# Master Thesis

# Experiments in In Silico Evolution with Aevol

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# Abstract

Not all aspects of evolution are fully understood, and one area of active interest is reductive evolution, in which the genome of an organism evolves to become significantly smaller over time. Among the more well-known examples of this phenomena is *Prochlorococcus*, a marine cyanobacteria whose genome is up to 50% smaller than its closest living relative, *Synechococcus*. To study the mechanisms behind reductive evolution in the lab would be too costly, expensive, and slow, so we turn instead to *in silico* evolution. This method seeks to simulate organisms and their evolution in software, allowing for greater control of the environment, mutation rates, and other variables, as well as providing a full record of all organisms in a lineage. In this thesis we use the in silico tool *Aevol* to study reductive evolution, particularly by looking at how varying parameters (e.g. mutation and selection rates, population size) impacts the structure of the genome as well as measures such as robustness, evolvability, and fitness. Through these methods we hope to shed some light on the underlying mechanisms which lead to reductive evolution.

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# Chapter 1

# Introduction

Reductive evolution is the process of the genome of an organism shrinking over time, with respect to both the number of base pairs and genes. Some species of bacteria have experienced reductive evolution over the course of millions of years, and this reduction in their genome has lead to a loss of genes, certain regulatory abilities, etc. For example, some strains of the marine cyanobacteria *Prochlorococcus* have experienced a reduction of nearly 40% of their base pairs when compared to larger strains of their closest living relative, *Synechococcus*[9]. Despite being extensively studied, the mechanisms and full impact of reductive evolution are not fully understood and are an area of ongoing research.

Although it would provide more conclusive evidence, performing *in vivo* experiments is often impractical because of the difficulty or impossibility of reproducing natural environmental conditions in a lab. Such experiments are often too costly in terms of both time and resources. As an alternative, *in silico experimental evolution* is one option that can be used to study the conditions under which an organism's genome may become reduced. In this method, organisms and their evolution over thousands or millions of generations are simulated in software. In this manner, one can control and evaluate every aspect of their evolution over time and a full record of their lineage may be maintained and studied, allowing one to go back and closely examine every step of the evolutionary period for a greater understanding of the factors that lead to specific effects on the genome. The in silico tool *Aevol* is one such platform which realistically models bacterial genomes and evolution, allowing one to draw conclusions about their real-world counterparts. In the following thesis, we present the results of our experiments in artificial evolution which aim to identify and evaluate several factors which potentially lead to changes in genome structure and a reduced genome in simulated bacteria using the Aevol platform.

## 1.1 Problem Statement

Among the difficulties of studying reductive evolution with in vivo evolutionary experiments, one of the most difficult obstacles to overcome is the lack of a full ancestral record. This lack of a full phylogeny can make it difficult or impossible to tell exactly when and how a specific event occurred, or a trait evolved or was lost, as illustrated in Figure 1.1 below. In this exam-

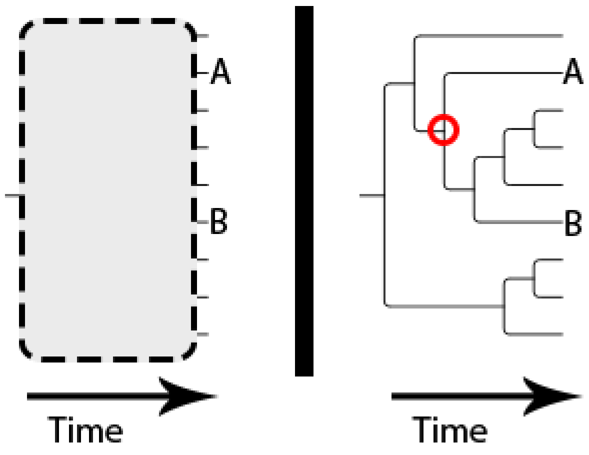


Figure 1.1: An illustration of unknown phylogeny. Since the phylogenetic information under the shaded box is typically not known, the point of divergence (red circle) can't be determined.

ple, we are comparing two related organisms A and B and we are trying to determine when and how a specific trait was gained or lost by one of the organisms. This may be useful, for example, if we are attempting to estimate the relative importance (due to conservation over many generations) of some trait. Without the phylogenetic information (under the shaded box) we may not be able to identify the point in their evolutionary history at which the two organisms diverged, making time estimates difficult or impossible.

