

**THE STATISTICAL MECHANICS OF EVOLUTION:
THE THERMODYNAMICS OF RNA-PROTEIN CO-EVOLUTION AND
A STOCHASTIC BIT-STRING MODEL FOR THE SELECTION OF SEX**

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101327

Date of submission : 11 June 2001

Date of defence examination: 26 June 2001

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JUNE 2001

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**EVOLÜSYONUN İSTATİSTİKSEL MEKANİĞİ:
RNA VE PROTEİNLERİN BİRLİKTE SEÇİLİMİNİN TERMODİNAMİĞİ VE
EŞEYLİ ÜREMENİN STOKASTİK BİT-DİZİ MODELİ**

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**Tezin Enstitüye Verildiği Tarih : 11 Haziran 2001
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HAZİRAN 2001

ACKNOWLEDGEMENTS

I am greatly indebted to my thesis supervisor Prof. Ayşe Erzan for her guidance and help in all the years I have been involved in research. Without her willingness to help, and the motivation she had instilled in me, it would not be possible to complete this work.

I am very much grateful to Volkan Sevim for his collaboration in every stage of this study. Without his original ideas and computational abilities Chapter 3 of this thesis would not be possible.

It is a great pleasure to thank Prof. Naeem Jan, who has introduced us to the subject “Evolution of Sex”, and Prof. Dietrich Stauffer for his continuing interest and many useful discussions. I also would like to thank Prof. Aslıhan Tolun, Dr. Benan Dinçtürk and Dr. Candan Tamerler Behar who have helped us battle our ignorance of biology and biochemistry and Dr. Ahmet Togo Giz for his very much useful suggestions.

I also would like to thank Nazım Ziya Perdahçı for his kind help and computational wisdom that he has shared with me.

Finally, I would like to very much thank my dear friend Vasfiye Hande Metin for her support and tolerance that made all this work possible.

June, 2001

Erkan Tüzel

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LIST OF SYMBOLS

S	: Entropy
ΔS	: Entropy gap
T	: Temperature
r	: RNA molarity
p_u	: Unfolded protein molarity
p_f	: Folded protein molarity
F, U	: Temperature dependent folding/unfolding rates
σ	: Protein synthesis rate (Chap.2), constant conversion probability (Chap.3)
D_P, D_R	: Protein and RNA diffusion coefficients
κ	: Thermal conductivity
C	: Heat capacity
k_u, k_f	: Folding/Unfolding rate constants
$[N], [D]$	: Native and denatured state protein molarities
k_{obs}	: Observed rate constant
k_{max}	: Rate constant maximum
N	: Total number of residues (Chap.2), total population (Chap.3)
ψ, θ	: Hierarchical model state variables
ϵ, J	: Coupling constants
λ	: Guiding parameter
q	: Number of degrees of freedom at each node
Z	: Partition function
η, ξ	: Order parameters
τ, a	: Time and space intervals
L	: Pore side length (Chap.2), length of coarse-grained genome (Chap.3)
Γ	: Mutation rate
m	: Number of deleterious mutations
$P(m)$	: Fermi function for survival
μ	: Survival/Conversion to sex threshold
$n_H(m), n_s(m)$	: Number of asexual/sexual (haploid/diploid) individuals with m mutations
$n_D(m)$	: Number of diploid strands with m mutations
N_A, N_S	: Total asexual/sexual population
$T_{m,m'}(\Gamma)$	: Transition probability from a state of m mutations to one with m' mutation
d_m	: The number of gametes with m mutations which get removed
D_D	: The number of diploid individuals who die

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SUMMARY

In recent years, physicists started to apply the tools of statistical mechanics to problems of biology such as protein folding, evolution, etc.

In this study we are going to propose a scenario for the co-evolution of fast folding proteins with large entropy gaps and of RNAs observed today, by means of a refrigeration mechanism, within the porous rocks of prebiotic earth. This is analogous to the process known as magnetic cooling, which is a standard procedure used to attain very low temperatures. We are going to quantify this scenario by mass and heat transport, and chemical reaction equations, and develop a two-state Hamiltonian to model the folding of proteins on rock surfaces. One might speculate that the accidental folding of proteins on nearby rock surfaces within pores, leading to a reduction in the temperature of the medium, provided an evolutionary advantage to those RNA molecules that are able to synthesize fast folding proteins with large entropy gaps.

Later, we will go forward in time one billion years and investigate the evolution of sexual reproduction from a pure asexual population of billions of bacteria. We will develop some models and present the results of both simulations and mean-field equations for our scenarios of conversion to sex. We will also investigate the behaviour of this system of equations from a nonlinear dynamics of point of view.

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TERMODİNAMİĞİ
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EŞEYLİ ÜREMENİN SEÇİLİMİNİN STOKASTİK BİT-DİZİ
MODELİ**

ÖZET

Son yıllarda, fizikçiler istatistiksel mekaniğin araçlarını protein katlanması, evrim, vb. biyolojik problemlere uygulamaya başlamışlardır.

Bu çalışmada, hızlı katlanan ve entropi farkları büyük olan proteinler ve RNA ların birlikte seçilimi için yaşam öncesi dünyada kaya gözenekleri içinde gerçekleşen bir soğutma çevrimi senaryosu önerilmektedir. Önerilen çevrim, literatürde manyetik soğutma olarak bilinen ve malzemeleri çok düşük sıcaklıklara indirmek için kullanılan bir yöntemin benzeridir. Bu senaryo madde ve ısı taşınım, ve kimyasal reaksiyon denklemleriyle tasvir edilmekte, proteinlerin kaya yüzeylerinde katlanmasını modelleyen iki durumlu bir Hamiltonyen önerilmektedir. Gözenekler içerisindeki proteinlerin kaya yüzeyleri üzerinde katlanıyor olması ve bunun sonucu olarak ortamın sıcaklığının azalmasının, hızlı katlanan ve entropi farkı büyük olan proteinler sentezleyebilen RNA molekülleri için evrimsel bir avantaj sağlayacağı öne sürülebilir.

Daha sonra, zamanda bir milyar yıl kadar ileriye gidilerek, sadece eşeysiz üreyen milyarlarca bakteriden oluşan bir topluluktan eşeyli üremenin evrimsel süreçte ortaya çıkması incelenmektedir. Eşeyli üremeye geçisi betimleyen senaryolar sunulmakta ve bu modellerin davranışları hem bilgisayar simülasyonları, hem de ortalama alan denklemleri ile araştırılmaktadır. Ayrıca, bu denklem sistemlerinin davranışları lineer olmayan bir yaklaşımla da incelenmiştir.

1. INTRODUCTION

The emergence of life can be regarded as the prime example of self-organization in an open system driven by currents of mass and energy. Spontaneous pattern formation in dissipative open systems [1] has occupied center stage in the last thirty years in the study of non-equilibrium phenomena.

Eigen [2] in a pathbreaking line of research that led to his receiving the Nobel Prize, has demonstrated that fluctuations in open non-equilibrium systems need not be exponentially damped, as they would be in equilibrium systems, but may in fact grow and come to dominate the whole system. The mechanism that paves the way for the spontaneous evolution of higher degree of organization in such an open system, is that such fluctuations be coupled in an autocatalytic cycle.

In order to understand the process of evolutionary change that can influence the observed properties of proteins, it seems reasonable to consider such processes in conjunction with the evolution of “pre-living” [3] concentrations of RNA. After all, if we define life as starting with self-replicating molecules, which eventually evolved into the modern genes [4, 5], it is natural to look for mechanisms whereby the present biological proteins evolved together with RNA, which provides the genetic code for protein synthesis. This we believe is a better strategy than trying to understand the evolution of protein populations [2] by themselves.

The crucial idea linking RNA stability and protein synthesis is that the translation of RNA code means picking out a specific sequence of amino acids from a random jumble and transforming them into an ordered chain. Once the peptide chain is formed and wiggles itself free from the RNA molecule, the chain tries to fold into its native state. However, at high temperatures, it seems a most unlikely process for the chain to fold on its own without any help [6]. The

surface of rocks within the pores in which these processes take place, may serve as guides for these peptide chains to fold more easily. Upon folding, as a result of the reduction in entropy, heat is given out mostly to the surface of rock. When the protein chain detaches itself from the rock surface, it is effectively at a lower temperature than its surroundings, and in coming to equilibrium with its surroundings will absorb heat and lower the temperature in its vicinity.

This is identical, in principle, to the process known as “magnetic cooling,” which is a standard procedure used to attain very low temperatures (milli Kelvin). Here, the magnetic moments in a sample are ordered by applying an external magnetic field, the spins equilibrate to a temperature that is in keeping with the much reduced disorder.

One may now hypothesize that the accidental folding of proteins on nearby rock surfaces, leading to a reduction of the effective temperature of the protein and eventually of the medium, provided an evolutionary advantage to those RNA molecules who were able to synthesize “foldable” proteins. The fates of the selected RNA chains were from then on forever bound with the synthesis- and eventual evolution- of polypeptides. Clearly we are making no claims that the present proteins are identical to the end products of this particular selection mechanism. Once the temperature was sufficiently lowered, other, more complex evolutionary pressures would come into play.

Another important move in the course of evolution is the emergence of sexual reproduction from a pure asexual world. Although the higher organisms we see today mainly reproduce sexually, there is a very large population of unicellular organisms, which still use asexual reproduction. For a large period of time, all of the organisms were reproducing only asexually. At the time sexual reproduction has evolved, in a much shorter period of time many different types of living organisms that are present today were formed.

In order to study the evolution of sex, we introduce a bit-string model to represent the genome of the unicellular organisms. We have performed Monte

Carlo simulations based on this model and found that the hypothesis made by Jan, Stauffer and Moseley [7] for the evolution of sex, namely a strategy devised to escape extinction due to too many deleterious mutations, is sufficient but not necessary for the successful evolution of a steady state population of sexual individuals within a finite population [8]. Simply allowing for a finite probability for conversion to sex in each generation also gives rise to a stable sexual population, in the presence of an upper limit on the number of deleterious mutations per individual. For large values of this probability, we find a phase transition to an intermittent, multi-stable regime. On the other hand, in the limit of extremely slow drive, another transition takes place to a different steady state distribution, with fewer deleterious mutations within the asexual population. We compare the results of simulations with the numerical solutions of the mean-field equations obtained for different densities involved [8].

We have also shown that [9], if the diploid cells, once formed, are also allowed to multiply by mitosis, as indeed they do in a haploid-diploid cycle, the whole population is taken over by diploid cells, who perform facultative sex if they are once more threatened by extinction due to too many deleterious mutations. Moreover we show that even an episodic conversion to sex, involving as few as only two individuals who survive to mate, leads to a steady state made up solely of sexual types.

2. A THEORETICAL SELECTION MECHANISM FOR NON-DEGENERATE PROTEINS IN PREBIOTIC RNA-PROTEIN CO-EVOLUTION

2.1 Role of Proteins and Temperature in Biological Selection

The evolution of proteins by a random choice of individual amino acids seems a most unlikely process. For example, the number of different sequences possible for a protein containing 250 amino acid residues (e.g., trypsin or chymotrypsin) is 20^{250} or 2×10^{325} , a quantity greater than the estimated number of atoms in the universe (9×10^{78}) [10]. This paradox known as the Levinthal Paradox [11] is still unsolved. The number of possible configurations might be reduced by introducing energetics; however, the number would still be large. This result indicates that there should be a strong selection mechanism in order to reduce the number of conformations possible to total population of proteins present today ($\sim 15,000$ *proteins*).

One of the most important properties that proteins possess today are their unique ground states. The energy landscapes of proteins are generally shown in schematic pictures, like the one in shown Figure 2.1. If one designs a toy protein consisting of a random sequence of amino acids, the ground state for this protein would be highly degenerate as shown in Figure 2.1a. Therefore, the entropy difference between folded and unfolded states would be very small. On the contrary, real proteins having “selected” amino acid sequences have unique ground states. This results in a large entropy gap, since there is only one protein conformation possible in the ground state (see Figure 2.1b).

The fact that proteins observed today have large entropy gaps and reach their

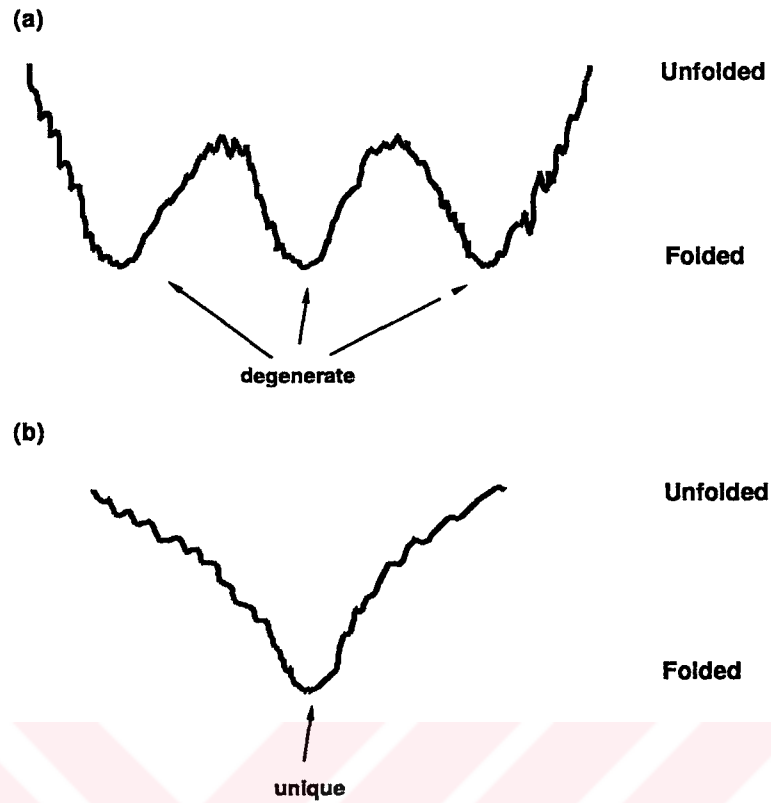


Figure 2.1: Energy landscapes of proteins; a) consisting of random configuration of amino acids, b) having a unique amino acid sequence dictated by the RNA molecules. A randomly generated configuration results in a system having degenerate ground states, however, a real protein chain has a unique ground state.

ground states very rapidly (order of millisecond range for small proteins) forms the basis of the scenario we are going to propose for selection. Another important factor in biological selection is the effect of temperature. Most of the proteins that are observed today best perform their functions at temperatures around 310 K. There are some small number of heat shock proteins that can resist higher temperatures [6]. Therefore, in the prebiotic earth, proteins that find themselves within a cooler environment might have advantages in selection, together with their template RNA molecules. Also, RNA molecules themselves can replicate and increase their populations. Better replication rates might have been achieved at lower temperatures due to the presence of a large variety of protein molecules,

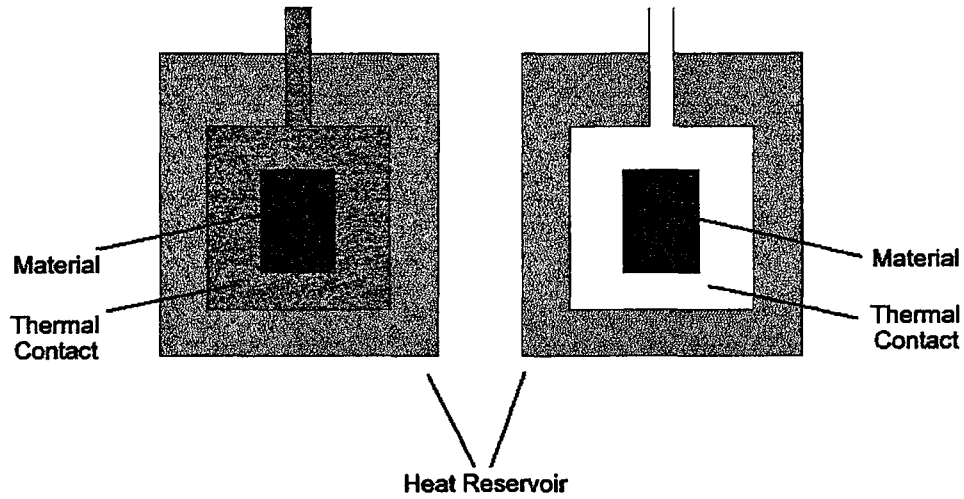


Figure 2.2: A magnetic refrigeration system.

a few of which can aid in the replication process, i.e. act as enzymes. Considering all of the above facts, we propose a scenario, in which a refrigeration cycle takes place to lower the temperature locally within porous rocks.

In the next section we are going to review magnetic refrigeration, and in the later sections we are going to describe the refrigeration scenario we propose for the selection and co-evolution of RNA and protein molecules, and investigate its applicability in detail with the aid of chemical reaction and diffusion equations.

2.2 Magnetic Refrigeration

It is possible to cool a magnetic sample by ordering it isothermally under a magnetic field, and allowing it to come adiabatically to a new equilibrium, and the method can be described as follows[12]. The material shown in Figure 2.2 is a magnetic sample initially in thermal contact with a heat reservoir at temperature T_i . In cooling experiments, this heat reservoir is liquid helium near $1^\circ K$ and thermal contact of the sample with the reservoir is established with the help of helium present in between. If one switches on the magnetic field, the material

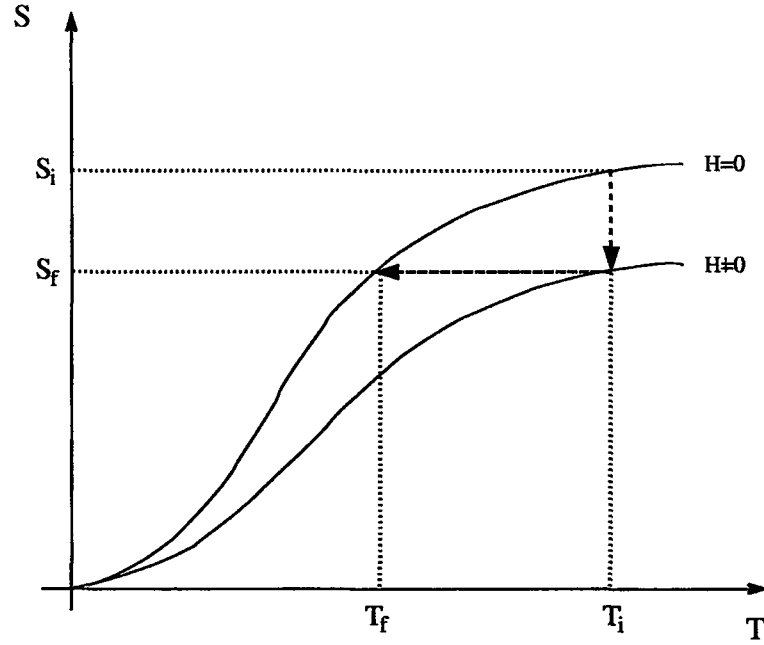


Figure 2.3: The entropy change in a spin system in the presence of magnetic field. The path of a refrigeration cycle is also shown.

sample is magnetized and work is done, but the sample gives off heat to the reservoir and remains at temperature T_i , therefore the process is isothermal as shown in Figure 2.3. The material is then thermally insulated by removing the thermal contact, e.g. helium gas, and the magnetic field is reduced to its initial value, $H = 0$ quasi-statically. As a result of this adiabatic demagnetization the temperature of the sample material falls to a final temperature T_f . Using this method, one can cool down the sample to very low temperatures. The temperature difference $T_i - T_f$ is a function of entropy difference between the two cases $H = 0$ and $H \neq 0$. The most important property of the material being cooled is the entropy difference between ordered and disordered phases. A larger entropy gap, which can be controlled by the external field, results in better cooling of the sample.

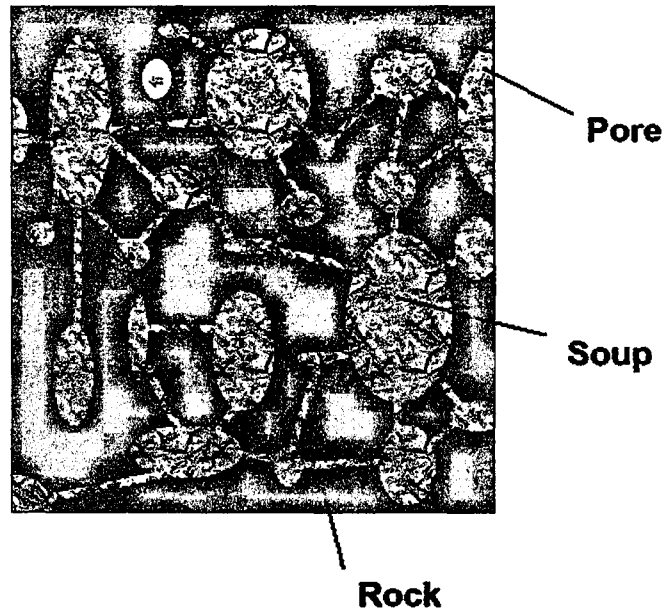


Figure 2.4: A cross section from the porous network within rocks. Each pore contains the prebiotic soup consisting of RNA, protein molecules, amino acids, etc. and material transport is made through the channels.

2.3 RNA in a Refrigeration Cycle

Before explaining the refrigeration scenario, let us describe how we implement the prebiotic conditions in porous rocks. Throughout the whole text, the word “environment” will be used to denote a picture consisting of pores of various sizes embedded in rocks. These pores within rocks may be connected to each other and they may be of any size. The surrounding rock will serve as an infinite heat reservoir for these pores since their sizes will be small when compared with the whole volume of rock. The channels between these pores aid in the transport of materials and the whole system might be thought of as a “porous network” (see Figure 2.4). We assume the pores contain a “prebiotic soup” namely, mixture of both organic and inorganic materials, that are assumed to be present in the prebiotic earth. However for simplicity, we are going to concentrate on a single sample pore that contains only the RNAs, protein molecules and amino acids

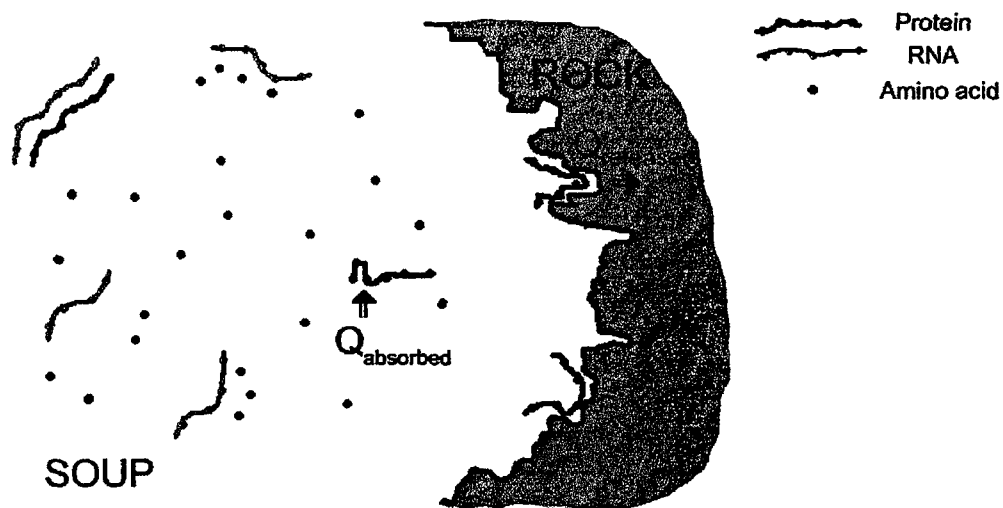


Figure 2.5: A cross section from a pore involved in a refrigeration cycle.

which are the relevant contents of the soup, and water, which does not actually enter our considerations, but determines some characteristics of the medium such as heat capacity, transport properties, etc.

Let us now consider a refrigeration cycle consisting of 3 different steps:

- Synthesis of proteins by RNA
- Partial folding of proteins on rock
- Unfolding of proteins in soup.

This refrigeration cycle is pictured in Figure 2.5. The amino acids are free to diffuse anywhere in the soup and we assume every RNA molecule can find enough amino acids around to use in the synthesis of proteins. The RNA molecules gather up amino acids and synthesize their own proteins according to the code present in their sequence. Here, the RNA molecule can be thought of as a “power supply” since it provides the information, i.e. determines the particular amino acid sequence, which it has built into the protein, giving it the tendency

to fold into a low entropy state. It is this information which enables the rock to “do work” (i.e. compress it) on the “coolant”, in other words guide the proteins in folding. The synthesized proteins are initially unfolded due to the high temperature of the soup. These unfolded proteins move within the soup trying to find a surface to attach their hydrophobic residues which dislike being together with water. After attaching on the surface of surrounding rock, the second step of refrigeration begins. If the molecule is able to fold around its hydrophobic residues, as a region shielded from water, the rock helps the unfolded protein in folding. Upon folding the coiled protein reduces its surface area, and takes some of its hydrophobic residues inside, therefore detaches from the rock. The last step of refrigeration is the unfolding of these folded proteins, within the soup, again due to higher temperature.

In the above scenario, two different heat transfers occur. When the unfolded protein folds, it gives up heat mainly to the rock proportional to its entropy gap. However, when it unfolds again inside the soup, the protein absorbs heat from its environment. This refrigeration cycle is very similar to the magnetic cooling cycle explained above, if one thinks of the rock as being both the thermal contact and the magnetic field. For the surrounding materials and soup content we consider, the thermal conductivity of rock is higher than the soup, and therefore much of the heat given out during folding will be transferred to the rock which is also the heat reservoir in the system (i.e., remains at constant temperature, at least far from the boundary).

