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Haris Gavranović1,∗, Cedric Chauve2, Jérôme Salse3 and Eric Tannier4,∗

1Faculty of Natural Sciences, University of Sarajevo, Bosnia and Herzegovina, 2Department of Mathematics, Simon Fraser University, Burnaby (BC), Canada, 3INRA, UMR 1095, Laboratoire Génétique, Diversité et Ecophysiologie des Céréales, 234 avenue du Brézet, 63100 Clermont Ferrand and 4INRIA Grenoble Rhône-Alpes, LBBE, UMR CNRS 5558, Université de Lyon 1, France

ABSTRACT

Motivation: Ancestral genomes provide a better way to understand the structural evolution of genomes than the simple comparison of extant genomes. Most ancestral genome reconstruction methods rely on universal markers, that is, homologous families of DNA segments present in exactly one exemplar in every considered species. Complex histories of genes or other markers, undergoing duplications and losses, are rarely taken into account. It follows that some ancestors are inaccessible by these methods, such as the proto–monocotyledon whose evolution involved massive gene loss following a whole genome duplication.

Results: We propose a mapping approach based on the combinatorial notion of “sandwich consecutive ones matrix”, which explicitly takes gene losses into account. We introduce combinatorial optimization problems related to this concept, and propose a heuristic solver and a lower bound on the optimal solution.

Availability: Upon request to the authors.

Contact: haris.gavranovic@gmail.com; eric.tannier@inria.fr

1 INTRODUCTION

Mapping ancestral genomes consists in ordering ancestral markers into chromosomess, according to the organization of the descendants of these markers in sequenced extant genomes. In the absence of a good model for genome structural evolution, mapping techniques for ancestral genomes, introduced by Bergeron et al. (2004), have given the most reliable ancestral configurations on animals (Chauve and Tannier, 2008; Ma et al., 2006; Ouangraoua et al., 2009), yeast (Bertrand et al., 2010; Chauve et al., 2010a; Tannier, 2009), or plant genomes (Murat et al., 2010), and even on a wide eukaryote dataset (Muffato, 2010; Muffato et al., 2010). These works also raised new methodological issues and stimulated a recent stream of algorithmic studies related to genome mapping (Adam et al., 2007; Chauve et al., 2010b; Dom, 2009; Dom et al., 2010; Manuch and Patterson, 2010; Stoye and Winters, 2009; Winters and Stoye, 2010), which had taken the back seat with the development of massive genome sequencing.

The general principle of ancestral genome mapping is:

(i) Define ancestral genome markers, constructed either from homologous gene families or from aligned chromosome segments;

(ii) Infer, from the structure of extant genomes, a collection of relations between ancestral markers which are believed to be ancestral; and

(iii) Assemble this collection into an ancestral genome.

The relations between ancestral markers can take several forms, such as adjacency or distance between pairs of markers, or contiguity/synteny of a subset of markers for example. The combinatorial nature of these relations defines the abstract representation of the ancestral genome, from totally ordered proto–chromosomal segments (Ma et al., 2006; Muffato et al., 2010) to contiguous ancestral regions (Chauve and Tannier, 2008; Ouangraoua et al., 2009) and ancestral linkage groups (Chauve et al., 2010b).

Up to now, most published methods require unique and universal ancestral markers, that is, each ancestral marker has exactly one descendant in every considered extant genome. This constraint, common to many genome–mapping methods (Bergeron et al., 2004; Chauve et al., 2010a; Chauve and Tannier, 2008; Ma et al., 2006; Ouangraoua et al., 2009; Tannier, 2009) and genome rearrangement studies (Aleksseyev and Pevzner, 2009; Zhao and Bourque, 2009), results in general in more tractable algorithmic problems for the assembly phase. Recently, several works tried to account for the possibly complex history of markers, by integrating whole genome duplication and gene loss either at the level of ancestral markers definition (Chauve et al., 2010a; Murat et al., 2010; Tannier, 2009), or in the whole mapping process, allowing duplicated markers arising from a whole genome duplication (Ma et al., 2008), or genes with duplications and losses (Bertrand et al., 2010; Muffato, 2010; Muffato et al., 2010). In these works, either a backbone of universal makers is used, or only adjacencies between genes were considered. It means that gene loss is neglected, and is expected to produce a reasonable amount of noise in the assembly phase.

