UNIVERSIDAD DE MÁLAGA
DEPARTMENT OF COMPUTER ARCHITECTURE

DOCTORAL THESIS

ALGORITHMS AND METHODS FOR LARGE-SCALE GENOME REARRANGEMENTS IDENTIFICATION

Presented by
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Algorithms and methods for large-scale genome rearrangements identification

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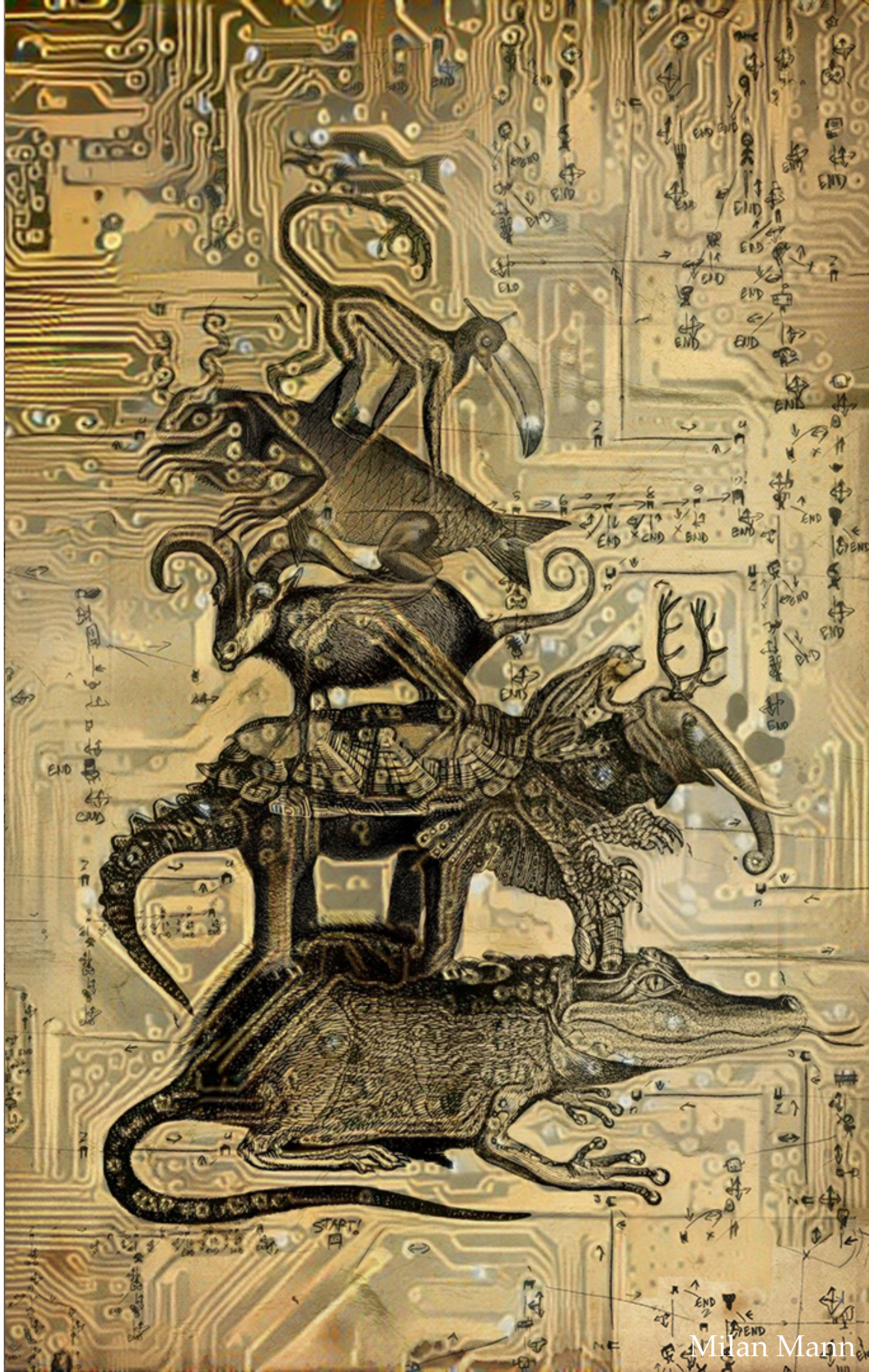


Milan Mann

Publications supporting the thesis

- **“Computational Synteny Block: A Framework to Identify Evolutionary Events”**, (*IEEE Transaction in Nano Bioscience,* 2015)
- **“Refining borders of genome-rearrangements including repetitions”**, (*BMC Genomics,* 2016)
- **“Computational workflow for the fine-grained analysis of metagenomic samples”**, (*BMC Genomics,* 2016)
- **“A multiple comparison framework for Synteny Block detection”** (IWBBIO, 2017)
- **“Ancestral sequence reconstruction: A framework to detect Synteny Blocks and revert rearrangements”** (in progress)

Overview

- Introduction
- Background
- Methods
- Results
- Conclusions and future work

Introduction

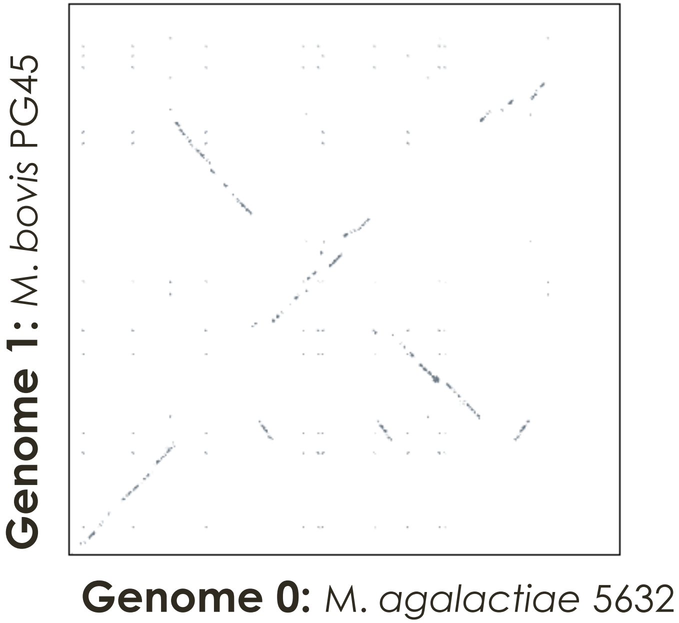
Synteny Blocks,
Large-Scale Genome
Rearrangements and
Break Points

General Overview

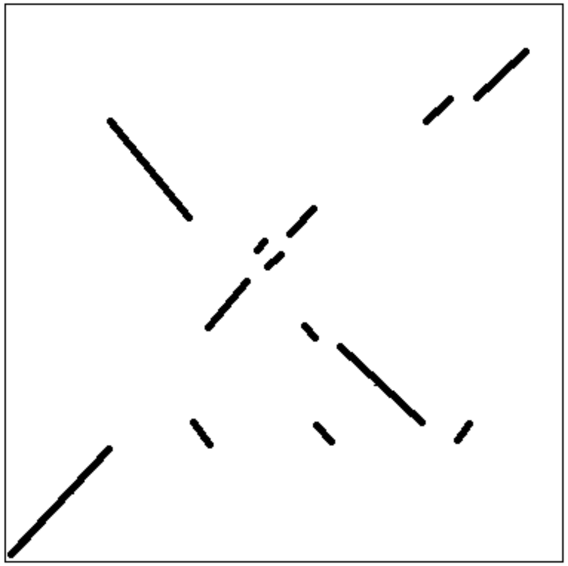
Synteny Blocks

- The idea: Conserved blocks that share the same order and *strand*

High Score segments Pairs (HSPs) produced by GECKO

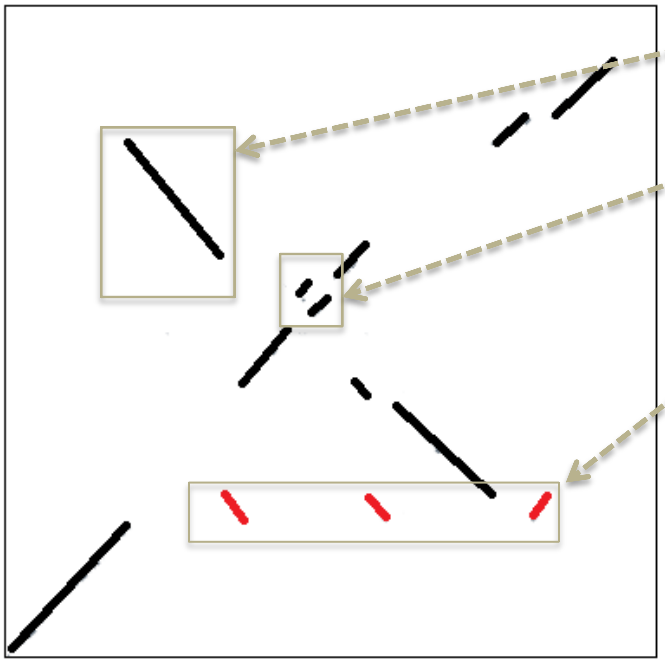


Synteny Blocks (SBs)



Large-Scale Genome Rearrangement

- **A LSGR is an operation that changes the order or the *strand* of a SB**



- Inversion

  Change the strand
- Transposition

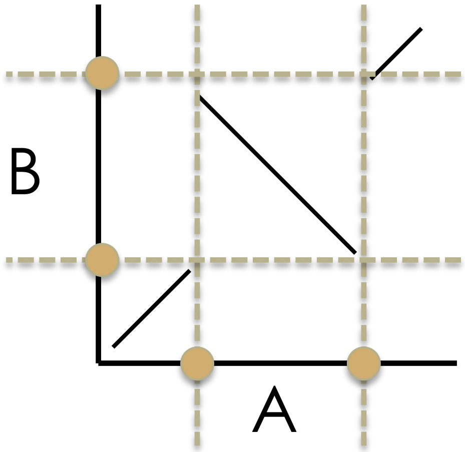
  change the order: moves the block to another position within the chromosome
- Duplication

  copy the block
- Translocation

  change the order: moves the block to another position in another chromosome

Break Point

- The point (or the region) in the sequence between two SBs that have suffered a LSGR

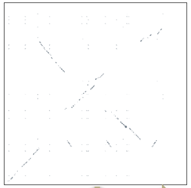


The SB in the middle has suffered a LSGR (inversion)

Dots represent BPs in the sequence

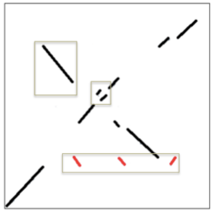
General Overview

HSPs
GECKO
(Torreño and Trelles, 2015)

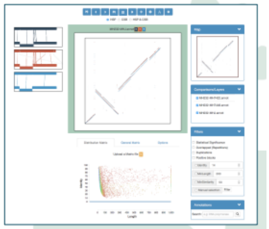


Starting point

SB and rearrangements pairwise detection

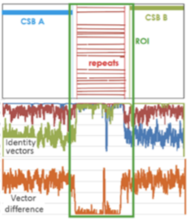


GECKO-CSB
Arjona and Trelles, 2015

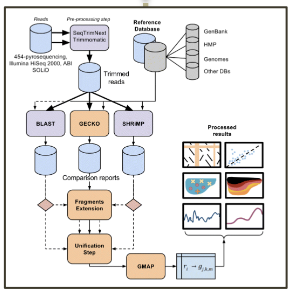


GECKO-MGV
Diaz del Pino, Arjona, Torreño, Benavides and Trelles, 2016

Refining SB borders and BPs

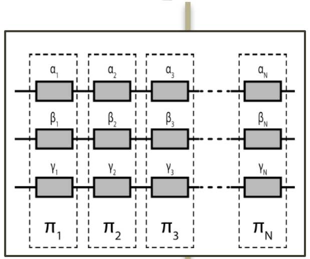


GECKO-Refinement
Arjona and Trelles, 2016



Meta-GECKO
Perez, Arjona, Torreño, Ulzurrun and Trelles, 2016

Rearrangements reconstruction (multi comparison)



(in progress)
GECKO-Evol
Arjona, Perez and Trelles, 2018?