For this refrigeration cycle to work and cool down the soup, there are two major parameters: entropy gap and folding/unfolding rates. Proteins with large entropy gaps and/or faster folding/unfolding rates will be more successful in cooling. In order to quantify the proposed cycle, one can write down the following heat and mass transport, and chemical reaction equations:

$$\begin{aligned}\frac{\partial r(\vec{x}, t)}{\partial t} &= D_R \nabla^2 r(\vec{x}, t) \\ \frac{\partial p_u(\vec{x}, t)}{\partial t} &= D_P \nabla^2 p_u(\vec{x}, t) - F(T(\vec{x}, t)) p_u(\vec{x}, t) \delta(\vec{x} - \vec{x}_b)\end{aligned}\tag{2.1}$$

$$\begin{aligned}
& +[U(T(\vec{x}, t))p_f(\vec{x}, t) + \sigma r(\vec{x}, t)](1 - \delta(\vec{x} - \vec{x}_b)) \quad (2.2) \\
\frac{\partial p_f(\vec{x}, t)}{\partial t} = & D_P \nabla^2 p_f(\vec{x}, t) + F(T(\vec{x}, t))p_u(\vec{x}, t)\delta(\vec{x} - \vec{x}_b)
\end{aligned}$$

$$\begin{aligned}
& -U(T(\vec{x}, t))p_f(\vec{x}, t)(1 - \delta(\vec{x} - \vec{x}_b)) \quad (2.3) \\
\frac{\partial T(\vec{x}, t)}{\partial t} = & \frac{\kappa(\vec{x})}{C(\vec{x})} \nabla^2 T(\vec{x}, t)
\end{aligned}$$

$$\begin{aligned}
& +F(T(\vec{x}, t))p_u(\vec{x}, t) \frac{T(\vec{x}, t)\Delta S(T(\vec{x}, t))}{C(\vec{x})} \delta(\vec{x} - \vec{x}_b) \\
& -U(T(\vec{x}, t))p_f(\vec{x}, t) \frac{T(\vec{x}, t)\Delta S(T(\vec{x}, t))}{C(\vec{x})} (1 - \delta(\vec{x} - \vec{x}_b)) \quad (2.4)
\end{aligned}$$

where $\vec{x}_b$ denotes a position on boundary, $T(\vec{x}, t)$ is the temperature and $r(\vec{x}, t)$, $p_u(\vec{x}, t)$ and $p_f(\vec{x}, t)$ denote the RNA, unfolded and folded protein molarities respectively. Now, let us investigate each equation term by term. The RNA molecules are allowed to diffuse only, resulting in the Laplacian term in Eq.(2.1), where D_R is the diffusion coefficient. The protein molecules, classified as unfolded and folded can both diffuse, so both Eqs.(2.2) and (2.3) have Laplacian diffusion terms, where D_P is now the diffusion coefficient for protein molecules. $F(T(\vec{x}, t))$ and $U(T(\vec{x}, t))$ denote the rate constants of the folding and unfolding reactions respectively. As explained above, when the unfolded protein folds, at a rate of $F(T(\vec{x}, t))$, the molarity of unfolded proteins decreases by an amount proportional to their molarity. Similarly, when the folded protein unfolds at a rate of $U(T(\vec{x}, t))$, the molarity of unfolded proteins increases proportional to the molarity of folded proteins, $p_f(\vec{x}, t)$. We allow folding to occur only at the boundaries, and unfolding to occur only inside the soup, these are guaranteed by the δ functions in the equations. Finally, σ is the rate at which new proteins are synthesized by the RNA molecules at a given instant of time.

The equation for the temperature change contains two extra terms besides the heat diffusion term. In the pure diffusion term $\kappa(\vec{x})$ and $C(\vec{x})$ denote the thermal conductivity and heat capacity of medium respectively. The first term in Eq.(2.4) is for the increase in temperature due to the folding of a protein molecule at the boundary. The second term represents the decrease in the temperature as a result of an unfolding event at any place except boundaries.

These equations model the refrigeration scenario without any further assumptions. All the coefficients involved in the equations should be replaced by their experimental values, in order to check whether such a refrigeration cycle is possible thermodynamically. One should especially focus on the unfolding entropies and effect of the presence of the rock surface during folding. These coupled equations comprise terms with a nonlinear temperature dependence and their solutions can not be obtained analytically. In order to solve these numerically one should apply the finite volume method to discretize these equations and perform iterations. We postpone the discussion of this method and stability of these equations until section 2.7, and have a closer look at physical and biochemical parameters.

2.4 Physical and Biochemical Parameters

The pre-biotic soup may consist of any type of inorganic or pre-organic material; however as previously stated we will only consider amino acids, proteins, RNAs and water. The composition of these materials are given in terms of the volume ratios. Water occupies minimum 50% of the volume of a pore, initially amino acids take up 30% of the pore. As RNA molecules synthesize proteins, we assume that same volume is occupied when the amino acids become a part of a protein chain. The highest concentration of RNA molecules which we allow is 20% volume ratio, and we have considered different values of the molarity of RNA molecules up to this maximum value. The amino acid molarity can be written in the following form

$$A(\vec{x}, t) = A(\vec{x}, 0) - L_p(p_u(\vec{x}, t) + p_f(\vec{x}, t)) \quad (2.5)$$

where $A(\vec{x}, 0)$ is the initial amino acid molarity and L_p is the length of the protein chain. The densities of biomolecules were calculated using their specific volumes [13, 14], and taken to be equal to the approximate value $\simeq 1.3 \times 10^3$ g/lt. For simplicity, we assume that all of the proteins and RNA molecules in the prebiotic soup consist of 100 residues.

2.4.1 Thermal Conductivities and Heat Capacities

Granite, marble, shale and sandstone have been considered as materials with different thermal conductivities. The percent errors in thermal conductivities given in handbooks may be as high as 50%. In order to reduce the amount of error, we have averaged over different values given in various references [15, 16]. The values given in tables are interpolated to give correct values in the temperature range we are interested in, and are shown in Figure 2.6. The heat capacities of the surrounding materials listed above are also investigated. As a function of temperature the heat capacities of these materials are interpolated in the appropriate temperature range and given in Figure 2.7 except for sandstone. In the handbooks we were able to access, the heat capacity of sandstone was only given for 373 Kelvin, therefore we have used this value as constant for the temperature range we are interested in, namely [323,360] K. This assumption is valid in this temperature range since the heat capacities vary only slightly with temperature.

We take the thermal conductivity of the soup to be approximately equal to the thermal conductivity of water. Its variation with temperature is given by Weast et al. [15] and plotted in Figure 2.8. In order to calculate the heat capacity of the soup, one has to take into account the contributions from water, RNA and protein molecules. The total heat capacity of the soup can be written in terms of the partial heat capacities,

$$C = C_{\text{RNA}} + C_{\text{protein}} + C_{\text{amino}}. \quad (2.6)$$

Let us now have a closer look at the heat capacities of each biomolecule:

Heat capacity curves for small proteins generally have characteristic peaks at the transition point signifying a rounded first order transition. The heat capacity of the unfolded protein is approximately 1.5 times the folded heat capacity. The transition temperature can be obtained easily from the heat capacity graphs, however, it depends strongly on the pH of the medium. Figure 2.9 shows the heat capacity curves of lysozyme at different pH values. Increasing the pH value

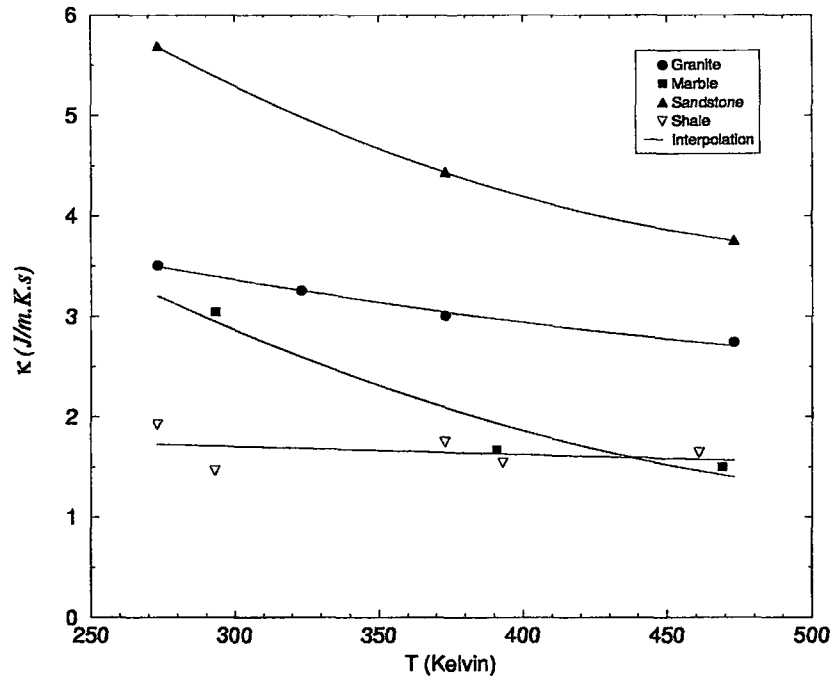


Figure 2.6: Thermal conductivities of granite, marble, shale and sandstone as a function of temperature. The solid lines represent the interpolated data [15, 16].

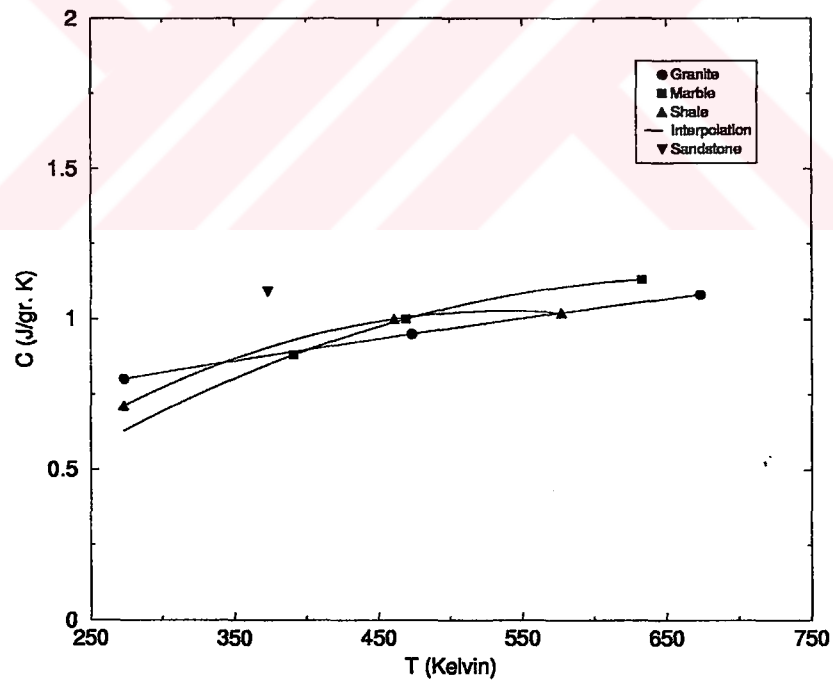


Figure 2.7: Heat capacities of granite, marble, shale and sandstone as a function of temperature. The solid lines represent the interpolated data [15, 16].

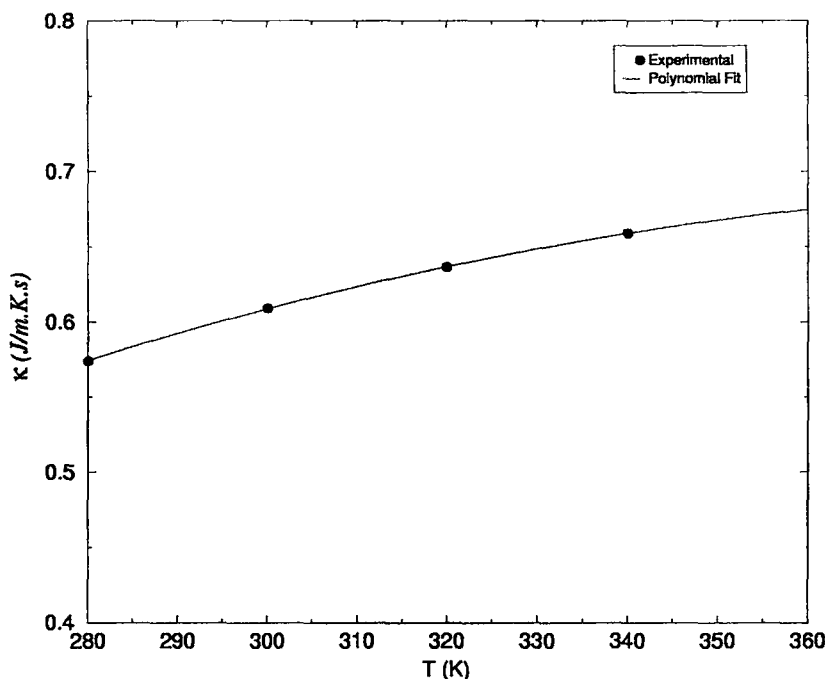


Figure 2.8: Thermal conductivity of liquid water. Points are the experimental values [15, 17]. Solid line is the polynomial interpolation of the data.

results in a shift in the transition temperature and the stability of lysozyme depends on the pH of the medium [18]. For most of the small proteins, the unfolded heat capacities per residue are of the order ~ 2 J/g.K. As it will be seen in further sections, we will be using proteins that partially folded or unfolded. Therefore, we are going to approximate the partial heat capacity of these molecules with the above value.

RNA molecules also have temperature dependent heat capacity curves. There are many softwares for analyzing structure and heat capacity of RNA molecules. We have used the package RNAdraw [19] in order to obtain the heat capacity curves of a given sequence of RNA. The analyzed RNA molecules with 75-125 residues are listed in Table 2.1. The heat capacity curves we have obtained using the sequence data for these RNAs are shown in Figure 2.10.

We have averaged the heat capacity curves, and used this average partial heat

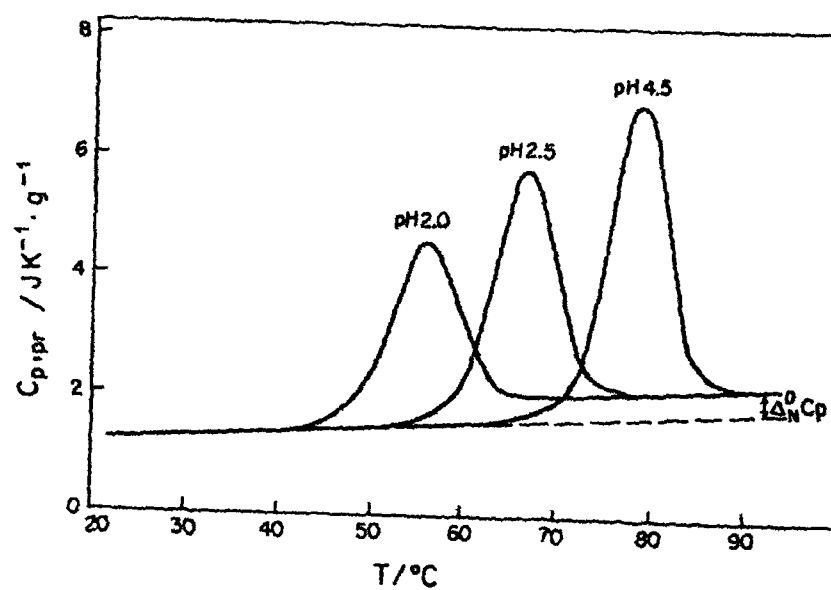


Figure 2.9: Temperature dependence of the partial heat capacity of a typical globular protein, egg-white lysozyme at different pH values (according to Privalov et al [20]). Taken from "Protein Folding", T. E. Creighton, ed., pg. 90 [21].

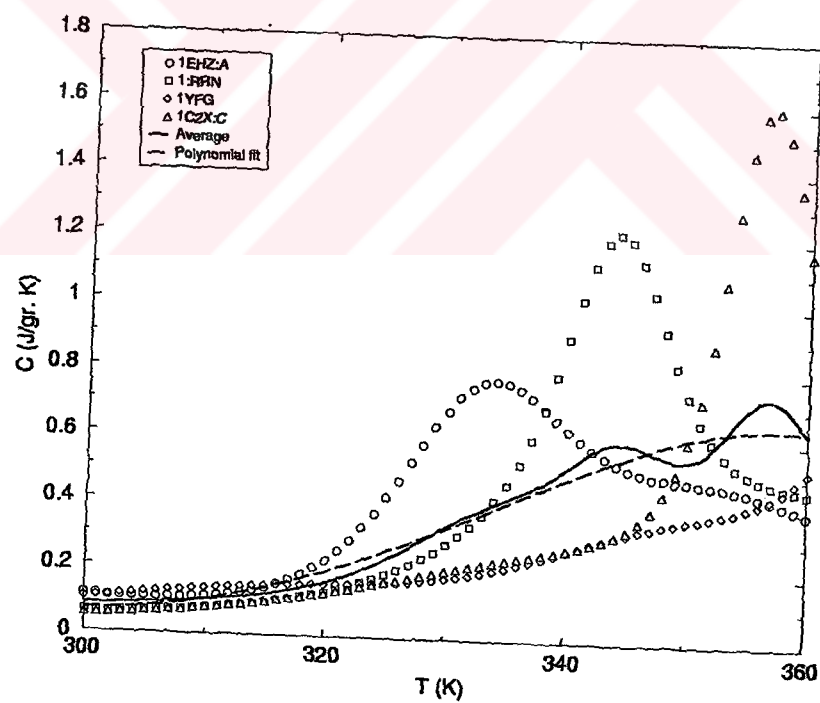


Figure 2.10: Heat capacity curves for various RNA molecules calculated using RNAdraw package [19].

Table 2.1: Molecular Weights of RNA' s having residues between 75-125. Source: Protein Data Bank [22].

PDB-ID	Compound	Molecular Weight (gr/mol)	Number of residues	Molecular Weight per residue
1EVV:A	Phenylalanine t-RNA	21178	76	278.66
1FIR:A	Hiv-1 tRNA	20837	76	274.17
2TRA	Yeast tRNA (A Form)	22899	75	305.32
5TRA	Yeast tRNA	24288	85	285.74
1RRN	5S Ribosomal RNA	40241	118	341.03
1YFG	Yeast Initiator tRNA	21845	75	291.27
1C2X:C	5S Ribosomal RNA	40996	120	341.63
Average				302.55

capacity in the iterations. Since the volume ratio of RNA molecules will be 20% maximum, the error introduced by this averaging is negligible.

Heat capacities per residue estimated for proteins are used as the heat capacities of amino acids. The heat capacity of water is constant in the temperature range we are interested in and has the value $\simeq 4.18$ J/g.K.

At each point in the soup, the heat capacity of the whole mixture can be calculated by multiplying the above molar heat capacities with the total mole of molecules present at that volume.

2.4.2 Molecular Weights, Diffusion Constants

The 20 different types of amino acids present in nature have molecular weights ranging from 89 g/mol to 204 g/mol. Since we are not considering here certain proteins having specific amino acid sequences, we are going to approximate the molecular weight of an amino acid with the average molecular weight ~ 137 g/mol. Chemical decomposition temperatures of amino acids are around 573K therefore in our modeled soup the amino acids have stable structures.

The diffusion coefficients [13] for various biomolecules are given in Table 2.2. These molecules are globular proteins that can be treated as if they were of

Table 2.2: Diffusion constants of various biomolecules in water at 293 K [13].

Molecule	Molecular Weight (gr/mol)	D $m^2/s(\times 10^{-11})$
Ribonuclease	13683	12
Lysozyme	14500	10.6
Myoglobin	17200	11.3
β -lactoglobulin	35000	7.82
Hemoglobin	68000	6.9

roughly spherical shape [23, 24, 25], and with a diameter large compared to that of the water molecule. One can use the Einstein-Stokes equation in order to calculate the diffusion constants of these molecules given by

$$D = \frac{k_B T}{6\pi\eta_0 R} \quad (2.7)$$

where η_0 is the viscosity of the solvent, T is the temperature and R is the radius of the molecule assumed to have a spherical shape. In all the numerical calculations, we will be considering the temperature interval [323,360] in Kelvins. One can take the diffusion coefficient for RNA molecules to be $\sim 8 \times 10^{-11} m^2/s$ assuming that the RNA molecule has a compact structure in this temperature range. According to the cycle explained above, the diffusion coefficient of protein molecules is subject to change due to changes both in temperature and average radius upon folding. However, since the proteins in our model can never fold completely (only about %50 folding can occur), and the changes in T and R affect the diffusion coefficient in reciprocal ways, we have taken the diffusion coefficient of protein molecules as constant in this range. This assumption brings only a $\sim 10\%$ change in the effective radius R if one considers the change in T as 37 K, a change one can expect at the early times of folding. Since we consider proteins consisting of 100 residues, $D \sim 1.2 \times 10^{-10} m^2/s$. (See Table 2.2).

2.4.3 Folding/Unfolding and Synthesis Rates

Protein folding/unfolding rates are widely investigated in the literature [26]. Depending on the type and size of the protein used in the experiments they generally exhibit different behaviours and most of these can be explained using “transition state” theories [27]. For small proteins folding is thought of as a two-state transition and this reversible reaction can be written as



The equilibrium condition for this reaction is

$$K_{eq} = \frac{[D]}{[N]} = \frac{k_u}{k_f}, \quad (2.9)$$

where $[N]$ and $[D]$ are the molarities of proteins in native and denatured states. Here k_f and k_u are the folding and unfolding rate constants respectively. Since the reaction equation does not contain any source or sink terms, for the native state molarity one can write the following equations

$$\frac{d[N]}{dt} = -k_u[N] + k_f[D] \quad (2.10)$$

$$\frac{d[D]}{dt} = k_u[D] - k_f[N], \quad (2.11)$$

subject to the initial condition

$$[N] + [D] = [N]_0, \quad (2.12)$$

if all the proteins are folded at the beginning of the reaction. These rate equations can be solved for both molarities easily. Direct substitution of Eq. (2.12) into Eq. (2.10) yields

$$\begin{aligned} \frac{d[N]}{dt} &= -k_u[N] + k_f([N]_0 - [N]) \\ \frac{d[N]}{dt} + (k_f + k_u)[N] &= k_f[N]_0 \end{aligned} \quad (2.13)$$

If one multiplies both sides of the above equation by the exponential factor $e^{(k_u+k_f)t}$ and combines the two terms on the left side of the equation it takes the form

$$\frac{d}{dt} ([N]e^{(k_u+k_f)t}) = k_f[N]_0e^{(k_u+k_f)t}. \quad (2.14)$$

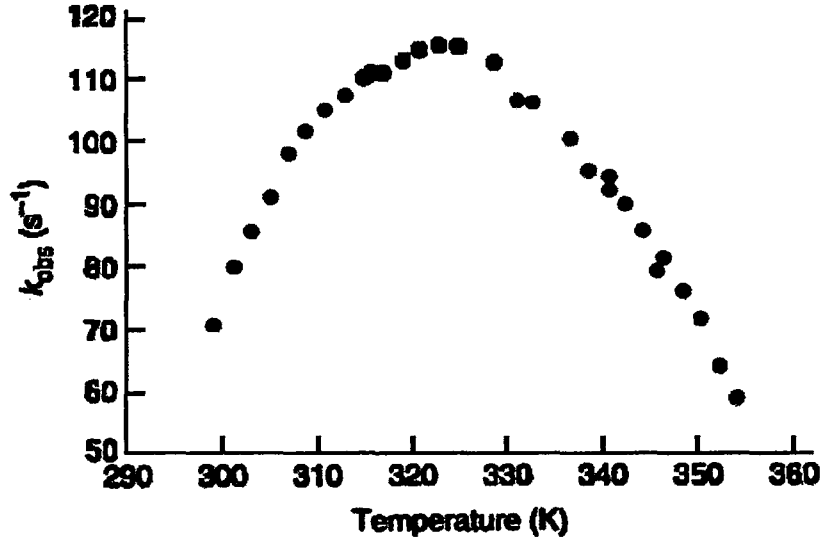


Figure 2.11: Plot of observed rate constant for protein CI2 against temperature. Taken from reference [26].