The above assumptions are not appropriate anymore if there is a highly heterogeneous marker content within the extant descendants considered to reconstruct an ancestral genome. For example, in the early monocotyledon evolution, a whole genome duplication is believed to have occurred (Abrouk et al., 2010; Salse et al., 2009), followed by numerous gene losses, representing...
Traveling Salesman Problem (TSP) is described in the PhD thesis of an ancestral genome. It is a binary matrix: there is an entry if the groups of markers that are believed to be contiguous in the matrix is built, whose columns are the ancestral markers and rows (see Chauve and Tannier, 2008 for a detailed description). A binary matrix is built, whose columns are the ancestral markers and rows represent groups of markers that are believed to be contiguous in the ancestral genome. It is a binary matrix: there is a 1 in an entry if the marker belongs to the group, and 0 otherwise. Then the assembly phase infers an order of the columns (i.e. markers) such that on all rows the entry ones are consecutive. If such an order, which satisfies the consecutive ones property, does not exist, then a combinatorial optimization approach is used, such as extracting a maximum subset of rows or flipping a minimum number of entries. Groups of markers defining the rows of the matrix are obtained from the comparison of all pairs of extant genomes whose evolutionary path contains the ancestor of interest.

The framework described above can be extended to handle the fact that a marker can be missing when comparing a pair of extant genomes, because of gene loss for example. It simply calls for a third type of entry: if a marker is missing, then it is not possible to say if it belongs or not to a given group of markers, so we may mark it with an entry $X$ in the matrix row associated to this group of markers. In the assembly phase, it can play the role of a 1 or a 0. The assembly problem is now to find an order of the columns such that on all rows, there is no entry 0 between two entries 1, or, if such an order does not exist, to approximate this property while optimizing a given combinatorial criterion. Note that in the absence of $X$ entries, the problem is equivalent to the consecutive ones described above. This problem was introduced by Golumbic and Wassermann (1998) and Golumbic (1998), under the names ‘sandwich interval hypergraph’ or ‘sandwich consecutive matrix’. It was proved that deciding if the columns of a matrix with entries 0, 1 or $X$ (a ternary matrix) can be ordered such that no 0 is between two 1s is NP-complete and the possibility to use this concept in physical mapping to account for missing or uncertain data was already mentioned in these articles.

In Section 2, we describe several computational results for the problem of ordering the columns of a ternary matrix. We define combinatorial criteria associated to matrices that do not have the sandwich consecutive ones property, a heuristic based on the technique of partition refinement, a reduction to the TSP, a local search principle and a lower bound that is used to assess the quality of the solutions computed with these methods. Next we apply these algorithmic results to the reconstruction of the gene order of the protocromosomes of the monocotyledon angiosperms before they underwent a whole genome duplication in Section 3.1. We discuss the robustness of the results and the multiplicity of solutions in Section 3.3. The framework we present is not restricted to plant genomes. It generalizes several of the previous methods, accounting for gene loss as an additional feature. It can be used for any ancestral genome reconstruction. We demonstrate that former results, like the boreoeutherian ancestor, can be retrieved with this new framework with a good accuracy and an increased coverage (Section 4).
The heuristic we describe now is based on the C1P matrix solver. The C1P matrix solver is based on a software to solve it and assess the qualities of the solutions. Our problem. We present below several techniques, a lower bound and attempting the resolution of a consecutive ones sandwich matrix for a minimum number of bad zeros.

It is used to assess the quality and, in some cases, to prove optimality. The Hamiltonian cycle problem. We can also define several objective functions in terms of bad zeros: minimum number of bad zeros among equivalent matrices, \((\mu_\text{tot}(M))\), maximal number of bad zeros in a row, number of rows having at least \(k\) bad zeros for example.