Objectives

- Formal definition of and detection of SBs
- Detection of LSGR and BP
- Refinement of SBs borders
- Reversion of LSGR

Background

"If I have seen further, it is by standing on the shoulders of giants"

State of the art

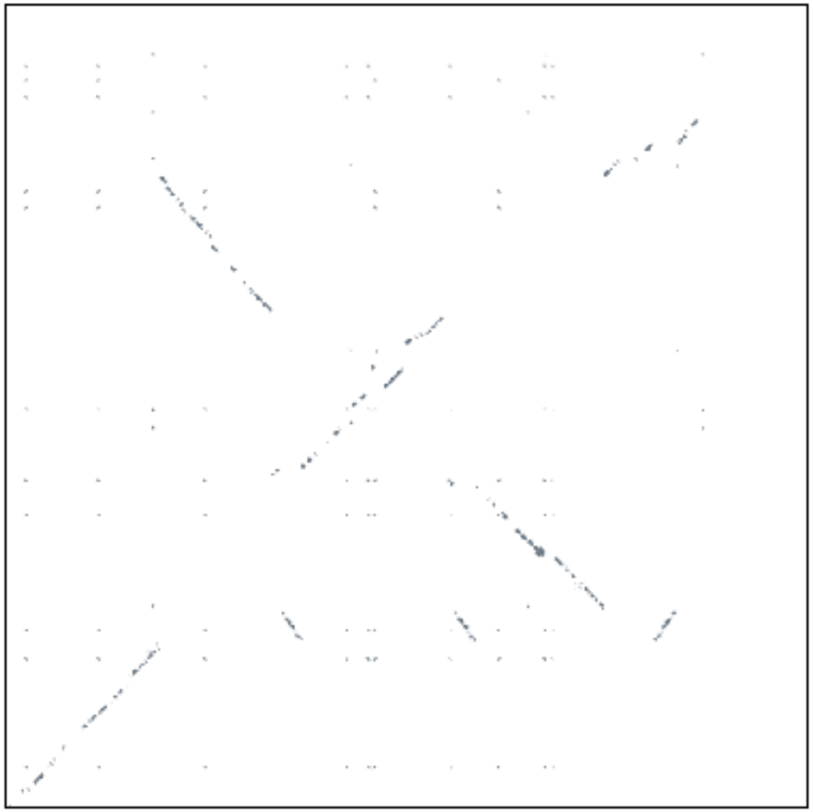
- **SB and BP detection**
  - No formal definition (difficult to compare methods)
  - The granularity problem
  - The BP contradiction
  - Dealing with repetitions

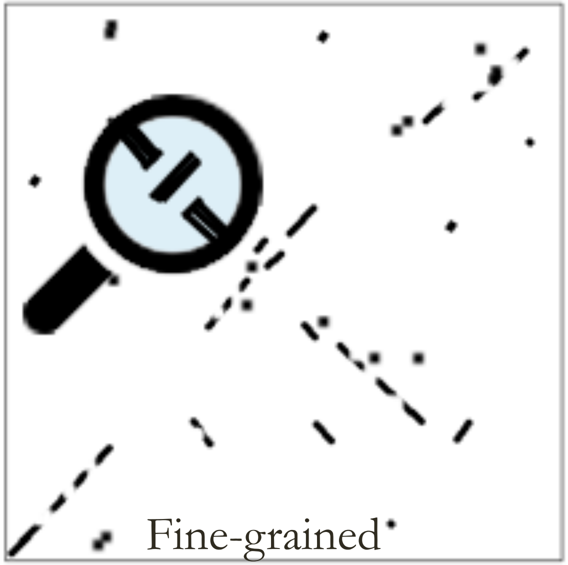
- **Methods to reverse LSGR**
  - Oriented to the "sorting permutation problem"
  - Reference depended
  - Not designed for dealing with repetitions

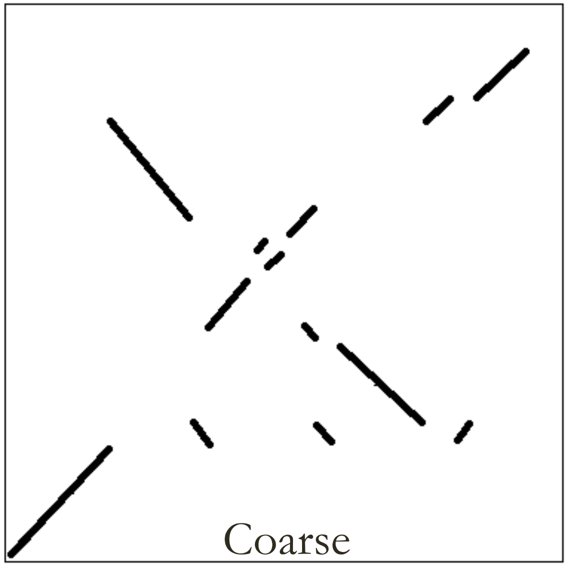
The granularity problem

Granularity	**SB**	**BP**	**LSGR**
Fine-grained	*Many (shorter and well conserved)*	*Many (shorter and better quality)*	*Small subset of total LSGR (short cycles)*
…	…	…	…
…	…	…	…
…	…	…	…
Coarse	*Few (larger and low percentage of identity)*	*Few (larger and noisy: Many short SB are included)*	*Small subset of total LSGR (Big picture)*

An example







The break point contradiction

- **Rearrangements do not occur randomly**
- **Fragile regions in the sequence, predispose to suffer a LSGR (hotspots)**
  - BP should not be defined as a relation between two genomes
  - Although comparison is the only way (so far) to detect them
  - Most methods to refine SB take for granted that BPs are not conserved regions.

Dealing with repetitions

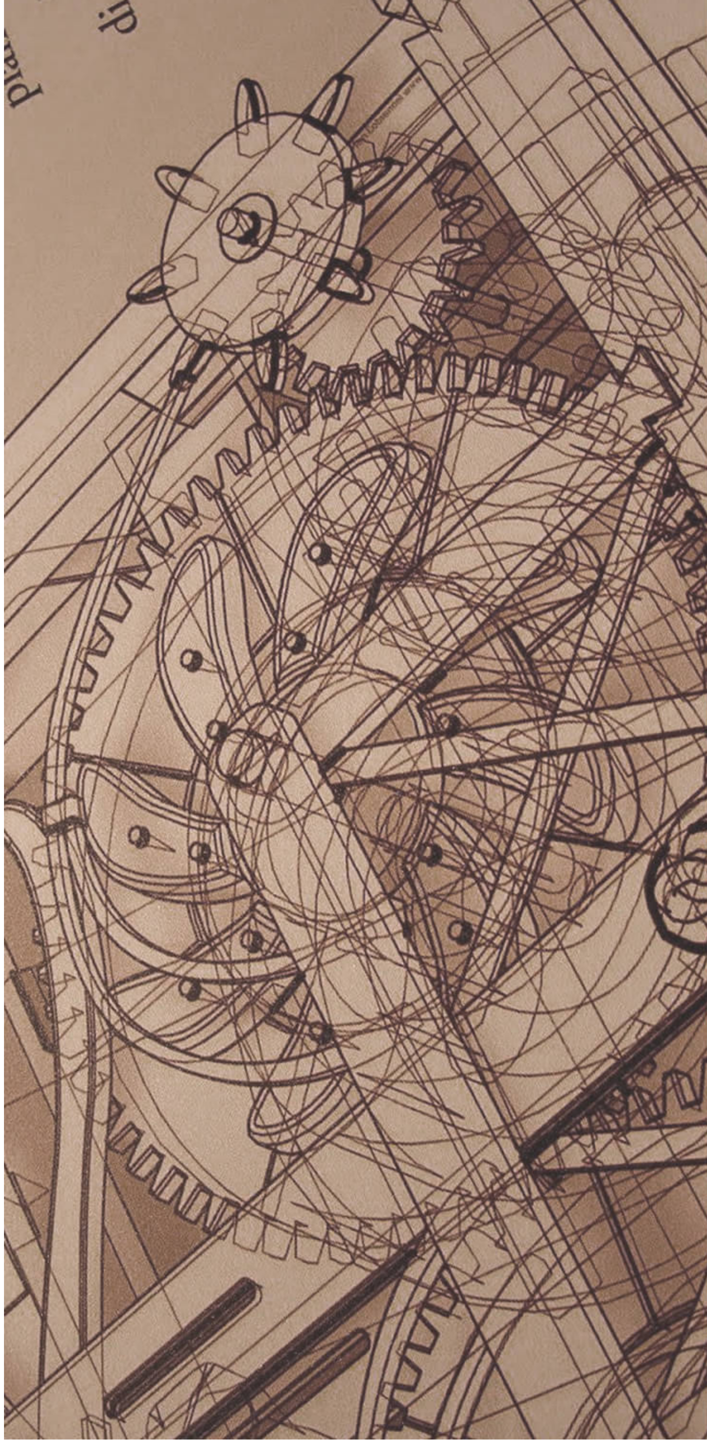
- Driven the evolution in many ways
- Mostly associate with mobile elements
- Repetitions increase the model complexity
    - Most methods to detect SBs avoid repetitions

The sorting permutation problem

- **Transform one sequence into another (the reference) through operations.**
- **Proven to be NP-hard**
  - A reference is needed
  - No "natural" way to include repetitions in the model
  - No use of inside-block information

Methods

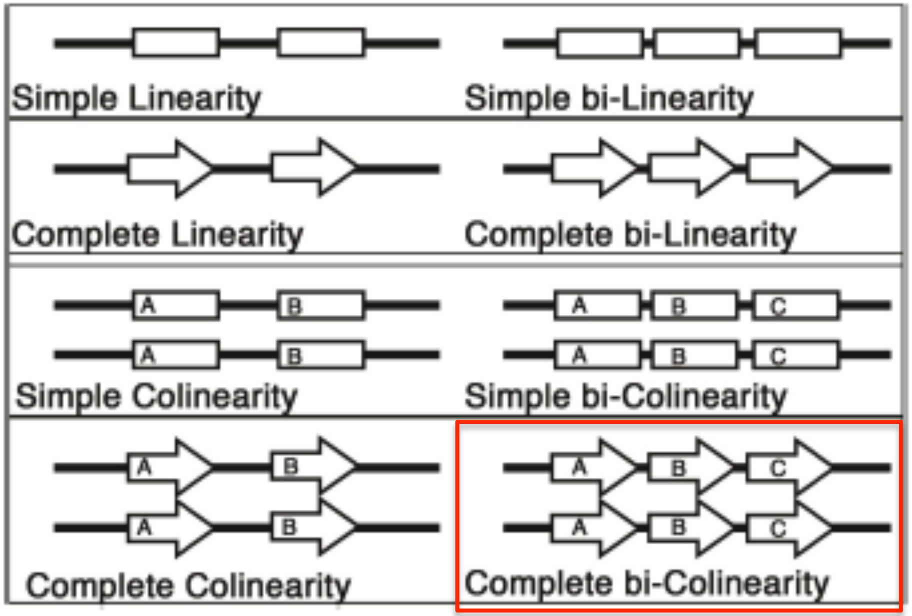
Pair-wise comparison method, refining blocks and multiple comparison framework: definitions and methods



Methods Overview

- **1) Pairwise SB and LSGR detection (GECKO-CSB)**
- **2) SB refinement**
- **3) Multi-genome SB and LSGR detection and reconstruction**

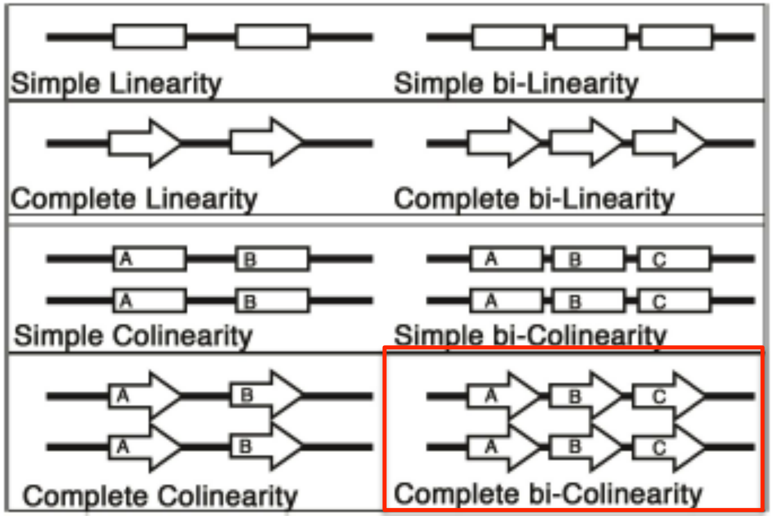
1-Computational Synteny Blocks: A pair-wise framework to detect LSGR

