

A MODEL FOR THE EVOLUTION OF NUCLEOTIDE POLYMERASE

DIRECTIONALITY

by

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A DISSERTATION

Submitted to the Faculty of the Stevens Institute of Technology  
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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2010



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## ABSTRACT

It is my goal to use the fundamental principles of thermodynamics to understand life. Specifically, it is my desire to use this approach to investigate evolution as an classical optimization problem. This approach is inspired by the observation that life is, at its most fundamental level, an elaborate collection of chemical reactions. Individually, each of these reactions is governed by the laws of thermodynamics, and thus far there is no known upper limit to the scale at which thermodynamic principles may be applied.

In this work I present a model systems which is inspired by this hypothesis. In this model, I will look at the directionality of nucleotide polymerases, all of which synthesize new nucleotide polymers in a 5' to 3' direction. This phenomenon could be the consequence of a very early founder effect. Alternatively, it could be that this directionality evolved due to an inherent evolutionary advantage. The model I have constructed, based on the fundamentals of nucleic acid chemistry, reveals that under certain conditions there is, in fact, an advantage to  $5' \rightarrow 3'$  synthesis. These results provide an insight into the forces that may have driven early biological evolution.

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Date: March 26th, 2010

Department: Chemistry, Chemical Biology, and Biomedical Engineering

Degree: Doctor of Philosophy

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## Chapter 1

### Introduction

Evolution has classically been a science of the past. That is, the primary sources of evidence used in the formulation and validation of evolutionary theory have been the fossil record, geology, and observations of the current form and distribution of species as a result of events that took place in the past. Where evolution does exhibit predictive power, it is primarily in the ability to predict what new evidence from the past should eventually be revealed, such as the many fossils of transitional forms discovered over the past centuries. In this regard, evolution has been wildly successful.

Where the study of Evolution has been thus far lacking is in its ability to predict the future. That is, looking at a collection of fossils and historical records of environmental conditions, evolutionary theory gives us the ability to identify which selective pressures acted on past populations. What evolutionary theory cannot do, at present, is predict which environmental conditions or physical forms will act as selective pressures in the future. At best, we can make educated guesses based on past evidence, but we lack the ability to assign a concrete measure of confidence in such predictions. In the end, the only sure way to determine which individuals are best fit is to wait and see which individuals ultimately survive and reproduce.

This is not an inherent failing of evolution, but rather a sign of a young and developing field. The inability to predict the future course of evolution is tied directly to the fact that selective pressures can come from practically any feature of an organism. Everything from diet to behavior to physical form can lead to a selection for or against the eventual reproductive success of an organism. The situation in which evolution finds itself is akin to what physics would be like if every projectile had its

own unique laws of motion. What evolution desperately needs is a fundamental set of principles from which the success of any organism can be derived, much as the path of every projectile can ultimately be derived from Newton's laws of motion.

The implications of a coherent method for deriving the evolutionary success of an individual undergoing natural selection would be far reaching. Perhaps the most obvious implication would be an improved ability to predict patterns of disease emergence and transmission. Cancer treatment would be another potential area of benefit, as cancer is an evolutionary process. With each round of chemotherapy or radiation treatment, those cancer cells which are better adapted to resist treatment will survive. These cells will then seed a reemergent, more difficult to treat, tumor. Understanding the dynamics of evolution, and how to predict the future course of evolution, would improve our ability to design effective treatments for both microbial diseases and cancer.

Even non-biological processes are driven by evolution and obey many of the same laws that Darwin first laid out 150 years ago. Both languages and economies undergo evolutionary processes, driven by the same math as cancer or the origin of species[4]. That said, we must make a distinction between biological and non-biological evolution. The reason for doing so lies in the approaches that can be taken to investigate each type of evolutionary system. We know far more about biological organisms than merely that they evolve. The past 50 years has seen an explosion of knowledge about the chemistry of life and the operation of those molecular systems which compose cells and influence the phenotypes of complex multicellular organisms.

For this reason, biological systems undergoing evolution present a unique opportunity to attempt to derive the fundamental principles of evolution from the individual mechanics of the evolving systems. Specifically, with biology we can explore the feedback mechanism which drives evolution: the "Central Dogma" of biology.

This is the pathway by which information flows from an organism's nucleic acids, where it is stored, to the organism's proteins which then drive fundamental biochemical processes[7]. These biochemical processes determine the phenotypes eventually selected for or against in the process of natural selection. From this flow of information, it is understood that those organisms which contain the information for generating the better fit traits will become enriched in the population, and therefore will pass on this information in greater numbers than their less fit peers[2].

The biochemical processes that ultimately result in phenotypic traits can be very complex, and their ultimate effect may be indirect. This makes it difficult to study the biochemical evolution of all but the simplest of traits in the simplest of organisms. There is, however, at least one biochemical process that is relatively simple, and whose impact has a very direct effect on the eventual transmission of genetic information: the process of nucleotide synthesis. Since a prime requirement for reproduction, especially in an era when all life was unicellular, is the ability to replicate genetic information, one would expect this to be a process under heavy selective pressure. Also, since the phenotype of reproduction rate is very closely tied to and dependent upon the specific molecular mechanisms, this process provides a prime opportunity to study how organism-scale selection manifests itself at the molecular scale.

## **Nucleic Acid Polymerization**

The two classes of biologically important nucleic acids are Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA). These two classes are very similar, differing only by the presence or absence, respectively, of a 2' hydroxyl group on the ribose sugar of their individual monomers and the specific nitrogenous bases attached at the 1'

position. Both types of polymer are formed by a process of dehydration synthesis catalyzed by a class of enzymes known as nucleotide polymerases. The dehydration reaction takes place between one of the hydroxyl groups on the  $\alpha$ -phosphate of a nucleotide triphosphate and the 3' hydroxyl group of the terminal monomer on the growing nucleic acid chain[8].

What is phenomenal about this process is that, first, it occurs in all biological organisms and, second, that it always occurs in the same manner. To understand why the consistency of directionality is notable, it helps to understand the catalytic process that occurs at the active center of nucleotide polymerases. All known nucleotide polymerases share a common chemical mechanism. In this mechanism two divalent metal cations, coordinated by a number of acidic amino acids, facilitate the transfer of an electron pair from the free 3' hydroxyl group of one nucleotide to the  $\alpha$  phosphate, which is attached to the 5' hydroxyl of the other nucleotide[1] (see Figure 1.1).

Importantly, the catalytically active cations of the polymerase active site have no knowledge of whether the 5'-phosphate is attached to the incoming nucleotide or the growing chain. If one were to imagine a nucleotide polymerase that proceeded in the reverse direction of all currently known nucleotide polymerases, the only aspect of the naturally occurring enzymes that would need to be modified are those portions which attach to the growing nucleotide polymer or the incoming nucleotide, all of which are distinct from the catalytic center. That is, there should be nothing preventing the chemical reaction at a hypothetical  $3' \rightarrow 5'$  polymerase active site from occurring.

The fact that all biological organisms polymerize both DNA and RNA with the same directionality implies that this aspect of nucleotide polymerization was decided extremely early on in the genesis of life on earth, most likely even before the biological distinction between DNA and RNA functionality arose. If the active

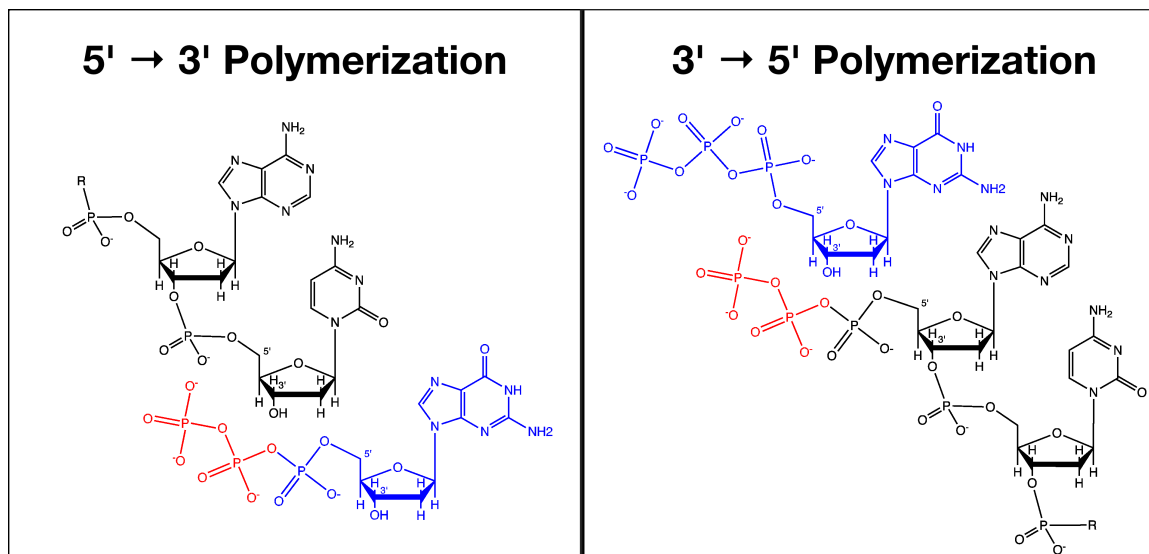


Figure 1.1: **Schematic of the Known  $5' \rightarrow 3'$  and the Proposed  $3' \rightarrow 5'$  Polymerization Reactions.** In each panel, the incoming nucleotide is colored blue and the pyrophosphate leaving-group is colored red. The  $3'$  and  $5'$  carbon atoms on the sugars of the incoming nucleotide and the terminal nucleotide of the growing chain are labeled (R represents the remainder of the growing polymer). Note that the pyrophosphate leaving-group is found on the incoming nucleotide in the  $5' \rightarrow 3'$  reaction, but the leaving-group in the  $3' \rightarrow 5'$  reaction is on the growing chain itself.

site mechanism of nucleotide polymerases cannot explain the bias of all life toward one polymerization directionality over the other, what alternative explanations might exist? There are two possibilities. The first is that the unique directionality is the consequence of a founder effect. A founder effect is when a small subset of a larger population is evolutionarily isolated and goes on to give rise to a new population. This new population would be expected to contain an oversampling of the traits of the small founding population as compared to the gene frequencies of the original population[6]. In the case of nucleotide polymerization, this would imply that at some point early on in the development of life on earth the population contained both forms of nucleotide polymerase, those that polymerize by extension  $3' \rightarrow 5'$  and  $5' \rightarrow 3'$ . Then, at some later point a subgroup of this population, consisting entirely

of organisms with a  $5' \rightarrow 3'$  polymerase, was isolated and subsequently gave rise to all life on earth today.

The other possibility is that there is an evolutionary advantage to the  $5' \rightarrow 3'$  directionality, and that all life polymerizes nucleotides in this direction as the result of a selection event. Unfortunately, the chances of finding any evidence directly supporting the hypothesis of a founder effect determining polymerase directionality are essentially nil. Short of finding some remnant modern population with reversed polymerases, the only evidence of an ancient  $3' \rightarrow 5'$  polymerase would be the enzymes or other early replicator molecules themselves, and individual molecules do not fossilize. The only way to shed light on which of these two competing hypotheses explains the current state of nucleotide polymerases would be to identify an evolutionary advantage imparted by a  $5' \rightarrow 3'$  polymerase over the reverse.

What might be the source of such an advantage? Looking again at the chemical reactions that would be carried out in each case (fig. 1.1), a major difference between the  $5' \rightarrow 3'$  case and the  $3' \rightarrow 5'$  case is the location of the pyrophosphate leaving-group relative to the other components of the polymerization reaction. This is important because it is known that triphosphate groups attached to nucleotides can spontaneously hydrolyze[5]. In the event that the triphosphate leaves prematurely with the  $5' \rightarrow 3'$  polymerase, it would be possible for the enzymatic machinery to discard the (now useless) incoming nucleotide and replace it with a new nucleotide containing an active triphosphate. In the event of a spontaneous hydrolysis with the  $3' \rightarrow 5'$  polymerase, because the active triphosphate in the reaction is on the growing chain the only choice the enzymatic machinery has is to discard all of its progress thus far or wait for some secondary machinery to replace the triphosphate group. Given that the former of these two choices represents significant waste, we can assume that the later choice is the only one that would be even remotely sustainable, but this

choice involves introducing a delay in reproduction.