We consider here linear combinations of these functions while creating optimization priority order among them. We ran the local search of Section 2.4 with a function \(\lambda_{\text{tot}}(\pi, M)\), and process to the following row. Else add two sets: \(\{X_a, X_b\}\) to the possible assignments of \(c\)

\begin{align*}
\text{Initialization:} & \quad \text{at first, all columns spanned by the component are assigned to } X_0. \text{ The first row } r_1 \text{ is treated the ones in } r_1 \text{ are assigned to } X_1, \text{ the zeros to } X_0 \text{ and the X entries to both } X_0 \text{ and } X_1. \\
\text{Induction:} & \quad \text{for a row } r_j, \text{ processed after rows } r_1, \ldots, r_{j-1}, \text{ assume the current columns partition is } \{X_1, \ldots, X_k\}. \text{ Let } \{X_{i_1}, \ldots, X_{i_k}\} \text{ be the largest interval of the partition such that (1) for every column with a 1 in } r_j, \text{ one of its possible assignments is in } X_0 \text{ or } X_{i_1}, \ldots, X_{i_k}, \text{ and (2) for every column with a 0 in } r_j, \text{ some of its possible assignments are outside } \{X_{i_1}, \ldots, X_{i_k}\}. \\
\text{If such interval does not exist, skip row } r_j \text{ and process the following row. Else add two sets: } X_0 \text{ before } X_{i_1}, \text{ and process to the following row. Let } X_0 \text{ (respectively } X_{i_1}) \text{ be the set containing all columns of } c, \text{ (3) if it has a 1 in } r_j \text{ and a possible assignment in } X_0 \text{ (respectively } X_{i_1}) \text{, replace the assignment of } c \text{ to } X_0 \text{ (respectively } X_{i_1}) \text{ by an assignment to } X' \text{ (respectively } X''), \text{ (2) if it has a 1 in } r_j \text{ and a possible assignment in } X_0 \text{ (respectively } X_{i_1}) \text{, add } X' \text{ (respectively } X'') \text{ to the possible assignments of } c, \text{ and (3) if it has a 1 in } r_j \text{ and some possible assignments in } \{X_1, \ldots, X_k\}, \text{ then remove all its other possible assignments. Finally, remove empty sets.} \\
\text{If there is a column with a 1 in } r_j \text{ and without a possible assignment within } \{X_0, \ldots, X_k\}, \text{ then this column is currently assigned to } X_0. \text{ In this case, either } a = 1 \text{ or } b = i, \text{ as the rows are processed according to a breadth first search of the current component. If } a = 1 \text{ (respectively } b = i), \text{ then } X_0 \text{ (respectively } X_{i_1}) \text{ is empty (in the opposite case skip row } r_j \text{ and process to the following row). Let } X_0 \text{ (respectively } X_{i_1}) \text{ be the set containing all columns with a 1 in } r_j \text{ but without an assignment within the sets } \{X_1, \ldots, X_k\}. \text{ Insert it before } X_0 \text{ (respectively after } X_{i_1}). \text{ If a column has a 1 in } r_j \text{ and a possible assignment to } X_0, \text{ then we add } X_0 \text{ (respectively } X_{i_1}) \text{ as a possible assignment for this column.} \\
\text{The result of this algorithm is then a partition of the columns into a totally ordered set } \{X_1, \ldots, X_k\}. \text{ From a theoretical point of view, this heuristic has the important property that, if } M \text{ does not contain any entry } X_0, \text{ this partition is the one computed by the algorithm of McConnell (2004) to decide the CIP. If } M \text{ does not satisfy the CIP, then the columns order defined by this partition induces bad rows or bad zeros.} \\
\text{2.3 Reduction to the TSP} \\
\text{It is well known that some variations of CIP can naturally be reduced to the TSP. We describe here such an approach, bearing
some similarity with the method of Muffato (2010) applied to teleost fishes: (i) construct a complete graph \( G=(V,E) \) where the set of vertices corresponds to the columns of the matrix and (ii) assign to every edges an appropriate cost/weight [Hamming distance, Jaccard coefficient, see Garriga et al. (2008) for example]. A Hamiltonian path in this graph represents a permutation of the columns, i.e. an order of the markers along ancestral chromosomes, and the shortest Hamiltonian path represents a solution tending to optimize the objective function defined by the cost model.