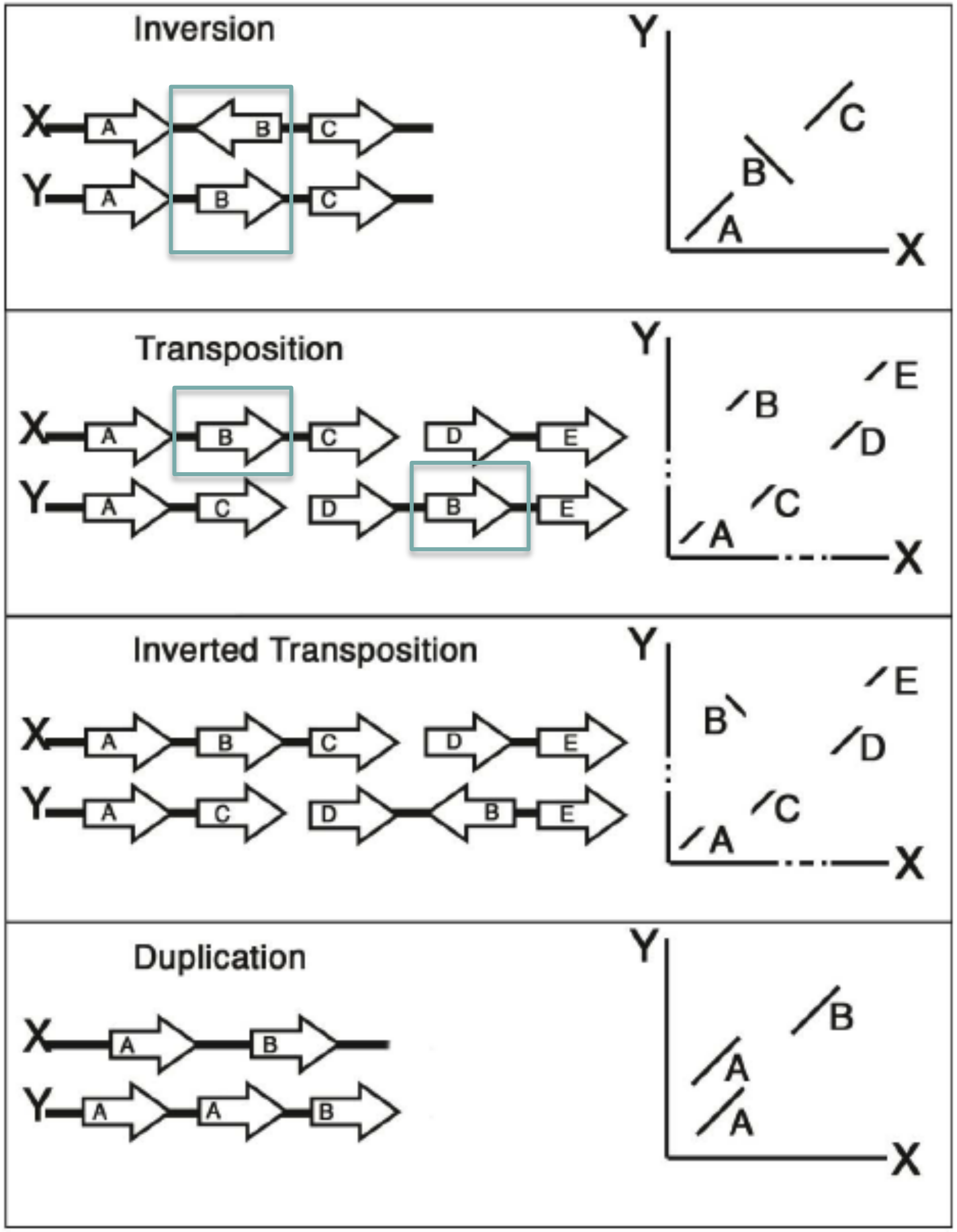


- Set of properties to detect SBs
- Arrows represent *strand*

1-Computational Synteny Blocks: A pair-wise framework to detect LSGR

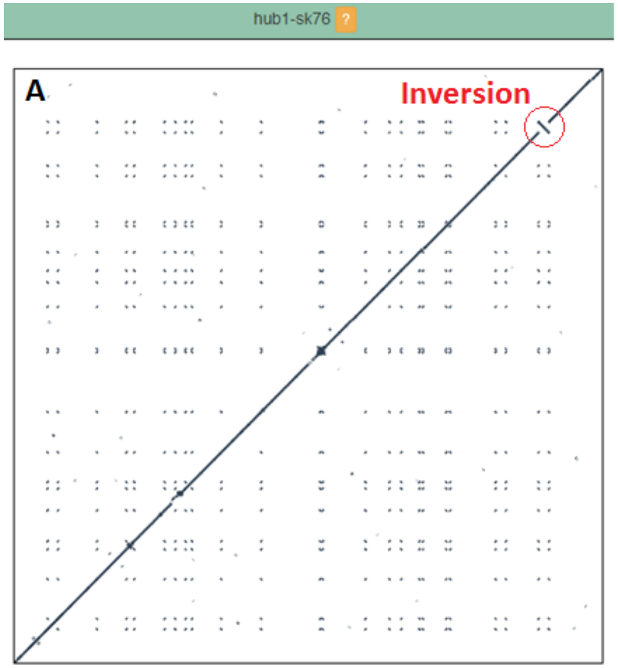
- These properties also describe rearrangements

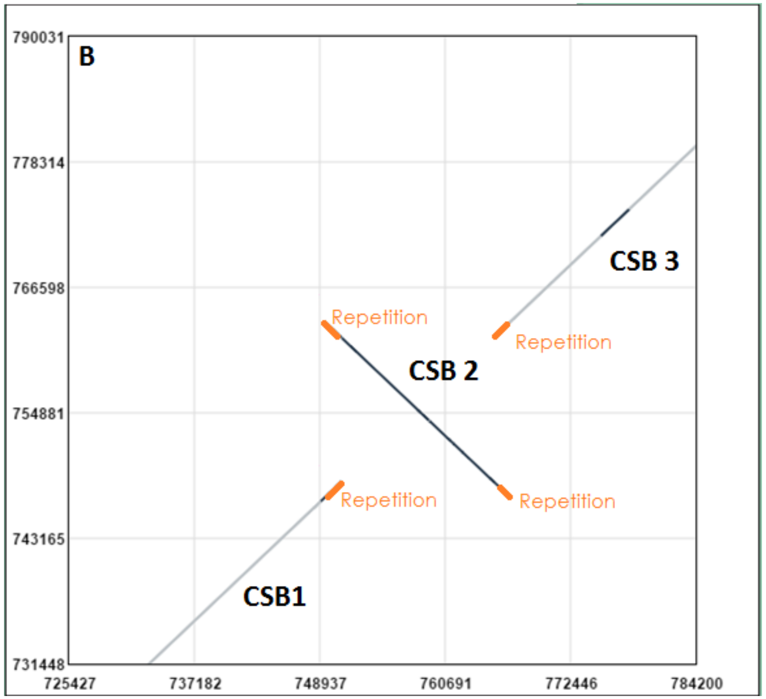




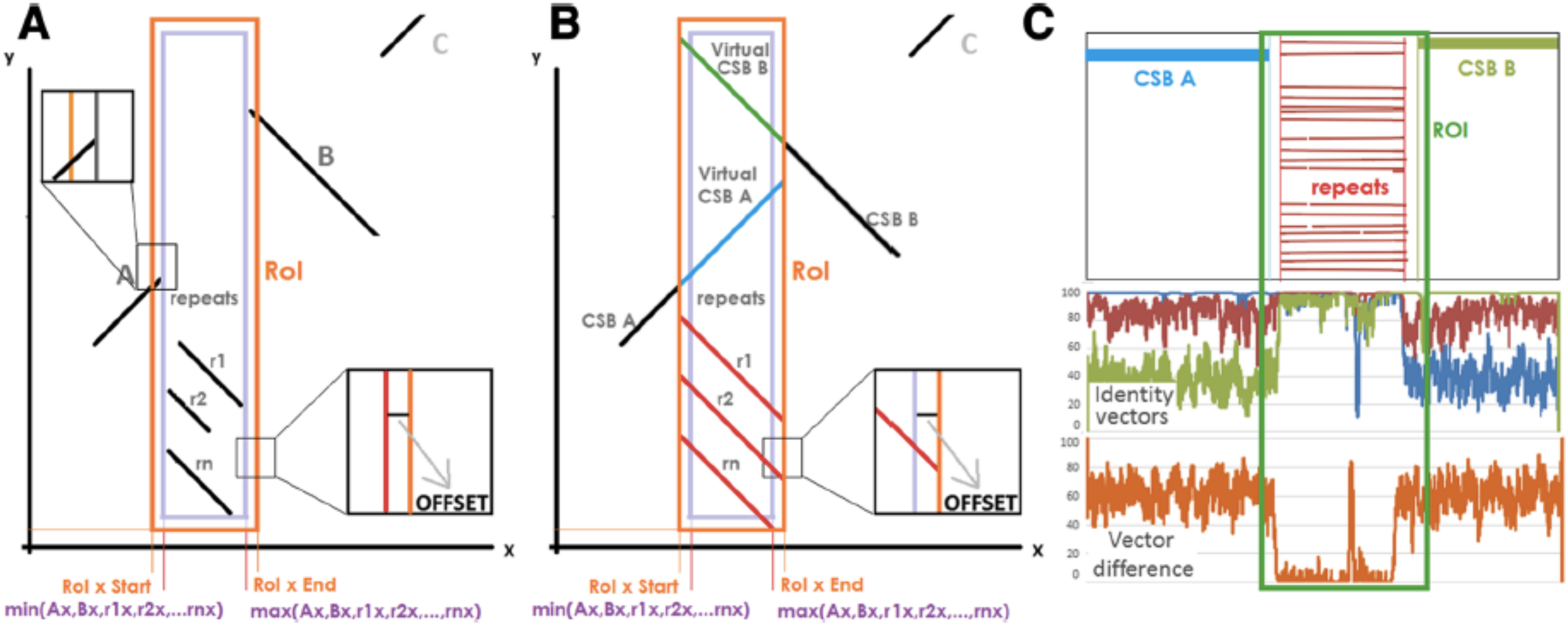
2-Synteny Block refinement

- Using repetitions to refine (if any)
- Does not force the BP to be a point or region



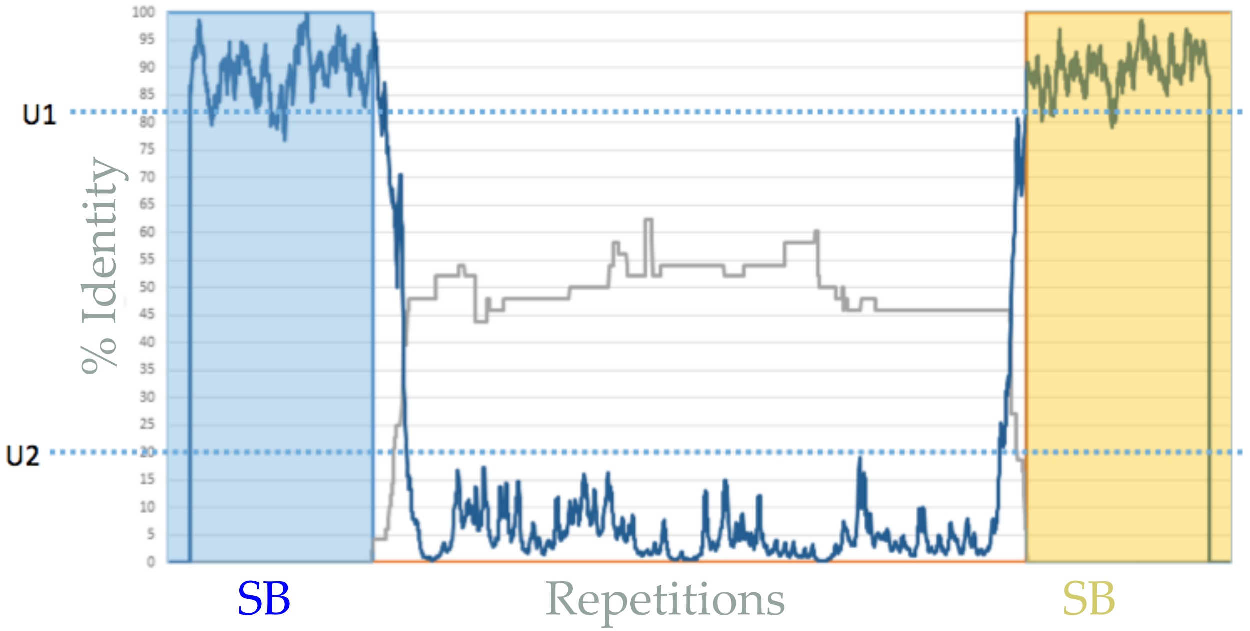


Refining based on transitions including repeats

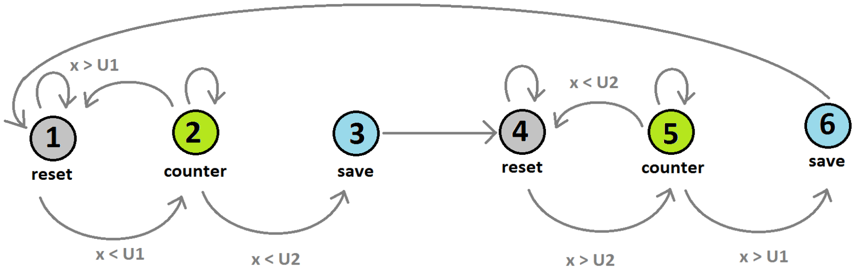


Illustrative representation of the Region of Interest (ROI). a ROI region in an inversion event (CSB B). (b) Virtual CSBs and repetitions. (c) Same representation but including identity vectors and vector difference graphs

