Ancestral Reconstruction and Investigations of Genomic Recombination on some Pentapetalae Chloroplasts

1 Introduction

Chloroplasts are one of the main organelles in plant cells. They are considered to have originated from cyanobacteria through endosymbiosis, when an eukaryotic cell engulfed a photosynthesizing cyanobacterium, which remained and became a permanent resident in the cell.

Chloroplasts have the ability to convert water, light energy, and carbon dioxide ($\text{CO}_2$) in chemical energy by using carbon-fixation cycle. This key role explains why photosynthetic organisms are at the basis of most trophic chains and are thus responsible for evolution and speciation. Moreover, as photosynthetic organisms release atmospheric oxygen when converting light energy into a chemical one, and simultaneously produce organic molecules from carbon dioxide, they are at the origin of the breathable air and represent a mid to long term carbon storage medium. Consequently, exploring the evolutionary history of chloroplasts is of great interest, and we propose to investigate it by the mean of ancestral genome reconstruction. This reconstruction will be achieved in order to discover how the biomolecules (proteins and DNA) have evolved over time due to mutations or recombination. It will be useful to compute the mutation rate, and to determine whether evidences of their cyanobacteria origin can be presented in this way.

This article thus aims at exploring the possibility to reconstruct the Last Universal Common Ancestor (LUCA) of all available chloroplastic genomes, for a large variety of reasons encompassing the comparison with cyanobacterial genomes. The goal of this paper is not to provide a definitive answer to this ambitious question, but to investigate scientific and technical obstacles that may potentially appear when trying to reach such a difficult goal. In this proposal, the ancestral reconstruction has been achieved in two stages. Firstly, after having obtained a large collection of complete chloroplastic genomes, their coding sequences have been extracted and automatically annotated following the approach detailed in [1], [2]. Using the genes shared in
common by these species, a well-supported phylogenetic tree has been obtained. However, the core genome of the whole species is too small to produce an accurate tree. A strategy was then applied which consisted in grouping subsets of sequences according to their similarity, inferring their phylogenies, and then merging the whole forest of trees, following the approach investigated in [3], [4], [5]. This first step being achieved, the second stage was to design algorithms that study the evolution of gene content and ordering among the supertree, and the latter must be validated with the naked eye on well chosen plant families. Our proposal in this article focuses on this second stage, and illustrates the kind of results that can be obtained on three small groups of Pentapetalae species. They have been selected due to the fact that, at the time of this study, only these groups of organisms had a sufficient number of complete genomes of good quality on NCBI. It will be completed in future work, by obtaining ancestral nucleotide sequence of each gene, and by filling intergenic regions using either state-of-the-art or novel algorithms.

Ancestral genome reconstruction has already been investigated several times in the literature [6], [7], [8]. Over the past decade, many methods have been exploited to reconstruct phylogenies from gene-order data. The first algorithm of this kind was established by Fitch [8]. It assumed a binary alphabet and is based on the maximum parsimony (MP) approach. It finds the label to the internal nodes of a tree that reduces the number of changes or modifications along tree edges. Usually, state-of-the-art algorithms deal with the permutations of integers, each integer corresponding to the position of the associated gene in the lexicographic ordering of the pan-genome gene names. In other words, tools like Badger [9] do not support genomes of various lengths and with repeated/missing genes. Our problem applied to chloroplasts may appear as more difficult, as we relax the permutation hypothesis. However, in the classical Multiple Genome Rearrangement Problem [10], targeted genomes are either bacterial or nucleus ones, which have many 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. Note that a new tool, called MLGO, can be applied for phylogenetic and ancestral genome reconstruction regarding gene-order data. MLGO relies on two methods: MLWD [11] for phylogenetic analysis and PMAG+ [12] for ancestral genome reconstruction. This tool uses the advantage of binary encoding on gene-order data, supports a relatively general model of genomic evolution (including not only rearrangements but also gene insertions, deletions, inversions, and duplications), and successfully accommodates itself into the framework of maximized likelihood.

2 Presentation of the problem

Let us consider a set of complete chloroplastic genomes for close plant species, like the ones presented in Table 1.