Another major downside to *in vivo* evolutionary experiments is that they are slow. For example, the well-known E. coli Long-Term Evolution Experiment (LTEE) by Profesor Lenski at Michigan State University has been ongoing since February of 1988 and only passed generation 65,000 in 2016, 28 years later.

As an alternative to in vivo experiments, *in silico* evolutionary experiments are well-suited to the task of studying reductive evolution. Generations of organisms may be evolved within a very short time period, and a full "fossil record" of each lineage may be kept on disk for further analysis.

The in silico tool Aevol has a realistic artificial chemistry model which was developed specifically to study genome structure. It contains tools to analyze the robustness, fitness, and evolvability of digital organisms over time.

## 1.2 Report outline

This chapter serves as the introduction to the thesis and the research problem we are facing. In Chapter 2, we provide some necessary background information on in silico evolution in general and Aevol in particular. Chapter 3 describes our experimental setup. Chapter 4 provides the results and analysis of the experiments, and Chapter 5 presents our conclusions.

Chapter 2

# Background

As discussed in Chapter 1, reductive evolution is a

## 2.1 Experimental Evolution

As discussed in Section 1.1, in vivo experiments, although sometimes more realistic, have their own set of difficulties. Some examples of these difficulties include recreating challenging environmental conditions (e.g. simulating the open ocean in a lab) and identifying and/or simulating the multiple selection pressures acting on genomes in the real world [1]. These challenges add enormous difficulty and complexity to conducting proper experiments and isolating the specific factors which lead to particular outcomes.

In silico evolution simulates organisms in software, thus allowing for far greater control and analysis of the environment and other experimental conditions. In contrast to in vivo experiments, a greater amount of control is also available with regard to the way organisms may interact, reproduce, and evolve. For example, a genome may be created completely from scratch or an existing genome may be fed into the simulation. Reproduction rates can depend on overall fitness, on relative fitness, or some other criterion.

Factors such as the mutation rates or selection pressure are then parameters for the model and may be kept constant or allowed to vary over time. Given that these are parameters of the system, they may be tightly controlled, leading to a clearer picture of the factors influencing different outcomes. An underlying deterministic model can also allow for a reconstruction of the system from any given point, allowing one to easily create a record of events, including phylogenetic trees.

**Why use Aevol**

The in silico tool *Aevol* was developed in the early 2000s to "study the evolution of the size and organization of bacterial genomes in various scenarios" [1]. The program has been expanded upon and tested in a variety of scenarios over the years. Examples of such experiments include: testing the predictability of evolution with high mutation rates as in viruses, [2], determining whether selection is able to overcome evolution's drive towards more complex organisms [5], examining the role of mutators in reorganizing the genome in order to overcome mutational load [7], examining the effects of population shape on levels of cooperation [6], modeling regulatory networks [8] and more.