Here, we define a modified Hamming distance \( \delta_H \) and modified Multiply Transpose distance \( \delta_{MT} \) for two columns \( a \) and \( b \) of a ternary matrix \( M \), parametrized by a real number \( \alpha \), as follows:

\[
\delta_H(a,b) = \sum_{\text{rows of } M} |a \oplus b| \\
\delta_{MT}(a,b) = 10000 - \sum_{\text{rows of } M} \frac{|a \oplus b|}{\alpha} 
\]

To solve the obtained TSP instances, we use the publicly available TSP solver Concorde, with Ilog CPLEX 11.0. Results of calculation to improve the solutions obtained by one of the heuristics described the objective function defined by the cost model.

An order of the markers along ancestral chromosomes, and the coefficient, see Garriga et al. (2008) for example. Every edges an appropriate cost/weight [Hamming distance, Jaccard coefficient, see Garriga et al. (2008) for example]. A Hamiltonian path in this graph represents a permutation of the columns, i.e. an order of the markers along ancestral chromosomes, and the shortest Hamiltonian path represents a solution tending to optimize the objective function defined by the cost model.

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\delta_{MT}(a,b) = 10000 - \sum_{\text{rows of } M} \frac{|a \oplus b|}{\alpha} 
\]

To solve the obtained TSP instances, we use the publicly available TSP solver Concorde, with Ilog CPLEX 11.0. Results of calculation are reported in Table 2. We tried both distances with several parameters, and chose the best solution. Except for huge matrices, the solution is not very sensitive to the parameter variation.

### 2.4 Local search

To improve the solutions obtained by one of the heuristics described above, we devised a simple local search that modifies the order of the matrix columns. The five basic moves we considered here are:

- move one column from a position to another one,
- move a set of consecutive columns to another position,
- swap the position of two columns,
- reverse the order of a set of consecutive columns, and
- move and Reverse, i.e. move a set of consecutive columns to another position and in reverse order.

We implemented the local search with an objective function first tending to minimize the number of bad rows and secondarily trying to minimize the number of bad zeros in bad rows.

### 2.5 Lower bound

To measure the quality of the solutions given by the combination of a constructive heuristic followed by a local search, we define a lower bound for the objective function that counts the number of bad rows in a solution, i.e. a lower bound on the minimal number of rows to remove from the matrix so that, for the remaining rows, there is an order of the columns with no bad row. Our approach is based on the notions of forbidden substructure characterization and incompatibility graph developed by McConnell (2004).

#### 2.5.1 The incompatibility graph and forbidden configurations

We first recall the construction of the incompatibility graph. Let \( a \) and \( b \) be two columns of matrix \( M \) and denote by \( (a,b) \) the fact that \( a \) appears before \( b \) in the order of columns. Let \( G \) be an undirected graph whose vertices are the elements of \( \{(a,b)|a,b \text{ are columns and } a \neq b\} \). Edges of \( G \) indicate the incompatibility between column orders with respect to the SC1P.

- In any given order, \( (a,b) \) and \( (b,a) \) cannot appear simultaneously. Thus, a first set of edges connects every pair \((a,b),(b,a)\).

Suppose now that \( a,b,c \) are three columns and there exists a row \( r \) such that \( M_{ar}=M_{br}=1 \) and \( M_{br}=0 \). In an order with the SC1P, \((a,b)\) and \((b,c)\) cannot hold both. We, therefore, say that \((a,b)\) and \((b,c)\) are incompatible and define an edge between these two vertices. We associate the row \( r \) to this edge; therefore, the graph \( G \) can have multi-edges between two pairs of vertices but these edges are distinguished by the associated rows. Note, however, that if any of these value is \( X \), then we can not say anything about incompatibility.

McConnell (2004) proved that the incompatibility graph of a binary matrix is bipartite if and only if the matrix itself has the C1P, which provides a key ingredient for a certificate for the C1P. A similar, although weaker property, also holds for the SC1P. A third forbidden configuration is a column vector which has two columns with at least one bad row and no bad row.