Integrating this expression and employing the B.C. ($[A] = [A]_0$ at $t = 0$) yields

$$[N] = \frac{[N]_0}{k_u + k_f} (k_f e^{-(k_u + k_f)t} + k_u). \quad (2.15)$$

One can also obtain an expression for $[D]$ using a similar procedure. The characteristic time of the exponential factor is usually denoted by τ_c where

$$\tau_c = \frac{1}{k_u + k_f}. \quad (2.16)$$

Usually the experimentally observed rate constant is the sum of folding and unfolding rate constants, namely $k_{obs} = k_u + k_f$. The temperature dependence of the observed rate constant highly depends on the type of protein used and nature of the transition. Although the rates of most chemical reactions become faster with increasing temperature, the rate constant for folding initially increases with temperature, goes through a maximum, and then decreases [27]. An example of such a measurement is given in Figure 2.11. The individual rate constants are functions of the ΔG of the folding/unfolding transition, mostly determined

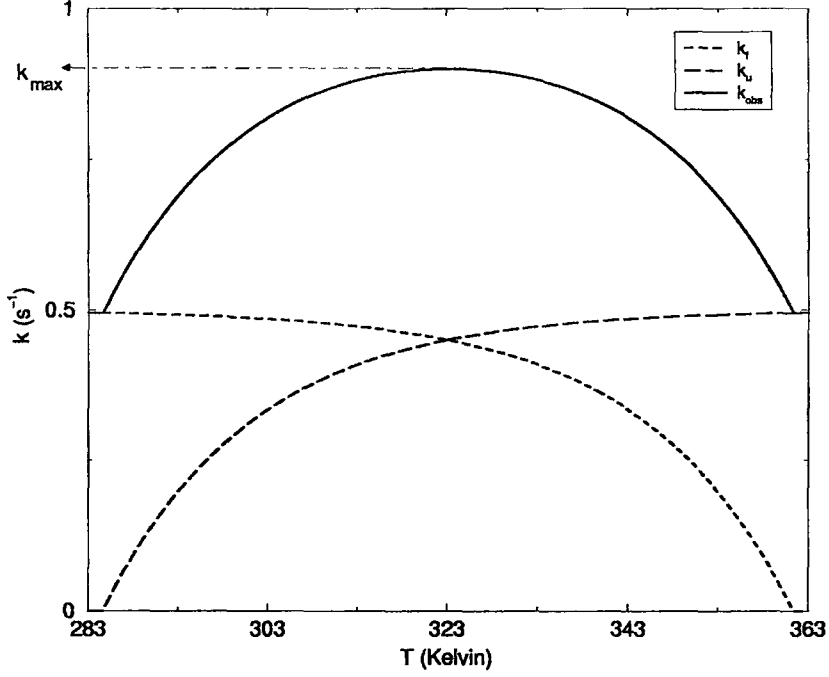


Figure 2.12: Folding and unfolding rate constants given by Eq.(2.17-2.18) are plotted for $b_1 = 0.1$, $b_2 = 0.06$ and $T_m = 323$ K. k_{obs} which is the sum of forward and backward rate constants is also drawn in the graph. k_{max} is the observed rate constant maximum.

experimentally. However, in our prebiotic soup we do not take specific proteins and do not explicitly know the other contents of the soup that might effect the rate constants. Furthermore, our proteins do not fold or unfold completely. In order to model the temperature dependence of these rate constants qualitatively, we have taken the following exponential functions

$$k_u = \frac{1}{2}k_{max} \left(1 - b_1 e^{-b_2(T-T_m)}\right) \quad (2.17)$$

$$k_f = \frac{1}{2}k_{max} \left(1 - b_1 e^{b_2(T-T_m)}\right) \quad (2.18)$$

where b_1 and b_2 are constants, and T_m is the temperature in which the observed folding rate is maximum. The above forms of the rate constants yield the observed rate constant shown in Figure 2.12. It is also seen from this figure that the behaviour of the observed rate constant shown in Figure 2.11 can be captured at least qualitatively. Most of the single domain proteins fold usually

in milliseconds [26], and larger proteins at the most in seconds. Therefore, the magnitude of the rate constants in Eqs.(2.17-2.18) can be very large. There is also recent study by Mayor et al [28] of an extremely fast protein whose unfolding lifetime is ~ 7.5 ns. The protein they have studied, namely the Engrailed Homeodomain protein has the highest folding and unfolding rate constants directly observed. Since the magnitude of these rate constants can vary depending of the type of protein, we have taken the maximum of these rate constants, namely k_{max} as a variable parameter.

The second important rate for our equations is the protein synthesis rate. There is no experimental evidence for the RNA's protein synthesis rate under prebiotic conditions. A plausible assumption would be to take this rate to be equal to the synthesis rates of simple organisms, such as E. coli. A typical E. coli synthesizes 1,400 proteins in a second [29]. Further assuming that this synthesis is made by all the of RNA molecules in the cell, typically 15,000 RNA molecules per cell, one can get a protein synthesis rate of 0.1 proteins per RNA per second per cell. We have used this value in the iterations of our rate equations.

2.4.4 Model and Experimental Entropy Changes of Protein Folding

As expressed earlier the most significant features of observed proteins today are their entropy gaps. When the protein folds, there is a considerable loss of conformational entropy. In order to calculate the entropy difference between the folded and unfolded states, knowing the unfolded state entropy will be enough for real protein chains. Since their ground state is not degenerate, the entropy of the folded state would be zero (modulo the entropy associated with the vibrations of the residues around this folded conformations).

A first approach to calculate the unfolded chain entropy would be to model the chain as a random walk. For simplicity let us assume that the chain lies on a lattice. The entropy of such a random walk of N steps would be

$$S = k_B \ln z^N \quad (2.19)$$

where z is the coordination number of a site on the lattice. For a three dimensional lattice $z = 6$, therefore for a chain of 100 residues

$$S = k_B \ln 6^{100} = 100k_B \ln 6 \simeq 1489 \text{ J/mol.K} \quad (2.20)$$

However, a real protein chain is not a random walk. There are many constraints in the system, such as bond and dihedral angles, and there is also the excluded volume effect which is very important. Therefore, a better approach would be to consider protein molecules as self avoiding walks. For a self avoiding walk of N steps, the entropy is

$$S \sim k_B \ln \tilde{z}^N N^{\gamma-1}. \quad (2.21)$$

Here the exponent γ takes the value $\simeq 7/6$ for all three dimensional lattices [30]. The modified coordination number is $\tilde{z} = 4.68$ for the three dimensional cubic lattice. Therefore, Eq. (2.21) yields

$$S \sim k_B \ln 4.68^{100} 100^{1/6} \simeq 1289 \text{ J/mol.K} \quad (2.22)$$

However, these estimates for protein entropies are not exactly correct since they do not take into account the degrees of freedom due to side chains. Suppose that a side chain has 3 additional degrees of freedom. Then for a chain of length N this would cause an extra $\Delta S = k_B N \ln 3$ increase in the entropy of the chain. Calculations of the residual entropy changes during folding are much more complicated and have sequence dependence. A table showing the residual and hydrational entropy changes for lysozyme is given below [31].

Based on the table data, a protein consisting of 100 residues would have an entropy difference of $\sim 1756 \text{ J/mol.K}$, much more higher than the SAW calculation. Therefore, in order to have realistic energy gaps, we have used these experimental data on protein entropy gaps.

When a protein folds, it loses its hydrogen bonded water molecules, therefore the decrease in the entropy of the protein+water system may be less than the expected value. This effect is called de-hydration. The effect of hydration

Table 2.3: Thermodynamics of lysozyme denaturation [31]. Entropies are given in units J/mol.K Mean ΔS_{res} per residue is ~ 17.56 J/mol.K. The lysozyme protein has 129 residues.

T (K)	ΔS_{obs}	ΔS_{hyd}	ΔS_{res}
283	247	-2026	2273
298	586	-1688	2274
333	1318	-964	2282
373	2067	-224	2291

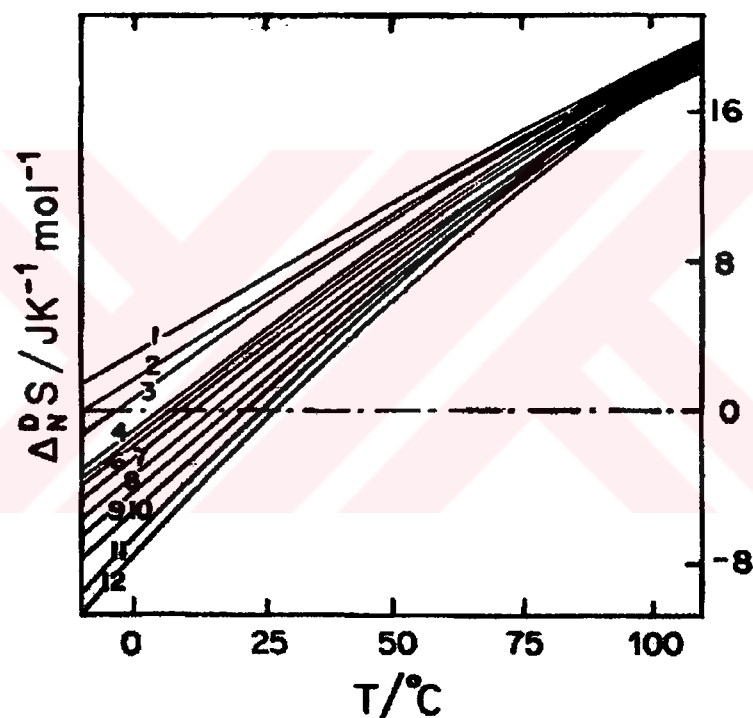


Figure 2.13: Temperature dependence of observed entropy gaps for various globular proteins (according to Privalov et al [32]). Investigated proteins are in order of numbers; ribonuclease A, parvalbumin, egg-white lysozyme, fragment X4 of plasminogen, trypsin, chymotrypsin, papain, staphylococcal nuclease, carbonic anhydrase, cytochrome c, pepsinogen and myoglobin. Taken from “Protein Folding”, T. E. Creighton, ed., pg. 90 [21].

becomes much more important at low temperatures as seen from Table 2.3. Figure 2.13 shows the experimentally observed entropy differences for various proteins by Privalov et al. [32]. Almost all of the proteins have qualitatively same ΔS values, reaching an asymptotic value at high temperatures. The contribution of hydration to free energy is shown in Figure 2.14. In the model we are going to describe in the next section the proteins do not completely fold therefore we

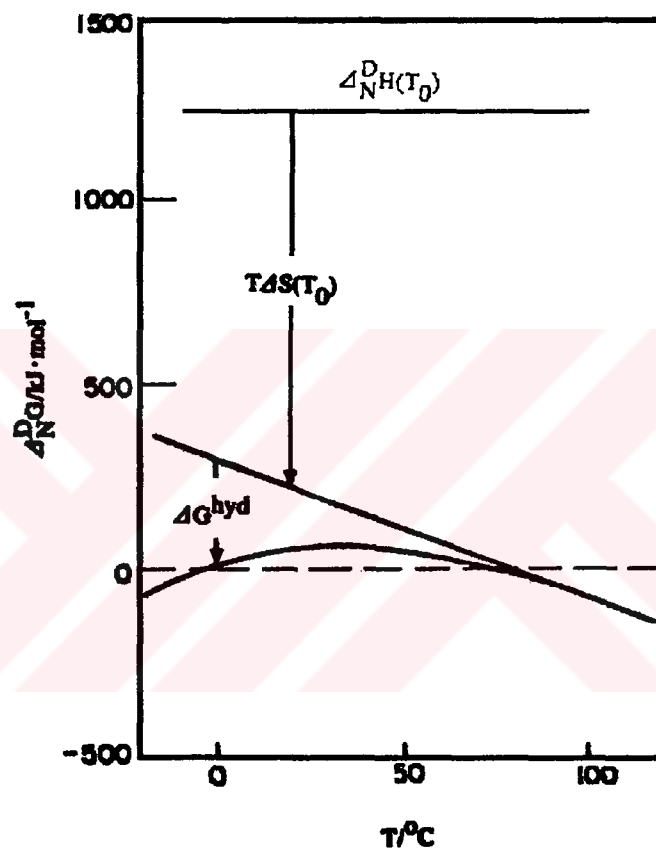


Figure 2.14: Contribution of hydration effect to the stabilization of a globular protein according Privalov et al. [33]. Taken from “Protein Folding” edited by T. E Creighton [21].

will ignore their hydrational entropy changes.

2.6 A Hierarchical Two-state Process to Represent Protein Folding During Refrigeration

There is experimental evidence that the folding transition is like a two-state system for many single-domain proteins [20, 26, 34]. For example, ribonuclease, lysozyme, chymotrysin, cytochrome c and myoglobin all have nearly two-state transitions. Therefore, we are going to assume that protein denaturation is cooperative (or all-or-none) transition in which denaturation occurs in a single step without any intermediates.

The model we are going to consider, is based on the hierarchical zipper-like model proposed by Bak et al. [35], in order to investigate the effect of guiding in the folding process. In the model, proteins are assumed to fold via a folding pathway consisting of nodes representing contacts in the native state. The existence of a unique pathway means that an ordered sequence of binding events occur between different parts of the protein [36]; and if this particular sequence is not followed, the protein can not fold.

Our model can be briefly described as follows. Let us consider a folding event, following a certain pathway as shown in Figure 2.15. One might assign a variable σ_i to each value of a dihedral angle (or a contact point). If a dihedral angle can take q different values, then σ_i can take values between 0 and q . At each node, a decision should be about a dihedral angle. Only one of the σ_i values, namely σ_i^* will be the correct one that will help the chain make a folding move. If one defines

$$\psi_i = \delta_{\sigma_i, \sigma_i^*}, \quad (2.23)$$

then the state variable ψ_i can be assigned to each node on the folding pathway. Now, the values of ψ can be either 0 or 1, depending on whether the correct path is taken at the junction or not. If the correct branch is chosen in the folding pathway, ψ takes the value 1, otherwise 0. Once a wrong branch is chosen, the protein can no longer fold as expected. For instance, the sequences $\{\psi\} = \{1, 1, \dots, 1\}$ and $\{\psi\} = \{0, 0, \dots, 0\}$ correspond to the native and fully

unfolded states respectively. In the model, no unfolding can occur inside an already folded part of the protein. If one desires a strictly two-state behaviour, then the Hamiltonian would be like

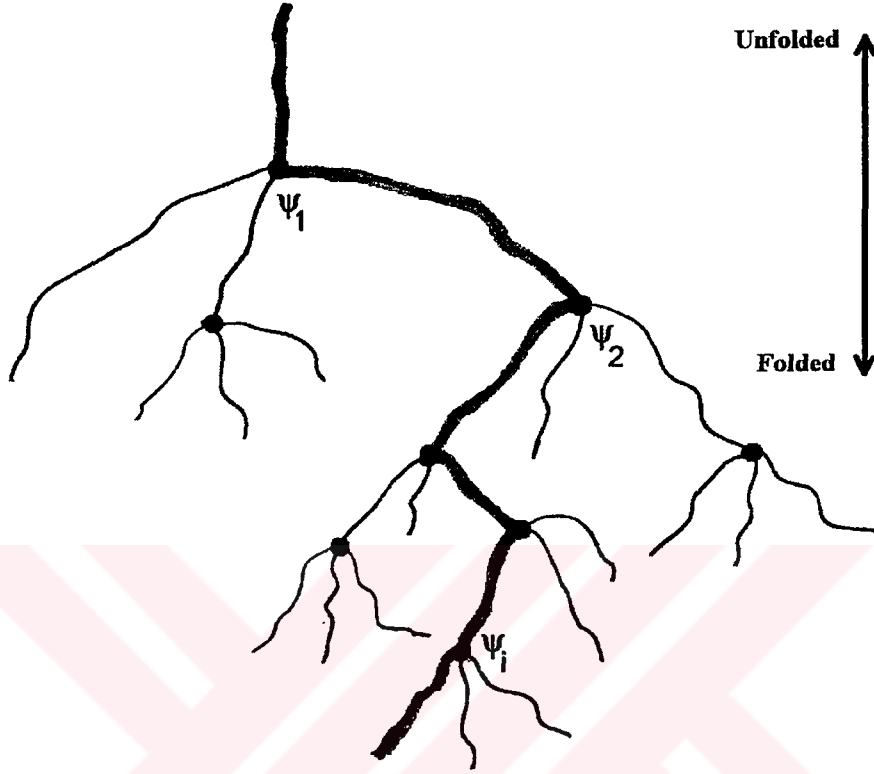


Figure 2.15: A folding pathway. The state variable ψ_i indicates whether the correct choice has been made at the i th node in the folding pathway.

$$-\mathcal{H} = \epsilon \Psi_N N \quad (2.24)$$

where

$$\Psi_N = \psi_1 \dots \psi_N, \quad (2.25)$$

and ϵ is the energy loss due to each folding event. This Hamiltonian allows the protein to be in two distinct states only, native and unfolded states. Although the proteins fold via a two-state folding pathway, especially at higher temperatures the presence of some intermediate states might aid in the folding process to find the native state. It has been experimentally observed [37] that the concentration

of chaperons in *E. coli* rises as the temperature increases, indicating that *E. coli* needs help in order to fold its proteins. A new Hamiltonian giving rise to intermediate states can be constructed in the same manner as follows

$$-\mathcal{H} = \epsilon \sum_{i=1}^N \Psi_i \quad (2.26)$$

where

$$\Psi_i = \psi_1 \dots \psi_i. \quad (2.27)$$

These two Hamiltonians can be combined by means of a parameter λ as follows [35]

$$-\mathcal{H} = \lambda \epsilon \sum_{i=1}^N \Psi_i + (1 - \lambda) \epsilon \Psi_N N. \quad (2.28)$$

For $\lambda = 1$ there are some intermediate states introduced, therefore the process can be named as “guided”, eventually $\lambda = 0$ corresponds to the “unguided” situation. In order to introduce the effect of ordering employed by RNA molecules one can introduce a second set of variables. If we assign a variable τ_i to each amino acid, then

$$\tau_i = 1, \dots, N_{AA}, \quad (2.29)$$

where N_{AA} is the total number of types of amino acids present in nature, namely 20. If the amino acid at a certain position is the correct amino acid as coded by the RNA, then $\tau_i = \tau_i^*$. We can again define a state variable θ_i which is

$$\theta_i = \delta_{\tau_i, \tau_i^*}, \quad (2.30)$$

Each amino acid on a protein chain then can be assigned a θ value, either 0 or 1, depending on the survival (or correct synthesis) of the corresponding amino acid residue. If the template dictated on the chain at a certain residue is preserved (or correct) then $\theta = 1$, if the residue is absent (or wrong) due to the thermal noise present in the system then θ is assigned a value 0. In this study we will take a coarse grained representation of the different amino acids, as hydrophobic and polar residues, so that $N_{AA} = 2$. The new Hamiltonian would then have the form

$$-\mathcal{H} = \lambda \epsilon \sum_{i=1}^N \Psi_i + (1 - \lambda) \epsilon \Psi_N N \Theta_N + J \Theta_N N. \quad (2.31)$$

where J is the energy cost of amino acid ordering and

$$\Theta_N = \theta_1 \dots \theta_N. \quad (2.32)$$

Θ_N is inserted in the second term to make sure that a strictly two-state folding can occur only if the sequence of the protein is completely preserved (or correct). We expect two phase transitions to occur in this system; first transition, namely the order-disorder transition of the amino acid sequence, occurring at a higher temperature between the preserved (or correct)- damaged (or incorrect) order of amino acids, and the second one is a folding-unfolding transition. Since ordering transition must occur before folding, the condition $\frac{\epsilon}{J} > 1$ must be satisfied.

Considering our previous discussion of the refrigeration cycle, “the guiding” here is introduced by the rock in order to aid folding and is controlled by the λ parameter. Since we follow a pathway, the partition function of the system can easily be calculated. For a protein consisting of N residues, the partition function would be

$$Z = \sum_{\{\psi, \theta\}} e^{-\beta \mathcal{H}} = \sum_{\{\psi, \theta\}} e^{\beta \lambda \epsilon \sum_{i=1}^N \psi_i + \beta (1-\lambda) \epsilon \Psi_N N \Theta_N + \beta J \Theta_N N}. \quad (2.33)$$

This partition function can be separated into two sums since the $\{\Theta\}$ set can have only two different values, 0 and 1. Then one gets

$$Z = \underbrace{(2^N - 1) \sum_{\substack{\{\Psi\} \\ \{\Theta\} \neq \{1,1,\dots,1\}}} e^{\beta \lambda \epsilon \sum_{i=1}^N \psi_i}}_A + \underbrace{1 \sum_{\substack{\{\Psi\} \\ \{\Theta\} = \{1,1,\dots,1\}}} e^{\beta \lambda \epsilon \sum_{i=1}^N \psi_i + (1-\lambda) \beta \epsilon N \Psi_N + \beta J N}}_B \quad (2.34)$$

If i labels the first variable where $\psi_i = 0$ then $-\mathcal{H} = (i-1)\epsilon$ and the number of degenerate states at this energy is q^{N-i} . Therefore, the first term A can be written as

$$A = \sum_{i=1}^N q^{N-i} e^{\beta \lambda \epsilon (i-1)} + e^{\beta \lambda \epsilon N} = q^N e^{-\beta \lambda \epsilon} \sum_{i=1}^N e^{(\beta \lambda \epsilon - \ln q)i} + e^{\beta \lambda \epsilon N}$$

Since the series above is a geometric series, one can write the sum as

$$A = q^{N-1} \left(\frac{1 - e^{N(\beta \lambda \epsilon - \ln q)}}{1 - e^{(\beta \lambda \epsilon - \ln q)}} \right) + e^{\beta N(\epsilon + J)} \quad (2.35)$$

The second term has the form

$$\begin{aligned} B &= \sum_{i=1}^N q^{N-i} e^{\beta\lambda\epsilon(i-1)+\beta JN} + e^{\beta N(\epsilon+J)} \\ &= e^{\beta JN} q^N e^{-\beta\lambda\epsilon} \sum_{i=1}^N \left(e^{\beta\lambda\epsilon - \ln q} \right)^i + e^{\beta N(\epsilon+J)} \end{aligned}$$

Once more using the geometric series formula

$$B = e^{\beta JN} q^{N-1} \left(\frac{1 - e^{N(\beta\lambda\epsilon - \ln q)}}{1 - e^{\beta\lambda\epsilon - \ln q}} \right) + e^{\beta N(\epsilon+J)} \quad (2.36)$$

If we define $\nu \equiv \beta\lambda\epsilon - \ln q$, then the partition function is

$$Z = q^{N-1} (2^N - 1 + e^{\beta JN}) \left(\frac{1 - e^{\nu N}}{1 - e^{\nu}} \right) + (2^N - 1) e^{\beta\epsilon\lambda N} + e^{\beta N(\epsilon+J)} \quad (2.37)$$

where

$$\beta = \frac{1}{kT}. \quad (2.38)$$

One can now calculate the Helmholtz free energy

$$F = -kT \ln Z, \quad (2.39)$$

the entropy

$$S = - \left(\frac{\partial F}{\partial T} \right) \Big|_V, \quad (2.40)$$

and heat capacity

$$C = T \left(\frac{\partial S}{\partial T} \right) \Big|_V \quad (2.41)$$

of the system. As seen from Eq.(2.37), the partition function does not have any volume dependent terms, therefore the partial derivatives above can be treated as ordinary derivatives. The entropy and heat capacity functions are not given here, however are plotted in Figures 2.16 and 2.17. We have plotted these for both the unguided and guided cases for comparison. It is obvious from the entropy curves that the system exhibits two first-order transitions in the thermodynamic limit, i.e. $\lim N \rightarrow \infty$, as expected from this two-state model.

One can also define an order parameter, measuring the extent of folding in the system. If we define η as the average number of folded nodes, then it would be

$$\eta = \frac{\sum_{\{\psi, \theta\}} \left(\frac{1}{N} \sum_{i=1}^N \Psi_i \right) e^{-\beta \mathcal{H}}}{Z}. \quad (2.42)$$

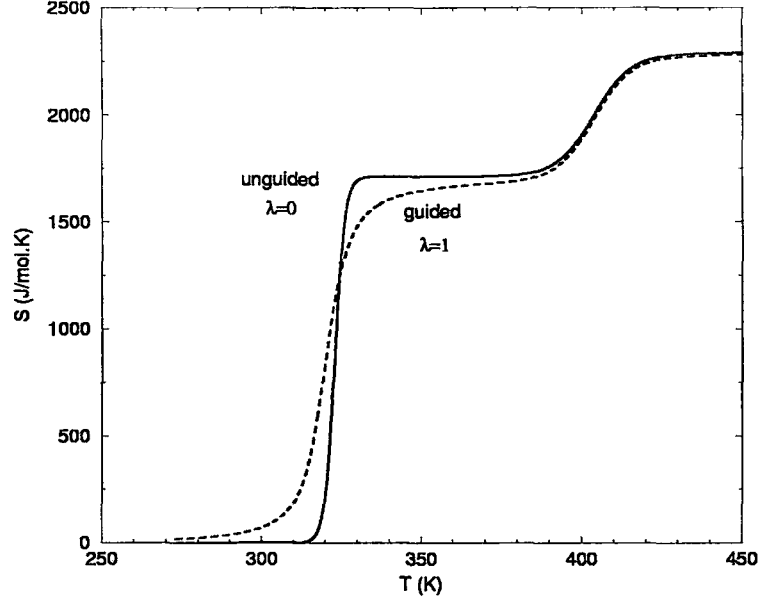


Figure 2.16: The entropy change with respect to temperature for the guided and unguided cases, for $q = 8$ and $\frac{\epsilon}{J} = 2.375$. Both the folding-unfolding and order-disorder transition regions are shown. The effect of guiding is negligible for the order-disorder transition. The folding-unfolding transition temperature is taken to be equal to 323 K.