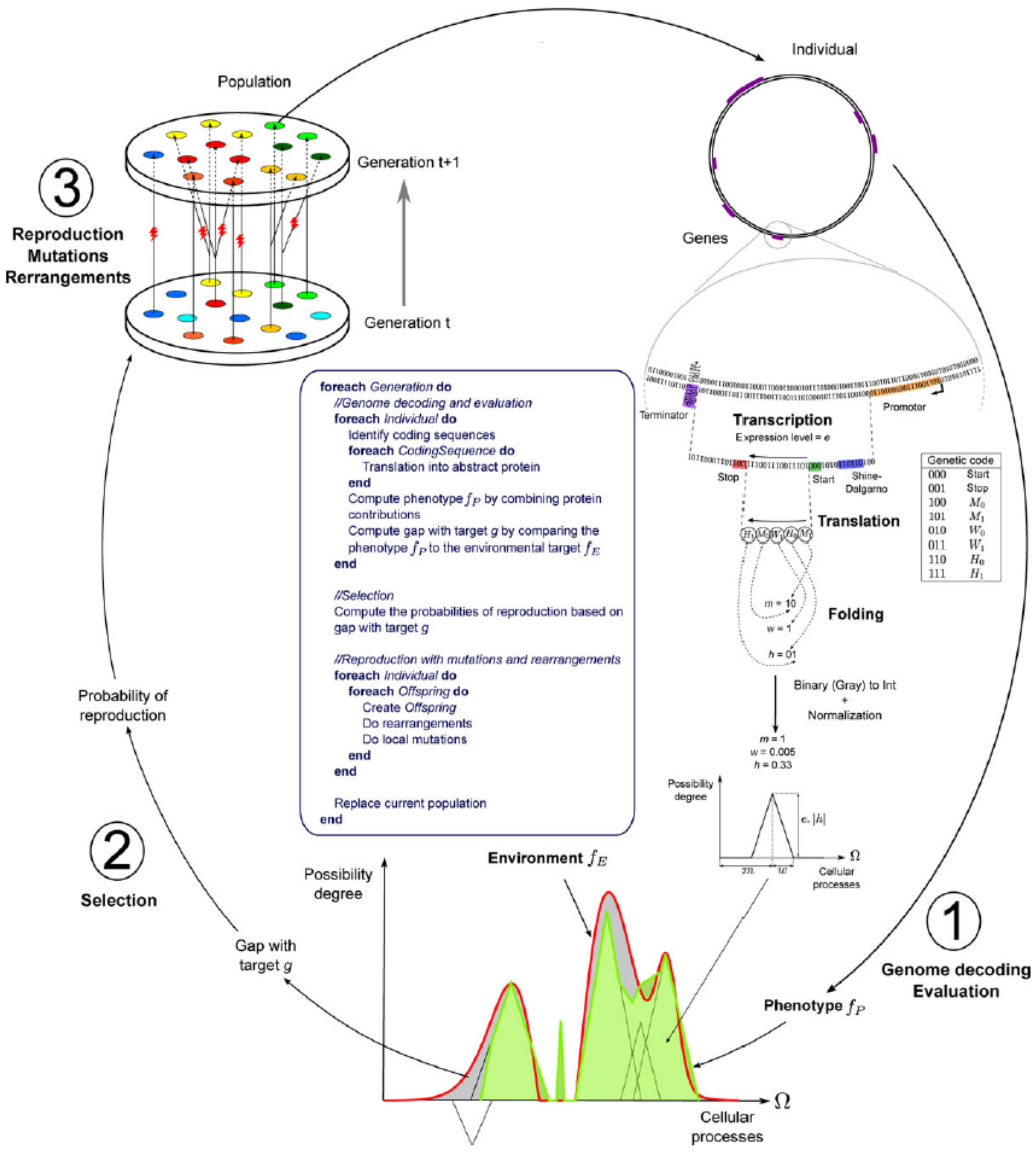
In the following sections, the in silico experimental evolution tool Aevol will be examined in greater detail.

## 2.2 Aevol

Organisms are simulated with a binary genome which can either be generated at random or input as a previously-generated sequence. Aevol essentially consists of three steps: 1) decoding the genome of these organisms to produce artificial proteins, 2) selecting the most fit individuals and 3) reproduction of these fittest individuals with possible variations (mutations, rearrangements, etc.). The population size $N$ is kept constant and a record of each generation is kept so that the phylogenetic lineages can be recreated.

### 2.2.1 Aevol's Architecture

Aevol's three steps—decoding the genome, selection, and reproduction—will be examined in more detail in this section. These steps are illustrated in Figure 2.1.



| Genetic code | |
|---|---|
| 000 | Start |
| 001 | Stop |
| 100 | $M_0$ |
| 101 | $M_1$ |
| 010 | $W_0$ |
| 011 | $W_1$ |
| 110 | $H_0$ |
| 111 | $H_1$ |

```
foreach Generation do
  //Genome decoding and evaluation
  foreach Individual do
    Identify coding sequences
    foreach CodingSequence do
      Translation into abstract protein
    end
    Compute phenotype $f_P$ by combining protein
    contributions
    Compute gap with target g by comparing the
    phenotype $f_P$ to the environmental target $f_E$
  end

  //Selection
  Compute the probabilities of reproduction based on
  gap with target g

  //Reproduction with mutations and rearrangements
  foreach Individual do
    foreach Offspring do
      Create Offspring
      Do rearrangements
      Do local mutations
    end
  end

  Replace current population
end
```