A delay, on its own, does not necessarily mean that the  $3' \rightarrow 5'$  case is at enough of a disadvantage to be completely eliminated from a population through natural selection. After all, polymerase rates are variable and a hypothetical  $3' \rightarrow 5'$  polymerase could simply evolve to polymerize faster. Unfortunately, such modifications come with a cost. There is a relationship between speed of nucleotide polymerization and error rate[3], and polymerizing faster would cause the polymerase to make more mistakes. A higher frequency of errors during reproduction would introduce a higher rate of mutation, making it more difficult for the  $3' \rightarrow 5'$  polymerizing organisms to maintain their speed advantage. Again, on its own this is not reason enough to dismiss the possibility of a  $3' \rightarrow 5'$  polymerase. There are many factors at play, and to understand how all of these combined contribute to the fitness of an organism that synthesizes its nucleic acids in a  $3' \rightarrow 5'$  direction, we will need to model the competitive evolution of the two polymerase types.

## Modeling Evolution

Because nucleotide polymerases are responsible for replicating the genetic information of an organism, and have a direct influence on both the rate at which an organism can reproduce as well as the fidelity with which genetic information is conveyed from one generation to the next, the approach I will take is relatively straightforward. The model presented here involves simplified model organisms designed to represent the earliest forms of self-replicating life. It is assumed these organisms exist in an environment rich with nutrients and an excess of available energy. The result of this being that the rate of genome duplication serves as the lone limiting factor on reproductive rate.



Since polymerase directionality would have been fixed extremely early on in the origin of life, it is reasonable to assume that the recombination of genetic elements would contribute only a negligible amount of variation to the model organisms. Additionally, the simplest of nucleotide polymerases observed in nature are the products of single genes. Even if there was recombination of individual genetic elements, the exclusive focus of this model is the polymerase. Whether a product polymerase gene is transferred intact to the offspring of one organism or transferred horizontally will not affect the conclusions that can be made. This is a result of the first assumption made that the model organisms have an excess of all resources aside from the polymerase. That is, whatever organism a polymerase may end up in, it will always be the single determining factor for both reproductive rate and genetic fidelity.

Ultimately, the goal of this model is to investigate the link between a well understood molecular process, nucleotide polymerization, and an evolutionary outcome, the selection for polymerase enzymes which proceed  $5' \rightarrow 3'$ . If there are general rules to be found that govern the process of evolution all the way from the molecular level to the scale of entire organisms and even populations, hopefully starting with a simple model like the one presented here will serve as an important first step in their eventual discovery.

## Chapter 2

### Design of the Polymerase Evolution Model

This chapter provides a detailed description of the design of the model system used to investigate the question of the evolution of nucleotide polymerase directionality. The goal of this model is to have organisms containing either a  $5' \rightarrow 3'$  or a  $3' \rightarrow 5'$  nucleic acid polymerase compete with each other in order to see which of the strategies, if either, are evolutionarily dominant. In the model, evolutionary pressure is applied by the rate at which organisms can reproduce and the fidelity with which they duplicate their genomes. Evolutionary momentum is introduced through simple genetic mutation. The model is also designed such that the influence of temperature on the outcome of this competition can be investigated.

### Overview

In simplest terms, this model consists of a number of individual organisms in competition with each other. The model can be largely divided up into, and thought about most clearly as, four interacting components. These are the environment, the organisms, the genomes, and the polymerases. The relationship between these components begins with the environment. Each run of the simulation involves exactly one environment. An environment may contain many organisms, up to a predefined carrying capacity, and at the beginning of a simulation run the environment is pre-populated organisms according to a set rules.

Each organism contains one genome. At the start of an organisms “life”, it uses its genome to create a polymerase. The polymerase replicates either  $5' \rightarrow 3'$  or  $3' \rightarrow 5'$  and may not change direction. The polymerase then replicates the genome

at a predetermined rate and with a random chance of introducing errors (mutations). When the polymerase is finished duplicating the genome, the organism will attempt to divide by binary fission using the newly created genome. If it is successful in doing so, then the new organism is added to the environment and begins a fresh life cycle while the original organism resets itself and also begins a fresh cycle.

A number of generalizations and assumptions have been made in order to render the problem being investigated tractable, but a consistent effort has been made to remain as true to life as possible. The motivation behind this is that, while the exact values generated by this model may not be precisely those that would be observed in the laboratory, the trends observed should hold true when transferred to the bench. For each piece decisions have been made regarding which aspects of the component are explored in depth, and which aspects are neglected, with an eye to the larger goal of investigating the role of polymerase directionality. Following is a detailed look at each component in turn.

## **Environment**

At the start of a simulation run, the environment for that run is initialized with a starting population of organisms. To create this starting population, a set of organisms with their genome length, polymerase rate, and polymerase directionality specified is divided according each organism's designated frequency in the starting population. The starting population size can be any number up to the specified maximum population of the environment.

A key defining feature of this model is that the environment is constrained in some ways, but not in others. Specifically, the number of organisms that can simultaneously co-exist has a hard numerical limit; a carrying capacity. On the other hand,

the amount of available energy, the quantity of activated nucleotide triphosphates, and the other raw materials required for forming a cell are all considered unlimited. In reality, the carrying capacity is a generalization that could correspond to physical space limits, but it could just as easily correspond to the aggregate limitations on the other resources not explicitly modeled.

The choice of limitation based on carrying capacity was driven by two factors. The first relates to the fact that selection during an exponential growth phase could potentially operate differently than during stationary phase growth. By placing an upper limit on the size of a population, it is possible to investigate selection during both growth phases. Second, while attempting to explicitly model all of the various resource and space constraints that might ultimately limit growth might yield a more complete model, it would also significantly increase the complexity of the model without adding much insight into the question at hand. Finally, numerically or density limited growth is a common observation across a wide range of living creatures from single cells to large populations of complex animals.

In order to model density dependent growth inhibition as the number of organisms approaches the carrying capacity, a random death probability is introduced to the environment. In keeping with observations that the pressure of density dependant inhibition is greater as the population of an environment approaches the carrying capacity, the death probability is calculated with an inverse function of the remaining capacity

$$P_{death} = \frac{1}{(N - n) + 1}$$

where  $N$  is the carrying capacity,  $n$  is the number of organisms currently in the environment, and 1 is added so that the probability of at least one organism being culled from the environment when the carrying capacity is reached is  $P_{death} = 1$ .

The model iterates its environment in a stepwise fashion. During each step, each of the organisms contained within the environment is allowed to carry out one time-step of its life cycle, followed by a population culling. Culling is carried out by calculating the death probability as above, and then randomly applying that probability to the environment to decide if an organism should be removed. If the decision is made to remove an organism, one is chosen from the environment randomly, removed, and the death probability is recalculated and reapplied. This process will repeat until the decision is made not to remove an organism. In this way, the number of organisms removed at each time step follows a Poisson distribution with an expectation value of  $P_{death}$ .

## Organism

Organisms are created and added to the environment either at the start of a simulation run, as part of the starting population, or during the simulation run as the result of binary fission of an existing organism. If the organism is created at the start of the simulation then its genome and polymerase properties are determined by the values used to seed the population. If it is created during a simulation run, then its properties are derived from those of its parent, with the possibility of introduced variation. This variation is embodied by, and in the model determined by, the genome contained within the organism. Each organism contains exactly one genome and one polymerase. Having only one polymerase is a simplification, but it is justified by considering this one polymerase as an exemplar of the various polymerase enzymes that would be found in a real organism.

Each organism is modeled as a state machine. The two states in which they can exist are: *Polymerizing* or *Duplicating*. At creation, each organism starts in the

*Polymerizing* state. When an organism is in the *Polymerizing* state, each simulation time-step is used to allow the organism's polymerase to add nucleotides to the genome copy being constructed. At the end of each time-step, the polymerase is queried as to whether or not it is finished with constructing the nascent genome. If it is, then the organism shifts to the *Duplicating* state. Otherwise, it remains in the *Polymerizing* state.

When an organism reaches the *Duplicating* state, the first task is to determine if the genome copy constructed by the polymerase is viable. This determination is made by comparing the fraction of errors made by the polymerase during polymerization against the empirically observed upper tolerance of 76%. If the genome copy is not viable, then it is discarded and the organism returns to the *Polymerizing* state to create a new genome copy. That is, no allowance is made for damage repair. This is yet another simplifying assumption, but considering that the model aims to simulate the earliest forms of life, it is doubtful that complex *post facto* error correcting mechanisms would have existed (or that they would have contributed significantly to the evolution of such early organisms).

If the genome is viable, the next determination that must be made is whether or not there is available capacity in the environment for a new organism. If the number of organisms in the environment is not at the environment's carrying capacity when the environment tells the organism in the *Duplicating* state to step, then a new organism containing the newly synthesized genome is created and inserted into the environment. In this case, the parent organism will return to the *Polymerizing* state to begin construction of another new genome. If, instead, the environment is at capacity when an organism, with a viable genome product, in the *Duplicating* state is told by the environment to proceed one time-step, the organism will remain unchanged, in the *Duplicating* state. It will next attempt to add a new organism with the synthesized

genome during the next environment step, but nothing will change until then.

## Genome

Genomes are created before the organism in which they are contained. In the case of a running simulation, the new genome is created by the parent organism before binary fission. For the starting population of organisms used to seed a simulation, the genomes are created ahead of time with predefined properties. The model genomes perform three important functions for the simulation: they generate polymerases with properties defined by the genome, they track the progress of the organism's polymerase during polymerization, and they return a copy of themselves once polymerization has completed.

Each genome codes for either a  $5' \rightarrow 3'$  polymerase or a  $3' \rightarrow 5'$  polymerase, and this will not change through subsequent generations. There is the possibility that this is not perfectly reflective of how early organisms may have functioned, as it is conceivable that, through acquired mutations, a genome which coded for a  $3' \rightarrow 5'$  polymerase may evolve in subsequent generations into a genome that codes for a  $5' \rightarrow 3'$ . Were this to occur, it would either have to be through a gradual mechanism or a small mutation affecting global properties of the polymerase. A gradual mechanism would be one where the forward or reverse speed of the polymerase is reduced to nearly zero, and then eventually the directionality flipped. It is safe to discount this possibility in the model, as such a transition would require the survival of an organism with an especially slow polymerase. As a consequence of the starting assumption that polymerase speed is the primary determining factor in the speed of replication, such organisms would be quickly out-competed in the simulation.

The alternative mechanism of a small mutation leading to a global change can

also be neglected for two reasons. First, if such a mutation were possible, the fidelity of communicating polymerase directionality from one generation to the next would be diminished. This would imply that selecting against a  $3' \rightarrow 5'$  polymerase would effectively be impossible, as such polymerases could appear at almost any time. This would also narrow the window of possible founder populations were the alternate founder effect hypothesis considered. In the case that polymerase directionality were trivially reversible, the hypothetical founder population would not only have to be homogenous in their polymerase directionality, but they would also have to have a unique restriction on the directionality reversing randomly. This also highlights the second reason that the possibility of such a mutation need not be considered: if such mutations were possible, it would be expected that they could be observed today. Certainly, it is possible that such a mutation might have existed in the past but have subsequently been locked out of the population of current polymerase sequences, but this seems unlikely. Nucleotide polymerases are wildly variable in their sequence and structure throughout all forms of life, and yet none exhibit reversed directionality.

When a genome is asked by its organism to create a polymerase, it will imbue the polymerase with a fixed polymerase rate in addition to a fixed directionality. The precise value the polymerase speed will take is determined when the genome is created from its parent (or from predefined values at the beginning of a simulation). The variance from its parent genome's polymerase speed is derived from the amount of acquired mutations as described below. Once the polymerase has been generated and polymerization begun, the polymerase will inform the genome during each simulation time-step how many nucleotides have been added to the copy being created, and how many of those are erroneous base pairs. The genome keeps track of this information for later reporting and calculations.

Finally, a genome can be queried as to the viability of the copy constructed



and asked to return that copy. The determination of viability is simply calculated by taking the percent errors ( $\frac{E}{L} * 100$ ) where  $E$  is the number of errors and  $L$  is the length of the full genome). If this percentage is above the threshold, set at 34% based on empirical observations, then the genome is deemed inviable. Given a viable copy was constructed, the genome can then return the copy but, in doing so, it must determine the properties of this new genome. As mentioned above, the directionality is inherited exactly. The polymerase speed coded for by the copy genome is determined in a two step calculation based on the number of errors (mutations) introduced. First, the variance from the parent genome's encoded polymerase rate is calculated by taking

$$\Delta = \frac{r_{max} - r_{min}}{2} * \frac{M}{M_{max}}$$

where  $r_{max}$  and  $r_{min}$  are the maximum and minimum possible rates, respectively,  $M$  is the number of errors made during replication, taken as a fraction of total genome length, and  $M_{max}$  is the maximum tolerated fraction of errors. The maximum and minimum rates were chosen as 1 and 10 nucleotides added per simulation time-step to reflect the roughly 10-fold range of empirically observed polymerase rates. The logic behind this metric is that more mutations will, on average, lead to greater alteration of enzyme function. Certainly there are individual mutations that could, on their own, have a large effect on polymerase rate, but averaged over many polymerases and many possible mutations, this general trend should hold.

