Ancestral Reconstruction and Investigations of Genomics Recombination on Chloroplasts Genomes

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SPIM

Thèse de Doctorat

école doctorale **sciences pour l'ingénieur et microtechniques**

UFC UNIVERSITÉ DE FRANCHE-COMTÉ

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Ancestral Reconstruction and Investigations of Genomics Recombination on Chloroplasts Genomes

By
Bashar Talib Hameed **Al-Nuaimi**

Thèse présentée et soutenue à Besançon le 13 octobre 2017
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DEDICATION

Throughout my life, one person has always been there during those difficult and trying times. I would like to dedicate this thesis and everything I do to my wife. In addition to my family, I have always been surrounded by strong supportive friends. I would not be who I am today without the support of my supervisor Jean-François COUCHOT

I also dedicate this work to my friends ; Bassam Alkindy and Abbas Abdul Azeez, who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started.

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ABSTRACT

Ancestral reconstruction and investigations of genomics recombination on chloroplasts genomes

Bashar Talib Hameed Al-Nuaimi
Université de Bourgogne Franche-Comté, 2017

Supervisor : Jean-François Couchot

The evolution theory underpins on modern Biology. All new species emerge from an existing species. This results in different species are sharing a common ancestry, as represented in phylogenetic classification. The common ancestry can explain the similarities between all living organisms, such as general chemistry, cell structure, DNA as the genetic material and genetic code. The individuals of a species share the same genes but (ordinarily) different sequence of alleles of these genes. An individual inherits alleles from their ancestry or parents. The purpose of phylogenetic studies is to analyze the changes that occur in different organisms during the evolution by identifying the relationships between genomic sequences and determining the ancestral sequences and their descendants. A phylogeny study can also estimate time of divergence between groups of organisms that share a common ancestor. Phylogenetic trees are useful in fields of biology, such as bioinformatics, for a systematic and comparative phylogenetic. Evolutionary tree or phylogenetic tree is a branching exhibit the evolutionary relationships among various biological organisms or other existence based upon differences and similarities in their genetic characteristics. Phylogenetic trees are built from molecular data like DNA sequences and protein sequences. In a phylogenetic tree, nodes represent genomic sequences and are called taxonomic units. Each branch connects any two adjacent nodes. Every similar sequence will be a neighbor on the outer branches, and a common internal branch will connect them to a common ancestor. Internal branches are called hypothetical taxonomic units. Thus the taxonomic units joined together in the tree are implied to have descended from a common ancestor. Our research performed in this dissertation focuses on improving appropriate evolutionary prototypes and robust algorithms for solving the phylogenetic and ancestral inference problems applying on gene order and DNA data under the whole-genome evolution, along with their applications.

Ancestral genome reconstruction can be described as a phylogenetic study of species of interest to extra details than what is provided by a standard phylogenetic tree. It may include information on ancestor species such as their gene content, the configuration of

these genes in the genome, the nucleotide sequence itself. Such information can help to understand the history of evolutionary a set of organisms better and through shed light on the genomic basis of phenotypes.

In this thesis, we are interested in both theoretical and practical problems in phylogenetic tree reconstruction and genome rearrangements. We propose a heuristic approach to ancestral genome reconstruction, and we implement one of the practical tools applicable to the analysis of real datasets spanning a complex phylogeny and accommodating a variety of genome architectures. We demonstrate the efficiency of our approach on the well-studied data set of chloroplast genomes and apply it to the reconstruction of rearrangement histories of complete, and really accurate reconstruction of some specific bacteria lineages such as *Mycobacterium* Genus. The reconstructing ancestral genomes problem of in a given phylogenetic tree stands in different comparative genomics domains. In this work, we focus on reconstructing ancestral genomes by the gene order, accessibility to reconstruction a whole genome DNA sequences. Ancestral genome reconstruction in this sense and for chloroplastic genomes and specific bacteria strains is the topic of this thesis.

KEY WORDS : Ancestral reconstruction, nucleotide sequence, taxonomic units, phylogenetic tree, *Mycobacterium* Genus, *Chloroplastic* genomes

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ABBREVIATIONS

AlignSeqs Align a Set of Unaligned Sequences.

BLAST ... Basic Local Alignment Search Tool.

BPSO .... Binary Particle Swarm Optimization.

BP ..... Bootstrap Probability.

DOGMA .. Dual Organellar GenoMe Annotator.

DPSO .... distributed Particle Swarm Optimization.

DNA ..... Deoxyribonucleic Acid.

EMBL .... European Molecular Biology Laboratory.

GA ..... Genetic Algorithm.

Gap ..... Absence of a homologous character in either sequence could be a reason of deletion in the comparing sequence or an insertion in the sequence to which it is compared to. In either case a gap is added and a value of -1 is added to the score. Gap indicated an arbitrary number of null characters (represented by dashes).

GSA ..... Global Sequence Alignment.

GTR ..... GTRGAMMA : GTR model of nucleotide substitution with the Γ model of rate heterogeneity. All model parameters are estimated by RAxML.

Indel ..... An insertion/deletion polymorphism, commonly abbreviated “indel,” is a type of genetic variation in which a specific nucleotide sequence is present (insertion) or absent (deletion).

JModelTest jModelTest is a tool to carry out statistical selection of best-fit models of nucleotide substitution.

LSA ..... Local Sequence Alignment.

LUCA .... Last Universal Common Ancestor.

MUSCLE . MUltiple Sequence Comparison by Log- Expectation.

MSA ..... Multiple Sequence Alignment.

ML ..... Maximum Likelihood.

MP ..... Maximum Parsimony.

MTBC .... *Mycobacterium tuberculosis complex.*

MLGO .... Maximum Likelihood for Gene-Order analysis.

MLWD .... Maximum Likelihood on Whole-genome Data.

NCBI ..... National Center of Biotechnology Information.

NW ..... Needle-man Wunsch Alignment.

PHAST ... Phylogenetic Analysis with Space/Time Models.

PSA ..... Pairwise Sequence Alignment.

PSO ..... Particle Swarm Optimization.

RAxML ... Randomized Axelerated Maximum Likelihood.

RNA ..... Ribonucleic acid.

rRNA ..... ribosomal RNA.

SW ..... Smith-Waterman Alignment.

T-COFFEE multiple sequence alignment that provides a dramatic improvement in accuracy with a modest sacrifice in speed as compared to the most commonly used alternatives.

Topo ..... Topology.

TU ..... Taxonomy Unit

tRNA ..... Transfer RNA.

I

General introduction

1

General Presentation

1.1/ Introduction

Why to reconstruct ancestral genomes ? From a fundamental point of view, studies of contemporary biological systems using, for example, approaches related to anatomy, biochemistry, physiology, and molecular biology are seriously limited by the absence of a mechanism of evolution description that would explain their establishment, organization, and functioning. The long-term aim of possessing ancestral genomes is thus to establish a broad framework for studying evolution despite the cruel lack of historical data. To achieve this goal, many algorithmic developments are necessary to efficiently and systematically process the large volumes of available data following a rigorous methodology.

Genomics studies are a typical case of very large volume data investigations : they are of very high resolution (until nucleotide level) and very reliable (many genome sequences contain less than one error every 10,000 bases), very abundant (100 sequenced *eukaryotic* genomes for instance, and more than 1,000 *prokaryotes*), and centralized in public databases. Genomes also provide fundamental entry points to the functional properties of organisms, such as the presence or absence of genes, the expansion or regression of gene families, the topology of the cis-regulatory elements. In other words, it informs about the likelihood of certain metabolic or developmental pathways that may exist in an organism, and the importance of functions specific to each species. Genomes thus represent the foundation on which many advances can be achieved, and accessing such information in an ancestral genome provides a broad spectrum of these properties.

From a more practical point of view, given the astronomical amount of genomic data supplied to the community, the pace of which is likely to accelerate further in the coming years, it is critical to maintain a substantial degree of organization for distribution and presentation of data. Ancestral genome reconstructions will allow the sequences and annotations of modern species to be linked naturally with those of ancestral species in the direction of Evolution, following the phylogeny of species. The ancestral genomes will serve as single reference points for comparing descending genomes, which will greatly facilitate the identification of ancestral genomics properties, and therefore specific lineage gains or losses. Conversely, the results that will continue to be obtained with different organization models will enrich them in return. To sum up, ancestral genomes are part of the foundations that will help us decipher the different molecular components contributing to the evolution of species, and that have led to such a variety of species and biological systems we can currently observe.

The aim of this thesis is to participate to the development of tools able to reconstruct the successive ancestors in several given lineages, thus providing a dynamic view of the evolution of genomes

1.2/ Presentation of the Problems

Recently, many approaches have been developed to solve the ancestral reconstruction problem [2, 3, 4, 5, 6], but either they are limited to the evolution of one given character (for instance, a particular nucleotide), or conversely they theoretically focus on large-scale nuclear genomes (several billions of nucleotides) facing multiple recombination events. Large-scale genomic evolution problem can't be tackled with same approaches than one-character methods : when considering the set of all possible recombinations on large genomes, the problem is indeed NP-hard. As far as we know, there is no directly applicable solution solving the evolution of large DNA sequences. Conversely, in this thesis, we focus on genomes that have a reasonable size and who faced a rational number of recombination like in the chloroplast case. Even if the problem becomes *a priori* tractable for such cases, it however requires the design of *ad hoc* solutions, and various difficulties remain to circumvent when dealing with such a specificity. For illustration purpose, the solutions will be applied on mid-scale genomes of chloroplasts first, and then of bacteria, growing so bit by bit the complexity of the problem we consider.

Let us recall the importance of understanding well the evolution of such mid-scale genomes. Chloroplasts are one of the main organelles in the plant cell. *They are considered to have originated from cyanobacteria through endosymbiosis when an eukaryotic cell engulfed a photosynthesizing cyanobacterium, which further remained and became a permanent resident in the cell*[1]. The term of chloroplast comes from the combination of chloro and plastid : it is an organelle (found in plant cells) that contain the chlorophyll. Chloroplast has indeed the ability to convert water, light energy, and carbon dioxide in chemical energy by using carbon-fixation cycle [7]. As this conversion releases oxygen, chloroplasts originated the breathable air and represent a mid to long-term carbon storage medium. Consequently, exploring the evolutionary history of chloroplasts is thus of great interest and *we propose to investigate it by the mean of ancestral genomes reconstruction*.

This reconstruction will be realized with the desire to explain how molecules have evolved over time, and to validate (or not) that this way can present evidence of their cyanobacteria origin. This long-term objective necessitates numerous intermediate advanced methods. For instance, it requires the ability to apply the ancestral reconstruction on a well-supported phylogenetic tree of a representative collection of chloroplastic genomes. Moreover, it necessitates the ability to detect content evolution (modification of genomes like gene loss and gain) along this accurate tree. These two prerequisites (gene content evolution, accurate phylogeny inference) have already been investigated in the literature, as reported briefly in the next section.

1. cf. Wikipedia

1.3/ STATE OF THE ART : A GENERAL OVERVIEW

There exist two main computational methods to handle gene order and to propose ancestral genome architectures : rearrangement-based and homology-based methods. The rearrangement-based methods typically search for the set of ancestral gene orders that minimizes the sum of rearrangement distances over all branches of the given phylogeny [3, 4]. Homology-based methods are used to solve the small phylogeny problem that consists of the ancestral gene orders reconstruction of a species tree at the internal nodes from extant genomes. This process takes gene adjacency into account and handles them as binary characters with present and absence states. In this way, by observing the gene order as a set of adjacency genes, the aim is to discover which adjacency is contained in the ancestral genomes [2, 8]. These methods offer a better understanding of the genome evolution history and can further improve our knowledge of the mechanisms linking organic sequences to their functions. Despite this, ancestral genetic sequence reconstruction suffers from several limits such as those involved in the regulation of genes (insertion, deletion, duplication). Along with the examination of molecular evolution, it relies on the validity of models and their fundamental hypothesis [9].

Accordingly, a critical component of ancestral reconstruction of genomes is the understanding of the phylogenetic tree relations between the species being examined [10]. It is crucial to identify the most suitable tree topology by using for instance bootstrap estimations in a maximum likelihood approach [11, 12]. Moreover, calculating the lengths of its branches is essential for a perfect reconstruction, as well as for evaluating the exactness of that reconstruction through simulations [13]. The lengths are related to the number of recombination and mutations that are likely to occur between an ancestor and its child nodes. This is why any error in the inferred phylogenetic relation will have obvious dramatic effects on the reconstructed ancestors.

So ancestral genome reconstruction (AGRC) can be described as an extension of phylogenetic study of species of interest : it provides extra details than what is usually obtained by a classical phylogenetic tree [11]. It may include information about ancestor species such as their gene content, the organized of these genes in the genome, the nucleotide sequence itself, and so on [14]. Such information can help to understand better the evolutionary history of a set of organisms and through shed light on the genomic basis of phenotypes [15]. The observation of species is described by the peak or last nodes of the tree (leaves) that are gradually correlated by branches to their common ancestor [16], while nodes are represented by the branching points of the tree, which are usually designated to as inner nodes or the ancestors [17].

The ancestral reconstruction problem is as old as the field of molecular evolution. For instance, over past decades, many methods were proposed to reconstruct phylogenies from gene-order data. The prior algorithm has been established by Fitch [18] : Fitch's parsimony algorithm first assumes a binary alphabet and is based on maximum parsimony (MP) patterns : it finds the labels to the internal nodes of a tree that reduce the number of changes or modification along tree edges. The most modern methods for phylogeny reconstruction from genome rearrangements include GRAPPA [3] and MGR [4]. However, these approaches are limited to cases where gene content are similar or when only a few deletions are expected [5]. Among the most recent methods which are based on gene adjacency, InferCARsPro is comparatively faster, but often produces an excessive number of chromosomes [2]. This problem is tackled by newer methods such as GapAdj [8], but it

is achieved by sacrificing a significant part of accuracy. Recently was proposed a faster and more accurate method called PMAG [5], which is a probabilistic framework method to infer ancestral gene order, and which involves gene insertions and deletions in addition to rearrangements. PMAG not only accurately infers ancestral genomes but also does an excellent job in assembling adjacencies into logical gene order [6]. All the aforementioned approaches, if we except PMAC, only deal with gene permutations, and they discard for instance the possibility of gene duplication or deletion. There is so an obvious lack of tools that concretely deal with ancestral reconstruction of mid-scale genomes, which consider all possible recombination – and we propose to fill this gap.

1.4/ Organization of the thesis manuscript

This current chapter is devoted to a general introduction of the thesis, providing the problematics and a brief description of both thesis subject and objectives. The introduction aims to place the work presented here in a more general context and provides essential data necessary for nonspecialists to understand the scope and development of the analysis presented here. The second part proposes a brief state-of-the-art about phylogenetic reconstruction, with existing methods that are related to this work.

Chapter 2 introduces some notions of biology that are essential for understanding the various problems addressed during this thesis. It gives a brief overview on how a phylogenetic tree can be generated from a set of DNA sequences, and some concepts regarding phylogenetic analysis and algorithms used for phylogenetic reconstruction. The concepts of local and global alignments and the most common implementations are detailed in this chapter too. Multiple alignment algorithms are additionally given. It is moreover explained why small divergences in given sequences may lead to a hard alignment problem. To analyze aligned sequences, we describe various phylogenetic concepts and terminologies. Methods for constructing phylogenetic trees are finally summarized (such as distance and character based methods), together with bootstrap analysis.

Chapter 3 illustrates essential resources jointly introduced in an artificial intelligence algorithm for phylogenetic tree reconstruction. In this chapter, we study first the relevance of the Simulated Annealing (SA) algorithm to fulfill the optimization task. Then, various metaheuristics have been executed in a distributed manner using supercomputing facilities. Our proposal is based on genetic algorithm and a particle swarm optimization approach that are developed in both linear and parallel fashions, in order to reconstruct a well supported phylogenetic tree while removing genes that blur the phylogenetic signal. An improved simulated annealing method is finally added, and a comparison of 3 given metaheuristics on a large number of new groups of species is proposed.

Chapter 4 discusses other methods that are used in our ad-hoc algorithm to generate ancestral genomes. Investigations of genomic recombination on *campanulides chloroplasts* are further detailed, depending on all provided information obtained with previously presented tools in Chapter 3. Then, in Chapter 5, we describe the relations that can be found between the phylogeny of a large set of 845 complete chloroplast genomes, and the evolution of gene content inside these sequences. Core and pan genomes have been computed on *de novo* annotations of these genomes, the former being used for producing well-supported phylogenetic trees while the latter provides information regarding the evolution of gene contents over time, and illustrates the specificity of some branches

of the trees.

In Chapter 6, we propose to reconstruct all ancestors of all complete available genomes of *Mycobacterium tuberculosis* and *M. canettii*. Doing so allows us to consider complete genomes that are more complex than the chloroplasts. The study starts by investigating the single nucleotide polymorphism level, while insertions-deletions (indels) and large scale recombinations are regarded in a second stage. By mixing automatic reconstruction of obvious situations with human interventions on signaled problematic cases, we prove that it is possible to achieve a concrete, complete, and really accurate reconstruction of lineages of the *Mycobacterium tuberculosis* complex.

Finally, the conclusion of the manuscript provides a summary of researches that have been realized during this thesis. A discussion about possible future work in this area is proposed too, to open the debate and introduce putative further investigations.

1.5/ PUBLICATIONS

All the objectives of this thesis have been investigated, even though a lot of work still remain to be realized. These investigations have been validated by the following publications.

1.5.1/ PUBLICATIONS IN INTERNATIONAL CONFERENCES AND JOURNALS

1. **CIBB 2015** Bassam Alkindy, Bashar Al-Nuaimi, Christophe Guyeux, Jean-François Couchot, Michel Salomon, Reem Alsrraj, and Laurent Philippe. "Binary Particle Swarm Optimization Versus Hybrid Genetic Algorithm for Inferring Well Supported Phylogenetic Trees". In Computational Intelligence Methods for Bioinformatics and Biostatistics : 12th International Meeting, CIBB 2015, Naples, Italy, September 10-12, 2015, pp. 165-179, Springer International Publishing, 2015.
2. **IJBBB 2017** Al-Nuaimi Bashar, Christophe Guyeux, Bassam AlKindy, Jean-François Couchot, and Michel Salomon. "Relation between Gene Content and Taxonomy in Chloroplasts". in International Journal of Bioscience, Biochemistry and Bioinformatics (IJBBB) 2017 Vol.7(1) : 41-50 ISSN : 2010-3638.
3. **IWBBIO 2017** Christophe Guyeux, Bashar Al-Nuaimi, Bassam Alkindy, Jean-François Couchot and Michel Salomon. "On the Ability to Reconstruct Ancestral Genomes from *Mycobacterium Genus*". In International Conference on Bioinformatics and Biomedical Engineering (IWBBIO 2017) pp. 642-658. Springer International Publishing, Granada, Spain, April 26-28,2017.
4. **JIB 2017** Bashar Al-Nuaimi, Roxane Mallouhi, Bassam AlKindy, Christophe Guyeux, Michel Salomon, and Jean-François Couchot. "Ancestral reconstruction and investigations of genomic recombination on Campanulids chloroplasts". Integrative Bioinformatics (JIB). Date of submission : 7th of November, 2016.
5. **BMC 2017** Régis Garnier, Christophe Guyeux, Jean-François Couchot, Michel Salomon, Bashar Al-Nuaimi and Bassam AlKindy. "Comparison of Metaheuristics to Measure Gene Effects on Phylogenetic Supports and Topologies". Submitted to Special Issue on BMC Bioinformatics Supplement. Date of submission : 23th of May, 2017.

6. **BMC 2017** Christophe Guyeux, Bashar Al-Nuaimi, Bassam Alkindy, Jean-François Couchot and Michel Salomon. "Investigating the ancestral reconstruction of bacterial genomes". Submitted to Special Issue on BMC Bioinformatics Supplement. Date of submission : 22th of July, 2017.

1.5.2/ Publications in national seminars and workshops

1. **SeqBio'2015** Bashar Al-Nuaimi, Roxane Mallouhi, Bassam AlKindy, Christophe Guyeux, Michel Salomon, and Jean-François Couchot. "Ancestral reconstruction and investigations of genomic recombination on Campanulides chloroplasts". Workshop of SeqBio 2015, Orsay, November 2015.
2. **Femto-st'2015** Bassam Alkindy, Bashar Al-Nuaimi, Huda Al'Nayyef, Panisa Treepong, Christophe Guyeux, Jean-François Couchot, Michel Salomon, and Jacques Bahi. "Bioinformatics Approaches on Genomic Evolution in Femto-ST (Core Genome, Phylogenetic Analysis, Transposable Elements, and Ancestral Reconstruction)". Workshop of Femto-ST, June 2015, Besancon, France. Note : Poster.
3. **Femto-st'2016** Bashar Al-Nuaimi, Bassam Alkindy, Christophe Guyeux, Jean-François Couchot, and Michel Salomon. "Ancestral reconstruction and investigations of genomic recombination on Campanulids chloroplasts and *Mycobacterium Genus*". Workshop of Femto-ST, August 2016, Besancon, France.

II

SCIENTIFIC BACKGROUND

2

Scientific Background

In this chapter, we introduce some notions of biology which are sufficient for the understanding of problems addressed in this thesis. Indeed, the challenges of reconstructing chromosomal rearrangements and ancestral genomes requires to know the structure of genomes. Obviously, phylogenetic tree reconstruction is a first step in the understanding of the ancestral relationship among a set of biological sequences. It includes the construction of a tree, where the nodes indicate separate evolutionary paths, and the branch lengths give an approximation of how distant the sequences represented by those branches are. Additionally, in this chapter, we will present various sequence alignment algorithms, which are a fundamental step in molecular phylogenetics to explain the evolution of speciation, quantification of substitution patterns and gene duplication events, but also a useful tool for identifying mutations leading to genetic diseases. This chapter covers the pairwise global and local alignments by dynamic programming with various scoring schemes, multiple sequence alignments that are reduced to pair-wise alignment, and profile alignment by using a guide tree. This chapter presents also a brief information on how a phylogenetic tree can be constructed from a set of DNA sequences, and how to evaluate the generated tree. Finally, some concepts concerning phylogenetic analysis and algorithms used for phylogenetic reconstruction will be reported. Note that the state-of-the-art part related to multiple sequence alignment and their use for phylogenetic analysis has been studied in common with my colleague Panisa Treepong, and written "four hands" as our investigations in this field have been performed together, in team.

2.1/ Chromosomes and genomes

2.1.1/ A short overview

Genomes contain the complete genetic material of an individual or a species, encoded in its DNA, except certain viruses whose genome is carried by RNA molecules. From one organism to another, the genome organization may differ. It can be composed of one or more DNA molecules, which will have a significant impact on the complexity of the problem of reconstructing chromosomal rearrangements and ancestral genomes. In *prokaryotes* (*bacteria and archaea*), the genome is located in the cytoplasm of the cells, which is usually contained in a circular DNA molecule. However, there are many exceptions : some species may have several circular chromosomes, or a single linear chromosome, or a linear chromosome and a circular one [19]. There may also be an extra-chromosomal component contained in plasmids and episomes.

In *eukaryotes*, we can distinguish the following :

1. Nuclear DNA composed of several linear chromosomes, contained in the nucleus of the cells (an element which indeed characterizes *eukaryotic* cells).
2. Non-nuclear DNA, contained in organelles, *i.e.*, the chloroplastic chromosome contained in the chloroplasts of photosynthetic organisms (*algae and plants*), or the mitochondrial chromosome contained in mitochondria of all the other *eukaryotes*.

In *eukaryotes*, linear chromosomes are characterized by a centromere and two telomeres in most organisms. The centromere shares the chromosome in two arms (left and right) and is essential for the smooth unfolding of the cell divisions. The telomeres are the two ends of a chromosome [20]. The number of chromosomes contained in the cell of an organism varies according to the considered species [1]. The size of the genome is mainly measured according to its number of nucleotides, or bases (in *bp* for base pair, since the majority of the genomes is made up of double strands of DNA). Multiples are also used, like *kb* for kilobase or *Mb* (megabase), which are respectively equal to 1,000 and 1,000,000 bases. Note that the size of a genome may vary from a few kb in viruses to several hundreds of thousands of Mb in some *eukaryotes*, as shown in Table 2.1.

The quantity of DNA is not proportional to the complexity of an organism. Some ferns, for example, have genomes more than ten times larger than the human one [21, 7, 22].

TABLE 2.1 – Some examples of genome varieties

Species	Kingdom	Genomes size	Number of genes
Mycobacterium tuberculosis	*Bacteria*	4.41Mb	4,008
Brucella abortus(chromosome 2) *Brucella abortus(chromosome 1)*	*Bacteria*	2,12 Mb 1,16 Mb	2200 1156
Actinidia chinensis	*Plantae*	616.1 Mb	39,040
Takifugu rubripes	*Animalia*	390 Mb	22–29,000
Plasmodium falciparum	*Alveolata*	22.9 Mb	5,268
Drosophila melanogaster	*Animalia*	122.6 Mb	17,000
Homo sapiens	*Animalia*	3.2 Gb	18,826

2.1.2/ GENOME AND DNA MUTATIONS

The cell is the 'building block' of life. It mainly performs functions for maintaining daily life and passing the genetic instructions to the next generation. The former function is particularly facilitated by proteins whereas the latter is mainly achieved through *Deoxyribonucleic acids* (DNA).

DNA is a polymer, where its monomer units are nucleotides. Each nucleotide in a DNA has three parts : a pentose sugar (desoxyribose), a phosphate, and one "base". Indeed, nucleotides can be classified into four types corresponding to their distinct bases : Adenine (A), Cytosine (C), Guanine (G), and Thymine (T). A and G are called purines, having a two-ring structure, while C and T are called pyrimidines and they conversely have a

1. For example, man has 23 pairs of linear chromosomes whereas *Escherichia coli*, an intestinal bacterium, has only one circular chromosome.

one-ring structure (see Figure 2.1). For the sake of concision, DNA is simply represented as a sequence over the alphabet (A, C, G, T).

During organism evolution, its DNA is replicated and passed on to its offspring. And through DNA replication, changes can occur in the sequence, which is referred as mutation. These variations in the DNA sequences occur at the base level, as depicted in Figure 2.2. These modifications change the characteristics of the generations and eventually, may lead to the production of new species. This evolutionary manner of DNA mutations can be represented, in a certain way, by a phylogenetic tree, introduced later in this chapter.

Fig. 2.1 – DNA has a double-helix shape. Bases are found in pairs inside the double helix. The bases in DNA are named A, T, G, and C. Pyrimidine T (resp. C) forms pairs with purine A (resp. G), and vice versa, https://www.slideshare.net/AmyHollingsworth/lab5dnaextractionfromstrawberriesandliverfall2014.

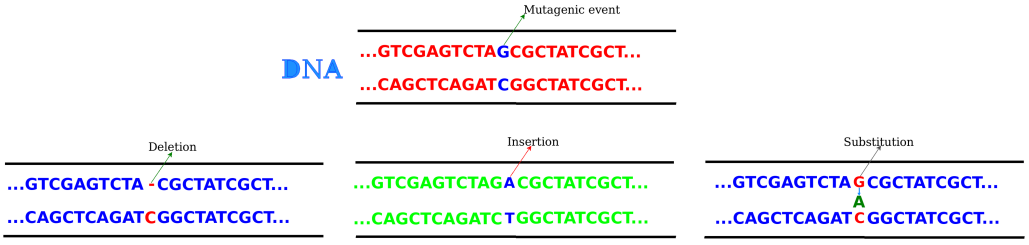


Fig. 2.2 – A mutation occurs when a DNA sequence is damaged or changed, which may for instance alter the genetic message carried by a gene.

These genomic mutations are now easily accessible via modern sequencing technologies, making it possible to discover single nucleotide polymorphisms [2], short insertions and deletions (INDELs) as well as other genomic mutations like duplication and inversions.

2.1.3/ Model of Nucleotide Substitution

As previously stated, over time, nucleotide sequences can “evolve” through substitution. This process can cause a nucleotide (A, C, T or G) to change into another nucleotide, and this is one of the most central driving force behind evolution. This modification in a DNA sequence may lead to an inactivation of a gene or to a mutation in the protein that

2. Let us recall that a single nucleotide polymorphism, usually abbreviated to SNP, is a mutation in a single nucleotide (A, T, C, or G) that occurs at a particular position in the genome, as shown in Figure 2.3. Each mutation is present to some appreciable degree within a population. Each organism has several single nucleotide polymorphisms that together create a unique DNA pattern for that.

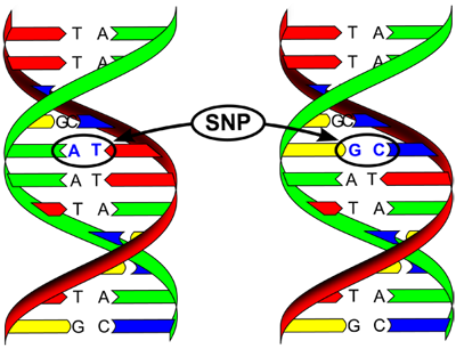


Fig. 2.3 – Single Nucleotide Polymorphism (SNP).

the sequence codes. As proteins are the building blocks of organic life, this may cause significant variations in an organism's characteristics. Alternatively, this modification may have no effect at all, being silent.

Commonly, this type of mutation can take place once or twice each million years on a given sequence location. Estimating the evolution of organisms over hundreds of millions of years, models of nucleotide evolution are helpful in speculating how one sequence of nucleotides may have evolved from another. These models can be inferred by either assuming that two given sequences had shared a common DNA ancestor or by assuming that one sequence evolved into the other.

At the simplest level, the proportion can be used for defining such a matrix P of nucleotide substitution :

$$P_d = \frac{n_d}{n} \tag{2.1}$$

where n indicates the total number of nucleotides in the sequence, and n_d is the number of base d, $d = \{A, C, G, T\}$. Other richer probabilistic models have been proposed in the literature, to provide a more accurate estimation of the mutation matrix P, like Jukes and Cantor [23], Kimura [24], and Tamura and Nei [25].

2.2/ Sequence alignment

One of the principal problems in computational molecular biology is sequence alignment. A sequence alignment is a process of aligning blocks of provided sequences (of DNA, RNA, or protein) to recognize similar regions – that may be a consequence of functional or evolutionary relationships between the sequences. Aligned sequences of amino acids or nucleotides are reproduced as rows within a matrix. Gaps are inserted between the deposits so that residues with identical or similar sequences are arranged in successive columns.

Let us for instance consider two sequences which are homogeneous except that the first sequence contains one other residue (e.g., a given nucleotide). When we view the alignment of these two sequences, the other residue will be matched to a gap. This corresponds to an insertion event in the first sequence or a deletion event in the second. On the other hand, if we note that an insertion event has occurred in the first sequence (concerning the second) then we know how to match that residue to a gap in the second.

Thus one way to build a sequence alignment is to find a series of insertions, deletions, or replacements collectively called mutation events, which will transform one sequence into the other. The number of mutation events needed to transform one sequence into the other is called the edit distance.

Indeed, in sequence alignment, there are two broad categories, namely the local and the global one. A local alignment returns the best matching subsequence, while in a global alignment, we obtain the best match of both sequences in their totality. Local sequence alignments intend to reveal similar regions in a given pair of sequences. In other words, they find an optimal local alignment by searching for two segments with maximum similarity score by discarding weak initial and terminal fragments, see. Figure 2.4 for an illustrative example.

Many algorithms have been developed for these two kinds of alignments, some of the most popular ones being summarized in the next subsections.

Fig. 2.4 – Global alignments are applied for comparing homologous genes whereas local alignment can be used to locate homologous regions in otherwise non-homologous genes.

2.2.1/ BLAST

Basic Local Alignment Search Tool (BLAST) is a database sequence search engine proposed by the National Center for Biotechnology Information (NCBI). The first version of BLAST was published in 1990 and it supported only ungapped searches. The second version, releazed in 1997 [26], has been designed to determine high-scoring local alignments between sequences, without discrediting the speed of such searches. BLAST addresses thus an essential problem in bioinformatics research. It uses a heuristic process that attempts local as crossed to global alignments and, therefore, it is suitable to identify relationships between sequences (amino-acid sequences of proteins or the nucleotides of DNA sequences) which share only isolated regions of similarity [27].

Table 2.2 displays the different BLAST programs available on the NCBI web server.

2.2.2/ Local Sequence Alignment : Smith–Waterman algorithm

The Smith-Waterman algorithm was developed by Temple F. Smith and Michael S. Waterman in 1981 [28]. Using a dynamic programming approach, it is able to provide the optimal local alignment between two strings. The algorithm estimates the alignment that

TABLE 2.2 – BLAST programs. http://www.ncbi.nlm.nih.gov/BLAST/

Program	Comparison	Application
BLASTN	DNA vs. DNA. Compares a nucleotide query sequence against a nucleotide sequence database.	Find DNA sequences that match the query
BLASTP	Protein vs. Protein. Compares an amino acid query sequence against a protein sequence database.	Find identical (homologous) proteins
BLASTX	DNA vs. Protein. Compares a nucleotide query sequence translated in all reading frames against a protein sequence database.	Find protein databases using a translated nucleotide query
TBLASTN	Protein vs. DNA. Compares a protein query sequence against a nucleotide sequence database dynamically translated inall reading frames.	Find genes in unknown DNA sequences
TBLASTX	DNA vs. DNA. Compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.	Find degree of homology between the coding region of the query sequence and known genes in the database.

minimizes the costs provided by a certain distance function. It is used to produce conserved regions between the two sequences, and one can align two partially overlapping sequences. Also, it is able to align the subsequence of the sequence to itself. This powerful dynamic programming approach was designed to discover the highly preserved fragments by discarding poorly conserved initial and terminal segments.

In this algorithm, a two-dimensional scoring matrix D of size $(m+1) \times (n+1)$ is formed from the two provided nucleotide, RNA, or protein sequences A and B of lengths n and m respectively. One extra column and one row containing zeros are added to the matrix, for score computation. The score in each cell is computed based on the scoring function presented in Equation 2.2,

$$D(i,j) = \max \begin{cases} D(i-1, j-1) + S(A_i, B_j), \\ D(i, j-1) - \text{gap penalty}, \\ D(i-1, j) - \text{gap penalty}, \\ 0. \end{cases} \tag{2.2}$$

where $D(i, j)$ is the value at line i and column j of the scoring matrix of A_i and B_j. The value $S(A_i, B_j)$ is provided by a standard substitution matrix, like those detailed in Section 2.1.3.

To sum up, the main algorithm steps are thus (the second step will consume the most significant part of the total calculation time) :

1. Initialize the matrix.
2. Being at position i in the first sequence A and at position j in the second one B, calculates the mutation score, and fill the matrix with the appropriate optimal value in $D(i, j)$.
3. Once the matrix is filled, trace back the optimal path within this matrix, to find the proper alignment.

Smith-Waterman is a tad more useful for tasks such as locating the difference between DNA sequences, because usually, a researcher is more interested in the change in the sequence of the gene, allowing them to determine the variation over time. For more details on the Smith-Waterman algorithm, see [28], or [29] for an improved version.

2.2.3/ GLOBAL SEQUENCE ALIGNMENT : THE NEEDLEMAN WUNSCH EXAMPLE

In global alignments, the alignment is carried out from the beginning until the end of the sequence to find out the best potential solution, which is more appropriate with

closely related sequences that have approximately the same length. S.A.Needleman and C.D.Wunsch [30] have developed the first method of this category, naturally called the Needleman-Wunsch algorithm. The objective of this latter is to maximize the number of matches between the sequences along the entire length of the sequences. The original Needleman-Wunsch algorithm computes the minimal edit distance of two sequences under a very general scoring scheme, taking $O(n^3)$ time and $O(n^2)$ space.

This algorithm is constituted by the following steps, similar to the Smith-Waterman ones :

— **Initialization :** A two-dimensional matrix D must be firstly initialized. The row vector represents the first sequence A, while the column one corresponds to the second sequence B.

— **Matrix scoring :** We fill the matrix D in the same manner than in Smith-Waterman. $D(i, j)$ is computed recursively according to a dynamic programming approach. If $S(i, j)$ is the substitution score for residues A_i and B_j and g is the gap penalty, then we have :

$$D(i,j) = \max \begin{cases} D(i-1, j-1) + S(A_i, B_j) \text{ match } A_i \text{ with } B_j \\ D(i-1, j) - g(\text{insertion in A}) \\ D(i, j-1) - g(\text{insertion in B}) \end{cases} \tag{2.3}$$

— **Traceback and alignment :** Tracing back process starts from the lowest right position in the scoring matrix. We then follow the maximum scores until reaching the upper left position. The path drawn in this matrix is considered to correspond to the most optimal global alignment for the two given sequences.

The main differences between Needleman-Wunsch and Smith-Waterman algorithms are :

— The zero condition : in Smith-Waterman, we insert a 0 in the cell i, j if $D_{i,j}$ is negative, which is not the case in the Needleman-Wunsch case.

— Sequences in scoring matrix are ordered in an opposite direction.

2.2.4/ Multiple Sequence Alignment (MSA)

Multiple sequence alignment is an expansion of pairwise alignment to combine more than two sequences at a time. They are implemented to identify conserved regions among a set of sequences, evaluating by doing so if they are evolutionarily related. Alignments are also used to help in building evolutionary relationships on phylogenetic trees construction, as described in the next section.

The goal of MSA is to align all of the sequences in a given set if possible. A MSA is thus a collection of three or more nucleotide or amino acid sequences that are aligned partially or entirely. Identical residues are aligned in columns across the length of the sequences. These aligned residues are homologous in a fundamental sense or even in an evolutionary sense : they are probably derived from a common ancestor.

Figure 2.5 is an example of the result of MSA applied on *Apiales* order. However, as soon as the sequences exhibit some divergence, the problem of multiple alignments becomes extraordinarily difficult to solve. And if exact approaches produce optimal alignments, they are not feasible in time or space for more than a few sequences. Let us finally notice that

the alignment accuracy can be hard to estimate and their actual biological significance can be ambiguous.

In practice, a very popular progressive sequence alignment tool is the Clustal family [31], in particular the weighted variant ClustalW that is incorporated in many web tools like GenomeNet[3] or EBI[4]. Another important progressive alignment approach is called T-Coffee [32], which operates as a post processing on various MSAs of the same set of sequences that are provided by other existing methods like Clustal. Due to its principle of conception, T-Coffee is slower than Clustal and its derivatives but, in general, it yields more accurate alignments for distantly related sequence sets.