- **Property**: if the incompatibility graph of a ternary matrix \( M \) contains an odd-length cycle, then \( M \) does not satisfy the SC1P.

Note that the vertices and edges of an odd-length cycle \( C \) define a set of columns and rows and, therefore, define a sub-matrix of \( M \), that we call a forbidden configuration and that we denote \( M_{Ki} \), which does not have the SC1P by the property above. Examples of such sub-matrices are given in Figure 1. They are sub-matrices of the matrix obtained from the monocot proto-chromosome A11 shown on Figure 6. The incompatibility graphs for those two sub-matrices are drawn on Figures 2 and 3.

**2.5.2 Disjoint forbidden configurations** Two odd cycles are called strongly disjoint in the incompatibility graph if they are disjoint in the graph, and if they do not share any row in the matrix. If there exists a set of \( k \) odd cycles that are pairwise strongly disjoint odd cycles, this clearly implies that there is at least \( k \) bad rows, as each sub-matrix associated with a cycle contains at least one bad row. We then introduce the problem of computing the maximum number of odd cycles that are pairwise, strongly disjoint, and we describe below an efficient heuristic for this problem.

First, we compute a set of odd cycles as follows. One odd cycle is found by searching the graph, and, in the associated sub-matrix, we flip the zero entries to \( X \). In this way, this odd cycle vanishes...
and we start again the search for another odd cycle, until the graph is bipartite. The set of odd cycles so obtained are still not strongly disjoint. So we find the maximum subset of strongly disjoint ones by solving an independent set problem on the graph which vertices are the odd cycles and two vertices are joint by an edge if they are not strongly disjoint. We solve this problem using an integer programming formulation and solver.

For the matrix $M$ with $m$ columns, the number of vertices in the incompatibility graph is $m \times (m - 1)$ but all previous calculations remain valid if we choose the suitable subset of columns, for example the set columns comprising one or several bad zeros and repeat the procedure to cover whole matrix. This is how, if needed, we can reduce the size of the graphs we deal with. The number of odd cycles can be very large, and this leads to rather big integer programs to solve. But all these programs were easily solved for small and medium instances (monocot ones) as it is reported here.

### 3 RECONSTRUCTING THE ANCESTRAL MONOCOTYLEDON GENOME

Monocotyledons are a branch of angiosperms whose genome has undergone a global duplication at an early stage of its evolution (Salse et al., 2009). We describe here how to define ancestral markers for this genomes, then how to compute ternary matrices representing putative features of the ancestral monocotyledon genome, and finally the result of the computational methods described in Section 2 on these matrices.

#### 3.1 Ancestral markers and ternary matrices

Two paralogous chromosomes or genes arising from a whole genome duplication are called ohnologous. Ohnologous chromosomes and chromosome segments were identified in Murat et al. (2010), and we use the gene homologies computed in the same study. For example, Rice chromosomes 1 and 5 are ohnologous, as well as Sorghum chromosomes 3 and 9. As all four arise from the whole genome duplication (Rice 1 is orthologous to Sorghum 3 and Rice 5 to Sorghum 9, due to a speciation posterior to the whole genome duplication), Rice 1 and Sorghum 9 are ohnologous, as well as Rice 5 and Sorghum 3. This gives four ohnologous relations on these four chromosomes summarized in Figure 4, where ohnology relationships between genes are drawn with gene positions in chromosomes. In the present work, we define a relation between genes as follows: an ohnologous pair of genes is a pair of paralogous genes that are located on two ohnologous segments. All ohnologous pairs on Rice chromosomes 1 and 5 and Sorghum chromosomes 3 and 9 are drawn in Figure 4. Genes are grouped into families defined by the transitive closure of the relation, and each family defines an ancestral marker.

We then define a matrix $M$, whose columns are the ancestral markers and rows are defined in terms of adjacencies and common intervals. Then for each pair of ohnologous segments $A$ and $B$, we computed common intervals (Chauve and Tannier, 2008) on the set of ancestral markers which have descendants in both segments. For each common interval $I$, a row of $M$ is constructed as follows:

- there is a 1 in column $i$ if the ancestral marker $i$ is in $I$,
- there is a 0 in column $i$ if the ancestral marker $i$ has descendants in $A$ and $B$ but is not in $I$, and
- there is an $X$ in column $i$ if the ancestral marker $i$ has no descendant in either $A$ or $B$, or both.