Once the variance is determined, it must be applied to the parent genome's encoded polymerase rate in such a way that the child's genome does not code for an invalid polymerase. So, if the variance would generate a polymerase rate above the maximum, then it is subtracted from the parent rate, and vice versa. If the variance would not result in an invalid rate being either added or subtracted, then it is applied

to the parent rate with an equal probability of either outcome.

## Polymerase

The single most significant component of the model is the model nucleotide polymerase itself. The other three components described above serve primarily to ensure that selection and descent with modification will occur, but it is the polymerase which embodies the trait that is ultimately being selected for or against: the polymerase's directionality. As with the genomes, the directionality and rate of a polymerase does not change subsequent to its creation. These properties are determined by the genome from which the polymerase is generated. The polymerases only have one function during a simulation: to add nucleotides to a growing genome copy. How this occurs depends on the polymerase directionality and the temperature of the simulation.

When an organism in the *Polymerizing* state carries out one step of simulation time, it requests of its polymerase for nucleotides to be added to the genome being synthesized. The polymerase does this by running through a loop with a maximum number of iterations determined by its rate. So, for example, a polymerase with a rate of 7 can carry out its nucleotide addition loop a maximum of 7 times. During each loop, two determinations must be made. First, the polymerase must determine whether the nucleotide about to be added is activated, and second it must determine whether a properly base-paired nucleotide is being added, or whether the nucleotide being added will generate a replication error.

The reason that the polymerase must determine whether or not the nucleotide being added is activated is due to a consequence of chemistry. All known nucleotide polymerases in nature use, as their substrate, nucleotide triphosphates. They use the energy released by cleaving the bond between the  $\alpha$  and  $\beta$  phosphates attached to

their 5' carbon to drive the polymerase reaction forward. However, this bond can be cleaved through spontaneous hydrolysis by water. The equilibrium between active and inactive nucleotides can be described by a Boltzmann distribution:

$$K = e^{-\frac{\Delta G^0}{RT}}$$

where  $\Delta G^0$  is the standard free energy of the hydrolysis reaction. To simplify the simulation calculations, the entire  $\frac{\Delta G^0}{RT}$  term is expressed as a generic simulation temperature  $\frac{1}{t}$ .

Where this process of inactivation becomes important is in how it will differentially affect a  $5' \rightarrow 3'$  polymerase vs a  $3' \rightarrow 5'$  polymerase. In the  $5' \rightarrow 3'$  case, the one that is observed in all known forms of life, the triphosphate is attached to the incoming nucleotide about to be added to the chain. If this incoming nucleotide becomes inactive before the polymerase has a chance to join it to the growing nucleic acid polymer, then this represents a wasted addition step. That is, if a polymerase with a rate of 7 has one of its nucleotides become inactive before phosphodiester bond formation, then it will only add 6 nucleotides during this simulation time-step.

On the other hand, in the case of a hypothetical  $3' \rightarrow 5'$  polymerase, the triphosphate group would be attached to the 5' carbon of the nascent nucleic acid polymer. If, during an addition step, this triphosphate is cleaved the polymerase will not be able to compensate by drawing in a new nucleotide from the pool of available nucleotides. Rather, the polymerization process will necessarily halt while the terminal end of the growing chain is reactivated. In the simulation, that process is modeled by terminating the polymerase's addition of new nucleotides when an inactivation event occurs. That is, if a  $3' \rightarrow 5'$  polymerase with a rate of 7 experiences inactivation during the addition of the 3rd nucleotide in this time step, then only 2

new nucleotides will be added to the growing genome during this particular step.

Furthermore, whereas a  $5' \rightarrow 3'$  polymerase will be constantly drawing in new activated nucleotides from the pool of all available nucleotides, a  $3' \rightarrow 5'$  polymerase depends on the nascent chain remaining activated. The consequence of this is that the probability of a  $5' \rightarrow 3'$  polymerase experiencing a deactivation event depends only on the free energy of the reaction and temperature, irrespective of polymerase rate. Alternatively, a  $3' \rightarrow 5'$  polymerase runs a greater risk of the triphosphate on the nascent chain becoming deactivated the longer it waits before adding a new nucleotide. To model this, the probability of deactivation is multiplied by the difference between the rate of each  $3' \rightarrow 5'$  polymerase and the maximum possible polymerase rate.

Finally, each time a new nucleotide is added by a polymerase, there is a risk that the nucleotide will be of the wrong sort. The primary driving force discriminating properly base-paired nucleotides from improperly paired nucleotides is the formation of hydrogen bonds between the incoming nucleotide and the corresponding nitrogenous base of the template nucleic acid. This process can be modeled by a similar Boltzmann distribution as used for determining the probability of a nucleotide becoming deactivated, but the  $\Delta G^0$  of base-pair hydrogen bond formation is approximately half that of spontaneous triphosphate hydrolysis. So, in the model the probability of an improper nucleotide addition event is calculated using  $\frac{1}{2}$  the simulation temperature.

Recently, research has indicated that hydrogen bond formation is not sufficient to completely explain the fidelity of correct pair formation by nucleotide polymerases. It has been suggested that polymerases also take advantage of geometric differences between proper and improper pair formation to improve their ability to discriminate. In the model, this additional factor is treated as a simple flow problem. If a polymerase is roughly represented as a cylindrical tube, the narrower the tube is the greater will be

its ability to reject geometrically unfavorable nucleotide pair configurations. However, the rate of the polymerase will also be affected by the radius of the tube (see figure 2.1 for an illustration of this concept). If rate is taken as the flux of nucleic acid through the tube, then we end up with a simple square root relationship between the ability of the polymerase to reject based on geometry and the number of nucleotides it can add in one simulation time-step. Specifically

$$\Gamma = \sqrt{r}$$

describes the geometric discrimination and

$$P_{error} = K * \Gamma$$

is the probability of making a mistake during each nucleotide addition event, calculated as the product of the temperature dependent Boltzmann distribution,  $K$ , and the geometric discrimination function,  $\Gamma$ .

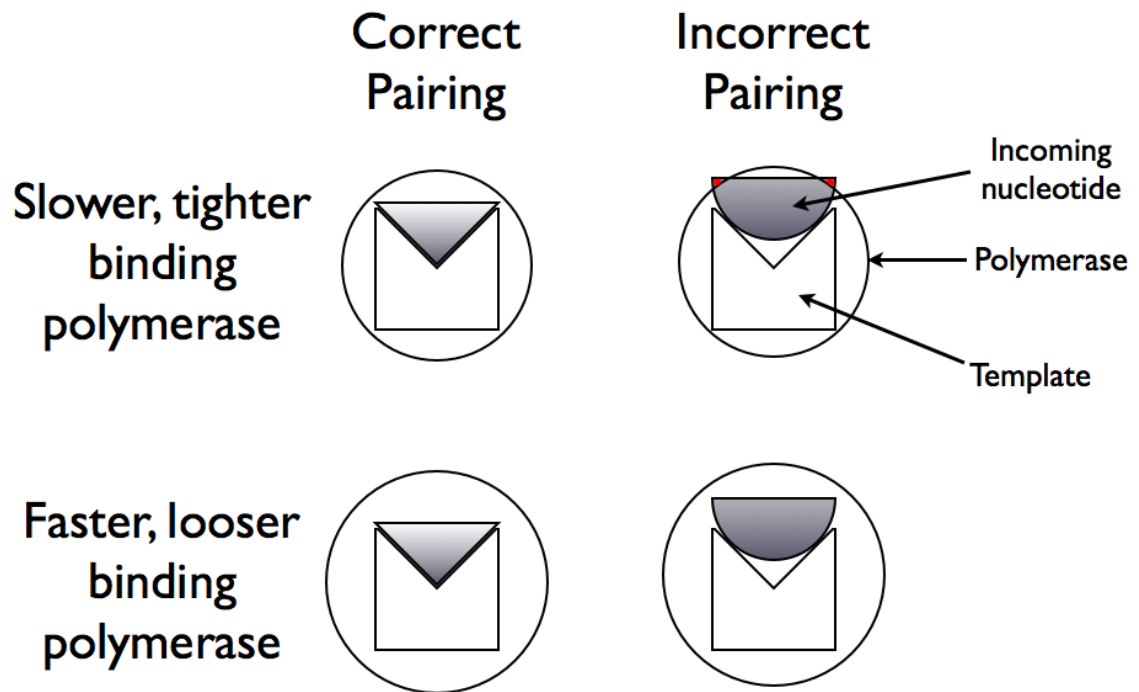


Figure 2.1: **A schematic diagram of geometry based polymerase discrimination.** The simplistic model of geometry based discrimination, and its relationship to polymerase rate, assumes that a tighter binding polymerase will be better able to exclude incorrect nucleotides based on shape. However, being tighter binding will restrict the rate at which the polymerase can translocate along the nucleic acid.

## Chapter 3

### Experimental Procedure and Results of Polymerase Modeling

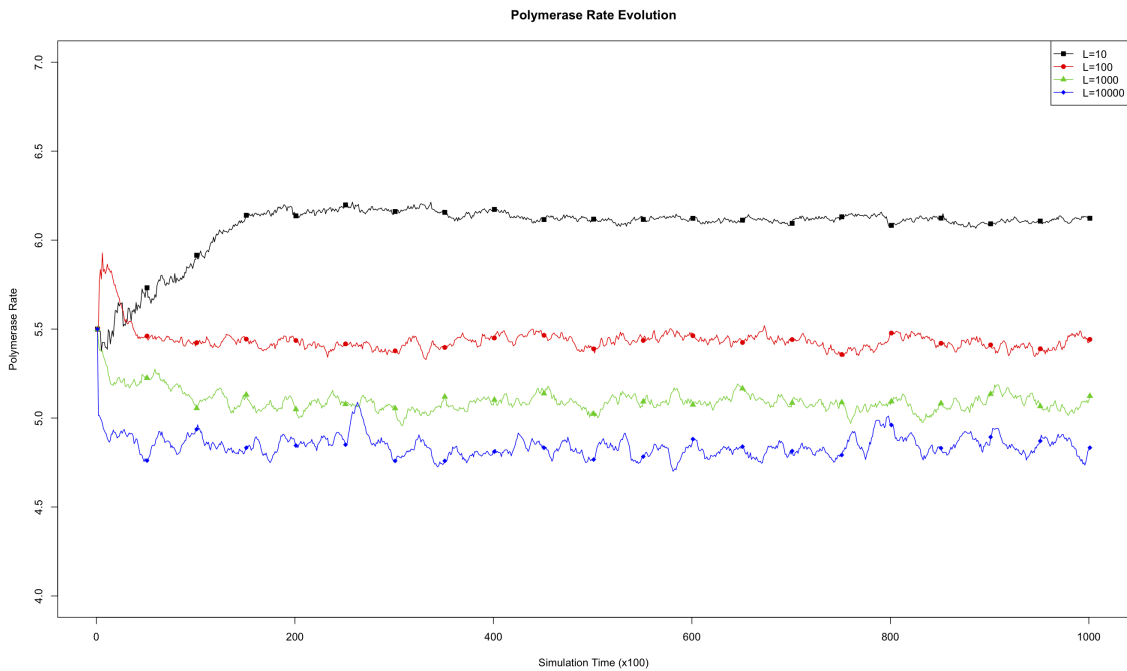
The procedure for performing an experiment with the model for polymerase directionality evolution consists of constructing an environment for the model to operate on, then setting the model running for a set number of time steps. Various parameters of the simulation are reported at set intervals during the run. Because a number of the dynamical systems in the model depend on randomly generated numbers, each experiment was carried out in 10 copies to smooth fluctuations, and all of the values reported represent a numerical average of all 10 runs. All plots were generated using the R software package.

The dynamics included in the model for polymerase directionality should generate scale free results. That is, the evolution of traits should show the same dynamics regardless of population size or genome length. In this model, the only trait capable of evolving is polymerase rate, so in order to verify that the dynamics were indeed scale free, the first experiments carried out were designed to investigate simple dynamics of polymerase rate evolution over a range of scales. One experiment consisted of starting populations of 10 organisms and a maximum population (environmental carrying capacity) of 1000 organisms combined with 4 different genome lengths as described in table 3.1.

Experiment	Temp. ( $t$ )	Starting Pop.	Max Pop.	Genome Length
#1	0.40	10	1000	10
#2	0.40	10	1000	100
#3	0.40	10	1000	1000
#4	0.40	10	1000	10000

Table 3.1: Determining genome length scale effects.

