the initial stages of the simulation can change the dynamics of the system. In order to see how the new model behaves we set the initial number of adult individuals belonging to non-apoptotic population to 1000 and decreased the number of adult individuals belonging to the apoptotic population to just 1. The number of senile individuals was kept the same (1 each).

From Fig. 6.10 it is clear that the behaviour is quite similar to the behaviour observed in the previous model. However, in the previous model (game theory-based), the features of the model interfered with the dynamics of the system. In that case, since the nutrients were randomly distributed and the number of individuals belonging to the non-apoptotic population was significantly higher, the members of apoptotic population essentially died out. In the new model, this is not the case. In view of the fact that we are using differential equations (as opposed to agent based modelling), there is no such random element.
Figure 6.10: The effect of initial number of individuals present. The initial number of adult individuals belonging to non-apoptotic population is set to 1000 and the number of adult individuals belonging to the apoptotic population was decreased to 1.

Figure 6.11: The effect of the initial number of individuals present. The initial number of adult individuals belonging to non-apoptotic population was set to 1000 and the number of adult individuals belonging to the apoptotic population was decreased to 1. The system was simulated up to 40,000 generations.

Even after 1000 generations we found that the apoptotic population had not died out; but they were still low in number (which is similar to the results we obtained in the previous model). In order to understand the long term dynamics, we simulated the model for 40,000 generations. The results were quite striking (see Fig. 6.11). After about 7000 generations, we found that the number of apoptotic individuals increased considerably. After 15,000 generations their numbers was higher than the number of non-apoptotic
individuals present. Towards the end of the simulation we found that the apoptotic ones reached a saturation level and the non-apoptotic ones died off. This behaviour is remarkable. It shows that the apoptotic organisms can invade a population of non-apoptotic cells and this could imply that apoptosis is an Evolutionary Stable Strategy (ESS).

![Graph](image1)

(a) The change in nutrients when the initial quantity was set to 100.

![Graph](image2)

(b) Magnified version of figure (a) from the 1st time-step onwards.

Figure 6.12: The change in nutrients when the initial quantity was set to 100.

One other parameter that we studied is the initial amount of nutrients added to the system. For this, we change the value of the initial amount of nutrient supplied to 100. The results can be seen in Fig. 6.12(a). This is quite similar to the behaviour we have
seen in the original unmodified model.

IV Analysis

IV.I Steady State Analysis

In the simulation we have seen that the populations were about to reach a stable state. In order to find the actual values, we use the original equations. For steady state we should have

i) For non-apoptotic population \((P_1)\):

\[
\frac{dA_1(t)}{dt} = 0.8A_1(t)N(t) - 0.01A_1(t) + 0.2S_1(t)N(t) - 0.4A_1(t)N(t) = 0 \quad (6.18)
\]

\[
\frac{dS_1(t)}{dt} = 0.4A_1(t)N(t) - 0.05S_1(t) = 0 \quad (6.19)
\]

ii) For apoptotic population \((P_2)\):

\[
\frac{dA_2(t)}{dt} = 0.8A_2(t)N(t) - 0.01A_2(t) + 0.6S_2(t)N(t) - 0.4A_2(t)N(t) = 0 \quad (6.20)
\]

\[
\frac{dS_2(t)}{dt} = 0.4A_2(t)N(t) - 0.1S_2(t) = 0 \quad (6.21)
\]

iii) And for nutrient:

\[
\frac{dN(t)}{dt} = 1 - 0.001(P_1 + P_2) = 0 \quad (6.22)
\]

Solving the above equations will give us the following sets values for \(A_1, S_1, A_2, S_2\) and \(N\) (as we can express one of the parameters as a function of all the other four parameters, we will only have 4 sets of values):
where the values in each row corresponds to the values of $A_1$, $S_1$, $A_2$, $S_2$ and $N$ in each of the four cases.

Out of these four sets of values, we can only take 2 sets because the other sets provide values for some parameters that are negative and none of our parameters can be negative. The two sets that agree with this condition are:

\[
A_1 = 8451.54255 \\
S_1 = 1548.45745 \\
A_2 = 0 \\
S_2 = 0 \\
N = 0.0229019946
\]

and

\[
A_1 = 0 \\
S_1 = 0 \\
A_2 = 9188.6117 \\
S_2 = 811.388301 \\
N = 0.022075922
\]

The first set of values indicate that in that steady state (as we put the rates of change of $A_1$, $S_1$, $A_2$, $S_2$ and $N$ to zero to obtain the above result) we will only have non-apoptotic cells (no matter what the initial number of populations are) and at steady state we will only have around 10,000 organisms. We tested this and the result (see Fig. 6.13) is found to be in agreement as the numerical evaluation gave the same result.
Figure 6.13: Steady state of non-apoptotic population where final numbers were found to be in agreement with what we have obtained from the analysis.

The second set of values is of more importance to us. Here we will only have an apoptotic population (approximately 10,000 cells). We have already tested this in our model and found that an apoptotic population is the one that remains at steady state (the number of non-apoptotic individuals were negligible). It is interesting to see that even in our model at an almost steady state the numbers were in agreement with what has been predicted here.

### IV.II Stability Analysis

Using the values we obtained for the steady state we can now check if the steady state is stable or not. In order to investigate this, we find the Jacobian of the original set of equations used. We get the Jacobian matrix as (using Matlab):
Now we can plug in the values we obtained for the steady state into the above matrix and we will then get two matrices corresponding with each set of values:

i) For the case where there is no apoptotic population (Please note the result provided below are from Matlab and the values are approximated to 0):

\[
1.0e+04 \times \begin{pmatrix} 0 & 0 & 0 & 0 & 1.1690 \\ 0 & 0 & 0 & 0 & 0.3381 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}
\]

ii) For the case where there is no non-apoptotic population:

\[
1.0e+03 \times \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 4.1623 \\ 0 & 0 & 0 & 0 & 3.6754 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}
\]

In order to find the steady state we need to find the modulus of the eigen values of the above matrices. For the first matrix we get

-0.0019 + 1.2276i
-0.0019 - 1.2276i
-0.0471 + 0.0000i
-0.1013 + 0.0000i
0.0004 + 0.0000i

The modulus of these values would give us:

1.2276
1.2276
0.0471
0.1013
0.0004

And for the second one we can have

-0.0182 + 0.8838i
-0.0182 - 0.8838i
-0.0004 + 0.0000i
-0.0508 + 0.0000i
-0.0647 + 0.0000i

The modulus of these values would give us:

0.8840
0.8840
0.0004
0.0508
0.0647

Since the modulus of the *eigen values* is less than 1 in this case, we can say that it is a stable equilibrium.
V Discussion and Conclusion

We have seen in the previous chapter that the apoptotic population dominates over the non-apoptotic population in a resource-restricted environment. Using the model discussed in this chapter, we get similar results to the ones obtained in the previous chapters. The new model also allows us to study the dynamics of the system analytically. The most striking observation we made is that given the right conditions, a single apoptotic organism can invade a colony of non-apoptotic organisms. However it should be emphasised that the following conditions needs to be met:

1. The nutrient quantity should be restricted (i.e.: the organisms should not be in a resource-rich environment) as we found that introducing too much nutrients into the system changes the dynamics;

2. The level of apoptosis should not be high (i.e.: the point at which apoptosis is triggered should be relatively high) as higher levels of apoptosis can result in killing of a large amount of population; and

3. The effect of ageing should be significant (i.e.: older cells affect the fitness of the younger generation and this in turn affects the average fitness of the group) as AFs impacts on efficiency of an organism to acquire nutrients.

We know that competing for food is a very common behaviour in the biological world. Accordingly, most organisms live in a resource-restricted environment. It is also documented that the apoptotic machinery is tightly controlled and is allowed to operate only when multiple conditions are met (see Chapter 2). This essentially makes it difficult for a process like apoptosis to kick-in easily. We have already seen in Chapter 4 that it is optimal for an organism to undergo ageing and that there is a trade-off between maintenance and reproduction. The conditions mentioned before are met in most biological scenarios.