Figure 2.1: Overview of Aevol's architecture, from [1]

### Decoding the Genome

In Aevol, a genome consists of a string of binary characters where 0 is complementary to 1. Each organism in the (initially clonal) population has a double-stranded circular genome which is either generated randomly or which was provided as input. To decode the genome and produce the phenotype, the sequence is searched for transcribed regions. Transcribed regions are denoted by promoter and terminator sequences. The promoter sequence is a sequence whose Hamming distance $d$ is within $d_{max} = 4$ mismatches of the predefined consensus sequence 0101011001110010010110. Terminators are sequences which can form a stem-loop structure with a stem size of 4 bases and a loop length of 3 bases (i.e. $abcd{*}{*}{*}\overline{dcba}$ where a is complementary to $\overline{a}$, b is complementary to $\overline{b}$, etc.). Lastly, the initiation and termination signals are sought, which are simply Shine-Dalgarno-like signals (i.e. $011011 * * * *000$ to start and $011011 * * * *001$ to stop). Lastly, an expression level $e$ is assigned to each coding region, following the formula $e = 1 - \frac{d}{d_{max}+1}$ where $d$ is again the Hamming distance between the coding region and the consensus sequence given above and $d_{max}$ is the maximum allowable distance (i.e. 4).

Once an initiation sequence is found, the following bases are read three at a time (codon by codon) until a stop codon (by default 001) is found. If a stop codon is not found, then no protein is produced. Since a transcribed region may have multiple initiation signals, operons are therefore allowed. The codons following an initiation signal encode for three parameters according to the genetic code given in Figure 2.1: $m$ (mean), $w$ (half-width), and $h$ (height), which together define a triangle representing a "cellular process".

A cellular process is simply an abstract representation of some phenotypic function and is represented by the ordered set $\Omega = [a, b] \subset \mathbb{R}$. To keep things simple, $\Omega$ is a one-dimensional space in the interval $[0, 1]$, i.e. a "cellular process" is simply a real number, and the genomic encoding of each cellular process determines the function $f(x) : \Omega \to [0, 1]$. The mean $m$ gives us the specific cellular process in the range $[0, 1]$. The width $w$ describes the "scope" of the process, i.e. the *pleiotropy* of the process, meaning the subset of the protein that is in the interval $(m - w, m + w) \subset \Omega$ . The height determines the degree of possibility of the process, i.e. its relative strength.

The codons are read one after the other and their Gray codes[1] are used to compute the real numbers $m$, $w$, and $h$ as follows. Each parameter ($m$, $w$,

[1]A binary encoding such that two successive values (e.g. 2, 3) only differ by at most one bit (e.g. 0011, 0010). See `https://en.wikipedia.org/wiki/Gray_code`

$h$) is assigned two codons in the genetic code (see Figure 2.1), for example $w_0 = 010$ and $w_1 = 011$. Any $w_0$ codons become a 0 in the Gray code, and vice versa with 1s. So if, for example, when reading the coding sequence, the codons $w_1$, $w_0$, $w_1$, $w_0$ are read, the Gray code becomes 1010, which is 12 in decimal. This is done for $m$, $w$, and $h$, and the resulting values are then normalized to be in the proper range. $w$'s range is specified in the parameter file (`MAX_TRIANGLE_WIDTH`), $h$ must be in the range $[-1, +1]$ (indicating that both activating and inhibitive processes are allowed) and $m$ must be in the range $[0, 1]$ (the range of $\Omega$).