Table 1: Genomes information of all considered species.

| Organism name                       | Accession | NCBI Nucleotide database ids | Sequence length, pb | Number of genes | Order | Lineage           |
|-------------------------------------|-----------|------------------------------|---------------------|-----------------|-------|-------------------|
| **Ageratina adenophora**            | NC_015621.1 | 334701780                   | 150,698             | 183             | Asterales | Eupatorieae    |
| **Anthriscus cerefolium**           | NC_015113.1 | 323149061                   | 154,719             | 166             | Apiaces  | Apiaceae        |
| **Aralia undulata**                 | NC_022810.1 | 563940258                   | 15,633              | 169             | Apiaces  | Araliaceae      |
| **Artemisia frigida**               | NC_020607.1 | 470227687                   | 151,076             | 164             | Asterales | Artemisiineae  |
| **Brassica oleracea**               | NC_028111.1 | 558602891                   | 156,459             | 168             | Apiaces  | Araliaceae      |
| **Castanea mollissima**             | NC_014674.1 | 313183972                   | 160,799             | 165             | Fabids   | Fabaceae        |
| **Castanopsis echinocarpa**         | NC_023801.1 | 595789916                   | 160,647             | 165             | Fabids   | Fabaceae        |
| **Chrysanthemum indicum**           | NC_020320.1 | 452849029                   | 150,972             | 167             | Asterales | Artemisiineae  |
| **Chrysanthemum morifolium**        | NC_020929.1 | 441403271                   | 151,033             | 165             | Asterales | Artemisiineae  |
| **Chrysobalanus icaco**             | NC_020461.1 | 63076125                    | 162,775             | 166             | Fabids   | Chrysobalanaceae|
| **Corynocarpus laevigata**          | NC_014807.1 | 317046152                   | 159,202             | 165             | Fabids   | Corynscarpaceae |
| **Cucumis melo**                    | NC_015983.1 | 346578170                   | 156,017             | 163             | Fabids   | Benincaseae     |
| **Cucumis sativus**                 | NC_007144.1 | 68164782                    | 155,293             | 168             | Fabids   | Benincaseae     |
| **Daucus carota**                   | NC_008325.1 | 114107112                   | 155,911             | 166             | Apiaces  | Apiaceae        |
| **Eleutherococcus senticosus**      | NC_016430.1 | 35922122                    | 156,768             | 169             | Apiaces  | Araliaceae      |
| **Fragaria chiloensis**             | NC_019601.1 | 428697178                   | 155,603             | 137             | Fabids   | Fragariineae    |
First, we assume that:

1. Each genome has been annotated with Dogma [13]. By doing so, the same gene prediction and naming process has been applied with the same quality of annotation. In particular, when a gene appears twice in the considered set of genomes, it receives twice the same name. At this level, each genome is described by an ordered list of gene names, with possible duplications. Other approaches are possible, see, e.g. [2], [14], [15].

2. The sequences inside the core genome (genes present everywhere in the considered set of species) have been multialigned, and a well supported phylogenetic tree has been obtained based on this alignment as shown in Figure 1 for Apiales order. This stage may necessitate the deletion of a few core genes that possibly blur the phylogenetic signal (for various reasons encompassing homoplasy, incomplete lineage sorting, horizontal gene transfers, etc.), for instance by using methods detailed in [1], [4], [5].
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Figure 1: Most supported phylogenetic tree obtained from Apiales order.
In this figure, the values at the top of the lines represent the branch lengths, while the values at the bottom correspond to the bootstrap supports (when it makes sense).

Our objective is then to reconstruct ancestral genomes at each node of the phylogenetic tree until the root node (last common ancestor). Such a reconstruction requires to first find the 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, a set of authorized operations are provided, which are:

– insertion, deletion, duplication, or inversion of one or a block of genes, at gene lists level;
– operations commonly considered in the Needleman-Wunsch edit distance [16] (insertion, modification, or deletion of a nucleotide, together with opening and enlarging a gap), at DNA sequence levels.

The operations listed above allow a parsimonious approach to be used so that the number of leaf nodes is as small as possible. Note that the global optimum over the tree may be obtained with a few local solutions (one ancestor of two genomes) that are not optimal.

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.

3.1 Method I: Naked eye investigation

As stated above, this method is not an algorithm that automatically builds the ancestors of the provided genomes, but it is a method applied manually, as follows. Some python codes were produced in order to graphically represent each triplet constituted by two sister species and their closest cousin as three parallel lines, as described in Figure 2 (the three lines at the top of the figure). On each line are located numerous equidistant vertices, one per coding sequence in the associated genome, and all the sequences having the same gene name according to Dogma are linked by a red edge.
Figure 2: Simulation of ancestral reconstruction process between two genomes. ADD means that the considered sequence has been added to the list of genes in the ancestral genome.