From the analysis (see ‘Analysis’ section of this chapter), it is clear that the steady state achieved (with only apoptotic individuals present) is a stable strategy. This indi-
cates that the process of apoptosis itself is likely to be evolutionarily stable provided the non-apoptotic cells’ behaviour remains unchanged (i.e.: by not becoming a tumour population). From the simulations we can say that apoptosis does serve an altruistic purpose. By reducing the number of very old cells, the fitness of the group is maintained above a minimum value. This allows the group to compete against non-apoptotic populations and thrive.

It is necessary to emphasise that, although the effects of some parameters such as \( r^*_A \) - which has a positive impact on the growth of the population and \( \delta_A \) - which has a negative impact on the growth of the population, it was necessary to simulate the effects of the parameters especially when we wanted to study the effect of more than one parameter simultaneously. It should also be noted that we have selected the values for the parameters for which we have seen interesting behaviours. Though the effects of some of the parameters seem similar we decided to list at least an ideal case for each scenario so that the reader can get a better understanding of the effects produced. We have used the same reasoning in the next chapter (Chapter 7) as well.

Using the two models we have developed, we can now model the behaviour of tumour cells. Tumour cells are essentially non-apoptotic cells with special characteristics. Since the behaviours of tumour cells are distinct from that of the non-apoptotic cells that we have discussed so far, we expect the population dynamics to change as well. Both of the models (game theory-based and differential equation-based) allow us to tweak parameters accordingly so as to account for the changes in behaviour. This idea is investigated in the next chapter.
Modelling of Tumour Populations

Neoplasms (tumour growth) are widely affected by the effects of apoptosis and angiogenesis (Evan and Vousden, 2001; Carmeliet and Jain, 2000). In this chapter these two factors, apoptosis and angiogenesis, along with other parameters like nutrient availability, are considered. We studied the effect played by these two factors in the dynamics of the tumour population. We have found that apoptosis and angiogenesis give neoplasms a significant advantage over normal cells; hence the tumour cells can easily invade a normal tissue. We also show that by changing the effects of these two, one can also change the dynamics. It was found that nutrient quantity plays a key role in the population dynamics. We believe that our model would be of interest in the field of oncology and may aid in devising strategies to suppress the growth of tumour populations by manipulating the impact of apoptosis, angiogenesis and nutrient availability; and help in producing new procedures that are less toxic than the current ones (for example, by limiting the amount of nutrients available to the cells).

I Introduction

In this chapter two factors, apoptosis and angiogenesis along with parameters like nutrient quantity are considered to study the growth of tumour populations and apoptotic populations.
I.I Hypothesis

In the previous chapter we have seen that apoptotic organisms have an advantage over non-apoptotic organisms in a resource-restricted environment. Due to the fact that tumour cells will have access to a greater number of nutrients because of angiogenesis, we hypothesise the following:

_Tumour cells will have a higher growth rate due to the fact that they have better access to nutrients. As a result, the tumour cells may outgrow apoptotic cells, and will have an advantage over apoptotic cells in certain situations._

II Model

Gradients in chemical concentrations may have a substantial effect on the qualitative dynamics of a community of populations. This concept has also been proved to a considerable extent by experiments as well. In order to study the effect of apoptosis and angiogenesis, we consider two populations - one is non-apoptotic and can promote angiogenesis and the second one is a normal population. (apoptotic somatic population)

Using this model we can simulate the effect of these two factors by varying the associated parameters and studying the change in dynamics.

The model makes the following assumptions:

1. There are two populations - neoplasms \((c)\) and apoptotic \((i)\);

2. We start with a fixed number of \(c\) and \(i\) (this is later changed to study the effect of initial numbers);

3. The changes in the number of individuals belonging to tumour populations are governed by equations (7.1) and (7.2) given in the next section;

4. The effect of angiogenesis is modelled by adding the parameters \(\alpha_c\) and \(\alpha_i\). The net nutrients received by the neoplasm population increase since they can promote angiogenesis. In the case of population A, the net result will be negative.
5. Nutrients are constantly added to the system and the rate of addition of nutrients is governed by the number of individuals present at time $t$ (according to equation (7.5));

6. Nutrients are expressed in terms of biomass (where 1 unit of biomass is equivalent to 1 organism);

7. The rate of consumption of nutrients is higher than the rate of replication, as a part of the energy obtained is spent on maintenance;

8. All the nutrients added to the system are consumed by the organisms present (similar to the model in Chapter 6);

9. The death rate is higher for the apoptotic population as the neoplasms do not undergo apoptosis;

10. It is assumed that the accumulation of DNA errors will have an impact on the reproduction of the cells. This effect will be severe in the case of tumour populations as they tend to have more DNA errors accumulated over time; and

11. An increase in the number of cells present will have a negative impact on the number of new cells produced.

Using these we can modify the model we developed in the previous chapter:

1) For tumour (cancer) cells:

$$
\frac{dA_c(t)}{dt} = (r_{A_c} + \alpha_c)A_c(t)N(t) - \delta_{A_c}A_c(t) + r_{S_c}^*S_c(t)N(t) - r_{C_c}^*A_c(t)N(t) \quad (7.1)
$$

Since tumour cells can undergo angiogenesis, $\alpha_c$ is added to the rate as the net nutrients available to tumour cells will be much higher than apoptotic cells.

$$
\frac{dS_c(t)}{dt} = r_{C_c}^*A_c(t)N(t) - \delta_{S_c}S_c(t) \quad (7.2)
$$
ii) For apoptotic cells:

\[
\frac{dA_i(t)}{dt} = (r_{A_i} - \alpha_i)A_i(t)N(t) - \delta_i A_i(t) + r_{S_i}^* S_i(t)N(t) - r_{C_i}^* A_i(t)N(t)
\]  

(7.3)

As apoptotic cells will have access to fewer nutrients because of angiogenesis amongst tumour cells, we have deducted \(\alpha_i\) from the rate of replication.

\[
\frac{dS_i(t)}{dt} = r_{C_i}^* A_i(t)N(t) - \delta_i S_i(t)
\]  

(7.4)

iii) For nutrient quantity:

\[
\frac{dN(t)}{dt} = I_n - \alpha(P_c + P_i)
\]  

(7.5)

where \(\alpha_C\) is the effect due to angiogenesis in tumour populations, \(P_c\) and \(P_i\) are the tumour and apoptotic populations respectively. Every other parameter used here has the same meaning as used in the previous chapter. Some of the other parameters can also be changed in order to account for specific characteristics exhibited by tumour populations. This is done whilst simulating the model (see Section III of this chapter).

## III Simulations

We simulated the model using equations (7.1), (7.2), (7.3), (7.4) and (7.5). We started the simulation with 10 adult individuals and 1 senile individual from each population together with 50 units of nutrients. This sample size was later varied to determine whether the initial number of individuals or nutrient quantity had any impact on the population dynamics of the system.