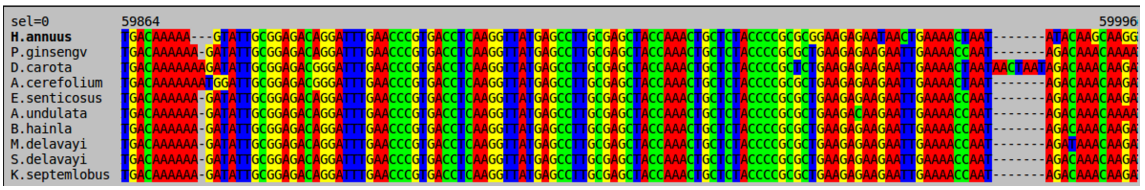


Fig. 2.5 – Multiple sequence alignment of various sequences of *Apiales* order.

2.3/ About phylogenetic trees

An evolutionary or phylogenetic tree is an acyclic graph (or branching diagram, see Figure 2.6) that is used to emphasize evolutionary relationships among groups of biological species, that is, their phylogeny based upon similarities and variations in their genetic or physical characteristics. The nodes are connected in the tree by branches. The latter highlight the relationship between taxonomic units (TU) at the leaves of the tree and their ancestors, corresponding to the internal nodes of the graph. Each branch has a length that represents, for example, the number of expected mutations per site (in amino-acids or nucleotide sequences) that have probably happened in these branches, in a sequence based phylogeny. Thus, branch lengths provide the time of variation between two organisms and their common ancestor. There are basically two kinds of phylogenetic trees :

— **The unrooted Phylogenetic Trees :** evaluate the relationships between all the given TUs. However, they usually do not provide sufficient information to deduce the evolution from the last common ancestors.

— **The rooted Phylogenetic Trees :** embed a root node that represents the last common ancestor of all TUs in the tree. The main way to root a tree is to specify an *outgroup*, which is a TU known to be outside the group of TUs under consideration. This latter can be a species known to have diverged before the divergence of the considered TUs.

It can be noticed that the time of evolution of a rooted species represented by a rooted phylogenetic tree can be computed from each sub-ancestor to the last common one when either the date of divergence or the divergence rate are known. Until now, however, this question is still an intensive subject of research.

3. http://align.genome.jp/
4. http://www.ebi.ac.uk/clustalw

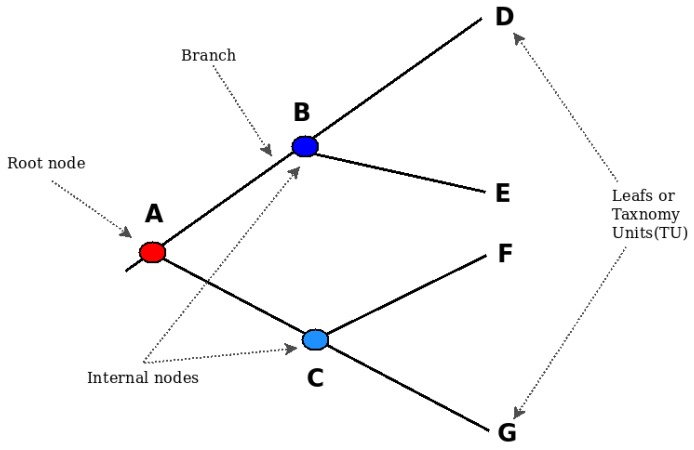


Fig. 2.6 – Example of a phylogenetic tree structure.

2.4/ PHYLOGENY CONSTRUCTION METHODS

The most known and commonly used methods of tree construction can be classified into two central divisions : *distance-based* and *character-based* methods.

Distance-based methods begin by transforming the original data into a matrix of pairwise distance values. The next stage is to infer a tree either by sequential joining approaches, or by estimating a set of candidate trees and applying a type of optimality criterion technique to select the best one. Under the minimum evolution criterion, the tree that has a minimum sum of branch lengths is selected as the best estimate. Distance-based algorithms encompass *UPGMA* and *Neighbor-Joining*, this latter being explained in the next subsection.

Character-based methods can depend on a divergence of phylogenetic characters such as genetic and molecular attributes to construct phylogenetic trees. As long as that there is divergence among taxa in the characteristic and that the characteristic is heritable, it could probably be accepted as a phylogenetic character. In this context, molecular phylogenetics, attempt to estimate the modification rates and patterns occurring in the sequences (protein, DNA, or RNA) and to reconstruct the evolutionary history of organisms using such characters.

Algorithms used to create phylogenetic trees using characters are more complicated than distance-based methods [33]. The algorithms are based on an optimization criterion such as *Maximum Likelihood*, *Maximum Parsimony*, or *Bayesian* methods in order to find the best tree according to the considered characters. For the sake of illustrations, we will detail such methods at the end of this chapter.

2.4.1/ NEIGHBOR-JOINING ALGORITHM

As previously said, the neighbor-joining algorithm constructs unrooted phylogenetic trees using distance methods. Both topology and branch lengths are computed by iteratively specifying (based on a distance matrix) a neighbor as a pair of TUs that are joined in a single internal node X in an unrooted tree, depending on the previously computed distance

matrix. An iteration of the neighbor-joining algorithm consists of the following steps :

1. Construct an unresolved tree with all TUs in a starlike structure with no hierarchy.
2. Construct a distance matrix by pairwise comparison and calculate the value of branch lengths, to identify the two most related sequences (TUs) : NJ seeks to build a tree which minimizes the sum of all branch lengths.
3. Determine which TUs are connected to an internal node X. They are treated now as one TU.
4. Join the closest neighbors (TUs with similar characters), the base pair that has the smallest sum-of-branch-lengths.
5. The algorithm is repeated until the topology of the tree is obtained.

The neighbor-joining method produces an unrooted tree. The sum of the branch lengths of N TUs in the tree is calculated as follows. Let us define D_{ij} and L_{AB} as the distance between TUs i and j and the branch length between nodes a and b respectively. The sum of branch lengths of the tree is defined based on the following formula :

$$S = \sum_{i=1}^{N} L_{iX} = \frac{1}{N-1} \sum_{i<j} D_{ij/}$$

The distance between nodes X and Y is calculated as follows :

$$L_{XY} = \frac{1}{2(N-2)} \left[\sum_{k=3}^{N} (D_{1k} + D_{2k}) - (N-2)(L_{1X} + L_{2X}) - 2 \sum_{i=3}^{N} L_{iY} \right]$$

The term inside the brackets is the sum of all distances including L_{XY}, and the outer term $\frac{1}{2(N-2)}$ is to eliminate unrelated branch lengths.

Neighbor-joining [34] is a method which is especially suited for datasets comprising lineages with broadly varying rates of evolution. It can be used in combination with techniques that allow correction for superimposed substitutions.

2.4.2/ Maximum Parsimony

The Maximum parsimony method [35] aims at minimizing branch lengths by reducing the number of mutations. This approach predicts the evolutionary tree that minimizes the number of actions needed to generate the marked variation in the sequences from common ancestral sequences. In a maximum parsimony phylogenetic study, the best tree is specified as the tree with the lowest branch lengths. More precisely, for given sequences, a MSA algorithm is used to align the sequences, and to identify the informative positions, that is, columns in the multiple sequence alignment with no gap and at least two characters.

The next step is to count the number of changes and assign this cost to each generated phylogenetic tree. The method then computes the total length L for each tree, which is calculated according to the following formula :

$$L = \sum_{j=1}^{C} w_j l_j$$

where l_j is the cost for character j, C is the total number of characters, and w_i is the assigned weight for each character, which is set to 1 in most cases. The tree that maximizes this L value is finally selected.

2.4.3/ BAYESIAN METHODS

For the sake of completeness, we evoke here the well-known and frequently used Bayesian methods [36], which estimate the phylogeny by calculating the conditional probability given the model, based on the following formula :

$$Pr[Tree|Data] = \frac{\Pr[Data|Tree] \times \Pr[Tree]}{\Pr[Data]}$$

where $Pr[Tree|Data]$ is called a posterior probability distribution [5].

Bayesian methods can thus apply a model of sequence evolution and are ideal for building a phylogeny using sequence data.

2.4.4/ MAXIMUM LIKELIHOOD

The Maximum Likelihood (ML) criteria requires a probabilistic model for the evolutionary process and finds the most likely tree, given the probabilistic model and the known sequences at the leaves. In other words, ML techniques are used to determine the topology and branch lengths that have the largest likelihood to produce the aligned data, providing the substitution model and the tree. The likelihood value is computed after the alignment stage and by considering some DNA or amino acids substitution models.

In ML method, the searching space is fulfill using a quartet program. This latter finds all possible sequence combinations for tree reconstruction, while the Maximum Parsimony criteria prefers solutions that minimize the number of mutations along the tree edges [37]. Bayesian methods and Maximum likelihood can apply a model of sequence evolution and are ideal to construct a phylogeny using data sequences. The main drawback of these methods is that they are computationally expensive. However, with today's computers, this is not too much a problem.

One of the most common tests used to evaluate the reliability of a deduced tree is the so-called Felsenstein's bootstrap test [38], which is usually estimated using Efron's bootstrap resampling technique [39]. It is accomplished in practice by sampling the input data [40] and measuring the proportion of deduced trees that support each branch of the best tree previously obtained. As a global rule, if the bootstrap value for a given internal branch is 95% or higher, the topology will be considered "valid" at that branch [6].

2.4.5/ ANCESTRAL GENOME RECONSTRUCTION

Ancestral reconstruction may focus at sequence level or at gene order level, the former being quite resolved [41, 8, 42, 43, 6, 13, 10, 44, 45], at least if we do not consider

5. A posterior probability is the probability that the tree is considered to be correct, if it has the maximum probability.

6. In Bayesian approaches, this is the posterior probability itself that gives an evaluation of robustness : the support of a branch increases with its probability.

indels and mutation neighborhood, while the latter is more difficult in general, due to its combinatorial complexity. More precisely, given an alignment of DNA sequences and a tree, ancestral nucleotides of extant species can be obtained by modeling the evolution of a trait through time as a stochastic process (Markov chain). Using it as the basis for statistical inference, both maximum likelihood or Bayesian inference approaches can be applied to estimate ancestral configuration.

Well known software like RAxML [46], BEAST2 [47], or PAML [48] can be used for such reconstructions. However, most of the time, like in the R package [49], indels are not considered in such ancestral state reconstruction, even if researches have recently been realized via the so-called “Poisson Indel Process” [50]. Such process is a significant improvement, if we compare it with the parsimony approach that can be found in PHAST software, or with the Thorne-Kishino-Felsenstein model of indel evolution. Large scale modifications, for its part, is most of the time regarded in a combinatorial framework by modeling genomes as permutations of genes or homologous regions. Indeed, this genome rearrangement problem [51] is usually formulated as follows : “given two genomes (permutations) and a set of allowable operations (like inversion, deletion, or transposition), what is the shortest sequence of operations that will transform one genome into the other ?”. As stated previously, even in the case of three genomes, such a problem is NP-hard [52], although it has received much attention in mathematics and computer science [53].

III

CONTRIBUTIONS

3

Comparison of Metaheuristics to Measure Gene Effects on Phylogenetic Supports and Topologies

A huge and continuous increase in the number of completely sequenced chloroplast genomes, available for evolutionary and functional studies in plants, has been observed during the past years. Consequently, it appears possible to build large-scale phylogenetic trees of plant species. However, building such a tree that is well-supported can be a difficult task, even when a subset of close plant species is considered. Usually, the difficulty raises from a few core genes disturbing the phylogenetic information, due for example to homoplasy problems. Fortunately, a reliable phylogenetic tree can be obtained once these problematic genes are identified and removed from the analysis. Therefore, in this chapter we address the problem of finding the largest subset of core genomes which allows to build the best supported tree. As an exhaustive study of all core genes combination is redhibitory, since the combinatorics of the situation made it computationally infeasible, we investigate three well-known metaheuristics to solve this optimization problem. More precisely, we design and compare distributed approaches using genetic algorithm, particle swarm optimization, and simulated annealing. The last approach is a new contribution and therefore described in details, whereas the two former ones have been already studied in a previous PhD/thesis. They have been designed *de novo* in a new platform, and new experiments have been achieved on a larger set of chloroplasts, to compare together these three metaheuristics. Finally, the ways genes affect both tree topology and supports are assessed using statistical tools like Lasso or dummy logistic regression, in an hybrid approach of the genetic algorithm. This chapter is the concatenation of a previous work published in 2015 [54] with Bassam Al Kindy, a former PhD student at the University of Franche-Comté.

3.1/ Introduction

These last years, the investigation of the evolutionary relationship between different plants has benefited from the multiplication of newly available chloroplast sequences. Indeed, thanks to the tools presented in the previous chapter of this thesis, it is possible to process

these sequences in order to build a phylogenetic tree that accurately characterizes the evolutionary lineages among the chloroplasts. Efficient coding sequence prediction and annotation tools have been developed to deal specifically with chloroplasts, for example DOGMA [55], and as can already been explained, there is also a great choice for the alignment of sequences. Moreover, given a set of sequences or characters, many well-established bioinformatics programs based on Bayesian inference or maximum likelihood, like BEAST or RAxML [46], can be used to reconstruct a phylogenetic tree. The objective is to obtain the most reliable and robust phylogeny, for instance in order to perform ancestral analysis with a high confidence level. As stated previously, several methods can be used to estimate the robustness of the produced tree, the most widely used are the bootstrap and the decay (or Bremer) analyses.

Obviously, a first condition to be able to build a phylogenetic tree for a given set of close plant species is to identify as precisely as possible the corresponding core genome (the set of genes in common). However, even if the core genome is large and accurate, the resulting phylogeny is not necessarily well-supported. In fact, the core genome genes are not constrained through evolution in a similar way. On the one hand some evolve under strong evolutionary constraints and thus reflect the story of the species while, on the other hand, other genes evolve more freely due to a lower role in the survival and adaptability of a species. The latter tell their own history and thus disturb the phylogenetic information. Furthermore, the way the robustness and accuracy of the obtained phylogenetic tree are altered by the amount of used data for the reconstruction process is not completed understood. Nevertheless, if we consider a set of species reduced to lists of gene sequences, an obvious dependence between the chosen subset of sequences and the obtained tree (topology, branch length, and/or robustness) can be observed. This dependence is usually regarded by the mean of gene trees merged in a phylogenetic network. In fact, phylogenetic networks are necessary to represent events like horizontal gene transfers, but statistical methods to infer such networks are still limited and under development.

In this chapter, we consider the situation from a dual point of view, that consists in starting with the complete core genome and then to remove the genes responsible for inconsistent phylogenetic signal. In other words, the objective is to find the largest part of the core genome that produces a phylogenetic tree as supported as possible, and which therefore gives the fairest view of the relationships between most of the sequences under consideration. Searching the problematic genes by exhaustively testing the combinations of core genome genes is nonsense due their huge number. Therefore, to speed up the finding of a satisfactory combination we rather consider metaheuristics. The first one, introduced in a previous work [1], is an ad hoc Genetic Algorithm (GA) which in some cases is not able to converge towards a suitable solution. Next, a Binary Particle Swarm Optimization (PSO) approach has been published in the the CIBB proceedings book [56]. Finally, in this chapter, which extends and improves the two former ones, we study the relevance of the Simulated Annealing (SA) algorithm to fulfill the optimization task. Also notice that the different metaheuristics have been executed in a distributed manner using supercomputing facilities. To sum up, the contribution of this chapter is threefold : first, it proposes a new simulated annealing approach, second a new version of the PSO, and third a comparison of the three metaheuristics on a large number of new groups of species.

3.2/ PRESENTATION OF THE PROBLEM

Let us introduce the problem of determining a phylogeny (evolution tree) for a given set of species by considering a set of chloroplast genomes that have been annotated using DOGMA [55] (the approach we applied is detailed in Section 3.6). To start we need to pick one or several genes on which the phylogeny will be based. Therefore we use the restricted core genome [57, 58], which consists of conserved genes present everywhere, whose size is larger than one hundred genes when the species are close enough. Then multiple sequence alignments are performed using muscle [59] and finally a phylogenetic tree is inferred thanks to the maximum-likelihood tree builder RAxML [46].

The relevance of the obtained tree is then assessed by its bootstrap values : if these ones are all above 95 the tree is well-supported. In this case we can reasonably estimate that the phylogeny of these species is solved. Bootstrapping is a random sampling technique commonly used to estimate the significance of branches of a phylogenetic tree. It consists to randomly select columns in the aligned DNA core sequences to be neglected during the tree building process and to check whether the same nodes are recovered. A large number of bootstrap repetitions, usually between 50 and 1000, are used to assess the tree reliability. As an illustration, a node which appears 95 times out of 100 by dropping a column means that the node is well-supported. Conversely, a low support value claims that a reduced part of the alignment supports the node, since by removing columns the node is reconstructed in different ways.

When such a well-supported tree is not built, but rather a tree having some branches exhibiting low supports, some genes of the core genome can be responsible of this lack of support. The objective is then to identify the most supported tree using the largest subset of core genes, a typical optimization problem. Obviously, the optimization problem we face cannot be solved by a brute force approach checking all possible combination of genes, due to the resulting combinatorial explosion. Indeed, for a core genome of n genes there would be 2^n trees to infer and that is clearly intractable in practice. To overcome such a combinatorial situation, a typical choice is to use a metaheuristic method.

In [1], we have first investigated the mixing of a genetic algorithm with Lasso tests to find problematic genes. Unfortunately, thorough and careful experimental investigations have led to results, recalled in Table 3.1, showing that this proposal is not able to predict the phylogeny of some particular plant orders. As can be seen, the lowest bootstrap value (or bootstrap score) obtained for 15 group of species is below 95 (column b in the table). The relevance of binary particle swarm optimization to find the largest subset of core genes has been studied in [56], producing slightly better bootstrap scores than GA with Lasso tests. In this chapter we introduce a third well-known metaheuristic method, namely simulated annealing, and we compare the three approaches considering new sets of species. Like the two former ones, the computations with SA algorithm will be done in a distributed manner. Multiple algorithm instances will be launched using a same cooling schedule and at the end of each Markov chain, for a same temperature, a centralized communication scheme will take place.

To sum up, Figure 3.1 gives an overview of the proposed pipeline to obtain the ancestral history of a set of species.

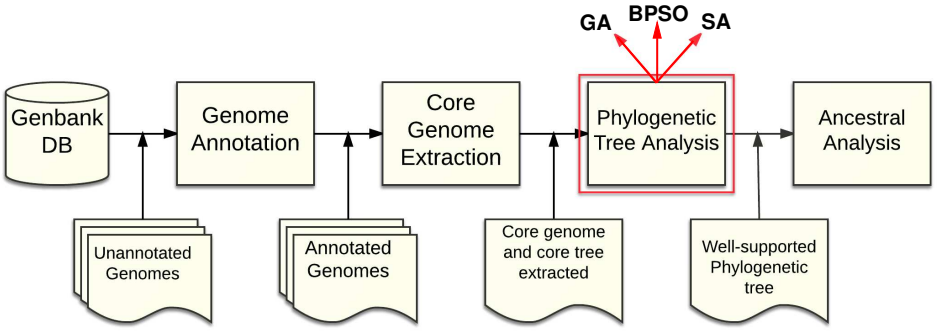


Fig. 3.1 – Overview of the proposed pipeline.

TABLE 3.1 – Results of genetic algorithm approach on various families.

Group	occ	c	# taxa	b	Terminus	Likelihood	Outgroup
Gossypium_group_0	85	84	12	26	1	-84187.03	*Theo_cacao*
Ericales	674	84	9	67	3	-86819.86	*Dauc_carota*
Eucalyptus_group_1	83	82	12	48	1	-62898.18	*Cory_gummifera*
Caryophyllales	75	74	10	52	1	-145296.95	*Goss_capitis-viridis*
Brassicaceae_group_0	78	77	13	64	1	-101056.76	*Cari_papaya*
Orobanchaceae	26	25	7	69	1	-19365.69	*Olea_maroccana*
Eucalyptus_group_2	87	86	11	71	1	-72840.23	*Stoc_quadrifida*
Malpighiales	422	78	10	96	3	-91014.86	*Mill_pinnata*
Pinaceae_group_0	76	75	6	80	1	-76813.22	*Juni_virginiana*
Pinus	80	79	11	80	1	-69688.94	*Pice_sitchensis*
Bambusoideae	83	81	11	80	3	-60431.89	*Oryz_nivara*
Chlorophyta_group_0	231	24	8	81	3	-22983.83	*Olea_europaea*
Marchantiophyta	65	64	5	82	1	-117881.12	*Pice_abies*
Lamiales_group_0	78	77	8	83	1	-109528.47	*Caps_annuum*
Rosales	81	80	10	88	1	-108449.4	*Glyc_soja*
Eucalyptus_group_0	2254	85	11	90	3	-57607.06	*Allo_ternata*
Prasinophyceae	39	43	4	97	1	-66458.26	*Oltm_viridis*
Asparagales	32	73	11	98	1	-88067.37	*Acor_americanus*
Magnoliidae_group_0	326	79	4	98	3	-85319.31	*Sacc_SP80-3280*
Gossypium_group_1	66	83	11	98	1	-81027.85	*Theo_cacao*
Triticeae	40	80	10	98	1	-72822.71	*Loli_perenne*
Corymbia	90	85	5	98	2	-65712.51	*Euca_salmonophloia*
Moniliformopses	60	59	13	100	1	-187044.23	*Prax_clematidea*
Magnoliophyta_group_0	31	81	7	100	1	-136306.99	*Taxu_mairei*
Liliopsida_group_0	31	73	7	100	1	-119953.04	*Drim_granadensis*
basal_Magnoliophyta	31	83	5	100	1	-117094.87	*Ascl_nivea*
Araucariales	31	89	5	100	1	-112285.58	*Taxu_mairei*
Araceae	31	75	6	100	1	-110245.74	*Arun_gigantea*
Embryophyta_group_0	31	77	4	100	1	-106803.89	*Stau_punctulatum*
Cupressales	87	78	11	100	2	-101871.03	*Podo_totara*
Ranunculales	31	71	5	100	1	-100882.34	*Cruc_wallichii*
Saxifragales	31	84	4	100	1	-100376.12	*Aral_undulata*
Spermatophyta_group_0	31	79	4	100	1	-94718.95	*Mars_crenata*
Proteales	31	85	4	100	1	-92357.77	*Trig_doichangensis*
Poaceae_group_0	31	74	5	100	1	-89665.65	*Typh_latifolia*
Oleaceae	36	82	6	100	1	-84357.82	*Boea_hygrometrica*
Arecaceae	31	79	4	100	1	-81649.52	*Aegi_geniculata*
PACMAD_clade	31	79	9	100	1	-80549.79	*Bamb_emeiensis*
eudicotyledons_group_0	31	73	4	100	1	-80237.7	*Eryc_pusilla*
Poeae	31	80	4	100	1	-78164.34	*Trit_aestivum*
Trebouxiophyceae	31	41	7	100	1	-77826.4	*Ostr_tauri*
Myrtaceae_group_0	31	80	5	100	1	-76080.59	*Oeno_glazioviana*
Onagraceae	31	81	5	100	1	-75131.08	*Euca_cloeziana*
Geraniales	31	33	6	100	1	-73472.77	*Ango_floribunda*
Ehrhartoideae	31	81	5	100	1	-72192.88	*Phyl_henonis*
Picea	31	85	4	100	1	-68947.4	*Pinu_massoniana*
Streptophyta_group_0	31	35	7	100	1	-68373.57	*Oedo_cardiacum*
Gnetidae	31	53	5	100	1	-61403.83	*Cusc_exaltata*
Euglenozoa	29	26	4	100	3	-8889.56	*Lath_sativus*

3.3/ PHYLOGENETIC PREDICTIONS USING METAHEURISTICS

3.3.1/ BINARY PARTICLE SWARM OPTIMIZATION

Particle Swarm Optimization (PSO) [60] is a stochastic optimization technique which has been successfully applied on various problems like function optimization, artificial neural network training,.... In this method, each particle has to learn from the success of neighboring individuals. An emergent behavior enables individual swarm members, particles, to learn from the discoveries, or from previous experiences, of the other particles that have obtained the most accurate solutions.

In the case of the standard binary PSO model [61], the particle position is a vector of N binary parameters. Next, a score (real number) is associated to each vector according to the optimization problem. The approach defines then how to move the particles in the N dimensional binary search space so that the produced optimal binary vector with respect to the highest score.

In more details, each particle i is represented by a binary vector X_i whose length N is the dimension of the search space. A 1 in coordinate j of this vector means that the associated j-th parameter is selected. A swarm of n particles is then a list of n vectors of positions $(X_1, X_2, \ldots, X_n)$ together with their associated velocities $V = (V_1, V_2, ..., V_n)$, which are N-dimensional vectors of values in $[0, 1]$ and which are initially randomly set. At each iteration, the velocity vector is updated as follows :

$$V_i(t+1) = wV_i(t) + \phi_1 \left(P_i^{best} - X_i\right) + \phi_2 \left(P_g^{best} - X_i\right) \tag{3.1}$$

where w, ϕ_1, and ϕ_2 are weighted parameters setting the level of each three trends for the particle, which are respectively to continue in its adventurous direction, to move in the direction of its own best position P_i^{best}, or to follow the gregarious instinct to the global best known solution P_g^{best}. Both P_i^{best} and P_g^{best} are computed according to the scoring function.

The position of the particle is then updated as follows :

$$X_{ij}(t+1) = \begin{cases} 1 & \text{if } r_{ij} \leq \mathit{Sig}(V_{ij}(t+1)), \\ 0 & \text{otherwise,} \end{cases} \tag{3.2}$$

where r_{ij} is a threshold that depends on both the particle i and the parameter j, while the *Sig* function is the sigmoid one [61], that is :

$$\mathit{Sig}(V_{ij}(t+1)) = \frac{1}{1 + e^{-V_{ij}(t+1)}} \tag{3.3}$$

Let us now recall how the BPSO approach has been used to solve our optimization problem related to phylogeny [56].

3.3.1.1/ BPSO APPLIED TO PHYLOGENY

The *Rosales* order, which has already been analyzed in [1] using a hybrid genetic algorithm and Lasso test approach has been retained here also. The *Rosales* order is constituted by 9 ingroup species and 1 outgroup (*Mollissima*), as described in Table 3.2. They have been annotated using DOGMA and their core genome has been computed according to the

method described in [57, 58]. Its size is equal to 82 genes. Unfortunately, the phylogeny cannot be resolved directly neither by considering all these core genes nor by considering any of the 82 combinations of 81 core genes.

TABLE 3.2 – Genomes information of *Rosales* species under consideration

Species	Accession	Seq.length	Family	Genus
Chiloensis	NC_019601	155603 bp	*Rosaceae*	*Fragaria*
Bracteata	NC_018766	129788 bp	*Rosaceae*	*Fragaria*
Vesca	NC_015206	155691 bp	*Rosaceae*	*Fragaria*
Virginiana	NC_019602	155621 bp	*Rosaceae*	*Fragaria*
Kansuensis	NC_023956	157736 bp	*Rosaceae*	*Prunus*
Persica	NC_014697	157790 bp	*Rosaceae*	*Prunus*
Pyrifolia	NC_015996	159922 bp	*Rosaceae*	*Pyrus*
Rupicola	NC_016921	156612 bp	*Rosaceae*	*Pentactina*
Indica	NC_008359	158484 bp	*Moraceae*	*Morus*
Mollissima	NC_014674	160799 bp	*Fagaceae*	*Castanea*

As some branches are not well supported, we can wonder whether a few genes can be incriminated in this lack of support, for a large variety of reasons encompassing homoplasy, stochastic errors, undetected paralogy, incomplete lineage sorting, horizontal gene transfers, or even hybridization. If so, we face the optimization problem presented previously : *find the most supported tree using the largest subset of core genes*.

Genes of the core genome are now supposed to be lexicographically ordered. Each subset S of the core genome is thus associated with a unique binary word w of length n : for each i, $1 \leq i \leq n$, w_i is 1 if the i-th core gene is in S and 0 otherwise. Any n-length binary word w can be associated with its percentage p of 1's and the lowest bootstrap b of the phylogenetic tree we obtain when considering the subset of genes associated to w. Each word w is thus associated with a fitness score value $\mathcal{F} = \frac{b+p}{2}$.

In the BPSO context the search space is then $\{0,1\}^N$, where $N = 82$ in *Rosales*. Each node of this N-cube is associated with the set of following data : its subset of core genes, the deduced phylogenetic tree, its lowest bootstrap b and the percentage p of considered core genes, and, finally, the score $\frac{b+p}{2}$. Notice that two close nodes of the N-cube have two close percentages of core genes. We thus have to construct two phylogenies based on close sequences, leading with a high probability to the same topology with close bootstraps. In other words, the score remains essentially unchanged when moving from a node to one of its neighbors. It allows to find optimal solutions using approaches like BPSO.

During swarm initialization, the L particles (set to 10 in our experiments) of a swarm are randomly distributed among all the vertices (binary words) of the N-cube that have a large percentage of 1's. The objective is then to move these particles in the cube so that they will converge to an optimal node.

At each iteration, the particle velocity is updated by taking into account its own best position and the best one considering the whole particle swarm (both identified according to the fitness value). It is influenced by constant weight factors as expressed in Equation (3.1). In this one, we have set $\phi_1 = c_1 \cdot r_1$ and $\phi_2 = c_2 \cdot r_2$ where $c_1 = 1$ and $c_2 = 1$, while r_1, r_2 are random numbers belonging to [0.1,0.5], and w is the inertia weight that is computed based on the following formula :

$$w = w_{max} - \frac{w_{max} - w_{min}}{I_{\max}} \times I'_{\text{cur}} \tag{3.4}$$

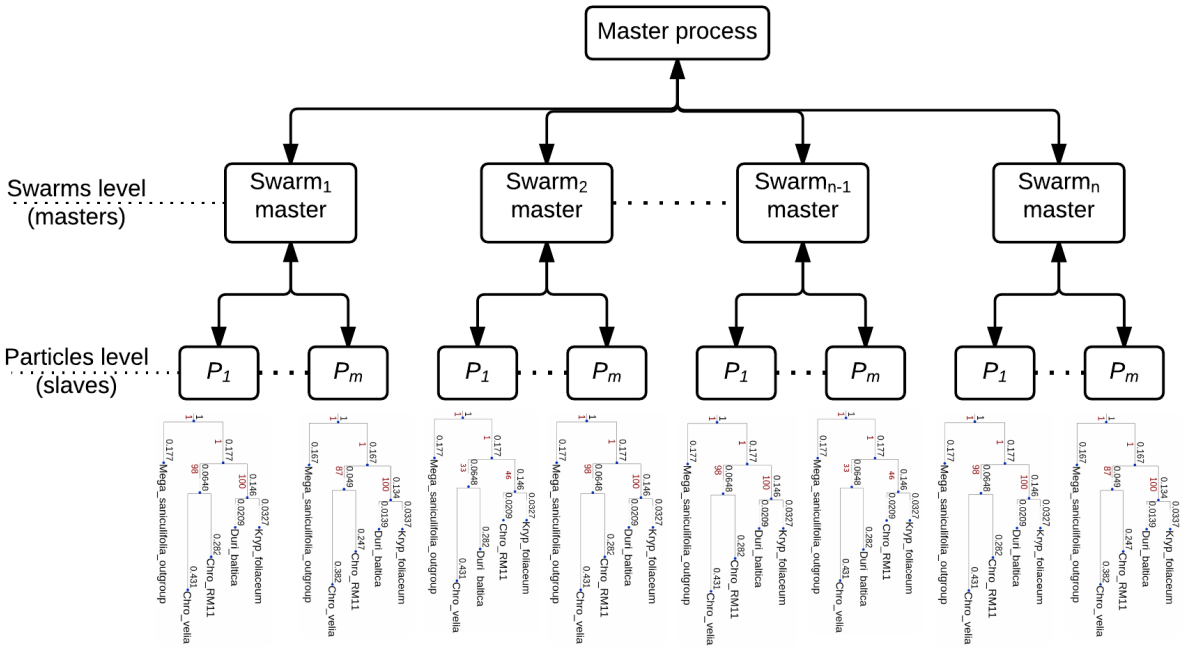


Fig. 3.2 – The distributed structure of BPSO algorithm.

where $I_{\max}$ represents the maximum number of iterations (or time step) and I'_{cur} is the current iteration. This equation determines the contribution rate of a particle's previous velocity and is determined as in [62].

To increase the number of included components in a particle, we reduce the interval of Equation (3.1) to [0.1, 0.5]. For instance, if the velocity V_{ij} of an element is equal to 0.51 and $r_{ij} = 0.83$, then $Sig(0.51) = 0.62$. So $r_{ij} > Sig(V_{ij})$ and this leads to 0 in the vector element j of the particle i. By minimizing the interval, we increase the probability of having $r_{ij} < Sig(V_{ij})$ and consequently the number of 1s, which means more included elements in the particle (a larger number of core genes).

Note that a large inertia weight facilitates a global search, while a small inertia weight tends more to a local investigation [63]. In other words, a larger value of w facilitates a complete exploration, whereas small values promote exploitation of areas. This is why Eberhart and Shi [64] suggested to decrease w over time, typically from 0.9 to 0.4, thereby gradually changing from exploration to exploitation. Finally, each particle position is updated according to Equation (3.2).

3.3.1.2/ Distributed BPSO with MPI

Traditional PSO algorithms are time consuming in sequential mode. The distributed version shown in Figure 3.2 has thus been proposed to minimize the execution time as much as possible. The general idea of the proposed algorithm is simple : a processor core is employed for each particle in order to compute its fitness value, while a last core called the master centralizes the obtained results. In other words, if we have a swarm of ten particles, we use ten cores as workers and one core as master (or supervisor).

More precisely, the master initializes the particles of the swarm and distributes them to the workers. When one worker finishes its job, it sends a "terminate" signal with the fitness

value to the master. This latter waits until all the workers have finished their jobs. Then, it determines the position of the particle that has the best fitness value as the global best position and sends this information to the workers that update their respective particle velocity and position. This mechanism is repeated until a particle achieves a fitness value larger than or equal to 95 with a large set of included genes. In the following, two distributed versions of the BPSO described previously are considered : in version I the equation used to update the velocity is slightly changed as shown below, and in version II we use the equations of Section 3.3.1.

3.3.1.3/ Distributed BPSO Algorithm : Version I

In this version Equation (3.1), which is used to update the velocity vector, is replaced by :

$$V_i(t+1) = x \cdot [V_i(t) + C_1(P_i^{best} - X_i) + C_2(P_g^{best} - X_i)] \tag{3.5}$$

where x, C_1, and C_2 are weighted parameters setting the level of each three trends for the particle.The default values of these parameters are $C_1 = c_1 \cdot r_1 = 2.05$, $C_2 = c_2 \cdot r_2 = 2.05$, while x which represents the constriction coefficient is computed according to formula [65, 66] :

$$x = \frac{2 \times k}{|2 - C - (\sqrt{C \times (C-4)})|}, \tag{3.6}$$

where k is a random value between [0,1] and $C = C_1 + C_2$, where $C \geq 4$. According to Clerc [66], using a constriction coefficient results in particle convergence over time.

3.3.1.4/ Distributed BPSO Algorithm : Version II

This version is a distributed approach of the sequential PSO algorithm presented previously in Section 3.3.1.

3.4/ Genetic algorithm

A genetic algorithm (GA) is a well-known metaheuristic algorithm which has been described by a rich body of literature since its introduction [67, 68]. In the following, we will only discuss the choices we made regarding operators and parameters. For further information and applications regarding the genetic algorithm, see for example [69, 70, 71, 72].

3.4.1/ Genotype and fitness value

Genes of the core genome are supposed again to be lexicographically ordered. At each subset s' of the core genome corresponds thus a unique binary word w of length n : for each i lower or equal to n ($i \in \{1, \ldots, n\}$), w_i is 1 if the i-th core gene is in s', else w_i is equal to 0. At each binary word w of length n, we can associate its percentage p of 1's and the lowest bootstrap b of the phylogenetic tree we obtain when considering the subset of

genes associated to w. At each word w we can thus associate the score $b + p$ as fitness value, which must be as large as possible.

3.4.2/ GENETIC PROCESS

Until now, binary words (genotypes) of length n that have been investigated are :

1. the word having only 1's (systematic mode) ;
2. all words having exactly one 0 (systematic mode) ;
3. at least 200 [1] words having between 2 and 10 (ten) 0's randomly located (random mode).

To each of these words is attached its score $b + p$. This latter is used to select the 50 best words, or fittest individuals, in order to build the initial population (see the upper part of Figure 3.3). After that, the genetic algorithm loops during 200 iterations or until discovering a word such that its score is larger than 190 (corresponding approximately to a case where at least 95% of core genes are used, which produces a tree whose bootstraps are larger than 95).

During an iteration the algorithm applies the following steps to produce a new population P' given a population P (see Figure 3.4) :

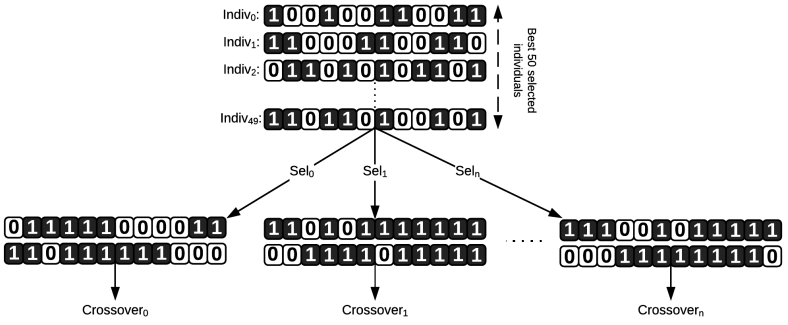


Fig. 3.3 – Random pair selections from given population.

— Repeat five times a random pickup of a couple of words and mix them using a crossover approach. The obtained words are added to the population P, as described in Section 3.4.3, resulting in population P_c.

— Mutate 5 words of the population P_c, the mutated words being added too to P_c, as detailed in Section 3.4.4, leading to population P_m.

— Add 5 new random binary words having less than 10% of 0's (see Section 3.4.5) to P_m producing population P_r.

— Select the 50 best words in population P_r to form the new population P'.

Let us now explain with more details each step of this genetic algorithm.

1. 200 is a parameter that has been specified according to our experiments : it seems to offer the best trade-off between computation time and quality of the initial population.