Given the fact that there are likely multiple coding sequences in a genome, several triangles (cellular processes) are translated from the genome, each parameterized by its own $m$, $w$, and $h$. These triangles form the phenotypic function $f_P$. *Fuzzy logic* is used to find the overall contribution of each cellular process, using the Lukasiewicz fuzzy operators[2]. Roughly speaking, the activating proteins are added up, as are the inhibiting proteins, and the difference between these two totals represents the final function $f_P$. More formally, if $f_i$ is the possibility distribution of the $i$-th activator protein and $f_j$ is the possibility distribution of the $j$-th inhibitor protein, then the phenotype of the individual is defined as:

$$f_P = max\left(min\left(\sum_i f_i(x), 1\right) - min\left(\sum_j f_j(x), 1\right), 0\right)$$

## Selection

After the genome is decoded, the organisms are tested for their fitness. Fitness in Aevol is related to the gap between the phenotype of a sequence $f_P$ and the environmental target function $f_E$, as illustrated in Figure 2.1. This environmental target function $f_E$ is a user-defined set of Gaussians which are specified in a parameter file, with each Gaussian being identified by three parameters: its height, its location along the axis, and its width. The difference between the phenotype (as calculated above) and the environmental function is the "metabolic error", labeled $g$ in the Figure and is more formally defined as: $g = \int_a^b f_E(x) - f_P(x)dx$. The idea is illustrated in Figure 2.2

[2]See `https://en.wikipedia.org/wiki/Lukasiewicz_logic` for an introduction.

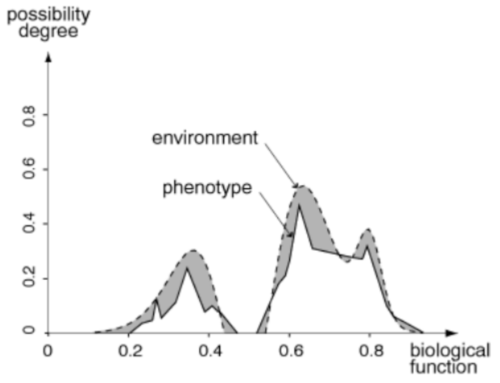


Figure 2.2: Overview of Aevol's conception of fitness, from [4]

Aevol contains several selection schemes but here we will only consider the `fitness_proportionate` scheme, since this was the only selection scheme employed in our experiments. In this scheme, the probability of reproduction for each organism $i$ is proportionate to its fitness, namely:

$$P(\text{reproduction}) = \frac{e^{-k*g}}{\sum_{i=1}^{N} e^{-k*g_i}}$$

where $k$ is a user-definable parameter which determines the selection intensity and $g$ is the metabolic error.

### Reproduction

Once the fittest organisms in the population are found and their probabilities of reproducing are calculated as described in the previous Section, new organisms are produced. This is done for each potential parent organism by drawing from a multinomial distribution with the probability of reproduction given above. Since the population size is held constant, this implies that a single organism with a high probability of reproduction may produce multiple offspring and an organism with low probability of reproduction may produce none.

When new organisms are created and their genomes are copied from their parent organisms, it is at this stage that some of the driving forces in evolution occur, namely the possibility for variation through mutation, indels, and frameshifts. Offspring will receive their parents' genome but their genome may be subject to perturbations due to stochastic effects. Mutation rates are set in the parameter file and include point mutations, insertions and deletions (indels), and rearrangements (duplication, deletions, translocations, and inversions).

The mutation, indels, rearrangement, etc. events are carried out by first determining the number $\mu$ of such events which will occur, based on the mutation rate specified in the parameter file and drawing from a binomial distribution (e.g. $B(L, \mu_{\text{point}})$ for point mutations, $B(L, \mu_{\text{large deletions}})$ for large deletions, etc. where $L$ is the size of the genome). Then a random point (or points, in the case of e.g. rearrangement) is chosen and the event is carried out, with the order of these events shuffled randomly.

## 2.3 Analyzing with Aevol

Once the experiments have completed, Aevol by default produces several statistics files which include information about genome size, the percentage of coding DNA, number of genes, average metabolic error, and many other statistics. It further includes a number of post-treatments that allow one to analyze specific individuals or the population at large, including tools for determining robustness, evolvability, coalescence, and the lineages.

One of the key features of Aevol is the ability to look back in time at the "archaeological record" of previous organisms, which is stored on disk, in order to perform various analyses. This is primarily done with a myriad of post-treatments", i.e. supplementary programs. These post-treatments generally require a `lineage` file, which shows a record back in time of the line of descent for an individual. One may specify either the best-ranked individual (i.e. the fittest) or a specific individual by their unique identification number. The basic idea is illustrated below in Figure 2.3.