This can be written as

$$\eta = \frac{1}{NZ} \left[(2^N - 1) \sum_{\substack{\{\Psi\} \\ \{\Theta\} \neq \{1,1,\dots,1\}}} e^{\beta \lambda \epsilon \sum_{i=1}^N \Psi_i} \left(\sum_{j=1}^N \Psi_j \right) + 1 \sum_{\substack{\{\Psi\} \\ \{\Theta\} = \{1,1,\dots,1\}}} \left(\sum_{j=1}^N \Psi_j \right) e^{\beta \lambda \epsilon \sum_{i=1}^N \Psi_i + (1-\lambda) \beta \epsilon \Psi_N N + \beta J N} \right] \quad (2.43)$$

and similar to the the partition function calculation

$$\eta = \frac{1}{NZ} \left[(2^N - 1) \left[\sum_{i=1}^N (i-1) q^{N-i} e^{\beta \lambda \epsilon (i-1)} + N e^{\beta \lambda \epsilon N} \right] + \left[\sum_{i=1}^N (i-1) q^{N-i} e^{\beta \lambda \epsilon (i-1) + \beta J N} + N e^{\beta N(\epsilon + J)} \right] \right]. \quad (2.44)$$

If one defines

$$\tilde{B} = \sum_{i=1}^N (i-1) \left(\frac{1}{q} \right)^{i-1} e^{\beta \lambda \epsilon (i-1)} \quad (2.45)$$

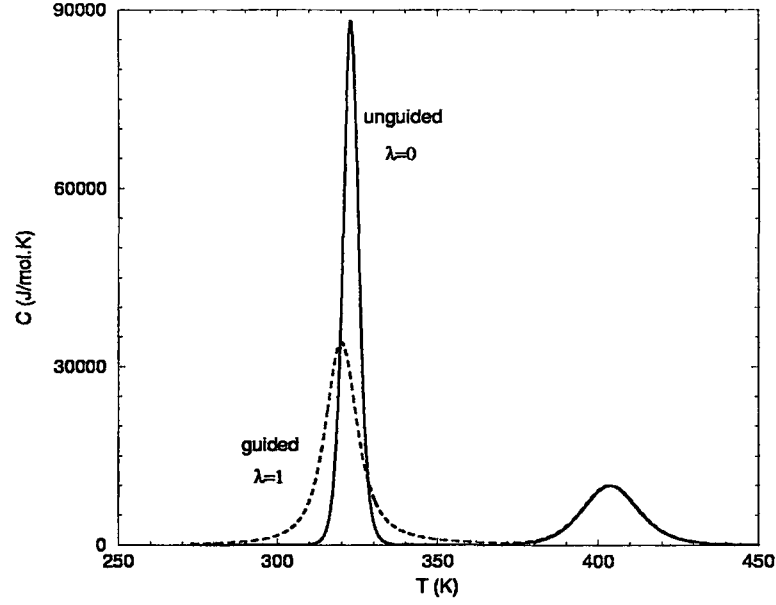


Figure 2.17: The heat capacity curves with respect to temperature for the guided and unguided cases, for $q = 8$ and $\frac{\epsilon}{J} = 2.375$. Both the folding-unfolding and order-disorder transition regions are shown. The effect of guiding is negligible for the order-disorder transition. The folding-unfolding transition temperature is taken to be equal to 323 K.

then the order parameter η can be written in terms of $\tilde{B}$ as

$$\eta = \frac{1}{NZ} \left[(2^N - 1) N e^{\beta \lambda \epsilon N} + N e^{\beta N(\epsilon + J)} + q^{N-1} \tilde{B} (2^N - 1 + e^{\beta J N}) \right]. \quad (2.46)$$

Here $\tilde{B}$ can be written in the form of a geometric series

$$\tilde{B} = \sum_{i=1}^N (i-1) \left(e^{\beta \lambda \epsilon - \ln q} \right)^{i-1} \quad (2.47)$$

which can then be summed as follows

$$\tilde{B} = \frac{e^\nu}{(1 - e^\nu)^2} \left[e^{\nu N} (N - 1) - N e^{(N-1)\nu} + 1 \right] \quad (2.48)$$

or

$$\tilde{B} = \frac{e^{\nu N}}{2 (\cosh \nu - 1)} \left[(N - 1) + e^{-\nu N} - N e^{-\nu} \right]. \quad (2.49)$$

The expectation value of Θ_N can be defined as another order parameter, namely

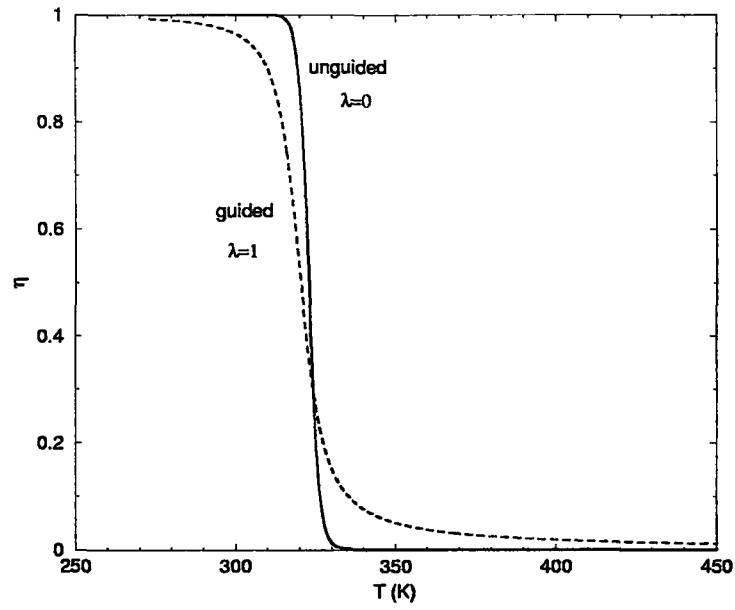


Figure 2.18: The order parameter η is plotted with respect to temperature for the unguided and guided cases ($\lambda = 0$ and $\lambda = 1$) respectively.

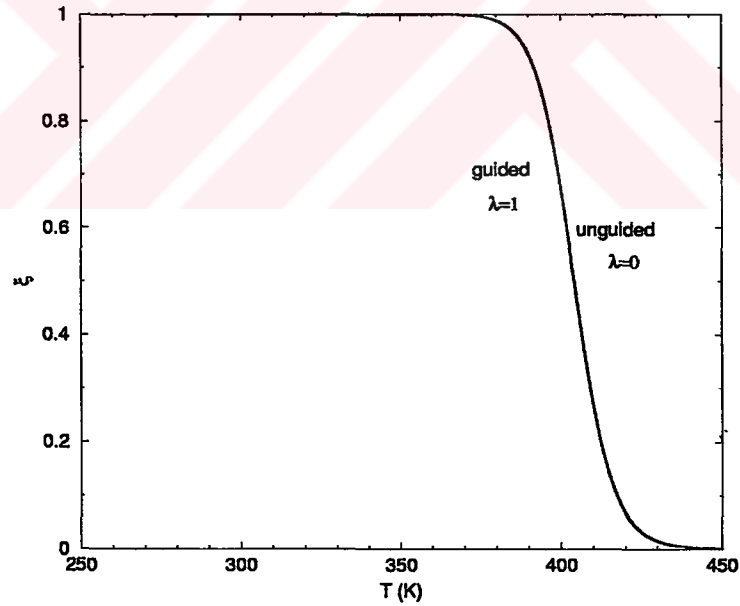


Figure 2.19: The order parameter ξ is plotted for $\lambda = 0$ and $\lambda = 1$. The two curves coincide showing that the presence of guiding, namely rock, does not have an effect on keeping amino acid order.

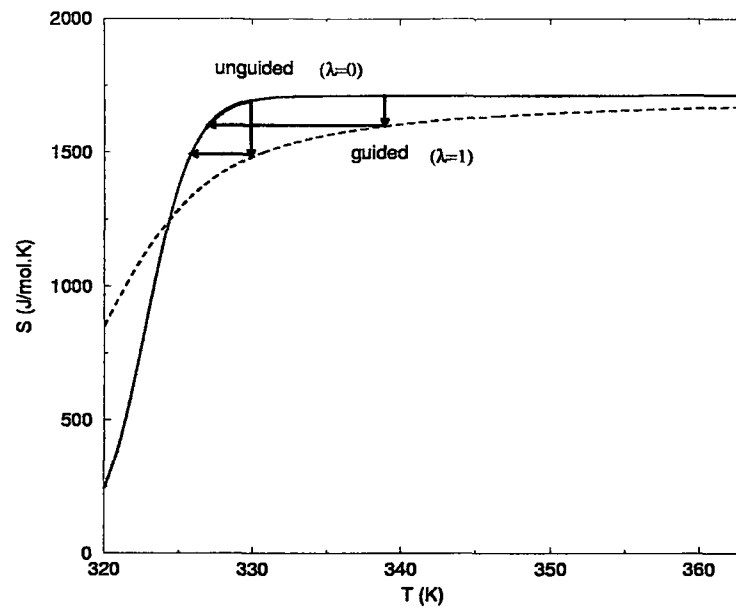


Figure 2.20: A closer look at the entropy changes that occur during a refrigeration cycle. The path of the refrigeration cycle is also drawn.

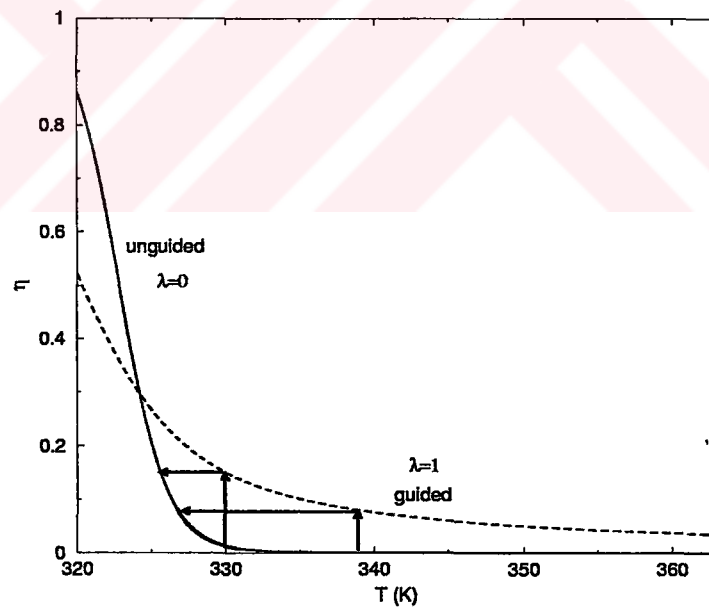


Figure 2.21: A closer look at the change in η as the cycle works. The paths of two following cycle steps are shown by arrows, however the steps on a real cycle will be much smaller.

ξ . Then ξ is

$$\xi = \frac{\sum_{\{\psi, \theta\}} \Theta_N e^{-\beta \mathcal{H}}}{Z}. \quad (2.50)$$

Evaluating the sums above gives

$$\xi = \frac{1}{Z} \left[(2^N - 1) \sum_{\substack{\{\Psi\} \\ \{\Theta\} \neq \{1,1,\dots,1\}}} 0.e^{\beta \lambda \epsilon \sum_{i=1}^N \Psi_i} + 1 \sum_{\substack{\{\Psi\} \\ \{\Theta\} = \{1,1,\dots,1\}}} e^{\beta \lambda \epsilon \sum_{i=1}^N \Psi_i + (1-\lambda) \beta \epsilon \Psi_N N + \beta J N} \right] \quad (2.51)$$

which can be further simplified as

$$\xi = \frac{1}{Z} \left(e^{\beta J N} q^{N-1} \left(\frac{1 - e^{vN}}{1 - e^v} \right) + e^{\beta N(\epsilon + J)} \right). \quad (2.52)$$

As seen from the figures above the effect of guiding, i.e. setting $\lambda = 1$ changes the behaviour of transition, i.e. smoothing out the entropy curves. An example T-S plot for the refrigeration cycle outlined above and the change in the order parameter η during the cycle is given in Figures 2.20 and 2.21. As figures indicate, the cycle will work until the entropy difference between guided and unguided cases tends to zero. At this point, the proteins will be $\sim 30\%$ folded, some of their hydrophobic residues taken inside, therefore they will have less tendency to stick to the rock surfaces. Figure 2.21 also shows how much folding occurs when the unfolded protein sticks to the surface of the rock ($\eta = 1.0$ corresponds to fully folded state).

2.6 Discretization of Rate Equations

Consider the following one dimensional diffusion equation for a scalar quantity u ,

$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2}, \quad (2.53)$$

where D is the diffusion coefficient. The straightforward approach to finite difference this equation is to choose equally spaced points along both time and

position, namely x and t axes respectively. Let us define

$$x_\ell = x_0 + \ell a, \quad \ell = 0, 1, \dots, \ell_{max} \quad (2.54)$$

$$T_t = T_0 + t\tau, \quad t = 0, 1, \dots, t_{max} \quad (2.55)$$

where a and τ represent the grid spacings in position and time respectively. We can denote the value of diffusing quantity as $u_\ell(t)$ at the ℓ th node on the one dimensional grid. There are several choices for representing the time derivative. The obvious way is to set

$$\left. \frac{\partial u}{\partial t} \right|_{\ell, t} = \frac{u_\ell(t+1) - u_\ell(t)}{\tau} \quad (2.56)$$

This is called forward Euler differencing [38]. Although it has first-order accuracy in τ , one can calculate quantities at timestep $t+1$ in terms of only quantities known at timestep t . For the position derivative, one can use a second-order representation still using only quantities known at timestep t

$$\left. \frac{\partial u}{\partial x} \right|_{\ell, t} = \frac{u_{\ell+1}(t) - u_{\ell-1}(t)}{2a} \quad (2.57)$$

The resulting finite difference approximation to Eq. (2.53) would be

$$\frac{u_\ell(t+1) - u_\ell(t)}{\tau} = D \left[\frac{u_{\ell+1}(t) - 2u_\ell(t) + u_{\ell-1}(t)}{a^2} \right] \quad (2.58)$$

or for $u_\ell(t+1)$

$$u_\ell(t+1) = u_\ell(t) + \frac{D\tau}{a^2} [u_{\ell+1}(t) - 2u_\ell(t) + u_{\ell-1}(t)] \quad (2.59)$$

This representation is called the Forward Time Centered Space scheme. It is now easy to generalize this to three dimensions. Assuming that the diffusion coefficient is the same in all directions and the grid is uniform, one can get

$$\begin{aligned} u_{\ell, m, n}(t+1) &= u_{\ell, m, n}(t) + \frac{D\tau}{a^2} [u_{\ell+1, m, n}(t) - 2u_{\ell, m, n}(t) + u_{\ell-1, m, n}(t) + \\ &\quad u_{\ell, m-1, n}(t) - 2u_{\ell, m, n}(t) + u_{\ell, m+1, n}(t) + \\ &\quad u_{\ell, m, n+1}(t) - 2u_{\ell, m, n}(t) + u_{\ell, m, n-1}(t)] \\ &= u_{\ell, m, n}(t) + \frac{D\tau}{a^2} \sum_{\ell', m', n' \in \{nn\}} (u_{\ell', m', n'}(t) - u_{\ell, m, n}(t)), \end{aligned} \quad (2.60)$$

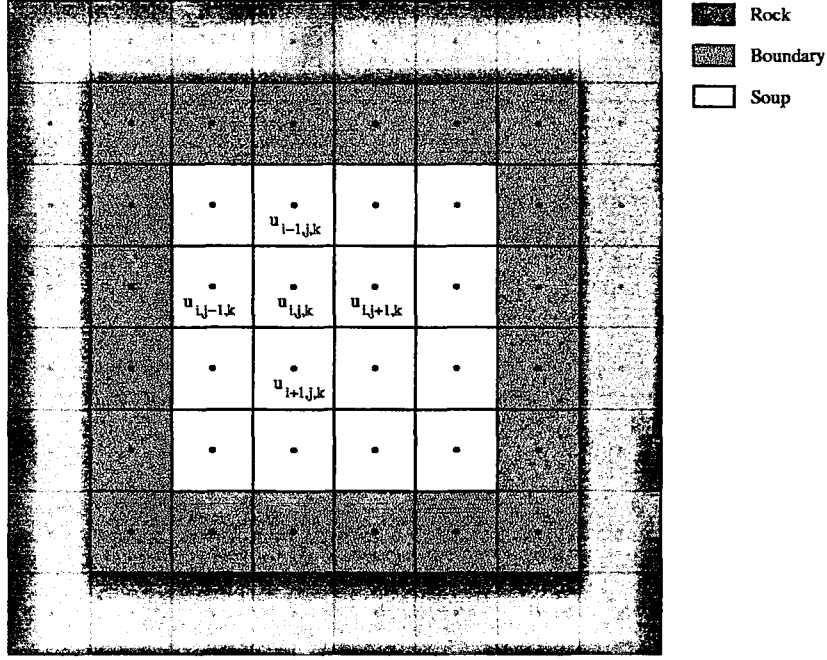


Figure 2.22: A cross section from the three-dimensional grid used in the iterations.

or in shorter notation,

$$u_{\vec{\ell}}(t+1) = u_{\vec{\ell}}(t) + \frac{D\tau}{a^2} \sum_{\vec{\ell}' \in \{nn\}} (u_{\vec{\ell}'}(t) - u_{\vec{\ell}}(t)), \quad (2.61)$$

where $\{nn\}$ denotes nearest neighbors to $\vec{\ell}$. The above discretization of the heat equation is applicable to any type of diffusion equation, by choosing appropriate values for τ and a . The stability analysis of this equation is carried out in the next section. Figure 2.22 shows a cross-section from the three dimensional grid that is used to model the prebiotic soup. At each lattice site there might be one or more fields, i.e. temperature, molarity, etc. Now, one can write the Eqs. (2.1-2.4) for the refrigeration cycle in discretized form as follows

$$\begin{aligned} \frac{r_{\vec{\ell}}(t+1) - r_{\vec{\ell}}(t)}{\tau} &= \frac{D_R}{a^2} \sum_{\vec{\ell}' \in \{nn\}} (r_{\vec{\ell}'}(t) - r_{\vec{\ell}}(t)) \\ \frac{p_{u,\vec{\ell}}(t+1) - p_{u,\vec{\ell}}(t)}{\tau} &= \frac{D_P}{a^2} \sum_{\vec{\ell}' \in \{nn\}} (p_{u,\vec{\ell}'}(t) - p_{u,\vec{\ell}}(t)) - F(T_{\vec{\ell}}(t))p_{u,\vec{\ell}}(t)B(\vec{\ell}) \end{aligned} \quad (2.62)$$

$$\begin{aligned} & +[U(T_{\vec{\ell}}^t)p_{f,\vec{\ell}}(t) + \sigma r_{\vec{\ell}}(t)](1 - B(\vec{\ell})) \quad (2.63) \\ \frac{p_{f,\vec{\ell}}(t+1) - p_{f,\vec{\ell}}(t)}{\tau} = & \frac{D_P}{a^2} \sum_{\vec{\ell}' \in \{nn\}} (p_{f,\vec{\ell}'}(t) - p_{f,\vec{\ell}}(t)) + F(T_{\vec{\ell}}(t))p_{u,\vec{\ell}}(t)B(\vec{\ell}) \\ & - U(T_{\vec{\ell}}^t)p_{f,\vec{\ell}}(t)(1 - B(\vec{\ell})) \quad (2.64) \end{aligned}$$

$$\begin{aligned} \frac{T_{\vec{\ell}}(t+1) - T_{\vec{\ell}}(t)}{\tau} = & \frac{\kappa_{\vec{\ell}}}{C_{\vec{\ell}} \cdot a^2} \sum_{\vec{\ell}' \in \{nn\}} (T_{\vec{\ell}'}(t) - T_{\vec{\ell}}(t)) \\ & + F(T_{\vec{\ell}}(t))p_{u,\vec{\ell}}(t) \frac{T_{\vec{\ell}}(t)\Delta S(T_{\vec{\ell}}(t))}{C_{\vec{\ell}}} B(\vec{\ell}) \\ & - U(T_{\vec{\ell}}(t))p_{f,\vec{\ell}}(t) \frac{T_{\vec{\ell}}(t)\Delta S(T_{\vec{\ell}}(t))}{C_{\vec{\ell}}} (1 - B(\vec{\ell})) \quad (2.65) \end{aligned}$$

where

$$B(\vec{\ell}) = \begin{cases} 1 & : \text{ if } \vec{\ell} \text{ is on boundary} \\ 0 & : \text{ if } \vec{\ell} \text{ is not on boundary.} \end{cases}$$

For the above set of equations the stability is yet another problem. The von-Neumann stability analysis, that we will discuss in the next section only applies to linearized equations, therefore deriving an exact stability condition for these equations is not easy.

2.7 Numerical Stability

2.7.1 Biological and Physical Limits on Stability

Before discussing the numerical stability of the rate equations describing the refrigeration cycle, one should also emphasize the biological limits that might affect stability. Since these equations have to be solved for pores having certain sizes, the pore size is an important limit in choosing the lattice spacing. For small pore sizes, eventually the lattice spacing a has to be decreased by the proper amount. The second important factor is the folding time of proteins. As stated earlier, we have modeled folding transition as a two-state process which occurs very fast. However, there is also an upper limit found for folding time experimentally. For most of the proteins, folding occurs at the order of milliseconds. For smaller proteins of 50-60 residues, this can be a thousand

times or more faster. The time increment τ used in the iterations can not be smaller than the folding time of proteins. Therefore, for very small pores of size $< 0.1\text{mm}$ these equations can not be solved by simple iteration. One should go to stochastic equations.

2.7.2 Von-Neumann Stability Analysis and Its Application to Simple Diffusion

The von Neumann stability analysis is a local method. One should assume that the coefficients of the difference equations are so slowly varying that they can be considered constant in space and time. In that case, the independent solutions of the difference equations are all of the form [38]

$$u_\ell(t) = \zeta^t e^{i\tilde{k}\ell a}. \quad (2.66)$$

where $\tilde{k}$ is a real spatial wave number and can have any value and ζ is a complex number. The difference equations are unstable, in other words have exponentially growing modes, if $|\zeta| > 1$ for some $\tilde{k}$. The number ζ is called the amplification factor at a given wave number $\tilde{k}$. For a scheme to be stable, $|\zeta| \leq 1$ condition should be satisfied. Let us first investigate the stability of the simple diffusion equation given by (2.59). Substituting Eq.(2.66) into Eq.(2.59) yields

$$\zeta^{t+1} e^{i\tilde{k}\ell a} - \zeta^t e^{i\tilde{k}\ell a} = \frac{D\tau}{a^2} [\zeta^t e^{i\tilde{k}(\ell+1)a} - 2\zeta^t e^{i\tilde{k}\ell a} + \zeta^t e^{i\tilde{k}(\ell-1)a}]. \quad (2.67)$$

Simplifying the above equation by canceling the exponential factors and solving for ζ gives

$$\zeta = 1 + \frac{D\tau}{a^2} [e^{i\tilde{k}a} + e^{-i\tilde{k}a} - 2] \quad (2.68)$$

$$= 1 + \frac{2D\tau}{a^2} [\cos(\tilde{k}a) - 1] \quad (2.69)$$

Since the cosine function can take values in the interval $[-1,1]$, the maximum and minimum values of ζ are

$$\zeta_{max} = 1 \quad (2.70)$$

$$\zeta_{min} = -\frac{4D\tau}{a^2}. \quad (2.71)$$

Applying the stability condition gives the following inequality for the stability of the difference scheme

$$\frac{D\tau}{a^2} \leq \frac{1}{2}. \quad (2.72)$$

In order to satisfy this stability condition one has to choose the time and space intervals appropriately. Failing to satisfy this condition, results in blow ups in the equations. For the three dimensional grid, same approach can be used and substitution of Eq. (2.66) into the discretized equation yields the following expression for ζ

$$\zeta = 1 + \frac{2D\tau}{a^2} [\cos(\tilde{k}_x a) + \cos(\tilde{k}_y a) + \cos(\tilde{k}_z a) - 3]. \quad (2.73)$$

Similarly the maximum and minimum values of ζ are

$$\zeta_{max} = 1 \quad (2.74)$$

$$\zeta_{min} = -\frac{12D\tau}{a^2}. \quad (2.75)$$

giving the condition

$$\frac{D\tau}{a^2} \leq \frac{1}{6}. \quad (2.76)$$

for the stability of the solutions.

In order to reach a stable solution, one has to obey such stability conditions in choosing a and τ , however, derivation of such a condition is not always trivial especially for nonlinear and coupled equations.

2.7.3 A Simplified Approach to the Stability of Refrigeration Cycle Equations

The rate equations modeling the refrigeration cycle are a set of coupled nonlinear equations, having complex temperature dependence. Von-Neumann stability analysis only works for linear or linearized equations. However, in order to have an idea of which factors affect stability, one can consider only the equation for temperature and derive a local stability condition for each lattice site, instead of a global stability condition.

Let us assume that the rates and entropy differences appearing in Eq.(2.65) are constants independent of the temperature. Von-Neumann stability analysis does not take into consider the effects on boundaries. Therefore, one can write the temperature equation for a point inside the soup as

$$\begin{aligned} \frac{T_{\ell}(t+1) - T_{\ell}(t)}{\tau} = & \frac{\kappa_{\ell}}{C_{\ell} \cdot a^2} \sum_{\tilde{\ell} \in \{nn\}} (T_{\tilde{\ell}}(t) - T_{\ell}(t)) \\ & - U p_{f,\ell}(t) \frac{T_{\ell}(t) \Delta S}{C_{\ell}}. \end{aligned} \quad (2.77)$$

Substituting Eq.(2.66) into Eq.(2.77) yields the following expression for ζ

$$\zeta = 1 + \frac{2\kappa_{\ell}\tau}{C_{\ell}a^2} [\cos(\tilde{k}_x a) + \cos(\tilde{k}_y a) + \cos(\tilde{k}_z a) - 3] - \frac{\tau \Delta S}{C_{\ell}} U p_{f,\ell}. \quad (2.78)$$

Once more substituting maximum and minimum values of the cosine function one can get

$$\zeta_{max} = 1 - \frac{\tau \Delta S}{C_{\ell}} U p_{f,\ell} \quad (2.79)$$

$$\zeta_{min} = 1 - \frac{12\kappa_{\ell}\tau}{C_{\ell}a^2} - \frac{\tau \Delta S}{C_{\ell}} U p_{f,\ell} \quad (2.80)$$

giving the condition

$$\frac{\kappa_{\ell}\tau}{C_{\ell}a^2} + \frac{\tau \Delta S}{12C_{\ell}} U p_{f,\ell} \leq \frac{1}{6}. \quad (2.81)$$

for the stability of the solutions for a given folded protein molarity. Since our equations are both coupled and nonlinear we do not expect Eq.(2.81) to give a correct bound for stability, however, it gives a rough estimate of the τ values one can use for a given pore size.