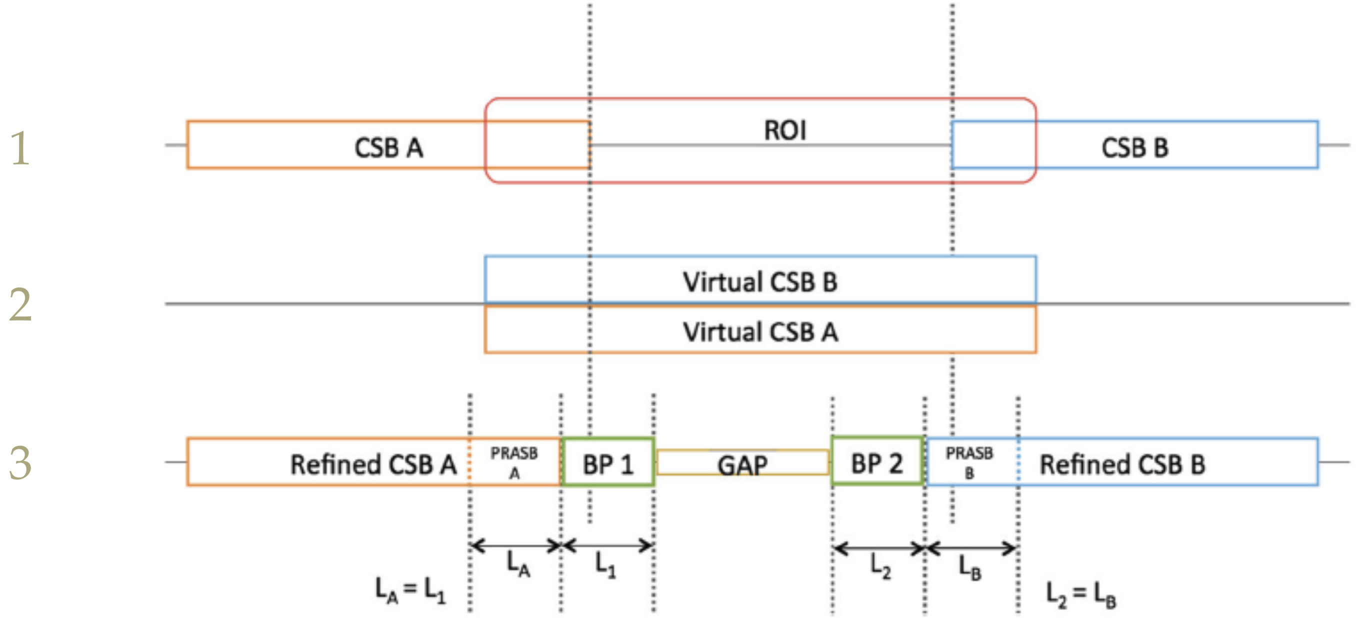
Finite State Machine to detect identity transitions



FSM detects the coordinates where the vector difference value was the last time at a certain threshold (U1) before reaching the second threshold (U2)



Result of the refinement



CSBs before and after the refinement. At the end of the refinement process, we detect BPs. We also extract PRASB and GAP sequences to analyse accuracy of the method. PRASB and BP have the same length

3-Multiple comparison framework

- **Motivation**
  - Formal SB definition
  - Solve the BP contradiction
  - Solve the granularity problem
  - No reference-based
  - Combine sequence information and rearrangements

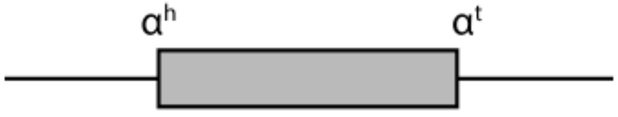
The Synteny Block concept

- **SB has two categories**
  - Block: The sequence
  - Synteny: The relation with other blocks

Block Element

- Subsequence in the sequence

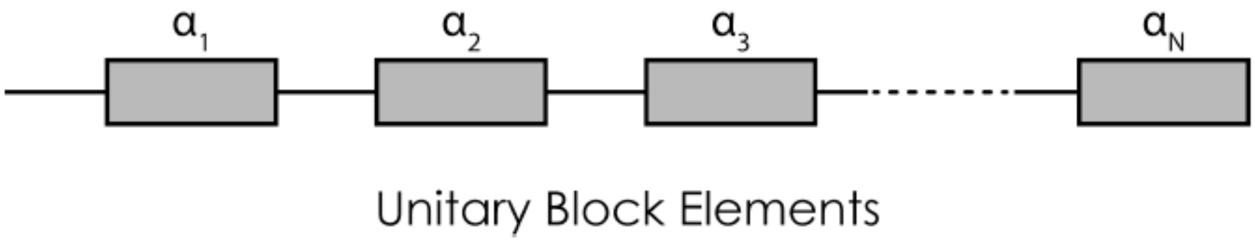
1. $\alpha^h < \alpha^t$
2. $|\alpha| = \alpha^t - \alpha^h$
3. $|\alpha| \geq 0$ *(As a consequence of 1 and 2)*



Block Element α

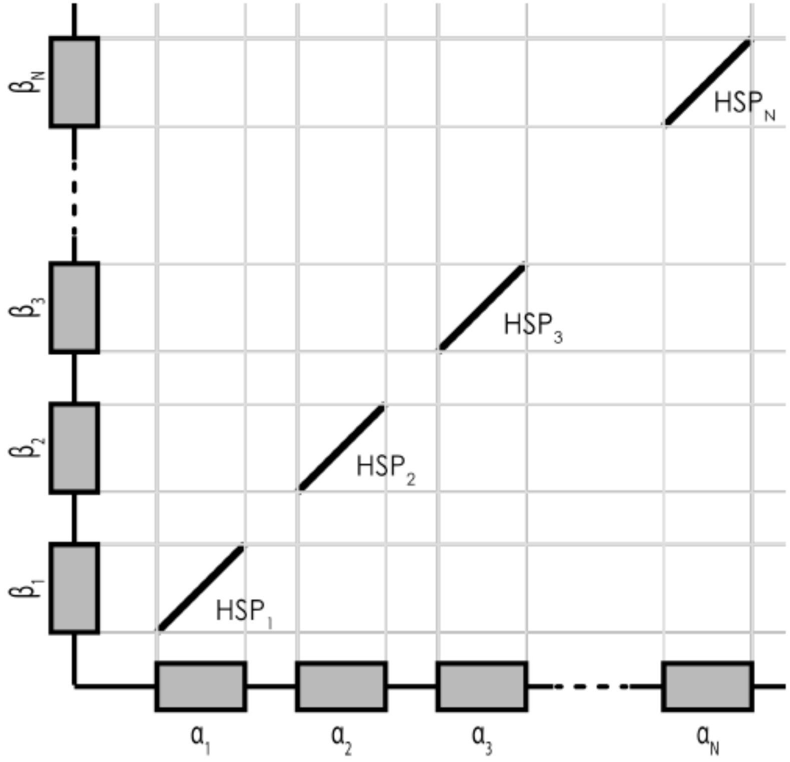
Unitary Block Element

- A Block Element that does not overlap with others Unitary Block Elements

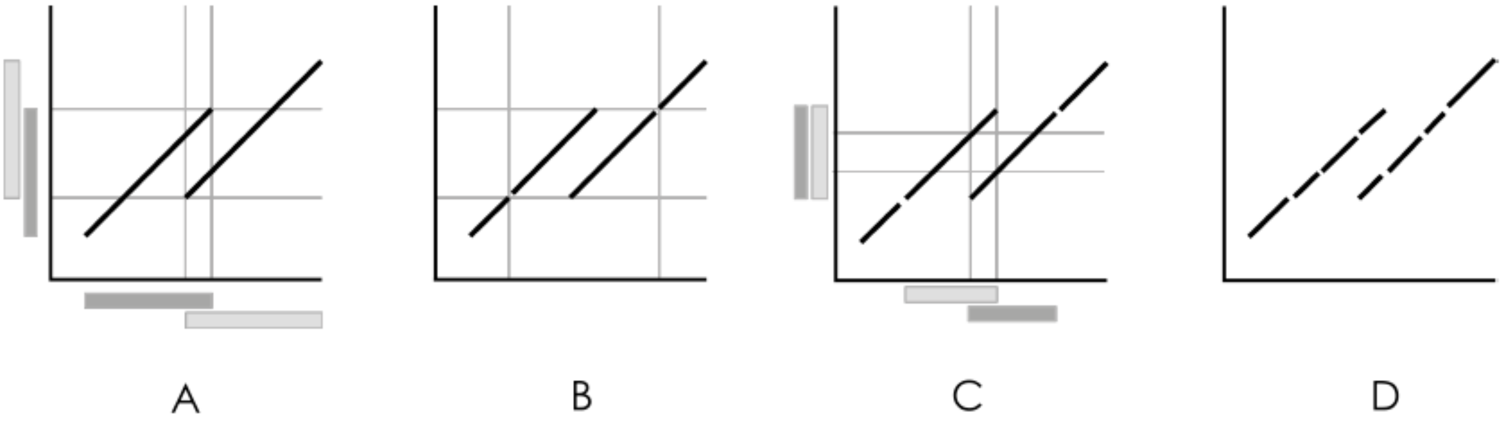


Unitary Conserved Element

- **A Block Element originate from comparison**



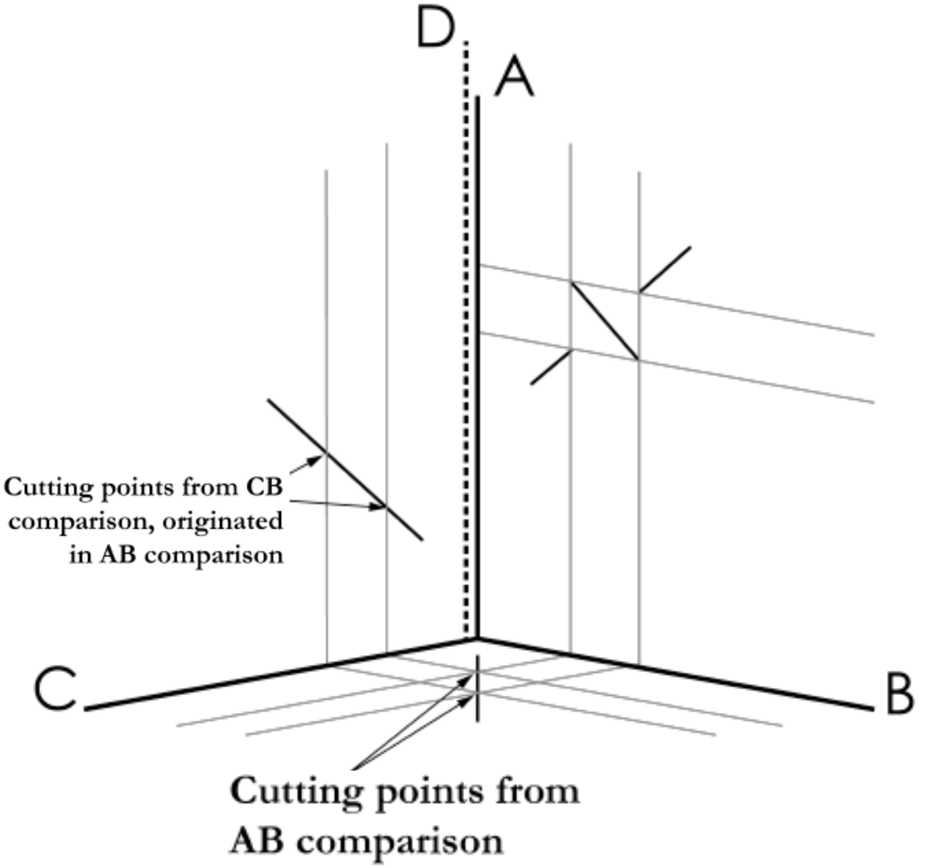
The Unitary Conserved Element problem

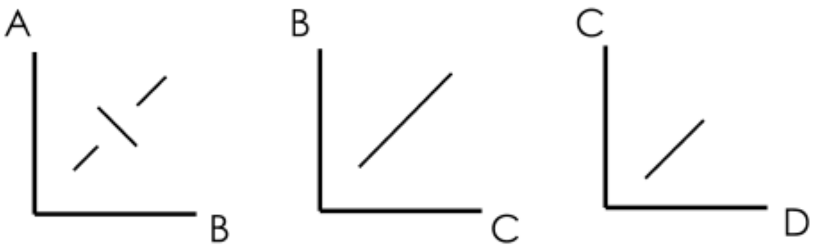


A) Two overlapped HSPs.
B) Result of the trimming process. Two fragments are still overlapped.
C) New overlapped Conserved Elements trigger a new trimming process.
D) Final result of the recursive trimming process.

The final pairs of Conserved Elements do not overlap.

The Unitary Conserved Element problem (II)


D
A
C
B
Cutting points from CB comparison, originated in AB comparison
Cutting points from AB comparison



Representation of the trimming process in a multiple comparison.

In the comparison AB there is an inversion, that triggers a trimming process in the comparison BC.

As a result, another trimming process is triggered in comparison DC.