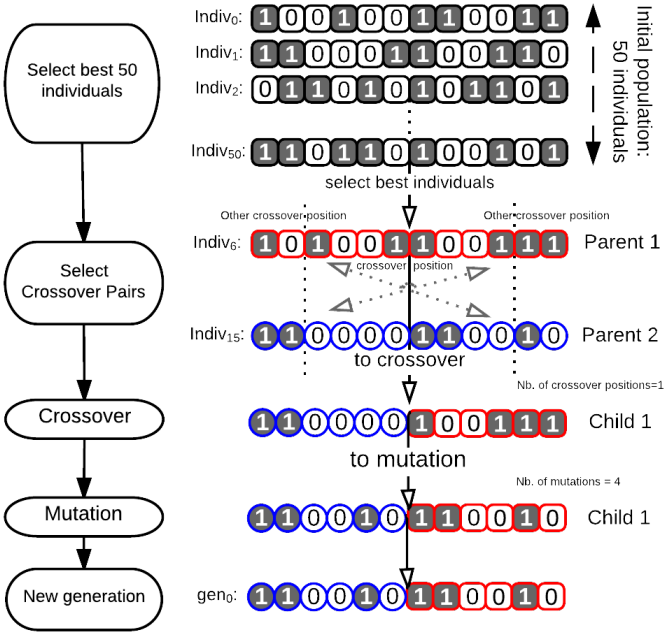


Fig. 3.4 – Outline of the genetic algorithm.

3.4.3/ Crossover step

Given two words w^1 and w^2, the idea of the crossover operation is to mix them, hoping by doing so to generate a new word w having a better score (see Figure 3.5(a)). For instance, if we consider a one-point crossover located at the middle of the words, for $i < \frac{n}{2}$, $w_i = w_i^1$, while for $i \geqslant \frac{n}{2}$, $w_i = w_i^2$: in that case, for the first core genes, the choice (to take them or not for phylogenetic construction) in w is the same than in w^1, while the subset of considered genes in w corresponds to the one of w^2 for the last 50% of core genes.

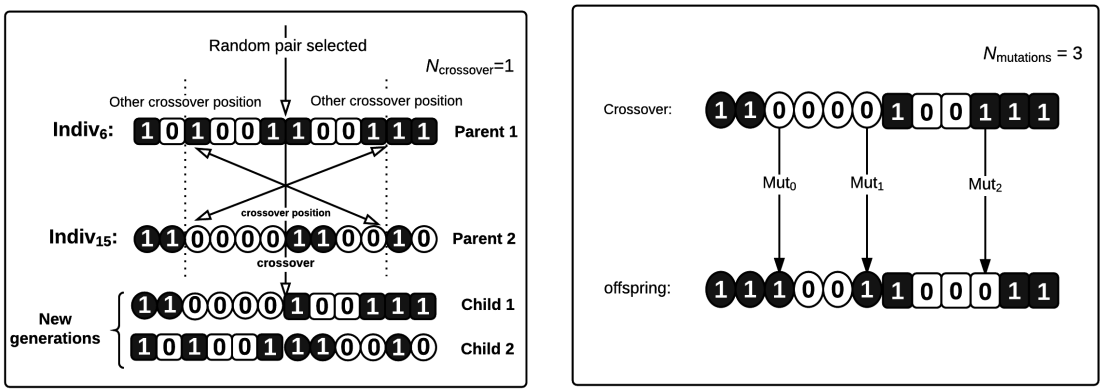
More precisely, at each crossover step, we first pick randomly an integer $N_{crossover} = k$ where $k < \frac{n}{2}$, and randomly again k different integers $i_1, \ldots i_k$ such that $1 < i_1 < i_2 < \ldots < i_k < n$. Then w^1 and w^2 are randomly selected from the population P, and a new word w is computed as follows :

- $w_i = w_i^1$ for $i = 1, ..., i_1$,
- $w_i = w_i^2$ for $i = i_1 + 1, ..., i_2$,
- $w_i = w_i^3$ for $i = i_2 + 1, ..., i_3$,
- etc.

Then the phylogenetic tree based on the subset of core genes labeled by w is computed, the score S of w is deduced, and w is added to the population with the fitness value of S attached to it. Note that, as a parametric option, one word instead of two is generated from this step.

3.4.4/ Mutation step

In this step, we ask how small changes in a given subset of genes (removing and/or adding few genes) may by chance improve the support of the associated tree. Similarly speaking,



(a) Crossover operation.

(b) Mutation operation.

Fig. 3.5 – (a) Two individuals were selected from given population. The first portion from determined crossover position in the first individual is switched with the first portion of the second individual. The number of crossover positions is determined by $N_{crossover}$. (b) Random mutations are applied depending on the value of $N_{mutation}$, changing randomly gene state from 1 to 0 or vice versa.

we try here to improve the score of a given word by replacing a few 0's by 1 and/or a few 1's by 0 as shown in Figure 3.5(b).

In practice, an integer $N_{mutation} = k$ where $k \leqslant \frac{n}{4}$ corresponding to the number of changes, or "mutations", is randomly picked. Then k different integers $i_1, \ldots, i_k$ lower or equal to n are randomly chosen and a word w is randomly extracted from the current population. A new word w' is then constructed as follows : for each $i = 1, ..., n$,

— if i in $\{i_1, \ldots, i_k\}$, then $w'_i = (w_i + 1) \mod 2$ (the gene is mutated),

— else $w'_i = w_i$ (no modification).

Again, the phylogenetic tree corresponding to the subset of core genes associated with w' is computed, and w' is added to the population together with its score.

3.4.5/ Random step

In this step, new words having a large amount of 1's are added to the population. Each new word is obtained by starting from the word having n 1s, followed by k random selection of 1s which are changed to 0, where k is an integer randomly chosen between 1 and 10. The new word is added to the population after having computed its score thanks to a phylogenetic tree inference.

3.4.6/ Genetic algorithm evaluation on a large group of plant species

The proposed pipeline has been tested with the genetic algorithm on various sets of close plant species. 50 subgroups, including on average from 12 to 15 chloroplasts species, encompassing 356 plant species, and already presented in this chapter (*c.f.* Table 3.1) have been used with our formerly published genetic algorithm. Obtained results with details are

contained too in Table 3.1. Column *Occ* represents the amount of generated phylogenetic trees from the corresponding search space for each group. The column c represents the number of core genes included within each group. The *# taxa* column is the amount of species corresponding to the considered group. b is the lowest value from bootstrap analysis. The *Terminus* column contains the termination stage for each subgroup, namely : the systematic (1), random (2), or optimization (3) stage using genetic algorithm and/or Lasso test. These stages, which have been proposed in [1], correspond to the systematic deletion of 0 or 1 gene ($N+1$ computations for N core genes), random suppression of core genes (ranging from 2 to 5 genes), and the so-called genetic algorithm on binary word populations improved by the use of a statistical test. Finally, the *Likelihood* column stores the likelihood value of the best phylogenetic tree (*i.e.*, according to the lowest bootstrap value b). A large occurrence value in this table means that the associated p-value and/or subgroup has its computation terminated in either penultimate or last pipeline stage. An occurrence of 31 is frequent due to the fact that 32 MPI threads (one master plus 31 slaves) have been launched on our supercomputing facility.

Notice that the groups in Table 3.1 can be divided in four parts :

— Groups of species stopped in systematic stage with weak bootstrap values. This is due to the fact that an upper time limit has been set for each group and/or subgroups, while each computed tree in these remarkable groups needed a lot of times for computations.

— Subgroups terminated during systematic stage with desired bootstrap value.

— Groups or subgroups terminated in random stage with desired bootstrap value.

— Finally, groups or subgroups terminated with optimization stages.

A majority of subgroups has its phylogeny satisfactorily resolved, as can be seen on all obtained trees which can be downloadable at http://meso.univ-fcomte.fr/peg/phylo. However, some problematic subgroups still remain to be investigated, which explains why the distributed BPSO is considered in the next section.

3.4.7/ First experiments on *Rosales* order

In a first collection of experiments, we have implemented the proposed BPSO algorithm on a supercomputing facility. Investigated species are the ones listed in Table 3.2. 10 swarms having a variable number of particles have been launched 10 times, with $c_1 = 1, c_2 = 1$, and w linearly decreasing from 0.9 to 0.4. Obtained results are summarized in Table 3.3 that contains, for each 10 runs of each 10 swarms : the number of removed genes and the minimum bootstrap of the best tree. Remark that some bootstraps are not so far from the intended ones (larger than 95), whereas the number of removed genes are in average larger than what is desired.

Seven topologies have been obtained after either convergence or $maxIter$ iterations. Only 3 of them have occurred a representative number of times, namely the Topologies 0, 2, and 4, which are depicted in Figures 3.7, 3.8 and 3.9 (see details in Table 3.4).

These three topologies are almost well supported, except in a few branches. We can notice that the differences in these topologies are based on the sister relationship of two species named *Fragaria vesca* and *Fragaria bracteata*, and of the relation between *Pentactina rupicola* and *Pyrus pyrifolia*. Due to its larger score and number of occurrences, we tend to select Topology 0 as the best representative of the *Rosale* phylogeny.

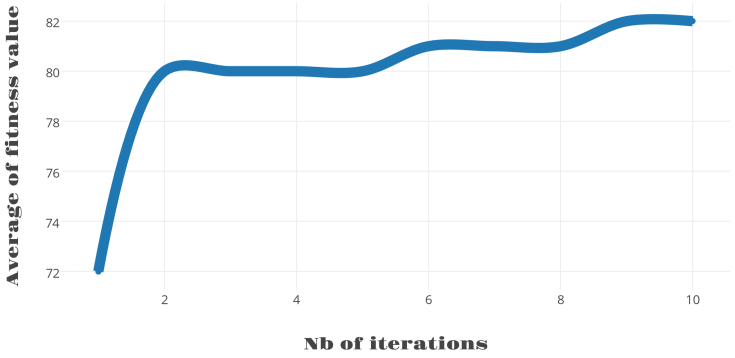


Fig. 3.6 – Average fitness of *Rosales* order

TABLE 3.3 – Best tree in each swarm

Swarm	Removed genes	$\mathcal{F}$	b
1	4	75.5	73
2	6	75.5	76
3	20	75	88
4	52	59.5	89
5	3	75.5	72
6	19	77.5	92
7	47	63.5	92
8	9	73.5	74
9	10	72.5	73
10	13	76.5	84

TABLE 3.4 – Best topologies obtained from the generated trees, b is the lowest bootstrap of the best tree having this topology, p is the number of considered genes to obtain this tree.

Topology	Swarms	b	p	$\mathcal{F}$	Occurrences
0	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	92	63	77.5	568
1	1, 2, 3, 4, 5, 6, 10	63	45	54	11
2	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	76	67	71.5	55
3	8, 1, 2, 3, 4	56	41	48.5	5
4	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	89	30	59.5	65
5	1, 3, 4, 5, 6, 9	71	33	52	9
6	5, 6	25	45	35	2

To further validate this choice, CONSEL [73] software has been used on per site likelihoods of each best tree obtained using the RAxML [46]. The CONSEL computes the p-values of various well-known statistical tests, like the so-called approximately unbiased (au), Kishino-Hasegawa (kh), Shimodaira-Hasegawa (sh), and Weighted Shimodaira-Hasegawa (wsh) tests. Obtained results are provided in Table 3.5, they confirm the selection of Topology 0 as the tree reflecting the best the *Rosales* phylogeny.

After having verified that BPSO can be used to resolve phylogenetic issues thanks to the

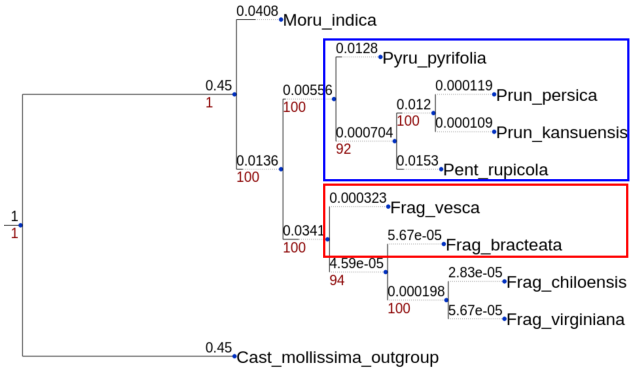


Fig. 3.7 – The best obtained topologies for *Rosales* order,Topology0

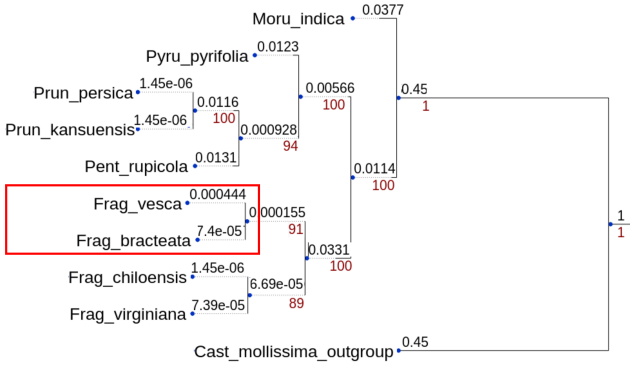


Fig. 3.8 – The best obtained topologies for *Rosales* order,Topology0

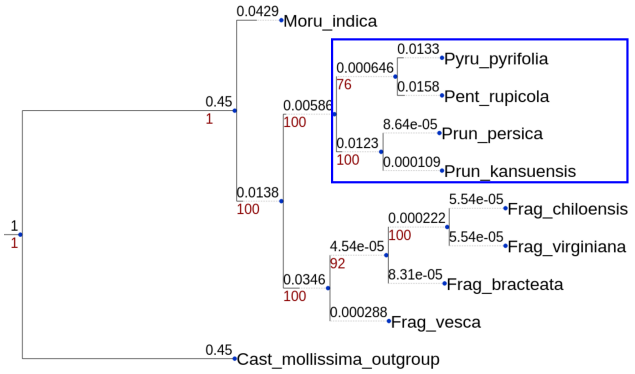


Fig. 3.9 – The best obtained topologies for *Rosales* order,Topology0

Rosales order, we now intend to deeply compare the genetic algorithm versus the swarm particle optimization. In order to do so, a large collection of group of plant species have been selected, on which we have successively launched the genetic algorithm and the BPSO one in distributed mode.

TABLE 3.5 – The CONSEL results regarding best trees

Rank	item	obs	au	np	bp	pp	kh	sh	wkh	wsh
1	0	-1.4	0.774	0.436	0.433	0.768	0.728	0.89	0.672	0.907
2	4	1.4	0.267	0.255	0.249	0.194	0.272	0.525	0.272	0.439
3	2	3	0.364	0.312	0.317	0.037	0.328	0.389	0.328	0.383

3.5/ A SIMULATED ANNEALING APPROACH

3.5.1/ GENERAL PRESENTATION

The original Simulated Annealing (SA) method is a local search based threshold class algorithm. Basically, a threshold algorithm is a loop in which a move is either done or not, according to a given criterion and until reaching a freeze [74]. Specifically, after an initialization step, this loop is composed by (a) a move in the neighborhood of the current solution, (b) an evaluation of this new position by a real-valued scoring function, then (c) a test, given a well chosen criterion, to store this position as the new best one. Various criteria can be considered. For instance, if a position is evaluated as a better solution than the best existing one, it becomes the reference solution for next iterations when the acceptation criterion is “only if best cost (score)” algorithm, which is a variant of a classical greedy local search [75]. The “all is accepted” algorithm produces, for its part, a random walk. Finally, between these two extremal situations, an acceptation criterion allows to store sometimes too positions with poorer scores than the best solution, which is an upward move via a stochastic component to avoid local minima. Such a stochastic approach facilitates theoretical analysis of asymptotic convergence. As such algorithms can be successfully used for a broad range of optimization problems, SA has been largely covered in the literature during the last decades [76, 75], for both empirical [77, 78] – typically on NP-hard problems – and theoretical perspectives [79, 75].

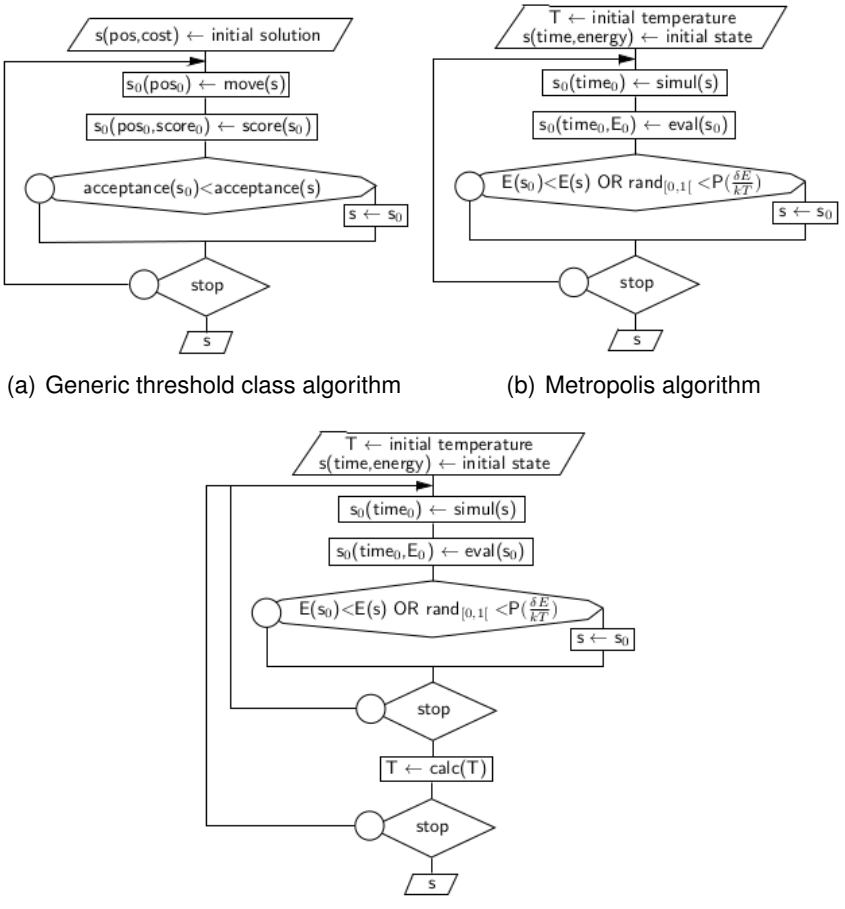
In simulated annealing, the criterion is inspired by the Metropolis-Hastings statistical (Markov chain Monte Carlo) thermodynamics algorithm [75]. SA simulates the cooling of a material in a heat bath until a steady (frozen or thermodynamic equilibrium) state. When the solid material is heated over its melting point, its solidification rate induces its structural properties. Two major antagonistic strategies are commonly used. On the one hand, after a fast cooling (quenching), the steady state is constituted by different thermodynamic free level areas. This corresponds to a local minimum for a local search, when considering energy as a score. On the other hand, after a slow cooling (annealing), almost one sole thermostatic level is expected, which corresponds to a global minimum. As feasible solutions of SA are system states, the structural proximity of the latter leads to the concept of solution neighborhood.

Thermodynamic laws show that at temperature t, the probability to increase in energy of the value δE is given by $p(\delta E) = \exp(-\delta E/kt)$ with k equal to the Boltzmann’s constant. Metropolis simulations [80] consist in the generation of a state perturbation, in the evaluation of energy modification, and finally in the decision to reject or not the new state according to the probability $p(\delta E)$. That is, the probability to keep a better (lower) level of energy is 1, while the one to keep an infinitely worst level of energy is equal to 0. Or, in other words, the likelihood to save a given state decreases as the energy level increases. A best global solution is reached by searching series of equilibria. Each equilibrium is obtained by series of Metropolis thresholds. The stop condition is typically an arbitrary duration or a number

of loop iterations. Then the temperature is decreased and the last obtained equilibrium becomes the starting state for a new series of thresholds. The final stop is triggered if no improvement has been found since an arbitrary number of equilibria.

Let us finally notice that, as a large set of temperature cooling schedules (decreasing function [81, 82]), of moving functions, of criteria, of strategies regarding initial values, of improvements on score function, of stop criteria, and even of theoretical modeling [83, 84, 75, 85] have been proposed in the literature [86, 84, 87], simulated annealing should be regarded more as a large family of algorithms than as a single one. Some members of the family including Basin Hopping [88] are themselves described as frameworks for ad-hoc global optimization algorithms.

A general overview of our proposal can be found in Figure 3.10, while algorithm details are provided hereafter.



(a) Generic threshold class algorithm (b) Metropolis algorithm

(c) Simulated annealing algorithm

Fig. 3.10 – Simulated annealing as a threshold class algorithm.

3.5.2/ Designing SA for phylogenetic studies

The objective is now to apply the simulated annealing method to find the largest subset of core genes that leads to the most supported phylogenetic tree. Intermediate computations

of subsets will help to understand, using regressions, the effects of given genes on both topology and supports. However, SA is complex to set up in practice, and finding new optima in finite time cannot be guaranteed, as related by Aarts, Korst, and van Laarhoven [74]. To enlarge the probability of success, we targeted the following requirements during our experiments :

— a concise representation for the problem under consideration ;

— a cooling schedule fitting with complexity, time, convergence, and precision considerations ;

— a moving function adapted to the state (solution) space ;

— and, similarly, an acceptation function adapted to the state space.

These four requirements are discussed hereafter.

Temperature scheduling. A criterion to increase the probability to reach convergence is the so-called logarithmic fading of control parameter (*i.e.*, temperature). The most simple choice is $t_{n+1} = C \cdot t_n$, where $C \in [0, 1]$ is a constant. However, according to our experiments, such a solution is not able to produce relevant results in the phylogenetic problem under consideration. This is why the control parameter has been updated following a tiered approach, leading to an inhomogeneous Markov model : the temperature decreases only after the end of its associated Markov chain. Additionally, near an equilibrium, the Markov chain length must increase when the control parameter decreases. But, as above, at low temperature the computation time may become prohibitive without any synchronisation between the control parameter and the Markov chain characteristics. To solve such an issue, various schedule solutions proposed in the literature link these two parameters. After having tested classical benchmarking functions like the well known three-hump camel, Levi [89], and Booth, we finally have chosen :

$$t_{n+1} = \left(\frac{t_f}{\frac{1}{t_i^{\frac{n_m - 1}{n_m - 1}}}} \right) \times t_n$$

where t is the control parameter, t_i and t_f are respectively the maximum (initial) and minimum (final) of allowed control parameter values for the SA computation, while n_m is the maximal number of Markov chains (equal to the temperature steps) allowed during computation.

About a relevant configuration of SA according to the state space. As in the other methods, the state space is constituted by Boolean vectors X_i of the form $(X_{i1}, \ldots, X_{in})$, where n is the number of core genes. X_{ij} is equal to 1 if and only if gene number j in alphabetic order is in the alignment provided to the phylogenetic tool. We thus navigate again on the n-cube on which each node (that is, each state) corresponds to a subset of core genes and has additionally a labeled value provided by the subset scoring function – which is again the average between the lowest bootstrap and the number of selected core genes. We can easily define a distance between two points inside this cube, like an Hamming distance between Boolean vectors, and the node score can be considered as the altitude of the current position.

To sum up, there is a topology on the state space, with neighborhood notion between two states, while the altitude (the score of a subset of genes, which is related to the SA

energy) is varying between two locations. Both the density and the form of energy peaks are varying through the landscape. Neighborhoods and moves, acceptation probability, temperature scheduling functions, and their related initial values are dependant on the characteristics, or the topology, of this state space. Obviously, there is no general way to set up the parameters of the simulated annealing in this situation, as usually with such heuristics. Even choosing close configurations of closely related problems like similar chloroplasts is not a guarantee of success.

Having these considerations in mind, we have stated some hypotheses at the basis of the neighboring notion. First of all, we assume that a solution is better if it is closer to the whole core genome, so improving the number of 1's in the Boolean vector is a desired trend. Secondly, we assume no correlation between genes, and so removing (or adding) one gene cannot modify so much the scoring function. As a consequence, the next investigated state should be near the previous one, in terms of Hamming distance, and most likely with a similar or larger number of active genes. In particular, moves in the state space cannot be randomized as what occurs in the original SA algorithm. Furthermore, the starting state must be the Boolean vector constituted by 1's (that is, the whole core genome), while the scoring function must preferably tend to add genes in the considered subset (if possible). With such requirements, the neighborhood function has been designed as follows :

— A number between 1 and $move\_distance_{max}$ (a parameter to set) is randomly chosen, following a Gaussian law. It corresponds to the number of coordinates that may possibly change.

— A subset of distinct coordinates are chosen accordingly, defining this move.

— For each Boolean coordinate, if the associated gene is inactive (0), it is activated (1). Otherwise, the gene is inactivated with a probability equal to $\frac{nz}{nc} \times \alpha$, where nz is the number of inactivated genes in the best current solution, nc is the total number of core genes in the problem, and α is a user-defined parameter.

Proposed SA optimization. Scores in this proposal are obtained using RAxML [90]. As an inference of a bootstrapped and rooted phylogenetic tree may take times, and as we need to compute several trees, each calculated state is tagged so that it is never recomputed without an explicit user demand. Associated and detailed results are buffered on disk. Then a simple, reliable, and not really space-characteristics dependent solution is the synchronization of some SAs after the end of a Markov chain [91]. In order to do so, a batch of SAs is launched with the same configuration. After a chain, each running SA shares its own best known solution to a server. Then, it demands to this server if a better state has been found before starting the next chain. Finally, each SA halts after n local non optimizing chains. So a stopped SA is not restarted, even if a better solution is found elsewhere (*i.e.*, the proposed SA stops as soon as possible).

Acceptance function is also selected to take advantage of previous moves, to allow some (not too large) jumps. This is an adaptation of the so-called Tsallis acceptance probabilities [86] with a control parameter normalization :

$$\left(1 - \frac{(1-q) * \Delta}{\bar{\Delta} * t}\right)^{\frac{1}{1-q}},$$

where Δ is the score difference between the previous and current states, $\bar{\Delta}$ their mean, t is a control parameter, and q is a user-defined factor.

How to stop the SA. To fix a predefined control (temperature) value needs to know some state space characteristics, so we choose an end criterion related to the absence of progression in scores. In other words, the proposed simulated annealing algorithm stops after t consecutive Markov chains without any score improvement. As SA is very slow on low temperatures, the choice has been to choose a small value for t. Then, a greedy local search can be launched on SA best states.

3.6/ Comparison of the metaheuristics

3.6.1/ Data generation

3.6.1.1/ Genomes recovery and annotations

At this stage, 780 complete genomes of chloroplasts have been downloaded from the NCBI, constituting the set of all available complete chloroplastic genomes at the date of the beginning of our study [56]. Various gene prediction methods have been previously tested, in order to translate these complete genomes in lists of annotated coding sequences. These methods encompass the single use of NCBI annotated genomes, the use of automatic annotation tools specific to organelles like DOGMA [55], and the mix of both.

Indeed, annotations from NCBI website are of very variable quality : humanly well-curated genomes go together with genomes having a lot of annotation errors, concerning either the gene names (classification or spelling errors) or DNA sequences (start and stop position, length). As the number of well annotated genomes was not enough to constitute a testing set for our experiments, we are then left to find an acceptable way to annotate the whole 780 complete genomes. As stated above, we tested various ways to annotate the genomes, and we evaluated them by checking their ability to recover the annotations (sequence positions and gene names) of the subset of humanly, well-curated genomes.

According to our experiments, there was no way to improve enough the quality of NCBI annotations [57]. Neither by cross-validating them using automatic annotation tools, nor by trying to correct errors in gene names and positions with these tools and some edit distances [58, 12]. Furthermore, to cluster the whole NCBI DNA sequences fail in separating well annotated genes in well separated clusters, due to junk DNA in the NCBI sequences. The large number of obvious errors in the NCBI annotated complete chloroplastic genomes can be explained by the large variety of annotation tools used during sequence submission, most of them being not specific to this kind of genomes (unlike DOGMA), to a misuse of these tools, or due to errors in manual annotations. The absence of a clear norm in the gene naming process adds difficulties, so that the sole method to provide accurate annotations to these 780 complete genomes was to constitute a basis of knowledge, with a subset of well curated genomes that represent well the plant diversity. And, to blast each genome against the basis, which is indeed what is done by DOGMA.

We finally have written a script that automatically send requests to the DOGMA web service, and recovers the annotated genomes. Due to this automatic process, the gene name spelling issue is resolved, and we can recover the clusters of homologous coding sequences by simply considering gene names. By applying the same tool for coding sequence prediction and naming process, we have resolved the problem of quality variability in annotations. And as DOGMA has been specifically designed for chloroplasts, errors in

sequence positions have been reduced as possible. At this stage, and using our script on DOGMA web service, we have then a collection of 780 complete and "well" annotated chloroplastic genomes, from which gene names can be used to recover core and pan genomes of any subset of genomes.

3.6.1.2/ Extracting subsets of genomes for simulations

To test the ability, for the three proposed metaheuristics methods, to find the largest subset of core genes that leads to the most supported trees, we needed to extract, from the set of annotated genomes, various distinct subsets that are such that :

- Using the whole core genome in the alignment, we cannot obtain a well supported tree.
- The time to compute this tree is reasonable, as we want to compute a lot of trees using a lot of subsets of core genes. For a given subset of core genes, this computation time encompasses :
  1. the multi-alignment of each core gene using Muscle [59],
  2. the concatenation of each aligned sequence to reconstruct the "sub" genome of each considered species (*i.e.*, the part corresponding to the considered subset of core genes),
  3. the computation of the best phylogenetic tree corresponding to this alignment (with RAxML [90]),
  4. the addition of bootstrap supports to this best tree using RAxML again,
  5. and finally the verification that one of these supports is lower than 95 at least. If so, this tree is considered as not well supported.

Given a subset of genomes, the multi-alignment of each core gene can be computed only once, prior to the research of the best subset of core genes leading to the most supported tree. So we do not have to consider the alignment stage when searching subsets of genomes with : (a) problematic phylogenies and (b) a time to infer their tree as low as possible. We stopped the process above before Stage 4 and we randomly pick another subset of species if the time to find their best phylogenetic trees using their whole core genome (*i.e.*, Stage 3) exceeds 10 seconds. If this computation time is below this threshold, we then compute 50 bootstraps and we check if the best bootstrapped tree has a problem of supports. If so, we have found a convenient subset of annotated genomes, on which we can test the three metaheuristics.

3.6.1.3/ A simple comparison in small dimensions

After having executed the three metaheuristics previously described, we have validated them on test examples. We have first performed a 1D/2D comparison of the three proposals, to obtain an easy-to-understand representation of the convergence of the optimization algorithms. Obtained results are depicted in Figure 3.11, circles denote successive positions given by SA, points are for GA, while PSO corresponds to triangles. Figure 3.12 represents the output evolution of the simulated annealing, with the consecutive ends of the Markov chains and the evolution of acceptation density. From the results, we can deduce that the desired convergence behavior is well obtained, and that the comparison

seems fair : no algorithm seems to underperform the other ones, and the general evolution of the energy seems to be comparable for the three algorithms. Such results allow us to further investigate simulated annealing, particle swarm optimization, and genetic algorithm for their ability to find the largest subset of core genes that leads to the most supported tree.

Fig. 3.11 – Successive positions given by the three metaheuristics : circles, points, and triangles are respectively for SA, GA, and PSO.

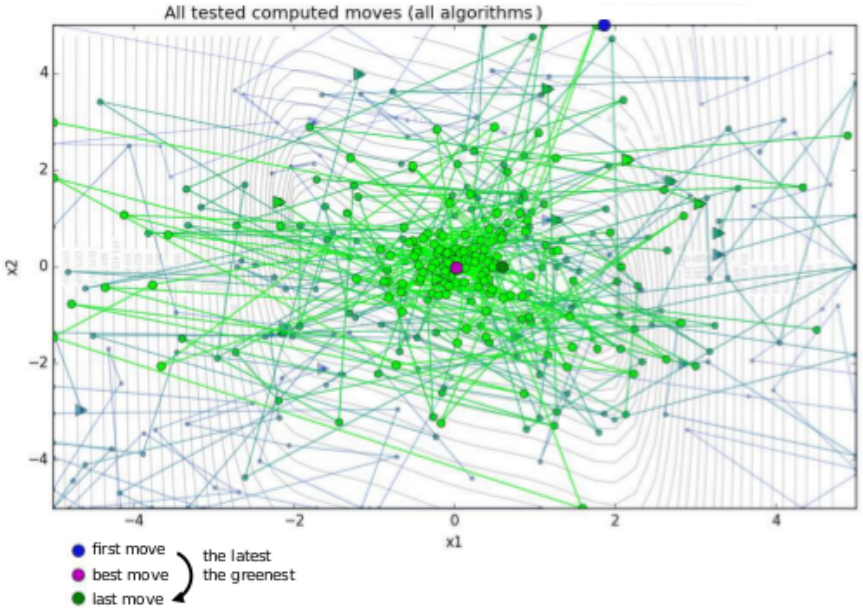


Fig. 3.12 – Illustration of output provided by simulated annealing approach : three-hump camel function, one instance of parallelled SA with final greedy local descent.

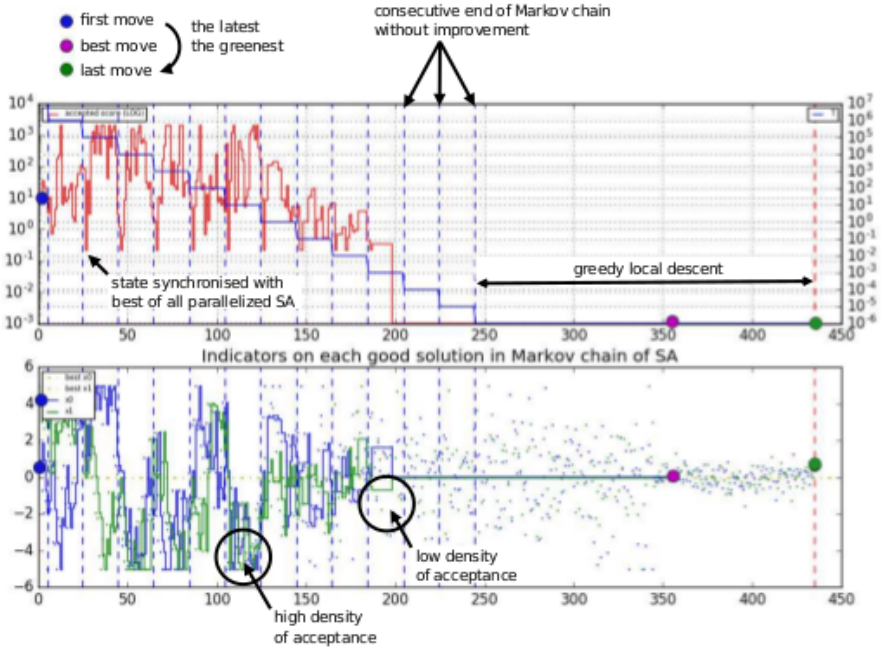


TABLE 3.6 – Family number 1 (Pelargonium cotyledonis as outgroup).

Accession Nb	Name	Nb. of genes	Length (nuc.)
NC_024082.1	Cylindrotheca closterium	257	165,809
NC_014808.1	Thalassiosira oceanica CCMP1005	138	141,790
NC_025313.1	Cerataulina daemon	195	120,144
NC_028052.1	Pelargonium cotyledonis	271	166,111
NC_015403.1	Fistulifera solaris	192	134,918
NC_024084.1	Leptocylindrus danicus	155	125,213

3.6.2/ EXPERIMENTING THE HEURISTICS ON SMALL COLLECTIONS OF GENOMES

We first focus on small sets of species with unresolved phylogenies, for computational reasons and because small trees are easier to compare. Even in such small sets, as the core genome contains more than 100 genes, the number of combinations to test is far from what is tractable using a brute force approach. We will see that it is easy to obtain various opposed but very well supported trees using large subsets of core genes, leading to the necessity to optimize both parameters.

3.6.2.1/ A FIRST FAMILY OF ALGAE

We have first considered the family listed in Table 3.6. The detailed taxonomy information is provided hereafter.

— **Cylindrotheca closterium.** Stramenopiles ; Bacillariophyta ; Bacillariophyceae ; Bacillariophycidae ; Bacillariales ; Bacillariaceae.

— **Thalassiosira oceanica CCMP1005.** Stramenopiles ; Bacillariophyta ; Coscinodiscophyceae ; Thalassiosirophycidae ; Thalassiosirales ; Thalassiosiraceae.

— **Cerataulina daemon.** Stramenopiles ; Bacillariophyta ; Mediophyceae ; Biddulphiophycidae ; Hemiaulales ; Hemiaulaceae.

— **Pelargonium cotyledonis.** Viridiplantae ; Streptophyta ; Embryophyta ; Tracheophyta ; Spermatophyta ; Magnoliophyta ; Eudicotyledons ; Gunneridae ; Pentapetalae ; Rosids ; Malvids ; Geraniales ; Geraniaceae.

— **Fistulifera solaris.** Stramenopiles ; Bacillariophyta ; Bacillariophyceae ; Bacillariophycidae ; Naviculales ; Naviculaceae.

— **Leptocylindrus danicus.** Stramenopiles ; Bacillariophyta ; Coscinodiscophyceae ; Chaetocerotophycidae ; Leptocylindrales ; Leptocylindraceae.

This family is constituted by 6 genomes, of length ranging from 120,144 to 166,111 nucleotides. The number of detected genes, for its part, ranges from 138 to 271, with a core genome of 122. The phylogeny with the alignment of these core genes leads to a small weakness in one branch (bootstrap of 94), as depicted in Figure 3.13. Indeed, inside this *bacillariophyta* phylum (eukaryotic algae), *C.closterium*, and *F.solaris* are naturally in the same clade, being both in the same class of *bacillariophyceae*, while the three other species are in three different classes inside this phylum.

To wonder whether some genes may be responsible of such weak uncertainty, we have firstly launched the genetic algorithm : its systematic mode (in population initialization

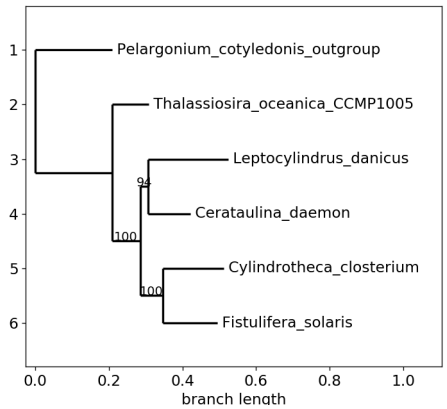


Fig. 3.13 – Phylogeny of family Number 1 with the whole core genome.

stage) indeed first tries to remove each core gene separately. This GA has stopped after 29 iterations, in systematic mode, leading to 2 topologies :

— Topology 0, depicted in Fig. 3.14(a), has occurred 27 times. The best obtained tree has a lowest bootstrap of 96, while in average the lowest bootstrap is equal to 86.

— Topology 1, for its part (see Figure 3.14(b)), has occurred twice, with a non supported branch of 64 in its best tree.