This defines the monocot ternary matrix. A toy example is given in Figure 5, based on two segmental ohnologous relations.

Common intervals are sets of ancestral genes which descendants are seen contiguous in two branches arising from the whole genome duplication, so they are believed to define a set of contiguous markers in the ancestor. In the example of Figure 5, moving column $A$ into the first position results in a matrix where no entry 0 is located between two entries 1, which means it satisfies the sandwich consecutive ones property.

We applied the technique described above to define five ternary matrices defining 5–7 proto-chromosomes of the monocotyledon ancestral genome (Murat et al., 2010) (proto-chromosomes A4, A5, A7, A8 and A11). See Table 1.

#### 3.2 Proto-chromosomes of the monocot ancestor

Figures 6 and 7 give examples of the shape of the instances and of the solutions. The shown ternary matrices represent the input used to compute proto-chromosomes A11 and A5 (Murat et al., 2010). Entries 1 are red, 0 are blue and $X$ are green. All rows are represented, even the bad ones. Red segments represent common intervals. For A11, only 35 ancestral genes were considered, whereas 120 genes were considered in A5.

A11 has only one bad row and as shown in the previous section the matrix does not have the SC1P. So this solution is optimal in terms...
Fig. 5. (Top) Three segments sharing two ohnologous relations, together with 8 ohnologous pairs of genes. (Bottom) The ternary matrix obtained by computing all common intervals. The three first rows encode the common intervals between the two upper segments, and the last three rows encode the common intervals between the two right segments.

Table 1. Characteristics of the five considered instances: name, number of rows, number of columns, ratio of 1s and Xs in the matrix

<table>
<thead>
<tr>
<th>Instances</th>
<th>No. of rows</th>
<th>No. of columns</th>
<th>%1</th>
<th>%X</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>748</td>
<td>139</td>
<td>5.79</td>
<td>59.02</td>
</tr>
<tr>
<td>A5</td>
<td>548</td>
<td>120</td>
<td>7.43</td>
<td>42.67</td>
</tr>
<tr>
<td>A7</td>
<td>144</td>
<td>164</td>
<td>2.00</td>
<td>55.43</td>
</tr>
<tr>
<td>A8</td>
<td>548</td>
<td>48</td>
<td>18.40</td>
<td>30.63</td>
</tr>
<tr>
<td>A11</td>
<td>50</td>
<td>35</td>
<td>14.06</td>
<td>34.55</td>
</tr>
</tbody>
</table>

of the number of bad rows, and in the bad row there is a solution with only one bad zero, then optimality is guaranteed also for the number of bad zeros. For A5, the lower bound gives at least 27 bad rows, whereas we find a solution with 34 ones (over a total of 548 rows). Statistics for the other chromosomes are given in Table 2.

To assess what we gained by modeling the result of gene loss instead of not taking it into account and consider it as a possible source of errors in the data, we also computed the solutions by replacing X entries by 0. We used the method of Chauve and Tannier (2008) to solve the problem of the minimum number of rows to discard, in order for the remaining matrix to have the C1P. In the A11 matrix, 11 rows have to be discarded (C1P) instead of only 1 (SC1P). For A5, 351 rows are bad instead of 59. So the noise due to some errors in the data is considerably reduced by paying attention to gene loss.

Table 2. Results obtained for two objective functions: number of bad rows and number of bad zeros

<table>
<thead>
<tr>
<th>Name</th>
<th>Mc</th>
<th>TSP</th>
<th>LS</th>
<th>LB</th>
<th>Mc</th>
<th>TSP</th>
<th>LS</th>
<th>LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>27</td>
<td>100</td>
<td>11</td>
<td>8</td>
<td>299</td>
<td>1045</td>
<td>57</td>
<td>–</td>
</tr>
<tr>
<td>A5</td>
<td>59</td>
<td>170</td>
<td>34</td>
<td>27</td>
<td>749</td>
<td>1859</td>
<td>432</td>
<td>–</td>
</tr>
<tr>
<td>A7</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>50</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A8</td>
<td>99</td>
<td>161</td>
<td>93</td>
<td>55</td>
<td>514</td>
<td>250</td>
<td>133</td>
<td>–</td>
</tr>
<tr>
<td>A11</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>15</td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>