The ancestral genome of each couple of sister species has then been manually deduced by a consensus approach. Each part shared in common in its genomes is put in the ancestor (see, for instance, the cases of YCF68 and YCF68_1: they are both in Genome-1 and Genome-2, so they were put on the ancestor). In case of a difference, both brother genomes are compared locally with their closest cousin. If the latter agrees with one of the two brothers, the agreement (sequence of genes) is put on the ancestor and this part of the lines is considered as resolved. For instance, PSBI is in Genome-1 but not in Genome-2. As it is in Genome-3, the parcimonious hypothesis is that it was in the ancestor of the 3 species, and that Genome-2 lost it.

If the closest cousin cannot help us to resolve the situation, because the cousin presents locally a third pattern different from the two brothers, then one or more new close cousins are considered recursively. It is the solution that reduces the largest number of rearrangement operations that is finally chosen, leading to the local ancestor gene list (again a parsimonious approach).
Figure 3: Names of the ancestors in the Apiales and Asterales phylogenetic tree, provided with supports and branch lengths: the numbers shown at each branch are bootstrap scores computed by RAxML [4], while branch lengths are indicated (cf. Br Lengths). A letter has also been associated to each internal node (the ancestors are in red), while the number of genes per genome is indicated under the label “Genome length”.

By doing so and verifying our results three times, a trustworthy ancestral list of genes at each internal node was obtained. Our next objective was to recover these ancestors automatically.

3.2 Method II: Ancestor Prediction based on Gene Contents

This method encompasses the following general steps:

- **Step 1: Preliminary stage.** In this step, all internal nodes from leaf nodes to the root one are named following the alphabetical order. Each letter in an internal node represents an ancestor genome. This latter can be the ancestor of two leaves, of an internal ancestor and a leaf node, or of two internal ancestors. The result of this step can be seen in Figure 3. In this tree, a bottom-up procedure was applied to predict the ancestor genome at each internal node.

- **Step 2: Genome Selection.** Figure 3 presents the most supported topology for two Campanulids subgroups. The algorithm starts by automatically selecting 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), defining what can be called an ordered list of cousins.

- **Step 3: Genes Investigation.** In the easiest situation, all gene couples completely match between the two sister species (which thus have the same length). In this case, the frequency of occurrences depends on the considered family, the ancestor is easily deduced as being the same as its children. If there is at least one problematic situation between the selected genomes (that is, if there is at least one deleted, duplicated, or inserted gene in one genome), as for example between E. senticosus and B. hainla, then a deeper investigation is initiated using one or more cousin genome(s). For instance, in this example, M. selavai and K. septemlobus...
will be considered first as cousin genomes to take the final decision in the treatment of such problematic situations.

In this case, all genes are iterated in the considered two brother 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 it is added in the ancestor genome $\gamma$ 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 $\gamma$ in position $i$, else this gene is considered as an insertion.

Figure 4 offers a simulation example of the considered procedure. In this figure, suppose that A, B, C, D, and E in the leaves are genes, and the objective is to predict the ancestor $\alpha_1$. Note that genes A, B, and D match in positions. Concerning the problematic C gene between these two genomes, a cousin will be needed to determine whether it is present in the $\alpha_1$ ancestor genome or not. One or both genomes in $\alpha_2$ 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 $\alpha_1$ ancestor and a delete operation is recorded (that is, AB_D). An insert state is also marked in $\alpha_2$ subtree, where gene E did not appear in either cousin genomes of $\alpha_1$ tree, nor in its brother. Such a deletion is illustrated in Figure 5a and b.

The simple conflict resolution presented above has been refined by considering the Gestalt pattern matching method [17] based on dynamic programming like Needleman-Wunsch, as it is implemented in the SequenceMatcher method of the difflib Python library.