As during these experiments, we have not leaved the initialization phase, it is useless to detail here the parameters set to configure the GA. The PSO, for its part, has been configured as follows : 3 particles, a fitness lower than 0.05 to freeze the runs, and all constants that define the velocity equal to 1. This heuristics has rapidly found a first well supported phylogenetic tree in a third different topology, and with all supports equal to 100, see Figure 3.14(c). However, the PSO has used only 47.5% of the core genes to reach such a tree. According to our stop criterion, this tree has not been returned by the algorithm. Indeed, this example illustrates the ability of the particle swarm optimization algorithm to more globally visit the whole space at the beginning, in order to discover regions of interest.

If we compare for instance the behavior of the PSO during the same time than the one required to finish the GA (29 iterations), we discovered 5 topologies, two of them having all their supports equal to 100 (Topologies 0 and 2 in Figure 3.14, occurring respectively 17 and 7 times). They however used only between 44.26% and 48.36% at this starting point in the PSO. Bit by bit, over iterations, the percentage of core genes is enlarging, and the swarms tend to prefer the Topology 0. Finally, after 350 computed trees (which was the stopping condition), this topology has been obtained in 53.42% of the cases, and its best tree has a lowest bootstrap of 100 using 66.39% of core genes. The number of occurrences of the other topologies has growth more slowly and, even if all the bootstraps of their best representatives exceed the value of 98, the latter fails in the attempt to significantly increase the number of considered core genes in these representatives (always lower than 55.8%).

The simulated annealing, for its part, raised 3 topologies, exactly the ones depicted in Figure 3.14. It has been launched with an initial temperature equal to 100, a final one of 1e-10, and an optimal exponential temperature function. Acceptation function was the Tsallis normalized one, with a q factor of 0.25, and initial (resp. final) acceptance of 0.7 (resp. 1e-05). A remarkable element is that these 3 topologies have the whole bootstraps

TABLE 3.7 – Family number 2 (Chromera velia as outgroup).

Accession Nb	Name	Nb. of genes	Length (nuc.)
NC_024082.1	Cylindrotheca closterium	257	165,809
NC_014808.1	Thalassiosira oceanica CCMP1005	138	141,790
NC_027721.1	Pseudo-nitzschia multiseries	267	111,539
NC_024084.1	Leptocylindrus danicus	155	125,213
NC_014340.2	Chromera velia	265	120,426

equal to 100. Furthermore, Topology 2 appears as the best one according to the produced result (it was Topology 0 according to the GA, while PSO has not succeeded in separating these two topologies). With details, the SA has stopped after 364 computed trees, with 6 occurrences of Topology 0, 43 of Topo. 1, and 315 for the Topology 2. Similarly, the percentage of core genes leading to the best representative in each topology is respectively of 56.56% (Topo. 0), 74.59% (Topo. 1), and 94.98% (Topo. 2), which thus outperforms the other ones according to these simulations.

Obviously, both PSO and SA have converged to local minima that are not global ones if we consider that both minimum bootstraps and proportion of core genes must be maximized. Launching them again with other initial values and parameters may select other optimal positions in the cube. The genetic algorithm with this family is emblematic, as during its initial population generation it has returned Topology 0 that is totally supported with 99.18% of the core genome. This topology seems to be an acceptable representation of the phylogenetic relationship between these chloroplasts. But it is remarkable that, using the same large proportion of core gene, we can break in the sister relationship between *L.danicus* and *C.daemon*. Indeed, this behavior has been obtained frequently with various collections of data, which will be illustrated below.

Up to now, we only have considered one problematic bootstrap, which may be easy to resolve when removing genes. New difficulties are added when there are at least two problems in the list of bootstraps, as improving the first one may lead to a decrease in the second value. We have investigated this point in the second tested family.

3.6.2.2/ A SECOND FAMILY WITH TWO PROBLEMATIC BOOTSTRAPS

The second small set of genomes is constituted by 4 *Bacillariophyta* plus an *Alveolata* as outgroup, as listed in Table 3.7. Taxonomic details are provided hereafter, while the phylogenetic tree based on the alignment of the core genome is provided in Figure 3.15(a).

— **Cylindrotheca closterium.** Stramenopiles ; Bacillariophyta ; Bacillariophyceae ; Bacillariophycidae ; Bacillariales ; Bacillariaceae.

— **Thalassiosira oceanica CCMP1005.** Stramenopiles ; Bacillariophyta ; Coscinodiscophyceae ; Thalassiosirophycidae ; Thalassiosirales ; Thalassiosiraceae.

— **Pseudo-nitzschia multiseries.** Stramenopiles ; Bacillariophyta ; Bacillariophyceae ; Bacillariophycidae ; Bacillariales ; Bacillariaceae.

— **Leptocylindrus danicus.** Stramenopiles ; Bacillariophyta ; Coscinodiscophyceae ; Chaetocerotophycidae ; Leptocylindrales ; Leptocylindraceae.

— **Chromera velia.** Alveolata ; Chromerida

The phylogenetic tree is not well-supported, having two bootstrap values of 86. Furthermore, *T.oceanica* and *L.danicus* are not sisters in this tree, while they belong in the *Coscinodiscophyceae* class of *diatom*. More seriously, the two other species belong to the *Bacillariaceae* family, which is in contradiction with this tree. It is not a necessity to recover exactly the known taxonomy, as we focus on chloroplasts, but this tree is at least suspicious if we consider both supports and taxonomy. This example illustrates the fact that to use the largest common subset of sequences is not sufficient enough to guarantee a well conducted phylogenetic study. Conversely, and obviously, to have good supports is not enough, as all best trees in the different topologies of the previous family are well supported in the SA case : the largest number of core genes must be thus coupled with the research of the best supports.

Once again, the genetic algorithm has stopped rapidly, in the systematic mode. The 22 first genes have been tested (*i.e.*, removed) before finding Topology 0 of Figure 3.15(a) with a lowest bootstrap equal to 96 (and 99.18% of the genes), thus stopping the GA, while a new topology (Topology 1, see Fig. 3.15(b)) has occurred three times (best tree having twice 94 as bootstraps). Compared with the first family, the genetic algorithm stops here before succeeding to reinforce the confidence put in Topology 0, which justifies to test the two other approaches.

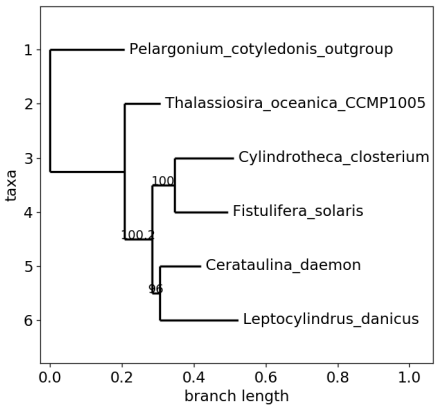
PSO heuristics produces the same two topologies after 1,165 computed trees, with all supports equal to 100, and approximately the same number of trees (632 for Topo. 0 and 533 for Topo. 2) and of genes (70.49% versus 74.59%). We stopped the swarm manually, as these two scores have not been improved during the last 500 iterations. Obviously, the 3 particles have been blocked in two local extrema, and the way we configured their velocity (0.9 and 0.8 for ϕ_1 and ϕ_2) does not allow them to leave these optima. So we still cannot choose definitively the topology number 0.

Finally, the simulated annealing has produced 400 trees before convergence. They all belong to the two topologies detailed above. However, produced results show that Topology number 1 must be preferred, according to the SA, and this latter is neither the one obtained with the whole core genome, nor the best one according to GA. Indeed, after convergence, all bootstraps here are equal to 100 in the best tree found inside each topology. But topology of Figure 3.15(b) has been obtained in 88.5% of the cases. More significantly, best tree in Topology 1 is obtained using 96.72% of the core genome, while for Topology 0, the best tree uses 90.98% of it. Remark that using the nine-tenths of the core genome, you can obtain a first topology with all supports equal to 100, while using more than 96% you can find a different topology with again all supports equal to 100. And, if we consider the average between the lowest bootstrap and the proportion of core genes as a score, the best topology according to GA has a score of 97.59/100, while it is of 98.36 for Topology 1 found by the SA.

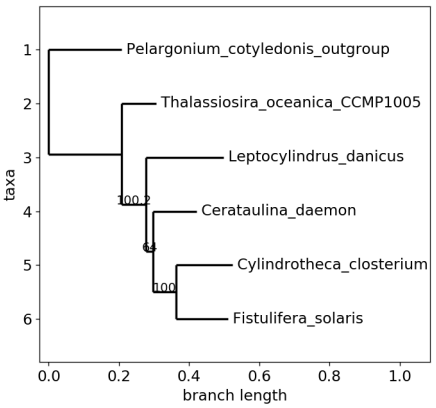
We will now further investigate the simulated annealing convergence process, before studying more deeply the two other algorithms in a next section.

3.6.3/ Early analysis on SA computed problem : an illustration

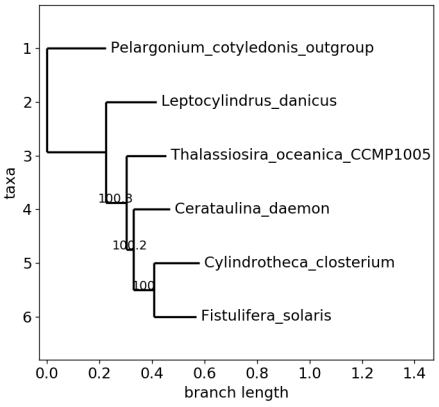
An example of a SA batch run (three clients on the first family described previously) is depicted in Figure 3.16. For easy understanding, only some outputs have been reported in the figure.



(a) Topology 0

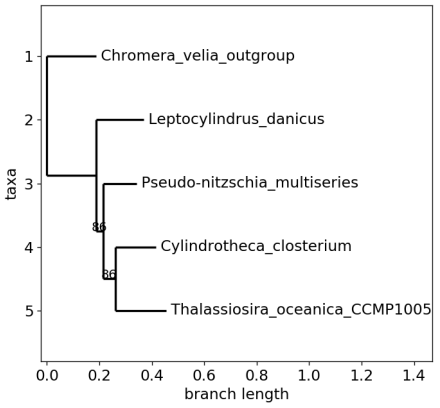


(b) Topology 1

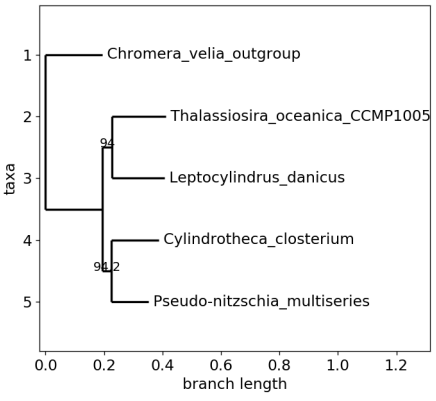


(c) Topology 2

Fig. 3.14 – Obtained topologies with the first family.



(a) Topology 0 with the whole core genome



(b) Topology 1 obtained by GA

Fig. 3.15 – Obtained topologies with the second family.

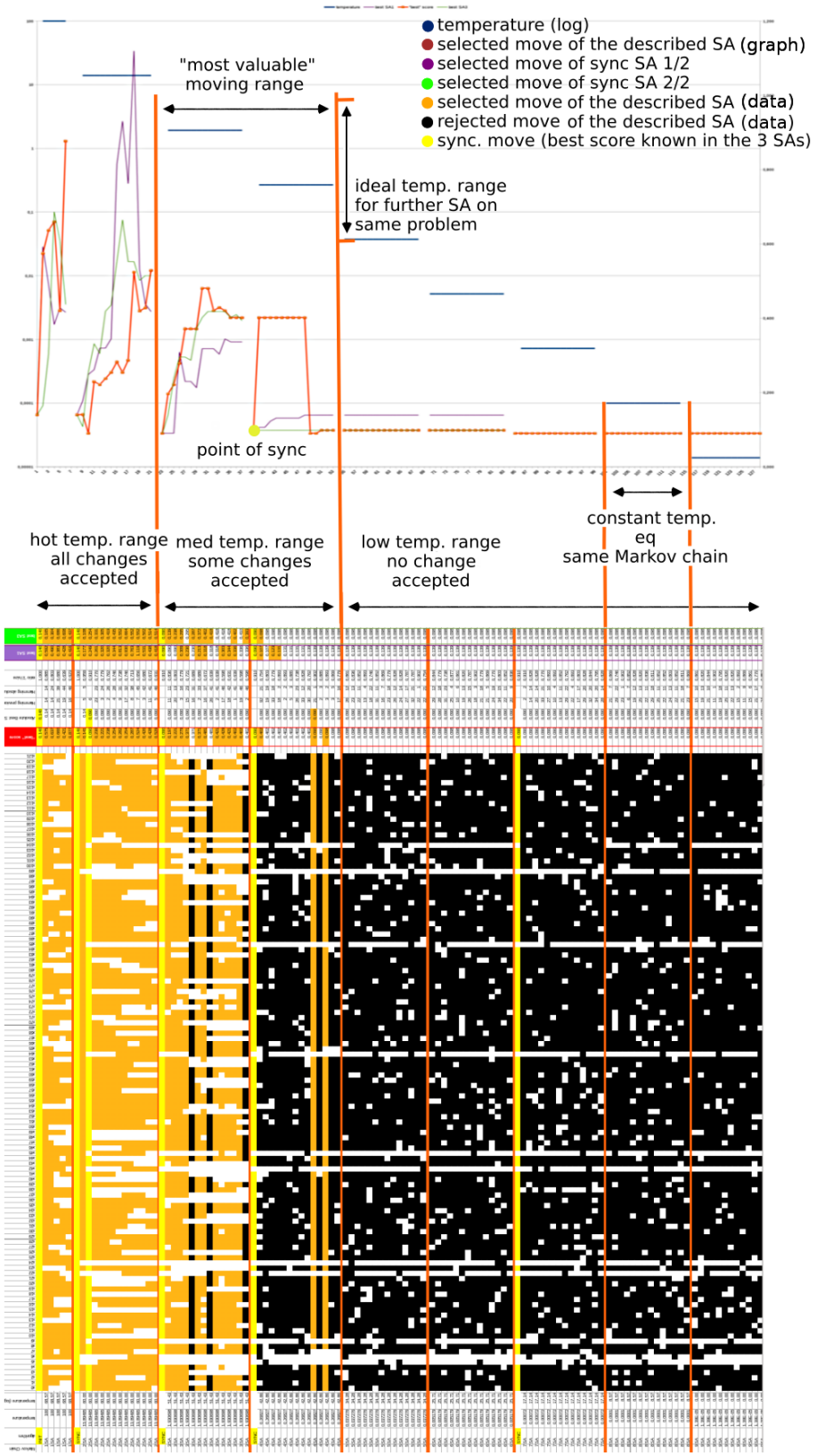


Fig. 3.16 – Illustration of clade analysis with a 3-parallelized SA.

On the lower part, all moves of the simulated annealing are reported with their nature : synchronized move in yellow (*i.e.*, copy, from a shared memory, of the best known solution found in the three SAs), move with an accepted status in orange, and rejected moves in black. Active genes are filled squares and not selected ones are white squares. Other important data for analysis are reported, such as : temperature, accepted score of other SAs (green and purple), and Hamming distance between two consecutive positions (moving behavior indicator).

On the upper part, a graph of accepted scores from the three SAs is provided, with the temperature variations due to move iterations (a lower score is a better one). As we represented the first run on a new collection of genomes, no previous configurations were available to set up the parameters. Consequently, a broad range of temperatures has been considered. The Markov chains are short, in order to reduce the computation time. From this beginning of an experiment, it can be deduced that :

— the temperature ranges well, allowing further experiments on the same set of data ;

— even with a poor configuration, SAs have found a score “not so bad”, which is associated to a topology that other heuristics have considered as a good one.

Another SA evolution is provided in Figure 3.17, in which the three main curves do not represent moves, but “moves of locally selected moves”, which are stabilized over time.

3.6.4/ A FURTHER COMPARISON OF THE DISTRIBUTED VERSIONS OF GA AND BPSO PERFORMANCE

During the experiments of the previous section, it was impossible to evaluate in practice the behavior of the genetic algorithm, as this latter found an optimum during the initialization stage. Similarly, BPSO has underperformed the two other algorithms, while SA always produced interesting results. This is why we decided, after having studied the SA evolution on the first family, to further investigate both BPSO (with its two velocity versions) and GA in large collections of experiments, distributed in a supercomputer facilities. To do so, 12 groups of plant genomes have been extracted from our set of annotated genomes. They have been applied on our two swarm versions, and results have been compared to the genetic algorithm ones.

Comparisons are provided in Tables 3.8 and 3.9. In these tables, *Topo.* column stands for the number of topologies, *NbTrees* is the total number of obtained trees using 10 swarms, b is the minimum bootstrap value of selected w, $100 - p$ is the number of missing genes in w and *Occ.* is the number of occurrences of the best obtained topology from 10 swarms. As can be seen in these tables, the two versions of BPSO did not provide the same kind of results :

— In the case of *Chlorophyta*, *Pinus*, and *Bambusoideae*, the second version of the BPSO has outperformed the first one, as the minimum bootstrap b of the best tree is finally larger for at least one swarm.

— In the *Ericales* case, the first version has produced the best result.

We can also remark that *Malpighiales* has better b in GA than the two versions of BPSO. *Pinus* data set has got maximum bootstrap b larger than what has been obtained using the genetic algorithm, while *Picea* and *Trebouxiophyceae* have got the same values of b than with genetic algorithm. Further comparison results between GA and both versions of BPSOs are provided in Figure 3.18.

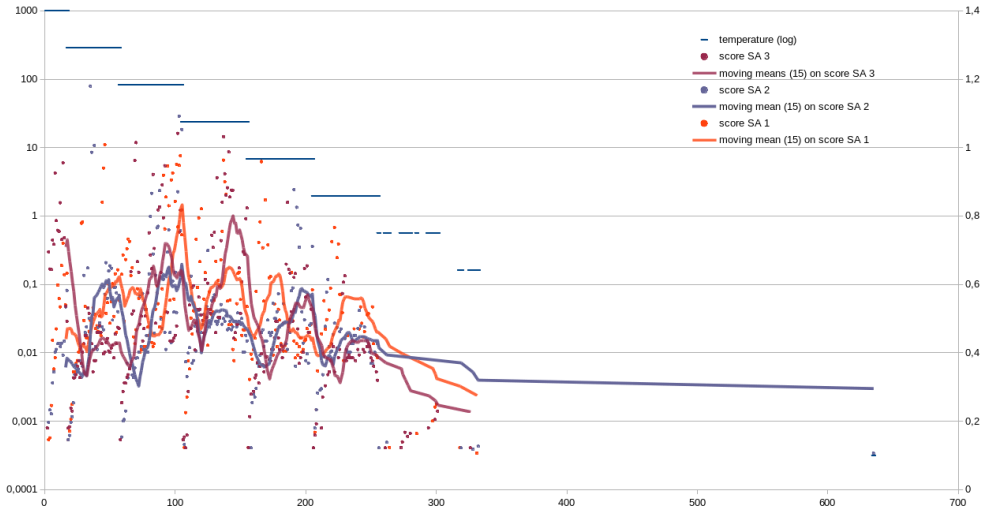
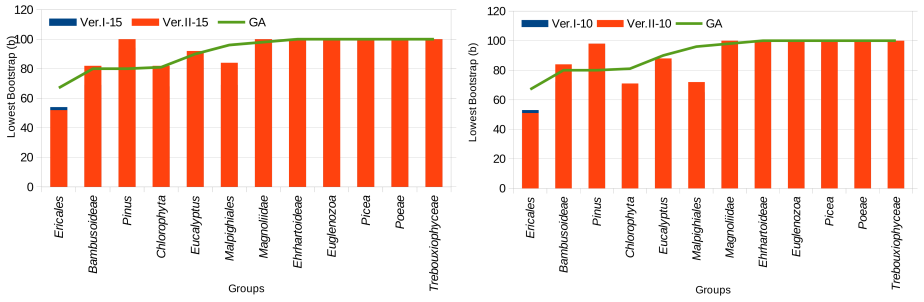


Fig. 3.17 – Illustration of convergence on 3-parallelized SA.



(a) BPSO with 15 particles vs. GA (b) BPSO with 10 particles vs. GA

Fig. 3.18 – BPSO with 10 and 15 particles vs. GA.

According to this figure, we can conclude that the two approaches lead to quite equivalent bootstrap values in most data sets, while on particular subgroups obtained results are complementary. In particular, BPSO often produces better bootstraps than GA (see *Magnoliidae* or *Bambusoideae*), but with a larger number of removed genes. Finally, using 15 particles instead of 10 does not improve so much the obtained results (see Figure 3.18 and Table 3.10).

TABLE 3.8 – Groups from BPSO version I.

Group	Topo.	NbTrees	b	$\|c\|$	$100 - p'$	Occ.	Swarms	Particles
Pinus	3	508	98	79	32	462	1,2,3,4,5,6,7,8,9,10	10
Pinus	3	530	94	79	11	129	1,2,3,4,5,6,7,8,9,10	15
Picea	1	100	100	85	42	100	1,2,3,4,5,6,7,8,9,10	10
Picea	1	428	100	85	13	428	1,2,3,4,5,6,7,8,9,10	15
Magnoliidae	3	750	100	79	20	613	1,2,3,4,5,6,7,8,9,10	10
Magnoliidae	3	845	100	79	19	707	1,2,3,4,5,6,7,8,9,10	15
Ericales	30	344	53	84	26	185	1,2,3,4,5,6,7,8,9,10	10
Ericales	34	555	54	84	5	363	1,2,3,4,5,6,7,8,9,10	15
Bambusoideae	8	496	72	94	37	456	1,2,3,4,5,6,7,8,9,10	10
Bambusoideae	11	694	69	94	18	621	1,2,3,4,5,6,7,8,9,10	15
Eucalyptus	16	828	86	83	7	632	1,2,3,4,5,6,7,8,9,10	10
Eucalyptus	20	1073	86	80	4	845	1,2,3,4,5,6,7,8,9,10	15
Malpighiales	34	327	65	78	35	233	1,2,3,4,5,6,7,8,9,10	10
Malpighiales	38	483	69	78	40	326	1,2,3,4,5,6,7,8,9,10	15
Chlorophyta	25	191	70	24	11	109	1,2,3,4,5,6,7,8,9,10	10
Chlorophyta	29	94	68	24	11	1	1,2,3,4,5,6,7,8,9,10	15
Euglenozoa	3	450	100	26	7	292	1,2,3,4,5,6,7,8,9,10	10
Euglenozoa	3	520	100	26	4	491	1,2,3,4,5,6,7,8,9,10	15
Ehrhartoideae	2	23	100	81	0	23	1,2,3,4,5,6,7,8,9,10	10
Ehrhartoideae	3	455	100	81	0	451	1,2,3,4,5,6,7,8,9,10	15
Trebouxiophyceae	3	409	100	41	2	405	1,2,3,4,5,6,7,8,9,10	10
Trebouxiophyceae	3	415	100	41	8	354	1,2,3,4,5,6,7,8,9,10	15
Poeae	1	971	100	80	9	971	1,2,3,4,5,6,7,8,9,10	10
Poeae	1	1399	100	80	20	1399	1,2,3,4,5,6,7,8,9,10	15

TABLE 3.9 – Groups from PSO version II.

Group	Topo.	NbTrees	b	$\|c\|$	$100 - p'$	Occ.	Swarms	Particles
Pinus	3	615	98	79	14	275	1,2,3,4,5,6,7,8,9,10	10
Pinus	3	628	100	79	12	558	1,2,3,4,5,6,7,8,9,10	15
Picea	1	635	100	85	14	635	1,2,3,4,5,6,7,8,9,10	10
Picea	1	821	100	85	15	821	1,2,3,4,5,6,7,8,9,10	15
Magnoliidae	3	494	100	79	16	73	1,2,3,4,5,6,7,8,9,10	10
Magnoliidae	3	535	100	79	42	384	1,2,3,4,5,6,7,8,9,10	10
Bambusoideae	6	952	84	81	23	94	1,2,3,4,5,6,7,8,9,10	10
Bambusoideae	9	1450	82	81	18	113	1,2,3,4,5,6,7,8,9,10	15
Eucalyptus	17	972	88	80	18	618	1,2,3,4,5,6,7,8,9,10	10
Eucalyptus	23	1439	92	80	10	843	1,2,3,4,5,6,7,8,9,10	15
Chlorophyta	25	529	71	24	6	397	1,2,3,4,5,6,7,8,9,10	10
Chlorophyta	46	1500	82	24	11	397	1,2,3,4,5,6,7,8,9,10	10
Ericales	30	97	51	84	11	56	1,2,3,4,5,6,7,8,9,10	10
Ericales	34	1257	52	84	7	800	1,2,3,4,5,6,7,8,9,10	15
Malpighiales	35	725	72	79	25	445	1,2,3,4,5,6,7,8,9,10	10
Malpighiales	86	1464	84	79	45	359	1,2,3,4,5,6,7,8,9,10	15
Euglenozoa	3	197	100	26	1	165	1,2,3,4,5,6,7,8,9,10	10
Euglenozoa	3	450	100	26	10	393	1,2,3,4,5,6,7,8,9,10	15
Ehrhartoideae	1	24	100	81	10	24	1,2,3,4,5,6,7,8,9,10	10
Ehrhartoideae	1	20	100	81	9	20	1,2,3,4,5,6,7,8,9,10	15
Trebouxiophyceae	3	319	100	41	1	313	1,2,3,4,5,6,7,8,9,10	10
Trebouxiophyceae	3	818	100	41	2	81	1,2,3,4,5,6,7,8,9,10	15
Poeae	1	991	100	80	22	991	1,2,3,4,5,6,7,8,9,10	15
Poeae	1	1490	100	80	26	1490	1,2,3,4,5,6,7,8,9,10	15

TABLE 3.10 – PSO vs GA.

	BPSO ver.I		**BPSO ver.II**		
Group	**10**	**15**	**10**	**15**	**GA**
Ericales	53	54	51	52	67
Bambusoideae	72	69	84	82	80
Pinus	98	94	98	100	80
Chlorophyta	70	68	71	82	81
Eucalyptus	86	86	88	92	90
Malpighiales	65	69	72	84	96
Magnoliidae	100	100	100	100	98
Ehrhartoideae	100	100	100	100	100
Euglenozoa	100	100	100	100	100
Picea	94	100	100	100	100
Poeae	80	80	100	100	100
Trebouxiophyceae	100	100	100	100	100

3.7/ CONCLUSION

This chapter has presented three metaheuristics to produce a well supported phylogenetic tree based on the largest possible subset of core genes. These methods are, namely, genetic algorithm, binary particle swarm optimization, and simulated annealing. They have been evaluated on various sets of chloroplast species and deployed on a supercomputer facilities. At this level, two main issues can be signaled when considering the objective to reconstruct the ancestral genomes of all existing chloroplasts.

On the one hand, given the average between the percentage of core genes and the lowest bootstrap as scoring function, we have shown on simple examples that, given a set of species, various global optima with contradictory topologies can be reached. These first experiments emphasize that sometimes the phylogeny of chloroplasts cannot perfectly be resolved using a tree : a phylogenetic network may be more close to the reality, branches within this network being as strong as the associated tree topology is frequent. In the first instance, this difficulty has not been considered : our current objective is to design algorithms making it possible to reconstruct ancestors through a tree. In case of success, we will then try to improve our pipeline, to become compatible with network topologies.

On the other hand, given a set of annotated genomes, we have proposed some methods to find the most supported tree based on the largest subset of core genes. In case where the considered species are close enough, their core genome is close to each genome alone : the intersection of sets that share a large amount of genes is large. In this situation, and in case of convergence of our method, the obtained well supported tree is based on a large part of each genome, and it can be considered as well representative : ancestral reconstruction can be reasonably applied on it. Conversely, when considering the chloroplasts of all possible plants, there is concern about the representativeness of the core genome, as the latter may be very small. This concerns passes from the phylogenetic tree to the ancestors reconstructed on it. To investigate this risk and the means to remove it, the evolution of core genome within the chloroplast taxonomy will be investigated in the next chapter.

4

Relation between Gene Content and Taxonomy in Chloroplasts

The aim of this chapter is to investigate the evolution of the core genome of chloroplasts when enlarging the set of considered species. Core and pan genomes have been computed here on *de novo* annotation of the 845 genomes available at the time of this study. We take the opportunity to investigate the specificity of some branches of the tree, when specificity is obtained on accessory genes. After having detailed the material and methods, we emphasize some remarkable relation between well-known events of the chloroplast history, like endosymbiosis, and the evolution of gene contents over the phylogenetic tree.

This study shows that taking simply the core genome of the whole 845 chloroplasts at the beginning of the pipeline presented in the previous chapter is not satisfactory, as this core genome is too small and not really representative. Enriching our method is thus a necessity to automatically obtain a well supported phylogenetic tree of the whole chloroplasts, on which the ancestral reconstruction process can operate. The content of this chapter has been presented in the ICBSB 2016 [92] conference and published in IJBBB journal [93].

Table 4.1 – Information on chloroplast sizes at highest taxonomic level

Taxonomy	nb. of genomes	min length length	max length	average	standart deviation
Alveolata	4	85535	140426	115714.2	19648.3
Cryptophyta	2	121524	135854	128689.0	7165.0
Euglenozoa	7	80147	143171	98548.7	19784.5
Haptophyceae	3	95281	107461	102683.6	5307.6
Rhodophyta	9	149987	217694	183755.5	18092.2
Stramenopiles	35	89599	165809	124895.1	15138.0
Viridiplantae	775	80211	289394	150194.9	20376.8

4.1/ Materials and methods

4.1.1/ Data acquisition

A set of 845 chloroplastic genomes (green algae, red algae, gymnosperms, and so on) has been downloaded from the NCBI website, representing all the available complete genomes at the date of March, 2016 (see Table 4.1). An example of such sequences, taken from the *Streptophyta* clade (a *Viridiplantae*), is provided in Table 4.2. Note that this set does not really constitute a very balanced representation of the diversity of plants, as plants of particular and immediate interest to us like *Viridiplantae* are first sequenced. We must however deal with such bias, as genomic data acquisition is most of the time human-centred. This set of sequences presents too a certain variability in terms of length, as detailed in Table 4.1.

Each genome has been annotated with DOGMA [55], an online automatic and accurate annotation tool of organellar genomes, following a same approach than in [57]. To apply it on our large scale database, a script that automatic send requests to the website has been used. By doing such annotations, the same gene prediction and naming process has been applied with the same average quality of annotation. In particular, when a gene appears twice in the considered set of genomes, it receives twice the same name (no spelling error). At this level, each genome is then described by an ordered list of gene names, with possible duplicates (other approaches for the annotation stage are possible, as explained in the previous chapter [57]). This description will allow us to investigate, later in this chapter, the evolution of gene content among the species tree, leading to the study of core and pan genomes described below.

4.1.2/ Core and pan genome

Given a collection of genomes, it is possible to define their core genes as the common genes that are shared among all the species, while the pan genome is the union of all the genes that are in at least one genome (*all* the species have each core gene, while a pan gene is in *at least one* genome). Shared genes are evidences of evolution from a common ancestor and of the relatedness of chloroplast organisms.

Table 4.2 – Example of genomes information of *Streptophyta* clade

Organism name	Accession number	Sequence length	Nb of CDS
Epimedium sagittatum	NC_029428.1	158273	85
Berberis bealei	NC_022457.1	164792	267
Torreya fargesii	NC_029398.1	137075	100
Lepidozamia peroffskyana	NC_027513.1	165939	93
Actinidia chinensis	NC_026690.1	156346	271
Quercus aliena	NC_026790.1	160921	259
Quercus aquifolioides	NC_026913.1	160415	176
Sedum sarmentosum	NC_023085.1	150448	99

TABLE 4.3 – Summarized properties of the pan genomes at the highest taxonomic level.

Taxonomy	Nb. genomes	Min N.b of pan genes	Max N.b of pan genes	Average Nb. of pan genes
Alveolata	4	253	266	262.25
Cryptophyta	2	258	259	258.5
Euglenozoa	7	193	267	253.428
Haptophyceae	3	251	266	258.333
Rhodophyta	9	156	267	246.222
Stramenopiles	35	73	271	238.971
Viridiplantae	775	85	271	229.827

To distinguish and determine the core genes may be of importance either to identify the specificity and the shared functionality of a given set of species, or to evaluate their phylogeny using the largest set of shared coding sequences (see the previous chapter). In the case of chloroplasts, an important category of genome modification is indeed the loss of functional genes, either because they become ineffective or due to transfer to the nucleus. Thereby, a small number of gene loss among species may indicate that these species are close to each other and belong to a similar lineage, while a significant loss means distant lineages. So core genome is obviously of importance when inferring the phylogenetic relationship, while accessory genes of pan genome explain in some extend each species specificity.

Three approaches have formerly been proposed (by members of the DISC department, FEMTO-ST) for eliciting core genomes. The first one uses correlations computed on predicted coding sequences [57], while the second one uses gene names provided during an annotation stage (*e.g.*, names found in NCBI database or automatically set by DOGMA [58]). The third method tries to take the advantages from the first two approaches, by considering gene information and DNA sequences, in order to find the targeted core genome [12]. Indeed, the naming process of genes is not standardized, and spelling errors may occur, while conversely start and stop positions of coding sequences are sometimes erroneous, which handicaps the two first approaches. This explains the reason to be of the last approach, that tries to take the best of the two other noisy approaches dealing with sequences or names. At this stage, we have definitively chosen the second approach described above, on names provided by DOGMA. Indeed, when checking all the possibilities on a few set of accurately annotated genomes, we have recovered at best the annotations on complete sequences by using this approach. Obtained results regarding gene content are discussed in the next section.

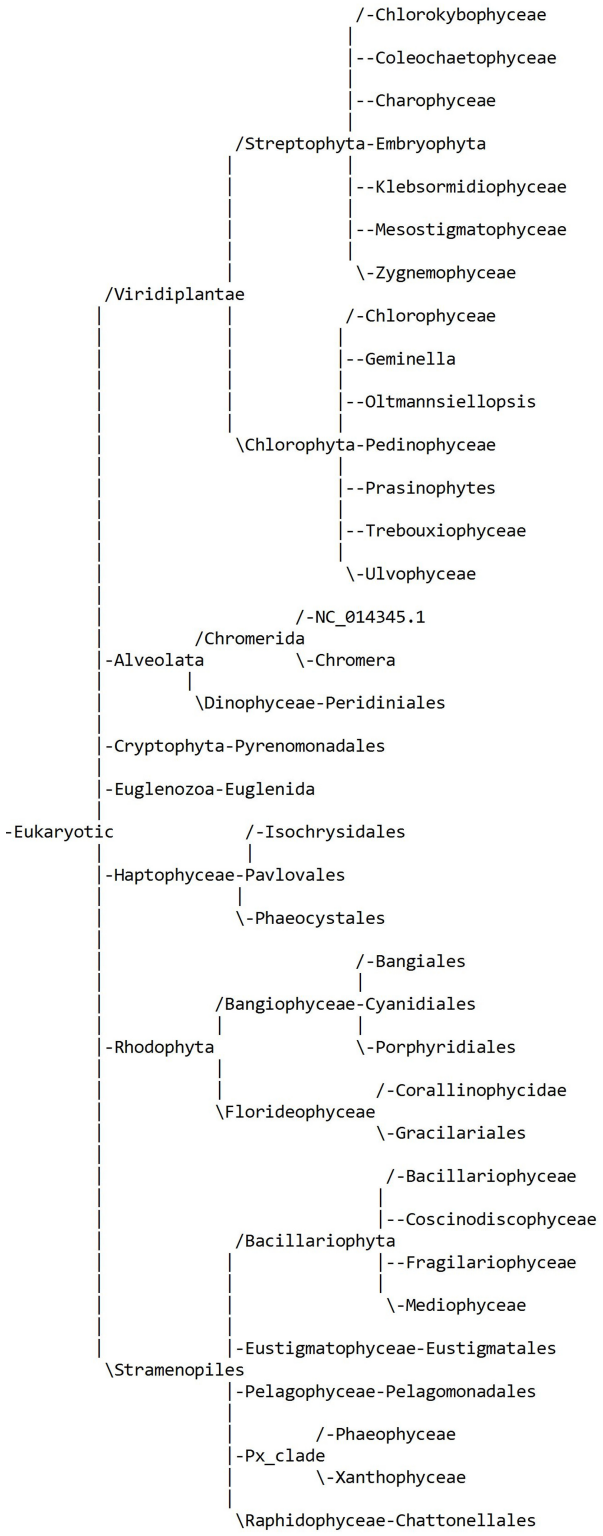


Fig. 4.1 – Taxonomy backbone tree

4.2/ Obtained results

4.2.1/ Gene content

Genes are rearranged in the genome by evolutionary events like insertion, deletion, transposition, and inversion, which are called genome rearrangements [94]. Such rearrangements can be studied by putting sets of genes at the leaves of the taxonomy tree downloaded on the NCBI website, which is partially reproduced in Figure 4.1.

It is true that this taxonomy tree is not fully accurate, as it is a general overview of plant species relationship based on dated information related to their geography, morphology, and so on. Nucleus DNA phylogeny has been used partially to recently update the taxonomy, but we have no information of support. And the nucleus evolution is not necessarily the same than the chloroplast one. However, such a taxonomic tree can be considered to have a rough, general overview of gene content evolution through various families of plants.

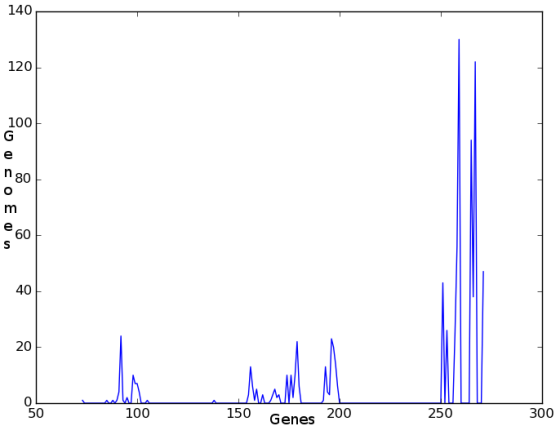


Fig. 4.2 – The distributions of chloroplast genomes depending on the genomes size.

A summary of obtained results, in terms of contents evolution at the top taxonomy level, is provided in Table 4.3. It is further detailed for the next taxonomic levels in Table 4.4. The core genome is constituted by 36 coding sequences, namely : *ATPA, ATPB, ATPH, ATPI, PETB, PETG, PSAA, PSAB, PSAC, PSAJ, PSBA, PSBC, PSBD, PSBE, PSBF, PSBH, PSBI, PSBJ, PSBL, PSBN, PSBT, PSI_PSBT, RBCL, RPL14, RPL16, RPL2, RPL20, RPL36, RPS11, RPS12, RPS12_3END, RPS14, RPS19, RPS2, RPS7*, and *RRN16*. The pan genome of the whole considered species, for its part, contains 268 genes. The core genome has thus 36 of the whole chloroplast genes, and it is too small to hope to infer a representative chloroplast phylogeny by using a subset of these 36 core genes.