Column Mc describes the results of the heuristic of Section 2.2. It reaches the optimal value for instances A7 and A11 and consistently provides solutions of quality for other instances. TSP reduction is numerically less competitive but still gives meaningful results. The local search often reaches the reported values even if it starts from a random order of columns but its efficiency is enhanced with good starting solutions from the previous two procedures. Finally, in the LB column we report the obtained lower bounds for the number of bad rows. Bold values point at optimal solutions obtained by heuristics.

Footnote:
A4, A7, A8 as named in Murat et al. (2010), even if two of them are split into at two segments in our reconstruction, leading to 5, 6 or 7 proto-chromosomes according to the branch in which the two probable rearrangements occurred.
Fig. 8. Representation of 950 good solutions for the A11 proto-chromosome.

Fig. 9. Representation of 950 good solutions for the A5 proto-chromosome.

3.3 Validation and robustness

Figures 8 and 9 represent a sample of locally optimal solutions obtained by repeatedly applying the program with a randomization (the local search is easily randomized for example, as well as the order in which rows are processed in the partition refinement heuristic). In these figures, the order of the columns we represent is the one of the best found solution, and a gray square at coordinates $i, j$ represents the proportion of solutions in which column $i$ is in position $j$: the darker the square is, the larger the proportion is.

The ‘X’ shape of the figures is explained by the symmetrical property of the problem: one order of the columns and its reverse have the same score according to all objective functions. Few columns have a compulsory position. But the distribution of the positions of most columns is far from uniform: there is a clear tendency to stay close to the position in the chosen best solution. In Figure 9, we can see an inversion for which it is impossible to decide whether the ancestral state carries it or not (the small ‘X’ shapes surrounded by a black ellipse). This is not surprising from the examination of Figure 4, where we can see that Rice chromosome 1 and Sorghum chromosome 3 carry one order on this part, while Rice 5 and Sorghum 9 exhibit the reverse order. So there has been an inversion along one evolutionary branch (the one leading to Rice1/Sorghum3, the other to Rice5/Sorghum9) after the duplication, but there is no way to decide on which one, so we cannot know the ancestral configuration.

4 RECONSTRUCTING THE MAMMALIAN ANCESTOR

The SCIP of ternary matrices generalizes the CIP for binary matrices. So the framework we present is not only a way to reconstruct ancestral genomes with massive gene losses as the monocot one, but also all problems solved by CIP techniques can be a fortiori solved in this case, with possibly more accuracy in the presence of unequal gene/marker content in extant species. Indeed, as discussed in Pham and Pevzner (2010) for example, when large datasets of extant genomes are considered, the number of universal markers naturally decreases, due, for example, to lineage-specific rearrangements that split the occurrence of a marker into several smaller genome segment. As a consequence usual methods to infer synteny blocks, either from genes or DNA alignments, are not adapted anymore.

Here we show that the SCIP can be used to handle such problems, and we illustrate this feature by reconstructing the boreoeutherian ancestor, which has been the subject of a large literature (including Chauve and Tannier, 2008; Ma et al., 2006; Muffato et al., 2010) and whose general architecture is mostly agreed upon by both computational and cytogenetics studies.

4.1 Ancestral markers

We used the Pecan alignments from Ensembl Compara (version 58, Paten et al., 2008), which is a set of non-universal homologous markers within 15 amniote genomes, including 12 placental mammals (Homo sapiens, Pan troglodytes, Gorilla gorilla, Pongo pygmaeus, Callithrix jaccus, Macaca mulatta, Mus musculus, Rattus norvegicus, Equus caballus, Canis familiaris, Bos taurus, Sus scrofa, Monodelphis domestica, Gallus gallus and Taeniopygia guttata). Alignments that were colinear in all genomes in which they are present were first grouped, and then alignments of length <100 kb in at least one species were discarded.