2.8 Results of Numerical Iterations

In order to find the best pore sizes and RNA volume ratios this cycle works, we have solved the equations for three different pore sizes, namely $L = 1\text{dm}$, 1cm and 1mm , and for RNA volume ratios ranging from 2×10^{-1} to $2 \times 10^{-5}\%$. We have substituted all the experimental values listed above, and iterated equations(2.1-2.4) for different pore sizes, with the rock taken as granite,

folding/unfolding rates and initial volume ratio of RNA molecules. We have used the ΔS values obtained from our two-state model normalized to experimental entropy gaps, which is obtained by the proper choice of q . For different pore sizes, we have to use different time steps in order to satisfy the stability condition which was approximated by equation(2.81). In all the iterations carried out we have used at least 5×10^5 time steps, and depending on the size of the time increment, τ , this value is increased. The steady state temperatures reached at the end of iterations are plotted in Figure 2.23.

As seen from the figure, the cycle works best for pores having a volume of 1cm^3 . For RNA volume ratios greater than a threshold which is approximately 2×10^{-3} , the temperature of the soup is sufficiently lowered. The biggest time increment allowed by stability for a pore of linear size 1mm is $\tau = 0.004$ s, therefore iterations are still being carried out to ensure that steady state is reached. Since the cycle best works for linear size 1cm, let us now investigate it in more detail. We have tried different folding/unfolding rate maxima, namely $k_{max} = 1, 10, 100$ and for the RNA volume ratio %2. The variation of temperature with respect to time is given in Figure 2.24.

The RNA molecules have a key role in the cycle. Without them, the cycle would not be able to work, therefore, the minimum amount of RNA molecules needed has to be investigated. We have also monitored the temperature change for RNA volume ratios in the interval $[2 \times 10^{-1}, 2 \times 10^{-5}]$. Volume ratios smaller than $\sim 2 \times 10^{-3}$ seem not enough to cool down the soup in the presented time interval in Figure 2.25. Longer iterations might be needed to attain low temperatures, however at longer time scales the effect of transport through the channels should also be considered.

The temperature profile of the pore, at given time intervals is plotted in Figure 2.26. As a first approach one might consider testing this cycle between two points only, one kept at the rock temperature, and the other corresponding to the soup. However, the profile shows this approach is not correct since the temperature of

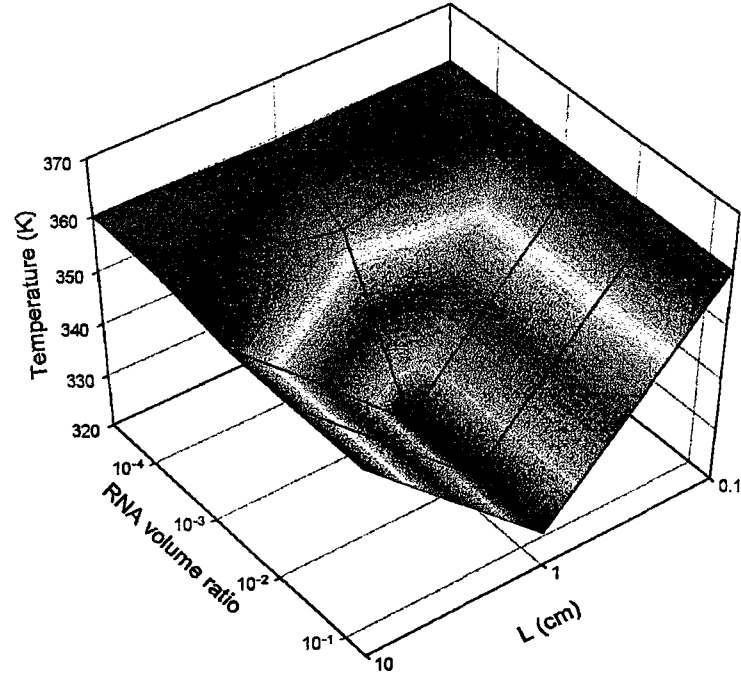


Figure 2.23: Steady state temperature reached for $k_{max} = 10.0$ for different pore sizes and RNA volume ratios. The mesh plane shown in figure is the linear interpolation between the temperature values obtained from iterations.

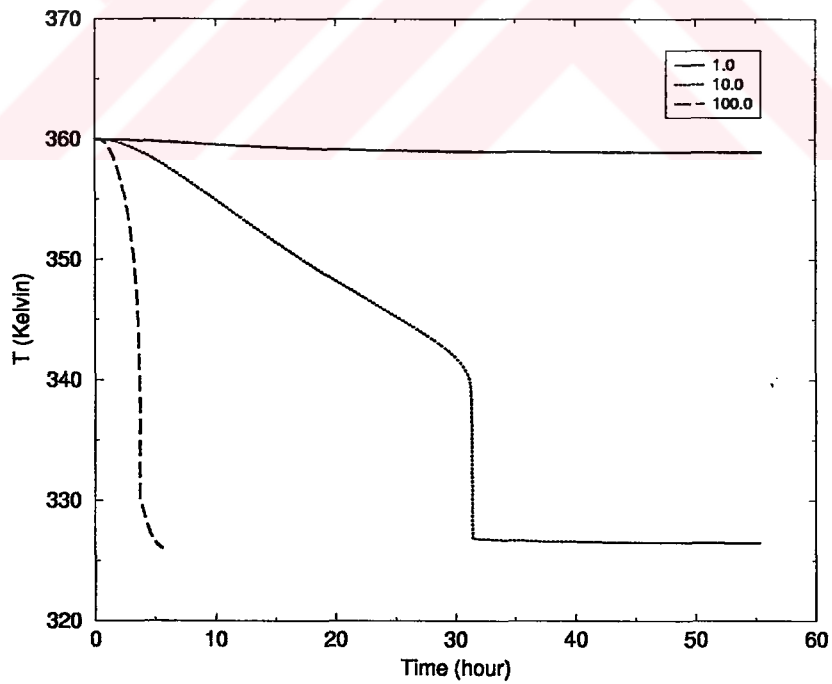


Figure 2.24: Temperature change with time for different values of k_{max} .

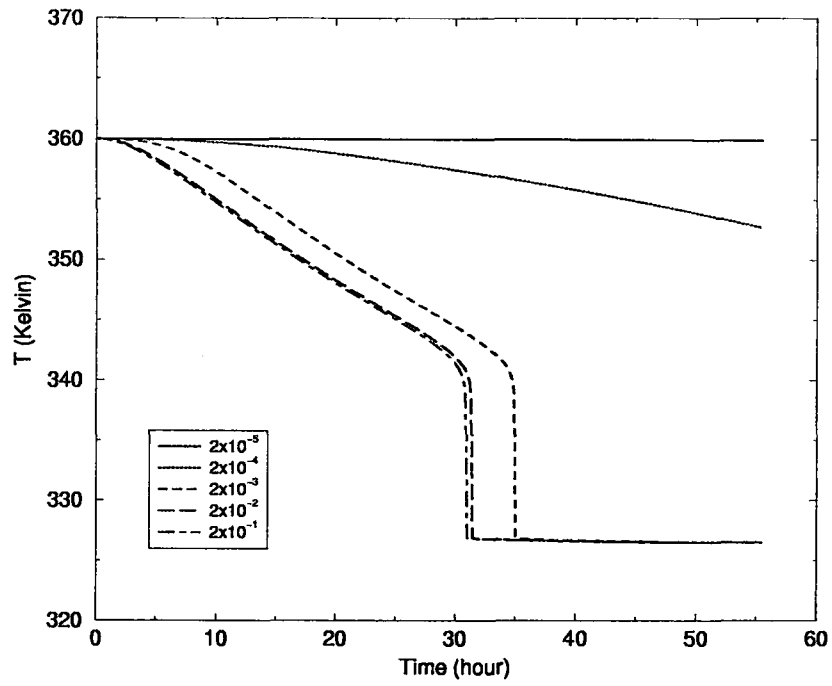


Figure 2.25: Temperature change with time for different values of RNA volume ratio in the interval $[2 \times 10^{-1}, 2 \times 10^{-5}]$.

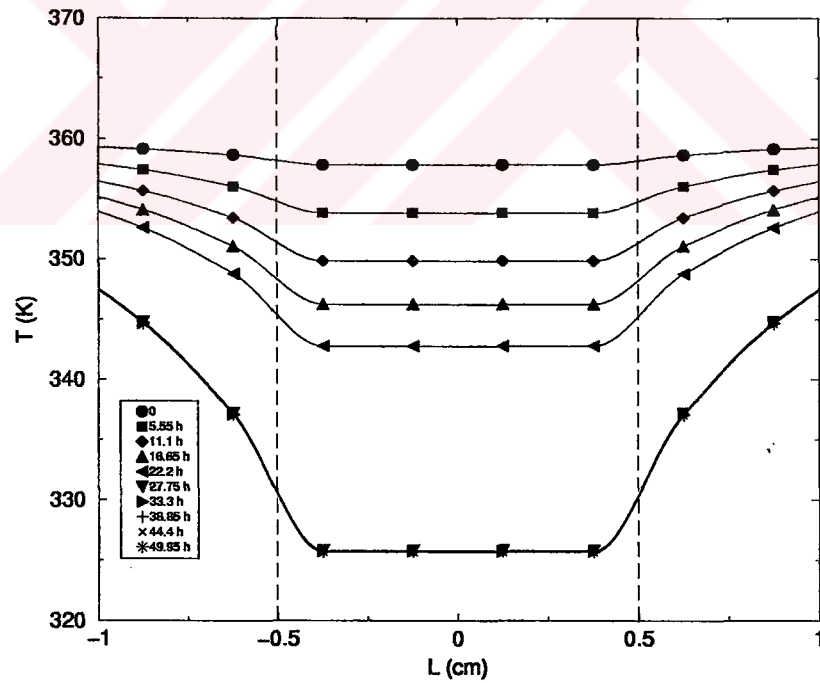


Figure 2.26: Temperature profiles from the center cross section of the pore of 1cm side length at different time intervals.

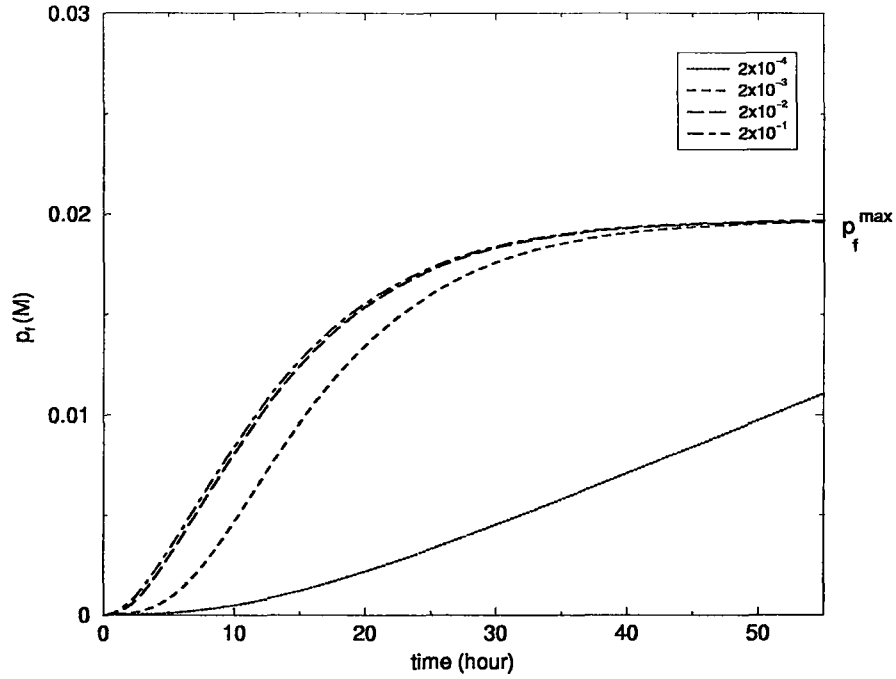


Figure 2.27: Change in folded protein molarities as steady state is approached for different RNA volume ratios.

the rock near the boundary is also changed in the steady state as seen from the steady state temperature profile.

The efficiency of the refrigeration cycle depends strongly on the molarities of the folded and unfolded proteins. Therefore, the steady state molarities of proteins are also of interest. For a pore of side length 1cm, for $k_{max} = 10.0$, the change in folded and unfolded protein molarities as steady state is approached are plotted in Figures 2.27 and 2.28. The figures represent the total molarities of proteins, summed over all the lattice points. The RNA molecules synthesize unfolded proteins. Therefore, depending on the volume ratio of RNA molecules, approach to steady state shows different behaviours. For RNA volume ratios below 2×10^{-3} , we have not been able to observe the steady state reached by the protein molarities (approach to p_u^{max} shown in Figure 2.29 is logarithmic).

We have also investigated the protein molarity profiles at different lattice points. Since, the lattice is symmetric, the values of molarities at two different lattice

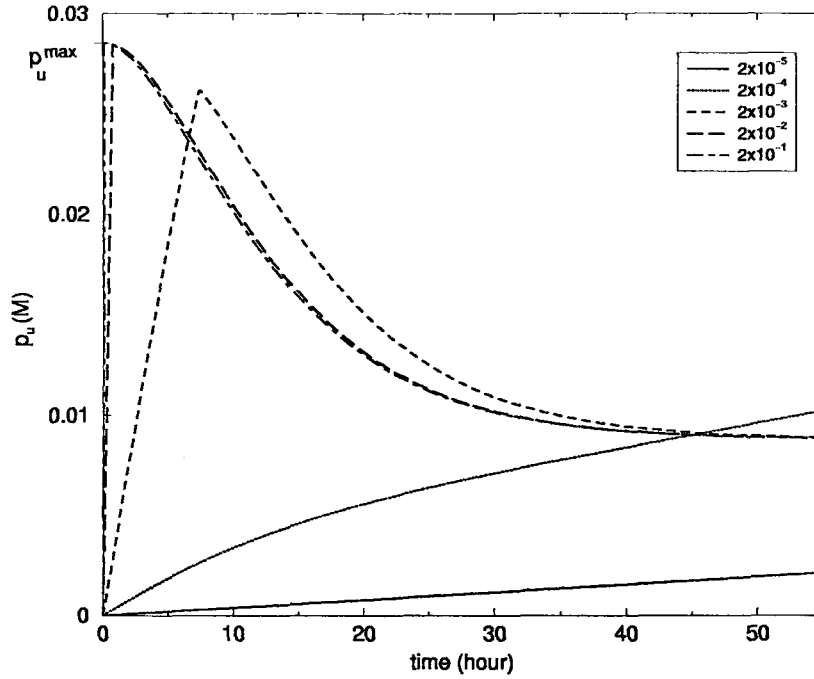


Figure 2.28: Change in unfolded protein molarities as steady state is approached for different RNA volume ratios.

points, one near the center and one at the soup boundary would be enough to understand the molarity profiles. Figure 2.29 and 2.30 shows the approach to steady state at these two points in the soup.

We have also tested the presence of hydration effect by using an engineering approach, namely adjusting the ΔS value to the average experimentally observed entropy gaps including the negative effect of hydration for different proteins at the transition temperature. This of course lowers the entropy gap, however, the results are qualitatively similar.

2.9 Discussion

The above results indicate the success of such a refrigeration scenario. We see for the present assumptions of RNA and protein molecules of moderate size, with 100 residues, that the cycle works best for the pores of linear size 1cm.

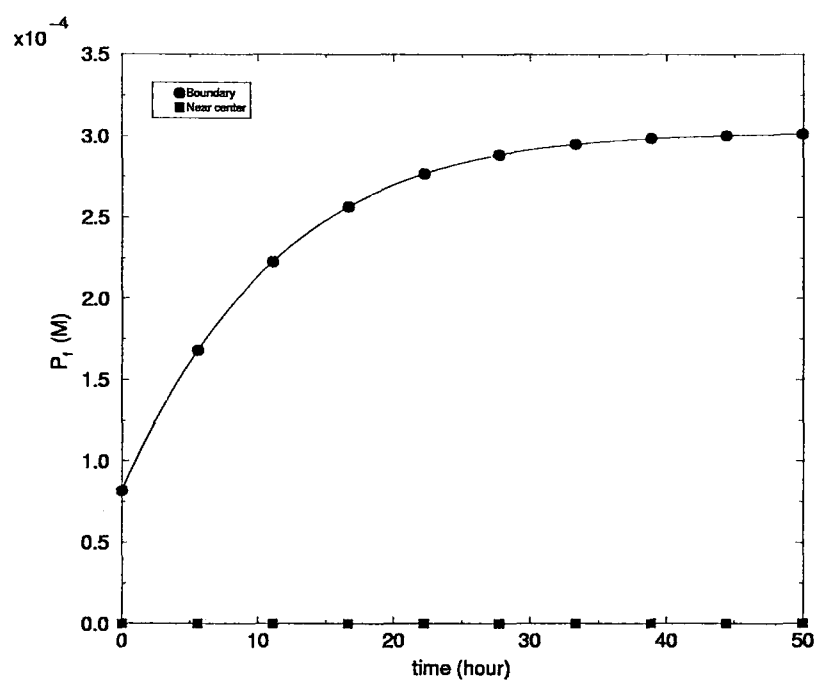


Figure 2.29: Change in folded protein molarities as steady state is approached for two points; one at a boundary and another near the center. RNA volume ratio is 2%.

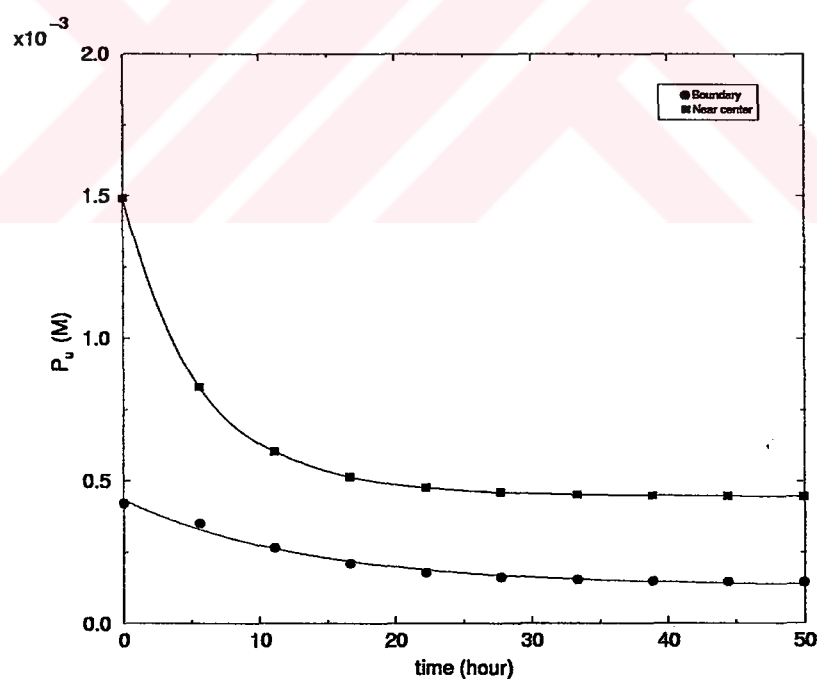


Figure 2.30: Change in unfolded protein molarities as steady state is approached for two points; one at a boundary and another near the center. RNA volume ratio is 2%.

If such a scenario took place at any time in the pre-biotic earth, this should have happened in pores of appropriate sizes. Besides, results also indicate the importance of the folding/unfolding rate constants. Proteins with larger rate constants (so called heat-shock proteins) and having larger entropy gaps observed today would better cool their soup resulting in an enhancement of their environment. One might also speculate that this is the reason for the presence of very fast folding proteins observed today under extremely hot conditions. The RNA molecules, synthesizing fast folding proteins, might have replicated more and become dominant in nature due to the lowering of the temperature in their vicinity by the help of their proteins.

The RNA molecules we have taken in the model can not replicate. Of course, we have in mind a scenario in which the RNA molecules can replicate and increase their population as a result of the cooling in their environment. It is clear that if the replication rates of RNAs are enhanced as a result of the decreasing temperature, those RNA molecules that can synthesize large gap-fast folding proteins would increase their populations noticeably after some time.

The efficiency of protein folding can be adversely affected if partially folded proteins aggregate in order to reduce exposed hydrophobic surfaces. Molecular chaperons bind reversibly to these partially folded chains preventing their aggregation and promoting their passage down the folding pathway. Therefore, one might also speculate that these chaperons have taken the role of rock surfaces in the course of higher evolution.

We do not claim that this cycle is the only mechanism that might have operated in the prebiotic earth. A different mechanism affecting the coevolution of proteins and RNA is that proposed by Eigen [2], namely the catalysis of RNA replication by certain proteins. Other studies besides ours have demonstrated that rock surfaces may have played a selection role in prebiotic conditions, c.f., the selection of right handed amino acids binding to optically active surfaces of calcite crystals with same chirality [39]. This work, and many others reveal the

possibility of other scenarios that might have taken part in biological selection. However, our calculations show that for realistic values of the physical constants involved, the proposed refrigeration cycle could very well have been responsible for the co-evolution of fast folding proteins with large entropy gaps and the RNA molecules which code them.



3. STRATEGIES FOR THE EVOLUTION OF SEX

3.1 Why Study Sex ?

Sexual reproduction is believed to have arisen already in unicellular organisms [5, 40] and such simple organisms who engage in sex, either habitually or facultatively, especially when the external conditions are unfavorable, such as certain yeasts, fungi or the green algae *Chlamydomonas* [29, 41, 42] are extant today. Since for such simple organisms, each act of fusion typically reduces the population by one (one offspring is produced at the expense of two parents) how such a mode of reproduction could have established a foothold in the evolutionary game remains a puzzle.[5, 40]

It is well known that the simplest unicellular organisms (prokaryotes, which include bacteria) multiply asexually, by cloning (mitosis) resulting, modulo possible errors in replication, in two copies of the original cell. These organisms possess only one set of genes, and are known as haploids. By contrast, eukaryotes, which comprise all the higher organisms, as well as many unicellular life forms, possess, at least in certain phases of their life cycle, two (not necessarily identical) sets of their complete genome. Although there is no firm evidence to support this fact, it is speculated that sexual reproduction may have arisen simultaneously with the emergence of diploidy, or at least soon thereafter.[40].

Many authors have argued [5, 40, 43, 44] that diploidy presents obvious advantages over haploidy, since such simple organisms face an even higher rate of random mutations, than more complex beings. Having an unimpaired copy of the damaged alleles and being able to make use of this copy in repairing the damage presents a clearcut advantage. It is generally assumed, moreover, that

deleterious mutations are recessive, so that in case an undamaged allele is present at some locus, this will be expressed in the phenotype rather than the damaged one, although it is not clear at what stage this mechanism of dominance has come into play as an inherited feature of genetic control.

The supposed advantages of fusion in giving rise to greater genetic variety, combined with these advantages of diploidy, have been studied via numerical models, by many groups [45, 46, 47, 48, 49, 50]. Notably Redfield[45] has shown, within a constant population model, that unless there is a much greater mutation rate for males, sex increases the mean fitness in a population, if a step-function like survival function in the number of deleterious mutations is assumed. The naturally occurring relative mutations rates for males, however, are apparently such that parthenogenesis would seem more favorable than sex. Modifying Redfield's model, Stauffer et al. [46, 47], Bernardes [48] and Cui et al. [50] have been able to show within "age structured" populations [51], that sexual reproduction can indeed lead to better results. Others [49, 52, 53] have discussed and modeled the proposition that a parasitic infestation could afford sex with an advantage over both asex and meiotic parthenogenesis in the greater genetic diversity it provides. Pekalski [54] has shown the superiority of sexual reproduction in adaptation to a periodically changing environment.

We would like to further investigate a very simple model for archaic sex, which does not have any of the more complex features discussed by the models cited above, such as sexual differentiation between males and females, or age structuring. We present computer simulations for several different strategies, and compare their outcomes with the iterations of a set of rate equations [8, 9].

In a previous study by Orcal et al. [55] we have shown that a finite steady state sexual population may arise from a purely asexual one, if an excess of deleterious mutations causes haploid individuals to perform syngamy and become diploid, and diploid organisms to engage in sexual reproduction as a means of escaping death [7]. We assumed, as is generally done, [45, 47] that mutations which

lead to departures from the “wild type” (the ideal type) are deleterious, and that deleterious mutations are recessive. Under various different assumptions regarding the subsequent mode of reproduction (i.e., whether sexual reproduction is hereditary or not) and of the number of offsprings, we found that the diploid population always persisted, and that it was consistently more successful in escaping the effects of deleterious mutations.

In this chapter we show the results of a threshold mechanism for switching to sexual reproduction, and test whether it is necessary for the successful establishment of a sexual population. We simulate two strategies for the evolution of sex within a fixed population N of simple organisms, who are all initially asexual (and haploid), and subject to a constant rate Γ of random mutations. Both haploid and diploid organisms die when the number of their expressed deleterious mutations exceed a certain number. We have also adopted a more realistic set of rules than in Ref. [55] for the mechanism of dominance, that is, the expression of mutated alleles. Here we allow a mutated gene to be expressed if the cell is homozygous for mutated alleles at this locus. Hence, the number of expressed deleterious mutations for diploid individuals is the number m of different loci at which the cell is homozygous for mutated alleles.

The first strategy, (Model *A*) is the adoption, with a certain probability, of syngamy and consequent diploidy when the number of mutations exceeds a threshold, threatening extinction. The second strategy (Model *B*) involves a small but constant probability σ for the accidental conversion to diploidy, independently of the number of mutations (or, equivalently, the fitness) of the individual. In model *A*, without habitual sex, the diploids then engage in sexual reproduction when they are similarly threatened by an excess of deleterious mutations. In Model *B* without habitual sex, they do so with a probability σ . The cloning of sexual individuals is not allowed in either Model *A* or *B*. We have also tested for the effect of habitual v.s. non-habitual sex.