The equations were altered to reflect the changes in the way that the tumour cells behave. Since one of the characteristics of tumour cells is that they replicate at a much faster rate, the replicative rate of the adult cells will be higher than that of the apoptotic cells. It
should be emphasised that the tumour cells also have access to more nutrients since they can trigger angiogenesis in tissue\textsuperscript{1}. Accordingly, the higher rate of growth amongst adult tumour cells can be maintained. However, one of the problems with tumour cells is that since their main focus is on replication, they hardly spend any energy on maintaining themselves. As a result, the replicative rate of the senile cells will be lower than that of apoptotic cells. Based on these deductions we modify the original set of equations as follows (the values of the parameters were later altered to study the impact of each parameter on the system):

i) For tumour population ($P_1$):

$$\frac{dA_1}{dt} = (0.7 + 0.2)A_1(t)N(t) - 0.01A_1(t) + 0.1S_1(t)N(t) - 0.4A_1(t)N(t) \quad (7.6)$$

$$\frac{dS_1}{dt} = 0.4A_1(t)N(t) - 0.05S_1(t) \quad (7.7)$$

ii) For apoptotic population ($P_2$)

$$\frac{dA_2}{dt} = 0.7A_2(t)N(t) - 0.01A_2(t) + 0.6S_2(t)N(t) - 0.6A_2(t)N(t) \quad (7.8)$$

$$\frac{dS_2}{dt} = 0.4A_2(t)N(t) - 0.1S_2(t) \quad (7.9)$$

iii) And for nutrient quantity

$$\frac{dN}{dt} = 1 - 0.001(P_1 + P_2) \quad (7.10)$$

One could also argue that, due to the fact that tumour cells are primarily interested in replication, the number of cells becoming senile might be higher in this population. Using the same logic one could also argue that when the death rate of senile individuals

\textsuperscript{1}This is represented in the model by having a higher rate of replication amongst adult tumour cells.
amongst tumour population is higher, the dynamics might be different as well. We have tested for all these cases in this chapter.

(a) Growth of tumour and apoptotic populations simulated over 10000 generations. Towards the end of the simulation, it was the tumour population that mainly survived rather than the apoptotic population.

(b) Magnified version of figure (a) up to the 450th time-step

Figure 7.1: Growth of tumour and apoptotic populations simulated over 10000 generations. Towards the end of the simulation, it was the tumour population that mainly survived rather than the apoptotic population.

The results of the simulation are shown in Fig. 7.1(a). From this figure it is clear that tumour cells have a clear advantage over apoptotic cells. This does not mean that the strategy adopted is evolutionarily stable. In order to confirm whether the strategy is stable or not we have to investigate what happens when the other population (apoptotic)
changes its strategies as a response to the behaviour of the tumour cells. This is done later in this chapter.

In the simulation we have kept the death rate of adult individuals ($\delta_A$) the same for both populations. This may not be the case in the biological world. Although the actual death rate depends on the behaviour of individual tumour cells, it is possible that the death rate of the adult cells is slightly higher than that of apoptotic adult cells.

![Simulation graph](image-url)

(a) Simulation with the value of the death rate of the adult cells ($\delta_{A_c}$) among tumour cells set to 0.013.

![Magnified graph](image-url)

(b) Magnified version of figure (a) up to the 600th time-step

Figure 7.2: Simulation with the value of the death rate of the adult cells ($\delta_{A_c}$) among tumour cells set to 0.013.
In order to test the sensitivity of this parameter ($\delta_{Ac}$), we have changed value to 0.013 (from $\delta_{Ac} = 0.01$) for the tumour population. The results are shown in Fig. 7.2(a). The results show that the tumour cells still have a clear advantage over apoptotic cells.

(a) Simulation where the value of death rate of the adult cells ($\delta_{Ac}$) among tumour cells set to 0.015.

(b) Magnified version of figure (a) up to the 600th time-step.

Figure 7.3: Simulation where the value of death rate of the adult cells ($\delta_{Ac}$) among tumour cells set to 0.015.

Now we vary the value of the parameter ($\delta_{Ac}$) to 0.0015 (from the original 0.01). Fig. 7.3(a) shows the result of this change. This is an interesting result. It shows that the model is very sensitive to relatively smaller changes to this parameter ($\delta_{Ac}$). It also
indicates that the tumour population will suffer when the young tumour cells are not maintained to a fitness level which is quite similar to that of the young apoptotic cells.

![Graph](image1)

(a) Case where the rate of replication of senile cells \( (r_{C_c}) \) amongst the tumour population set to 0.01.

![Graph](image2)

(b) Magnified version of figure (a) up to the 600th time-step.

Figure 7.4: Case where the rate of replication of senile cells \( (r_{C_c}) \) amongst the tumour population set to 0.01.

The replication of senile cells (in order to produce adult cells) is also equally important. We need to see how the dynamics change when this parameter \( (r_{C_c}) \) is altered. In order to understand this, we changed the value for the tumour cells from 0.1 to 0.01. The results are shown in Fig. 7.4(a). It is surprising to find that even when the ability to replicate
for senile cells is affected, it has hardly any impact on the dynamics of the population. The tumour population is still the dominant strain of the two.

(a) Case where the rate of conversion of the adult cells to senile cells ($r_{C_c}^*$) amongst tumour population was set to 0.6.

(b) Magnified version of figure (a) up to the 1400th time-step.

Figure 7.5: Case where the rate of conversion of the adult cells to senile cells ($r_{C_c}^*$) amongst tumour population was set to 0.6.

It is also vital to see how fast the adult cells are being converted into senile cells. We have seen that it is necessary for the tumour cells to maintain their young cells, but they cannot continue to do for a long period of time due to the inherent nature (to replicate faster) of the cells. To study the impact due to this, we change the rate of conversion of
adult cells to senile cells ($r_{cc}^*$) amongst the tumour population to 0.6 (from $r_{cc}^* = 0.4$). The results are shown in Fig. 7.5(a).

It is quite clear from the figure (Fig. 7.5(a)) that this change affects the dynamics of the populations. However, in this particular case the apoptotic population only has an advantage in the first few generations. By the 1000th generation the dominance has been reversed and this continues further (i.e.: the tumour cells acquire advantage over the
apoptotic population). This begs the question - what happens when this effect (due to higher rate of conversion) is severe? For this we set the conversion rate from the adult to senile population ($r^C_{C_s}$) amongst tumour cells to 0.8 (which is in fact a very high rate). Although it is a biologically extreme case, it is clear from Fig. 7.6(a) that the tumour population perishes at this rate.

(a) Here both the rate of conversion of the adult cells to senile cells ($r^C_{C_s}$) and the mortality rate of senile cells ($\delta_{s_e}$) in the tumour population are altered. They are set to 0.6 and 0.5 respectively.

(b) Magnified version of figure (a) up to the 800th time-step.

Figure 7.7: Here both the rate of conversion of the adult cells to senile cells ($r^C_{C_s}$) and the mortality rate of senile cells ($\delta_{s_e}$) in the tumour population are altered. They are set to 0.6 and 0.5 respectively.

For the purposes of simulating all biologically significant scenarios, we also decided to
test the effect of the above-mentioned parameter with the death rate of senile individuals. This is biologically more plausible as the senile tumour cells are likely to die faster due to the higher rate of replication amongst tumour cells (which causes more DNA errors). We keep the rate of conversion of adult cells to senile cells ($r^*_c$) amongst the tumour population at 0.6 (from $r^*_c = 0.4$) and set the death rate ($\delta_s$) of those senile cells to 0.5. The effect can be seen in Fig. 7.7(a).

We can see that in this case the surviving phenotype is the apoptotic one. The tumour population has almost died out towards the end of the simulation. This shows that the parameter (death rate of senile cells - $\delta_s$) is a key factor in determining the dynamics of the population. It is interesting to note that since the tumour population engages in aggressive replication, it can potentially adversely impact upon the survival rate of the older population. This is rate of death is too high for the older population; the high death rate combined with the effect of a high rate of conversion from adult to senile population has negatively affected the population growth of the tumour cells as a whole.

![Figure 7.8: Result of the simulation where the rate of conversion of the adult cells to senile cells ($r^*_c$) and the mortality rate of senile cells ($\delta_s$) in the tumour population were set to 0.6 and 0.01 respectively.](image)

We can also look at the effectiveness of the rate of conversion alone. For this, we set the mortality rate amongst tumour cells ($\delta_s$) to a very low 0.01 and keep the rate of conversion of adult to senile cells ($r^*_c$) amongst tumour population to 0.6. The net result
can been observed in Fig. 7.8. Comparing this to Fig. 7.5(a) we can see that the impact is similar. This shows that the effect of the rate of conversion is in fact quite significant.