Then an ancestral marker is defined by each alignment which has at least an occurrence in two extant species whose evolutionary path goes through the boreoeutherian node. This gives 1724 ancestral markers covering 35% of the human genome, whereas there was 990 universal markers (i.e. present in all 15 species) covering 24% of the human genome. So allowing non-universal markers results in a significant improvement of the coverage of extant genomes by ancestral markers.

4.2 Reconstructing proto-chromosomes

A ternary matrix for the boreoeutherian ancestral genome was constructed by performing pairwise comparisons of extant genomes. For each pair of species whose evolutionary path goes through the boreoeutherian ancestor, we computed conserved adjacencies and maximal common intervals on the set of ancestral markers which have a descendant in both genomes. The ternary matrix was then constructed in the same way than for the monocot ancestor, with entries $X$ used to represent markers that were missing (lost) in at
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Length is the cumulative length of the markers, taken on the human genome, composing the segments. Assoc. represents the chromosomal associations, again for the human genome, i.e. the human chromosomes containing the markers present on each proto-chromosomal segment.

At least one of the two considered genomes. This matrix contains 1724 columns and 89,023 rows.

After application of the SC1P solver, the resulting columns order implied 316 (0.3%) bad rows and 2753 bad zeros. Keeping the bad rows in the matrix defines 24 proto-chromosomal segments, while discarding the 316 bad rows split some of these segments into several sub-segments and resulted in 26 proto-chromosomal segments. Characteristics of these proto-chromosomal segments are given in Table 3.

It appears that the number of proto-chromosomes obtained when keeping the bad rows is comparable to what is usually expected for this ancestral genome (Froenicke et al., 2006). Moreover, the chromosomal associations that are observed are globally consistent with previous studies, but for ancestral segments 1 and 14. Segment 14 is clearly monochromic and joins two proto-chromosomes (one corresponding to human chromosome 11 and one corresponding to segments of human chromosome 19). It is interesting to see it is split into two segments when discarding the bad rows. Similar observation does not hold for segment 1, also probably monochromic. Understanding the signal that results in these CAR will be key to refine the SC1P approach. Finally, we can see that segment 8 seems to capture an association between human chromosomes 12, 22 and 10 (although lost when discarding bad rows) that has been described in cytogenetics studies (Froenicke et al., 2006) but never recovered in computational studies based on sequenced genomes until now.

In conclusion, we recover an ancestral genome which fully meets the standards of usual reconstructions, with some additional interesting features and a higher coverage than if only universal markers were used.

5 CONCLUSION

We introduce a general framework for ancestral genome reconstruction by genome mapping, which contains the principles of most former methods and adds the possibility to handle heterogeneous genome content and, in particular, gene losses. We apply it to the reconstruction of the pre-duplication monocot ancestor, which is inaccessible to other methods requiring universal genes or conserved adjacencies. We also show that it can be applied to other taxonomic groups as illustrated with the boreoeutherian ancestor, by providing a solution with a higher coverage than if markers were restricted to universal markers.

The solution we describe is based on a classical combinatorial problem, the sandwich consecutive ones matrix problem, the sandwich consecutive ones matrix. We propose a software based on several algorithmic techniques to solve related optimization problems. We also assess the quality of our solutions by computing a lower bound of the optimal solution, and by representing a large set of locally optimum solutions.

The results we obtain suggest that the framework is well adapted to handle a heterogeneous gene/marker content in paleogenomics studies. This should motivate further investigations on related methodological and algorithmic problems. In order to process large datasets, algorithms will have to be able to handle very large instances, with dozens of thousands of columns and possibly several hundreds of thousand of rows. Currently, we are limited to one order of magnitude below. Moreover, to decrease the number of entries X in the ternary matrices, and then the number of bad rows, better models of ancestral markers and common intervals with unequal gene/marker content should be investigated. It is also possible to generalise this framework by explicitly modeling gene duplications, adding the possibility of multiplicities as in Wittern and Stoye (2010). This addition will be the topic of a future work.

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REFERENCES


Matrix Sandwich