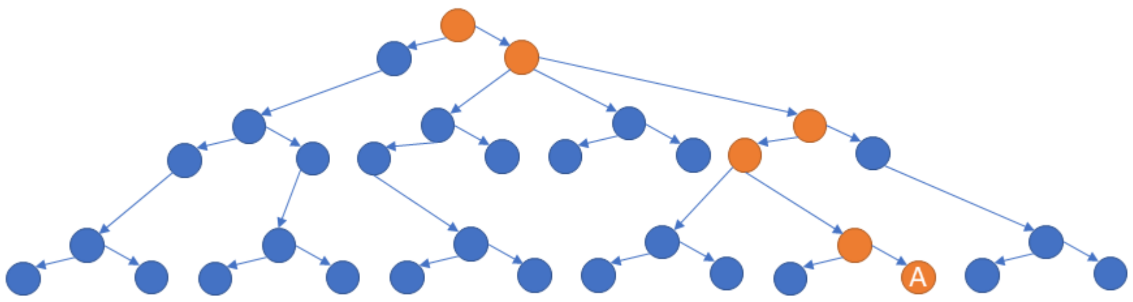


Figure 2.3: A basic illustration of a lineage. The ancestors of the individual (labeled 'A') can be traced back through the previous generations for all of its ancestors (all in orange).

In the following subsections we will examine other factors that Aevol calculates, as these factors play a major role in reductive evolution.

### 2.3.1 Evolvability

Evolvability is usually defined as the ability of a system (in this case an organism) to evolve. In other words, a system has evolvability "if mutations in it can produce heritable phenotypic variation"[10]. However, of critical importance is that this is not simply having a large amount of genetic diversity, but rather *adaptive* diversity which provides some benefit.

In Aevol, evolvability is calculated by generating a large set of offspring for a specific individual (one whose `lineage` was generated) at regular periods along their lineage and then determining the number of "positive offspring". Positive offspring are defined as those whose fitness is greater than their parent's. The evolvability of an individual is then the sum total of all improvement of all of the beneficial offspring, i.e.:

$$\text{evolvability} = \frac{|\text{positive offspring of } i|}{|\text{total offspring of } i|} * \sum \Delta_{fitness}^{\text{positive offspring}}$$

where $\Delta_{fitness}^{\text{positive offspring}}$ is the cumulative sum of the fitness increase for the positive offspring. Thus, evolvability in Aevol accounts for both the likelihood of a positive mutation and the average improvement provided by said mutation. Practically speaking, to find an organisms evolvability in Aevol one must give the post-treatment `misc_ancestor_robustness` a lineage file and then multiply the fraction of the number of positive offspring (column 2) by the cumulative total of the fitness gap $g$ of the positive offspring (column 10).

### 2.3.2 Robustness and Antirobustness

Robustness is broadly defined as the ability of an organism to withstand disruptions or perturbations without affecting its phenotype. One may describe robustness in terms of mutational robustness–which describes the extent to which an organism's phenotype is not affected by stochastic mutational events–or one may speak of environmental robustness, which describes the ability of an organism to maintain its phenotype in diverse environments with little or no loss in fitness.

Also important is the idea of antirobustness, wherein an organism may actually *thrive* on such perturbations. This has been suggested as a possible method of minimizing the effects of *Muller's ratchet*, wherein deleterious mutations accumulate in a population as a result of genetic drift[3]. A large number of deleterious mutations may provide fodder for selection to fix more beneficial mutations in the population[7].

Aevol calculates statistics for both reproductive and mutational robustness statistics, as well as antirobustness. Robustness in Aevol is calculated similarly to evolvability: a `lineage` file for an individual is fed to the post-treatment `misc_ancestor_robustness`, which generates a large number (specifiable by the user) of offspring, whose fitness is then measured. The percentages of positive, neutral, and negative offspring determine the robustness and antirobustness.

It is, then, a seeming trade-off between robustness and evolvability. The more robust a system is, the less phenotypic variation is generated by random mutation events, and thus less evolvability. However, a key factor is to distinguish between genomic and phenotypic robustness; a strong phenotypic robustness promotes structural evolvability, as the likelihood that a mutation is deleterious is smaller in populations with more robust phenotypes. For a full discussion, see [10].

