

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 most popular 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 looking at how varying conditions impact 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 50% of their base pairs when compared to their closest living relative, *Synechococcus*. 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 the most definitive evidence, *in vivo* experiments are often impractical because it is frequently difficult or impossible to reproduce the natural environmental conditions in a lab. 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 reduce. 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, potentially leading to 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 example,

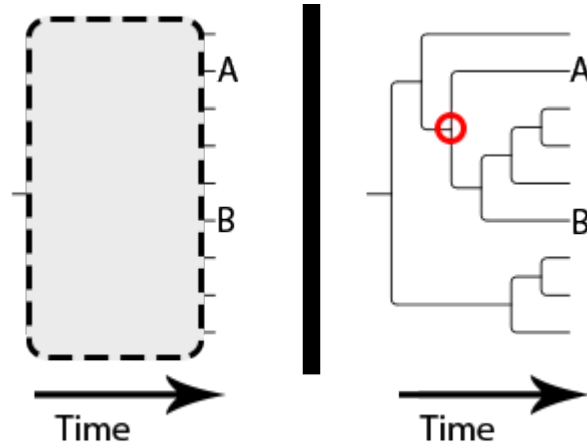


Figure 1.1: An illustration of unknown phylogeny. Since the phylogenetic information under the shaded box is typically not known, the point of divergence (red circle) can't be determined.

if we are comparing two related organisms, A and B, and we are trying to determine when a specific trait was gained or lost by one of the organisms (e.g. to determine its relative importance due to conservation over many generations), 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 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 Professor 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*, *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

2.1 In Silico Evolution

In addition to the difficulties discussed in Section 1.1, in vivo experiments, although sometimes more realistic, have their own set of difficulties, including challenging environmental conditions (e.g. simulating the open ocean in a lab) and simulating the multiple selection pressures acting on genomes in the real world [1], sometimes making it difficult or impossible to tell which factor(s) lead to which outcome.

In silico evolution simulates organisms in software, thus allowing for far greater control and analysis of the environment and other experimental conditions. Varying strategies can be used to create the organisms (e.g. random genes, explicitly providing a previously-evolved genome, etc.) and then the organisms may interact, reproduce, and evolve, with the fittest individuals surviving to produce the next generation.

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

In the following sections, each of the three steps of aevol will be examined in greater detail.

2.2 aevol

The in silico tool *aevol* was developed to "study the evolution of the size and organization of bacterial genomes in various scenarios"[1]. 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 essentially consists of three steps: decoding the genome, selection, and reproduction. These steps are illustrated in Figure 2.2.1 and described in more detail below.