We find that both strategies *A* and *B* lead to a finite steady state sexual

population, with typically a smaller average number of mutations (greater fitness) than the asexual population. Thus no threshold mechanism seems to be necessary for a successful sexual population to take hold. However, for habitual practice of sexual reproduction by diploid individuals (i.e., those that are not facing extinction in Model A) calls for unrealistically large mutation rates in order for a macroscopic sexual population to be established in the steady state.

To be able to distinguish the contribution of diploidy (just possessing two alleles of the same gene) from that of sexual reproduction (leading to greater variability via fusion), we have also performed simulations on a purely diploid population, with an initial population again consisting only of the wildtype. We found that the steady state distribution in this case was practically indistinguishable from the non-hereditary versions of both Model A and B. For reasonable values of the mutation rate Γ driving organisms to the edge of extinction in Model A, or σ , the probability to engage in sexual reproduction in Model B, the observed frequency of sexual reproduction for either of these models is so low that the establishment of a finite “sexual” population, in spite of the “2 $\rightarrow$ 1” disadvantage is really due to the success of diploidy in reducing deaths from an excess of deleterious mutations.

The organisation of the chapter is as follows. In the next section we explain in detail the two models and we report the results of our simulations. In Section 3.3, we display and examine the mean field evolution equations and discuss our findings in the light of these equations. In Section 3.4, we investigate the limits of strong and extremely weak driving of this system, for $\Gamma \rightarrow 1$ and $\Gamma \rightarrow 0$, as well as a transition to chaos via an intermittent route, found for large values of σ . In Section 3.5, we further investigate an episodic conversion to sex, involving as few as only two individuals who survive to mate, leading to a steady state made up of solely sexual types. A discussion of the results is provided in Section 3.6.

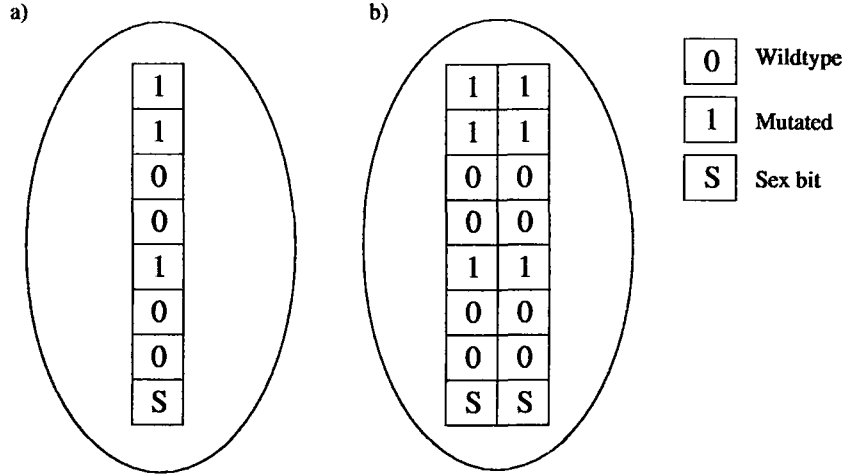


Figure 3.1: An example of haploid and diploid cells used in the simulations. The genome length in the figure is taken to be 8 for simplicity. The last gene, namely S , denotes the sex bit. We define the wildtype bit to correspond to “0”.

3.2 Models for Conversion to Sex

We represent the genetic code of each one-celled individual with a bit-string of “0”s and “1”s. At each locus, we have taken the value “0” to correspond to the wild type and “1,” to a deleterious mutation (which we will call “mutation,” for short, where this is not liable to lead to any confusion.) We use the bit defining the “sign”, to specify whether the individual is asexual (+) or sexual (-). For asexual, haploid, cells, we have one 15-bit string, whereas, for the sexual cells, we have two 15-bit strings which are allowed to be different, i.e., the individuals are now diploids. An example of haploid and diploid cells are given in Figure 3.1, where the genome is represented by 8 bits for simplicity. Since the genetic difference between individuals of the same species is typically less than 10% even for simple organisms [40], this rather short string for the genetic code may be considered as a coarse grained model for the complete genome of the individual, which we divide up into different zones, retaining a “0” where there are no mutations, and flipping the bit to “1” if there are one or more mutations in this

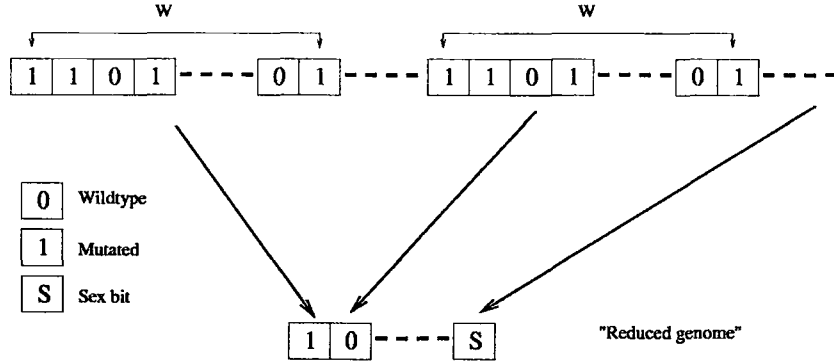


Figure 3.2: Coarse graining used in representing real genomes. Each genome segment of length W corresponds to a single bit in the coarse grained genome. The last gene, namely S denotes the sex bit.

zone. Figure 3.2 shows this coarse graining. A mutation consists of flipping a randomly chosen bit except the sign bit, and it is implemented by scanning all the individuals in the population, and, with probability Γ picking those to be mutated. Clearly there may be any number of mutated individuals at any one generation (time step), the number fluctuating around ΓN , where N denotes the total population.

The number of deleterious mutations m is simply the number of “1”s for a haploid individual. For a diploid, the number of “expressed” deleterious mutations is taken to be the number of loci at which both homologous alleles are set to “1.” This is how we model the mechanism of dominance of the wild type (or, equivalently, the recessiveness of deleterious mutations.) We will use the term “fitness,” loosely, for $L - m$; thus increasing m will decrease the fitness of the individual. The usual assumption made by biologists is that each deleterious mutation decreases the fitness by a fixed factor, so that the fitness would decay exponentially with $L - m$. Other functions have also been used [43], which assume a positive or negative correlation between the effect of successive deleterious mutations. The fitness, or survival, function we have chosen is of the “truncation type” [45], i.e., essentially a step function, to describe the threshold behaviour

in switching to diploidy and sex under an excess mutation load [7, 55, 56]. The probability of survival as a function of m is given by a Fermi-like distribution [56], $P(m)$,

$$P(m) = \frac{1}{\exp[\beta(m - \mu)] + 1} \quad . \quad (3.1)$$

For large β (or “low temperatures,” in the language of statistical mechanics), $P(m)$ behaves like a step function [55]. Individuals with $m > \mu$ die, those with $m < \mu$ survive, and those with $m = \mu$ survive with a probability of 1/2. In the present paper we have confined ourselves to the “zero temperature” limit, taking $\beta = 10$, which is large enough for practical purposes. The threshold was chosen as 4, which allows for just sufficient variability of the typical fitness of the steady state population without becoming totally unrealistic as to the percentage of mutated domains, for $L = 15$. A posteriori, it can be seen [57] that this choice for the survival function favors sex most strongly, as was also found by Redfield [45], since it is tolerant to genetic diversity for small deviations from the wildtype, while strictly punishing for $m > \mu$. For finite temperatures, this distribution interpolates between the step function and the exponential decay (i.e., the Boltzmann distribution).

We keep the total population constant, as in the Redfield model [45], by making up for the deficit in the population after all the bacteria have been either found fit for survival or killed off according to the survival probability in Eq. (3.1). Asexual individuals multiply by simply making another copy of themselves, namely by mitosis, while a pair of sexual organisms each contribute one bit-string to their offspring and die in the process.

We performed the simulations on a fixed population of $N = 1000$, for 16-bit strings. The total number of time steps in each simulation is taken to be much larger than the time necessary for the transients to die off and the system to settle down to a steady state. Since the probability to mutate a single gene in a diploid individual is $\Gamma/(2L)$, on the average the steady state is reached after $2L/\Gamma$ time steps, where $2L$ is the total number of genes in a diploid individual,

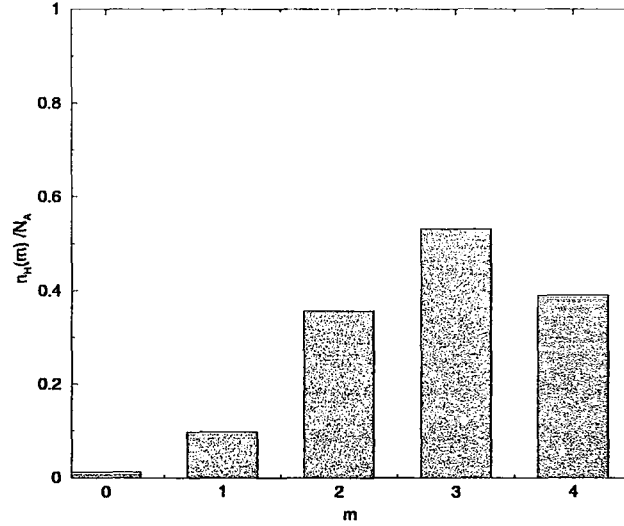


Figure 3.3: The distribution over m for a pure asexual population for $\Gamma = 10^{-3}$.

or, in other words, the number of mutated genes in the population is greater than the total number of genes of one individual. In all the simulations, the reported results are averages over 10 runs. The fluctuations depend on the model chosen, however the relative error estimate based on one standard deviation is typically less than about 6%, as long as there is only one fixed point for the dynamics. In the steady state, the distribution of the asexual and sexual populations over m , are independent of Γ , for $\Gamma > 1/N$. The cases where $\Gamma < \frac{1}{N}$ and $\Gamma \approx 1$ have interesting consequences, and are discussed in section 3.4.

3.2.1 Asexual Steady State

We start with a set of N initially identical asexual individuals, all identical to the wildtype, i.e., all 0's. Under the conditions outlined above, without introducing sex, and for $\Gamma \geq 1/N$, the population of asexuals settles down to the steady state given in [55], where the number $n_H(m)$ of asexual (haploid) individuals with m mutations is $n_H/N_A = 0.012, 0.098, 0.356, 0.531, 0.001$ for $m = 0, \dots, 4$, where N_A is the total number of asexuals (equal, at this stage to the total population, N). This steady state distribution is shown in Figure 3.3. In this region this

steady state distribution is independent of Γ , which only sets the scale of time. That this should be so, is not self-evident, and only follows from the form of the solution to the set of evolution equations, as shown in Section 3.3.

3.2.2 Triggering Sex

The alteration of the sex gene can be accomplished in two different ways. One can choose to trigger sex with a threshold mechanism or define a constant probability for each individual to become sexual. These mechanisms are further discussed in the following subsections. In either case, the haploid organism first makes a copy of its own set of genes, as if it were going to perform mitosis, but then forms two gametes instead. One of these gametes will pair up with a gamete from another individual who has been turned on to sex, and the other will be discarded. This conversion from haploidy to diploidy can be termed syngamy [43], or fusion. It should be noted that endomitosis (simple doubling of the genetic material without subsequent cell division) as a means of making the transition to diploidy would not help the organism in escaping the effects of an excess of deleterious mutations, since the two copies of the genome would be identical.

One should note that sexual reproduction may be implemented in different ways, resulting in different numbers of offsprings produced [55]. Here we will define sexual reproduction in such a way that when two sexual individuals mate they always give rise to one sexual offspring; thus, the population is reduced by one, each time an act of sexual reproduction takes place. Clearly, increasing the number of offsprings will increase the advantage that the sexual population enjoys. Indeed, judging from our previous results [55], the number of offspring exceeding two would lead to the takeover of the population by the diploid sexual types.

When two diploid cells engage in sexual reproduction, they each contribute one gamete towards a single diploid sexual offspring. Let us denote the two gametes as $\{Aa\}$ in one parent, and $\{Bb\}$ in the other parent. Then the genome of the

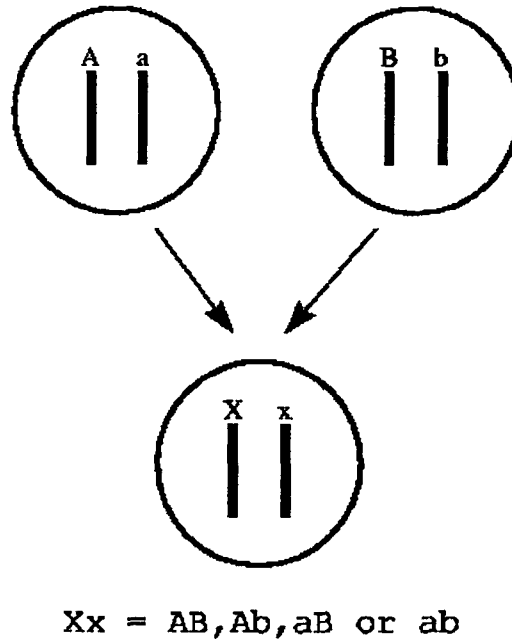


Figure 3.4: We illustrate how the two bit strings are shared between two sexual individuals as they beget one offspring.

offspring may be, $\{AB\}$, $\{Ab\}$, $\{aB\}$ or $\{ab\}$ (see Figure 3.4). We do not allow for crossover between the gametes during sexual reproduction. We are aware that recombination with crossover leads to enhanced variability in the genetic code. This presents sexual reproduction with yet a further advantage, and would only strengthen our results. However, we have refrained from introducing it at this stage to be able to see the contribution of diploidy and more clearly. Moreover, it is not known [40], whether recombination (i.e., with crossover) is a feature that existed in very early forms of sexual reproduction. With a reproduction rate of once every 12 minutes, mutation in unicellular organisms is a much more effective means of adaptation.

3.2.3 Sex at the Threshold of Extinction - Model A

In model A, alteration of the sex gene takes place only under special conditions, namely the threat of death due to too many mutations [7]. Once the asexual

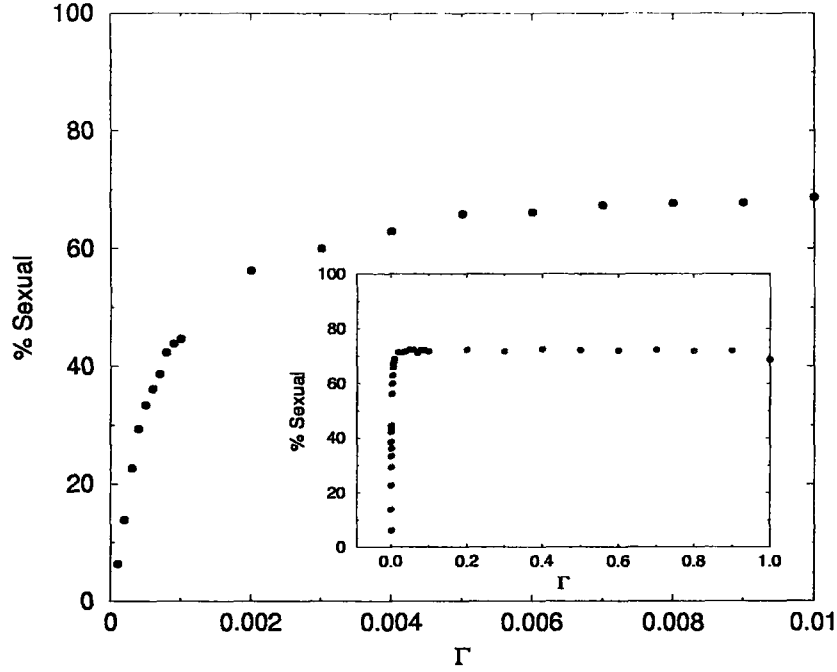


Figure 3.5: The percentage of sexual population v.s. Γ is plotted for Model A where hereditary sex is not allowed, for a population of 1000 individuals. The inset shows a larger range of Γ where the step-function like jump is more apparent. Both curves represent averages over 10 runs.

steady state is reached, we allow the sex gene to be “turned on” for the least fit members of the population. In any pass through the population, if those individuals that are in the tail of the distribution (i.e. those with $m \geq \mu$ mutations) survive, then they are turned sexual by deterministically and irreversibly switching their sign bits to one. Once their sex bit is turned on, these individuals will be labelled “sexually active” and mate with other sexually active individuals. If there is only one active sexual at a certain time step then it must wait subsequent generations until it finds a partner. After mating, the sexual individual becomes sexually inactive and the only way for it to become sexually active again is to face extinction once more. The deficit in the population due to deaths and to sexual reproduction is then made up by copying randomly selected asexual individuals.

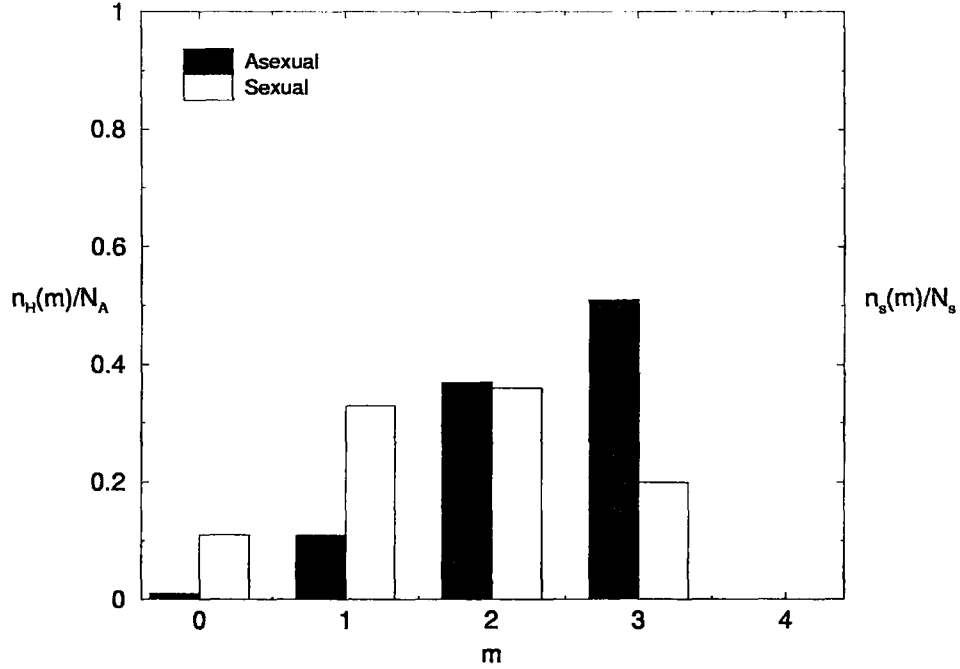


Figure 3.6: The distribution of both sexuals and asexuals over the number of expressed deleterious mutations m , for Model A. $\Gamma = 10^{-3}$. Hereditary sexuality is not allowed and the distributions are normalized to unity over each population separately.

In this model, therefore, there is no hereditary sexuality: there is, however, a hereditary transition to diploidy. This gives an unfair advantage to the sexuals in that they both enjoy the benefits of diploidy and escape the disadvantage of $2 \rightarrow 1$ reproduction.

We see that for $\Gamma \sim 10/N$ the proportion of the sexuals in the population saturates to $\sim 70\%$ as shown in Figure 3.5, and remains at this value independently of the value of Γ . In order to obtain points near $\Gamma \simeq 0$ one has to do very long runs to get accurate results, and these are discussed in Section 3.4, as well as the chaotic behaviour displayed when Γ becomes too close to 1.

The steady state distributions of both asexuals and sexuals with respect to m are also independent of Γ , (See Figure 3.6) for $\Gamma \geq \frac{1}{N}$ and sufficiently smaller than

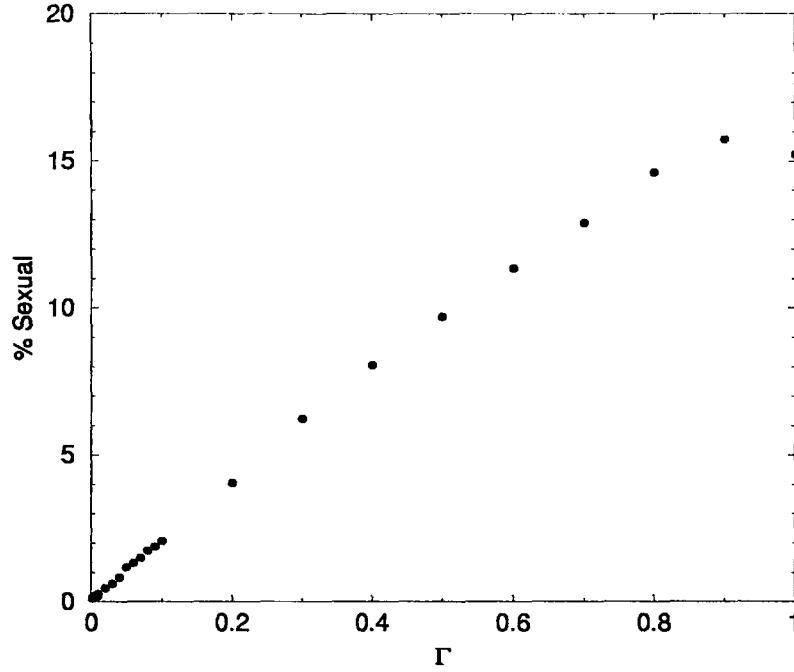


Figure 3.7: The percentage of the sexual population v.s. Γ for Model A with hereditary sexuality introduced. The total population is 1000 individuals and the results are averaged over 10 runs.

1. The peak of the distribution shifts towards lower m values for sexuals [55]. We have compared Model A with an asexual diploid population and found, for $\Gamma = 1/N$, that these two populations are indistinguishable with respect to their m -distributions.

Model A with Hereditary Sex: We have also tested the case of hereditary, or habitual, sex, in which sexually active individuals can mate randomly either with sexually active individuals who have been converted to sex in that generation, or with individuals who have already been converted in some previous generation. As in the case of non-hereditary sex, the population is allowed to grow back to its fixed value by cloning randomly selected asexual units.

This small difference results in a noticeable increase in the number of matings at each time step, and therefore leads to a decrease in the number of sexual individuals in the steady state. We have found that the steady state comprises

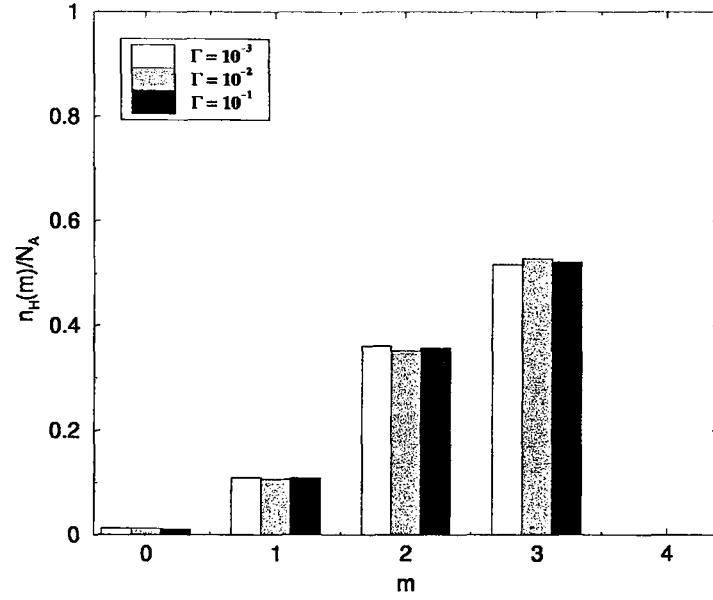


Figure 3.8: The distribution of the asexual population with respect to m , for different values of Γ for Model A with hereditary sexuality. The histograms are normalized to unity.

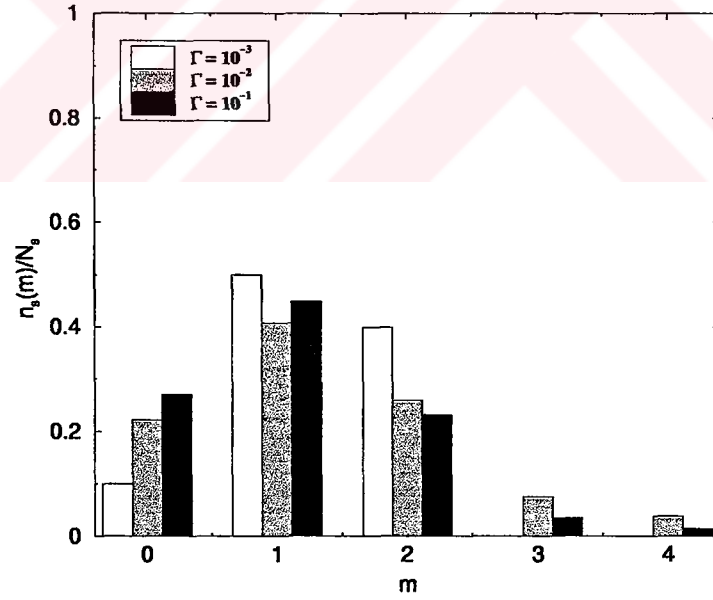


Figure 3.9: The distribution of the sexual population with respect to m , for different values of Γ for Model A with hereditary sexuality. The histograms are normalized to unity.

a macroscopic sexual population only for $\Gamma > 1/N$. For $\Gamma < 1/N$, the average number of sexual individuals drops to about 1%, or around 10 individuals in a population of $N = 1000$. The sexual population increases linearly with Γ and reaches only $\sim 15\%$ (as compared to 70% for non-hereditary sex) as $\Gamma \simeq 1$ (see Figure 3.7). The m -distributions are shown in Figures 3.8 and 3.9 for the asexual and sexual populations. The peak of the sexual population has shifted to 1 as a result of the greater number of mating events. Thus we may conclude that hereditary and habitual sex in this model is punished more severely; the relative improvement in the mean value of m does not compensate sufficiently for the loss of the parents. Nevertheless, one can see that the increased frequency of mating has lead to a further increase in the fitness over and above that afforded by diploidy without sex (which has a distribution identical to Model A without hereditary sex).