### 2.3.3 Fitness

As mentioned above, fitness in Aevol is closely tied in to the "metabolic error". This error, $g$, is calculated as the gap between the environmental function $f_E$ and the phenotype of the organism, $f_P$. Once $g$ is determined as described above in Section 2.2.1, it may be used to calculate the actual fitness of the organism according to the equation:

$$\text{fitness} = exp(-k * g)$$

where k is the *selection coefficient* variable set by the user in a parameter file. Aevol provides fitness statistics both for the fittest individual and for the population at large.

### 2.3.4 Structure

Another strength of Aevol is its ability to analyze changes in the structure of DNA. As with fitness, Aevol produces statistics about individuals and the population at large for many aspects of genome structure, including: the number of coding vs. non-coding RNAs (i.e. they have at least one gene), the average size of coding and non-coding RNAs, the number of genes, the number of "essential" base pairs (i.e. those that are part of a functional coding sequence), etc. The table below summarizes where these aspects are to be found in Aevol's myriad statistics files.

| **stat_genes_⟨best/global⟩** | **stat_bp_⟨best/global⟩** |
|---|---|
| number of coding RNAs | number of bp not in any CDS |
| number of non-coding RNAs | number of bp not included in any functional CDS |
| average size of coding RNAs | number of bp not included in any non-functional CDS |
| average size of non-coding RNAs | number of bp not included in any RNA |
| number of functional genes | number of bp not included in any coding RNA |
| number of non-functional genes | number of bp not included in any non-coding RNA |
| average size of functional genes | number of non-essential bp |
| average size of non-functional genes | number of non-essential bp including non-functional genes |

## 2.4 Related Work

# Chapter 3

# Methods

With an understanding of the basics behind us, in this chapter we provide an overview of our contributions and proposed solution.

## 3.1 Contributions

## 3.2 Experimental Designs

To test the conditions under which reductive evolution might occur, we conducted a series of experiments in which a “wild type” genome was allowed to evolve in differing conditions for 500,000 generations. To first create the wild type, it was originally generated randomly and allowed to evolve for 10 million generations. By the beginning of its use in our experiments, it comprises 13,237 base pairs with 132 functional genes (i.e. genes which produce a gene product, or “cellular process”). A total of 7 different conditions were used to test the wild type’s response, and to control for experimental bias we performed five runs of each condition, leading to a total of 35 experiments.

### 3.2.1 Inputs

In Table 3.1, our parameter values for all input parameters may be found. Please note that for $\mu$, this represents the rates for point mutations, small insertions, and small deletions. The rearrangement rates were not changed under any condition and were always $1e - 6$ for duplications, deletions, translocations, and inversions.

| **Condition** | **Parameter** | | |
|---|---|---|---|
| | $\mu$ | $k$ | $N$ |
| control | $1.00E{-}7$ | 1000 | 1024 |
| mutation up | $4.00E{-}7$ | 1000 | 1024 |
| mutation down | $2.50E{-}8$ | 1000 | 1024 |
| selection up | $1.00E{-}7$ | 4000 | 1024 |
| selection down | $1.00E{-}7$ | 250 | 1024 |
| population up | $1.00E{-}7$ | 1000 | 4096 |
| population down | $1.00E{-}7$ | 1000 | 256 |

Table 3.1: Table of input parameters. $\mu$ is the mutation rate, $k$ is the selection strength, and $N$ is the population size.

As can be seen in Table 3.1, only one parameter varied per condition. This was a critical starting point in order to isolate potential causes of reductive evolution.

### 3.2.2 Evaluation Strategy

#### Robustness

#### Evolvability

#### Time to Coalescence

## 3.3 Expected Results

# Chapter 4

# Results and Discussion

## 4.1 Results

In this section we present the results of our experiments.