In order to avoid a founder bias with regards to polymerase rate, in each experiment the ten organisms in each seed population consisted of one organism of each of the ten possible polymerase rates. The simulation temperature of 0.40 was chosen because it results in a significant amount of error, and therefore introduced variability, during growth but is not a high enough temperature that certain other temperature effects begin to alter growth and evolution. The results from this experiment are plotted in figure 3.1.



**Figure 3.1: Effects of Genome Length on Polymerase Rate Evolution.** The average polymerase rate for the entire simulation population is plotted against simulation time steps (each unit on the abscissa is equivalent to 100 simulation time steps). The organisms each had genomes of length 10, 100, 1000, or 10000.

Second, a set of experiments were carried out to investigate the effect of population size on the evolution of polymerase rate. In this experiment, the genome length of the organisms in each environment was held constant at 1000, the starting population was set to 10, and the maximum population was set to either 100, 1000,



Experiment	Temp. ( $t$ )	Starting Pop.	Max Pop.	Genome Length
#5	0.40	10	100	1000
#6	0.40	10	1000	1000
#7	0.40	10	10000	1000
#8	0.40	10	100000	1000

Table 3.2: Determining size scale effects.

10000, or 100000. As in the first set of experiments, the starting population in each environment was seeded with organisms with polymerase rates evenly distributed in the range 1-10 and the simulation temperature was set to 0.40. Table 3.2 summarizes these experiments.

Figure 3.2 is a plot of the average polymerase rate of the population in each environment against the simulation time. Based on these initial experiments, it was decided that a maximum population size of 1000 with a genome length of 1000 could serve as an adequate representative of the dynamics over the range of possible values. These values were chosen because they also keep the size of the simulations reasonable with regards to the amount of computational time required to run each simulation, since the run time of the simulations scale with the population size and organisms with longer genomes require more time-steps to achieve the same number of doublings as organisms with shorter genomes.

Finally, in order to validate the model and how reasonably it simulates the observed growth dynamics of biological organisms, a set of experiments were carried out starting with small populations of 10 organisms, as before, and following their growth at different temperatures. The first two sets of experiments were only carried out with forward polymerizing organisms. To be sure that model organisms with reverse,  $3' \rightarrow 5'$ , polymerases had growth dynamics similar to forward polymerizing organisms, these experiments were carried out using both types of organisms.

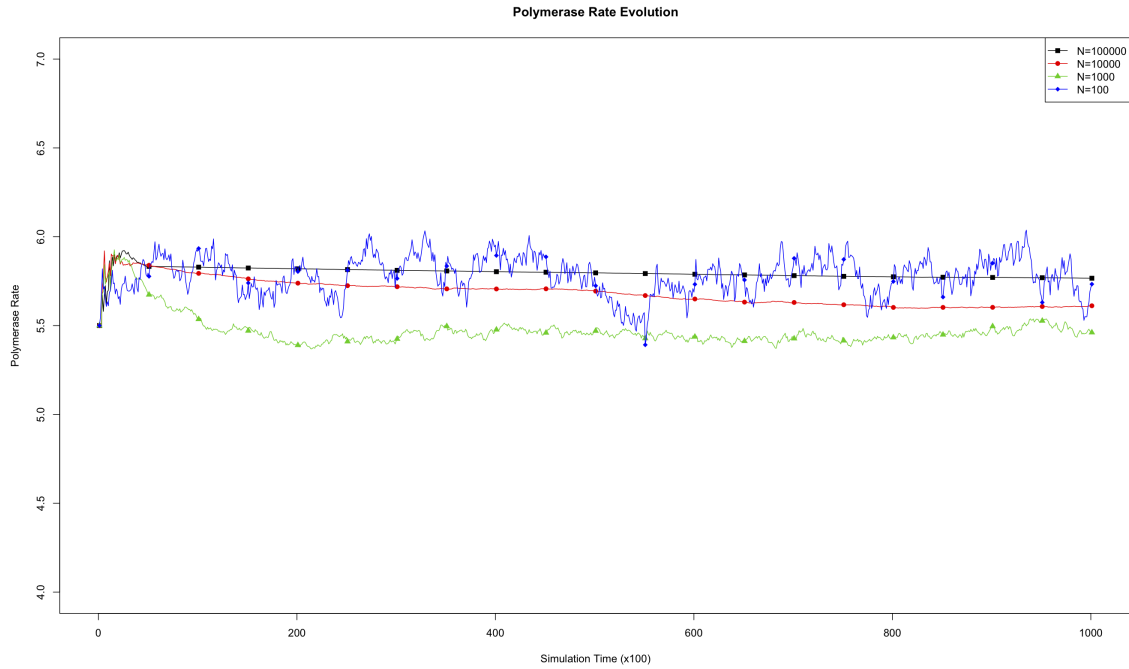


Figure 3.2: **Effects of Environmental Carrying Capacity on Polymerase Rate Evolution.** The average polymerase rate for the entire simulation population is plotted against simulation time steps (each unit on the abscissa is equivalent to 100 simulation time steps). The organisms each had a genome length of 1000, and the environments had a maximum capacity ( $N$ ) of 100, 1000, 10000, or 100000 organisms.

Table 3.3 summarizes the parameters used for these experiments.

For each experiment, the population was plotted against simulation time in figure 3.3 and the average polymerase rate of all the organisms in each environment is plotted in figure 3.4. Values for the forward polymerizing organisms are indicated with closed circles and values for the reverse polymerizing organisms are indicated with open circles.

In each of the experiments performed up to this point, forward or reverse organisms were allowed to grow in isolation. In order to gain some insight into the dynamics of polymerase evolution, it is necessary to set these two classes of model organisms in competition with each other. When considering the competition of

Experiment	Temp. ( $t$ )	Max Pop.	Genome Length	Directionality
#9-13	0.10	1000	1000	forward
	0.30			
	0.40			
	0.50			
	0.60			
#14-18	0.10	1000	1000	reverse
	0.30			
	0.40			
	0.50			
	0.60			

Table 3.3: Growth dynamics at various temperatures.

organisms, there are two domains which are interesting to probe. The first is the competition of the organisms as they explore a new ecological niche. That is, the way in which organisms compete during phases of exponential growth. The second is the competition that occurs when an environment is already at its carrying capacity. It would be expected that an evolutionary strategy which results in the most rapid growth should dominate during exponential growth. It is also conceivable that such a strategy might not represent the most efficient use of resources available and therefore might ultimately loose out to a different strategy when growth is limited by resources.

To understand both of these domains, two more sets of experiments were performed. In the first set, the simulation environments were seeded with 10 organisms, 5 each with forward or reverse polymerizing polymerases. Again, in order to avoid unnecessary founder bias in the polymerase rates, a cluster of polymerase rates was included in the starting population. Since the starting population was divided between the two types of model organisms, the starting population consisted of one each of organisms with polymerase rates of 3, 4, 5, 6, or 7 going forward or backward. The experimental conditions are summarized in table 3.4. Simulations were carried out at various temperatures in order to additionally probe the effect that temperature

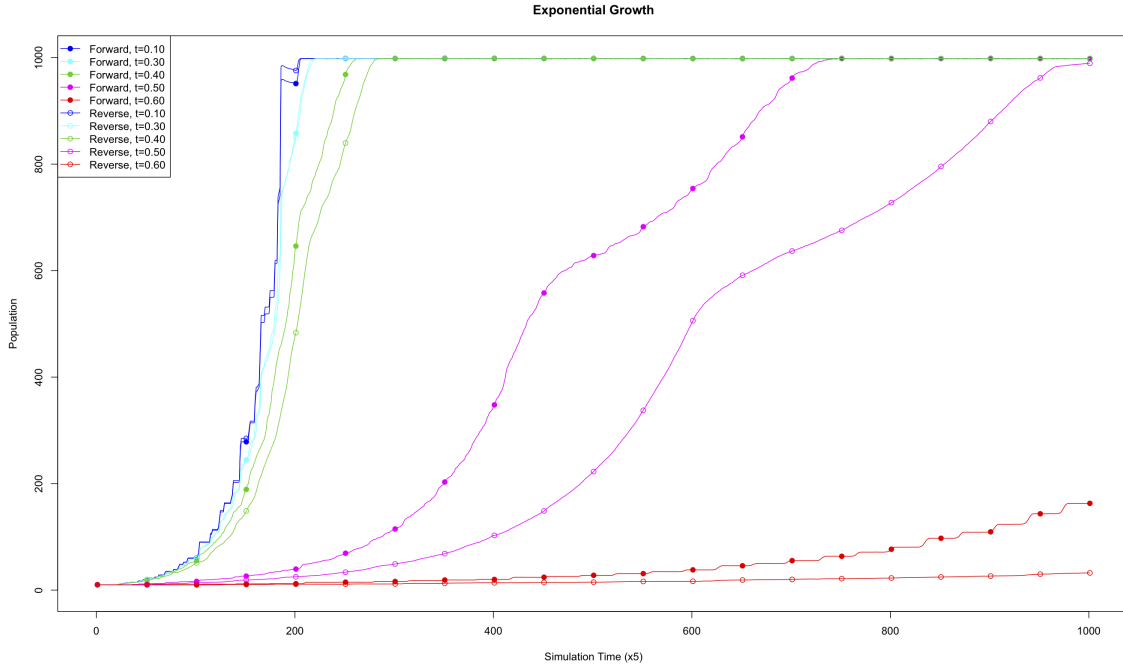


Figure 3.3: **Growth of model organisms at various temperatures.** The population size at each time point is plotted for the forward polymerizing organisms (closed circles) and reverse polymerizing organisms (open circles). The simulation time steps are plotted on the abscissa (each unit represents 5 simulation time steps).

would have on the competition between the two organism types.

A plot of how the polymerase rate evolved over time at each temperature is presented in figure 3.5. For each temperature condition, the forward rate is represented by the line with closed circles and the reverse rate is represented by the line with open circles. In addition to the way the polymerase rate evolves, it is also important to know how the organisms fared in terms of survival in the face of competition. So, the population of the forward and reverse polymerizing organisms at each temperature is presented in figure 3.6. Again, the lines with closed circles represent the forward polymerizing organisms and the open circles represent the reverse polymerizing organisms.

The final experiment consisted of seeding each environment with the maximum

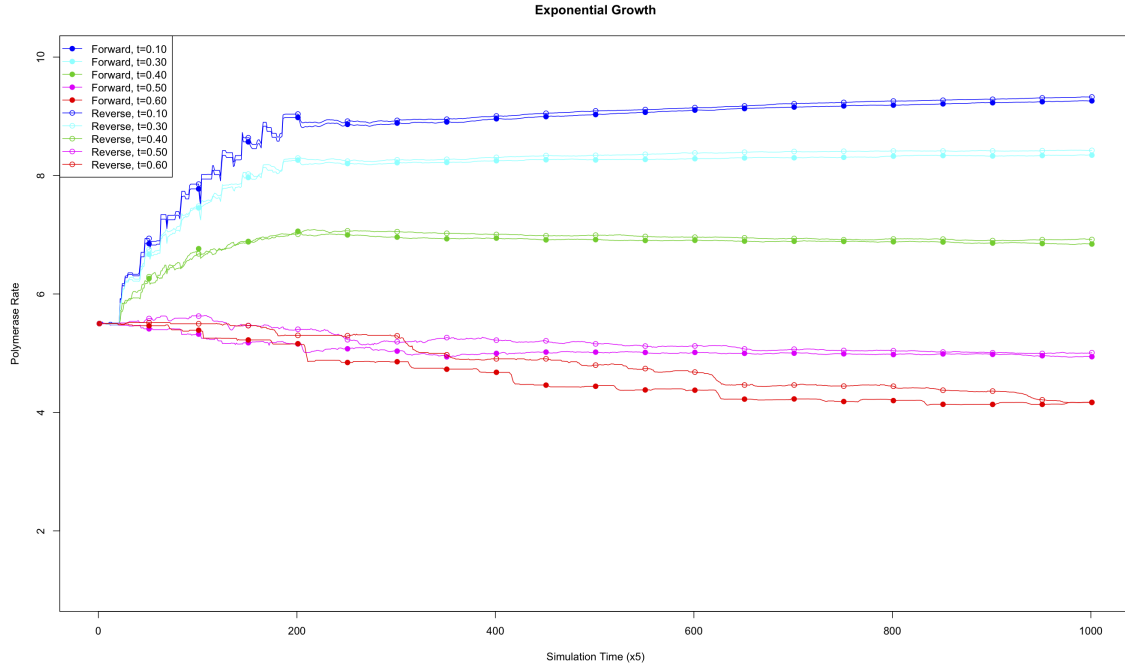


Figure 3.4: **Evolution of polymerase rate for exponentially growing model organisms at various temperatures.** The averages of the polymerase rates for all the model organisms in each environment is plotted for the forward polymerizing organisms (closed circles) and reverse polymerizing organisms (open circles). The simulation time steps are plotted on the abscissa (each unit represents 5 simulation time steps).

population for that environment. This means that each environment, with a carrying capacity of 1000, started with 500 forward polymerizing organisms and 500 reverse polymerizing organisms. Forward and reverse polymerizing organisms were equally distributed between all possible values, 1 to 10, for polymerase rate. The environments were all run at different temperatures. Table 3.5 summarizes the experimental conditions.