3.2.4 Mutating the sex gene with constant probability - Model B

Our second strategy for conversion to sex involves a constant probability σ for the accidental conversion to sex, independently of the distance, as expressed by m , from the wildtype. For this model (Model B), once the asexual steady state is reached, at each generation we allow the sex gene to be “turned on” irreversibly, with a small probability σ for each individual. Like in Model A, these individuals will be “sexually active” and mate with other sexually active individuals of that generation. (If there is only one active sexual at a certain time step then it has to wait till it finds a partner at a subsequent generation.) If we take sexual reproduction to be non-hereditary, after mating the sexual individual becomes sexually inactive. (Within some subsequent generation it can once more become sexually active with probability σ). The deficit in the population is made up by copying randomly selected asexual individuals.

We find, (see Figure 3.10) that this scenario again gives rise to a steady state macroscopic population of sexuals - but it is smaller than the one in Model A. The total percentage of sexuals is a function of σ/Γ , as can be seen from the

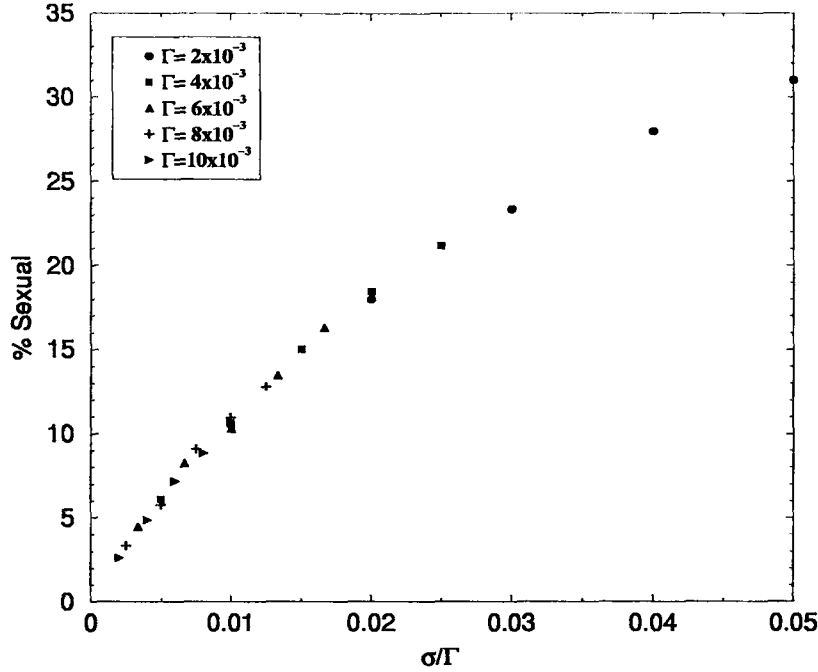


Figure 3.10: Percentage of the sexual population v.s. σ/Γ plots for various Γ values for Model B. Hereditary sexuality is not allowed. All the points collapse onto a single curve in the interval shown.

figure, and grows with σ/Γ . In Figures 3.11 and 3.12, we display the distribution of asexual and sexual individuals over the effective number of mutations m , for two small values of σ and Γ . The characteristic sandpile like [58] distribution of asexuals is accompanied by a distribution of sexuals which is again shifted towards smaller values of m . It is interesting to observe that raising σ increases the total number of sexuals, and therefore depresses the number of asexuals, as is to be expected. However, it is not immediately obvious why keeping σ fixed and decreasing the overall mutation rate should decrease the number of asexuals. Clearly, raising Γ increases the death rate of both types of organisms, but since the conversion to sex is not coupled to the increase in the number of mutations, an increased Γ only benefits the asexuals who get cloned to make up the deficit population. For large values of σ , a novel phase transition takes place, which is the subject of Section 3.4.

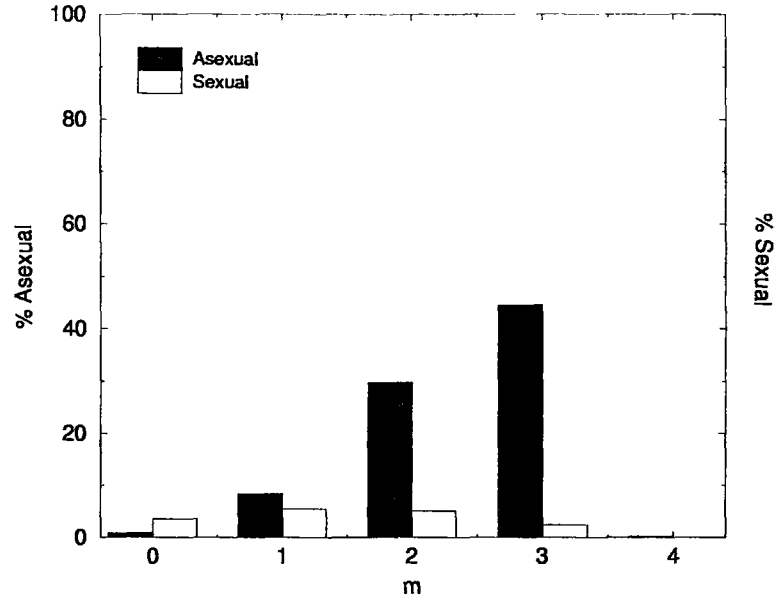


Figure 3.11: Distribution with respect to m , for both sexual and asexual populations for Model B , without hereditary sex. $\Gamma = 6 \times 10^{-3}$, $\sigma = 10 \times 10^{-5}$; the histograms represent averages over 10 runs for a population of 1000.

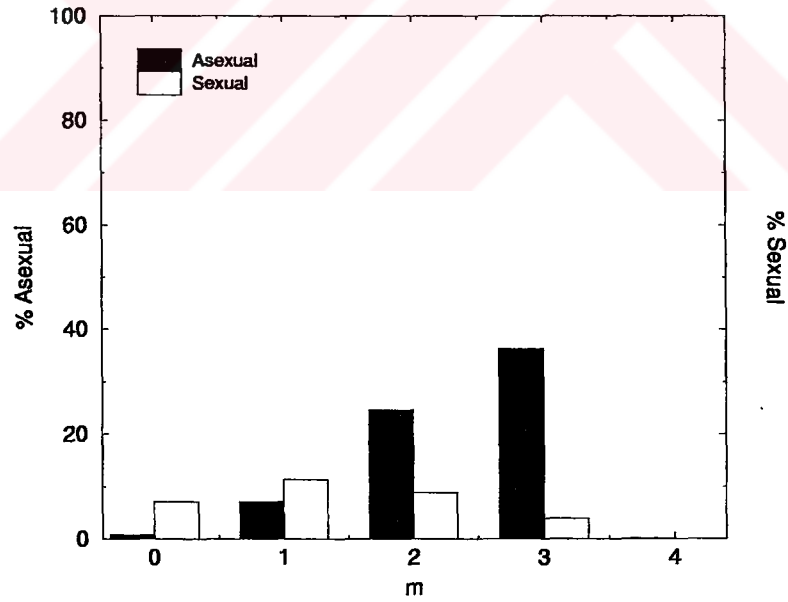


Figure 3.12: Distribution with respect to m , for both sexual and asexual populations for Model B , without hereditary sex. $\Gamma = 2 \times 10^{-3}$, $\sigma = 10 \times 10^{-5}$; the histograms represent averages over 10 runs for a population of 1000.

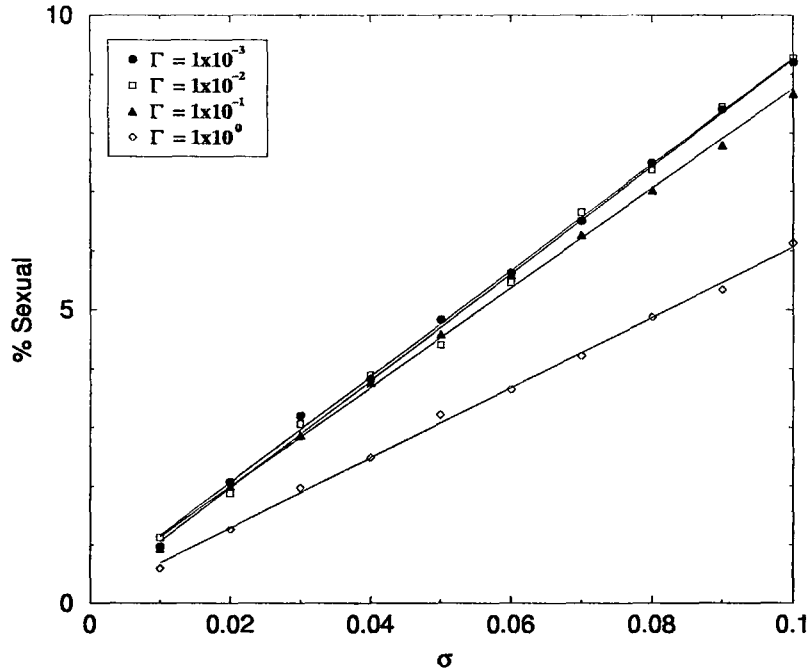


Figure 3.13: The percentage of the sexual population v.s. σ for various Γ values for Model B with hereditary sex. The growth with σ is linear for the different Γ values.

Model B with Hereditary Sex: If the conversion to sexual reproduction is hereditary, then at any given time step all the sexual individuals mate, except for the odd guy out. In Figure 3.13 we show the total percentage of the sexual population as a function of σ alone. One sees that the growth is very close to linear with σ , however the collapse as a function of σ/Γ does not occur here. The curves extrapolate to zero at $\sigma = 0$. As long as $\sigma > 1/N$ one may have a small but nonvanishing sexual population. For smaller values of σ , the number of sexual individuals again fluctuates very strongly and is of $O(1)$. (see Section 3.4).

Comparing the results of Model B with those from an asexual diploid population again reveals that they are indistinguishable for the non-hereditary case for small σ , whereas for hereditary sex, the fitness has improved as shown in Figures 3.11 and 3.12.

3.3 Mean Field Evolution Equations

To try to understand analytically some of the features found from the simulations, we have examined the behaviour of the iterative equations that can be obtained for the different densities involved. These equations follow directly from the definitions of the various rates and densities. The only assumptions needed are that *i*) the hydrodynamic limit obtains, i.e., that the number of events per generation are correctly given by the product of the relevant rates and the densities, and *ii*) each individual is able to sample the total population when it picks a mate. It is because of the latter assumption that we refer to the equations as “Mean Field.” If all the individuals can pair with each other at any instant, it means that the interactions are “infinitely long range.” The departure from the simulation results due to the failure of the first assumption, coming from the eventually discrete nature of the phenomena, is discussed below, and under Section 3.4.2 “the limit of infinitely slow driving.” Moreover, as we show in Section 3.4.1, the iterative equations tend to smooth out the intermittent route to chaos which we observe in the numerical simulations, in the limit of strong driving (very large mutation rates).

Given that the mutation rate per individual is Γ , and that any of the L bits have equal probabilities of being hit, we see that in each generation, a haploid makes a transition from a state with m mutations to one with $m + 1$ mutations with the probability $T_{m,m+1}(\Gamma) = \Gamma(L - m)/L$. If a mutation hits the same bit twice, its value will be reset to “0”, so that $T_{m,m-1}(\Gamma) = \Gamma m/L$ gives the probability per generation that a haploid with m mutated bits makes a transition to a state with one less. All other elements of this transition matrix T are zero, since each individual is tested only once to see if it will undergo a mutation (with probability Γ) and if yes, only one bit is mutated at random.

For low temperatures and for μ , the upper limit of the number of mutations tolerated by the haploid individual, being set to four, the survival probability is

given by,

$$P(m) = \begin{cases} 1, m = 0, 1, \dots, 3 \\ \frac{1}{2}, m = 4 \\ 0, m > 4 \end{cases} \quad (3.2)$$

3.3.1 Asexual Steady State

The time-evolution equations for the asexual population, with $n_H(m)$ being the number of individuals with m mutated genes, are, with the above definition of the mutation matrix,

$$\begin{aligned} n_H(m, t+1) = & (1 - \Gamma)n_H(m) + \sum_{\delta=\pm 1} T_{m+\delta, m} n_H(m + \delta, t) \\ & - [1 - P(m)]n_H(m, t) \\ & + \sum_{m'} [1 - P(m')]n_H(m', t)n_H(m, t)/N_A \quad . \end{aligned} \quad (3.3)$$

The first two terms describe the building up of the mutation load, i.e. “Muller’s ratchet,” the third term subtracts off the number of individuals with m mutations that die off with probability $1 - P(m)$. The last term is the source term, arising from the replacement of the deceased individuals by randomly cloning the extant ones and $N_A = \sum_m n_H(m)$ is the total number of asexual individuals.

For large β , one effectively has,

$$\begin{aligned} n_H(m, t+1) = & (1 - \Gamma)n_H(m, t) + \sum_{\delta=\pm 1} T_{m+\delta, m} n_H(m + \delta, t) \\ & + \frac{1}{2}n_H(4, t)n_H(m, t)/N_A \quad , \end{aligned} \quad (3.4)$$

for $m < 4$. The source term $[1 - P(4)]n_H(4)n_H(m)/N_A$ has been replaced by its value $\frac{1}{2}n_H(4)n_H(m)/N_A$, and it is assumed that $n_H(m > 4) \equiv 0$. This assumption is supported by numerical data in the steady state.

Note that for $\Gamma N \sim O(1)$, $n_H(4)$ will be small, i.e., of the order of unity. For $m = 4$, this enables us to put the source term in the last equation equal to zero, since it will be of $O(1/N)$ while the other terms are of $O(1)$, and we get,

$$n_H(4, t+1) = (1 - \Gamma)n_H(4, t) + \sum_{\delta=\pm 1} T_{4+\delta, 4} n_H(4 + \delta, t) - \frac{1}{2}n_H(4, t)$$

$$= (1 - \Gamma)n_H(4, t) + \Gamma(1 - 3/L)n_H(3) - \frac{1}{2}n_H(4, t) \quad . \quad (3.5)$$

Then we see that in the steady state, one may replace $n_H(4)/2$ appearing in the source terms by $\Gamma[(1 - 3/L)n_H(3) - n_H(4)]$. This leads to equations that are homogenous in Γ in the steady state, yielding, therefore, a steady state distribution of the population between sexual v.s. asexual individuals which are independent of Γ at least for $\Gamma \geq 1/N$. (see Figure 3.5) Iterating these equations leads to a steady state with an m -distribution that is in agreement with the simulation results [55] (see Figure 3.3).

3.3.2 Coexisting Asexual and Sexual Populations

We now define a new quantity, $n_D(m)$ as the number of m -mutation strings that belong to a diploid organism. The expected number of diploid organisms with m expressed deleterious mutations can be obtained, once the $n_D(m)$ are known.

The probability for two strings with m_1 and m_2 mutations (i.e., bit set to “1”) to give rise to m loci at which both bits are “1” can easily be calculated. It is given by

$$p(m; m_1, m_2) = \frac{m_1!m_2!(L - m_1)!(L - m_2)!}{L!m!(m_1 - m)!(m_2 - m)!(L - m_1 - m_2 + m)!} \quad , \quad (3.6)$$

for $L - m_1 - m_2 + m > 0$ and 0 otherwise. This expression is symmetrical in m_1 and m_2 , both of which must be $\geq m$. The number of diploid organisms with m expressed mutations is then,

$$n_s(m) = \frac{1}{2} \sum_{m_1=m}^L \sum_{m_2=m}^{L^*} p(m; m_1, m_2) n_D(m_1) n_D(m_2) / (2N_S) \quad , \quad (3.7)$$

where N_S is the number of diploid organisms, $\sum_{m=0}^L n_s(m)$, and $L^* = \min[L, L + m - m_1]$. The factor of $\frac{1}{2}$ out front comes from converting from the number of gametes that are members of diploid organisms with m expressed mutations, to the number of such diploid organisms. The factor $n_D(m_2)/(2N_S)$ in the sum is the probability of encountering a gamete with m_2 mutations as the other member of the pair making up the diploid organism.

A similar computation leads to the number of diploid individuals who die as a result of too many mutations,

$$D_D = \frac{1}{2} \sum_{m=0}^L \sum_{m_1=m}^L \sum_{m_2=m}^{L^*} [1 - P(m)] p(m; m_1, m_2) n_D(m_1) n_D(m_2) / (2N_S) \quad , \quad (3.8)$$

where L^* is defined as above.

The number of gametes with m mutations, which get removed because they happen to be members of diploid organisms which die, is

$$d_m = \sum_{m''=0}^L \sum_{m'=0}^{\min[m, m'']} [1 - P(m')] p(m'; m, m'') n_D(m, t) n_D(m'', t) / (2N_S) \quad . \quad (3.9)$$

We must also define the number of gametes with m bits set to “1,” that can take part in sexual reproduction, which is

$$\tilde{d}_m = \sum_{m'}^{\tilde{L}} p(4; m, m') n_D(m, t) n_D(m', t) / (2N_S) \quad (3.10)$$

where $\tilde{L} = \min[L, L + 4 - m]$. Since $\tilde{d}_m$ is only defined for $m \geq 4$, $\tilde{L} = L + 4 - m$. Note that $\sum_{m=4} \tilde{d}_m = 2n_s(4)$.

Here we have only considered the scenarios without habitual sex.

Model A: We now have, from (3.4,3.5), for sufficiently large β

$$\begin{aligned} n_H(m, t+1) = & (1 - \Gamma) n_H(m, t) + \sum_{\delta=\pm 1} T_{m+\delta, m} n_H(m + \delta, t) - \delta_{m,4} n_H(4, t) \\ & + \left[\frac{3}{4} n_H(4, t) + D_D(t) + \frac{1}{4} n_s(4, t) \right] n_H(m, t) / N_A \quad . \end{aligned} \quad (3.11)$$

The terms proportional to Γ are due to random mutation. The coefficient of the Kronecker delta $\delta_{m,4}$ is $n_H(4)$ since all of the asexuals with $m = 4$ are removed either due to death or conversion to sexuals. The final term represents the number of m -mutation haploids which get cloned to keep the population constant; the expression in the square brackets is the number of individuals which get removed from the population and determines the strength of this source term. The $(3/4)$ factor multiplying $n_H(4)$ comes from two parts: $(1/2)$ of the haploids with 4 mutations die; the other half is converted to sex, and mate, their number being once more halved as a result, contributing $(1/4)n_H(4)$ to the “removals.”

D_D (which is $= (1/2)n_s(4)$ for large β) is the number of diploids that die, and $(1/4)n_s(4)$ comes from half of the $m = 4$ diploid population being converted to sex, their number being once more halved when they mate.

The dynamics of the number of strands $n_D(m)$ that make up diploid organisms is,

$$\begin{aligned} n_D(m, t+1) = & \left(1 - \frac{1}{2}\Gamma\right)n_D(m, t) + \sum_{\delta=\pm 1} T_{m+\delta, m} \left(\frac{1}{2}\Gamma\right)n_D(m+\delta, t) \\ & - d_m(t) - \frac{1}{4}\tilde{d}_m + \delta_{m,4}P(4)n_H(4, t) \quad . \end{aligned} \quad (3.12)$$

For diploids, the probability of a mutation hitting any one gene is halved, because there are twice as many of them. The d_m term is the number of m -gametes that are removed as a result of death, and in practice (for large β) is nonzero only for $m \geq 4$. The next term gives the reduction in the number of m -gametes as a result of sexual reproduction. A factor of $(1/2)$ comes from the probability to engage in sex, and another from the fraction of gametes that are discarded as a result. Finally, there is a contribution from the conversion of haploids to diploids. We have neglected the situation where *a*) there is only one active sexual individual is present, so that no mating with concomitant discarding of a gamete, can take place; or *b*) a conversion from haploid to diploid is impeded because there is only one haploid strand with 4 mutations. It can be checked explicitly that Eqs.(3.11,3.12) conserve the total population.

Iterating these equations leads to a steady state distribution that is roughly comparable but not identical to the simulation results (see Table 3.1). For $\Gamma = 10^{-3}$ the percentage of the sexual population is 24% of the total, and saturates to 36% as Γ is increased, as compared to 70% from the simulations. This discrepancy seems to come from the fact that the dynamics is really driven by the strongly fluctuating small population at $m = 4$, and mean field theory is simply not able to capture this.

The distribution over m is also modified; one sees that the distribution of the asexuals is quite similar to the simulation results, while the peak of the sexual

Table 3.1: The distribution of the population with respect to the number of expressed mutations, obtained from an iteration of the mean field equations for Model A.

$\Gamma = 10^{-3}$			$\Gamma = 10^{-2}$		
m	Asexual%	Sexual%	m	Asexual%	Sexual%
0	0.9	8.5	0	0.8	9.4
1	7.8	11.0	1	6.5	16.2
2	26.7	4.4	2	22.3	8.8
3	40.1	0.6	3	33.8	1.9
4	0.0	0.0	4	0.1	0.2
Total	75.5	24.5	Total	63.5	36.5

distribution has shifted to $m = 1$, from $m = 2$. This indicates that the mean field theory overestimates the effect of remixing, as is to be expected, since the gametes, instead of being paired in a definite way at any given moment, are perpetually part of a single gene pool.

Model B: In this case we have a uniform probability for conversion to sex. The equations become,

$$\begin{aligned}
 n_H(m, t+1) = & (1 - \Gamma)n_H(m) + \sum_{\delta=\pm 1} T_{m+\delta, m} n_H(m+\delta) - [1 - P(m)]n_H(m, t) \\
 & - \sigma n_H(m) + \left\{ \sum_{m'} [1 - P(m')] n_H(m') \right. \\
 & \left. + \frac{1}{2}\sigma N_A + D_D(t) + \frac{1}{2}\sigma N_S(t) \right\} n_H(m) / N_A . \quad (3.13)
 \end{aligned}$$

Here, haploids are converted to diploids and removed at the rate of σ , and the reduction in the population due to mating of recent converts gives the $\frac{1}{2}\sigma N_A$ term in the source. The sexuals moreover mate among each other with probability σ , which leads to a further sink with strength $\frac{1}{2}\sigma N_S$. Apart from these, the terms are identical to Eq.(3.11). The dynamics of the m -gametes are,

$$n_D(m, t+1) = (1 - \frac{1}{2}\Gamma)n_D(m, t) + \sum_{\delta=\pm 1} T_{m+\delta, m} (\frac{1}{2}\Gamma)n_H(m+\delta, t) - d_m$$

Table 3.2: The distribution of the population with respect to number of expressed mutations, obtained from an iteration of the mean field equations for Model *B*.

$\sigma/\Gamma = 0.01$			$\sigma/\Gamma = 1.00$		
m	Asexual%	Sexual%	m	Asexual%	Sexual%
0	2.9	9.3	0	1.7	32.2
1	14.3	3.0	1	8.3	14.7
2	32.4	0.4	2	18.8	2.3
3	37.7	0.0	3	21.9	0.1
4	0.1	0.0	4	0.0	0.0
Total	87.3	12.7	Total	50.7	49.3

$$-\frac{1}{2}\sigma n_D(m, t) + \sigma n_H(m) \quad . \quad (3.14)$$

In this case, the iteration of mean field equations yield results (see Table 3.2) that are much closer to those found from the simulations.

The evolution equations, which we have written as difference equations, are of course nonlinear. In the simplest case of asexual reproduction (Eqs. (3.4, 3.5)) this second order nonlinearity comes purely from the condition of a fixed finite population, and appears in the source term for the restoration of the population to its fixed value by randomly sampling the asexual population and cloning it. With the introduction of sex, the source term in the equations for the asexual organisms (3.11, 3.13) acquires a contribution from the number of sexual individuals that are removed either through death or through sexual reproduction. Such terms contain nonlinearities up to third order. We expect to find nontrivial behaviour in the limit of large nonlinearities in these equations, and this turns out to be the case, as we explore in the next section.

3.4 Limits of Strong and Extremely Weak Driving, Chaotic Behaviour

3.4.1 The Limit of Strong Driving

After the discussion of the last section, it is natural to expect that the nonlinearities present in the problem should drive it to chaotic behaviour when their amplitude is sufficiently large.

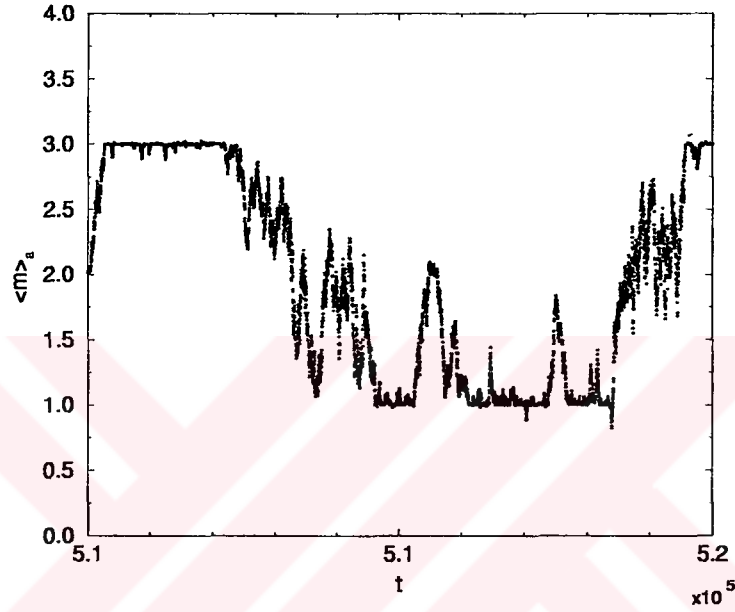


Figure 3.14: The intermittent variation with time of $\langle m \rangle_a$, the average number of mutations for the asexual population, in Model *B*, for $\sigma = 0.5$. The averages are taken over the population at time t . $\Gamma = 10^{-3}$. It is clearly seen that there are two metastable states. The picture shows a window of 10^4 time steps after the transients are dropped.