To raise this issue, we decided to consider that :

1. The taxonomy tree can be considered as trustworthy at the uppers classification levels (*e.g.*, class and order) as for instance brown algae are obviously separated from land plants.
2. Errors may appear when going deeper in the taxonomy tree, for instance when considering family or genus levels. But, at this stage, the core genome is large enough to accurately apply the method of the previous chapter for inferring a supported tree.

TABLE 4.4 – Taxonomy in the second level

Taxonomy		Nb. genomes	Min N.b of pan genes	Max N.b of pan genes	Avg N.b of pan genes
Alveolata	*Chromerida*	2	253	265	259.0
	Dinophyceae	2	265	266	265.5
Cryptophyta	*Pyrenomonadales*	2	258	259	258.5
Euglenozoa	*Euglenida*	7	193	267	253.428
Haptophyceae	*Phaeocystales*	1	266	266	266.0
	Isochrysidales	1	251	251	251.0
	Pavlovales	1	258	258	258.0
Rhodophyta	*Bangiophyceae*	6	156	266	240.166
	Florideophyceae	3	251	267	258.333
Stramenopiles	*Px_clade*	6	251	271	261.166
	Bacillariophyta	20	138	271	231.35
	Eustigmatophyceae	6	253	267	262.16
	Raphidophyceae	1	258	258	258.0
	Pelagophyceae	2	73	266	169.5
Viridiplantae	*Chlorophyta*	58	156	271	244.517
	Streptophyta	717	85	271	228.638

We then have proposed to construct our accurate phylogeny by using the taxonomy tree from NCBI as backbone structure, after removing all branches after the family level. Then, for each family, process of the previous chapter can be applied on our supercomputer facilities, providing so well supported trees based on large core sequences of each considered family. Such a procedure is justified if we can find a taxonomy level, like the family one, such that almost all core genomes are significantly bigger than the 36 coding sequences found everywhere in the 845 chloroplasts. To have it confirmed, we thus further examined the relations between gene contents and taxonomy.

4.2.2/ RELATIONS BETWEEN GENE CONTENT AND TAXONOMY

We have further investigated the distribution of number of genes according to the group of species. Obtained results are reproduced in Figures 4.2 and 4.3. Four groups have appeared among the 845 genomes, which are taxonomically coherent. As shown in Fig. 4.3, the cluster of largest genomes has a number of genes ranging from 229 to 271, while in the group of smallest genomes, the lowest number of genes is for the *Viridiplantae* case. In particular, among the genomes having less than 120 genes, we found accession number NC_012903.1 (*Eukaryota, Stramenopiles, Pelagophyceae, Pelagomonadales, Aureoumbra lagunensis*), and 63 *Spermatophyta* species : 3 *Pinidae*, 58 *Magnoliophyta*, one *Cycadidae*, and finally one *Gnetidae*. We finally obtain chloroplast genomes varying from 73 to 271 genes.

We can further note that (1) most of the organisms in green lineage (green algae and land plants) have a lower number of genes in their chloroplasts compared to the red algae. (2) Most land plants have genome sizes ranging between 120 and 160 kb [95]. (3) Most of the differences in genome size are due to the number of paralogous genes. (4) According to our computation, no gene was specific to a given clade (that is, present in only one clade).

When regarding more deeply the ordered list of genes to investigate the reasons of such differences of size, it appears to us that the gene content evolution can mostly be explained

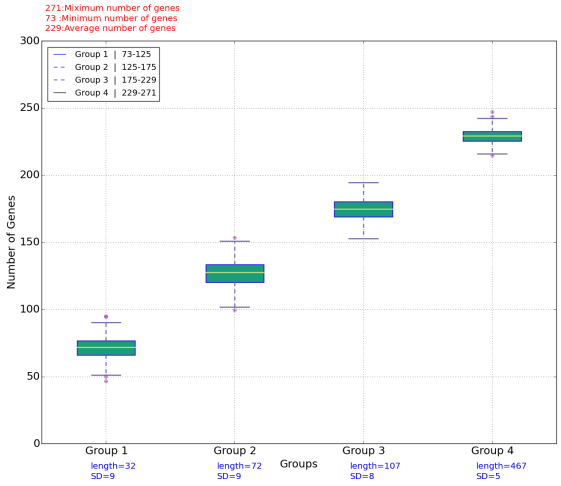


Fig. 4.3 – Classification of chloroplast genomes according to numbers of pan genes.

by repetitions of some genes and the loss of other ones : no large scale recombination is responsible of such variations. Usual case is as in Figure 4.4 for *ACCA* pan gene [1], on which single vulnerable genes are lost, possibly in various independent branches, due to delete mutations. Such results have been obtained by comparing, for each couple of close genomes, all gene names and positions, by practicing a naked eye investigation using homemade scripts. Some mutation and indel events are provided too in Table 4.5, for the sake of illustration.

TABLE 4.5 – Example of comparison between pairwise genomes from various species, to investigate the changes that occurred within branches of the tree.

Index	Clade	Sub-kingdom	Order/ Family	Genome name	N.b of pan genes	Deletion/ Insertion	Matching ratio
1	*Viridiplantae*	*Embryophyta*	*Camelineae*	*Camelina* *Barbarea*	92 267	173/0	48.46
2	*Viridiplantae*	*Embryophyta*	*Camelineae*	*Aquilaria* *Hibiscus*	101 267	164/0	28.26
3	*Viridiplantae*	*Embryophyta*	*Sapindales*	*Acer* *Azadirachta*	92 267	173/0	49.02
4	*Viridiplantae*	*Embryophyta*	*Zamiaceae*	*Lepidozamia* *Zamia*	92 267	172/0	51.66
5	*Viridiplantae*	*Embryophyta*	*Lamiaceae*	*Lavanduleae* *Perman*	92 267	173/0	49.91
6	*Stramenopiles*	*Pelagophyceae*	*Pelagomonadales*	*Aureoumbra* *Aureococcus*	73 267	193/0	27.13
7	*Viridiplantae*	*Eudicotyledons*	*Berberidoideae*	*Epimedium* *berberis*	85 267	180/0	33.52
8	*Viridiplantae*	*Eudicotyledons*	*Actinidia*	*NC_026690_1* *NC_026691_1*	92 271	174/0	48.63

1. http ://www.uniprot.org

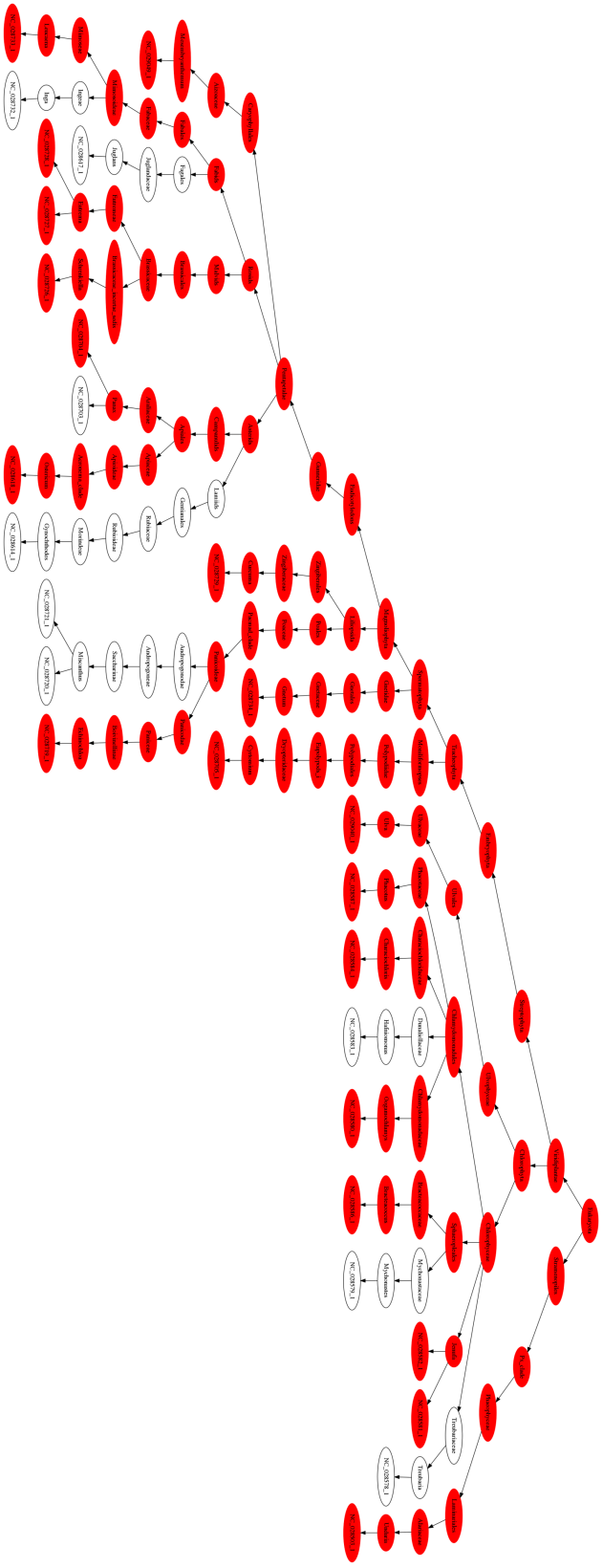


Fig. 4.4 – ACCA gene loss in various branches of the tree

4.3/ Through a well supported tree of chloroplasts

4.3.1/ How we computed our phylogenetic tree, and why

The next step to realize when trying to reconstruct the evolution of gene content over time is to deeply investigate the phylogeny of these chloroplasts, in order to obtain a tree as supported as possible. As stated previously, a branching error in the tree may lead to an erroneous transmission of an ancestral state, which is dramatically perpetuated until reaching the last universal common ancestor. However, as we considered all existing plant taxa, we faced chloroplastic sequences that have diverged a lot since two billion of years, so the core genome of these 845 sequences is very small when compared with sequence length of each representative, and inferring a tree on such a partial information will probably lead to numerous errors.

After investigations of the previous section, the approach finally chosen has been to group plant families per close packets (same family in the taxonomy). Such a grouping enlarges the number of shared gene sequences (core genes of the considered family) on which a more representative phylogeny can be computed [1]. After having aligned the core genes of each family using MUSCLE [59] on our supercomputer facilities, we then have inferred a phylogenetic tree per family. To obtain such a tree, the RAxML [46, 96] program has been employed to compute the phylogenetic maximum-likelihood (ML) function with the setup described hereafter. General Time Reversible model of nucleotide substitution, with Γ model of rate heterogeneity and hill-climbing optimization method. The outgroup, for its part, has been randomly picked from a close but different family of chloroplasts.

After such a computing, if all bootstrap values are larger than 95%, then we have considered that the phylogeny is resolved, as the largest possible number of genes has led to a very well supported tree. In case where some branches are not supported, we can wonder whether a few genes can be incriminated. Such problem has been resolved by finding the largest subset of core genes leading to the most supported tree, by the heuristic approach detailed in the previous chapter and published in [56, 1]. Obtained trees are then merged on a well-supported and representative supertree.

4.3.2/ Phylogenetic investigations

The approach detailed in the previous section has led to a well supported phylogenetic tree of the whole available chloroplasts, with the ordered list of genes at each leaf of the tree. An overview of the latter is provided in Figure 4.1. Obtained tree, available on our website, is in general coherent with the NCBI taxonomy, except in some specific locations.

By going into the details of the obtained tree, it is well known that the first plants endosymbiosis ended in a great diversification of lineages comprising Red Algae, Green Algae, and Land Plants (terrestrial). The interesting point in the production of our results is that the organisms resulting from the first endosymbiosis are distributed in each of the lineages found in the chloroplast genome structure evolution as outlined in Figure 4.1. More precisely, all Red Algae chloroplasts are grouped together in one lineage, while Green Algae and Land Plant chloroplasts are all in a second lineage. Furthermore, organisms resulting from the secondary endosymbioses, as listed in Table 4.4, are well localized in the tree : both the chloroplasts of Brown Algae and *Dinoflagellates* representatives are found exclusively in the lineage also comprising the Red Algae chloroplasts from which

they evolved, while the *Euglens* is related to Green Algae from which they evolved. This latter makes sense regarding biology, history of lineages, and theories of chloroplasts origins (and so photosynthetic ability) in different *Eucaryotic* lineages [97].

4.4/ CONCLUSION

In this chapter, we made significant progress in the study of chloroplastic sequence evolution, by providing material and methods required in the quest of the ancestral genome of the chloroplasts. A large set of complete chloroplast genomes has been studied *de novo* regarding both core and pan genomes, phylogenetic relationship, and gene content modifications. We then started to study the produced data, by emphasizing some remarkable relations between well-known events of the chloroplast history and the evolution of gene contents over the phylogenetic tree.

Our intention is now to investigate more systematically such relations between remarkable ancestral nodes in the tree, endosymbiosis events, and evolution of gene content. We will wonder whether some branches of the trees are statistically remarkable when considering gene content (for instance, do we have a correlation between the presence or absence of a subset of genes, and a particular taxonomy). To do so, we must investigate, in the following chapters, how gene ordering and content of each ancestral node can be computed using ad hoc algorithms, how ancestral DNA sequences can be inferred, and finally how ancestral intergenic regions can be deduced. By producing such ancestral genomes, it will then be possible to investigate hypotheses formulated by biologists, regarding the origin of chloroplasts, their recombination events, and the transfer of some material to the nucleus.

5

Ancestral Reconstruction and Investigations of Genomic Recombination on Campanulides Chloroplasts

In this chapter, we propose a semi-automated method to rebuild genome ancestors of chloroplasts by taking into account gene duplicate. Two methods have been used in order to achieve this work : a naked eye investigation using homemade scripts, whose results are considered as a basis of knowledge, and a dynamic programming based approach similar to Needleman-Wunsch. The latter fundamentally uses the gestalt pattern matching method of sequence matcher to evaluate the occurrences probability of each gene in the last common ancestor of two given genomes.

The two approaches have been applied on sets of sequences of reasonable sizes, making it possible to apply a manual inspection for cross validation. The chosen chloroplastic genomes are from *Apiales*, *Asterales*, and *Fabids* orders, the latter having inversions too. These closely related group of families have been chosen because *Apiales* species do not undergo insertions or deletions, while they slightly occur in the *Asterales* and *Fabids* orders. We then carried out a series of experiments to extensively verify and compare the obtained ancestral reconstruction results with the latest released approach called MLGO (Maximum Likelihood for Gene-Order analysis). The first part of this chapter has been presented in the 2015 SeqBio workshop [98] and is currently submitted to Journal of Integrative Bioinformatics (JIB).

5.1/ Introduction

This chapter starts with proposing a way to reconstruct the Last Universal Common Ancestor (LUCA) of the whole set of all available chloroplastic genomes. It aims at investigating scientific and technical obstacles that may appear when trying to answer this difficult question. The proposed ancestral reconstruction is twofold. Firstly, a few number of families is selected as running example, from the large collection of complete chloroplastic genomes presented in the previous chapter. Their coding sequences have been further extracted and automatically annotated them following the approach detailed in the previous chapter, and in [56, 57, 58]. Using the commonly genes by these species, a well-supported

phylogenetic tree has been obtained. However, as signaled previously, the core genome of the whole specie is too small to produce an accurate tree. We then have decided to apply the strategy of the two previous chapters to obtain a well supported tree of the chloroplasts we considered here, that are composed by combinations of a few number of close families.

This first step being achieved, the second stage is now to design algorithms that study the evolution of gene content and ordering among the tree, and the latter must be validated with naked eye on these small combinations of plant families. Our proposal in this chapter focuses on this second stage, and illustrates which kind of results can be obtained on three small groups of *Campanulides* species. It will be completed in further work, by obtaining ancestral nucleotide sequence of each gene, and by filling intergenic regions using either state-of-the-art or novel algorithms.

As previously stated, ancestral genome reconstruction has already been investigated in the literature [13, 10]. Usually, state of the art algorithms deal with permutations of integers. Our problem applied to chloroplasts may appear as more difficult, as we relax the permutation hypothesis. However, in the classical Multiple Genome Rearrangement Problem [45], targeted genomes are bacterial or nucleus ones, which have much more genes than a chloroplast. Furthermore, gene order and content do not evolve so much when considering related plant species. Such observations explain why state-of-the-art algorithms cannot be applied to our particular problem even if this latter should be solvable.

5.2/ Presentation of the problem

Let us consider a set of complete chloroplastic genomes for close plant species, like the *Apiales* order as shown in Table 5.1.

Taken into consideration results from the previous chapter, we assume first that :

1. Each genome has been annotated with Dogma [55]. By doing so, the same gene prediction and naming process has been thus applied with the same quality of annotation. At this level, each genome is described by an ordered list of gene names, with possible duplicates.
2. The sequences inside the core genome have been multialigned, and a well supported phylogenetic tree has been obtained based on this alignment as shown in Figure 5.1 for *Apiales* order. This stage may necessitate to remove a few core genes in each family, by using methods detailed in the previous chapters and in [56, 1, 99].

Our objective is then to reconstruct ancestral genomes at each node of the phylogenetic tree until the root node.Such a reconstruction is threefold : it requires first to find the

Table 5.1 – Genomes information of *Apiales* order.

Organism name	Accession	Genome Id	Sequence length	Number of genes	Lineage
Daucus carota	NC_008325.1	114107112	155,911 bp	138	Apiaceae
Anthriscus cerefolium	NC_015113.1	323149061	154,719 bp	132	Apiaceae
Panax ginseng	NC_006290.1	52220789	156,318 bp	132	Araliaceae
Eleutherococcus senticosus	NC_016430.1	359422122	156,768 bp	134	Araliaceae
Aralia undulata	NC_022810.1	563940258	156,333 bp	135	Araliaceae
Brassaiopsis hainla	NC_022811.1	558602891	156,459 bp	134	Araliaceae
Metapanax delavayi	NC_022812.1	558602979	156,343 bp	134	Araliaceae
Schefflera delavayi	NC_022813.1	558603067	156,341 bp	134	Araliaceae
Kalopanax septemlobus	NC_022814.1	563940364	156,413 bp	134	Araliaceae

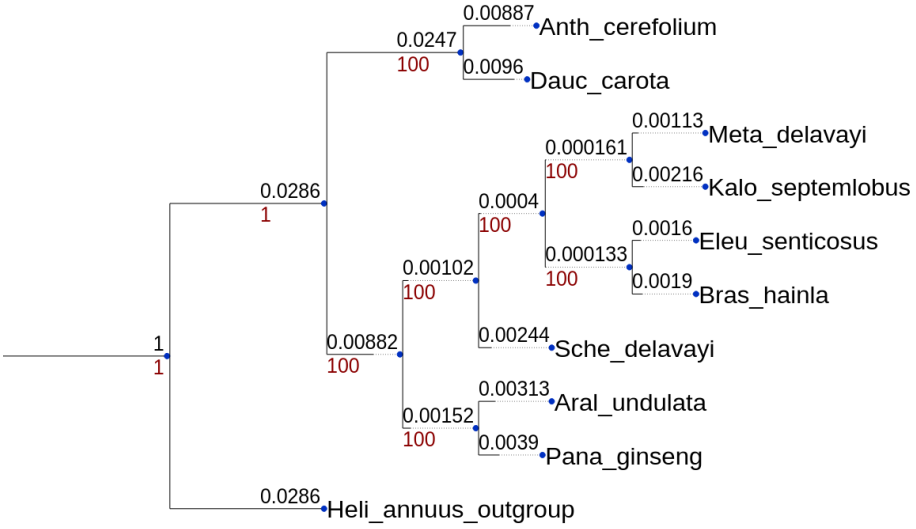


Fig. 5.1 – Most supported phylogenetic tree obtained from *Apiales* order.

ordered list of genes of each ancestor, then the DNA sequence of each ancestral gene, and finally to fill in intergenic regions.

For all three steps of reconstruction, the authorized operations are :

- insertion, deletion, duplicate, or inversion of one or a block of genes, at gene lists level ;
- operations which are commonly considered in the Needleman-Wunsch edit distance [30] (insertion, modification, or deletion of a nucleotide, together with opening and enlarging a gap), at DNA sequence levels.

The operations listed above allow to reduce the number of leaf nodes. Notice that the global optimum over the whole tree may be obtained with a few local solutions (one ancestor of two genomes) that are not optimal.

5.3/ Ancestral analysis methods

Two methods have been applied on our set of data : an automatic gestalt pattern based gene features matching process and a naked eye manual cross-validation. Let us begin by introducing the manual approach. This was completed first to determine which ancestor genomes our automatic algorithm should produce.

5.3.1/ Method I : Naked eye investigation

As stated above, this method was not an algorithm that automatically builds the ancestors of the provided genomes, but it was a method applied by hand, as follows. We have produced *ad hoc* software to represent each triplet constituted by two sister species and their closest cousin as three parallel lines, as described in Figure 5.2. On each line are located numerous equidistant vertices, one per coding sequence in the associated

genome, and all sequences having the same gene name according to Dogma are linked by an edge.

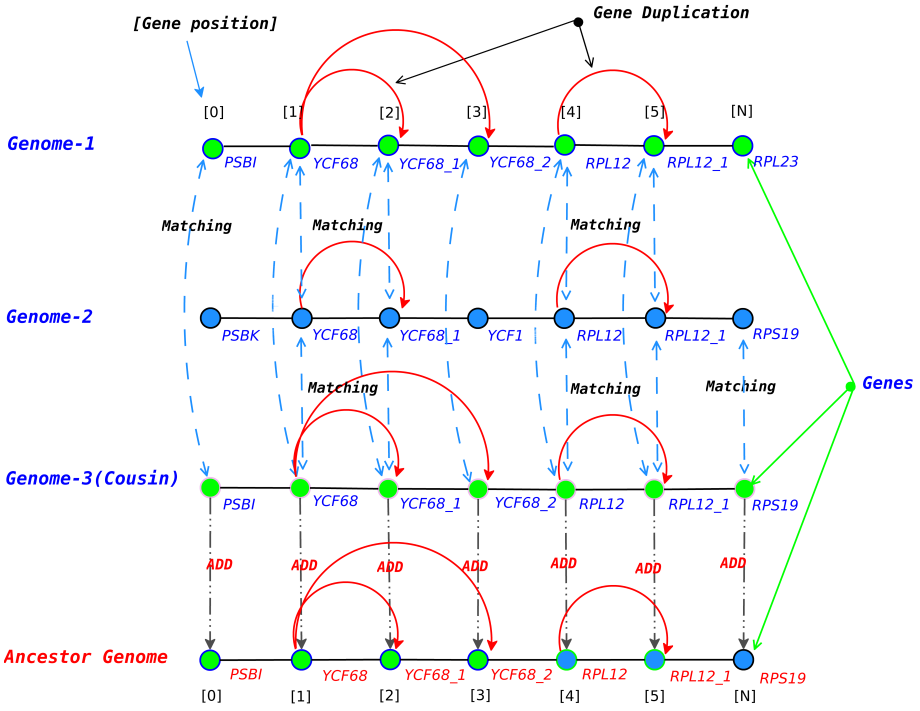


Fig. 5.2 – Simulation of ancestral reconstruction process between two genomes

We then have manually deduced the ancestral genome of each couple of sister species by a consensus approach. Each part shared in common in their genomes is put in the ancestor. In case of a difference, the two sister genomes are compared locally with their closest cousin. If the latter agrees with one of the two sisters, the agreement (sequence of genes) is put on the ancestor and this part of the lines is considered as resolved. If the closest cousin cannot help to resolve the situation, because it locally presents a third pattern different from the two sisters, then one or more new close cousins are considered, and the solution that minimizes the number of rearrangement operations is finally chosen, leading to the local ancestor gene list (parsimonious approach).

By doing so and verifying our results three times (by matching the gene contents of each genome with three cousin genomes in the same clade), we obtained a trustworthy ancestral list of genes at each internal node. Our next objective was then to automatically recover these ancestors.

5.3.2/ Method II : Ancestor Prediction based on Gene Contents

This method is fivefold :

— **Step 1.** In this stage, all the nodes are named following an alphabetical order. Each letter in an internal node represents an ancestor genome.

An example of the result of this stage can be seen in Figure 5.3 when applied on a Apiales and Asterales species tree.

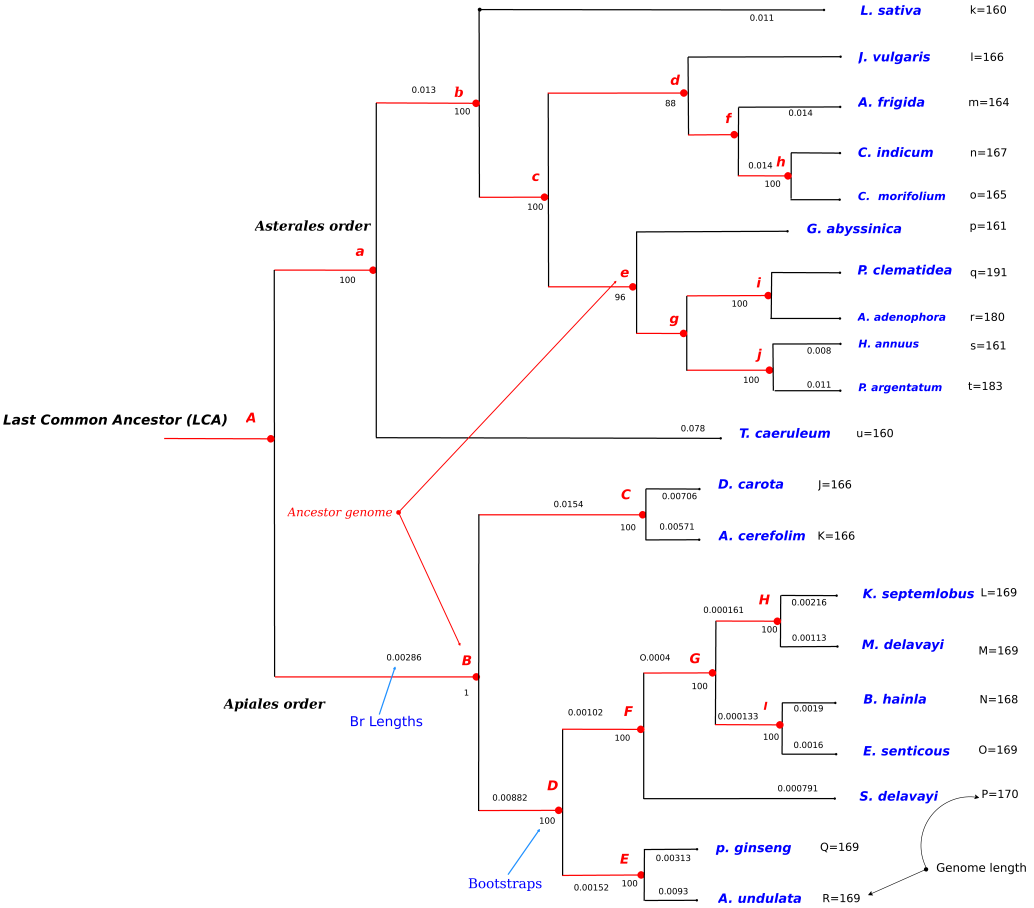


Fig. 5.3 – *Apiales* and *Asterales* species tree. The numbers shown at each branch are bootstrap values computed by RAxML [1]. A letter has also been associated to each internal node as defined in Step 1.

— **Step 2.** This stage automatically selects the two closest sister species according to the Needleman-Wunsch distance applied to lists of genes. The other species are then ordered according to their distance in the tree (number of nodes between it and one of the two sister species, and Needleman-Wunsch distance to solve ex-aequo cases), yielding to what is further denoted as an ordered list of cousins.

— **Step 3** In the simplest situation, all the gene couples between the two sister species completely match. In this case, the ancestor is directly deduced as being the same as its children. In any other situation (*i.e.*, there is at least one deleted, duplicated, or inserted gene. . .), then a deeper investigation is initiated using one or more cousin genome(s).

In this case, we iterate all genes in the two sister genomes U_1 and U_2. If gene g_i in U_1 matches properly in name, position, and orientation with g'_i in U_2, then we add it in the ancestor genome γ at position i. Otherwise, consider the gene g''_i at the same location in the first cousin genome : if g_i or g'_i is equal to g''_i then add the most frequent gene to the ancestor genome γ in position i, else this gene is considered as an insertion.

Figure 5.4 gives a simulation example of the considered procedure. We suppose that the leaves A, B, C, D, and E are genes, and the objective is to predict the ancestor α_1. Note that genes A, B, and D match in positions. Concerning the problematic C gene

between these two genomes, we need a cousin to determine whether it is present in the α_1 ancestor genome or not. One or both genomes in α_2 subtree are considered to be cousin(s) to treat the problem of gene C. The two cousin genomes have one copy of gene C in their gene lists. According to our voting system, gene C will be in α_1 ancestor and one delete operation is recorded (AB_D). An insert state is also marked in α_2 subtree, where gene E did not appear in either cousin genomes of α_1 tree, nor in its sister. Such a deletion is illustrated in Figures 5.5.

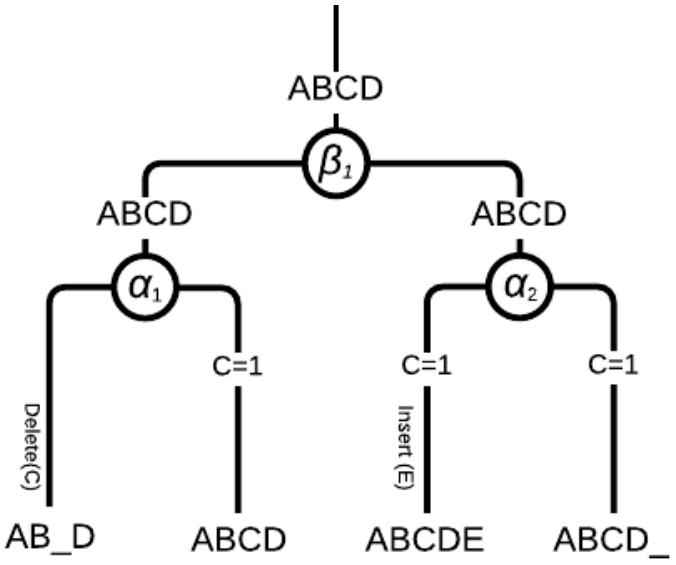


Fig. 5.4 – Simulation of gene investigation step between two genomes.

The conflict resolution presented above has been refined by considering the gestalt pattern matching method [100] based on dynamic programming like in Needleman-Wunsch.

— **Step 4.** After applying the previous step to all genes of the sister species, their ancestor is then reconstructed. The subtree of the two sister genomes is then replaced by the list of genes of their ancestor.

— **Step 5.** Repeat from Step 2 until the final root ancestor is constructed.

5.4/ Discussion

We performed the whole process of ancestral gene order reconstruction on two data sets, namely : *Apiales* and *Asterales*. The starting point is the phylogenetic tree already presented in Figure 5.3.

5.4.1/ The *Apiales* order

Let us first consider the *Apiales* order. We then apply the manual and the automatic approaches to infer ancestral states at each internal node of the tree. The results were convergent and they lead to the following conclusions regarding the evolution of gene content among the tree.

We first focused on the evolution of duplicates summarized in Table 5.2. For each duplicate, the number of copies is specified too. Let us now enter into details regarding the leaves of the phylogenetic tree shown in Figure 5.3.

TABLE 5.2 – Gene duplicate for each genome in *Apiales* order.

	Genome name								
Gene name	*B. hainla*	*E. senticosus*	*A. undulata*	*P. ginseng*	*M. delavayi*	*A. Cerefolium*	*D. carota*	*S. delavayi*	*K. septemlobus*
ACCD	0	0	0	0	0	2	2	0	0
RPS12	2	2	2	2	2	2	2	2	2
NDHA	2	2	2	2	2	2	2	2	2
NDHK	2	2	2	2	2	0	0	2	2
RP12	4	4	4	4	4	4	4	4	4
RPS7	2	2	2	2	2	2	2	2	2
RPOC1	2	2	2	2	2	2	2	2	2
YCF2	4	4	4	4	4	4	4	4	4
YCF3	3	3	3	3	3	3	3	3	3
RPL23	2	2	2	2	2	2	2	2	2
YCF1	3	2	2	2	2	2	2	3	2
CLPP	3	3	3	3	3	3	3	3	3
ATPF	2	2	2	2	2	2	2	2	2
ORF56	4	4	4	4	4	2	4	4	4
RRN23	2	2	2	2	2	2	2	2	2
YCF68	4	6	6	6	6	2	0	6	6
RRN5	2	2	2	2	2	2	2	2	2
RRN4.5	2	2	2	2	2	2	2	2	2
YCF15	2	2	2	2	2	4	4	2	2
RRN16	2	2	2	2	2	2	2	2	2
ORF42	2	2	2	2	2	0	2	2	2
RPS19	0	0	0	0	0	2	2	0	0
TRNV-GAC	2	2	2	2	2	2	2	2	2
TRNL-UAA	2	2	2	2	2	2	2	2	2
TRNL-CAA	2	2	2	2	2	2	2	2	2
TRNV-UAC	2	2	2	2	2	2	2	2	2
TRNR-ACG	2	2	2	2	2	2	2	2	2
TRNN-GUU	2	2	2	2	2	2	2	2	2
TRNA-UGC	4	4	4	4	4	4	4	4	4
TRNI-GAU	4	4	4	4	4	4	4	4	4
TRNI-CAU	2	2	2	2	2	2	2	2	2
RPS12_3END	2	2	2	2	2	2	2	2	2

- Sister species *E. senticosus* and *B. hainla* have been considered first, with *K. septemlobus* playing the role of the cousin. After manual and automatic comparisons, we found that the gene *YCF1* is present twice in *E. senticosus*, while it is in three copies in *B. hainla*. As the cousin has only two sequences of *YCF1*, we suggest that the latter is present twice in the ancestor : one gene has been inserted in *B. hainla*. Similarly, *YCF68* is in 4 copies in *B. hainla* and in 6 copies in the sister species. As the cousin presents 6 copies too, we can deduce that the common ancestor of *E. senticosus* and *B. hainla* contains 6 copies of this gene. In other words, two copies of *YCF68* have been removed in *B. hainla*. All the other genes are similar in both names and locations, and so we are able to deduce the ancestral genome *(I)*.
- The sister genomes *A. undulata* and *P. ginseng* have exactly the same ordered list of genes, which is thus assigned to their last common ancestor *(E)*.
- Similarly, all couples of sister species *A. undulata* and *P. ginseng*, *M. delavayi* and *K.septemlobus*, *S.delavayi* and *M. delavayi*, and finally *K. septemlobus* and *E. sentucosus* match perfectly when considering each couple of sister genomes. In other words, they have not deviated from their respective last common ancestors, which presents the same sequence as their children species. Selected genomes are aligned graphically as shown in Figure 5.5. We then identify, by using naked eyes investigation and human thinking, the most parsimonious scenario applied on a deduced ancestor, which can lead to these two children using the lowest number of edit operations (such as inserted and deleted genes). Figure 5.6 shows this

matching process applied on *Meta_Delavayi* and *kalo_septemlobus*, which have a core genome of 169 genes (only the 23 first genes are depicted). Note that, in this example, *Meta_Delavayi* (L) and *kalo_septemlobus* (M) match completely, so the ancestor H is very easy to obtain ($H = L \cap M$ has 169 genes).

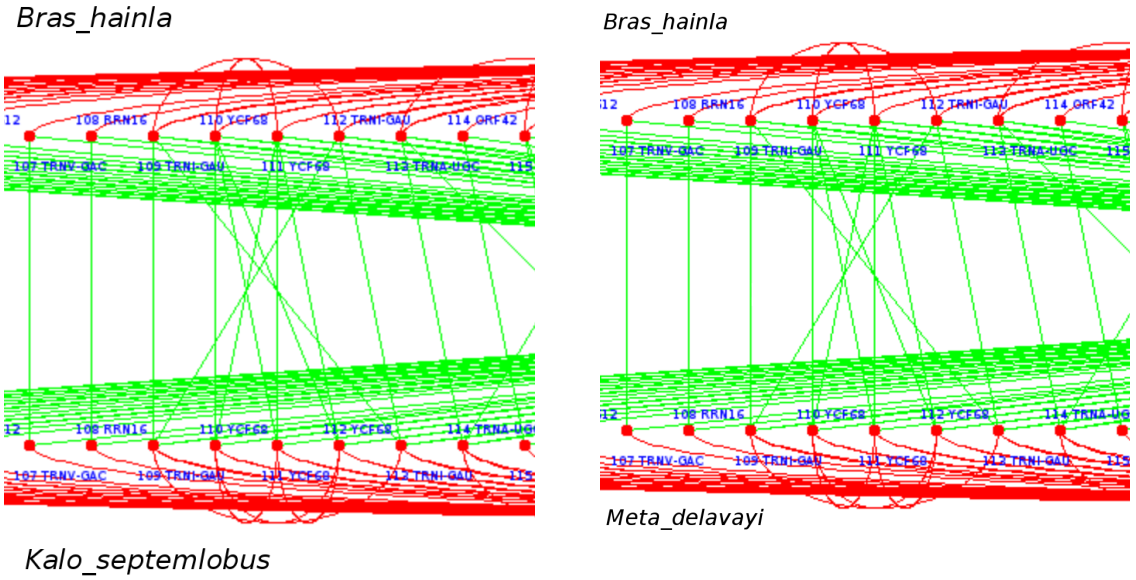


Fig. 5.5 – Graphical presentation of genes alignment between pairs of genomes (*Bras_hainla*, *Kalo_septemlobus*) and (*Bras_hainla*, *Meta_delavayi*)

- Let us finally compare *A. cerefolium* and *D. carota*. *YCF68* gene exists in 2 copies in *A. cerefolium* while it is missing in *D. carota*, as shown in Figure 5.7. The cousin, for its part, also contains the gene *YCF68* (in 6 copies), and so our algorithm concludes to the presence of this gene (in 2 copies) in the ancestor of *A. cerefolium* and *D. carota*. Additionally, *D. carota* contains 4 copies of *ORF56*, while this gene is only represented twice in its sister. As the cousin genome has 4 representatives of *ORF56*, we can reasonably deduce that this is the case too in the ancestor of these two sister species : two copies of the gene *ORF56* have been deleted from the genome *A. cerefolium*. Such decisions are depicted in Figure 5.7 (B), which shows a specific region of the ancestor genome *(C)*. This region has been generated by our algorithm, which has been applied on *A. cerefolium* and *D. carota*, and it has been cross manually validated.