A plot detailing the change in polymerase rate over time is presented in figure 3.7 for the 0.10, 0.30, 0.40, 0.50, and 0.60 simulation temperature experiments. As in figure 3.6, the forward values are represented with closed circles and the reverse with open circles. The number of forward and reverse polymerizing organisms in the

Experiment	Temp. ( $t$ )	Max Pop.	Genome Length	Seed organisms
#19-23	0.10	1000	1000	5 forward
	0.30			(rates: 3, 4, 5, 6, 7)
	0.40			and
	0.50			5 reverse
	0.60			(rates: 3, 4, 5, 6, 7)

Table 3.4: Competitive growth at various temperatures.

Experiment	Temp. ( $t$ )	Max Pop.	Genome Length	Seed organisms
#24-34	0.10	1000	1000	500 forward (rates: 1-10)
	0.15			
	0.20			
	0.25			
	0.30			
	0.35			and
	0.40			500 reverse (rates: 1-10)
	0.45			
	0.50			
	0.55			
	0.60			

Table 3.5: Competitive growth in a full environment at various temperatures.

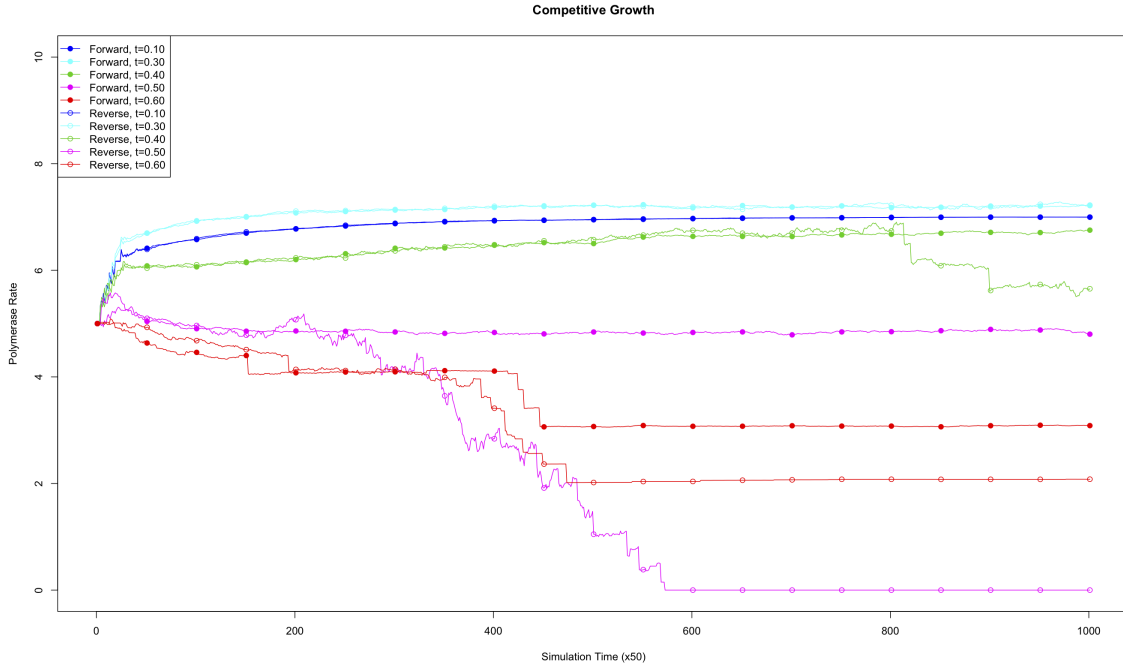


Figure 3.5: **Competitive evolution of forward and reverse polymerase rates.** The averages of the polymerase rates for all the forward polymerizing organisms (closed circles) and reverse polymerizing organisms (open circles) in each environment is plotted against simulation time. Each unit on the abscissa represents 50 simulation time-steps.

environments at the same subset of simulation temperatures is presented in figure 3.8. Additionally, since the primary goal of these experiments is to determine under what, if any conditions, the population of reverse polymerizing organisms drops to zero, the number of reverse polymerizing organisms at each of the simulation temperatures is presented as figure 3.9.

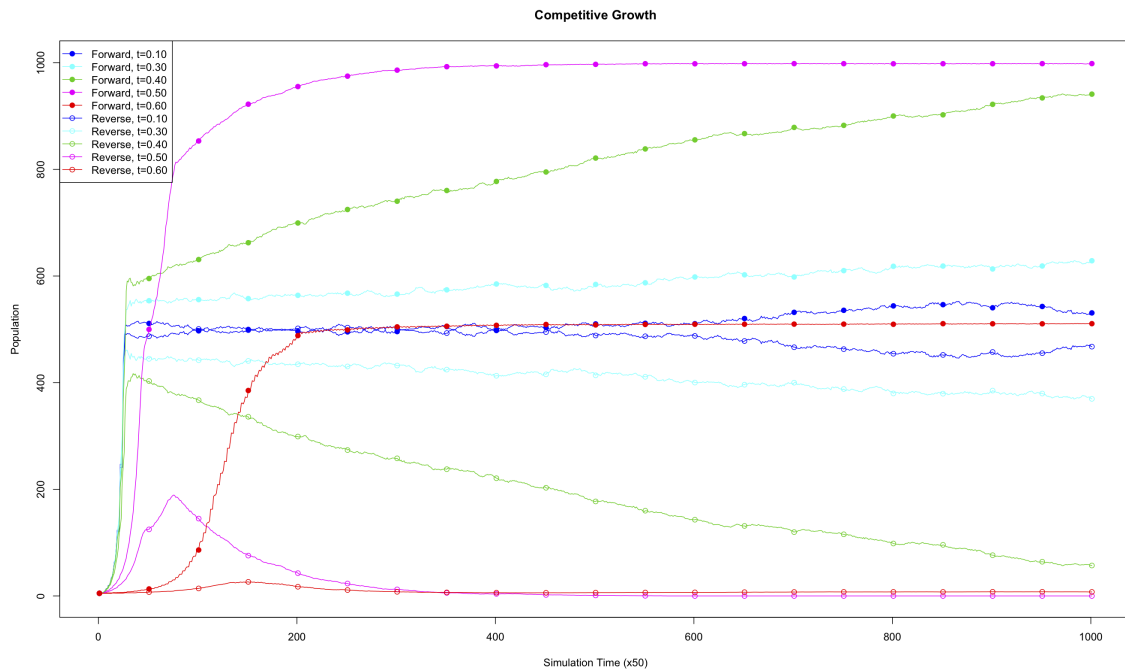


Figure 3.6: **Competitive growth of forward and reverse polymerizing organisms.** The total population of forward polymerizing organisms (closed circles) or reverse polymerizing organisms (open circles) for each environment is plotted against simulation time. Each unit on the abscissa represents 50 simulation time-steps.



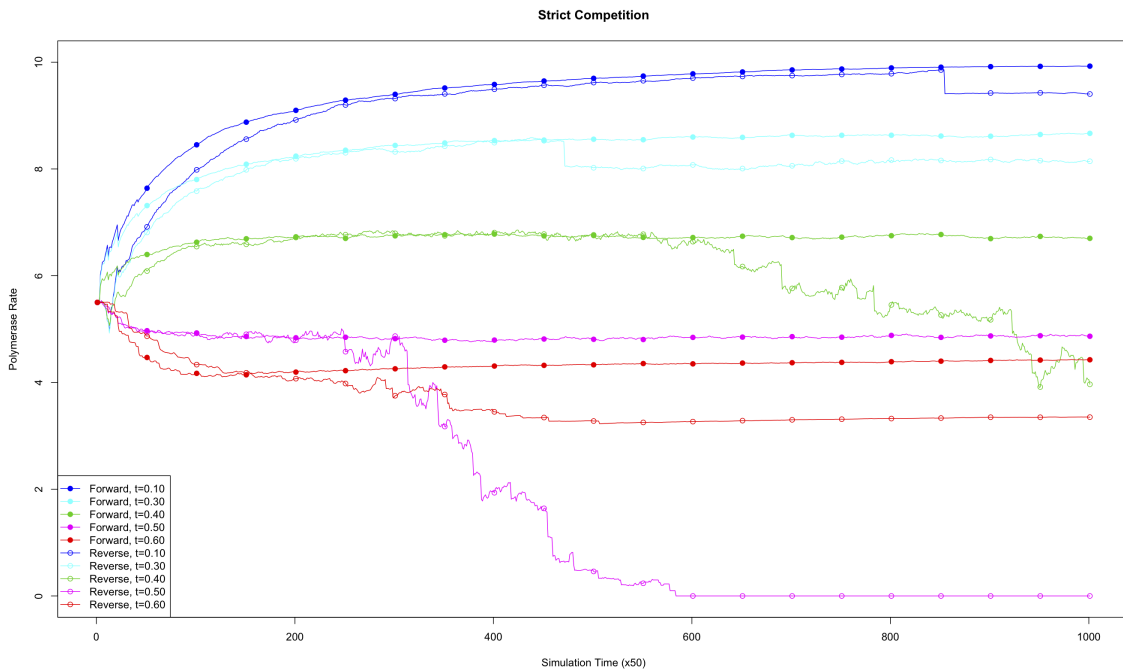


Figure 3.7: **Competitive evolution of polymerase rate for forward and reverse polymerizing organisms in a full environment.** The average of the polymerase rates for all of the forward polymerizing organisms (closed circles) and reverse polymerizing organisms (open circles) in each environment is plotted against simulation time. Each unit on the abscissa represents 50 simulation time-steps.

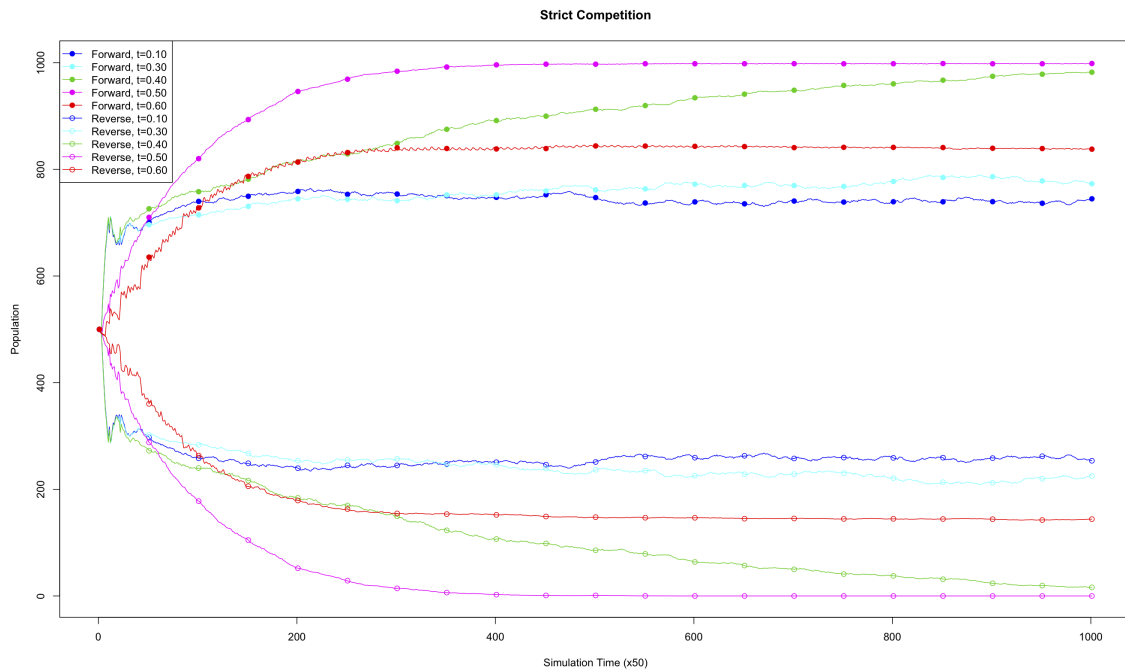


Figure 3.8: **Competitive growth of forward and reverse polymerizing organisms in a full environment.** The total population of forward polymerizing organisms and reverse polymerizing organisms in each environment is plotted against simulation time. Each unit on the abscissa represents 50 simulation time-steps.