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

**Step 5: Loop-back.** Repeat from Step 2 until the final root ancestor is constructed.

So all matching genes are directly assigned to the ancestor. For non-matching genes, the process consists of the selection of a third genome, among the cousins according to the provided tree. The selected cousin is the closest one to the two considered genomes, according to the chosen distance. It is then compared to the two sister species for each non-matching gene, if the cousin agrees with one sister, then the considered gene is added to the ancestor.
Figure 5: Graphical presentation of genes alignment between two genomes, useful in the naked eye investigation. Red curves indicate duplicated genes within a genome, while green lines indicate homology (same gene) between two distinct genomes. (A) At this particular location of the genome, a gene loss can be seen in *B. hainla* (or one gene gain in *K. septemlobus*), namely the YCF69. (B) As the same situation occurs between *B. hainla* and *M. delavayi*, we can conclude to a gene loss (as gaining twice the same gene at the same location is less likely). (C) At this location, the gene order is not modified between the genomes of the two considered sister species, namely *K. septemlobus* and *M. delavayi*.

4 Discussion

The whole process of ancestral gene order reconstruction was performed on three data sets, namely: *Apiales*, *Asterales*, and *Fabids*. Their phylogenetic relationship, obtained using RAxML [18] on multi sequence alignment of their core genes, is reproduced in Figure 3.

4.1 The *Apiales* order

Let us first consider the *Apiales* order for which both the phylogenetic relationship and the gene lists of leaves are known at the beginning of the study. 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 lead to the following conclusions regarding the evolution of gene content among the tree. We focused first on the evolution of duplications. Table 2 (supplementary material) contains all duplicated genes among the *Apiales* order. For each duplication, the number of copies is specified as well. Let us now enter into details regarding the leaves of the phylogenetic tree shown in Figure 3.

- 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 \textit{B. hainla} and in 6 copies in the sister species. As the cousin presents 6 copies as well, it can be deduced that the common ancestor of \textit{E. senticosus} and \textit{B. hainla} contains 6 copies of this gene. In other words, two copies of \textit{YCF68} have been removed in \textit{B. hainla}. All the other genes are similar in both names and locations, and thus the ancestral genome (I) can be deduced.

- The brother genomes \textit{A. undulata} and \textit{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 \textit{A. undulata} and \textit{P. ginseng}, \textit{M. delavayi} and \textit{K. septemlobus}, \textit{S. delavayi} and \textit{K. septemlobus}, \textit{S. delavayi} and \textit{M. delavayi}, and finally \textit{K. septemlobus} and \textit{E. sentucosus} match perfectly when considering each couple of brother 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. 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 5c shows this matching process applied on \textit{M. delavayi} and \textit{K. septemlobus}, which have a core genome of 169 genes (only the 23 first genes are depicted). Note that, in this example, \textit{M. delavayi} (L) and \textit{K. septemlobus} (M) match completely, so the ancestor \textit{H} is very easy to obtain (\textit{H} = \textit{L} \cap \textit{M} has 169 genes).

- Let us finally compare \textit{A. cerefolium} and \textit{D. carota}. The \textit{YCF68} gene exists in 2 copies in \textit{A. cerefolium} while it is missing in \textit{D. carota}, as shown in Figure 6a. The cousin, for its part, also contains the gene \textit{YCF68} (in 6 copies), and so our algorithm concludes the presence of this gene (in 2 copies) in the ancestor of \textit{A. cerefolium} and \textit{D. carota}. Additionally, \textit{D. carota} contains 4 copies of \textit{ORF56}, while this gene is only represented twice in \textit{A. cerefolium}. As the cousin genome has 4 representatives of \textit{ORF56}, we can reasonably deduce that this is the case too in the ancestor of these two sister species: two copies of the gene \textit{ORF56} have been deleted from the genome \textit{A. cerefolium}. Such decisions are depicted in Figure 6b, which shows a specific region of the ancestor genome (C). This region has been generated by our algorithm, which has been applied on \textit{A. cerefolium} and \textit{D. carota}, and it has been 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 	extit{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 cases, the disparity comes from the variation in numbers of gene copies. The obtained results are summarized in Figure 7.
Figure 7: Insertion and deletion events found during ancestor reconstruction on *Apiales* order.
Letters in red refer to ancestor genomes (their lengths are provided too).

4.2 The *Asterales* order

The *Asterales* order, which is close to the *Apiales* one, was then examined. Table 3 in supplementary material contains what has been deduced from our experiments on this order. As can be seen, *Asterales* genomes have undergone many more changes compared to the *Apiales* ones. This difference between both 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 8.