Unitary Synteny Element

- **A set of Unitary Conserved Elements from different sequences**
  - More than one block $\pi = \{\alpha, \alpha', \alpha'', ..., \beta, \beta', \beta'', ..., \gamma, \gamma', \gamma'', ..., \omega''\}$
  - Same length $|\alpha| = |\alpha'| = |\alpha''| = ... = |\beta| = |\beta'| = |\beta''| = \ ... = |\omega''|$
  - Every Unitary Conserved Block belong to one and only one Unitary Synteny Element

$$\forall \pi_i, \pi_j \in \Pi, j \neq i : \pi_i \cap \pi_j = \emptyset$$

and

$$\pi_1 \cup \pi_2 \cup \pi_3 \cup ... \cup \pi_{N_\Pi} = A_{\Phi_A} \cup B_{\Phi_B} \cup \Gamma_{\Phi_\Gamma} \cup ... \cup \Omega_{\Phi_\Omega}$$

Unitary Synteny Element

- Graphic representation

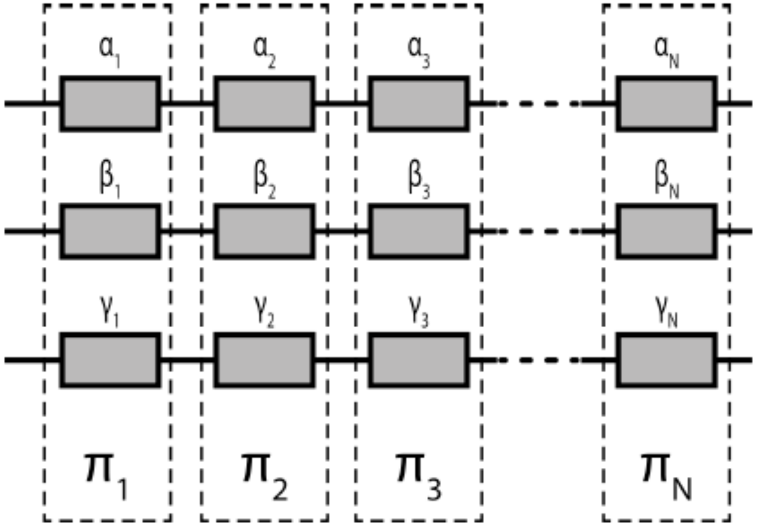
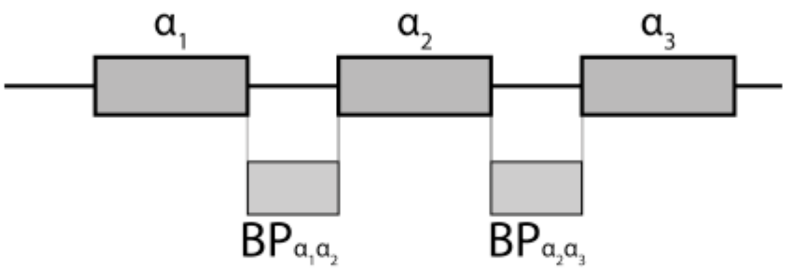


Fig. 3.6 Graphic representation of three Synteny Elements. Synteny Element π_1 links α_1,β_1 and γ_1 Unitary Conserved Elements.

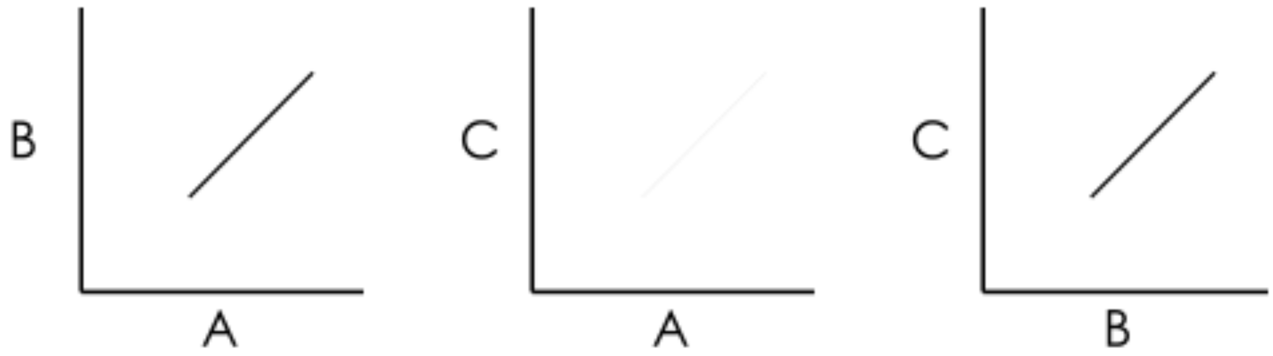
Break Point

- **Defined as the region (or point) between two Unitary Conserved Elements**



The transitivity property of Synteny Block: Inferred HSP

- This method does not increase the number of Unitary Conserved Blocks
- It just reveals *synteny* relations that have not been detected by the chosen comparison method.
  - Hence, this supports the evidence why SBs must be defined in a N-dimensional space.



Synteny Block concatenation

- If the succession is the same

$$\Pi(\alpha_{a+i}) = \Pi(\beta_{b+i}) = \Pi(\gamma_{g+i}) = \ldots = \Pi(\omega_{o+i}) = \pi_i : i = \{-1, 0, +1\}$$

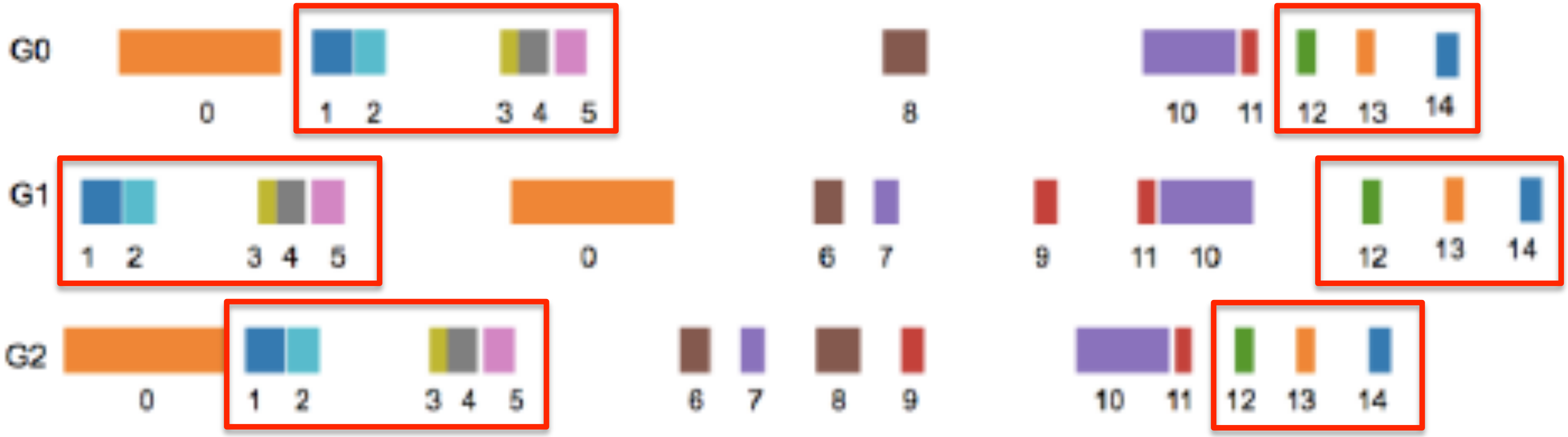
- All these Unitary Conserved Elements conform each a Unitary Synteny Element:

$$\begin{array}{llllll}
\pi_{-1} & = \alpha_{a-1} & \cup \beta_{b-1} & \cup \gamma_{g-1} & \cup \ldots & \cup \omega_{o-1} \\
\pi & = \alpha_{a} & \cup \beta_{b} & \cup \gamma_{g} & \cup \ldots & \cup \omega_{o} \\
\pi_{+1} & = \alpha_{a+1} & \cup \beta_{b+1} & \cup \gamma_{g+1} & \cup \ldots & \cup \omega_{o+1}
\end{array}$$

- and the sign relation between them is the same along adjacent Elementary Conserved Blocks

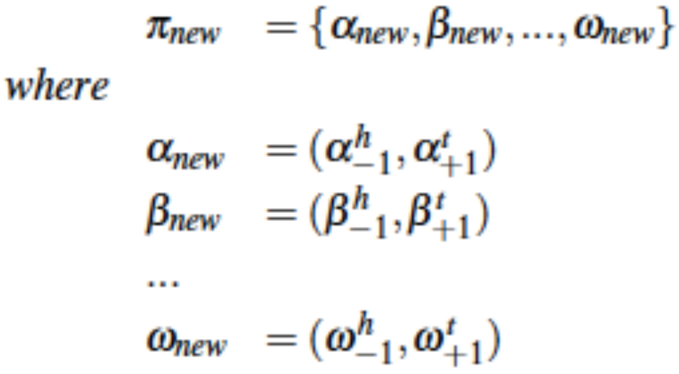
$$\begin{array}{lll}
sign(\alpha_{a-1}, \beta_{b-1}) & = sign(\alpha_{a}, \beta_{b}) & = sign(\alpha_{a+1}, \beta_{b+1}) \\
sign(\alpha_{a-1}, \gamma_{g-1}) & = sign(\alpha_{a}, \gamma_{g}) & = sign(\alpha_{a+1}, \gamma_{g+1}) \\
sign(\beta_{b-1}, \gamma_{g-1}) & = sign(\beta_{b}, \gamma_{g}) & = sign(\beta_{b+1}, \gamma_{g+1}) \\
\ldots & & \\
sign(\psi_{p-1}, \omega_{o-1}) & = sign(\psi_{p}, \omega_{o}) & = sign(\psi_{p+1}, \omega_{o+1})
\end{array}$$

SB concatenation: Example (I)



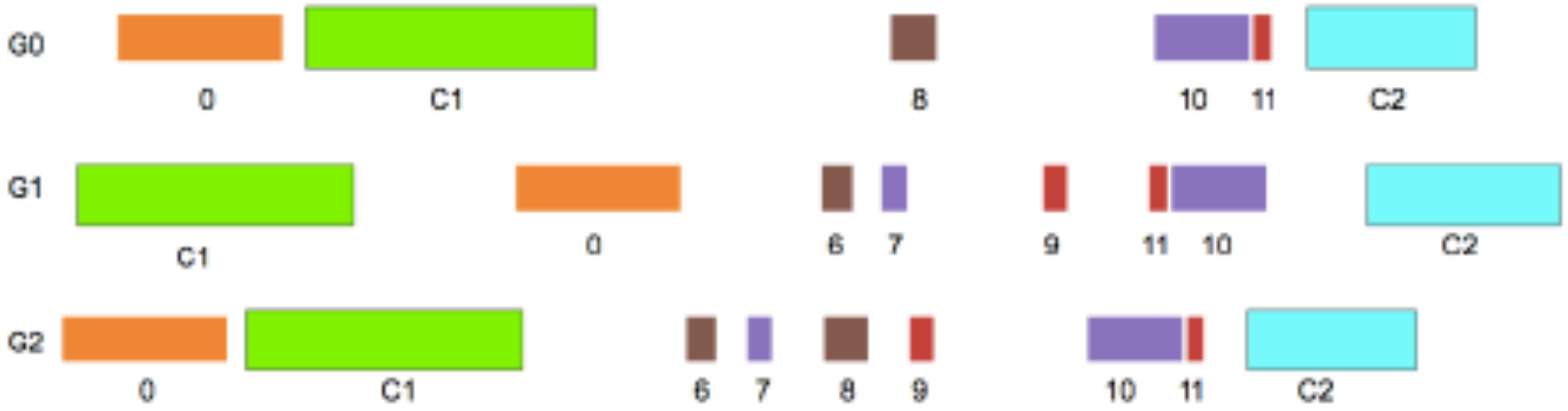
Synteny Block concatenation

- Then, Unitary Synteny Elements $\pi-1, \pi$ and $\pi+1$ can be merged into a single one by concatenating their Unitary Conserved Elements as follows:



$$
\begin{aligned}
\pi_{new} &= \{\alpha_{new}, \beta_{new}, \ldots, \omega_{new}\} \\
\textit{where} & \\
\alpha_{new} &= (\alpha^{h}_{-1}, \alpha^{t}_{+1}) \\
\beta_{new} &= (\beta^{h}_{-1}, \beta^{t}_{+1}) \\
\ldots & \\
\omega_{new} &= (\omega^{h}_{-1}, \omega^{t}_{+1})
\end{aligned}
$$

SB concatenation: Example (II)