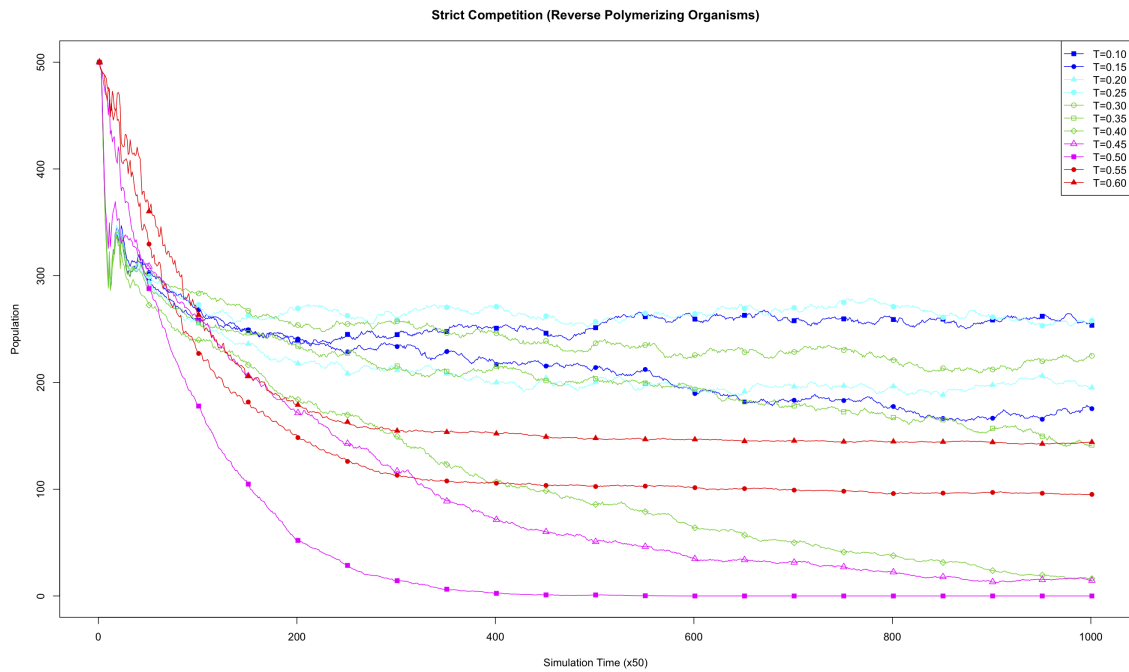


Figure 3.9: **Growth of reverse polymerizing organisms under competitive conditions.** The population of reverse polymerizing organisms present in each environment is plotted against simulation time. Each unit on the abscissa represents 50 simulation time-steps.

## Chapter 4

### Analysis and Discussion of Model Results

In the study of evolutionary dynamics there exists the concept of a stable equilibrium. The organisms in such a situation may not all represent the “best fit” mechanism for coping with a particular ecological niche, but at the same time none are sufficiently better off than the others that they will dominate the population. If organisms polymerizing nucleotides in a  $3' \rightarrow 5'$  direction represented a stable equilibrium with organisms polymerizing in a  $5' \rightarrow 3'$  direction, then it would be expected that organisms exhibiting such a trait should be found in nature. On the other hand, if  $3' \rightarrow 5'$  polymerization is a losing strategy when in competition with  $5' \rightarrow 3'$  polymerization, the lack of modern  $3' \rightarrow 5'$  polymerizing organisms would be an expected consequence of Darwinian evolution.

When modeling the competition between organisms with a  $5' \rightarrow 3'$  polymerase and organisms with a  $3' \rightarrow 5'$  polymerase, this is an important aspect of modern evolutionary theory to keep in mind. It is not sufficient to show that polymerizing nucleotides in a  $5' \rightarrow 3'$  direction is a better fit than, or produces organisms which reproduce faster than polymerizing nucleotides in a  $3' \rightarrow 5'$  direction. Rather, to discriminate between the possibility that the modern ubiquity of  $5' \rightarrow 3'$  polymerases is the result of a founder effect versus being a consequence of natural selection, we need to show that  $3' \rightarrow 5'$  polymerizing organisms cannot exist in a stable equilibrium. It is with this in mind that the model presented here was constructed.

In constructing this model, a number of explicit and implicit assumptions were made. The first, and perhaps largest, assumption is that polymerizing nucleotides to create new nucleic acid polymers by adding nucleotide triphosphates to the  $5'$  end of

the growing chain is physically possible. As there are no known polymerase enzymes which carry out the polymerization reaction in a  $3' \rightarrow 5'$  direction, it is impossible to say with certainty that such polymerases might exist. However, based on the nature of the active site chemistry of nucleotide polymerases, there is also no reason to believe that such  $3' \rightarrow 5'$  polymerases are not possible, beyond the fact that such polymerases do not currently exist. For this reason, we are comfortable with this assumption.

The second implicit assumption made in constructing this model is that the only viable building blocks for nucleic acid polymers are 5'-triphosphate nucleotides. If nucleotides phosphorylated on their 3' oxygen could be used by a hypothetical  $3' \rightarrow 5'$  polymerase, then the chemistry of such a polymerase would not, qualitatively, be any different than that of a  $5' \rightarrow 3'$  polymerase working with 5'-triphosphate nucleotides. That is, in the case that a  $3' \rightarrow 5'$  polymerase could work with a 3'-triphosphate nucleotide, then the activated triphosphate moiety would reside on the incoming nucleotide, instead of on the growing chain, in the same way that the triphosphate moiety is found on the incoming 5'-triphosphate nucleotide used by  $5' \rightarrow 3'$  polymerases.

We can discount the possibility of 3'-triphosphate nucleotides as raw material for a polymerase reaction for a number of reasons. If, in keeping with current scientific consensus, ribonucleic acids represent the more ancient form of nucleotides and the 2'-deoxyribonucleic acids are a more recent invention of biology, then a potential 3'-triphosphate nucleotide would have its triphosphate group attached on a carbon adjacent to a free hydroxyl. The free 2'-hydroxyl group of ribonucleic acids is known to polymerize hydrolysis reactions in a number of ribozymes, so it would not be unreasonable to assume that the 2'-hydroxyl would also catalyze a hydrolysis of the triphosphate group on a 3'-triphosphate nucleotide. This would serve to

significantly restrict the available primordial pool of 3'-triphosphate nucleotides as compared to 5'-triphosphate nucleotides, whose adjacent carbon's oxygen would normally be incorporated in the formation of the furanose ring. Indeed, it has been shown that ribose-3'-phosphate decomposes more readily under mild acid conditions than ribose-5'-phosphate.

The remaining assumptions made are the various explicit assumptions required for construction of the model itself as described in chapter 2. Of these, the two most important are the assumption that the dynamics of spontaneous nucleotide hydrolysis occur on a time-scale commiserate with the time-scale of nucleotide incorporation and the assumption of a geometry-based discrimination mechanism for incorrect nucleotide incorporation by the polymerase. As for the first of these two, we can surmise that the time-scale for spontaneous nucleotide hydrolysis cannot be significantly smaller than the time-scale for nucleotide incorporation, for if it were then nucleotide polymerization would, in effect, be impossible. If the time-scale were much larger, this would be the equivalent of removing a constant factor from the calculation for the probability that nucleotide inactivation occurs during polymerization. The effect of such an adjustment would be largely quantitative, raising the simulation temperature at which the effects reported would be observed. As for the assumption of geometry-based discrimination by the polymerase, the same experiments reported herein were reproduced without said mechanism incorporated in the model (data not shown). The only difference in the results from these experiments was that the viability of reverse polymerizing organisms at the highest temperatures (see figure 3.8) was not observed.

A note should be made on the significance of the simulation temperature used in the model. If we take the expression used in the model for the temperature dependence of the equilibrium between the polymerase-template-nucleotide complex for

the correct nucleotide, the free polymerase-template complex, and the polymerase-template-nucleotide complex for the incorrect nucleotide:  $e^{-\frac{1}{t}}$ , and compare it to the experimentally determined value of  $5\frac{kcal}{mol}$  for the  $\Delta\Delta G$  of this system, we can calculate the real temperature corresponding to each simulation temperature with the expression

$$T = t \frac{5\frac{kcal}{mol}}{R}$$

This gives us a real temperature of  $-22^{\circ}\text{C}$  for a simulation temperature of 0.10 and a real temperature of  $1200^{\circ}\text{C}$  for a simulation temperature of 0.60. Obviously this range of temperatures is unreasonably large for life, even when accounting for aqueous environments with high salinity or at large pressures.

As mentioned in the introduction, every effort was made when designing this model to remain faithful to the realities of the biochemical processes that a nucleotide polymerase must carry out. That said, a number of confounding factors limit the applicability of the precise quantitative temperature values used in the simulations to understanding real biological processes. It has already been mentioned that the need to reduce the probability of spontaneous nucleotide hydrolysis by a constant factor to account for a difference in time-scales would effect the absolute temperature values at which various simulation outcomes are observed. The same sort of constant factor consideration would, potentially, also need to be made to account for a difference between the precise effect of geometry-based discrimination of real nucleotide polymerases and the model values.

The primary model values impacted by simulation temperature are those for the equilibrium describing the incorporation of correct versus incorrect nucleotides during nucleic acid synthesis, and the equilibrium between active and inactive nucleotides. The chemical phenomena described by these values, however, are subject

to more influences than just temperature. For example, it is well documented that the energy associated with a correct nucleotide base-pairing is heavily influenced by the dielectric constant of the solvent and the concentration of nucleic acid counter-ions. The equilibrium of nucleotide triphosphates would, likewise, be subject to influence by effects of nucleotide concentration and pH. Thus, the value of simulation temperature can be thought of as a stand in for a number of thermodynamic factors that might influence the biochemical processes involved in the model processes. Therefore, the primary utility of the simulation temperature is in revealing the collected influence of thermodynamics on the system.

Before we consider these influences, let us look at the results of the initial experiments performed with the model. In figure 3.3, we can see that the model fairly accurately captures the exponential growth phase that is empirically observed in nature. At lower simulation temperatures, the gradual leveling off to a stationary phase population plateau is not as clean as we might like. Particularly, there seems to be a under-damping of growth by the simulation's population based culling probability. Still, an informative comparison can be made between the growth rates of forward and reverse polymerizing organisms. At the lower simulation temperatures of 0.10 and 0.30, there is effectively no difference between the two categories of organism. As the temperature is increased successively to 0.40, 0.50, and 0.60, the difference in population between the forward and reverse polymerizing organisms at each simulation time step becomes successively greater, to the point where, at a simulation temperature of 0.60, there is effectively no growth of the reverse polymerizing organisms. This difference in growth is expected due to the extra penalty suffered by reverse polymerizing organisms when a nucleotide triphosphate spontaneously hydrolyzes.

Remember that in these first experiments there is no competition between forward and reverse organisms. Each sort of organism is being allowed to grow in



isolation. This explains why, in figure 3.4 there is essentially no difference in the evolution of polymerase rates between the two. When growing in isolation, the only evolutionary pressures present are the deleterious effects of too many polymerization errors and density dependent culling, and these two factors will be experienced identically by either forward or reverse polymerizing organisms.

As we begin to put the organisms in competition with each other, figure 3.5 reveals that, at higher temperatures, the evolution of the polymerase rates of the forward and reverse polymerizing organisms begin to diverge from each other. At the lower temperatures of 0.10 and 0.30, there is still effectively no difference in the relative polymerase rates of the forward and reverse organisms. It is interesting to note, though, that the absolute value of polymerase rate for the populations in competition is lower than that of the populations in isolation at these temperatures. Another way of stating this is that the overall fitness of the organisms in the environment is reduced when they are in competition. This is an expected outcome based on current evolutionary dynamics theory.

At the higher temperatures, the average polymerase rate of the reverse polymerizing organisms begins to drop off as their population numbers, as seen in figure 3.6, begin to drop off. This drop is not, however, indicative of a reduction in the fitness of the reverse polymerizing organisms. Rather, as the reverse polymerizing organisms are gradually outcompeted by the forward polymerizing organisms their number is reduced. Since the only mechanism in the model for removing organisms from the population is the density dependent random culling, which members of the reverse polymerizing organism population will be removed is likewise random. Since the organisms with a faster polymerase rate will reproduce more rapidly, they will be present in greater number, and therefore will be more likely to be removed from the environment first. In other words, the rate of the decrease in average polymerase rate

of the reverse polymerizing organisms can be seen as a proxy measure for the rate at which the forward polymerizing organisms are preferentially occupying the available capacity in the environment created when a reverse polymerizing organism is culled.