The process detailed above continues with the obtained ancestors and is repeated until reaching the root of the tree : the Last Universal Common Ancestor (LUCA) of *Apiales*. By operating this reconstruction stage, we found that chloroplasts of this order have not faced so much deletion or insertion in their genomes. Indeed, in most of the cases, the disparity comes from the variation in numbers of gene copies. The obtained results are summarized in Figure 5.8.

5.4.2/ THE *Asterales* ORDER

We have then examined the *Asterales* order, which is close to the *Apiales* one. Table 5.3 contains what has been deduced from our experiments on this order. As can be seen, *As-*

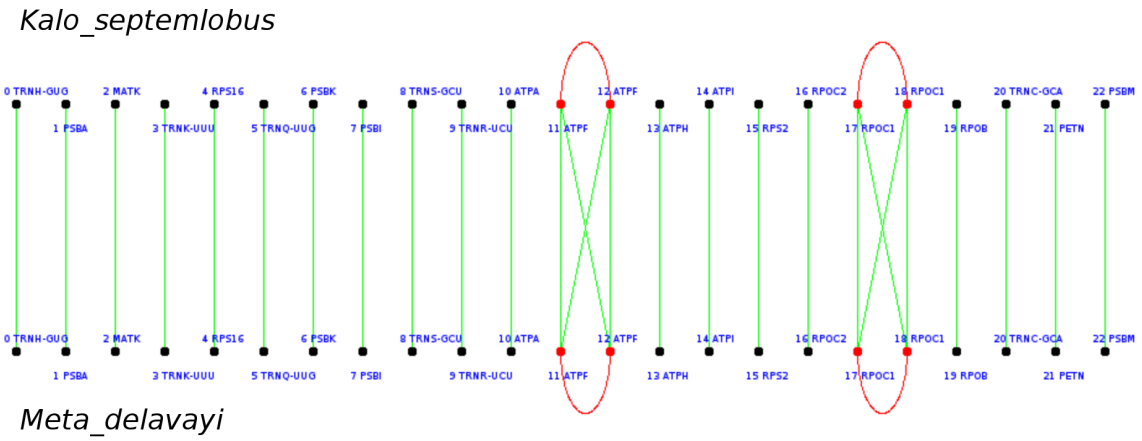


Fig. 5.6 – Graphical presentation of genes alignment between genomes *Kalo_septemlobus* and *Meta_delavayi*

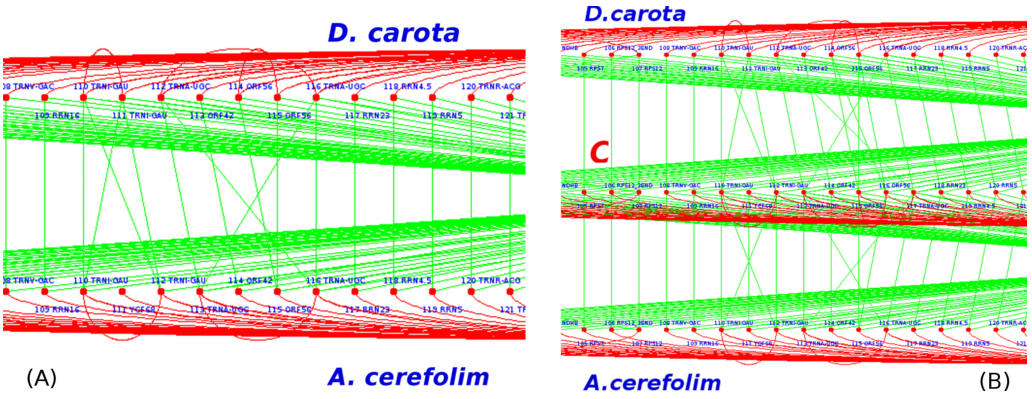


Fig. 5.7 – (A) : Example of gene correspondances in sister genomes *D. carota* and *A. cerefolium*. For instance *YCF68* is found in position 111 in *A. cerefolium* while it is missing from *D. carota*. Additionally, *ORF56* is in 2 copies, positions 114 and 115, in *D. carota*, while this gene is only represented once at position 115 in its sister. (B) Comparison between two sisters. The result is the ancestor genome *(C)*. We can reasonably deduce that two copies of *ORF56* have been deleted from genome *A. cerefolium*. The ancestor, for this part, contains too the gene *YCF68*, which has been deleted from the genome *D. carota*

terales genomes have faced much more modifications compared to the *Apiales* ones. This difference between the two orders lead to a larger variation in the lengths of *Asterales* genomes.

For the sake of illustration, let us consider for instance the chloroplast of *H. annuus*. It only contains 161 coding sequences while its sister species, namely *P. argentatum*, has 183 genes. The matching process previously described has led in this case to an ancestor of size 162. More precisely, 23 genes have been inserted and two other ones have been deleted in *P. argentatum*, while only one gene has been removed in *H. annuus*, as described in Figure 5.9.

In this second order and in most cases, genes are comparable in both names and locations,

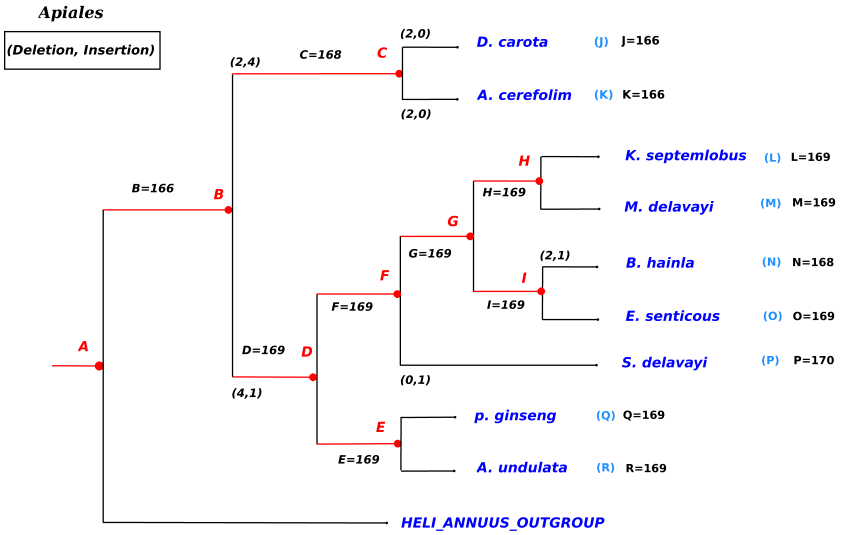


Fig. 5.8 – Insertion and deletion events found during ancestor reconstruction on *Apiales* order. Letters in red refer to ancestor genomes (their lengths are provided too).

having the same positions if we do not consider duplicates. Indeed, almost all differences in this set of chloroplastic genomes come from a variation in the number of copies, which has been inferred well by our automatic tool. Let us now investigate a third order, to compare it with *Apiales* (only a few variations of genomes) and *Asterales* (large variety in duplicates).

5.4.3/ THE *Fabids* ORDER

It was easy to deal with *Apiales* order, while *Asterales* improved the complexity of the ancestral reconstruction, due to duplicates. However, in both cases the proposed algorithm was able to recover results that have been inferred manually (naked eye investigation). We now consider a larger and more complicated order, namely the *Fabids*, to evaluate the performances of our proposal when facing a complex collection of genomes.

Indeed, the main problem with this new order is that it contains large scale inversions in some branches, while it was not the case with the two other orders previously studied. In this case, a single inversion detection algorithm has been able to highlight helpful information regarding such regions, like the beginning and the end (insertion or deletion) of reversals. However, the most difficult case where insertions or deletions are inside the inversion zone is difficult to handle.

In this situation, we proposed to select one of the two sisters to operate as a reference. We then search for the best cousin within the same clade, and we compare the status of each gene in this region (matching, or need insertion or deletion). Most of the reversal regions we considered match at the genes names level, but with reverse positions. Figure 5.10 presents our finding on *Fabids* order, with information about the length of each node. We provide also various rearrangement information like the number of insertion, deletion, and inversion.

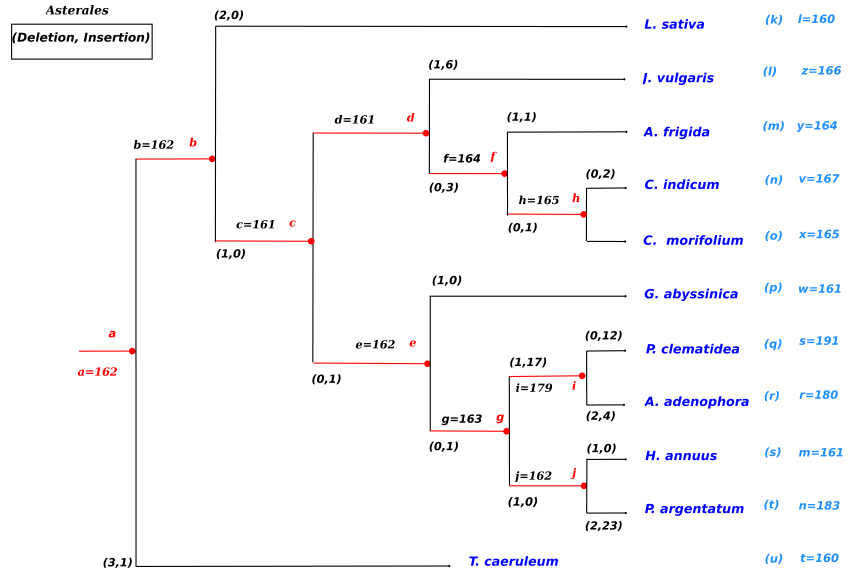


Fig. 5.9 – Summary of the complete ancestral genomes reconstruction of the *Asterales* order. Insertion and deletion events are provided, with names and length of each internal node.

5.4.4/ Comparison with MLGO

For the sake of comparison, we have examined the ancestral genome contents of both *Apiales* and *Asterales* species with MLGO tool, which stands for Maximum Likelihood for Gene Order Analysis [1]. This latter is, to the best of our knowledge, the first web tool for phylogeny and ancestral genomes reconstruction compatible with genome rearrangements [6].

On the one hand, the ancestors in the *Apiales* order, provided either by our approach or with MLGO, are very similar in terms of gene contents. However our method outperforms MLGO when investigating the specific location of genes and their number of duplicates. On the other hand, results are very different for some nodes in the *Asterales* case, as summarized in Table 5.4. For instance, when gene *YCF1* in internal node d has 2 copies in both of its two children and their closest cousin has 2 occurrences of this gene too. In this case, our algorithm proposes to set the number of *YCF1* in d to 2, while MLGO produced only one copy. Similar consequences can be outlined in the most difficult case, namely the *Fabids* order, as highlighted by Table 5.5. At each time, our algorithm outperforms the MLGO results by producing what is the most likely ancestral state (numbers of genes and their positions) in each situation. For instance, considering the ancestral node *M*, we found that gene *INFA* is missing in the first child while it is present in the second one. We also found it in the two closest cousins, with one copy at each time. The most reasonable scenario is to consider that the ancestral node under consideration also has a single copy of *INFA*. This result is produced by our algorithm, while MLGO considers that *M* must not have *INFA* in its genome. Other divergent results can be reported, as in the ordinary case of node *E* : gene *ATPF* is present once in each of the two children. So our algorithm considers that it is present once in *E*, while with MLGO, we found that this node must contain two copies of *ATPF*. Other nodes are problematic in the MLGO case, for example,

1. http://www.geneorder.org

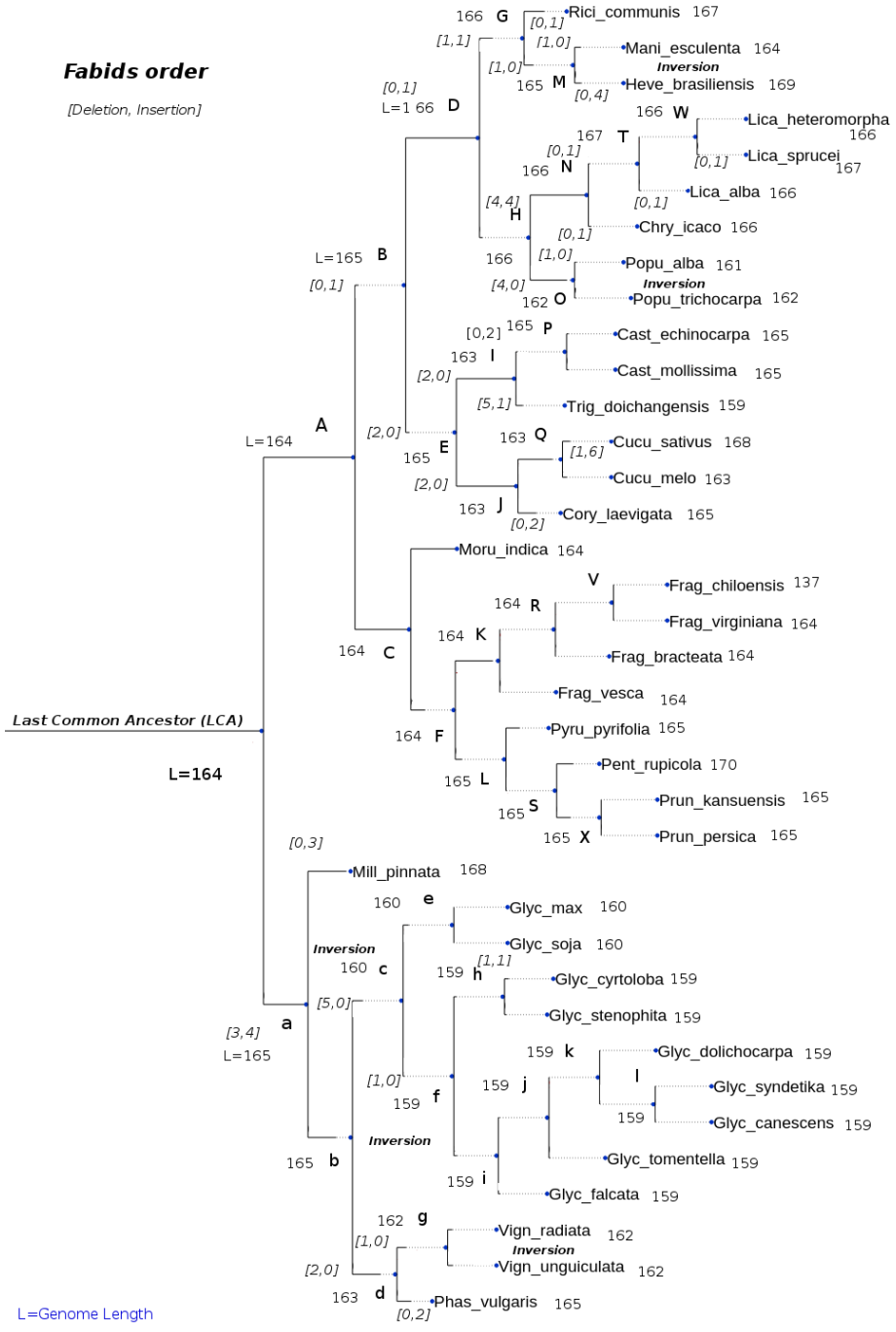


Fig. 5.10 – A phylogenetic tree in the reconstruction of the *Fabids* ancestor and the unambiguous reconstruction accuracy of our algorithms on this tree. Alphabetic characters represent the ancestors. L stands for the length of each node (number of genes), *[D, I]* describes the number of deletions and insertion, while inversions are indicated too.

{*a, b, c, d*} as can be seen in Table 5.5. Each time, our algorithm produces results in agreement with the one that have been deduced manually, while in some cases MLGO has yielded surprising results.

TABLE 5.3 – Gene duplicate for each genome in *Asterales* order.

	Genome name									
Gene name	*G. abyssinica*	*J. vulgaris*	*L. sativa*	*A. frigida*	*C. indicum*	*C. morifolium*	*P. clematidea*	*A. adenophora*	*H. annuus*	*P. argentatum*
ACCD	0	0	0	0	0	0	2	0	0	0
NDHB	4	4	4	4	4	4	4	4	4	4
RPS12	2	2	2	2	2	2	3	3	3	2
NDHA	0	0	2	2	0	0	0	0	0	0
NDHK	2	2	2	2	2	2	2	2	2	0
NDHF	0	0	0	0	0	0	0	0	0	2
RPL2	4	4	4	2	2	2	2	2	4	6
RPS7	2	2	2	2	3	3	3	3	2	4
YCF2	2	2	2	2	2	2	7	2	2	6
YCF3	3	3	3	3	3	3	3	3	3	3
RPL23	2	2	2	2	2	2	4	4	2	2
YCF1	0	0	0	0	2	2	4	3	0	4
CLPP	3	3	3	3	3	3	3	3	3	3
ATPF	2	2	2	2	2	2	3	2	2	2
ORF56	4	4	4	4	4	4	4	4	4	4
RRN23	2	2	2	2	2	2	2	2	2	2
YCF68	2	6	2	2	4	4	5	4	2	2
RRN5	2	0	2	2	2	2	2	2	2	0
RRN4.5	2	2	2	2	2	2	2	2	2	0
YCF15	2	4	2	2	2	2	2	2	2	0
RRN16	2	2	2	2	2	2	2	2	2	0
ORF42	2	2	2	2	2	2	0	2	2	0
RPS19	2	0	0	0	0	0	3	3	2	2
RPL14	0	0	0	0	0	0	2	2	0	0
RPS3	0	0	0	0	0	0	0	0	0	2
ORF188	0	0	0	0	0	0	0	0	0	0
CCSA	0	0	0	0	0	0	0	0	0	0
RPOB	0	0	0	0	0	0	0	0	0	3
RPOA	0	0	0	0	0	0	0	0	0	2
PSBD	0	0	0	0	0	0	0	0	0	3
TRNA-UGC	4	4	4	5	5	5	4	4	4	2
TRNI-GAU	4	4	4	4	4	4	4	4	4	4
TRNI-CAU	0	2	0	0	0	0	0	0	2	2
TRNI-ACG	2	0	2	2	2	2	2	2	0	0
TRNR-ACG	2	2	2	2	2	2	2	2	2	2
TRNN-GUU	2	2	2	2	2	2	2	2	2	2
TRNV-GAC	2	2	2	2	2	2	2	2	2	0
TRNL-UAA	2	2	2	2	2	2	2	2	2	0
TRNL-CAA	2	2	2	2	2	2	4	4	2	0
TRNV-UAC	2	0	2	2	2	2	2	2	2	0
TRNF-GAA	2	0	0	0	0	0	2	2	0	0
TRNT-GGU	0	0	0	2	2	2	0	0	0	0
TRAN-GCA	0	0	0	0	0	0	2	2	0	0
TRNS-GCU	0	0	0	0	0	0	2	3	2	2
TRNR-UCU	0	0	0	0	0	0	0	0	0	2
RPS12_3END	2	2	2	2	2	2	2	2	2	2

TABLE 5.4 – The variation in comparison results of ancestral genomes nodes on *Asterales* order with MLGO.

		Species genome				Ancestor results	
Ancestor node	Gene name	Genome 1 (Nb of genes)	Genome 2 (Nb of genes)	Cousin genome 1 (Nb of genes)	Cousin genome 2 (Nb of genes)	Ancestor	MLGO
j	*RPOA*	1	2	3	2	2	1
g	*RPOA*	2	2	1	1	2	1
g	*TRNF-GGA*	2	1	2	2	2	1
e	*TRNF-GGA*	2	2	1	1	2	1
d	*YCF1*	2	2	2	1	2	1

TABLE 5.5 – The variation in ancestral genomes nodes which were achieved by comparing our method results with *MLGO* tool on *Fabids* order.

		Species genome				Ancestor results	
Ancestor node	Gene name	Genome 1 (Nb of genes)	Genome 2 (Nb of genes)	Cousin genome 1 (Nb of genes)	Cousin genome 2 (Nb of genes)	Ancestor	MLGO
M	*INFA*	—	1	1	1	1	—
N	*ACCD*	2	2	2	1	2	1
T	*ACCD*	1	2	2	2	2	1
Q	*YCF1*	1	2	1	1	1	2
E	*NDHK*	2	2	1	1	2	1
E	*INFA*	2	1	—	—	1	—
E	*ATPF*	1	1	2	2	1	2
d	*RPS16*	2	—	1	1	1	2
c	*RPS19*	*2*	*1*	*2*	*2*	*2*	*1*
b	*RPS19*	*2*	*2*	*1*	*2*	*2*	*1*
a	*PSBG*	*1*	—	—	—	—	*1*
a	*TRNK-UUU*	*2*	*1*	*2*	—	*2*	*1*
a	*NDHK*	*1*	*2*	*2*	—	*2*	*1*

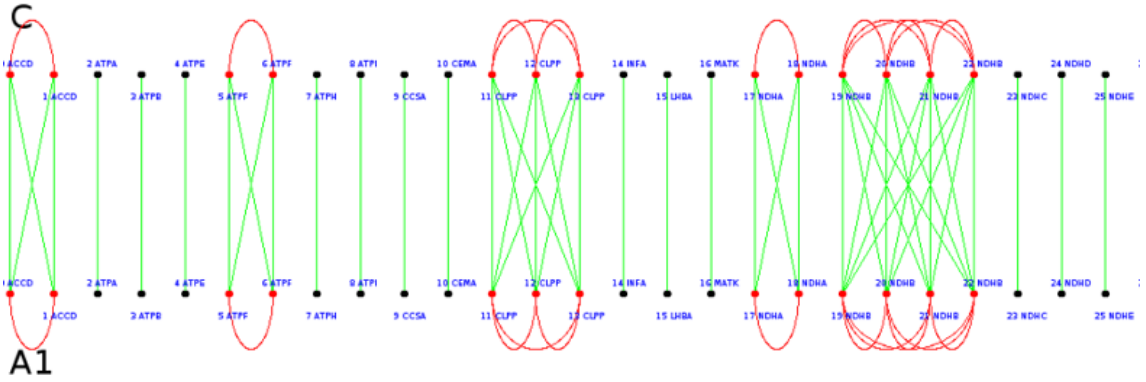


Fig. 5.11 – Example of comparison with MLGO on *Apiales* order. We show similarity in gene contents between our results, ancestor node *(C)*, and ancestor node *(A1)* from MLGO.

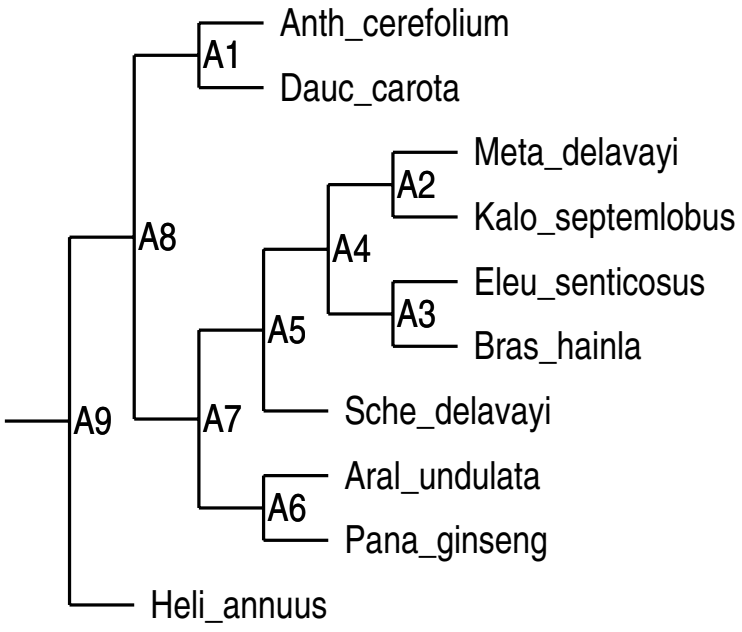


Fig. 5.12 – *Apiales* order tree produced by MLGO.

5.5/ CONCLUSION

This chapter has presented a first ancestral reconstruction of gene content and order and its application to *Apiales*, *Astrales*, and *Fabids* orders. The approach can be considered as a recursive tree reduction. Experiments have shown that this approach is more accurate than MLGO which is considered as the state of the art tool for genomes reconstruction.

6

On the Ability to Reconstruct Ancestral Genomes from *Mycobacterium* Genus

In this thesis, we state that, even if the ancestral reconstruction problem is NP-hard in theory, its exact resolution is feasible in various situations. The previous chapter focused on organelles that contain various genomic changes caused by rearrangements, like gene duplication and loss. However, we claimed that the small size of these genomes make it possible to deal in practice with the ancestral reconstruction problem. In this chapter, we enlarge the size of genomes by considering particular bacteria, namely some clonal ones. In the latter, the increase of the genome size is balanced by the decrease of recombination events, when compared with chloroplasts. This is the reason why we claim that the ancestral reconstruction problem is tractable too for such genomes, even if tools and algorithms introduced in the previous chapter must be adapted to the bacterial reign. Such accurate reconstruction, which identifies too some highly homoplasic mutations will be applied in this chapter, to two *Mycobacterium* pathogenetic bacterias. By mixing automatic reconstruction of obvious situations with human interventions on signaled problematic cases, we will indicate that it should be possible again to achieve a concrete, complete, and really accurate reconstruction of lineages of the *Mycobacterium tuberculosis* complex. Thus, it is possible to investigate how these genomes have evolved from their last common ancestors. Let us finally note that the content of this chapter has been presented in the IWBBIO conference, 2017 edition [101].

6.1/ Introduction

Mycobacterium tuberculosis is presently still one of the principal causes of death worldwide. Approximately one-third of the world population is infected by the *Mycobacterium tuberculosis complex* (MTBC), with about 9 million event cases annually, leading to estimated a million deaths each year. Due to their different host tropism and phenotypes, members of MTB complex display various pathogenicities ranging from particularly human (*M. tuberculosis*, *M. africanum*, and *M. canetti*) or rodent pathogens (*M. microti*) to *Mycobacteria* with a broad host spectrum (like *M. bovis*, see [102, 103, 104]). *Mycobacterium tuberculosis* has been in the human population for thousands of years, as fragments of the spinal column of Egyptian mummies from 2300 BCE show definite pathological signs of

tubercular decay. Since then, Robert Koch identified the bacterium responsible for causing consumption in 1882.

The MTB complex belongs to the slow-growing sublineage of *Mycobacteria*. Based on topographical characteristics, MTBC can be categorized into six clusters, including species such as *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti*, and *M. canettii*. Members in MTBC share 99.95% of their genomic sequences and a rigorously clonal population structure [105]. Compared to more ancient species (*e.g.*, *M. marinum*), MTBC has shorter but more virulent chromosomes [106, 107]. Considering that they all are derived from a common ancestor, it is interesting that some are human or rodent pathogens, whereas others have a wide host spectrum [108]. The genome of *M. tuberculosis* was studied using the strain *M. tuberculosis H37Rv*. It has a circular chromosome of about 4,200,000 nucleotides long, while containing about 4,000 genes [109]. The different species of the *Mycobacterium tuberculosis* complex show a $95-100\%$ DNA relatedness based on studies of DNA homology, and the sequences of the 16S rRNA gene are the same for all the species.

MTBC genomes have been modified during the evolution by mutation, insertion-deletion of nucleotides, by large-scale changes (inversion, duplication or deletion of large DNA strands), or by other modifications specific to repetition (insertion sequences, etc.). Being able to predict either its past or its future evolution may have multiple applications, *e.g.*, to reconstruct the past history and the ancestors of bacteria, to better understand their mechanism of virulence and resistance acquisition, or to predict outbreaks. The relatively short timescale (tuberculosis disease is relatively recent, as its most recent common ancestor evolved $\approx$ 40,000 years ago [110]), the relatively reasonable sizes of considered genomes, the relative rarity of recombination events, and the recent possibility to have access to old and present bacterial DNA sequences, may lead to the possibility to model the evolution of these genomes, in order to reconstruct and to understand their ancient history and to predict their future evolution.

To do so, new algorithms of detection and of evolution regarding genomic modifications must be written. They may be adapted from the chloroplast case, even if these two kinds of genomes have very different characteristics (length, percentage of shared genes between lineages, etc.). Indeed, researches on this subject mainly focus on predicting the evolution of nucleotide mutations, and by assuming specific forms for matrix mutations which seem incompatible with recent experimental measures [111]. These models for evolution must be differently designed, in order to better reflect the reality. Additionally, the serious impact of other modifications operating on the genomes (as insertions and deletions of nucleotides, inter and intra chromosomic recombinations, or modifications specific to repetition, see Fig. 6.1), must be taken into account more deeply, while a concrete ancestral reconstruction of bacterial lineage must be finally achieved.

The objective of this chapter is to prove that, given a set of close bacterial genomes, it is possible to reconstruct in practice their recent sequence evolution history, by mixing state-of-the-art tools with a pragmatic manual completion and cross-validation. We will illustrate that, in practice, it should be possible to reconstruct ancestral genomes for some lineages of the *Mycobacterium* genus, using all available complete genomes of such a lineage (for instance, 65 complete genomes of the MTB complex are currently available, and we have more than 1,000 archives of reads).

An important remark, motivating our proposal, is that the NP-hard character of this problem only appears if we consider a very large number of operations in very large sequences.

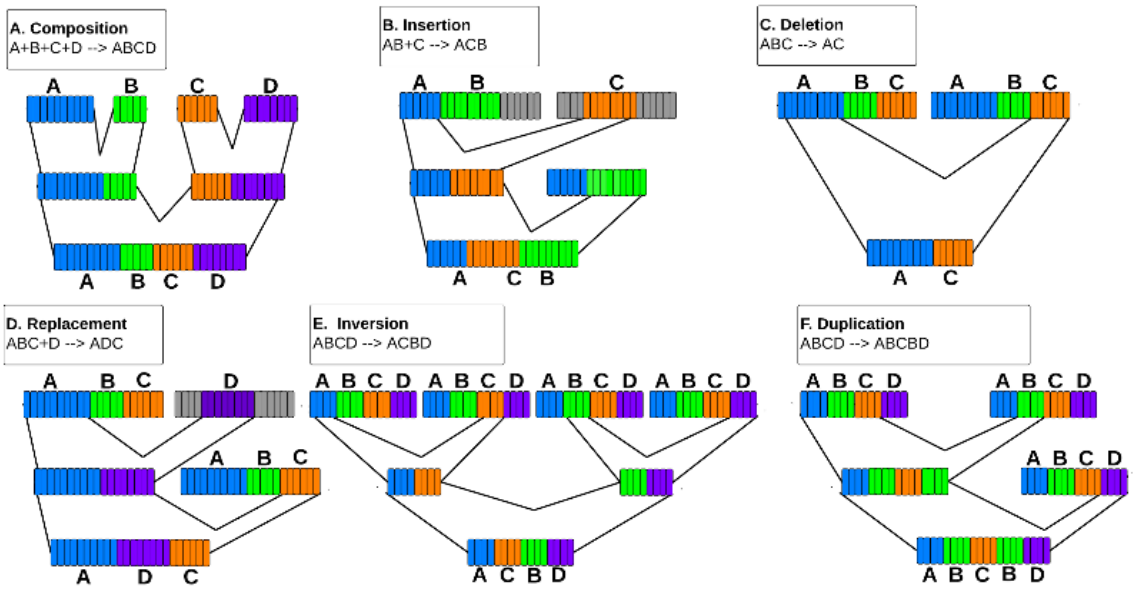


Fig. 6.1 – Various genome rearrangement events.

On our side and with the chosen bacteria, we will consider quite small sequences and a relatively small number of large scale recombinations. So we face tractable problems in various real situations, on which simple and pragmatic approaches may work.

6.2/ A CONCRETE SEMI-AUTOMATIC ANCESTRAL RECONSTRUCTION

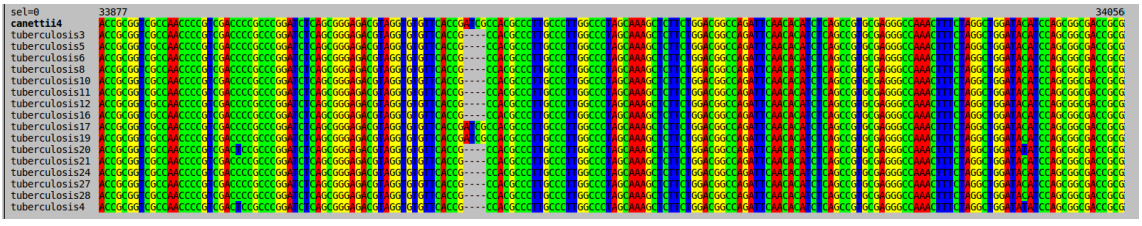


Fig. 6.2 – Representation of a multiple sequence alignment.

The complete sequences of the 65 *Mycobacterium* genomes available on the NCBI [1] have been downloaded. Listed according to their species, 42 genomes of *tuberculosis*, 15 *bovis*, 2 *africanum*, 5 *canettii*, and 1 *microti* have been recovered. Table 6.1 shows information about some of these *Mycobacterium* genomes. Among this MTBC, we particularly focused on *tuberculosis* and on *canettii* because the virulent *tuberculosis* species is supposed to have emerged from *canettii* forty thousand years ago. To verify such an evolutionary hypothesis, the first task of our approach, proposed to achieve an ancestral reconstruction of close genomes, is to perform a multiple sequence alignment of the sequences. This task is described in the next section.

1. ftp ://ftp.ncbi.nih.gov/genomes

TABLE 6.1 – Information about some *Mycobacterium* genomes.

Organism name	Accession	Sequence length	Number of genes
Mycobacterium tuberculosis W-148	NZ_CP012090.1	4,418,548 bp	4,133
Mycobacterium tuberculosis H37Rv	NC_018143.2	4,411,709 bp	4,132
Mycobacterium africanum GM041182	NC_015758.1	4,389,314 bp	4,089
Mycobacterium africanum strain 25	CP010334.1	4,386,422 bp	4,798
Mycobacterium microti strain 12	CP010333.1	4,370,115 bp	4,321
Mycobacterium canettii CIPT 140010059	NC_015848.1	4,482,059 bp	4,137
Mycobacterium canettii CIPT 140070008	NC_019965.1	4,420,197 bp	4,103
Mycobacterium bovis strain ATCC BAA-935	NZ_CP009449.1	4,358,088 bp	4,095
Mycobacterium bovis BCG str. Tokyo 172	NZ_CP014566.1	4,371,707 bp	4,076

6.2.1/ MULTIPLE SEQUENCE ALIGNMENT

The first problem of this alignment stage, is to identify a common starting point in these complete circular genomes. In order to do so, we searched for a reference sequence of 200 nucleotides from *M. tuberculosis H37Rv*, and we found it or its transconjugate in each genome using a local blast. Then, a circular rotation (together with a transconjugate operation if needed) has been performed on each complete genome, so that each sequence starts with the same 200 nucleotides, if we except SNPs. Once these sequences have been operated to share the same orientation and starting location, the overall alignment of each chromosome has been performed.

Alignment of large sets of sequences is a common task during biological investigations and has a wide variety of applications incorporating homology detection [112], finding evolutionarily relevant sites, and phylogenetics. A multiple sequence alignment, as depicted in Figure 6.2, may explain many aspects about a gene : which regions are constrained, which sites undergo positive selection [113], and potentially the structure of its gene product [114]. Furthermore, aligning sequences can help to detect events of mutations or recombination in couples of close genomes.

To achieve such an alignment, we thus have considered the *AlignSeqs* function from Decipher R package [115]. Indeed, after various tests on well known alignment tools that can perform a large MSA on several to tens of thousands of sequences, like MAFFT [116], we found that package was the only one that achieved to align complete bacterial genomes with a good accuracy. AlignSeqs performs group-to-group alignment [117, 118], and aligns a sequence established by merging groups along a guide tree until all the input sequences are aligned, as shown in Figure 6.2.

This *AlignSeqs* function takes as input two aligned sets of DNA sequences and returns a merged alignment. It can be used to achieve multiple sequence alignment on sequences of the same kind. Indeed, multiple alignments are accomplished by aligning two sequences, merging with another sequence, combining with another set of sequences, and so on until all the sequences are aligned [119, 117]. We thus obtained a first representation of synteny of the whole 65 *Mycobacterium* genomes, which is depicted in Figure 6.3. It can be observed that these 65 genomes have a high sequence similarity with low recombination events.

6.2.2/ PHYLOGENETIC STUDY

This first representation of synteny blocks, obtained thanks to the multiple sequence alignment of the whole *Mycobacterium* genus, has allowed us to detect the location of a few large scale inversions. We thus have been able to manually invert again these inversions, so that the multiple alignment became quite perfect, if we except small indels and SNPs. It is then possible to use all the 65 complete genomes in the next stage, namely the phylogenetic study.

Indeed, the evolutionary history of our population of genomes can be represented as a phylogenetic tree using the multiple sequence alignment combined with manual local inversions previously obtained. As previously recalled, various methods are well established in the literature to investigate the best phylogenetic tree for a given set of aligned sequences. On our side, we decided to consider the use of RAxML as a default phylogenetic tree reconstruction toolkit, a well known and reputed software based on maximum likelihood [1, 46].

As we reversed the inversions, our phylogenetic investigations are based on the whole genome. This leads to well supported and trustworthy trees of strains, on which we can reliably consider to reconstruct ancestral states. As an illustrative example, we represent the phylogenetic trees of *M. canettii* species with the *M. tuberculosis* outgroup in Figure 6.4. This very well supported tree has been obtained using RAxML with GTR Gamma model as advised by JModelTest 2.0. The *M. tuberculosis* phylogeny, for its part, leads to bootstrap supports larger than 98%, as shown in Figure 6.5.

Note that, with these bacteria, we have not to find the largest subset of core sequences that leads to the most supported tree, as aligning the whole complete genomes leads to a well supported tree : it is not possible to improve the results, which is nice as the core genome is many times greater than in the chloroplast case. Indeed, let us recall that *M. tuberculosis* species have 42 genomes with size 4Mb and 4000 genes, while in the case study on the chloroplasts, the genome has around 271 pan genes in maximum. So, it is not sure that the heuristic approach to find the core gene of the previous chapter can succeed to find the well-supported phylogenies).

Having an accurate representation of the general evolution of MTBC strains due to this phylogenetic study, we are then left to reconstruct the ancestral states of the alignment at each internal node of the tree. This final ancestral reconstruction will be applied in two stages, considering first the variants of length 1 in the alignment (namely, single nucleotide polymorphism and indels of 1 nucleotide), and then larger variants that mainly consist of insertion or deletion of a subsequence at a location in the tree.