In this second order and in most cases, genes are comparable in both names and locations, having the same positions if we do not consider duplications. Indeed, almost all differences in this set of chloroplastic genomes come from a variation in the number of copies. Let us now investigate a third order, to compare it with *Apiales* (only a few variations of genomes) and *Asterales* (large variety in duplications).
4.3 The Fabids order

It was easy to deal with Apiales order, while Asterales improved the complexity of the ancestral reconstruction, due to duplications. However, in both cases the proposed algorithm was able to recover results that have been inferred manually (naked eye investigation). Let us 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 both previously studied orders. In this case, a single inversion detection algorithm has been able to signal 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.

![Figure 8: 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.](image-url)
Figure 9: A phylogenetic tree in the reconstruction of the *Fabids* ancestor and the unambiguous reconstruction accuracies 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 also indicated.

In this situation, our proposition consisted in selecting one of the two brothers to operate as a reference. The best cousin was then searched within the same clade, and the status of each gene in this region (matching, or need insertion or deletion) was then compared. Most of the considered reversal regions match at the genes names level, but with reverse positions. Figure 9 presents our finding on *Fabids* order, with information about the length of each node. Various rearrangement information were also provided such as the number of insertion, deletion, and inversion.

Figure 10: The variation in comparison results of ancestral genomes nodes on *Asterales* order with MLGO.

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. This latter is, to the best of our knowledge, the first web tool for phylogeny and ancestral genomes reconstruction compatible with genome rearrangements [12].

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 duplications. On the other hand, the results are very different for some
nodes in the Asterales case, as summarized in Figure 10. For instance, when gene YCF1 in internal node d has 2 copies in both 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.

| 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             | INF A     | 1                      | 1                      | 1                           | 1                           |          |      |
| T             | ACCD      | 2                      | 2                      | 2                           | 2                           | 1        | 1    |
| Q             | YCF1      | 1                      | 1                      | 1                           | 1                           | 1        | 1    |
| E             | NOD1      | 2                      | 2                      | 1                           | 1                           | 2        | 1    |
| E             | INF A     | 2                      | 1                      | 1                           | 2                           | 1        |      |
| E             | ATPF      | 1                      | 1                      | 1                           | 1                           | 2        | 1    |
| d             | RPS16     | 2                      | 1                      | 1                           | 1                           | 2        | 1    |
| c             | RPS19     | 2                      | 2                      | 2                           | 2                           | 1        |      |
| a             | PSIG      | 1                      | 1                      | 1                           | 1                           | 1        | 1    |
| a             | TRNK-UUU  | 1                      | 1                      | 1                           | 1                           | 1        | 1    |
| a             | NOD1      | 1                      | 2                      | 2                           | 2                           | 1        |      |

Figure 11: The variation in ancestral genomes nodes which were achieved by comparing our method results with MLGO tool on Fabids order.

Similar consequences can be outlined in the most difficult case, namely the Fabids order, as highlighted by Figure 11, 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, it was found that gene INF A is missing in the first child while it is present in the second one. It was also found in both its 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 INF A. This result is produced by our algorithm, while MLGO considers that M must not have INF A 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, this node must contain two copies of ATPF. Other nodes are problematic in the MLGO case, for example, [a, b, c, d] as can be seen in Figure 11. 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. Indeed, these results were produced following a random extraction among the differences in the order of genes in the current species: on a large random extraction of our large set of reconstructed ancestral genes, leading to 18 different evolutionary scenarios according to our method and that of MLGO, we systematically discovered the right ancestral situation, while MLGO systematically misled itself.
Figure 12: Green lines indicate homologous genes while red vertices are for paraloguous ones. C line in figure (B) is the reconstructed ancestor of these two chloroplasts.

(A) 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. (B) Apiales order tree produced by MLGO.

5 Conclusion and future works

Given a set of close annotated chloroplastic genomes, we have extracted the largest subset of core genes that lead to the most supported phylogenetic tree. On such trees, we have proposed a first ancestral reconstruction of gene content and order. The algorithm is based on the SequenceMatcher method of the Python difflib library and the results obtained were verified with the naked eye on well-defined families. Ways to merge the forest of phylogenetic trees in a supertree have been considered as well, and the way gene content evolves through a tree of core genomes has finally been presented.

This proposal belongs to an ongoing project regarding the design of the ancestral reconstruction of chloroplastic genomes. Our objective in this article was to show the feasibility of the approach on simple and specific events: duplication, insertion or deletion of a gene. More complex recombinations or larger magnitudes such as inversion, large-scale duplications, or events related to repeated sequences, certainly require further developments, and are beyond the scope of this article. But we intend to continue both the theoretical investigations and their applications. The next steps of such research work are to reconstruct the ancestral DNA sequences, to extend the algorithms to larger genomes (of bacteria, for instance), to apply them to larger sets of species (e.g. the whole available complete genomes of chloroplasts), and to extract various knowledge from these ancestors regarding the evolution of genome sequences.