Based on this result, what seems to be the most interesting aspect of the simulation, then, is how available capacity is occupied by one or the other sort of organism following a random death. That is, competition between the organisms when the environment is at capacity will probably reveal more about the comparative fitness of the two polymerase strategies than following exponential growth from a small starting population. It is also worth noting that, especially at the higher temperatures, starting with a small population is not the best strategy for comparing forward and reverse polymerizing organisms since both sorts of organism experience significant hardship growing beyond their starting population. Indeed, while the population of forward polymerizing organisms at a simulation temperature of 0.60 appears to remain remarkably stable at approximately 500, what this actually represents is that in the 10 repetitions of this simulation carried out, there was a 50/50 chance of the initial population of forward polymerizing organisms surviving at all (and those that did maintained a nearly full environment of 1000 organisms).

It was for these reasons that the last set of experiments, starting with an already full environment, were performed. The justification in starting with an evenly split population relies on another aspect of stable evolutionary equilibria. Namely, if there is a stable equilibrium between forward and reverse polymerizing organisms, then any starting population containing these two types will converge on the equilibrium ratio of one to the other. If, on the other hand, one or the other sort of organism is dominant, then even a small starting population of the dominant organism will eventually overcome the other. This implies that any ratio of forward and reverse polymerizing organisms in the starting population would be valid, and

then the choice of an even split is made to reduce the possibility of an early random fluctuation causing an unrealistic culling of one or the other sort of organism.

Looking at figure 3.7, we can see that the dynamics of the evolution of polymerase rate at each temperature in the case of starting with a full environment are similar to those of starting with a small seed population. Now, though, we can look at figure 3.8 and see that, as we hypothesized, the decrease in rate does correspond to the inability of the reverse polymerizing organisms to sustain their population in the face of competition from forward polymerizing organisms at simulation temperatures of 0.40 and 0.50. Furthermore, at a simulation temperature of 0.50, where the average polymerase rate of the reverse polymerizing organisms drops off most rapidly, we can see that the decrease in population of the reverse polymerizing organisms seems to only be limited by the rate at which the forward polymerizing organisms can reproduce and occupy the available capacity freed by culling. In other words, at this temperature it appears as if nearly every culled organism, forward or reverse, is replaced by a forward polymerizing organism. This matches the expectation for a situation in which one organism is strictly dominant.

Starting with a full environment reveals a few other interesting aspects of the model. Focusing on the early time points in the simulations in figure 3.7, we can now clearly see that the evolution of polymerase rate of the reverse polymerizing organisms lags that of the forward polymerizing organisms at every temperature. At the lower simulation temperatures of 0.10 and 0.30, the reverse polymerizing organisms eventually catch up with the forward polymerizing organisms to set up a stable equilibrium as revealed by the stable populations of forward and reverse polymerizing organisms at later points in the simulations at these temperatures (figure 3.8). The one exception to this rule appears at a simulation temperature of 0.60.

Curiously, at a simulation temperature of 0.60, the reverse polymerizing or-

organisms are, again, able to set up a stable equilibrium with the forward polymerizing organisms. This aspect of the simulation could not have been predicted by the results from starting with a small seed population. In figure 3.6 at a simulation temperature of 0.60, the reverse polymerizing organisms never get a chance to build up any significant population. Yet, starting with a full environment, we can see that these organisms survive rather stably in competition with the forward polymerizing organisms. In this particular case, what we are observing is not any increase in fitness of the reverse polymerizing organisms, but rather a steeper decrease in the fitness of the forward polymerizing organisms. This is further emphasized by the fact that, unlike the lower simulation temperatures of 0.10 and 0.30 where the reverse polymerizing organisms are only able to achieve a stable equilibrium once they evolve their polymerase rates to match those of the forward polymerizing organisms, at a simulation temperature of 0.60 the reverse polymerizing organisms have a polymerase rate significantly lower than that of the forward polymerizing organisms once stable equilibrium is achieved.

It seems, then, as if there are multiple regimes of competition that the forward and reverse polymerizing organisms go through as simulation temperature is raised. We can see that this is the case by looking at a larger set of temperatures in figure 3.9. This figure reveals at least four competition regimes (see figure 4.1). The first is a stable equilibrium between the forward and reverse polymerizing organisms that occurs at low temperatures where the reverse polymerizing organisms have a chance to evolve their polymerases to match those of the forward polymerizing organisms before they are outcompeted. The second regime resembles a weak evolutionarily stable strategy. Such a situation is characterized by one organism which is able to dominate over another, but only through a process of selection. That is, over time, selection will favor the forward polymerizing organisms at this temperature. The third regime

resembles a pure evolutionarily stable strategy, one where the dominant organism is immune to invasion by the lesser organism. This is where the forward polymerizing organisms grow to occupy any vacancies left by dying reverse polymerizing organisms. The reverse polymerizing organisms aren't even given the chance to compete for these spots. The final regime represents the unexpected high-temperature stable equilibrium. This is the case we saw where the forward and reverse polymerizing organisms are able to co-exist while still having very different polymerase rates. In this regime, rate of reproduction is no longer the dominant selective force. Rather, the high rate of mutation, and the ability of the organisms to reproduce in the face of such, becomes more important.

To answer the question first posed, it is conceivable that the  $5' \rightarrow 3'$  nucleotide polymerases which exist, exclusively, in nature today were the result of a Darwinian competition between these and the alternative,  $3' \rightarrow 5'$  nucleotide polymerases. We cannot say for sure that this is the case without having a firmer grasp on the specific values for a number of model parameters, and the temperature at which life first originated. The possibility that the success of  $5' \rightarrow 3'$  nucleotide polymerases was the consequence of a founder effect still remains. However, by attempting to model this evolutionary process, we have come upon a potentially more interesting result. We have, as illustrated in figure 4.1, a model system where thermodynamic influences may determine the nature of the competition between two alternatives.

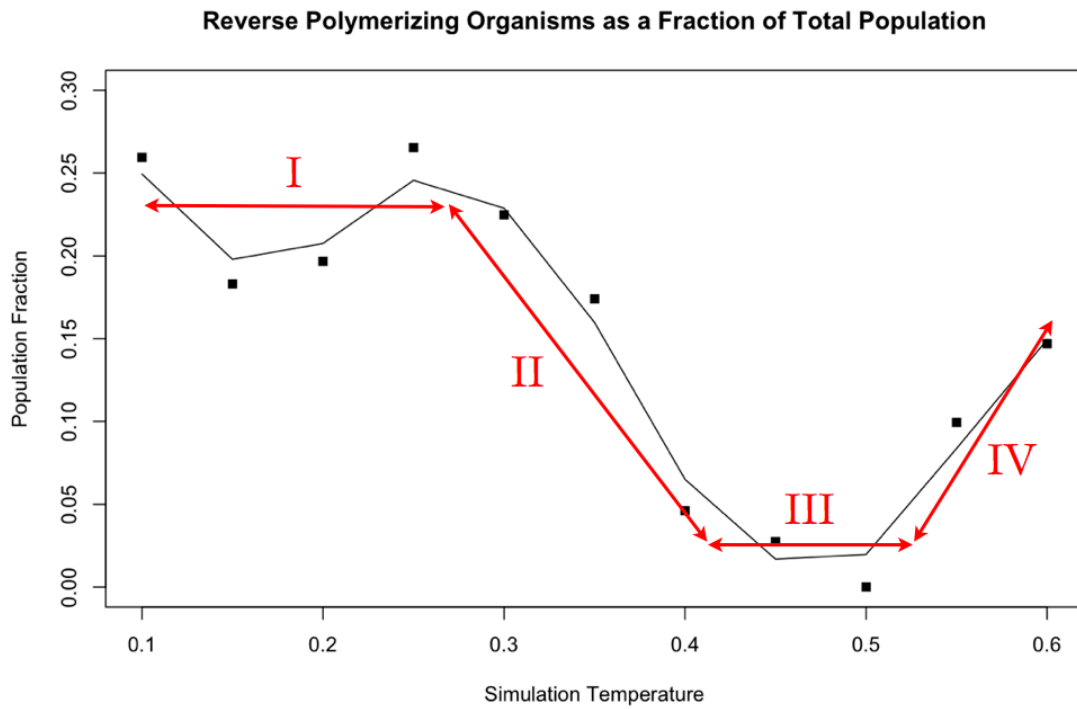


Figure 4.1: **Competition Regimes.** The average population fraction of reverse polymerizing organisms over the last 25000 simulation time steps from figure 3.9 is plotted against simulation temperature. The black line is a smoothed spline constructed from the data, and the red arrows highlight the four competition regimes observed: I. Stable Low-Temperature Equilibrium, II. Weak Evolutionarily Stable Strategy, III. Evolutionarily Stable Strategy, IV. Stable High-Temperature Equilibrium.

## Appendix A

### Source Code

Following is the Ruby source code for the four main classes and the executable Ruby script used to run simulations based on YAML input files.

#### constants.rb

```

1 # Copyright (c) 2009 Joshua Ballanco
2 #
3 # constants.rb
4 #
5 # This file contains constants used elsewhere in the evolver project.
6
7 MAX_TOL_MUT_RATE = 0.34
8 MAX_POLY_RATE = 10
9 MIN_POLY_RATE = 1

```

#### environment.rb

```

1 # Copyright (c) 2009 Joshua Ballanco
2 #
3 # class Environment
4 #
5 # Abstract: The Environment class contains the entire simulation. It is
6 # primarily responsible for tracking all of the organisms in the simulation,
7 # calculating the density dependent death probability, randomly culling
8 # organisms according to that probability, and stepping each organism at each
9 # time step of the simulation
10
11 # The GenomeForEnvironment struct is used to seed the starting population of
12 # the environment.
13 GenomeForSpecies = Struct.new(:genome, :population_frequency)
14
15 class Environment
16   attr_reader :temperature
17

```

```

18 # The Environment must be initialized with the temperature of the
19 # simulation, the maximum and starting populations, and a GenomeForSpecies
20 # array enumerating the genomes to be use in creating the initial organisms.
21 def initialize(temperature,
22               max_population,
23               starting_population,
24               *genomes_for_environment)
25
26   # Some simple sanity checks on passed in arguments
27   #unless genomes_for_environment.inject(0) {|total, gfe|
28   #   Rational(total + Rational(gfe.population_frequency))
29   #} == 1.0
30   #   raise ArgumentError, "population frequencies of genomes must total 1"
31   #end
32   if starting_population > max_population
33     raise ArgumentError, "The starting population must be less than the
34                           maximum population"
35   end
36
37   # Set the environments parameters
38   @temperature = temperature
39   @max_population = max_population
40   @organisms = []
41
42   # Populate the environment
43   genomes_for_environment.each do |genome_for_species|
44     (starting_population * genome_for_species.population_frequency)
45     .round.times do
46       @organisms << Organism.new(genome_for_species.genome.dup, self)
47     end
48   end
49 end
50
51 # Runs the environment for iterations steps (default is 1000).
52 def run(iterations=1000)
53   iterations.times { step }
54 end
55
56 # Step each organism, then calculate the culling probability based on the

```



```

57  # current population as a fraction the maximum population. The probability
58  # is calculated as  $\frac{1}{(N-n)+1}$  where  $N$  is the maximum population
59  # of the environment and  $n$  is the current number of organisms in the
60  # environment. This probability is applied iteratively until no organism is
61  # culled.
62  def step
63    @organisms.each(&:step)
64
65    while (rand < (1.0 / ((@max_population - @organisms.length) + 1)))
66      @organisms.delete_at(rand(@organisms.length))
67    end
68  end
69
70  # The add_organism method attempts to add an organism to the environment.
71  # If there adequate capacity, the organism is added and the method returns
72  # true. If the environment is currently full, then nothing is done and
73  # the method returns false.
74  def add_organism(organism)
75    if @organisms.length < @max_population
76      @organisms << organism
77      return true
78    else
79      return false
80    end
81  end
82
83  # The report method returns a hash containing the values for this
84  # environment as well as the results of iterating over the @organisms
85  # array and calling each organism's report method in turn.
86  def report
87    { :temperature => @temperature,
88      :max_population => @max_population,
89      :current_population => @organisms.length,
90      :organisms => @organisms.collect{|organism| organism.report} }
91  end
92  end

```

organism.rb

```

2 #
3 # class Organism
4 #
5 # Abstract: This is the base class for evolving organisms. It represents an
6 # abstract organism which is replicating its genome using a DNA polymerase
7 # and, when finished making the copy, replicating into two new organisms.
8 #
9 # The Organism class is implemented as a finite state machine with the
10 # following states:
11 #   -- replicate_genome: The organism is synthesizing a new genome using its
12 #                       existing genome as a template
13 #   -- divide:          Split into two, adding a new organism to the
14 #                       environment (if capacity for it exists)
15
16 class Organism
17   # Initialization consists of setting the genome, translating a polymerase
18   # from that genome, and passing in a reference to the environment in which
19   # this organism lives.
20   def initialize(genome, environment)
21     @genome = genome
22     @environment = environment
23     @polymerase = @genome.translate_polymerase(@environment.temperature)
24
25     # We'll start with replicating the genome:
26     @next_step = method(:replicate_genome).to_proc
27   end
28
29   # Step this organism and set the next step to the return value
30   def step
31     @next_step = @next_step.call
32     return self
33   end
34
35   # We're in the middle of creating a new genome. To do this, we allow the
36   # polymerase to add as many nucleotides as it will. Following that, we query
37   # the polymerase as to its current status, and proceed based on that
38   # information.
39   def replicate_genome
40     @polymerase.add_nucleotides