Inversions

- If

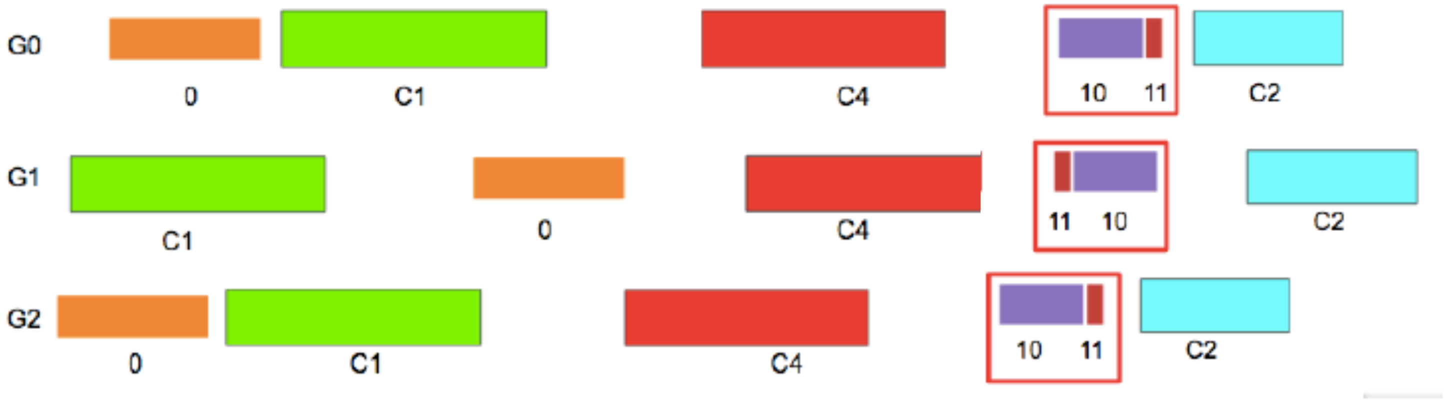
$$\begin{array}{llllll}
\Pi(\alpha_{a-1}) & = \Pi(\beta_{b-1}) & = \Pi(\gamma_{g-1}) & = \ldots = & \Pi(\omega_{o-1}) & = \pi_{-1} \\
\Pi(\alpha_{a}) & = \Pi(\beta_{b}) & = \Pi(\gamma_{g}) & = \ldots = & \Pi(\omega_{o}) & = \pi \\
\Pi(\alpha_{a+1}) & = \Pi(\beta_{b+1}) & = \Pi(\gamma_{g+1}) & = \ldots = & \Pi(\omega_{o+1}) & = \pi_{+1}
\end{array}$$

- And

$$\begin{array}{lll}
sign(\alpha_{a-1},\beta_{b-1}) & = sign(\alpha_{a+1},\beta_{b+1}) & = \boxed{-sign(\alpha_a,\beta_b)} \\
sign(\alpha_{a-1},\gamma_{g-1}) & = sign(\alpha_{a+1},\gamma_{g+1}) & = \boxed{-sign(\alpha_a,\gamma_g)} \\
\ldots & & \\
sign(\beta_{b-1},\gamma_{g-1}) & = sign(\beta_{b+1},\gamma_{g+1}) & = sign(\beta_b,\gamma_g) \\
\ldots & & \\
sign(\psi_{p-1},\omega_{o-1}) & = sign(\psi_{p+1},\omega_{o+1}) & = sign(\psi_p,\omega_o)
\end{array}$$

- Then, either α_a or β_b, γ_g,…, ω_o are inversions

Detection of an Inversion: Example



Transpositions

- If

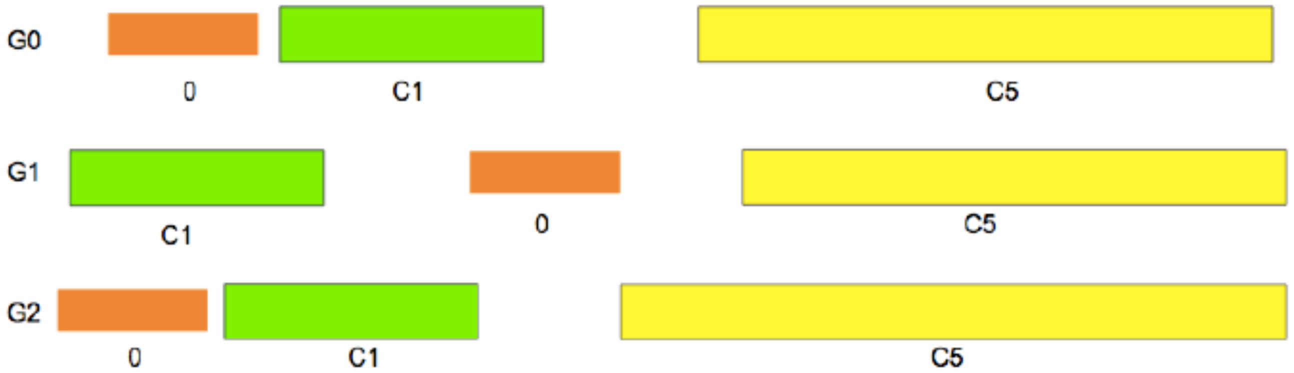
$$\begin{aligned}
\Pi(\alpha_{a-1}) &= \Pi(\beta_{b-1}) &&= \Pi(\gamma_{g-1}) &&= \ldots = \Pi(\omega_{o-1}) &&= \pi_{-1}\\
\Pi(\alpha_{a}) &= \Pi(\beta_{b+1}) &&= \Pi(\gamma_{g+1}) &&= \ldots = \Pi(\omega_{o+1}) &&= \pi_{+1}
\end{aligned}$$

- And

$$\begin{aligned}
\Pi(\alpha_{i-1}) &= \Pi(\beta_{j-1}) &&= \Pi(\gamma_{k-1}) &&= \ldots = \Pi(\omega_{l-1}) &&= \pi_{m-1}\\
\Pi(\alpha_{i}) &= \Pi(\beta_{b}) &&= \Pi(\gamma_{g}) &&= \ldots = \Pi(\omega_{o}) &&= \pi\\
\Pi(\alpha_{i+1}) &= \Pi(\beta_{j+1}) &&= \Pi(\gamma_{k+1}) &&= \ldots = \Pi(\omega_{l+1}) &&= \pi_{m+1}
\end{aligned}$$

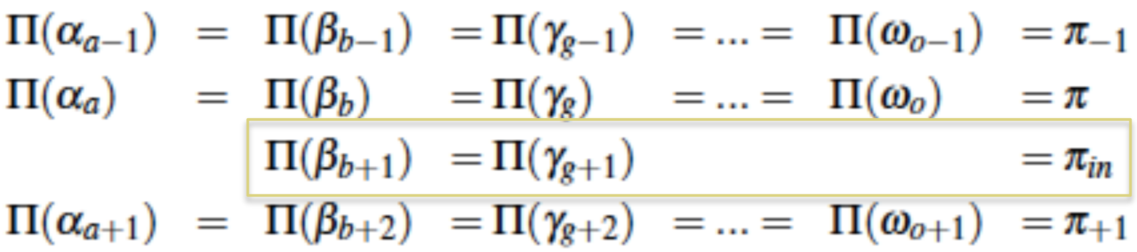
- Then, either α_a or β_b, $ɣ_g$,…, ω_o are transpositions

Detection of a Transposition: Example



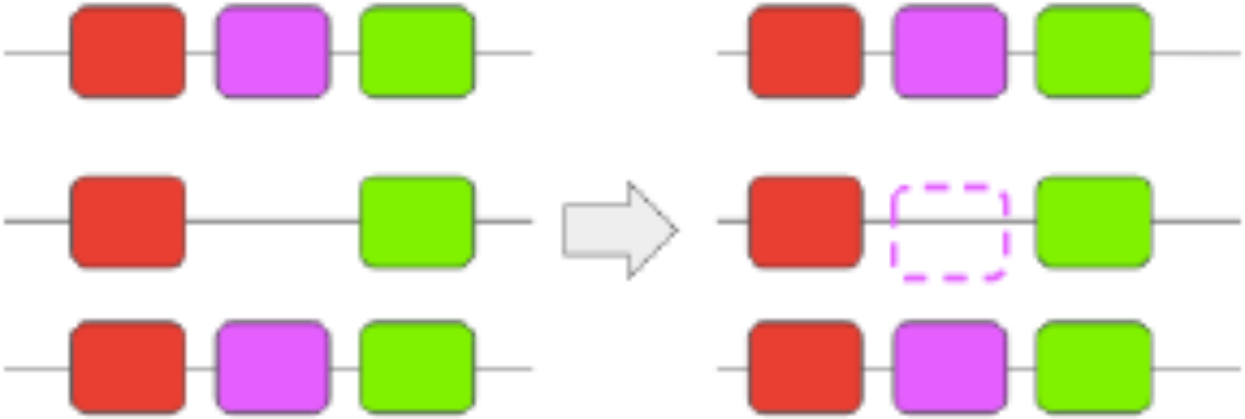
Insertions and deletions

- **When concatenating, not detected inserted blocks can be inferred if the length of the new Synteny Element is not the same.**
  - A multiple alignment is needed
- **An insertion can be detected as follows:**



$$\begin{array}{llllll}
\Pi(\alpha_{a-1}) & = \Pi(\beta_{b-1}) & = \Pi(\gamma_{g-1}) & = \ldots = & \Pi(\omega_{o-1}) & = \pi_{-1} \\
\Pi(\alpha_{a}) & = \Pi(\beta_{b}) & = \Pi(\gamma_{g}) & = \ldots = & \Pi(\omega_{o}) & = \pi \\
 & \multicolumn{5}{l}{\boxed{\Pi(\beta_{b+1}) \quad = \Pi(\gamma_{g+1}) \qquad\qquad\qquad\qquad = \pi_{in}}} \\
\Pi(\alpha_{a+1}) & = \Pi(\beta_{b+2}) & = \Pi(\gamma_{g+2}) & = \ldots = & \Pi(\omega_{o+1}) & = \pi_{+1}
\end{array}$$

Detection of an Insertion/ deletion: Example



Duplications

- If

$$\pi = \{\alpha_1, \beta_2, \gamma_3, \ldots, \alpha_4\}$$

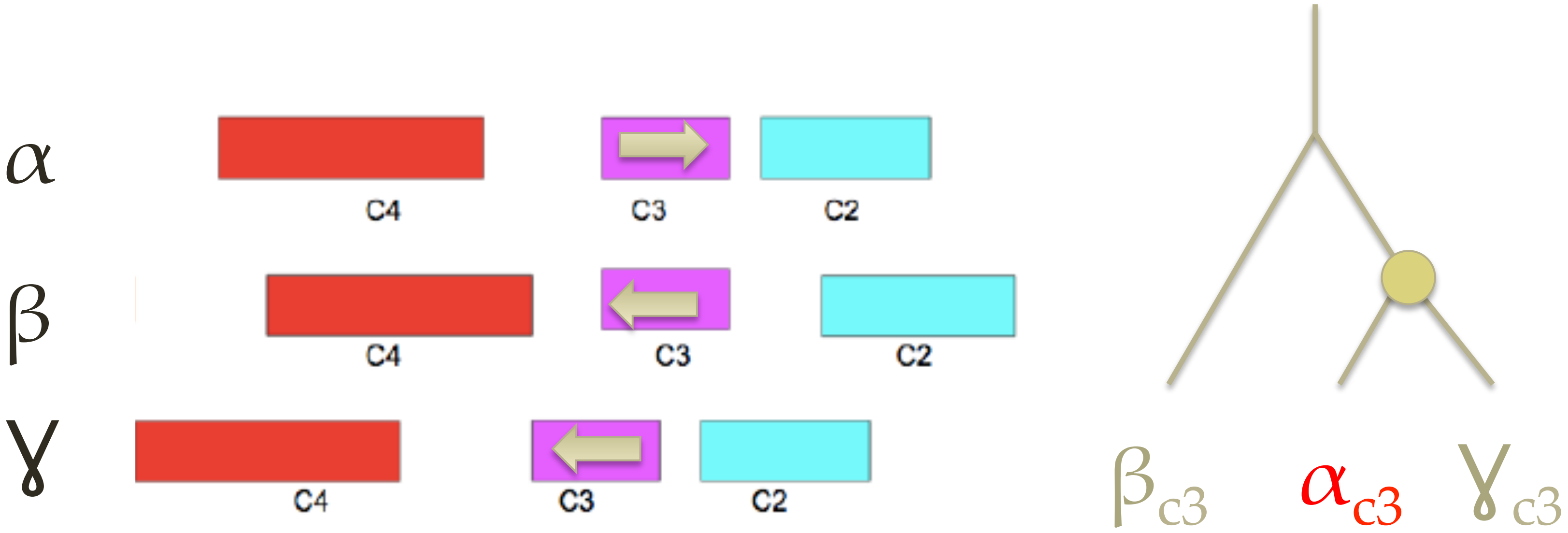
- And

$$\begin{array}{lllllll}
\Pi(\alpha_{a-1}) & = & \Pi(\beta_{b-1}) & = \Pi(\gamma_{g-1}) & = \ldots = & \Pi(\omega_{o-1}) & = \pi_{-1} & \neq \Pi(\alpha'_{d-1}) \\
\Pi(\alpha_{a}) & = & \Pi(\beta_{b}) & = \Pi(\gamma_{g}) & = \ldots = & \Pi(\omega_{o}) & = \pi & = \Pi(\alpha'_{d}) \\
\Pi(\alpha_{a+1}) & = & \Pi(\beta_{b+1}) & = \Pi(\gamma_{g+1}) & = \ldots = & \Pi(\omega_{o+1}) & = \pi_{+1} & \neq \Pi(\alpha'_{d+1})
\end{array}$$

- Then, α'_d is a duplication

How to select the genome to perform the reversion?