![Graph showing population over time with different markers for tumour and apoptotic populations.]

(a) Case where the mortality rate of senile ($\delta_{sc}$) tumour cells was set to 0.1.

![Magnified version of figure (a) up to the 800th time-step.]

(b) Magnified version of figure (a) up to the 800th time-step.

Figure 7.9: Case where the mortality rate of senile ($\delta_{sc}$) tumour cells was set to 0.1.

This encouraged us to test for the impact of the mortality rate amongst senile cells. In order to investigate this we set the parameter value ($\delta_{sc}$) for tumour cells to 0.1 (from ($\delta_{sc}$) = 0.05). Fig. 7.9(a) shows this scenario. It is very clear that simply doubling this value has had little impact on the end result.

Now we alter the value of the parameter ($\delta_{sc}$) to 0.2 (which is four times the original
value). The results obtained are shown in Fig. 7.10(a). Once again, there is hardly any change.

\[ \text{(a) Here the mortality rate of senile tumour cells (} \delta_{s_c} \text{) was set to 0.2.} \]

We further alter the value of \( \delta_{s_c} \) to 0.5 (from \( \delta_{s_c} = 0.05 \)) and Fig. 7.11(a) shows the results we obtained. Although the dynamics changed (the apoptotic population was dominant during the beginning stages), the end point still remains the same.

\[ \text{(b) Magnified version of figure (a) up to the 800th time-step.} \]

Figure 7.10: Here the the mortality rate of senile tumour cells (\( \delta_{s_c} \)) was set to 0.2.

To test for an extreme case (which is biologically unlikely), we set the parameter value (\( \delta_{s_c} \)) to 0.9. The results are shown in Fig. 7.12(a). It is very clear that even when we set
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(a) Resultant curve obtained when the mortality rate of senile tumour cells ($\delta_{sc}$) was set to 0.5.

(b) Magnified version of figure (a) up to the 900th time-step.

Figure 7.11: Resultant curve obtained when the mortality rate of senile tumour cells ($\delta_{sc}$) was set to 0.5.

the parameter to an extreme value, it cannot change the end state. This implies that this parameter can only alter the final state if it is considered along with the other parameters. For example, the combination of a moderately high mortality rate amongst senile members and a relatively high rate of conversion of adult to senile cells can negatively impact the population growth of tumour cells (as shown in Fig. 7.7(a)).

Another parameter that needs testing is the effect of the amount of nutrients added to the system. In order to study this we change the maximum amount of nutrients that can
(a) The mortality rate of senile tumour cells ($\delta_{sc}$) was altered to test for an extreme case where the value was set to 0.9.

(b) Magnified version of figure (a) up to the 700th time-step.

Figure 7.12: The mortality rate of senile tumour cells ($\delta_{sc}$) was altered to test for an extreme case where the value was set to 0.9.

be added to the system ($I_n$) to 100. The result obtained by running the simulation for 1000 generations is shown in Fig. 7.13. It shows that the tumour population has barely any advantage during the initial growth period. However this does eventually change. It appears as though like the tumour population gains the upper hand towards the end and the apoptotic population is almost entirely eliminated.
Figure 7.13: Case where the maximum amount of nutrients ($I_n$) that could be added to the system was set to 100. This sudden increase in nutrient quantity causes huge oscillations (similar to what we have seen in Chapter 6).

The results from Fig. 7.13 are inconclusive (though it helps us understand the initial growth phase). In order to understand the long term effects we extended the simulations to 20,000 generations. The results are shown in Fig. 7.14(a).

The reasons for the fluctuations in the graph have little to do with the actual dynamics of the system. They are in fact caused by the way we have implemented the system. The equation that governs the allocation of nutrients (equation (7.5)) is set in such a way that once the value of the population crosses a particular threshold, the nutrients are completely shut off. This affects the population growth and the net population decreases. Since the maximum amount of nutrients supplied to the system is 100 times higher than that of the original value (which was 1), every time a high level of nutrients are added it causes a huge fluctuations in the graph.

This reasoning can be tested and verified by changing the value of this parameter to 1000 (we would expect an even bigger fluctuation in this case). This is shown in Fig. 7.15 and as expected the fluctuations were higher here.

One might also be interested in seeing how the nutrient quantity stabilised in the initial experimental set up. This is shown in Fig. 7.16(a). This (changes in nutrient quantity)
(a) The maximum amount of nutrients ($I_n$) that could be added to the system was set to 100 and was simulated over 20000 generations.

(b) Magnified version of figure (a) up to the 1800th time-step.

Figure 7.14: The maximum amount of nutrients ($I_n$) that could be added to the system was set to 100 and was simulated over 20000 generations.

is quite similar to what we have seen in the previous chapter when we studied apoptotic and non-apoptotic populations.
Figure 7.15: Case where the maximum amount of nutrients ($I_n$) that could be added to the system was set to 1000.

### III.I Parameter Testing

We have tested the model extensively by varying the parameters over a wide range of values. Although accurate values of these parameters cannot be predicted for a hypothetical environment, we can estimate the range of values that we are interested in.
(a) The change in nutrient quantity in the system.

(b) Magnified version of figure (a) from the 200th time-step onwards.

Figure 7.16: The change in nutrient quantity in the system.

Based on the assumptions we discussed in this chapter, we simulated the model for a range of parameter values as shown in Table 7.1.
Table 7.1: Relationship between parameters used in differential equation-based model for simulating tumour and apoptotic populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Conditions</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_C^*$</td>
<td>0.001 - 0.9</td>
<td>Not applicable</td>
<td>Tested for a wide range</td>
</tr>
<tr>
<td>$r_A^*$</td>
<td>0.001 - 0.9</td>
<td>Not applicable</td>
<td>Tested for a wide range</td>
</tr>
<tr>
<td>$\delta_A$</td>
<td>0.01 - 0.9</td>
<td>$\delta_A &gt; \delta_C$</td>
<td>Since population A can undergo apoptosis, its death rate will be higher in that case.</td>
</tr>
<tr>
<td>$\delta_C$</td>
<td>0.001 - 0.1</td>
<td>$\delta_A &gt; \delta_C$</td>
<td>Since population A can undergo apoptosis, its death rate will be higher in that case.</td>
</tr>
<tr>
<td>$\alpha_A^<em>, \alpha_C^</em>$</td>
<td>0.001 - 0.1</td>
<td>$r_{S1} &lt; r_{S2}$</td>
<td>The net impact on the nutrients will affect both populations.</td>
</tr>
<tr>
<td>$\kappa_A, \kappa_C$</td>
<td>0.00001 - 0.001</td>
<td>$\delta_{A1} = \delta_{A2}$</td>
<td>The net impact on the nutrients will affect both populations.</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.0001-0.01</td>
<td>Not applicable</td>
<td>Tested for a wide range</td>
</tr>
<tr>
<td>$I_n$</td>
<td>1 - 500</td>
<td>Not applicable</td>
<td>Tested for a wide range</td>
</tr>
<tr>
<td>$r_{A_{con}}^<em>, r_{C_{con}}^</em>$</td>
<td>0.1 - 0.9</td>
<td>$\delta_{A1} = \delta_{A2}$</td>
<td>The rate of consumption of nutrients is higher than the rate of replication as a part of the energy obtained is spent on maintenance. Since the DNA errors are high in the case of neoplasm, they require more energy to be spent on maintenance.</td>
</tr>
</tbody>
</table>
IV Discussions