```

```

41     case @polymerase.status
42     when :polymerizing
43         return method(:replicate_genome).to_proc
44     when :finished
45         return method(:divide).to_proc
46     end
47 end
48
49 # In order to divide, we first extract the new genome. If the new genome has
50 # too many errors, then we reset and try again. Otherwise, we create a new
51 # organism from the genome and attempt to insert the new organism into the
52 # environment. If there is no room, we keep trying until there is (or we are
53 # randomly killed). Once we've put the new organism in the environment,
54 # reset and start replicating again.
55 def divide
56     @new_genome ||= @polymerase.new_finished_genome
57     unless @new_genome
58         @polymerase.reset
59         return method(:replicate_genome).to_proc
60     end
61
62     @new_organism ||= Organism.new(@new_genome, @environment)
63     if @environment.add_organism(@new_organism)
64         @new_genome = nil
65         @new_organism = nil
66         @polymerase.reset
67         return method(:replicate_genome).to_proc
68     end
69
70     return method(:divide).to_proc
71 end
72
73 # The report method returns details about the organism's genome and
74 # polymerase
75 def report
76     { :genome => @genome.report,
77       :polymerase => @polymerase.report }
78 end
79 end

```

## genome.rb

```

1  # Copyright (c) 2009 Joshua Ballanco
2  #
3  # class Genome
4  #
5  # Abstract: The genome class comprises the genome of an organism. It is
6  # initialized with a length and some information about the sort of polymerase
7  # enzyme that it codes for. As it is copied (by a polymerase), it tracks
8  # progress and a count of the errors made. When requested, the genome can
9  # generate a polymerase or a copy of itself.
10
11 class Genome
12   attr_reader :length, :added_nucleotides, :errors
13
14   # Initialization requires the length of the genome as well as the rate and
15   # directionality of the polymerase coded for by the genome.
16   def initialize(length, polymerase_rate, directionality, mutable = true)
17     @length = length
18     @polymerase_rate = polymerase_rate
19     @directionality = directionality
20     @mutable = mutable
21     @added_nucleotides = 0
22     @errors = 0
23   end
24
25   # This method gets called during a Genome#dup. The length of the original is
26   # left unchanged, but the properties of the polymerase generated are
27   # recalculated based on how many mutations occurred during replication.
28   def initialize_copy(orig)
29     if @mutable
30       high_dev = MAX_POLY_RATE - @polymerase_rate
31       low_dev = @polymerase_rate - MIN_POLY_RATE
32       max_dev = (MAX_POLY_RATE - MIN_POLY_RATE) / 2.0
33       mut_frac = (@errors / @length.to_f) / MAX_TOL_MUT_RATE
34       mut_frac = mut_frac > 1 ? 1 : mut_frac
35       change_in_rate = (mut_frac * max_dev).round
36
37       if change_in_rate > high_dev
38         @polymerase_rate -= change_in_rate

```

```

39         elsif change_in_rate > low_dev
40             @polymerase_rate += change_in_rate
41         elsif rand(2) == 0
42             @polymerase_rate -= change_in_rate
43         else
44             @polymerase_rate += change_in_rate
45         end
46     end
47
48     if (@polymerase_rate > MAX_POLY_RATE || @polymerase_rate < MIN_POLY_RATE)
49         raise RuntimeError, "Polymerase Rate out of Bounds"
50     end
51
52     # At the end, we reset @added_nucleotides and @errors to 0
53     self.reset
54 end
55
56 # Start over replicating the genome (e.g. if an unviable copy was made and
57 # discarded).
58 def reset
59     @added_nucleotides = 0
60     @errors = 0
61 end
62
63 # Add a new nucleotide to the genome replica. If there was an erroneous
64 # inclusion, add to the number of errors as well.
65 def add_nucleotide(error = false)
66     @errors += 1 if error
67     @added_nucleotides += 1
68 end
69
70 # Seed a new polymerase with the directionality and rate defined by this
71 # genome.
72 def translate_polymerase(temperature)
73     Polymerase.new(self, @directionality, @polymerase_rate, temperature)
74 end
75
76 # This method returns the viability of the replica genome being created. The
77 # organism can tolerate up to MAX_TOL_MUT_RATE of its nucleotides being

```

```

78   # mutated. It would also not be viable if it wasn't finished being
79   # duplicated.
80   def viable?
81     if @added_nucleotides >= @length
82       true
83     else
84       false
85     end
86   end
87
88   # The report method returns a hash of properties about the genome
89   def report
90     { :length => @length,
91       :added_nucleotides => @added_nucleotides,
92       :errors => @errors }
93   end
94 end

```

### polymerase.rb

```

1  # Copyright (c) 2009 Joshua Ballanco
2  #
3  # class Polymerase
4  #
5  # Abstract: The polymerase class describes a generic nucleotide polymerase.
6  # When asked to, it will add a number of new nucleotides to the nascent
7  # genome. The directionality of insertion depends on the directionality that
8  # the polymerase was initialized with. Each polymerase will always be in one
9  # of two states:
10 #   -- polymerizing: The genome is not yet finished.
11 #   -- finished:    The genome has been completely replicated.
12
13 class Polymerase
14   attr_reader :status
15
16   # The polymerase is created for a genome, and must specify a directionality,
17   # polymerization rate, and simulation temperature when it is created.
18   def initialize(genome, directionality, rate, temperature)
19     @status = :polymerizing
20     @genome = genome

```

```

21     @directionality = directionality
22     unless (@directionality == :forward || @directionality == :reverse)
23         raise ArgumentError, "directionality must be :forward or :reverse"
24     end
25     @rate = rate
26     @temperature = temperature
27 end
28
29 # This method adds a nucleotide to the genome. First, the polymerase checks
30 # to see if the genome is already finished being replicated. If not, then it
31 # will add a number of nucleotides up to the rate of this polymerase. When
32 # adding nucleotides, there is the chance that the nucleotide being added
33 # has become dephosphorylated. There is also a chance, dependent on the
34 # temperature and polymerase rate, that an error is made during replication.
35 def add_nucleotides
36     if @genome.added_nucleotides > @genome.length
37         @status = :finished
38         return
39     end
40
41     @rate.times do
42         thermal_prob = Math::E**(-1.0 / @temperature)
43         if ((@directionality == :forward) &&
44             (rand < thermal_prob**2))
45             next
46         elsif ((@directionality == :reverse) &&
47             (rand < (thermal_prob**2 * (MAX_POLY_RATE - @rate + 1))))
48             return
49         else
50             @genome.add_nucleotide(rand < (thermal_prob * Math.sqrt(@rate)))
51         end
52     end
53 end
54
55 # If we're done polymerizing and the genome that we've produced is viable,
56 # then return the genome. Otherwise, return nil
57 def new_finished_genome
58     if @status == :finished && @genome.viable?
59         @genome.dup

```

```

60     else
61         nil
62     end
63 end
64
65 # Resets the polymerase back to starting with a fresh genome to start
66 # polymerizing anew.
67 def reset
68     @genome.reset
69     @status = :polymerizing
70 end
71
72 # The report method returns a hash of properties about the polymerase
73 def report
74     { :status => @status,
75       :directionality => @directionality,
76       :rate => @rate }
77 end
78 end

```

## evolver

```

1  #!/usr/bin/env ruby1.9
2  #
3  # Copyright (c) 2009 Joshua Ballanco
4  #
5  # evolver - This is the command line utility to manipulate the Evolver model.
6
7  $LOAD_PATH << File.join(File.dirname(__FILE__), '..', 'lib')
8
9  require "optparse"
10 require "yaml"
11 require "evolver/constants"
12 require "evolver/organism"
13 require "evolver/environment"
14 require "evolver/genome"
15 require "evolver/polymerase"
16
17 options = {}
18 OptionParser.new do |opts|

```



```

19  opts.banner = "Usage: evolver [options] <environment file>.yaml"
20
21  options[:iterations] = 1000
22  opts.on("-n [iterations]",
23          "Number of iterations to run per environment") do |iter|
24    options[:iterations] = iter.to_i
25  end
26
27  options[:report_frequency] = 10
28  opts.on("-f [frequency]",
29          "Report frequency (iterations between reports)") do |freq|
30    options[:report_frequency] = freq.to_i
31  end
32
33  options[:snapshot_frequency] = 0
34  opts.on("-s [frequency]",
35          "Snapshot frequency (iterations between snapshots)") do |freq|
36    options[:snapshot_frequency] = freq.to_i
37  end
38
39  options[:threads] = 1
40  opts.on("-j [threads]",
41          "Number of concurrent threads to run for calculations") do |thr|
42    options[:threads] = thr.to_i
43  end
44
45  options[:report_values] = %W( forward_num
46                               reverse_num
47                               forward_rate
48                               reverse_rate
49                               organism_num
50                               organism_rate )
51  opts.on("-r [Value1,Value2,Value3]", Array,
52          "Values to report") do |values|
53    options[:report_values] = values
54  end
55  end.parse!
56
57  def run(options, environment)

```

```

58   genomes_for_env = environment[:genomes].collect do |genome|
59     GenomeForSpecies.new(Genome.new(genome[:length],
60                                     genome[:polymerase_rate],
61                                     genome[:directionality],
62                                     genome[:mutable_polymerase])),
63     genome[:freq])
64   end
65   env = Environment.new(environment[:temperature],
66                         environment[:max_population],
67                         environment[:starting_population],
68                         *genomes_for_env)
69
70   env.use_threads(options[:threads]) if options[:threads] > 1
71   # Run the simulation for the report frequency, then output the requested
72   # values. Keep doing this until we've hit the max iterations limit.
73   @iterations_complete = 0
74   report(environment[:name], env, true, *options[:report_values])
75   while (@iterations_complete + options[:report_frequency] < options[:iterations])
76     env.run(options[:report_frequency])
77     report(environment[:name], env, false, *options[:report_values])
78     if (options[:snapshot_frequency] > 0 &&
79         @iterations_complete % options[:snapshot_frequency] == 0)
80       File.open(environment[:name] + '_snapshots.txt', 'a') do |snapshotfile|
81         snapshotfile << YAML::dump(env.report) << "...\\n"
82       end
83     end
84     @iterations_complete += options[:report_frequency]
85   end
86   env.run(options[:iterations] - @iterations_complete)
87   report(environment[:name], env, false, *options[:report_values])
88 end
89
90 def report(name, env, print_header, *report_values)
91   File.open(name + '_out.csv', 'a') do |outfile|
92     outfile << report_values.join(',') << "\\n" if print_header
93     env_report = env.report
94     values = report_values.collect do |value|
95       send("report_#{value}".to_sym, env_report)
96     end

```

```

97     outfile << values.join(',') << "\n"
98   end
99 end
100
101 # Methods for reporting simulation parameters:
102 def report_forward_num(env_report)
103   env_report[:organisms].inject(0) do |total, org|
104     total + (org[:polymerase][:directionality] == :forward ? 1 : 0)
105   end
106 end
107
108 def report_reverse_num(env_report)
109   env_report[:organisms].inject(0) do |total, org|
110     total + (org[:polymerase][:directionality] == :reverse ? 1 : 0)
111   end
112 end
113
114 def report_forward_rate(env_report)
115   env_report[:organisms].inject(0.0) do |total, org|
116     total + (org[:polymerase][:directionality] == :forward ?
117              org[:polymerase][:rate] : 0)
118   end / report_forward_num(env_report)
119 end
120
121 def report_reverse_rate(env_report)
122   env_report[:organisms].inject(0.0) do |total, org|
123     total + (org[:polymerase][:directionality] == :reverse ?
124              org[:polymerase][:rate] : 0)
125   end / report_reverse_num(env_report)
126 end
127
128 def report_organism_num(env_report)
129   env_report[:current_population]
130 end
131
132 def report_organism_rate(env_report)
133   env_report[:organisms].inject(0.0) do |total, org|
134     total + org[:polymerase][:rate]
135   end / env_report[:current_population]

```

```
136 end
137
138 # After parsing the options, the only remaining argument should be the
139 # environment specification file. Load this and run the simulation for each
140 # environment described:
141 environments = YAML::load_file(ARGV[0])
142 environments.each do |environment|
143   run(options, environment)
144 end
```

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