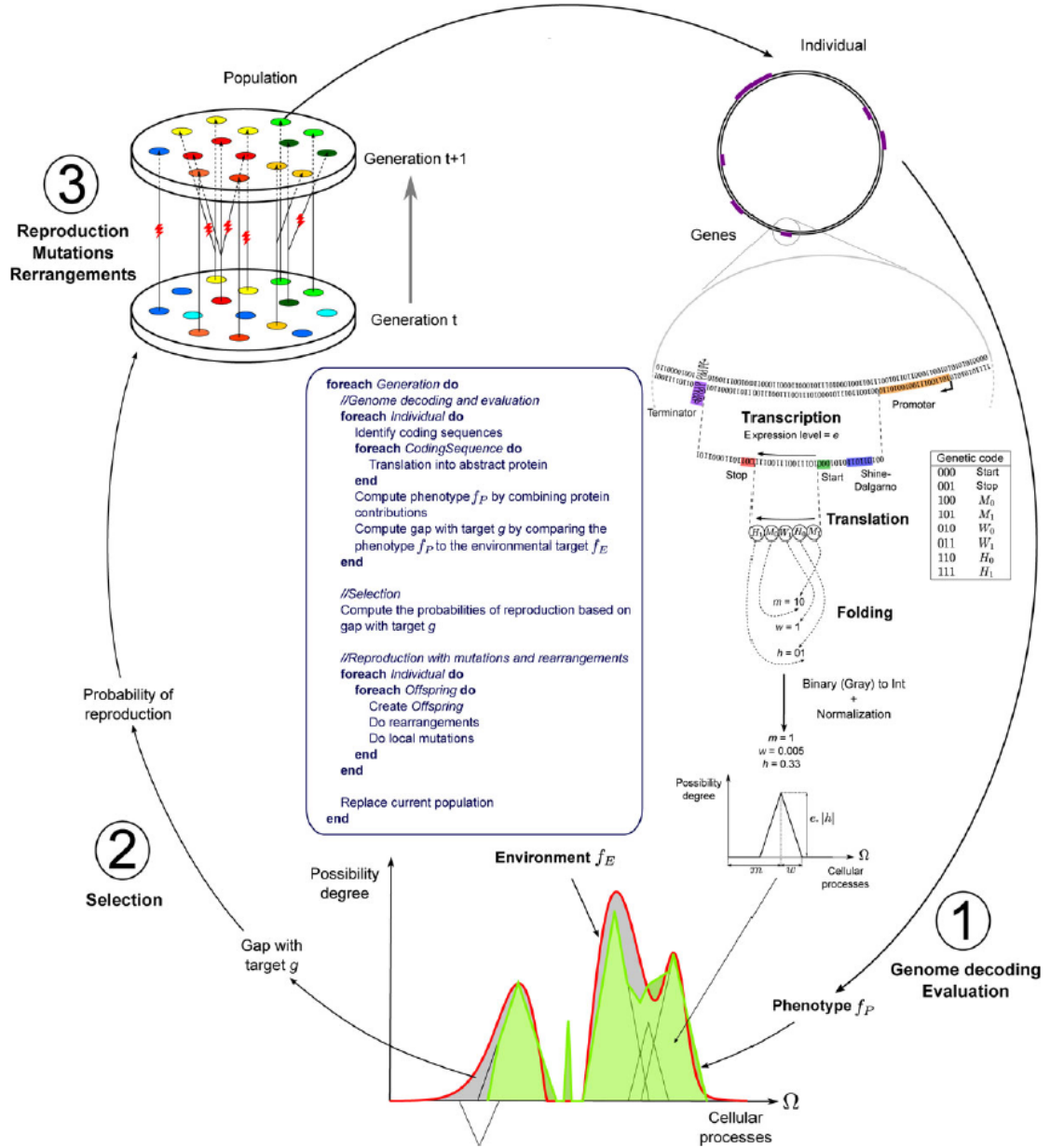


Figure 2.1: Overview of Aevol's architecture, from [1]

Decoding the Genome

In aevol a genome consists of a string of binary characters where 0 is complementary to 1. Each organism in the 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. 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).

Once an initiation sequence is found, the following bases are read three at a time according to the genetic code given in Figure 2.2.1 until a stop codon (by default 001) is found. These codons encode for three parameters, M (mean), W (half-width), and H (height), which together define a triangle representing a "cellular process" (i.e. some phenotypic function).

A cellular process is simply an abstract set of possible functions that can be performed and are represented by the set $\Omega = [0, 1] \subset \mathbb{R}$. The model of *fuzzy logic* is used to find the overall contribution of each cellular process. The mean is in the range $[0, 1]$ and represents the specific cellular process, i.e. the "location" of the process on the function $f \rightarrow 4$. The width describes the "scope" of the process, i.e. the *pleiotropy* of the process. Lastly, the height determines the degree of possibility of the process, i.e. its relative strength.

Selection

After the genome is decoded, the organisms are tested for their fitness. Fitness in Aeol is defined as the gap between the phenotype of a sequence and the environmental function f_E , as illustrated in Figure 2.2.1. This environmental target function f_E is a user-determined set of Gaussians which are specified in a parameter file. The difference between the phenotype (as calculated above) and the environmental function is the "metabolic error", labeled g in the Figure.

Aeol 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 is proportionate to its fitness, which is to

say $\exp(-k * g)$, where k is a user-definable parameter which determines the selection intensity and g is the metabolic error.

Reproduction

2.2.2 aevo1's Post-Treatments

aevo1_misc_ancestor_mutagenesis

This post-treatment generates and analyses mutants for the provided lineage. Specifically, this program generates evolvability, robustness, and antirobustness statistics for the mutants.

1. Generation
2. Fraction of positive mutants
3. Fraction of neutral mutants (reproductive robustness)
4. Fraction of neutral mutants (mutational robustness)
5. Fraction of negative mutants
6. Cumulative delta-gaps of positive mutants
7. Cumulative delta-gaps of negative mutants
8. Delta-gap for the best mutants
9. Delta-gap for the worst mutant
10. Cumulative delta-fitness of positive mutants
11. Cumulative delta-fitness of negative mutants
12. Delta-fitness for the best mutants
13. Delta-fitness for the worst mutants

aevo1_misc_ancestor_robustness

Generates mutants for a given lineage and analyzes their robustness, providing several statistics:

1. Generation

2. Fraction of positive offspring
3. Fraction of neutral offspring (aka reproductive robustness)
4. Fraction of neutral mutants (aka mutational robustness)
5. Fraction of negative offspring
6. Cumulation of delta-gaps of positive offspring
7. Cumulation of delta-gaps of negative offspring
8. Delta-gap for the best offspring
9. Delta-gap for the worst offspring
10. Cumulation of delta-fitness of positive offspring
11. Cumum of delta-fitness of negative offspring
12. Delta-fitness for the best offspring
13. Delta-fitness for the worst offspring

aevol_misc_ancestor_stats

Analyzes a lineage and produces the following outputs:

1. non-coding statistics (e.g. ancestor_stats_bp-b000000000-e000010000-i196-r1.out)
2. mutation statistics (e.g. ancestor_stats_mutation-b000000000-e000010000-i196-r1.out)
3. Each generation's M W H (currently not working? – e.g. ancestor_stats_envir-b000000000-e000010000-i196-r1.out)
4. Each generation's genome size and termination number (e.g. ancestor_stats_nb_term-b000000000-e000010000-i196-r1.out)
5. The lineage's fitness statistics
 - (a) Generation
 - (b) Population size

- (c) Fitness
 - (d) Genome size
 - (e) Metabolic error
 - (f) Parent's metabolic error
 - (g) Metabolic fitness
 - (h) Secretion error
 - (i) Parent's secretion error
 - (j) Secretion fitness
 - (k) Amount of compound present in the grid cell
 - (l) Int probe
 - (m) Double probe
6. Statistics about the how many genes are in 20 RNA (e.g. `ancestor_stats_operons-b000000000-e000010000-i196-r1.out`)
7. Individual gene statistics
- (a) Generation
 - (b) Number of coding RNAs
 - (c) Number of non-coding RNAs
 - (d) Average size of coding RNAs
 - (e) Average size of non-coding RNAs
 - (f) Number of functional genes
 - (g) Number of non-functional genes
 - (h) Average size of functional genes
 - (i) Average size of non-functional genes
8. Rearrangement statistics (e.g. `ancestor_stats_rear-b000000000-e000010000-i196-r1.out`)
- (a) Generation
 - (b) Actual duplication rate
 - (c) Actual deletion rate

- (d) Actual translocation rate
- (e) Actual inversion rate
- (f) Average alignment score

aevo_misc_coalescence

Prints coalescence statistics for a given lineage.

aevo_misc_create_eps

Creates a directory, analysis-generation_XXXXX with several EPS files. The EPS files are as follows:

- best_genome_with_CDS.eps
- best_genome_with_mRNAs.eps
- best_phenotype.eps
- best_pos_neg_profiles.eps
- best_triangles.eps

aevo_misc_extract

Extracts the genotype and/or data about the phenotype of individuals in the provided population and write them into text files for easy parsing by programs such as MATLAB. The program lets you specify whether you want the sequence, the triangle data, or both. By default (i.e. with no parameters) gives just the sequence, into a file called “sequence”.

aevo_misc_lineage

Using the tree files, recreates the lineage of an individual. By default, this is done for the best individual, but another individual can be specified by ID number or rank (-i or -r, respectively).

aevo_misc_mutagenesis

This generates and analyzes mutations for an individual from a population backup (as opposed to a lineage file for ancestor_mutagenesis). One can specify point mutations, small indels, duplications, large deletion, translocations, or inversions.

aevo_misc_protein_map

Creates a CSV file which analyzes the proteins generated by the specified lineage. The CSV file specifies:

1. generation – Which generation is being described
2. protein_id – An identifier for the protein.
3. shine_dal – The location of the Shine-Dalgarno initiator for the protein on the sequence.
4. length – The number of base pairs which encodes the protein.
5. concentration – The “amount” of a protein encoded, specified by the distance between the promoter and the consensus sequence.
6. hamming_dist – The Hamming distance between the promoter sequence and the consensus sequence.
7. dist_next_protein – The distance to the next protein?
8. nb_proteins – The number of proteins?
9. dna_length – The length of the DNA sequence.

aevo_misc_robustness

Calculates replication statistics for a given individual (specified by either ID or rank) at a given time. Specifically, the following information is saved in a new directory (analysis-generation XXXXXXXXXXXX):

1. Parent id – The identifier of this organism’s parent.
2. Parent metabolic error – The metabolic error rate of the parent.

3. Parent secretion – The secretion rate of the parent.
4. Mutant metabolic error – The metabolic error rate of the mutant.
5. Mutant secretion – The secretion rate of the mutant.
6. Mutant genome size – The genome size of the mutant.
7. Mutant number of functional genes – The number of functional genes in the mutant.

2.3 Changeable Factors

2.3.1 Fitness

2.3.2 Evolvability

2.3.3 Robustness

2.3.4 Structure

Percent Coding vs. Non-Coding DNA

Number of Genes

2.4 Related Work

Chapter 3

Approach

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

3.1 Contributions

3.2 Experiment Designs

3.3 Expected Results

Chapter 4

Experiments, Results, and Discussion

4.1 Experiments

In this section we describe the experiments generally.

4.2 Evaluation Strategy

In this section we describe how we evaluated the experiments, our metrics, etc.

4.2.1 Robustness

4.2.2 Evolvability

4.2.3 Coalescing Time

4.3 Experiment Results

In this section we describe the results of our experiments.

4.3.1 Discussion

4.3.2 Relation to Real-World Biological Entities

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.