6.2.3/ ANCESTRAL RECONSTRUCTION : MONONUCLEOTIDIC VARIANTS

Focusing on mononucleotidic variants, we separated the treatment of single nucleotide polymorphisms (SNPs) versus insertion-deletions (indels). For the former, the situation seems quite simple, the only problem being to prevent confusion between a “true” SNP and a SNP induced by a recombination of the indel kind. For the latter, future challenges encompass to determine which indels are related to tandem repeats, which are associated with mobile elements, or which are due to repeated sequences. Let us detail each case hereafter.

Regarding SNPs, the ancestral reconstruction is achieved as follows. The marginal probability distributions for bases at ancestral nodes in the phylogenetic tree are first calculated. These distributions are obtained using the sum-product message passing algorithm [120], assuming independence of sites. The ancestral reconstruction is done by using PHAST software [121], which reconstructs indels too by parsimony, also assuming site independence. Obtained results on mononucleotidic variants are then carefully visually checked, as the number of such variants is not excessive, see Tables 6.2 and 6.3.

At the end, 2,956 SNPs and 166 indels have been found in the alignment of the clade constituted by the 5 strains of *M. canettii*, as shown in Figure 6.6. The Figure 6.7, for its part, represents the location of the 394 SNPs and of the 25 indels that have been found in the alignment of the clade constituted by 8 genomes of *M. tuberculosis*.

6.2.4/ Ancestral reconstruction of larger variants

Mycobacterium species considered in this chapter are highly conserved, with really similar regions and without rearrangement. As previously evoked, we found only a few significant inversions, like the one at the last common ancestor of strains *CIPT 140010059*, *140070010*, *140060008*, *140070017*, and *140070008*, as shown in Figure 6.9. The Figure 6.10, for its part, is a dotplot representing these homologous regions, as identified by the FindSynteny function in R. All the Synteny blocks of the 42 *M. tuberculosis* are finally depicted in Figure 6.8, where we have obtained 99% of DNA sequence identity. To sum up, if we except a large scale inversion, we can only report some small indels at this recombination level.

Ad hoc algorithms have then been designed to deal with mid size variants. More specifically, we have written first a string algorithm that detects small and noisy inversions, but the latter, distributed on our supercomputer facilities, was only able to detect artifacts. So either the MTBC genomes have not faced inversion events during its recent history, or this recombination case still needs further investigations. Authors tend to prefer the first possibility, as *Mycobacterium* genomes evolve in a clonal manner (which is not the case, for instance, with *Yersinia* genus, in which a large amount of mobile elements has led to a large number of reported inversions [122]). Duplication, for its part, has not yet been investigated but, as for inversions, the analysis of synteny blocks tends to show that such events are rare, at least if we consider the large scale ones.

Both indels of midsize and SNPs have been deeply studied, using PHAST software as detection tool. From obtained results, we can conclude the following points. (1) Such events are quite rare in some lineages of the MTB complex like *tuberculosis*, as described in Table 6.4. (2) Most of the times, the situation is very easy to manually understand, leading either to an insertion or to a deletion at an obvious internal node of the tree, as illustrated in Figure 6.11 and 6.12. (3) Most of the times, the inserted motif has not faced mutations during evolution : leaves that contain the motif have no mutation in it, thereby contributing to an easy to resolve situation. (4) Surprisingly, ancestral states recovered by PHAST and its parsimony approach leads to disappointing results. Similarly, obviously wrong results have been obtained with state-of-the-art competitor software. To sum up, a manual reconstruction of mid size indels is possible, due to the low number of these recombinations that are mainly very easy to resolve, while automatic tools from the literature are not currently able to do it.

All these steps are summarized in Figure 6.15. In this one, gray boxes correspond to

TABLE 6.2 – Number of alignment columns with polymorphism, by pair of strains, on *M. canettii* genomes. Note that, when a large string is deleted at some location in the tree, all the characters of this deletion are counted here.

	canettii0	***canettii1***	***canettii2***	***canettii3***	***canettii4***	***tuberculosis1***
canettii0	0	3524	27256	60957	4833	3354
canettii1	3524	0	27260	61233	7971	1150
canettii2	27256	27260	0	62717	27468	27437
canettii3	60957	61233	62717	0	60987	61346
canettii4	4833	7971	27468	60987	0	7510
tuberculosis1	3354	1150	27437	61346	7510	0

TABLE 6.3 – Variations in the alignment of *M. tuberculosis*

	tuberculosis4	***tuberculosis19***	***tuberculosis17***	***tuberculosis16***	***tuberculosis27***	***tuberculosis28***	***tuberculosis24***	***tuberculosis10***
tuberculosis4	0	199770	214401	219205	216387	217235	216919	217186
tuberculosis19	199770	0	212403	219039	216908	216672	216726	216953
tuberculosis17	214401	212403	0	216808	216534	217011	216786	216882
tuberculosis16	219205	219039	216808	0	216669	216916	216251	216678
tuberculosis27	216387	216908	216534	216669	0	142974	189148	199505
tuberculosis28	217235	216672	217011	216916	142974	0	189460	199412
tuberculosis24	216919	216726	216786	216251	189148	189460	0	194315
tuberculosis10	217186	216953	216882	216678	199505	199412	194315	0

M. canettii SNPs

Fathers	**Children**	**No. of SNPs**
100.2	*canettii2*	1041
	canettii3	12398
100	*canettii0*	1
	canettii1	9
100.3	*100*	28
	100.2	735
100.X	*100.3*	111
	canettii4	438

M. tuberculosis SNPs

Fathers	**Children**	**No. of SNPs**
100	*tuberculosis19*	5
	tuberculosis17	14
100.2	*tuberculosis24*	1
	tuberculosis10	0
100.3	*tuberculosis27*	0
	tuberculosis28	0
98	*100.2*	1
	100.3	0
100.4	*98*	0
	tuberculosis16	1
100.X	100	5
	100.4	1

TABLE 6.4 – Number of SNPs in the considered species (100.X refers to an ancestral node, as in the tree)

manual steps whereas all the other ones are automatically executed. Indeed, obtained results on mononucleotidic variants have been carefully checked by naked eye, as the number of such variants is not excessive, while ad hoc algorithms were designed to deal with variants of larger size, see Figures 6.13 and 6.14.

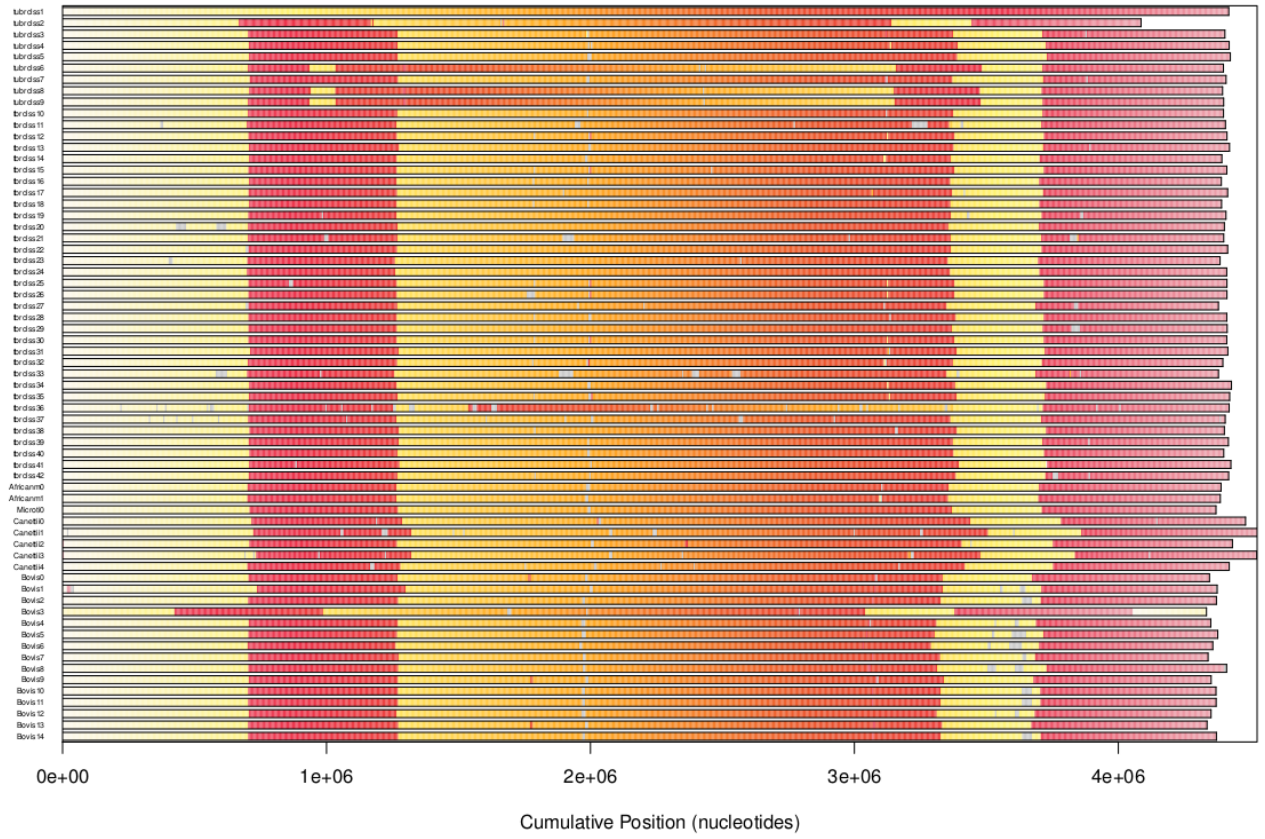


Fig. 6.3 – A synteny representation of all available *Mycobacterium* strains

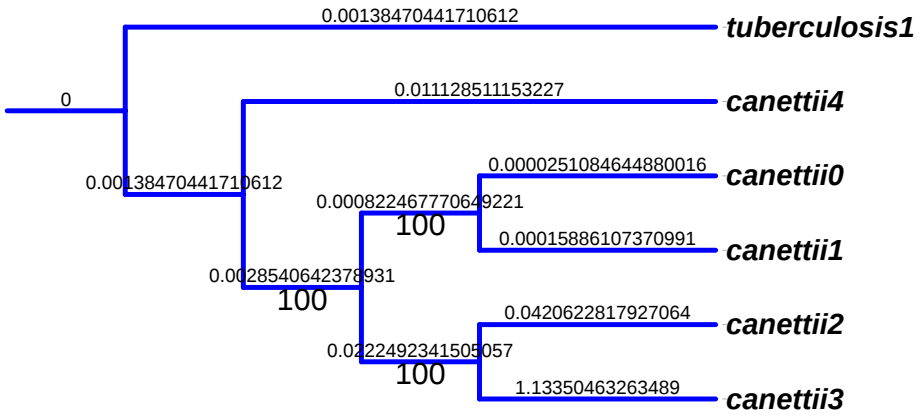


Fig. 6.4 – Well-supported phylogenies on *M. canettii* species using a *M. tuberculosis* as outgroup

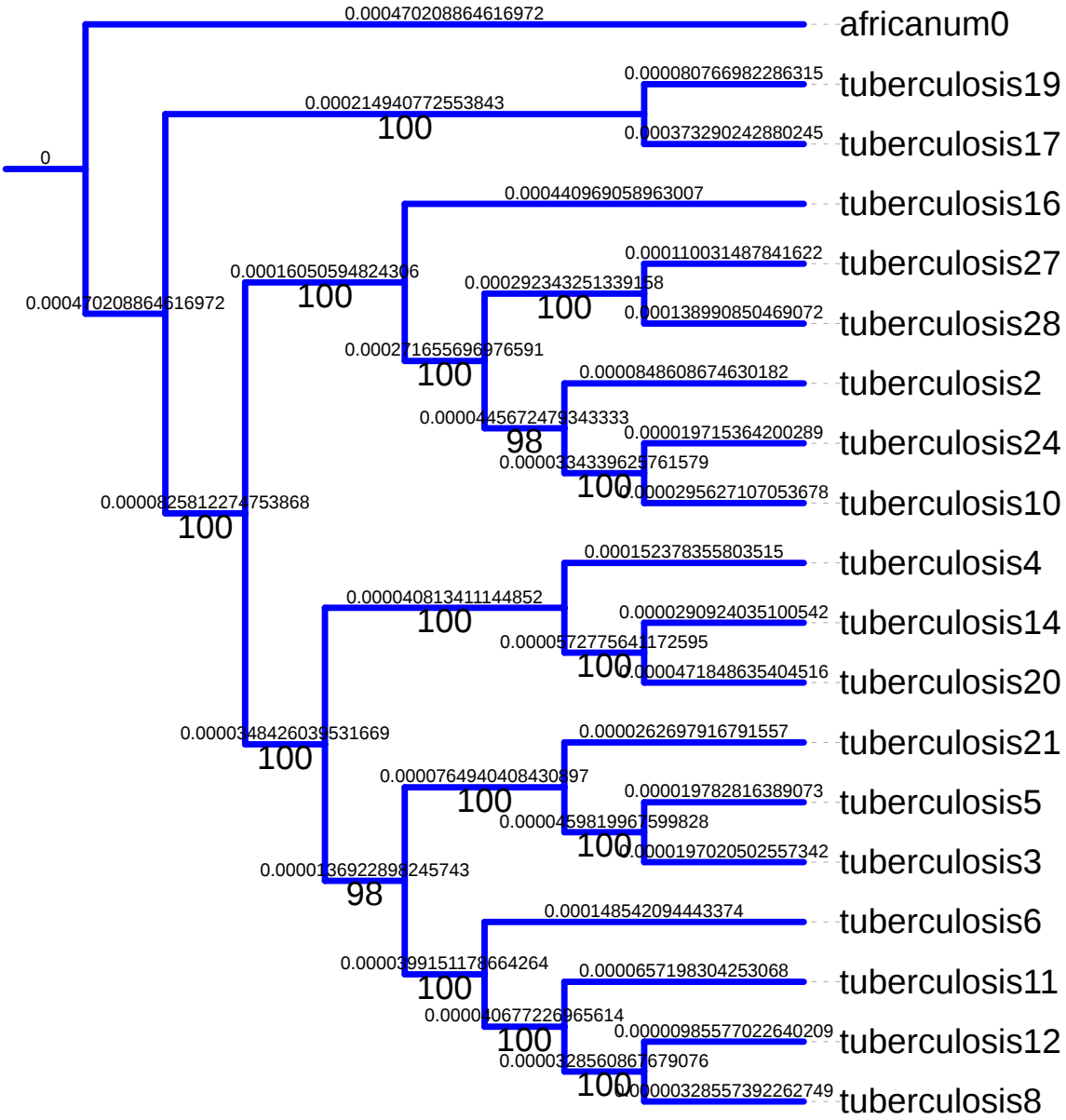


Fig. 6.5 – Well-supported phylogenies of *M. tuberculosis* species with *M. africanum* as outgroup. Phylogenetic trees have been calculated on the entire genomes with RAxML and GTR Gamma model

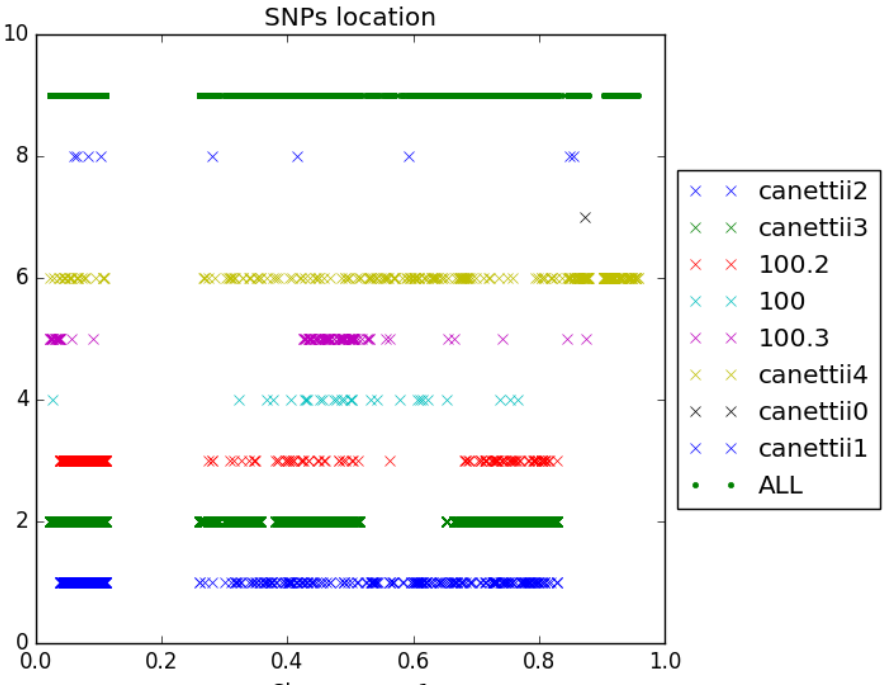


Fig. 6.6 – SNPs location of mononucleotidic variants of *M. canettii*.

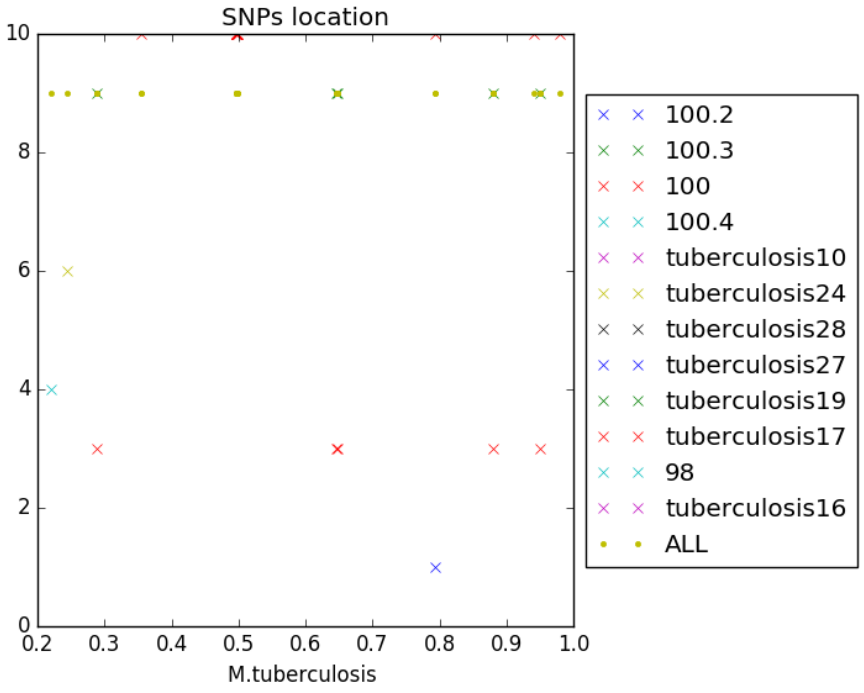


Fig. 6.7 – SNPs location of mononucleotidic variants of *M. turberculosis*.

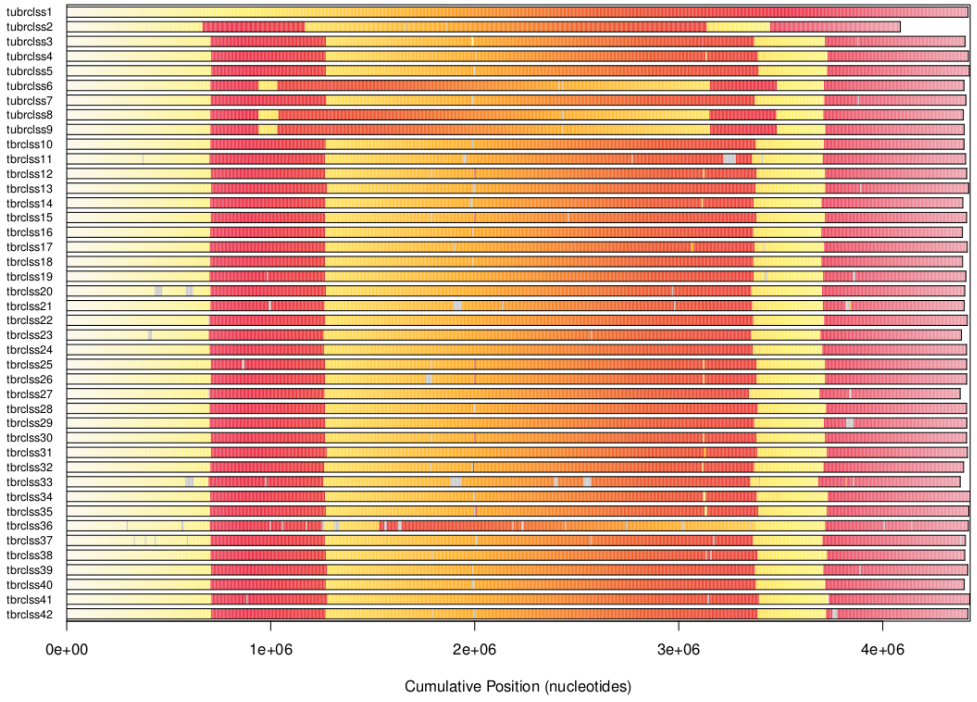


Fig. 6.8 – A representation of *M. tuberculosis* genomes species tends to show more than 95% nucleotide similarity with little recombination events.

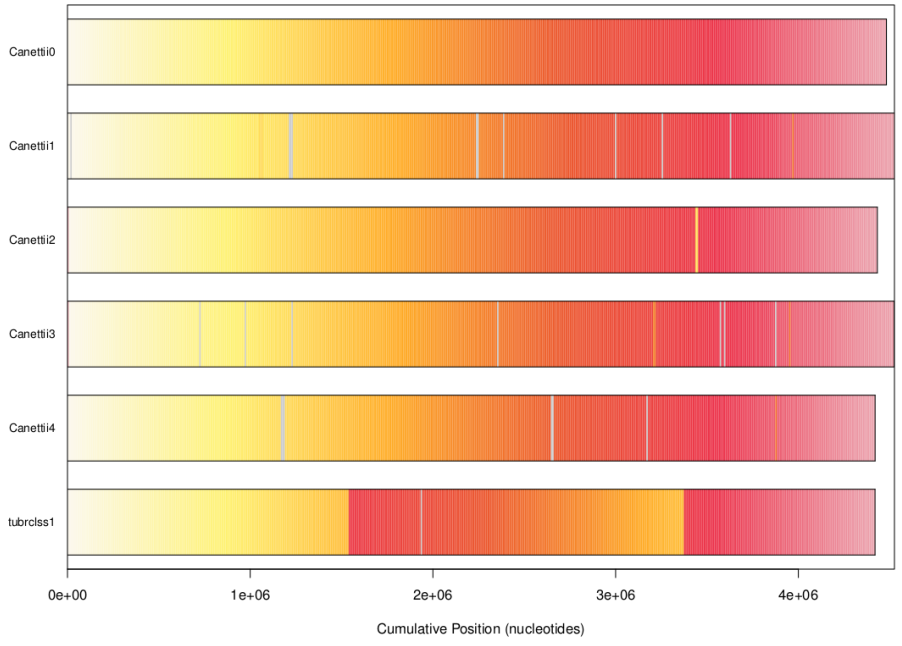


Fig. 6.9 – Synteny blocks in *M. canettii*. Each genome is colored according to the position of the corresponding region in the first genome (gray if a region is unshared).

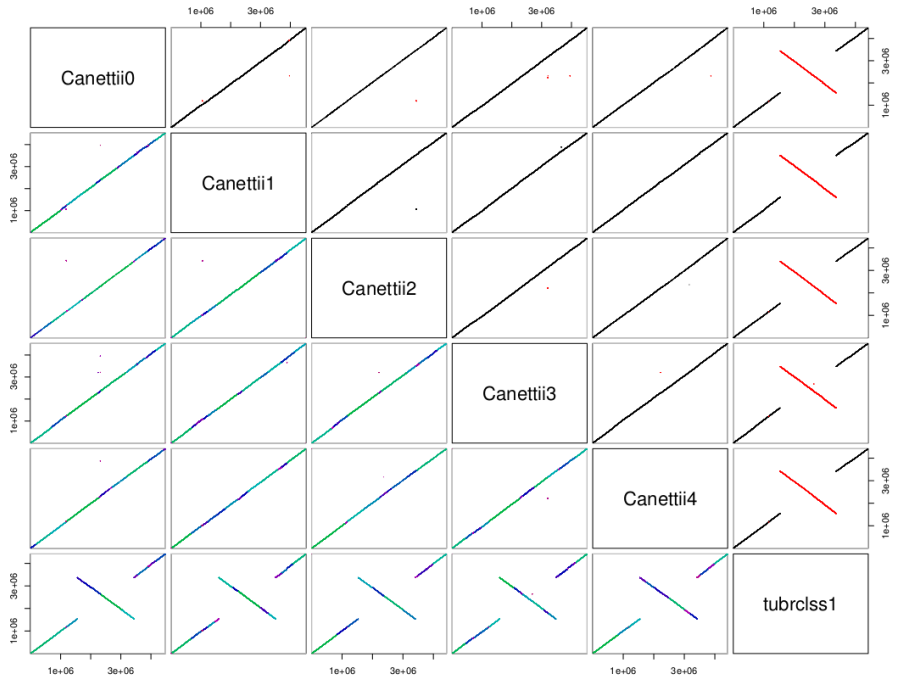


Fig. 6.10 – Dot plots provide an alternative representation of the synteny map of *M. canettii*. Black diagonal lines show syntenic regions sharing the same orientation, whereas red anti-diagonal ones represent blocks of synteny between opposite strands. The description of all of these species tends to show a high sequence similarity with little recombination events.

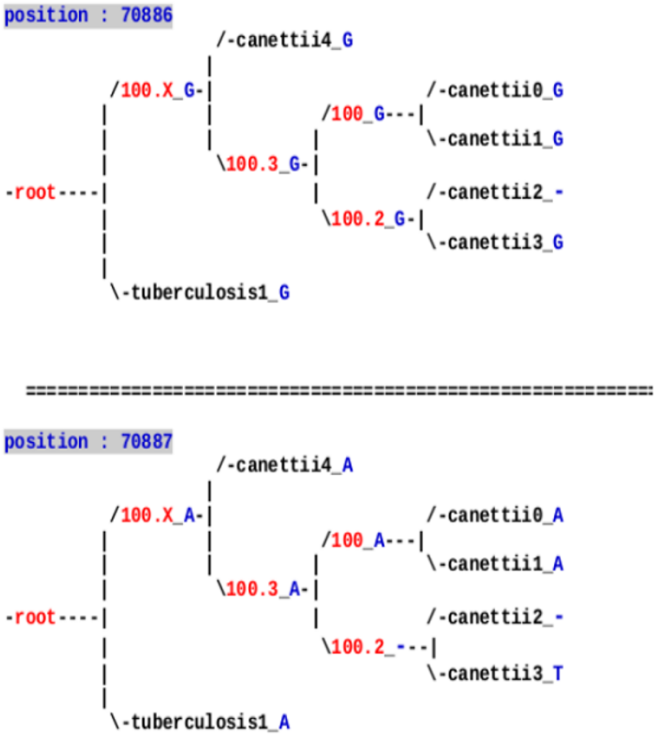


Fig. 6.11 – The insertions and deletions of nucleotides (indels) on the internal node of the tree (a) represent the nucleotides contain the ancestor nodes and their children on *M. canettii* species

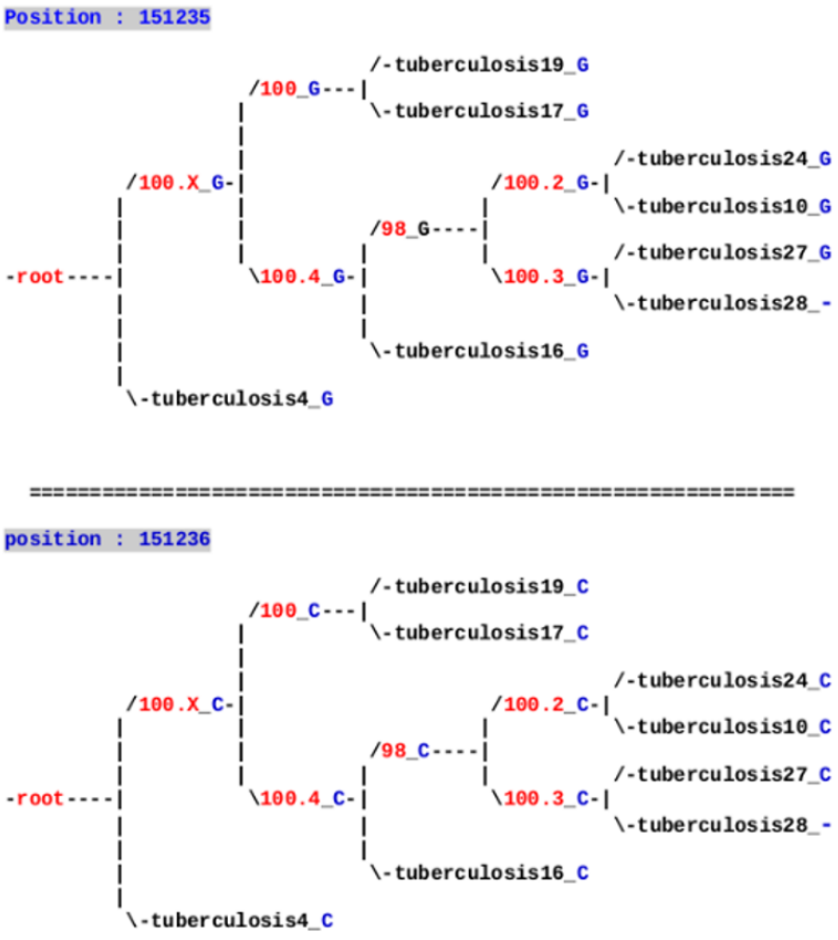


Fig. 6.12 – Example of an ancestral reconstruction of one problematic column in the alignment

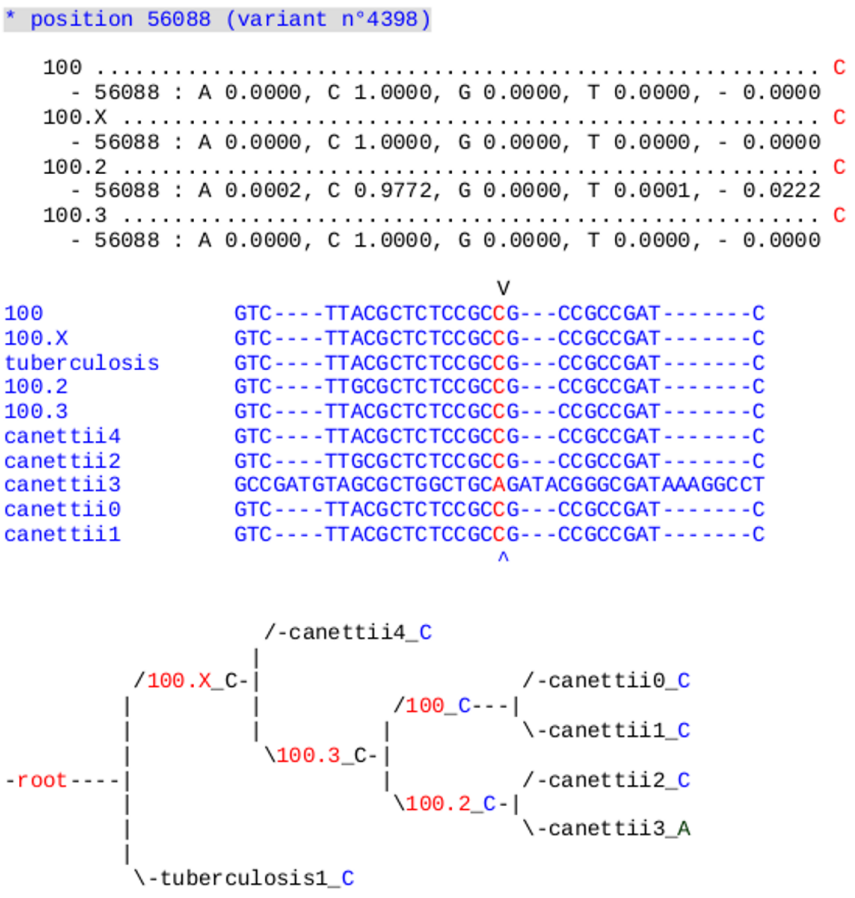


Fig. 6.13 – Ancestral reconstruction examples on *M. canettii* species

```
* position 289106 (variant n°507)
   98 ..................................................... T
     - 289106 : A 0.0000, C 0.0409, G 0.0000, T 0.9475, - 0.0114
   100.X ................................................... C
     - 289106 : A 0.0000, C 0.9990, G 0.0000, T 0.0000, - 0.0008
   100.2 ................................................... T
     - 289106 : A 0.0000, C 0.0000, G 0.0000, T 0.9999, - 0.0000
   100.3 ................................................... C
     - 289106 : A 0.0000, C 0.9850, G 0.0000, T 0.0002, - 0.0147
   100 ..................................................... C
     - 289106 : A 0.0000, C 0.9999, G 0.0000, T 0.0000, - 0.0000
   100.4 ................................................... T
     - 289106 : A 0.0000, C 0.0454, G 0.0000, T 0.9410, - 0.0134

                                    V
tuberculosis27     TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
tuberculosis28     TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
tuberculosis24     TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
tuberculosis10     TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
98                 TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
tuberculosis19     TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
tuberculosis17     TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
tuberculosis16     TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
100.X              TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
tuberculosis4      TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
100.2              TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
100.3              TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
100                TGGACCGCCTGGAACAGGCGCTGCGCGCCGGTGAATTGCCG
100.4              TGGACCGCCTGGAACAGGCGTTGCGCGCCGGTGAATTGCCG
                                    ^
```

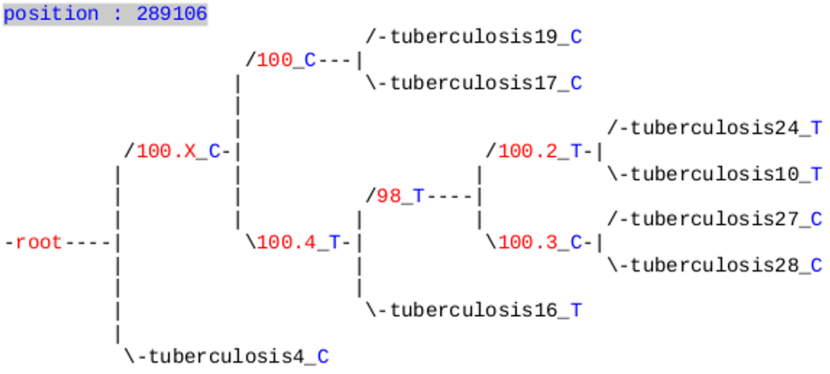


Fig. 6.14 – Ancestral reconstruction examples on *M. tuberculosis* species

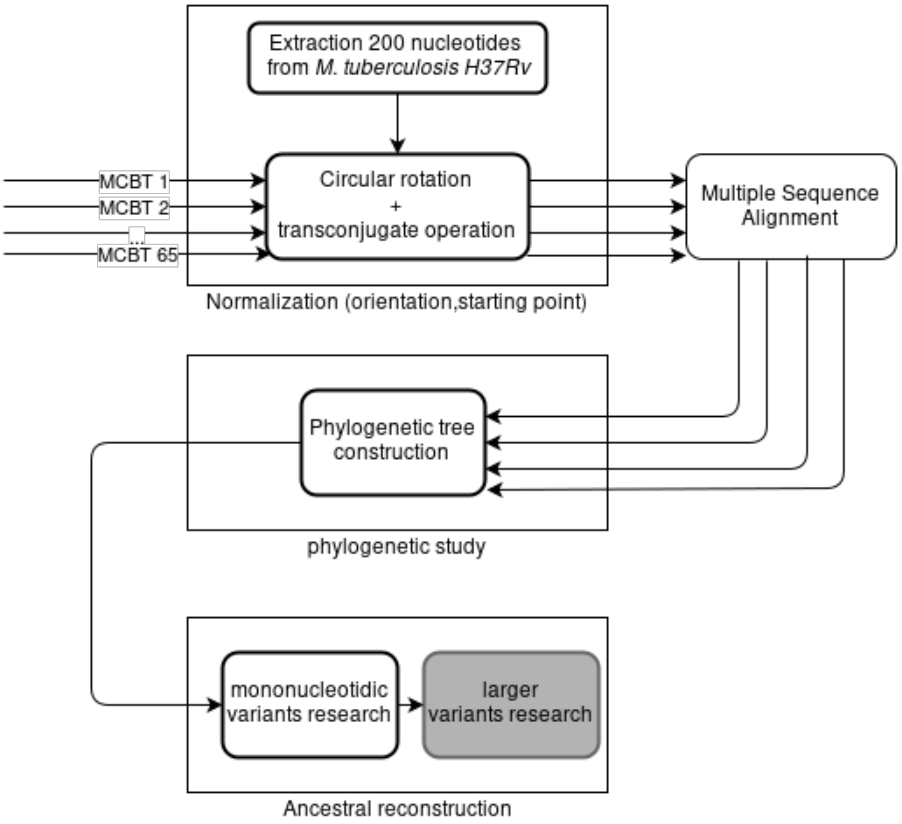


Fig. 6.15 – Flowchart of the proposed approach.

6.3/ DISCUSSION

The obtained ancestors have not yet been studied in this chapter. They will be investigated with updated and improved algorithms, encompassing mobile elements and gene content evolution analyzes. In order to do so, we will consider the phylogenetic tree whose leaves will contain sets of genes, and we will compute core and pan genomes at each internal node of the tree. Having this core and pan tree, we will design an algorithm to investigate more deeply the evolution of these pan and core genomes over the tree, to see if some branches can be related to hot spots of evolution. We thus intend to determine at which rate such loss or gain occurs, and which kinds of functionality are concerned. We will finally compute how much mutations fall inside a coding sequence, by studying which kind of genes has evolved on the phylogenetic tree, by wondering if the mutation rate has changed over time, and if such mutability can be related to environmental events. In other words, we will wonder which variations have been potentially significant among the numerous events that have been found when aligning these sequences.

With such a pipeline, we intend to investigate the following questions. Are some recombinations at the origin of severe tuberculosis epidemics ? Are transposases responsible of such recombinations like inversions [123, 124] ? Are transposases in general more present in *M. tuberculosis* (affecting humans) than in *M. africanum*, *M. bovis*, or *M. bovis BCG* ? Are they related to the virulence of the strain ? How core and pan genomes have evolved over time in this complex ? Finally, we will compare the last common ancestor of this complex to a *M. canettii*, to see if the *canettii* ancestor hypothesis can be verified by the ancestral reconstruction way.