### 4.1.1 Condition Analysis

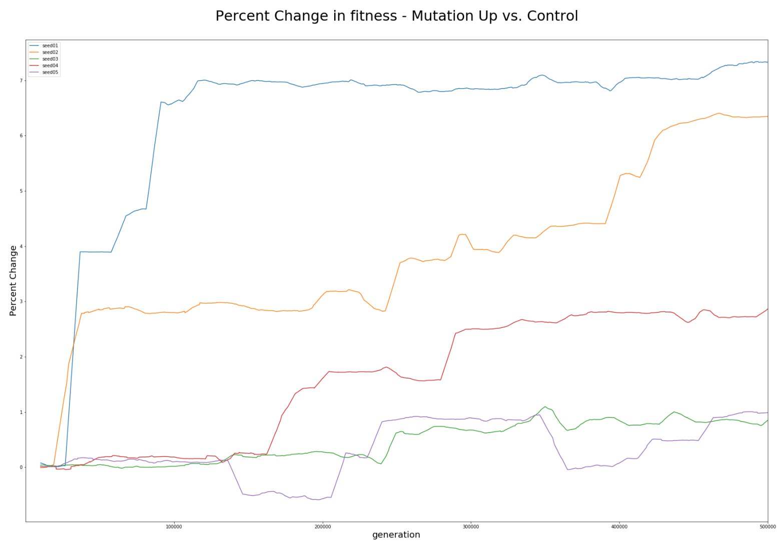
#### Mutation Up

## 4.2 Discussion

### 4.2.1 Relation to Real-World Biological Entities

### 4.2.2 Limitations of Results

One limitation to consider is that only one parameter varied per condition. It may be possible that only under a combination (e.g. low selection and high mutation rates) does reductive evolution occur.


Percent Change in fitness - Mutation Up vs. Control
seed01
seed02
seed03
seed04
seed05
Percent Change
0
1
2
3
4
5
6
7
generation
100000
200000
300000
400000
500000

Chapter 5

# Conclusion and Future Work

In this section we will summarize our overall conclusions drawn from the work and highlight a few possibilities for further research.

## 5.1 Conclusion

## 5.2 Future work

# Bibliography

[1] Bérénice Batut, David P. Parsons, Stephan Fischer, Guillaume Beslon, and Carole Knibbe. In silico experimental evolution: a tool to test evolutionary scenarios. *BMC bioinformatics*, 14 Suppl 15:S11, 2013.

[2] Guillaume Beslon, Vincent F Liard, and Santiago F Elena. Testing evolution predictability using the aevol software. 16th international meeting of the European Society of Evolutionary Biology (ESEB 2017) , August 2017. Poster.

[3] Isabel Gordo and Brian Charlesworth. On the speed of muller's ratchet. *Genetics*, 156(4):2137–2140, 2000.

[4] Carole Knibbe. *Evolution of genome structure by indirect selection of the mutational variability: a computational approach.* Theses, INSA de Lyon, December 2006.

[5] Vincent Liard, David Parsons, Jonathan Rouzaud-Cornabas, and Guillaume Beslon. The complexity ratchet: Stronger than selection, weaker than robustness. *Artificial Life Conference Proceedings*, 1(30):250–257, 2018.

[6] Octavio Miramontes, Franois Taddei, Ariel B. Lindner, Antoine Frenoy, and Dusan Misevic. Shape matters in cooperation. *Artificial Life Conference Proceedings*, (28):340–341, 2016.

[7] Jacob Rutten, Paulien Hogeweg, and Guillaume Beslon. Adapting the engine to the fuel: mutator populations can reduce the mutational load

by reorganizing their genome structure. *BMC Evolutionary Biology*, 19, 12 2019.

[8] Yolanda Sanchez-Dehesa, Loïc Cerf, Jose Maria Pena, Jean-François Boulicaut, and Guillaume Beslon. Artificial Regulatory Networks Evolution. In *Proc 1st Int Workshop on Machine Learning for Systems Biology MLSB 07*, pages 47–52, Evry, France, September 2007.

[9] Zhiyi Sun and Jeffrey Blanchard. Strong genome-wide selection early in the evolution of prochlorococcus resulted in a reduced genome through the loss of a large number of small effect genes. *PloS one*, 9:e88837, 03 2014.

[10] Andreas Wagner. Robustness and evolvability: a paradox resolved. *Proceedings of the Royal Society B: Biological Sciences*, 275(1630):91–100, 2008.