Building a phylogenetic tree, using the block information (subsequences)



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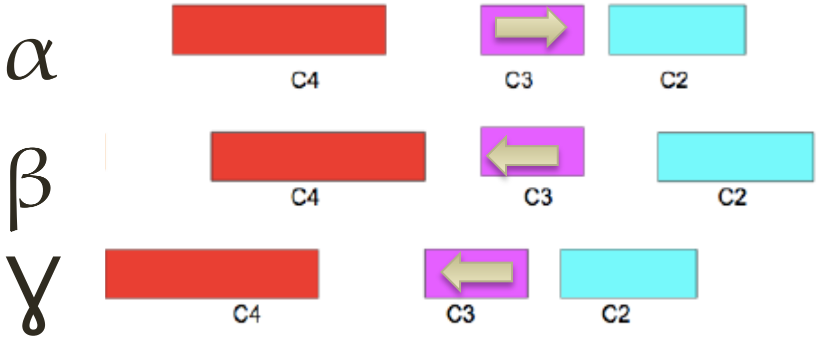
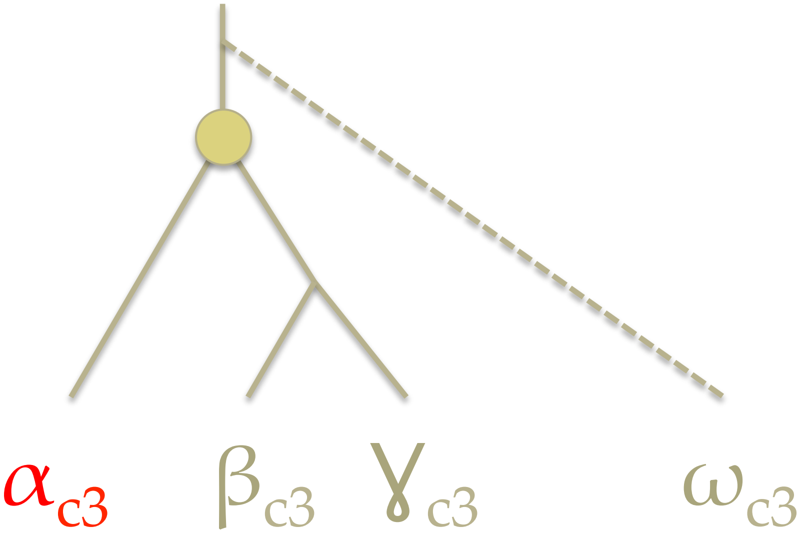




Summary

- 1) Pairwise SB and LSGR detection (GECKO-CSB)
- 2) SB refinement
- 3) Multi-genome SB and LSGR detection and reconstruction

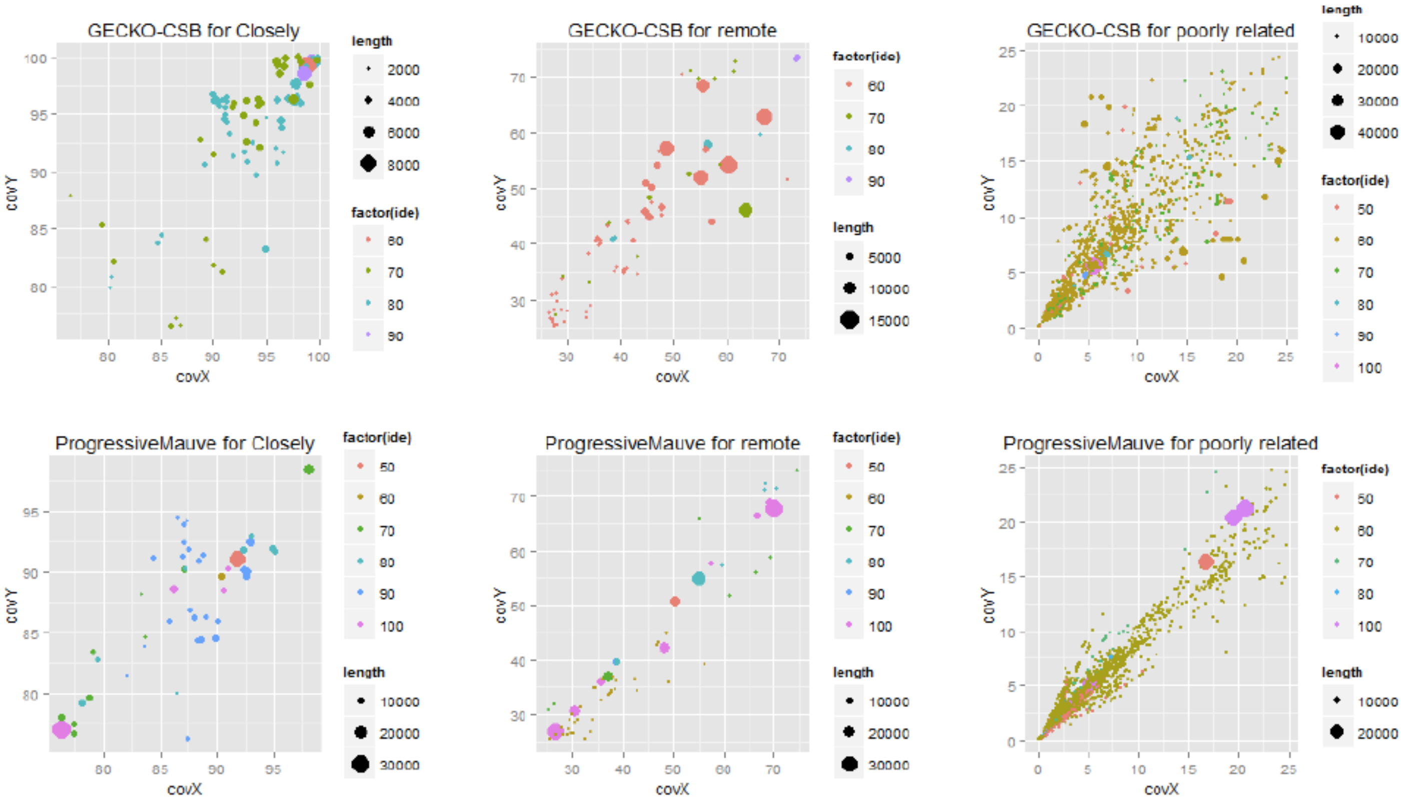
Results and discussion

Experiments

- **Our methods were compared with state-of-art methods, implemented by progressiveMauve, GRIMMsynteny and CASSIS.**
- **Data set of 68 Mycoplasmas, 2.278 pairwise genome comparisons.**

Pairwise framework

- Better % coverage at all levels of similarity, especially in the less related genomes

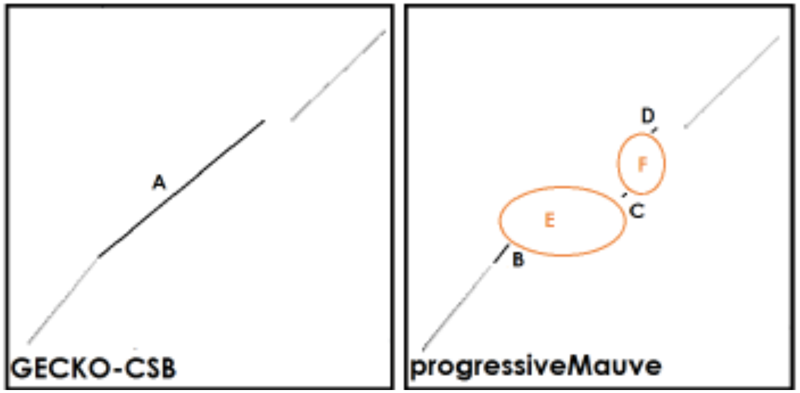


Pairwise framework

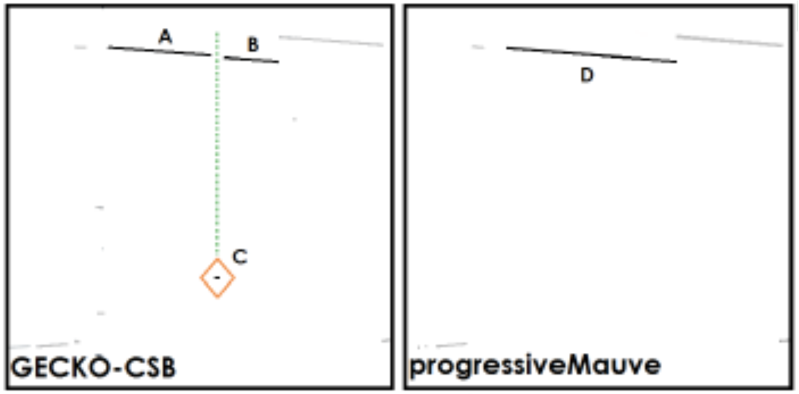
- More coverage over both types of regions
  - For coding regions, around 90% against 75%
  - For non-coding regions 76% against 60%

Pairwise framework

- **Differences of SB detection for a certain region in the genomes using Gecko-CSB and progressiveMauve methods**



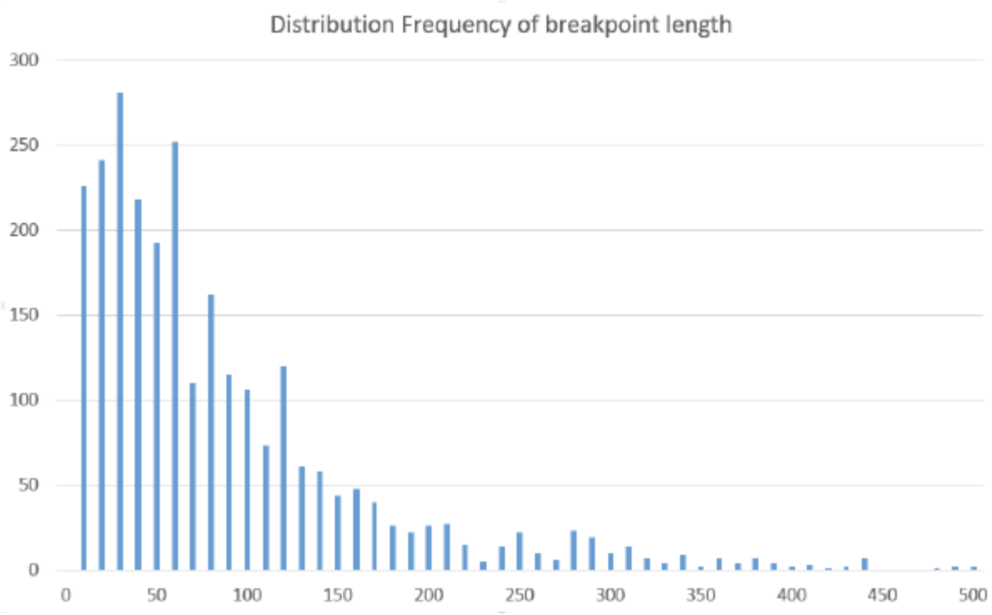
(a) Gecko-CSB detects one SB. (b) progressiveMauve detects three SBs (B,C and D).



(a) Gecko-CSB detects three SBs (A,B and C). (b) progressiveMauve detects one large SB.

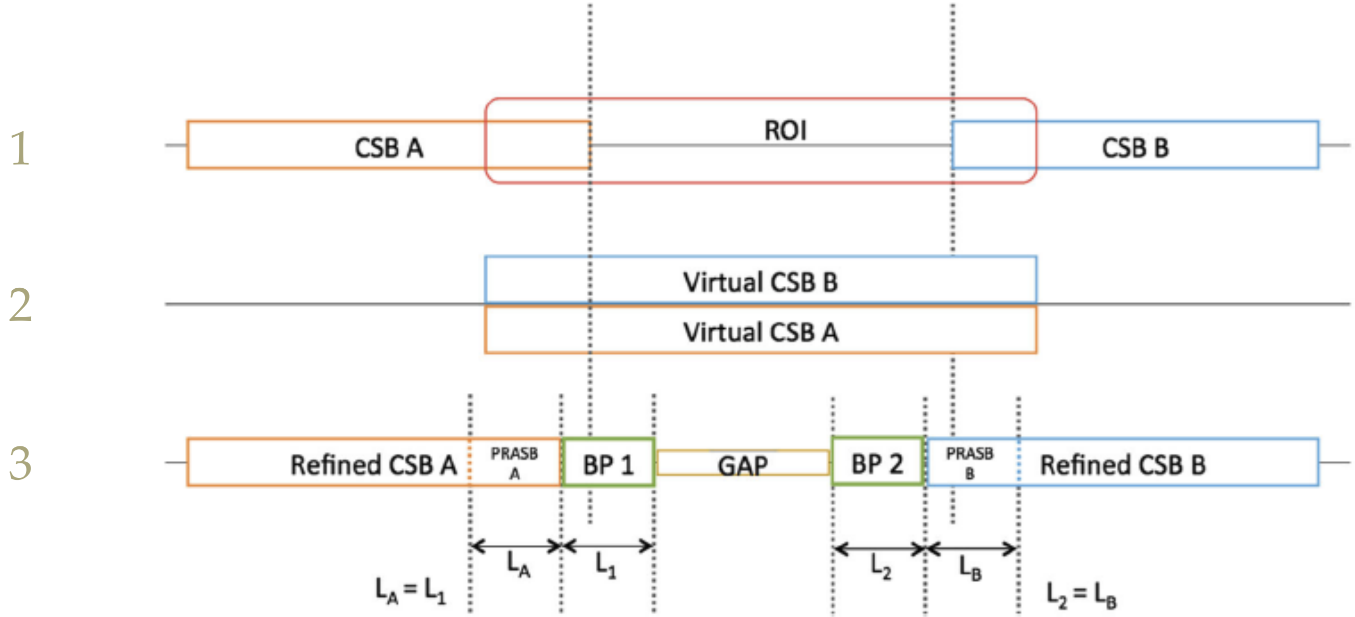
Refining Synteny Blocks

- In a massive comparison, around 70% of the BPs detected by our method are sized below 100 bps and 95% below 300 bps.