At this point, our partial conclusion is that the reconstruction of ancestral sequences is possible, at least in the case of close and clonal bacterias. Furthermore, elements being part of this reconstruction have already been designed, at least in their first revision (for instance to detect and deal with mononucleotidic variants). However, the MTB complex seems to be a little too complicated for a first deep investigation of semi-automatic reconstruction of ancestral sequences of bacteria, and a genus like *Brucella* may be more easy to deal with in a first concrete investigation of this problem.

6.4/ CONCLUSION

In this chapter, we have firstly emphasized that, even if various algorithms and software already exist to face the NP-hard character of the ancestral genome reconstruction problem, they do not work perfectly, in particular when SNPs or indels fall into repeated sequences. We have then argued that, when regarding the relatively low number of mutation and recombination events in such *Mycobacterium*, a pragmatic approach is possible. We have proposed to reconstruct all ancestors of all complete available genomes of *Mycobacterium tuberculosis* and of *M. canettii*. The study has started by investigating single nucleotide polymorphism level, while indels and large scale recombination are regarded in a second stage. Our conclusion is that, by mixing automatic reconstruction of obvious situations with human interventions on signaled problematic cases, it may be possible to achieve a concrete, complete, and really accurate reconstruction of some specific bacteria lineages. We can thus investigate how these genomes have evolved from their last common ancestors.

IV

Conclusion

7

Conclusion and Perspectives

7.1/ Conclusion

In this thesis work, we made significant progress in the quest of the ancestral genome of chloroplasts and of MTBC. A large set of complete chloroplast genomes has first been studied *de novo* regarding both core and pan genomes, phylogenetic relationship, and gene content modifications. We then started to explore the produced data, by emphasizing some remarkable relations between well-known events of chloroplast history and the evolution of gene contents over the phylogenetic tree.

Three metaheuristics have then been used (genetic algorithm, binary particle swarm optimization, and simulated annealing) to produce a well supported phylogenetic tree based on the largest possible subset of core genes. They have been evaluated on various sets of chloroplast species and deployed on a supercomputer facilities. Given the average between the percentage of core genes and the lowest bootstrap as scoring function, we have shown on simple examples that, given a set of species, various global optima with contradictory topologies can be reached. These first experiments emphasize that sometimes the phylogeny of chloroplasts cannot perfectly be resolved using a tree : a phylogenetic network may be more close to the reality, branches within this network being as strong as the associated tree topology is frequent.

We have first investigated the gene order problem and DNA ancestral coding sequence reconstruction in the case of organelles. We applied our designed methods to the case of chloroplasts, which is more complex than mitochondria due to the size of the genomes, gene prediction issues, and because two chloroplasts do not share the same genes. At each time, we have considered all currently available complete genomes, and we have applied ancestral state reconstruction algorithms coupled with careful human curation.

After having investigated our ability to reconstruct all ancestors of all complete available genomes of *Campanulids* chloroplasts, we then considered larger genomes by focusing on the ancestral reconstruction of *Mycobacterium* pathogenetic bacterias. The study started by investigating manually large scale recombination and then focusing on both single nucleotide polymorphism and indels using PHAST and human operations. Indeed, by mixing automatic reconstruction of obvious situations with human interventions on signaled problematic cases, we have shown that a concrete, complete, and really accurate reconstruction of the lineage ancestors can be achieved.

7.2/ FUTURE WORK

Genomes of chloroplasts have notably lost functional genes, either because they become ineffective or due to transfer to the nucleus. We thus first plan to determine more precisely the rate of such loss, whether this is indeed related to a nucleus transfer, and which kind of functionality is practically concerned. Indeed, this thesis is an ongoing work regarding investigating more systematically such relations between remarkable ancestral nodes in the tree, endosymbiosis events, and evolution of gene content. We will wonder whether some branches of the trees are statistically remarkable when considering gene content (for instance, do we have a correlation between the presence or absence of a subset of genes, and a particular taxonomy). Then, the gene ordering and content of each ancestral node will be computed using ad hoc algorithms. Ancestral DNA sequence reconstruction, which has been initiated here, will be totally inferred, and ancestral intergenic regions will be deduced, in order to have all ancestral genomes with confidence indications like probabilities.

We will then wonder if evidence of the *Cyanobacteria* origin of chloroplasts can be stated using our approach. In particular, the last universal common ancestor of all chloroplasts will be compared to available *cyanobacterial* genomes, regarding gene contents and DNA sequences, to see if a *cyanobacterial* origin of chloroplasts can be assessed by the mean of ancestral reconstruction. At this stage, it may be interesting to compare both ancestors (of chloroplasts on the one hand, and of *Cyanobacteria* on the other hand).

Concerning our heuristic approaches for detecting blurring genes in phylogenetic studies, we note that networks can be obtained by merging gene trees. In future work, we will propose a way to obtain such networks with large subsets of random core genes, and will show that such ways reinforce the stability and the confidence of the network. We intend to provide too criteria for deciding if either a tree or a network is preferable for a given set of DNA sequences. We will measure the impact of this choice and of the coexistence of different well-supported topologies on works like ancestral genome reconstruction. Finally, the various ways to set up the metaheuristics proposed here will be systematically investigated, to find the best manner to configure these ones when targeting the largest subset of core genes leading to the most supported tree or network.

Practically speaking, we intend to reconstruct all ancestors of all complete available genomes of specific bacteria strains. We plan to start to reconstruct the ancestors of the *Brucella abortus* lineage, in which the low number of indels, mutations, and inversions allow a human validation of the complete reconstruction. This first study will be enlarged to the whole *Brucella* genus, using all available complete genomes. More complex but still clonal genomes will then be investigated, to go deeper in the study and understanding of genomic recombinations. Targeted bacterias will be the *Yersinia pestis* on the one hand, and *Pseudomonas aeruginosa* on the other hand. Moreover, we intend to compare them with ancient DNA when available (like for *Y. pestis*). In parallel, original mathematical description of some recombination mechanisms will be proposed, encompassing branching process and partial differential equation approaches for modeling mobile elements. We will then study which kinds of genes (in terms of functionality) have mutated over time, if the mutation rate has evolved among branches of the phylogenetic tree, and if such mutability can be related to environmental events.

We want to correlate the evolutionary history of microorganisms to epidemiological data : events of genomic recombination may be related to epidemic outbreaks. Moreover, such

putative correlations may be learned by deep learning algorithms, leading to a new way to predict epidemic risks. Finally, we intend to develop and test new bioinformatic tools : Dummy logit regression and LASSO test to evaluate effects of genes on phylogeny ; automated core and pan genomes extraction ; Laplacian eigenmaps with Gaussian mixture model for sequence clustering ; deep learning algorithms to detect insertion sequences, virulent factors, and other sequences of interest... Finally, all the knowledge gained and tools developed regarding the evolution of genomes will be applied to cancer diseases, by comparing the evolution of genomes between healthy and tumorous cells.

V

APPENDIX

8

APPENDIX

Listing 8.1 – This code is to download genbank or unannotated fasta files.

```
from Bio import Entrez,SeqIO
from tabulate import tabulate
from sys import argv
from numpy import array,savetxt
import os
import xlsxwriter

Entrez.email = 'bbgak2002@yahoo.com'
gbk='Genomes_gb/'                       # the folder to store gbk files
fasta='Genomes_fasta/'                  # the folder to store fasta files
def saveGenome(genomeName,typ):         # fetch the files from genbank
    if typ=='gb':
        if not os.path.exists(gbk):
            os.mkdir(gbk)
        handle=Entrez.efetch(db='nucleotide',id=genomeName,rettype=typ) # Accession id works, returns genbank format, l
        #store locally
        local_file=open(gbk+genomeName,'w')
        local_file.write(handle.read())
        handle.close()
        local_file.close()
    else:
        if not os.path.exists('Genomes_fasta'):
            os.mkdir('Genomes_fasta')
        handle=Entrez.efetch(db='nucleotide',id=genomeName,rettype=typ) # Accession id works, returns genbank format, l
        #store locally
        local_file=open(fasta+genomeName,'w')
        local_file.write(handle.read())
        handle.close()
        local_file.close()

def getGenomes(listName,typ):           # input: give the list of genomes names to fetch them from genbank. typ: gb or f
    id_list=open(listName).read().split('\n')
    if not os.path.exists('Genomes_gb'):
        os.mkdir('Genomes_gb')
    act_Gen=os.listdir(gbk)
    if not os.path.exists('Genomes_fasta'):
        os.mkdir('Genomes_fasta')
    fasta_Gen=os.listdir(fasta)
    for i in range(len(id_list)-1):
        print 'Try␣to␣fetch␣%s␣from␣genBank...'%id_list[i],
        act_Gen=os.listdir(gbk)
        fasta_Gen=os.listdir(fasta)
        if id_list[i] not in act_Gen and typ=='gb':
            #print 'Try to fetch %s from genBank...'%id_list[i],
            saveGenome(id_list[i],typ)
            print 'done!'
        elif id_list[i] not in fasta_Gen and typ=='fasta':
            #print 'Try to fetch %s from genBank...'%id_list[i],
            saveGenome(id_list[i],typ)
            print 'done!'
        else: print 'Exist!'

def GeneCount(typ,mapp=[1,1,1,1,1,1,1,1,1,1,1,1,1]):
# extract information from each genome in the folder.
    if typ=='gb':
        i=1
        Data=[]
        v=os.listdir(gbk)
        print v
```

```
        for GID in os.listdir(gbk):
            gb_file=gbk+GID
            gene=0
            trna=0
            rrna=0
            orf=0
            print 'working␣with:␣',gb_file,'...',
            gb_record=SeqIO.read(open(gb_file,"r"), "genbank")
            for v in gb_record.features:
                if v.type=='gene':                  # count the number of genes
                    gene+=1
                elif v.type=='tRNA':                # count the number of tRNA
                    trna+=1
                elif v.type=='rRNA':                # count the number of rRNA
                    rrna+=1
                elif 'orf' in v.type.lower():
                    orf+=1
            Organism=gb_record.features[0].qualifiers['organism'][0]
            try:
                if 'plastid:' in gb_record.features[0].qualifiers['organelle'][0]:
                    Plastid=gb_record.features[0].qualifiers['organelle'][0].split('plastid:')[1]
                else: Plastid=gb_record.features[0].qualifiers['organelle'][0]
            except IOError, KeyError:
                print 'Error␣with␣plast␣ID..Passed'
                pass
            Accession=gb_record.id
            GenomeID=gb_record.annotations['gi']
            Seq_Version=gb_record.annotations['sequence_version']
            SeqLength=str(len(gb_record.seq))+'␣bp'
            Date=gb_record.annotations['date']
            Taxonomy='␣<--␣'.join(x for x in gb_record.annotations['taxonomy'])
            Data.append([str(i),Organism,Plastid,Accession,GenomeID,Seq_Version,SeqLength,str(gene),str(trna),str(rrna),str(or
            print 'done!'
            i+=1
        f=open('genomes_info_test.dat','w')     # store the data in list format, each row for one genome.
        f.write(str(Data))
        f.close()
        head=['No','Name','Plastid','Accession','GenomeId','Seq.Ver.','Seq.␣length','Nb.Genes','Nb.tRNA','Nb.rRNA','Nb.ORF','I
        head1=[head[idx] for idx in range(len(mapp)) if mapp[idx]==1]

        #print tabulate(Data,head1,tablefmt="grid")            # print table
        Data.insert(0,head)
        # Create a workbook and add a worksheet.
        workbook = xlsxwriter.Workbook(argv[1].split('.')[0]+'_info.xlsx')
        worksheet = workbook.add_worksheet()
        # Start from the first cell. Rows and columns are zero indexed.
        row = 0
        col = 0
        # Iterate over the data and write it out row by row.
        #f1=open('text_genomes.txt','w')
        for rec in Data:
            col=0
            for item in rec:
                if mapp[rec.index(item)]==1:
                    if rec.index(item)==len(rec)-1:
                        if '<--' in item[-1]:
                            fam=item.split('␣<--␣')
                            worksheet.write(row, col, fam[fam.index(argv[1].split('.')[0])+1])
                        else: worksheet.write(row, col,item)
                    else: worksheet.write(row, col,item)
                    col+=1
            row+=1
        #f1.write('%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\n'%(no,name,pid,access,gid,sv,sl,ng,ntrna,nrrna,norf,lup
        #f1.close()
        print head1

if argv[2].lower()=='gb' or argv[2].lower()=='gbk':
    getGenomes(argv[1],argv[2])
# argv[1]: list of genomes names, argv[2]: retreive format [gb or fasta]
    GeneCount(argv[2],mapp=eval(argv[3]))
# extract information from each genome.
elif argv[2].lower()=='fasta':
    getGenomes(argv[1],argv[2])
    GeneCount(argv[2],mapp=eval(argv[3]))
# argv[2]: retreive format [gb or fasta], argv[3]: save info. Map format
else: print 'Syntax:\npython␣S1_fetchgenome.py␣<list␣of␣genomes␣accessions>␣<fasta␣or␣gb>'

'''
retreive all genomes in a list of genomes names.
syntax:
      getGenomes(<list of genomes names>,<retreiving format: 'gb' or 'fasta'>)
'''
```

Listing 8.2 – PSO implementation.

```
#-*-coding:utf8-*-
from __future__ import division
from os import system, listdir,mkdir
from os.path import exists
from sys import argv
from commands import getstatusoutput
from cogent import LoadTree
from random import random, randint
from numpy import array
from numpy import *
#import matplotlib.pyplot as plt
import time
import numpy as np

GENOMES = listdir('Genomes')

GENES =  sorted([k.split(']')[0] for k in open('Genomes/'+GENOMES[0]).read().split('gene=')[1:]])
##GENES.remove('RRN4.5')

GENES_OU = {'noyau':['elp','ef1a','rpb2','pepck','pold'], 'ARN':['rrnL','rrnS'],'Myto':GENES}

dst=argv[1]

if not exists(dst):
    mkdir(dst)

def bootstraps(arbre):
    X = [k.split(':')[0] for k in arbre.split(')')[1:] if ':' in k]
    return [eval(x) for x in X if x != '']

def faite(texte):
    if texte in listdir(dst) and 'arbre' in listdir(dst+texte):
        boots = bootstraps(open(dst+texte+'/arbre').read())
        return (texte, boots)
    else:
        print texte+'-->␣not␣exists'
        fait([],[GENES[k] for k in range(len(GENES)) if particule['position'][k]==0],[k for k in listdir('Genomes') if

def fait(sans_genome = [], sans_gene = [], outgroup = 'E.vogeli'):
    if dst not in listdir('.'):
        system('mkdir␣%s'%dst)
    if 'alignements' not in listdir('.'):
        system('mkdir␣alignements')
    for k in sans_genome:
        assert k in GENOMES
    for k in sans_gene:
        assert k in GENES
    assert outgroup not in sans_genome
    assert outgroup in GENOMES
    sansGenome = sorted(sans_genome)
    sansGene = sorted(sans_gene)
    texte = ''
    for gene in GENES:
        if gene in sansGene:
            texte += '0'
        else:
            texte += '1'
    if texte in listdir(dst) and 'arbre' in listdir(dst+texte):
        print "␣␣␣=>␣deja␣fait"
        boots = bootstraps(open(dst+texte+'/arbre').read())
        return (texte, boots)
    else:
        try:
            system(r'\rm␣-fr␣%s'%dst+texte)
        except:
            pass
        system('mkdir␣%s'%dst+texte)

        ##############################################
        dd = [k for k in listdir('Genomes') if '~' not in k]
        dico = {}
        for k in dd:
            dico[k.split('.dogma')[0]]={}
            fic = open('Genomes/'+k).read().split('>')[1:]
            for l in fic:
                if l.split('\n')[0].split(']')[0].split('=')[1] not in sansGene and l.split('\n')[0].split(']')[0].spli
                    dico[k.split('.dogma')[0]][l.split('\n')[0].split(']')[0].split('=')[1]]=''.join(l.split('\n')[1:])

        for k in dico[dico.keys()[0]].keys():
            dd=open(dst+texte+'/gene_'+k+'.fasta','w')
            for l in dico.keys():
```

```
        dd.write('>'+l+'\n')
        dd.write(dico[l][k]+'\n')
    dd.close()
#print "Alignements"
################### ALIGNEMENTS #########################
for k in GENES:
    if k not in sans_gene:
        if 'gene_'+k+'.fasta_aln' not in listdir('alignements/'):
            print "␣*␣Alignements␣avec␣t_coffee␣de␣:",k
            #print 't_coffee %s'%dst+texte+'/'+k+' -mode mcoffee -multi_core 1 -n_core 6 -max_n_proc 6 -output fasta'
            #getstatusoutput('t_coffee %s'%dst+texte+'/gene_'+k+'.fasta -mode mcoffee -multi_core 1 -n_core 6 -max_n_p
            #getstatusoutput('t_coffee %s'%dst+texte+'/gene_'+k+'.fasta -mode mcoffee -multi_core 0 -output fasta')
            #getstatusoutput('mv gene_* alignements/')
            system('t_coffee␣%s'%dst+texte+'/gene_'+k+'.fasta␣-mode␣mcoffee␣-multi_core␣0␣-output␣fasta')
            system('mv␣gene_*␣alignements/')

#for k in listdir(texte):
#    print "   - alignement de",k.split('gene_')[1].split('.fasta')[0]
#    #getstatusoutput('muscle -in '+texte+'/'+k+' -out '+texte+'/'+k+'_aln')
for k in GENES:
    if k not in sans_gene:
        system('cp␣alignements/gene_'+k+'.fasta_aln␣%s'%dst+texte+'/')


##########################################
dico = {}

dd = [k for k in listdir('Genomes') if '~' not in k]

for k in dd:
    dico[k.split('.dogma')[0]]=''

for cle in GENES_OU.keys():
    for k in GENES_OU[cle]:
        if 'gene_'+k+'.fasta_aln' in listdir(dst+texte):
           fic = open(dst+texte+'/'+'gene_'+k+'.fasta_aln').read().split('>')[1:]
           for fi in fic:
               if fi.split('\n')[0] in dd:
                   dico[fi.split('\n')[0]]+=''.join(fi.split('\n')[1:])

s=''
for k in dico.keys():
    s += '>'+k+'\n'
    s += dico[k]+'\n'

fic = open(dst+texte+'/alignements.fasta','w')
fic.write(s)
fic.close()

##########################################
ddd=open(dst+texte+'/alignements.fasta').read()

ss=str(len(dd)-len(sans_genome))+'␣'
ss += str(len(''.join(ddd.split('>')[1].split('\n')[1:])))+'\n'
for k in ddd.split('>')[1:]:
    ss += k.split('\n')[0].rstrip('␣␣␣␣␣␣')+'␣'
    ss += ''.join(k.split('\n')[1:])+'\n'
ee=open(dst+texte+'/alignementsRAxML.fasta','w')
ee.write(ss)
ee.close()

##########################################
subs={}
for k in listdir('alignements'):
    if k.endswith('fasta_aln'):
        ee=open('alignements/'+k).read().split('>')[1]
        ee=''.join(ee.split('\n')[1:])
        subs[k.split('gene_')[1].split('.fasta_aln')[0]]=len(ee)


modele = open(dst+texte+'/modele.txt','w')
debut,fin = 1,0
for cle in GENES_OU.keys():
    for k in GENES_OU[cle]:
        if 'gene_'+k+'.fasta_aln' in listdir(dst+texte):
            fin += subs[k]
    if fin>debut:
        modele.write('DNA,␣'+cle+'␣=␣'+str(debut)+'-'+str(fin)+'\n')
    debut = fin + 1
modele.close()


##########################################
```

```
        # -f D: fast Hill-clambing with RELL Bootstraps from http://www.ncbi.nlm.nih.gov/pubmed/23418397
        # -f d: default fast hill... from http://link.springer.com/article/10.1007/s11265-007-0067-4#page-1
        getstatusoutput('raxmlHPC-SSE3 -d -f o -p 12345 -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+texte+'1 -o '+ou

        getstatusoutput('raxmlHPC-SSE3 -d -f o -p 12345 -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+texte+'2 -o '+ou

        getstatusoutput('raxmlHPC-SSE3 -f o -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+texte+'3 -o '+outgroup+' -f
        '''
        getstatusoutput('raxmlHPC-PTHREADS-SSE3 -d -T 6  -f o -p 12345 -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+t

        getstatusoutput('raxmlHPC-PTHREADS-SSE3 -d -T 6 -f o -p 12345 -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+te

        getstatusoutput('raxmlHPC-PTHREADS-SSE3 -f o -T 6 -m GTRGAMMA -q %s'%dst+texte+'/modele.txt -n '+texte+'3 -o '+
        #sleep(5)
        '''
        system('mv RAxML_bestTree.'+texte+'1 %s'%dst+texte)
        system('mv RAxML_info.'+texte+'1 %s'%dst+texte)
        system('mv RAxML_log.'+texte+'1 %s'%dst+texte)
        system('mv RAxML_randomTree.'+texte+'1 %s'%dst+texte)
        system('mv RAxML_result.'+texte+'1 %s'%dst+texte)
        system('mv RAxML_bipartitionsBranchLabels.'+texte+'3 %s'%dst+texte)
        system('mv RAxML_info.'+texte+'3 %s'%dst+texte)
        system('cp RAxML_bipartitions.'+texte+'3 %s'%dst+texte)
        system('mv RAxML_bipartitions.'+texte+'3 %s'%dst+texte+'/arbre')
        system('mv RAxML_bootstrap.'+texte+'2 %s'%dst+texte)
        system('mv RAxML_info.'+texte+'2 %s'%dst+texte)

        #system('rm %s'%dst+texte+'/*fasta')
        system('rm %s'%dst+texte+'/*_aln')

    boots = bootstraps(open(dst+texte+'/arbre').read())
    return (texte, boots)


def addition(mot1, mot2):
    return ''.join([str((int(mot1[k])+int(mot2[k]))%2) for k in range(len(mot1))])

'''
def plotxy(x,y):
    print "X",x
    print "Y",y
    coefficients = polyfit(x,y,0.05)
    poly = poly1d(coefficients)
    xs = arange(min(x)-1, max(x)+1,0.05)
    ys = poly(xs)
    #plot(xs, ys)
    plt.plot(x, y, 'o')
    plt.ylabel('Iteration')
    plt.xlabel('Fitness')
    plt.draw()
    time.sleep(0.05)
'''
POPULATION = 10#[k for k in listdir(dst) if 'arbre' in listdir(dst+k)]
POP = []
fitness = 0
Thresh=95
mx_iter=10
C1 = 2.05
C2 = 2.05
C = C1+C2
# Constriction Coefficient
k1 = random.random()
x1 = 2*k1/abs(2-C-(C*(C-4))**0.5)
print x1
i =0
x=[]
cur_iter=1
minor_results=[]
oldfit=0
oldgbest=[]
# Initiallisation de la population
y=[k for k in listdir(dst) if 'arbre' in listdir(dst+k)]
dead=[]
y2 = []
x2=[]
start_time= time.time()
y=[k for k in listdir(dst) if 'arbre' in listdir(dst+k)]
dead=[]
start_time= time.time()
for k in range(POPULATION):
    position = array([randint(0,1) for k in range(len(GENES))])
    vitesse =  array([random.random() for k in range(len(GENES))])
    POP.append({'position':position,
```

```
                'vitesse':vitesse,
                'score':0,
                'best':position})
while cur_iter<=mx_iter:
    Population=[k for k in listdir(dst) if 'arbre' in listdir(dst+k)]
    print "\n* New round %d"%(cur_iter)
    #For each particle
    #    Calculate fitness value
    #    If the fitness value is better than the best fitness value (pBest) in history
    #        set current value as the new pBest
    #End
    for item, particule in enumerate(POP):
        particle_starttime=time.time()
        print "  - particule %d: %s "%(item,''.join(str(a) for a in particule['position']))
        #print "  - Vitesse   %d: %s "%(item,', '.join(str(a) for a in particule['vitesse']))
        if ''.join(str(a) for a in particule['position']) not in Population:
            Population.append(''.join(str(a) for a in particule['position']))
            resultat = fait([],[GENES[k] for k in range(len(GENES)) if particule['position'][k]==0],[k for k in listdir('Genom
        else: resultat =(''.join(str(a) for a in particule['position']),bootstraps(open(dst+''.join(str(a) for a in particule[
        print resultat[1]
        Min=min(resultat[1])
        if Min>particule['score']:
            particule['score'] = Min
            particule['best']  = particule['position']
            particle_endtime=time.time()-particle_starttime
            print"particle_endtime",particle_endtime
            print "......position:%s, score:%d\n"%(''.join([str(u) for u in particule['position']]), particule['score'])
        else: print "......position:%s, score:%d\n"%(''.join([str(u) for u in particule['position']]), particule['score'])
        #x1.append(particule['score'])
        #y1.append(i)
        #plotxy(x1,y1)
    #print particule['score']
    #Choose the particle with the best fitness value of all the particles as the gBest
    fitness = max([k['score'] for k in POP])
    gBest = array([k['position'] for k in POP if k['score'] == fitness][0])
    i= i+1
    x2.append(fitness)
    y2.append(i)
    if cur_iter<=mx_iter:
        print 'End Round:%d\n..... Best position: %s\tFitness:%d\n'%(cur_iter,''.join(str(a) for a in gBest),fitness)
        for particule in POP:
            r1=random.uniform(0.1,0.5)
            r2=random.uniform(0.1,0.5)
            particule['vitesse']= x1*particule['vitesse']
            particule['vitesse']+= C*r1*(particule['best']-particule['position'])
            particule['vitesse']+= C*r2*(gBest-particule['position'])
            #    Update particle position according equation (b)
            l=[]
            for u in particule['vitesse']:
                 #print"U:",u
                 r=random.uniform(0.1,0.5)
                 #print "r",r
                 v=1/(1+math.exp(-u))
                 #print"expo:",v
                 if r<v:
                    l.append(1)
                 else: l.append(0)
            particule['position']=array(l)
            #particule['position']+= [int(round(u)) for u in list(particule['vitesse'])]
            #particule['position']%= 2
        cur_iter+=1
    else:
        print 'Iteration:%d\tBest position: %s\tFitness:%d...Done!'%(cur_iter,''.join(str(a) for a in gBest),fitness)

f=open(dst+dst.replace('/',''),'w')
print "--- %s seconds ---",time.time() - start_time
print"x2",x2
print"y2",y2
f.write('x2='+str(x2)+'\n')
f.write('y2='+str(y2)+'\n')
f.close()
```

Listing 8.3 – Graphical presentation of genes alignment between genomes.

```
from pickle import load
import PIL.Image as Image
import PIL.ImageDraw as ImageDraw
import PIL.ImageFont as ImageFont
from ALL_ANCESTORS import *
dico = load(open('Apiales_GenesList.pkl'))# Tree 1
r=3
d=40
```

```
dec=10
for (genome1,genome2,genome3) in [('Bras_hainla','K','Eleu_senticosus'),('Kalo_septemlobus','I','Meta_delavayi'),('I','

    print (genome1,genome2,genome3)
    Genome1 = dico[genome1]
    Genome2 = dico[genome2]
    Genome3 = dico[genome3]
    nb_genes=max(len(Genome1),len(Genome2),len(Genome3))

    im = Image.new('RGB',(d*nb_genes+2*dec, 600),(255,255,255))
    draw = ImageDraw.Draw(im)
    font = ImageFont.truetype("/usr/share/fonts/truetype/dejavu/DejaVuSans.ttf",30)

    draw.text((10,30),genome1,(0,0,0),font=font)
    draw.text((10,345),genome2,(0,0,0),font=font)
    draw.text((10,545),genome3,(0,0,0),font=font)

    font = ImageFont.truetype("/usr/share/fonts/truetype/dejavu/DejaVuSans.ttf",8)
    for item1, k1 in enumerate(Genome1):
        for item2, k2 in enumerate(Genome2):
            if k1 == k2:
                draw.line((d*item1+dec,100,d*item2+dec,300),(0,255,0))

    for item1, k1 in enumerate(Genome2):
        for item2, k2 in enumerate(Genome3):
            if k1 == k2:
                draw.line((d*item1+dec,300,d*item2+dec,500),(0,255,0))

    for item1, k1 in enumerate(Genome1):
        for item2, k2 in enumerate(Genome1):
            if k1 == k2 and item1 != item2:
                draw.arc((d*item1+dec,50,d*item2+dec,150),180,0,(255,0,0))

    for item1, k1 in enumerate(Genome2):
        for item2, k2 in enumerate(Genome2):
            if k1 == k2 and item1 != item2:
                draw.arc((d*item1+dec,250,d*item2+dec,350),0,180,(255,0,0))

    for item1, k1 in enumerate(Genome3):
        for item2, k2 in enumerate(Genome3):
            if k1 == k2 and item1 != item2:
                draw.arc((d*item1+dec,450,d*item2+dec,550),0,180,(255,0,0))


    for item, k in enumerate(Genome1):
        if Genome1.count(k)>1:
            draw.ellipse((d*item-r+dec,100-r,d*item+r+dec,100+r),(255,0,0))
        else:
            draw.ellipse((d*item-r+dec,100-r,d*item+r+dec,100+r),(0,0,0))
        if item%2==0:
            draw.text((d*item+dec-10,85),str(item)+'␣'+k,(0,0,255),font=font)
        else:
            draw.text((d*item+dec-10,115),str(item)+'␣'+k,(0,0,255),font=font)

    for item, k in enumerate(Genome2):
        if Genome2.count(k)>1:
            draw.ellipse((d*item-r+dec,300-r,d*item+r+dec,300+r),(255,0,0))
        else:
            draw.ellipse((d*item-r+dec,300-r,d*item+r+dec,300+r),(0,0,0))
        if item%2==0:
            draw.text((d*item+dec-10,285),str(item)+'␣'+k,(0,0,255),font=font)
        else:
            draw.text((d*item+dec-10,315),str(item)+'␣'+k,(0,0,255),font=font)


    for item, k in enumerate(Genome3):
        if Genome3.count(k)>1:
            draw.ellipse((d*item-r+dec,500-r,d*item+r+dec,500+r),(255,0,0))
        else:
            draw.ellipse((d*item-r+dec,500-r,d*item+r+dec,500+r),(0,0,0))
        if item%2==0:
            draw.text((d*item+dec-10,485),str(item)+'␣'+k,(0,0,255),font=font)
        else:
            draw.text((d*item+dec-10,515),str(item)+'␣'+k,(0,0,255),font=font)


    im.save('drawGenomes/'+genome1+'_'+genome2+'_'+genome3+'.png')
```

Listing 8.4 – Python code to alignment and construction the phylogenetic tree for specific species.

```
from os import system
from rpy2.robjects.packages import importr
from cogent import LoadTree
#from pyfaidx import Fasta
from Bio import SeqIO
import os
import sys
from optparse import OptionParser
from Bio.Seq import Seq
from Bio.SeqRecord import SeqRecord

esp = [('africanum',0)]
for k in [0,11,2,12,1,3,13,14,10]:
    #for k in range(13):
    esp.append(('tuberculosis',k))

dd=eval(open('dicoGenomes7.txt').read())


chaine = ""

for es in esp:
    acces = [i for i in dd.keys() if dd[i]['espece']==es[0]and dd[i]['number']==es[1]][0]
    print acces
    chaine += ">"+es[0]+str(es[1])+"\n"
    #chaine += open('Genomes/'+acces+'.fasta').read().split('\n')[1]+'\n'

    def chunks(l, n):
      """Yield successive n-sized chunks from l."""
      #for i in xrange(1, len(l), n):

      yield l[10000:n]
      #print len(l)


    if __name__ == '__main__':
      handle = open('Genomes/'+acces+'.fasta', 'r')
      records = list(SeqIO.parse(handle, "fasta"))
      record = records[0]

      for pos, chunk in enumerate(chunks(record.seq.tostring(),3100000)):
        chunk_record = SeqRecord(Seq(
            chunk, record.seq.alphabet),
            id=record.id, name=record.name,
            description=record.description)
        outfile = 'my_data/'+acces+'.fasta'# % pos

        SeqIO.write(chunk_record, open(outfile, 'w'), "fasta")
        chaine += ''.join(open('my_data/'+acces+'.fasta').read().split('\n')[1:])+'\n'


fic = open('Data/aligne.fasta','w')
fic.write(chaine)
fic.close()

bio=importr('Biostrings')
seqs=bio.readDNAStringSet("Data/aligne.fasta")
dec=importr('DECIPHER')
aligned = dec.AlignSeqs(seqs)
bio.writeXStringSet(aligned,file="Data/result.fasta")
exit()

raw_input("C'est␣bon␣?")

system("raxmlHPC-PTHREADS-SSE3␣-T␣8␣-s␣Data/result.fasta␣-m␣GTRGAMMA␣-n␣tuberculosis1␣-f␣o␣-p␣123␣-o␣africanum0")
system("raxmlHPC-PTHREADS-SSE3␣-T␣8␣-s␣Data/result.fasta␣-m␣GTRGAMMA␣-n␣tuberculosis2␣-f␣o␣-p␣123␣-b␣0123␣-N␣autoMRE␣-o␣africa
system("raxmlHPC-PTHREADS-SSE3␣-T␣8␣-m␣GTRGAMMA␣-n␣tuberculosis3␣-f␣b␣-t␣RAxML_bestTree.tuberculosis1␣-z␣RAxML_bootstrap.tuber

# On affiche l'arbre
arbre = LoadTree(treestring=open("RAxML_bipartitions.tuberculosis3").read())
print arbre.asciiArt()
```

Listing 8.5 – Python code to reconstruct Ancestral Clade by using PHAST

```
from os import system, listdir
from cogent import LoadTree
arbre = str(LoadTree(treestring=open('DataAncestor/RAxML_bipartitions.canettii3').read()))
arbre = arbre.replace('):',')100.X:')
output = open('DataAncestor/arbreClade.newick','w')
output.write(arbre)
output.close()
```

```
seq = "DataAncestor/result_canettii.fasta"
system('rm␣DataAncestor/*.fa')
system('rm␣DataAncestor/*.probs')
system("phyloFit␣--gaps-as-bases␣--tree␣DataAncestor/arbreClade.newick␣--out-root␣DataAncestor/mytree␣"+seq)
system("prequel␣--keep-gaps␣--no-probs␣"+seq+"␣DataAncestor/mytree.mod␣DataAncestor/anc")
system("prequel␣--keep-gaps␣"+seq+"␣DataAncestor/mytree.mod␣DataAncestor/anc")

s=open(seq).read()
for k in listdir('DataAncestor'):
    if k.endswith('.fa'):
        s+='\n'+open('DataAncestor/'+k).read()

fic = open('DataAncestor/aligneAvecAncetres.fasta','w')
fic.write(s)
fic.close()

system('seaview␣DataAncestor/aligneAvecAncetres.fasta')
```

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Résumé :

La théorie de l'évolution repose sur la biologie moderne. Toutes les nouvelles espèces émergent d'une espèce existante. Il en résulte que différentes espèces partagent une ascendance commune, telle que représentée dans la classification phylogénétique. L'ascendance commune peut expliquer les similitudes entre tous les organismes vivants, tels que la chimie générale, la structure cellulaire, l'ADN comme matériau génétique et le code génétique. Les individus d'une espèce partagent les mêmes gènes mais (d'ordinaire) différentes séquences d'allèles de ces gènes. Un individu hérite des allèles de leur ascendance ou de leurs parents. Le but des études phylogénétiques est d'analyser les changements qui se produisent dans différents organismes pendant l'évolution en identifiant les relations entre les séquences génomiques et en déterminant les séquences ancestrales et leurs descendants. Une étude de phylogénie peut également estimer le temps de divergence entre les groupes d'organismes qui partagent un ancêtre commun. Les arbres phylogénétiques sont utiles dans les domaines de la biologie, comme la bioinformatique, pour une phylogénétique systématique et comparative. L'arbre évolutif ou l'arbre phylogénétique est une exposition ramifiée les relations évolutives entre divers organismes biologiques ou autre existence en fonction des différences et des similitudes dans leurs caractéristiques génétiques. Les arbres phylogénétiques sont construits à partir de données moléculaires comme les séquences d'ADN et les séquences de protéines. Dans un arbre phylogénétique, les noeuds représentent des séquences génomiques et s'appellent des unités taxonomiques. Chaque branche relie deux noeuds adjacents. Chaque séquence similaire sera un voisin sur les branches extérieures, et une branche interne commune les reliera à un ancêtre commun. Les branches internes sont appelées unités taxonomiques hypothétiques. Ainsi, les unités taxonomiques réunies dans l'arbre impliquent d'être descendues d'un ancêtre commun. Notre recherche réalisée dans cette dissertation met l'accent sur l'amélioration des prototypes évolutifs appropriés et des algorithmes robustes pour résoudre les problèmes d'inférence phylogénétiques et ancestrales sur l'ordre des gènes et les données ADN dans l'évolution du génome complet, ainsi que leurs applications.

La reconstruction du génome ancestral peut être décrite comme une étude phylogénétique d'espèces d'intérêt pour des détails supplémentaires que ce qui est fourni par un arbre phylogénétique standard. Il peut s'agir d'informations sur les espèces ancêtres telles que leur contenu génétique, la configuration de ces gènes dans le génome, la séquence nucléotidique elle-même. Ces informations peuvent aider à mieux comprendre l'évolution de l'évolution d'un ensemble d'organismes et à travers la lumière générique sur les bases génomiques des phénotypes.

Dans cette thèse, nous sommes intéressés par des problèmes théoriques et pratiques dans la reconstruction des arbres phylogénétiques et les réarrangements du génome. Nous proposons une approche heuristique de la reconstruction ancestrale du génome et nous mettons en œuvre un des outils pratiques applicables à l'analyse des ensembles de données réels couvrant une phylogénie complexe et accueillant une variété d'architectures génomiques. Nous démontrons l'efficacité de notre approche sur l'ensemble de données bien étudié des génomes de chloroplastes et nous l'appliquons à la reconstruction des histoires de réarrangement de la reconstruction complète et très précise de certaines lignées de bactéries spécifiques telles que le genre *Mycobacterium*. Le problème de reconstruction des génomes ancestrales dans un arbre phylogénétique donné se situe dans différents domaines génomiques comparatifs. Dans ce travail, nous nous concentrons sur la reconstruction des génomes ancestrales par l'ordre des gènes, l'accessibilité à la reconstruction des séquences d'ADN d'un génome complet. La reconstruction du génome ancestral en ce sens et pour les génomes chloroplaciques et les souches de bactéries spécifiques est le sujet de cette thèse.

Mots-clés : Reconstruction ancestrale, séquence nucléotidique, unités taxonomiques, arbre phylogénétique, *Mycobacterium* Genre, *Chloroplastic* génomes

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