IV.I Steady State Analysis

In the simulation we have seen that the populations almost reach a stable state. In order to find their actual values, we use the original equations used. For steady state we should have:

i) For tumour population ($P_1$):

\[(0.7 + 0.2)A_1(t)N(t) - 0.01A_1(t) + 0.1S_1(t)N(t) - 0.4A_1(t)N(t) = 0\]  \(\text{(7.11)}\)

\[0.4A_1(t)N(t) - 0.05S_1(t) = 0\]  \(\text{(7.12)}\)

ii) For apoptotic population ($P_2$):

\[0.7A_2(t)N(t) - 0.01A_2(t) + 0.6S_2(t)N(t) - 0.6A_2(t)N(t) = 0\]  \(\text{(7.13)}\)

\[0.4A_2(t)N(t) - 0.1S_2(t) = 0\]  \(\text{(7.14)}\)

iii) And for nutrients:

\[1 - 0.001(P_1 + P_2) = 0\]  \(\text{(7.15)}\)

Solving the above equations will give us the following sets of values for $A_1$, $S_1$, $A_2$, $S_2$ and $N$ (Since we can express one of the parameters as a function of all the other four parameters, we will only have 4 sets of values):

i) $\frac{625\sqrt{705}}{3} + 3125, 6875 - \frac{625\sqrt{705}}{3}, 0, 0, \frac{\sqrt{705}}{80} - \frac{5}{16}$

ii) $3125 - \frac{625\sqrt{705}}{3}, \frac{625\sqrt{705}}{3} + 6875, 0, 0, -\frac{\sqrt{705}}{80} - \frac{5}{16}$
iii) \(0, 0, \frac{225000}{13} - \frac{5000 \sqrt{465}}{13}, \frac{5000 \sqrt{465}}{13} - \frac{95000}{13}, \frac{\sqrt{465}}{240} - \frac{1}{16}\)

iv) \(0, 0, \frac{5000 \sqrt{465}}{13} + \frac{225000}{13}, -\frac{5000 \sqrt{465}}{13} - \frac{95000}{13}, -\frac{\sqrt{465}}{240} - \frac{1}{16}\)

where the values in each row correspond to the values of \(A_1, S_1, A_2, S_2\) and \(N\) in each of the four cases.

Out of these four sets of values, we can only take 2 sets of values for \(A_1, S_1, A_2, S_2\) and \(N\) because the other sets provide values for some parameters that are negative and none of these parameters can be negative. The two sets that agree with this condition are:

\[
\begin{align*}
A_1 &= 8656.63252 \\
S_1 &= 1343.36748 \\
A_2 &= 0 \\
S_2 &= 0 \\
N &= 0.0193979512
\end{align*}
\]

and

\[
\begin{align*}
A_1 &= 0 \\
S_1 &= 0 \\
A_2 &= 9013.90052 \\
S_2 &= 986.099482 \\
N &= 0.0273494111
\end{align*}
\]

The first set of values indicate that, in the stable state we will only have tumour cells and no matter what the initial number of members are, in a steady state one will only have around 10,000 organisms. We have already seen that the result is in agreement with our simulations\(^2\) (hence further analysed).

\(^2\)The second set also yields a steady state but it is quite similar to the case we discussed in Chapter 6.
IV.II Stability Analysis

Using the values we obtained for the steady state we can now check whether the steady state is stable or not. In order to investigate this, we find the Jacobian of the original set of equations used. We get the Jacobian matrix as:

\[
\begin{pmatrix}
\frac{n}{2} - \frac{1}{100} & \frac{n}{10} & 0 & 0 & \frac{a_1}{2} + \frac{n_1}{10} \\
\frac{2n}{5} - \frac{1}{10} & 0 & 0 & 0 & \frac{2a_1}{5} \\
0 & 0 & 3n - \frac{1}{10} & 3n & \frac{3a_2}{10} + \frac{3a_2}{5} \\
0 & 0 & \frac{2n}{5} & -\frac{1}{10} & \frac{2a_2}{5} \\
-\frac{1}{10000} & -\frac{1}{10000} & -\frac{1}{10000} & -\frac{1}{10000} & 0
\end{pmatrix}
\]

Now we can substitute the values we obtained for the steady state into the above matrix and we get two different matrices corresponding to each set of values. For the case where there is no apoptotic population:

\[
1.0e + 04 \times \begin{pmatrix}
-0.0000 & 0 & 0 & 0 & 1.1690 \\
0 & -0.0001 & 0 & 0 & 3.4627 \\
0 & 0 & -0.0000 & 0.0000 & 0 \\
0 & 0 & 0.0000 & -0.0001 & 0 \\
-0.0000 & -0.0000 & -0.0000 & -0.0000 & 0
\end{pmatrix}
\]

This is the case we are interested in.\(^3\)

In order to find the steady state we need to find the eigen values of the above matrix. We get the following values:

-0.0084 + 0.8899i
-0.0084 - 0.8899i
-0.0335 + 0.0000i
-0.1009 + 0.0000i

\(^3\)The case where there is no tumour population exists can also be analysed (and we found that to be stable) but it will be quite similar to the case in the last chapter where we analysed apoptotic and non-apoptotic populations (see Chapter 6)
-0.0032 + 0.0000i

The modulus of the above values is

0.8899
0.8899
0.0335
0.1009
0.0032

Since the modulus of all eigen values is less than 1, we can say that the system is a stable equilibrium.

IV.III Using the Game Theory Based Model

Figure 7.17: Simulation result obtained using agent based modelling when the game theory-based model was used. The results were similar to the one obtained using the differential equation based model.

In Chapter 5, we used the model:
In order to study the dynamics of tumour and apoptotic populations we set the value for \( c \) to 0.05 for tumour individuals and 0.1 for the apoptotic population (as we had done previously in Chapter 5). Every other parameter and condition remained the same, as discussed in Chapter 5. The reason why we had to reduce the value of \( c \) (which is the cost of competition to obtain nutrients) is because of angiogenesis in the tumour population. This will effectively reduce the amount of energy required to secure nutrients. Reducing the value of \( c \) is one of the ways to account for this. The result of the simulation is shown in Fig. 7.17.

We can also change parameters such as \( f(t) \) to study the effect of faster ageing in the tumour population. We have already done this using the new model in this chapter and we also found that the differential equation-based model is far better (efficient and less complex) than the game theory based model.

V Discussion and Conclusion

One of the accepted and widely adopted methods of treating tumours is to promote apoptosis amongst tumour cells. It was also reported that it is relatively easy to induce apoptosis in tumour cells using chemotherapy or UV radiation as this method will trigger apoptotic pathways in many tumorous cells. In our experiments we have seen that the dynamics of the populations are affected when the death rate is increased for older tumour cells (as we do in radiotherapy) and when the rate of conversion of adult to senile cells is increased.

From our simulations, we understand that the tumour cells have the upper hand in governing the dynamics of the tumour and apoptotic populations provided that the parameter values are selected appropriately. We also saw that a minor alteration in the parameter...
values will affect the stability of the system (see the section ‘Parameter testing’). This finding could prove valuable in helping to find a way to suppress the growth of the tumour population.

In order to study the effect of nutrients, we set the maximum amount of nutrients added to the system to a higher value. It is evident that by providing ample nutrients to normal cells, they can flourish at the same rate as the tumour cells. This does not mean that the growth rate of tumour cells has decreased. One should consider other parameters as well when devising a way to minimise the growth of tumour populations. This idea could be helpful in developing new ways to counter tumour growth.

The last 40 years have seen an extraordinary improvement in our understanding of apoptosis and its importance in the area of cancer therapy. Although there is much more to discover, our current understanding of the role played by apoptosis in tumour growth can help us to develop new methods for cancer diagnostics, prognosis and therapy.

We have found that both apoptosis and angiogenesis provide neoplasms with a significant advantage over normal cells. Accordingly, the tumour cells can easily invade a normal tissue. We have also shown that by changing the effects of these two factors, one can also alter their dynamics.