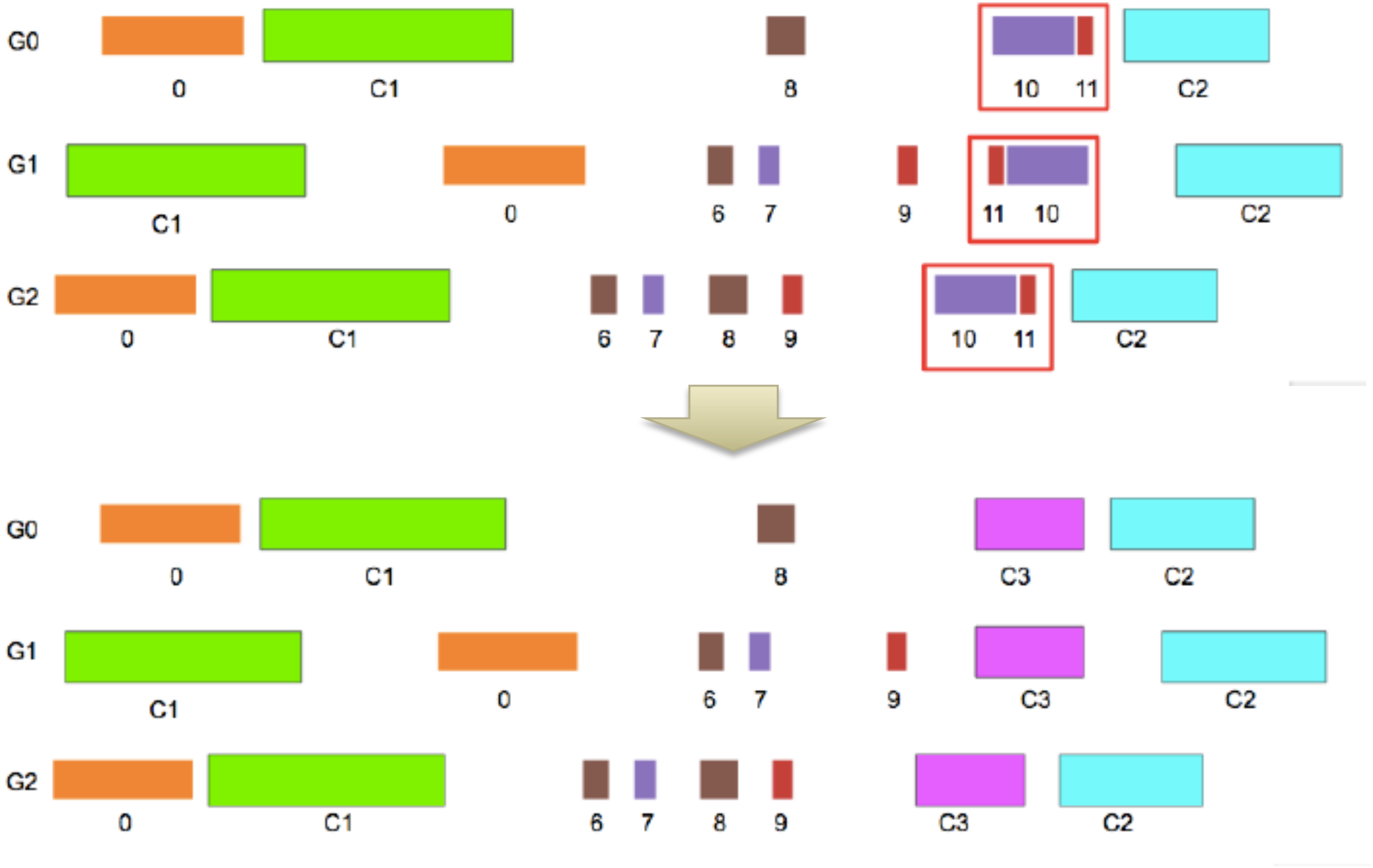
  - In a particular example of two genomes (~800Kbps) highly related, our method reports BPs sized below 100bps whereas CASSIS reports BPs sized up to 86.000 bps.

Result of the refinement

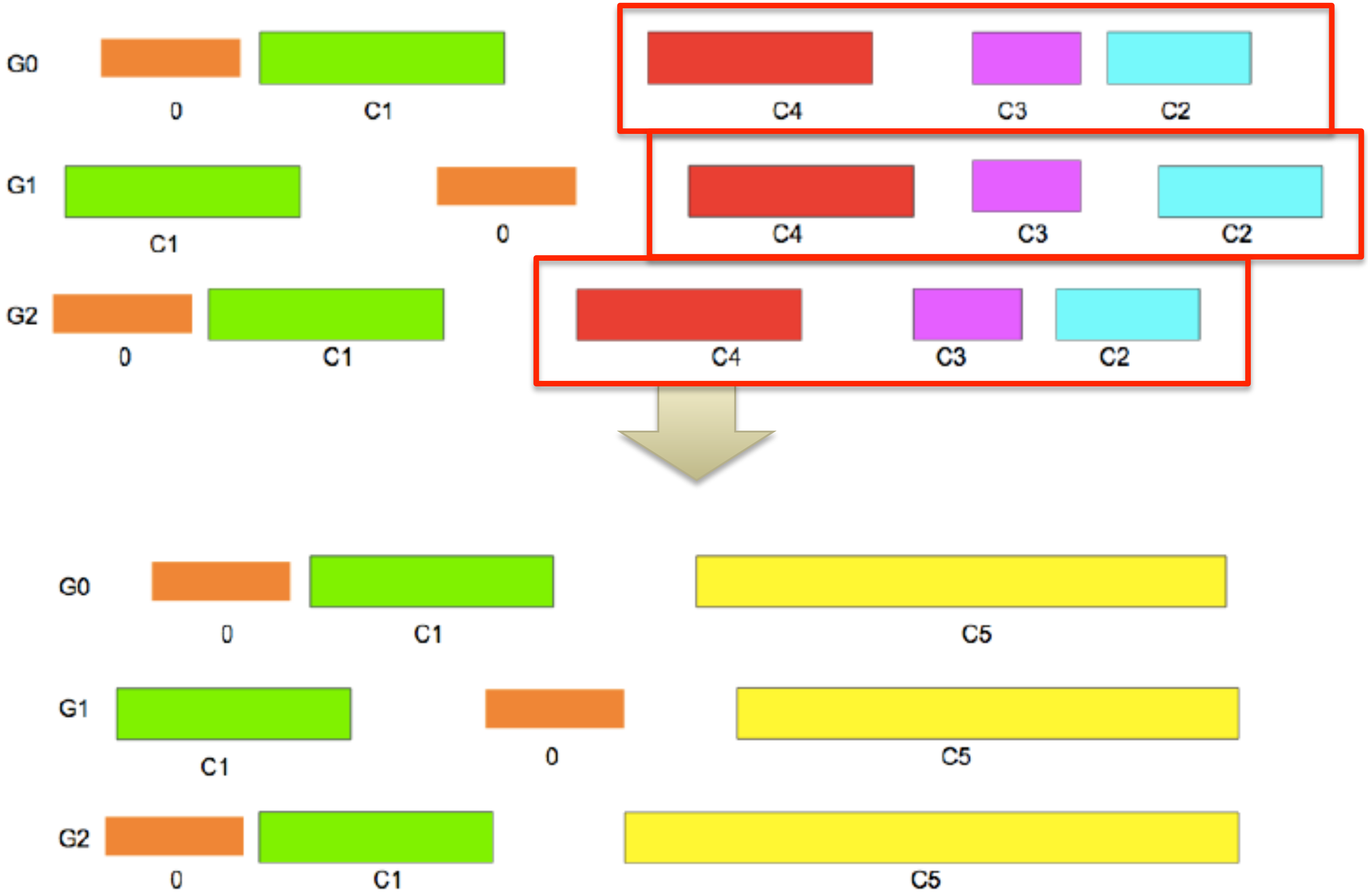


CSBs before and after the refinement. At the end of the refinement process, we detect BPs. We also extract PRASB and GAP sequences to analyse accuracy of the method. PRASB and BP have the same length

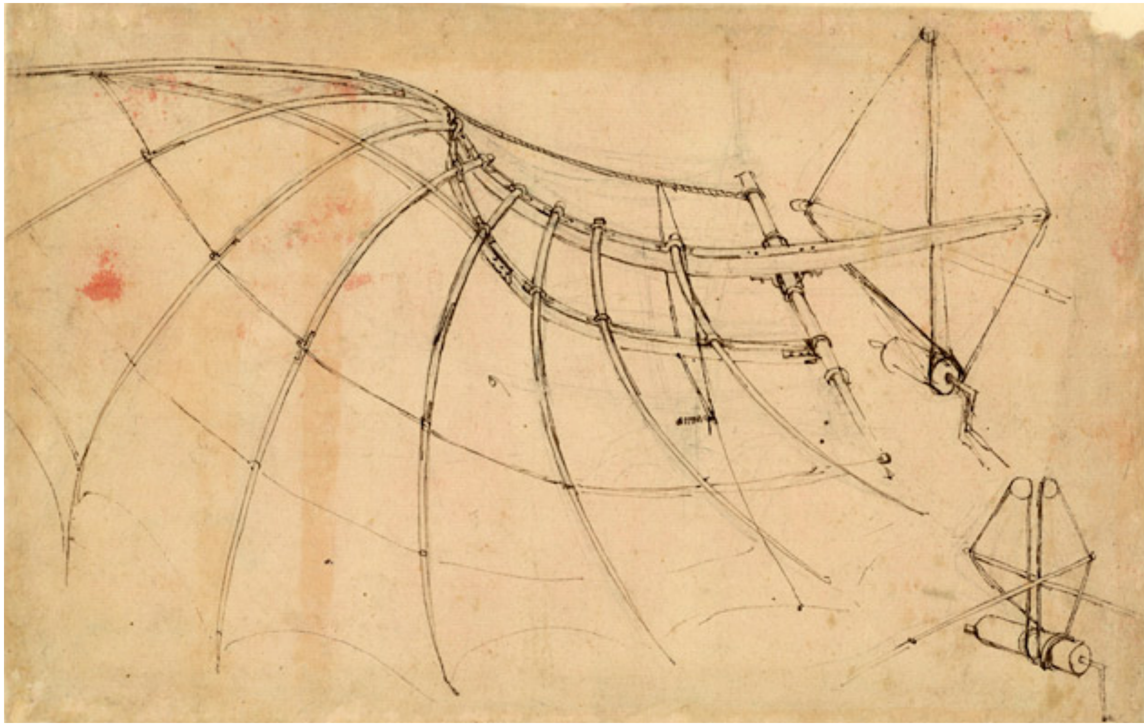
Reconstruction of LSGR solves the granularity problem



Reconstruction of LSGR solves the granularity problem



Conclusions, contributions and future work



Advances in the State of the art

- **SB and BP detection**
  - Formal definition of SB
  - The granularity problem solved
  - The BP contradiction solved
  - Repetitions included in the model

- **Methods to reverse LSGR**
  - Combined with the SB detection
  - No Reference depended
  - Designed for dealing with repetitions

Conclusions and contributions

- **More coverage**
- **Formal definition of SB and rearrangements**
- LSGR reversion and SB concatenation as **solution for the granularity problem**
- **Method to refine** SB and BPs

Open Research Lines

- Frequencies of LSGR to improve **inter-genome distances** and **phylogenetic organizations**
- The rearrangement history reconstruction could also be helpful for **ancestral genome reconstruction.**
- Refined BPs can be used as input to **find hidden patterns** or extract features in order to set up a formal definition of BP.
- BPs may help the understanding of LSGR and the **prediction** of future LSGRs

Acknowledgments

Questions?