We have tested the limit of $\Gamma = 1$ and found that for Model *A* with hereditary sex, the system becomes unstable. The total asexual population and sexual population display oscillations with a period of 2 time steps. The m -distributions also oscillate for both populations, with the same period, the amplitude of the oscillations being much larger for the asexuals. For such large values of Γ , at each time step a large number of asexuals are driven to large m values and are

converted to sexuals, they mate, and reduce their expressed mutations. This leads to a macroscopic fluctuation in the number of sexuals, with the halving of the mating population, which then causes a very large number of asexuals to be cloned in turn. The time average of the sexual population is depressed slightly below the saturation value as a result, as can be seen in Figure 3.7. These oscillations are not observed in the iteration of the mean field equations.

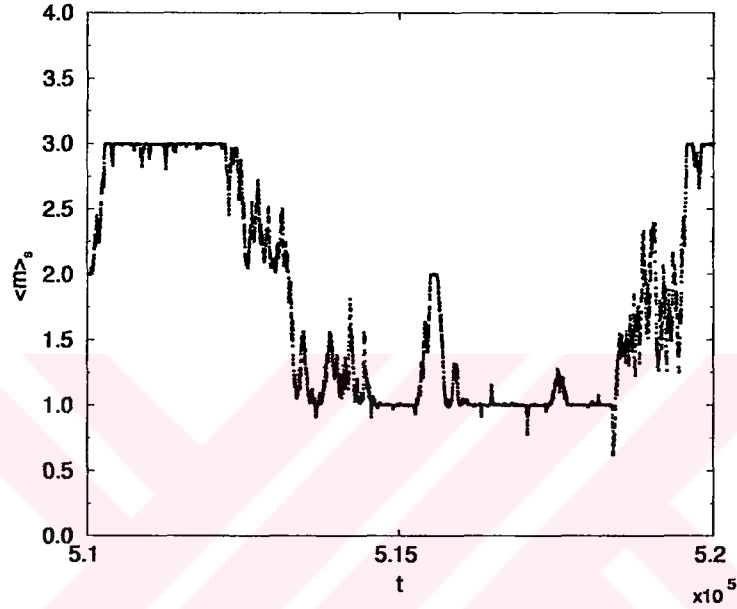


Figure 3.15: The intermittent variation with time of $\langle m \rangle_s$, the average number of expressed mutations for the sexual population, in Model *B*, for $\sigma = 0.5$. The averages are taken over the population at time t . $\Gamma = 10^{-3}$. It is clearly seen that there are two metastable states. The picture shows a window of 10^4 time steps after the transients are dropped.

A much more striking behaviour is found in Model *B* for large values of σ . As we increase the value of σ , the probability of random conversion to sex, beyond about 0.05, a spectacular transition takes place to a strange attractor for the dynamics of both the asexual and sexual populations. In place of the well converged m distributions for both asexual and sexual populations, shown in Figures 3.11-3.12 one observes that both distributions are intermittently switching between several

meta-distributions. The average value of m computed over the asexual and the sexual populations is shown in Figures 3.14-3.15, and displays this striking intermittent behaviour, where the distribution of the two populations becomes much more closely coupled than in the lower σ values. They now move more or less *in phase*, and their excursions take them all the way down to the wild type. Now it is only possible to talk about a distribution of distributions. To display this graphically, we have plotted the distribution of the average number of expressed mutations in the two populations, $\langle m \rangle_a$ and $\langle m \rangle_s$, as a function of σ . In Figures 3.16-3.17, we show three dimensional plots for these distributions, compiled over 10^4 time steps for each value of σ . In Figures 3.18- 3.19, a contour plot of the same distribution as in Figures 3.16-3.17 are shown. It is possible to read off from the contour plots that the transition is taking place around $\sigma_c \simeq 0.05$.

Besides being intermittent, this transition has a dramatic effect on the m distribution of the sexual population, in that it shifts it to much higher values. It can be seen in Figure 3.19 that for $\sigma < \sigma_c$, the mean m for the sexual population is $\langle m \rangle_s \sim 0.75$, while for large σ it is comparable to the corresponding value for the asexual population, closer to 3. The reason seems to be that with the great depletion of the population when too many individuals are being switched on to sex and engaging in sexual reproduction, the asexuals are cloning too many identical copies to make up for the deficit. When these are subsequently turned sexual and mate among each other, “inbreeding” takes place - there is not sufficient genetic diversity for sex to lead to sufficient mixing and therefore an amelioration of the effective fitness.

We have iterated the mean field equations (3.13,3.14) for Model *B* and found that this intermittent behaviour is suppressed. The sexuals simply evolve along the lower branch which in the simulations has the smaller weight, while the asexuals evolve along the higher (large m) branch, which has the greater weight in the simulations, and the evolution is completely stable. For $\sigma = 0.9$ and $\Gamma = 0.1$, $\langle m \rangle_a = 2.43$ and $\langle m \rangle_s = 0.47$.

3.4.2 The limit of infinitely slow driving ($\Gamma \rightarrow 0$)

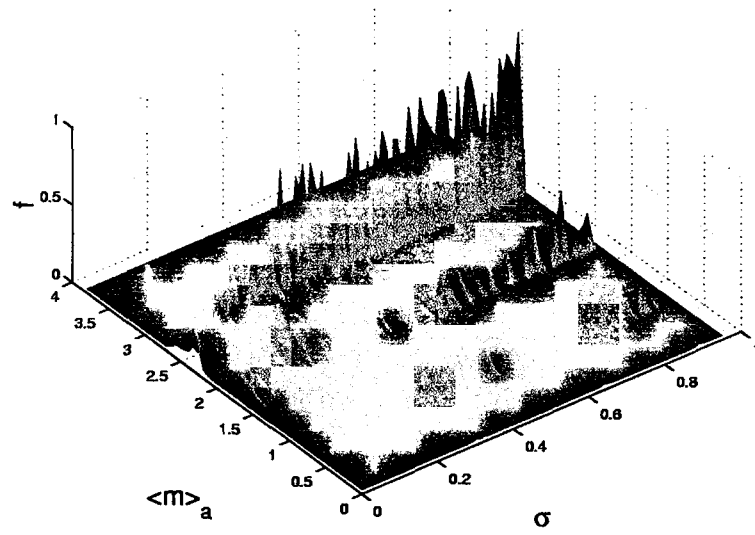


Figure 3.16: A 3D plot showing the branching distributions of $\langle m \rangle_a$ with respect to σ . After a threshold at $\sigma \sim 0.05$, the distribution displays more than one peak. The z-axis indicates the relative weights of these peaks. The total population is 1000 and the figure represents single runs of 10^4 steps after transients, for each σ value.

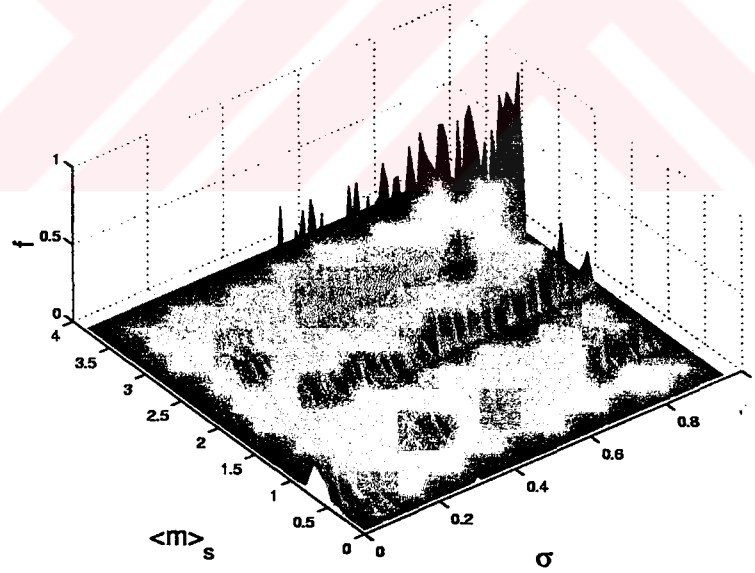


Figure 3.17: A 3D plot showing the branching distributions of $\langle m \rangle_s$ with respect to σ . After a threshold at $\sigma \sim 0.05$, the distribution displays more than one peak. The z-axis indicates the relative weights of these peaks. The total population is 1000 and the figure represents single runs of 10^4 steps after transients, for each σ value.

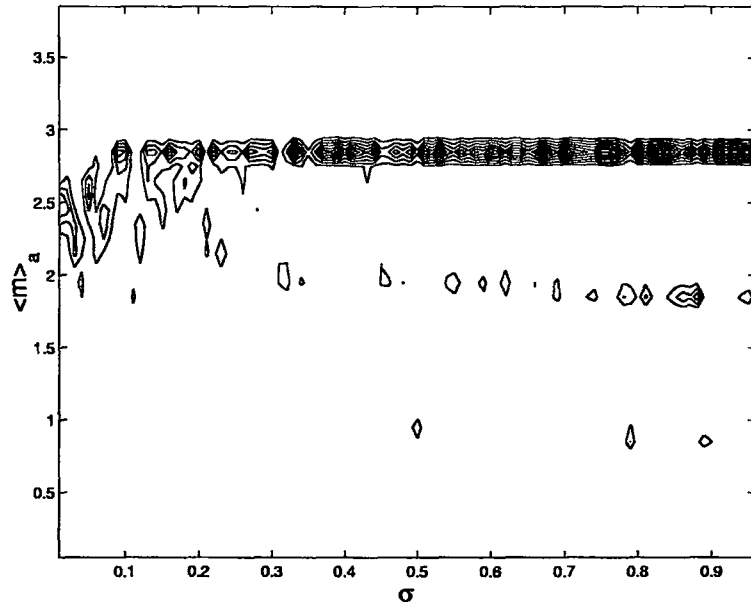


Figure 3.18: Contour plot showing the branching of $\langle m \rangle_a$, as σ increases, for a population of 1000, computed over 10^4 time steps.

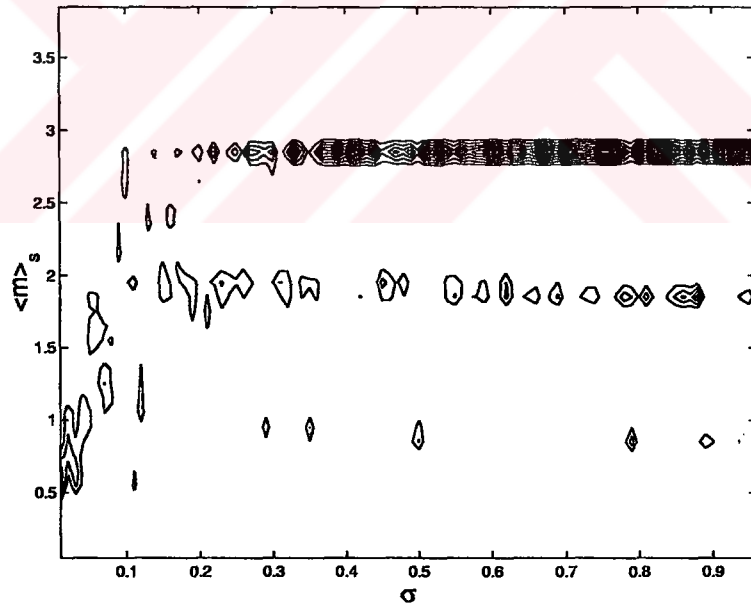


Figure 3.19: Contour plot showing the branching of $\langle m \rangle_s$, as σ increases, for a population of 1000, computed over 10^4 time steps.

or $\sigma \rightarrow 0$)

3.4.2 The limit of infinitely slow driving ($\Gamma \rightarrow 0$ or $\sigma \rightarrow 0$)

In the limit of infinitely slow driving, i.e., $\Gamma \rightarrow 0$ or $\sigma \rightarrow 0$, we observe a transition to a different phase.

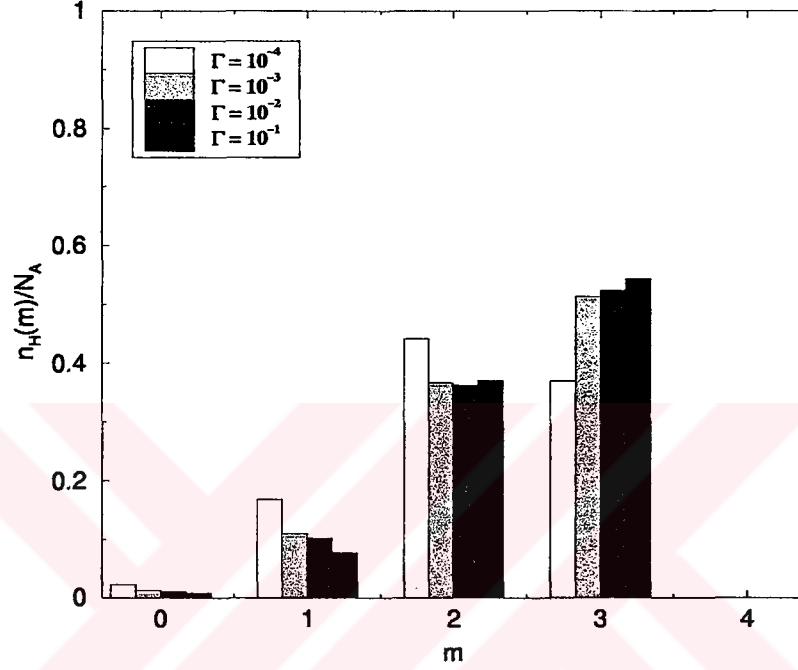


Figure 3.20: The distribution over m for asexual populations, for different values of Γ for Model A, without hereditary sex. The steady state distribution changes and the peak on the distribution shifts to a smaller m value as one lowers the Γ value below the threshold $1/N = 10^{-3}$.

For $\Gamma < 1/N$, we find a qualitatively different asexual steady state, where the m distribution has shifted to lower m values (compare with Figure 3.6) and no longer has the characteristic minimally stable sand-pile like [58] distribution. For $\Gamma = 10^{-4} = (10N)^{-1}$, over a run of 10^6 steps, we find $n_H(m)/N \simeq 0.03, 0.14, 0.44, 0.39$ for $m = 0, \dots, 3$ respectively, where the peak has moved to $m = 2$ from $m = 3$, or broadened towards the left. This does not seem simply to be due to a slowing down of the dynamics. Rather, once the mutation rate

drops below $1/N$, the flow over the $m = 4$ threshold which stabilizes the skewed distribution slows down to a dribble. This gives the m distribution time to get stabilized at $m = 2$ rather than being pushed to the $m = 3$ limit. The mechanism for the stabilization is provided by the dead bacteria being replenished from among the most prevalent extant ones.

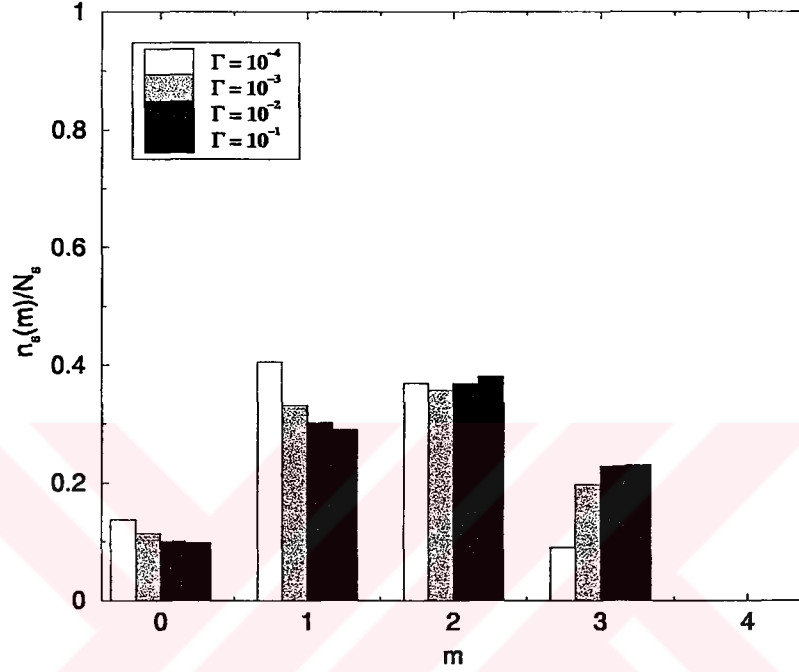


Figure 3.21: The distribution over m for sexual populations, for different values of Γ for Model A, without hereditary sex. The steady state distribution changes and the peak on the distribution shifts to a smaller m value as one lowers the Γ value below the threshold $1/N = 10^{-3}$.

Once sex is turned on in Model A, we similarly observe that the peaks in the distribution of the asexual and sexual populations have shifted to lower m values ($m = 1$ and $m = 2$ respectively), as shown in Figure 3.20-3.21. Although the total sexual population is relatively small here, we have checked that the fluctuations in the histogram over 10 different realizations stay small.

Iteration of the dynamical equations, on the other hand, reveal no such phase transition and, for the asexual steady state, converge to the same steady state

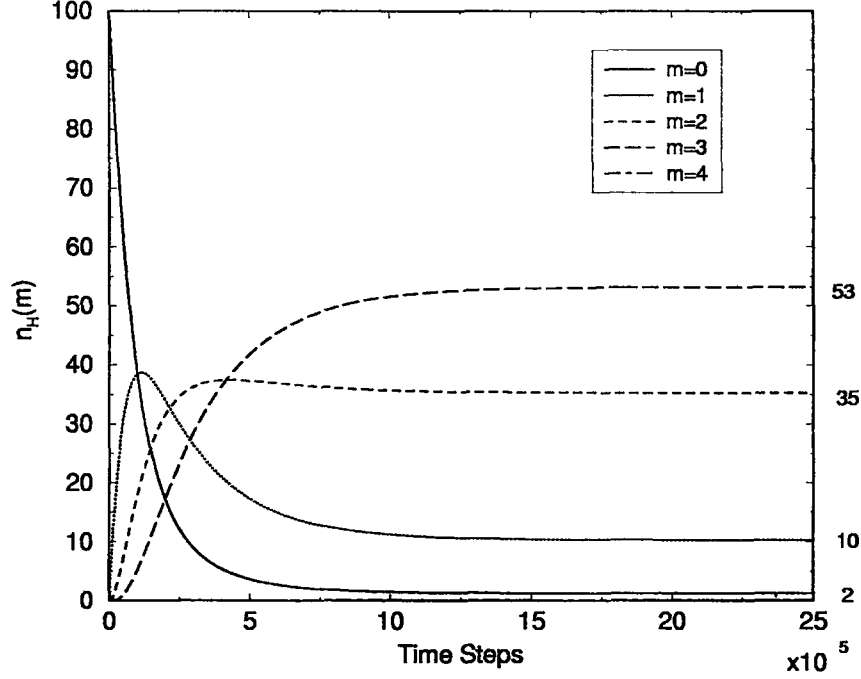


Figure 3.22: The iterated solutions of the equations for the purely asexual population, without the introduction of sex, as a function of time for different values of m . $\Gamma = 10^{-4}$.

distributions as found for $\Gamma > 1/N$. In Figure 3.22, we show the time series for $n_H(m)$ ($N = 100$) for the asexual population without conversion to sex. At time $t = 0$, the largest density is of course at $m = 0$, and then the maximum shifts successively to $m = 1, 2$ and finally to $m = 3$ where it stabilizes. Comparison with the simulation results seem to indicate that the simulations get stuck at an intermediate “metastable” state, while the peak is around $m = 2$. The fact that in the simulation one has to wait around until, with a very low probability, a discrete individual is pushed over the $m = 4$ barrier, dies, and is cloned from among the live bacteria, while in the mean-field equations, there is a weak but steady seepage, is what prevents this phase transition from taking place.

3.5 Haploid-Diploid Cycle and Episodic Conversion to Sex

In the previous sections we described scenarios where the haploids, who were allowed to multiply, also provided a source for the diploid sexuals, whose numbers were halved every time they mated. Now we want to test whether the diploid, sexual population can survive autonomously, without a source being provided by the rapidly multiplying haploids, if only they are also allowed to perform mitosis themselves.

Once a steady state with coexisting haploid and diploid populations is reached via Model A we switch off the conversion of haploid organisms to diploidy and sex. We now keep the total population constant by making up for the deficit in the population at each step by cloning randomly selected individuals, *regardless of whether they are sexual or asexual*. In colonies undergoing a haploid-diploid cycle, it is quite frequently the case [42] that the diploid phase of the cycle also involves multiplication by mitosis.

The result is that the diploid individuals completely win over the population. The haploids which now cannot compete with the sexuals, become extinct [44]. Here we find that the diploid phase of the HDC becomes abbreviated to the point where haploids appear only as gametes which do not perform mitosis. This is exactly the situation in highly evolved sexual organisms.

One can also think of a scenario in which conversion to sex takes place accidentally over a short period of time after the asexual population settles down to a steady state. In fact this is probably the most realistic situation, given the random nature of the mutations.

The way we actually implemented this in the computer code was by deterministically switching the sign bits of the first two asexual individuals to survive with $m = 4$, and then turning off the possibility of further conversion. These then form two sexual individuals by endomitosis, and if the first survives long enough so that it can mate with the second, will give rise to one sexual

offspring.

The rest of the rules are as explained above; in each generation we clone randomly chosen individuals to make up the deficit in the population, regardless of whether they are haploids or diploids. We allow the diploid individuals to mate when they face extinction in the course of their lives.

Suprisingly, the diploids capture the population in 95 percent of the performed runs. (In the rest, the single diploid, which is still at the threshold of extinction with an m value of 4, may not survive until a partner arrives.)

3.6 Discussion

The mechanism of random conversion to sex, in the presence of a constant rate of mutations, investigated here as a scenario for the maintenance of a macroscopic sexual production, is in fact very closely related to “coevolution of cell senescence and diploid sexual reproduction in unicellular organisms,” studied by Cui et al. [50]. In this paper a “senescence clock” ticks off a finite lifetime for each bit-string. Sexual reproduction (conjugation) resets the senescence clock; unless this happens after a number of generations of cloning, the offspring stop dividing and die.

Our Model B can be seen as a simpler version of the model proposed by Cui et al., with an intrinsic mechanism, provided by Muller’s ratchet [44], for cell senescence. The constant mutation rate sets the time scale for the survival of any given bitstring, unless it succeeds engaging in sex, with a given probability (our σ). A survival function (Eq.(3.1)) leads to the elimination of genomes carried by haploid individuals multiplying by asexual reproduction, once they have accumulated too many mutations as a result of prolonged exposure to the constant mutation rate [5, 44].

Our Model A goes one step further, in that it makes the number of mutations (the cell clock) provide the triggering mechanism for the transition to diploidy and

sex. It is gratifying to find that this is a more successful strategy for establishing a sexual population than a constant rate of conversion to sex.

Chopard et al. [59] have pointed out that care must be taken in the investigation of finite populations, amplifying and stabilizing small fluctuations which in the thermodynamic limit would be attenuated to zero. They emphasize the importance of spatial variations which cannot be captured by mean field theories. In this study we have demonstrated the relevance for finite populations of discrete stochastic events, whose effect in the very weak driving limit cannot be captured by the “mean field” equations. In the very weak driving limit the system is below the hydrodynamic regime, and exhibits a qualitatively different phase than which is described by the continuum approximations.

In a recent article Pekalski [54] has studied a model which is in many ways similar to ours. There the success of sexual reproduction, meiotic parthenogenesis and asexual reproduction, in maintaining a finite population in the face of periodically changing environmental conditions and a constant mutation rate, is studied in terms of the relative sizes of the populations. Age is included in the model as a parameter which reduces the fitness. The populations do not interact. The findings are that meiotic parthenogenesis and sexual reproduction are more favorable than mitotic reproduction, with slight differences between them depending on the precise conditions.

We may conclude that the advantage of sexual reproduction over pure diploidy, in leading to greater fitness and therefore to a reduced mortality rate, really comes into play only with a sufficiently large frequency of mating, as opposed to mitosis as the means of reproduction, as found for the hereditary and habitual practice of sex in both Models A and B. This frequency is driven by the mutation rate Γ in Model A, and by the probability σ in Model B, namely the same mechanism as the conversion from haploidy to diploidy. On the other hand, greater frequency of mating, with the fusion of two gametes, one from each parent, means a “ $2 \rightarrow 1$ ” reduction in numbers, and this effect competes with the advantage gained from

increased fitness, leading to a saturation of the sexual population at increased rates, to $\sim 15\%$ as $\Gamma \rightarrow 1$ for Model A, and $\sim 10\%$ as $\sigma \rightarrow 1$ for Model B.

We were also able to show that a pair of simple, unicellular organisms who have accidentally converted to diploidy, and which subsequently engage in sexual reproduction, begetting one sexual offspring, can give rise to a population of sexual types which totally take over a finite population, provided they are also allowed to multiply by mitosis, on an equal footing with the haploids in the population.



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