We believe that our model would be of interest in the field of oncology and may aid in devising strategies to manipulate the effects of apoptosis, angiogenesis and nutrient quantities present in the system and could also aid in producing new procedures that are less toxic and mutagenic than the current ones. Although we found that tumour populations can thrive over apoptotic populations in some cases, it does not necessarily mean that they are evolutionarily stable. The dynamics can easily change when the apoptotic population changes its strategies as a response to the tumour populations. This is discussed in the next chapter (Chapter 8).
Critical Analysis and Conclusion

"There are years that ask questions and years that answer." - Zora Neale Hurston

In the previous four chapters (Chapters 4-7) we saw how the simple modelling of senescence and apoptosis can potentially shed light on the evolution of the stable strategies adopted by an organism. In this chapter we critically analyse the models described in those chapters and see how they fit in with the existing literature.

I Causes of Ageing

As our research is not primarily concerned with the biological causes of ageing, we examined the phenomenon from the perspective of synthetic biology instead. Kirkwood et al. (1977) have shown that there is an optimal amount of energy that an organism needs to spend on maintenance. If the organism spends too much energy on maintenance, its reproductive capacity will be affected. This is critical in relation to the population dynamics. When we modelled ageing this aspect was considered. In the case of yeast (see section Model from General Principles from Chapter 4) we have used this to account for the fact that some older mother cells diffuse ERCs from their own cells to their daughter cells.
The original contribution of our research is not in understanding the causes of ageing but in understanding how a number of ageing factors present in an organism can affect its mortality. We subscribe to Kirkwood’s view that since it is optimal for the organism not to spend too much on maintenance, there is a good chance that the organism will continue to acquire an increasing amount of ageing factors over time. This has been reported in the literature as well and is discussed in Chapter 3. In Chapter 4 we show that by using a simple measure of the amount of AFs present one can predict the survival probability of the organism. It should be noted that modelling using the Gompertz equation has been applied to many populations including yeast and the resultant curves obtained from experimental data were in agreement with the Gompertz curve. It has also already been proved that the age of the organism affects its mortality (see Chapter 3). In fact what the first part of our research does is establish a direct link between the amount of AFs present in an organism and its survival probability. This link is required when we study the dynamics of apoptotic and non-apoptotic populations where the organisms are allowed to accumulate the AFs, which is in turn associated with the fitness of the organism.

One of the issues with the model (see section ‘Model from First Principles’ of Chapter 4) is that it had a number of parameters and optimization was possible even without considering all the biological aspects. For example, if one looks at Fig. 8.1 one can see that the graph is perfectly fitted with the data. However this can also be accounted for when one considers the fact that the diffusing of ERCs itself has very little impact in improving the survival probability of the organism (as reported in Chapter 3). It is also worth mentioning that in the case of existing models like Gillespie et al. (2004b) there are far too many parameters. It is also worth noting that once we simplified the equations many parameters lost their original biological meaning. However this is not a major concern as we are only interested in finding a relationship between the AFs and the survival probability of the organism.

This approach allowed us to formulate that one can express the survival probability of the organism as a function of the number of AFs present. This in turn means that a function
of the AFs is also a measure of the fitness of an organism by considering that senescence is associated with a decrease in the fitness of the organism. It has been shown that an increase in mortality caused by ageing is a representation of the fitness cost to organisms (Abrams, 1991). For the sake of simplicity we assume the relationship to be linear, and this need not to be the case in the real world. Since we are restricting ourselves to the area of synthetic biology this does not pose a problem.

In our model we allow the AFs to accumulate using a sigmoid curve (since it is the nature of the mortality curve). We have also experimented with a simple linear accumulation of AFs and have had similar results. In our models we considered only positive senescence. There are organisms that show hardly any senescence. Vaupel et al. (2004) have made a case for negative senescence. Despite the fact that Hamilton (Hamilton, 1966) ruled out the possibility of having negative senescence, this need not be the case. Many researchers have indicated that there are some organisms that have hardly shown any increase in their mortality rate (Finch, 1994; Strihler, 2012). There are many species that have shown negative senescence (and as a result their mortality rate fell with age) for a period following the start of reproduction (Vaupel et al., 2004). Vaupel et al. (2004) have also

Figure 8.1: Fitted data using Model from First principles (without ERC migration).
demonstrated that organisms that attain a size at reproductive maturity that is less than maximum size are likely to show negative senescence. Most of these studies focus on a specific period in the lifespan of the organism rather than the whole lifespan itself. Also, the number of organisms showing negligible senescence are also very few. Hence these should be considered as exceptions to the rule. This view is also consistent with the mathematical formalism of Kirkwood and Rose (1991a).

Although we started with a heuristic approach, we showed (using the model from first principles) that one can derive a relationship between AFs and the mortality rate of an organism. We have also shown the same using other methods as described in Chapter 4.

II Case for Apoptosis

Using the models we developed in Chapter 4 we created a game theory-based model to study the population dynamics of apoptotic and non-apoptotic populations. We assumed that the ageing factors would grow according to a Gompertz function. The reason behind this selection is to reflect the fact that the AFs have a direct relationship to the mortality of the organism and the mortality curve follows the Gomperz function. This could be simplified by assuming that the AFs would grow at a linear rate. Experiments carried out using this assumption also yielded similar results.

One of the key features of the game theory-based model is the presence of cooperation amongst similar individuals (i.e. if they belong to the same phenotype). Though we have seen the biological argument for this in Chapter 5, one could also model it without this assumption. Since we have implemented the game theory-based model using Agent Based Modelling, the effects of cooperation can easily be implemented. We have tested for the other case (where is no cooperation\(^1\)) using a differential equation-based approach and obtained similar results.

In the game theory-based model the nutrients were distributed randomly. This could potentially create a bias if one of the sets of populations is very low in number. This is\(^1\)

\(^1\)We have also removed many other potential skewed assumptions
the reason why the apoptotic population could not wipe out the non-apoptotic organisms when the apoptotic populations were very few in number. When we removed this limitation in the differential equation based approach, we saw that the apoptotic population could easily thrive in comparison to the other population. In some of the experiments we had to place the nutrients near the organisms in order to simulate the model at a faster pace. We ensured that the allocation of nutrients was fair to both populations.

The complexity of the game theory-based model also made it difficult to run simulations for a wide range of parameters. Although we have tested it for a set of parameters, we have had to restrict ourselves to a limited set of values and the intervals between the values were slightly higher than the ones we used in the differential equation-based approach. The random allocation of the initial number of individuals and the nutrient units also added to the complexity of the model. In order to maintain consistency we had to repeat the experiments multiple times to obtain the average values. However, even then some form of bias existed when the number of individuals in a particular group was much lower than the other. Since their numbers were lower, there was a lesser chance of them being placed next to a nutrient (which is also limited in quantity). This increased the chances of them being starved, thereby favouring the population with a greater number of initial individuals. Despite this, it is interesting to see that the apoptotic population did thrive compared to the non-apoptotic population when the size of apoptotic population was lower than the non-apoptotic population, but still comparable.

The concept of the nutrient reserve is biologically valid since many organisms can live through famine and they use up the fat reserves built-up in their bodies. Concepts such as the energy spent on competition (to obtain nutrients) and on migration (to look for food) are also biologically valid. Therefore, the model allows us to simulate the biological scenario without losing the biological meaning of the parameters.

In the model we have seen that in a resource-restricted environment, the apoptotic population has a clear advantage over the non-apoptotic population. However, unfortunately the model does not show how a single apoptotic cell can invade a population of non-
apoptotic organisms because of the bias in the system\textsuperscript{2}. Accordingly, the evolutionary stable strategy cannot be investigated. Yet, the dynamics suggest that the strategy (being apoptotic) is likely to be evolutionarily stable. This idea was investigated with the help of the second model we developed (differential equation-based model). Additionally, in the game theory model we had to assume many other parameters including the carrying capacity of lattice cells, growth of nutrient units in lattice cells and how the presence of a particular phenotype affects the behaviour of the other. Since we were doing simulations of a synthetic environment many of these parameters only added greater complexity to the model. As these were essential parameters, it was impossible to model without using them.