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Notes

1 http://www.geneorder.org.

References

[1] Alsrraj R, AlKindy B, Guyeux C, Philippe L, Couchot J-F. Binary Particle Swarm Optimization versus Hybrid Genetic Algorithm for Inferring Well Supported Phylogenetic Trees. Computational Intelligence Methods for Bioinformatics and Biostatistics, Lecture Notes in Bioinformatics LNBI series, Springer (Revised and extended journal version of the CIBB2015 conference). 9874, 165–179. 2016.
[2] AlKindy B, Guyeux C, Couchot J-F, Salomon M, Bahi JM. Gene similarity-based approaches for determining core-genes of chloroplasts. 2014 IEEE International Conference on Bioinformatics and Biomedicine (BIBM), 2014:71–74.
[3] AlKindy B, Al’Nayef H, Guyeux C, Couchot J-F, Salomon M, Bahi J. Improved core genes prediction for constructing well-supported phylogenetic trees in large sets of plant species. IWBBIO, 3rd Int. Work-Conf. on Bioinformatics and Biomedical Engineering, Granada, Spain, 2015:379–390.
[4] AlKindy B, Guyeux C, Couchot J-F, Hybrid Genetic Algorithm and Lasso Test Approach for Inferring Well Supported Phylogenetic Trees based on Subsets of Chloroplastic Core Genes. Vol. 9199. Proceedings of AlCoB 2015, 2nd International Conference on Algorithms for Computational Biology. Mexico City (Mexico); 2015:83–96.
[5] AlKindy B, Guyeux C, Couchot J-F, Salomon M, Bahi J. Using genetic algorithm for optimizing phylogenetic tree inference in plant species. MCEB15, Mathematical and Computational Evolutionary Biology, poster, 2015.
[6] Blanchette M, Diallo AB. Computational reconstruction of ancestral DNA sequences. Methods Mol Biol 2008;422:171–84.
[7] Rascal VL, Pontarotti P, Levasseur A. Ancestral animal genomes reconstruction. Curr Opin Immunol 2007;19:542–6.
[8] Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. Systematic zoology. 1971;20(4):406–416.
[9] Larget B, Simon DL, Kadane JB, Sweet D. A bayesian analysis of metazoan mitochondrial genome arrangements. Mol Biol Evol 2005;22:486–95.
[10] Hannenhalli S, Chappell C, Koonin EV, Pevzner PA. Genome sequence comparison and scenarios for gene rearrangements: a test case. Genomics 1995;30:299–311.
[11] Lin Y, Hu F, Tang J, Moret B. Maximum likelihood phylogenetic reconstruction from high-resolution whole-genome data and a tree of 68 eukaryotes. Pac Symp Biocomput 2013;285–96. https://doi.org/10.1142/9789814447973_0028.
[12] Hu F, Zhou J, Zhou L, Tang J. Probabilistic reconstruction of ancestral gene orders with insertions and deletions. IEEE/ACM Trans Comput Biol Bioinform 2014;11:667–72.
[13] Wyman SK, Jansen RK, Boore JL. Automatic annotation of organelar genomes with DOGMA. Bioinformatics 2004;20:3252–5.
[14] Alkindy B, Couchot J-F, Guyeux C, Mouly A, Salomon M, Bahi J. Finding the Core-Genes of Chloroplasts. International Journal of Bio-science, Biochemistry and Bioinformatics (IJBBB). 2014;4(5):361–368.
[15] AlKindy B, Al-Nayyef H, Guyeux C, Improved core genes prediction for constructing well-supported phylogenetic trees in large sets of plant species. Series Lecture Notes in Computer Science (LNCS) Vol. 9043. Granada, Spain: Bioinformatics and Biomedical Engineering, Springer; 2015:379–390.
[16] Needleman S, Wunsch C. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J Mol Biol 1970;48:443–53.
[17] Ratcliff JW, Metzener DE. Pattern-matching-the gestalt approach. Dr Dobbs J 1988;13:46.
[18] Stamatakis A, Ludwig T, Meier H. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 2005;21:456–63.

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