One of the striking features of the game theory-based model was that were able to model the growth of AFs along with the growth of each organism. This allowed us to model the effect of AFs on the fitness of the organisms. In the field of apoptosis modelling, this was a novel idea. The game theory model allowed us to implement it directly rather than obscure it through indirect parameters. In the model, the AFs were allowed to grow in each time-step and in the case of an organism belonging to the apoptotic population, the apoptotic machinery was activated once it had crossed a critical value. Since the organisms were actively seeking out nutrients, fitness played an important role. Organisms with higher AFs had to spend more energy on maintenance than the organisms with fewer AFs present.

In the biological world the threshold may not be as well-defined as it was in the model. However, the whole idea was to model a simpler version of the process and examine the population dynamics when one of set of populations was allowed to undergo apoptosis. Also, in the biological world it is more likely that the organisms with a greater number of AFs will have greater difficulty in securing nutrients in the first instance. This consideration will only reinforce our assumption.

The differential equation-based model uses an entirely different set of parameters to the game theory-based model. The differential equation-based model implements some of the

\textsuperscript{2}The system favours the individuals that are already higher in number
assumptions we used in the previous model indirectly. The effect of ageing is represented in showing how good an organism is at replicating itself. Apoptosis is introduced by increasing the death rate of older cells. In this model, we divided organisms belonging to the same phenotype into two subdivisions - adult and senile. Although we did this for ease of implementation of the model, this is also considered to be a valid approach from a biological point of view. There are many organisms that show various stages of development (Oppenheim, 1980). For simplicity we decided to split the population into three parts. In many cases, it would make sense if we split them into three - Young, Adult and Senile. However, for our purposes two was sufficient.

We used a set of parameters to define how the organisms progress from one stage to the other and how the replication and mortality rates change when they do so. In this way we were able to study the effect of AFs (organisms with a higher level of AFs had a lower replication rate) and apoptosis. The simplicity of the model also allowed us to implement it very efficiently. This in turn allowed us to test for a wide range of parameters and study how minor alterations to the parameters can influence the population dynamics in more detail.

Amongst the results we obtained, the most astounding one was the invasion of a single apoptotic organism over an entire population of non-apoptotic organisms. This allowed us to suspect that this strategy (apoptosis) is likely to be evolutionarily stable. Since the model used differential equations, it was possible to carry out stability analysis using analytical methods. We found that the strategy (apoptosis) was stable. As we have seen that a single apoptotic organism can invade a colony of non-apoptotic organisms the strategy is also evolutionarily stable. The model also allowed us to investigate how the parameters influence the dynamics of the populations. We found that apoptosis is a solution only if all the conditions are met. Since many of these parameters are intrinsic in nature, through natural selection the organism will evolve to select the right set of parameters.
Chapter 8 Section III

III Tumour Cells Versus Apoptotic Cells

The research we did in this area is of special interest to researchers working in the field of mathematical oncology. The results obtained when modelling apoptotic and non-apoptotic populations inspired us to investigate this special case. Tumour cells are essentially non-apoptotic cells and we found in Chapters 5 and 6 that apoptotic cells have a clear advantage over non-apoptotic cells in most naturally-occurring scenarios. However, we also know that tumour cells have the potential to invade apoptotic populations. In order to understand this we made alterations to the parameters we used in the game theory and differential equation based models to study the population dynamics of both tumour and apoptotic cells.

Two features we had to introduce were the ability of tumour cells to evade apoptosis and their ability to acquire nutrients more efficiently. The former was easy to implement as it simply meant that the cells would behave like non-apoptotic cells. For the latter, we had to change the parameter values specifically to meet the requirements. In the case of the game theory-based model we lowered the competition cost for tumour cells and in the differential equation-based model we increased their replicative rate. We also had to consider the implications of these changes as well. For instance, the tumour cells tend to have more errors accumulated over time.

There are a number of game theory-based models of tumour and apoptotic populations as described in Chapter 2. However hardly any of these models account for the effects of AFs carried by each organism. We simulated their dynamics using the game theory-based model and found that tumour cells in fact can invade the population of apoptotic cells. Since the model itself was complex, we decided to test for different subsets of parameter values using the differential equation-based model.

Our modelling results indicate that the tumour cells can invade a population of apoptotic organisms in the right situation. This also gave us guidance as to how tumour colonies ought to be treated. The model demonstrates what happens when the parameters are changed. As many extrinsic parameters can be altered, one could possibly find way to
treat tumours. However, this lies well outside the scope of our work and we have not explored this area in detail. It makes less sense to do this using a completely synthetic model.

There are many other factors that our model has ignored. These include: the pressure exerted by other cells, type of tumour and the stage the tumour has reached. These are areas where the model could be improved. We restricted ourselves to a simple model as we were working with a synthetic environment.

### III.I Evolutionary Stable Strategy

We have seen in Chapter 6 that apoptosis is a stable strategy if the conditions remain the same. In Chapter 7 we found that tumour populations can have the upper hand over apoptotic populations provided that the conditions are favourable. Does this mean that being tumorous is an evolutionary stable strategy? The answer to this question can be found by investigating what happens to the tumour populations once they age. One of the properties of tumour cells is that they accumulate errors (AFs) at a faster rate because of their very high rate of replication. One of the problems with this is that the daughters of the tumour cells over multiple generations will become very unfit and their mortality rate will increase considerably. Accordingly over a long period of time, this strategy would not have any advantage from an evolutionary point of view. This could be the reason why tumour-like aggressive activity is only seen in mutated somatic cells.

### IV Main Results

In Chapters 2 and 3 we discussed the key ideas and previous experiments carried out and how we would be using them for further research. All of our results have been reported in Chapters 4, 5, 6 and 7. In this section, we list the main conclusions we were able to draw from our research.

In our research we found the following:
1. Ageing Factors can be used as a measure of the mortality rate of an organism. The higher the quantity of AFs present, the lower the chances of survival for an organism as this is the only scenario where the experimental data agreed with the model in the trial and error method;

2. Apoptosis could be a strategic choice opted for by the organism during the process of evolution as it allows the population to thrive over non-apoptotic populations;

3. Given the right conditions (i.e.: relatively lower rate of apoptosis and relatively higher impact from ageing factors), apoptosis is a stable strategy in a resource-restricted environment; and

4. Tumour populations can invade a population of apoptotic cells if they have better access to nutrients as well as a higher replication rate. This is true even when the mortality rate is relatively high amongst seline tumour cells;

Even though it is not a result, in view of the fact that tumour populations tend to accumulate large amounts of DNA damage over multiple generations, we can suspect that being tumorous is not a viable long term strategy.

V Interpretations

In our models we have used a simple synthetic environment. However, we believe that the concept developed here can be extended to the real world. We were primarily interested in the impact of AFs on the population dynamics, and so we have restricted ourselves to a simpler version of the biological phenomena.

One of our key deductions\(^3\) is that apoptosis plays a key role in governing the population dynamics of the system. In the game theory-based model we used co-operation as a key feature as it has been in a wide range of species (as reported in Chapter 3). In the differential equation-based model, we found that even when this assumption (of

\(^3\)Some of the speculations we made are set out in Appendix V.
cooperation) is taken away, apoptosis still plays a key role in governing the population dynamics.

In our models we have made some assumptions that are based on empirical observations. For example, we assumed that older individuals will pass some of their AFs to the next generation. This may not be true in all cases. Although in many species (e.g. passing of ERCs from older mother cells to daughter cells and older mammals tend to give birth to defective babies) this is observed, this need not be the case with all species. One can modify our models to see if this alter the population dynamics.

VI Limitations

The main limitation of our model is that it is simplistic. We had to opt for such a model as it is difficult to study the dependencies of various parameters if we make the model more complex. We have seen this problem partly in our game theory-based model, which was more realistic than the differential equation-based model. However, it is possible to extend our model to include more complex features and parameters. This would make the model complex and possibly case-specific. In order to retain the generalised nature of the model, we decided to retain the simplistic approach.

The above-mentioned point is particularly valid when we analyse how AFs impact upon the fitness of the organism. In our models (game theory-based and differential equation-based) the relationship is linear. This is likely to be much more complex in reality (as we have seen in the yeast population). It is worth mentioning that some organisms do exhibit zero or negative senescence. In these cases, the fitness of the organism and number of AFs present will have a complex relationship. This is beyond the scope of this thesis. However, it is interesting to note that zero or negative senescence has only been noticed during a specific period of an organism’s life (rather than throughout its lifespan).

The effect of nutrients is another key aspect worth mentioning here. We limited ourselves to nutrient-restricted environments in most cases. Despite the fact that we have tested for extremes in some cases, we have not studied cases where the way nutrients are added is
complex (e.g. in some cases, an organism can have a symbiotic relationship with another organism that provides the nutrients). As these considerations will only add complexity to the model, we have not used them in our research.

VII Final Conclusion

Our research shows that apoptosis is a strategic behaviour (evolved strategy) when populations are growing in a resource-restricted environment. One of the major advantages of our model is that it identifies AFs as an important parameter. Since we can represent the mortality of an organism as a function of the AFs present (which can be measured directly) we managed to quantify the associated fitness of the organism. This in turn allowed us to study the population dynamics of ageing populations.

It is possible that evolution allowed organisms to develop apoptosis machinery and that natural selection favoured these organisms. This is particularly interesting considering the fact that the apoptosis is quite similar in both simple unicellular organisms and complex multicellular organisms.

Many of the parameters used in our synthetic models can be measured biologically. Although we have not seen any research where these are reported, one could test our model using parameters obtained from the biological world. Nevertheless, our synthetic model suggests that organisms that can undergo apoptosis have a clear advantage over those who do not. This alone makes our findings somewhat distinctive.

It is also clear that in certain situations some of the members of the apoptotic populations can mutate to become tumour cells and this can invade the entire population. From our simulations, it is also clear that this is not an evolutionary stable strategy. Oncologists can see that some of our experiments can be used to identify potential treatments of tumours.
Death may be the greatest of all human blessings.

SOCRATES
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I Differential Equations Used in Chapter 4

In this section we present some of the differential equations that we employed when deriving the model from first principles.

We are interested in solutions of equations of the form:

\[
\frac{dE}{dt} - r(t)E(t) = p(t)
\]  

(A.1)

where \( r(t) \) and \( p(t) \) are given functions. We proceed as follows:

1. Solution for the homogeneous case \((p = 0)\):

   For \( p = 0 \), the general solution is

   \[
   E(t) = ce^\int_{t_0}^t r(\tau)d\tau
   \]  

   (A.2)

   with an arbitrary constant \( c \) (which corresponds to the constraint \( E(0) = c \).)

2. Solution for a Dirac-\( \delta \): For \( p(t) = c\delta(t - t_0) \) (i.e. a Dirac peak of mass \( c \) at \( t_0 \)) one solution is:

   \[
   E(t) = \begin{cases} 
   0, & E < t_0 \\
   ce^\int_{t_0}^t r(\tau)d\tau, & E > t_0 
   \end{cases} = cH(t - t_0)e^\int_{t_0}^t r(\tau)d\tau
   \]  

   (A.3)

   This is the specific solution where \( E = 0 \) for \( t < 0 \) and \( E(t_0) = c \). The general solution is a sum of this and the general solution for the homogeneous case.

3. Solution for superposition of Dirac-\( \delta \)s:

   Now the LHS above is linear, so if \( p(t) = c_1\delta(t - t_1) + c_2\delta(t - t_2) \) and \( E_i \) is a solution for \( c_i\delta(t - t_i) \), then \( E = c_1E_1 + c_2E_2 \) must be a solution for \( p(t) \) by linearity.

4. In other words, for an arbitrary \( p(t) \) it follows (since \( p(t) = \int p(\tau)\delta(t - \tau)d\tau \)) that
the following $E(t)$ is a solution:

$$E(t) = \int_0^t p(\tau) e^{\int_{\tau}^{\tau'} r(\tau') d\tau'} d\tau$$  \hfill (A.4)

This is the formalism of the Green’s function broken down to the differential equation for $E$ which we started from.

**Reeh’s universal constant ($c$):** This is used in order to avoid carrying around too many integration constants and to simplify notation with “rules”:

$$c + c = c, \ c \times c = c$$  \hfill (A.5)

where $c$ is always greater than 0.

**Including Multiple Generations**

Gillespie et al. (2004a) included multiple generation (daughter and granddaughter cells) in their simulations even though Sinclair et al. (1998) considered only the original population of mother cells. We have also done experiments by altering our model to include multiple generations and found that there is a slight change in the survival probability values of the initial time period ($t=1-10$). The rest of the curve is unaffected.

**II High Nutrient Amount**

Though equations (7.1), (7.2) and (7.5) helped us to simulate the model, one of the problems with these modified equations is that the parameters lost their original biological meaning (but they still have biological significance). In order to fix this problem, let us assume that the value of $N(t)$ is significantly higher. This allows us to have:

$$\frac{N(t)}{K + N(t)} \approx 1$$  \hfill (A.6)
This means we can avoid including the nutrient part while analysing the model.

Equations (6.5) and (6.6) will therefore become:

\[
\frac{dA}{dt} = r_A A - \delta_A A(t) + r_S S(t) - r_C A(t) \tag{A.7}
\]

\[
\frac{dS}{dt} = r_C A(t) - \delta_s S(t) \tag{A.8}
\]

### III Speculations

In our research we speculated that AFs play a key role in determining the fitness of an individual. We subscribed to the idea of Kirkwood and Rose (1991a) that the organism would spend the optimal amount of energy on maintenance and its primary focus would be on reproduction. We have extended this concept to suggest that the AFs would accumulate in the organism’s body and would have a direct impact on the survival probability of the organism. Even though it is a simplistic view, we used data from Sinclair and Guarente (1997b) to show that this is biologically plausible.

One of the key problems with Kirkwood et al. (1977) is that their model cannot explain why Calorie Restriction (CR) improves the lifespan of the organisms. If we used their model, it would suggest that since the organism has access to fewer nutrients the optimal amount of energy spent on maintenance will also be lower. Accordingly, their mortality rate should increase. However, this is not the case.

The deduction would be different if we use our game theory-based model to explain why the lifespan is increased. According to our model, the organisms will not have sufficient energy to spend on reproduction. Therefore, it can use its nutrient reserve for finding more nutrients or enter into a hibernation mode. By entering into a hibernation mode the organism can extend its lifespan. But it should be emphasised that this idea is very speculative and this may not be what is happening in the biological world.
A fundamental question that needs to be addressed is how apoptosis can be naturally selected when most organisms in the wild die before reaching the latter stages of their lifespan. A plausible explanation for this can be given using our model; however, this is speculative in nature. In Chapter 3 we discussed the fact that the apoptotic machinery in multi-cellular organisms is quite similar to the machinery we find in uni-cellular organisms. There is a good chance that the apoptotic machinery was selected by the multi-cellular organisms from their uni-cellular ancestors. As most uni-cellular organisms are able to complete their full lifespan, apoptosis is highly relevant in their case. Due to the fact that apoptosis does not cause a negative effect on the population dynamics of organisms that tend to die early in their lifespan, apoptotic machinery can be retained generation after generation. However, as we mentioned before, this is a speculative answer and requires validation from further biological experiments.