Introduction to Bioinformatics for Computer Scientists

Lecture 5

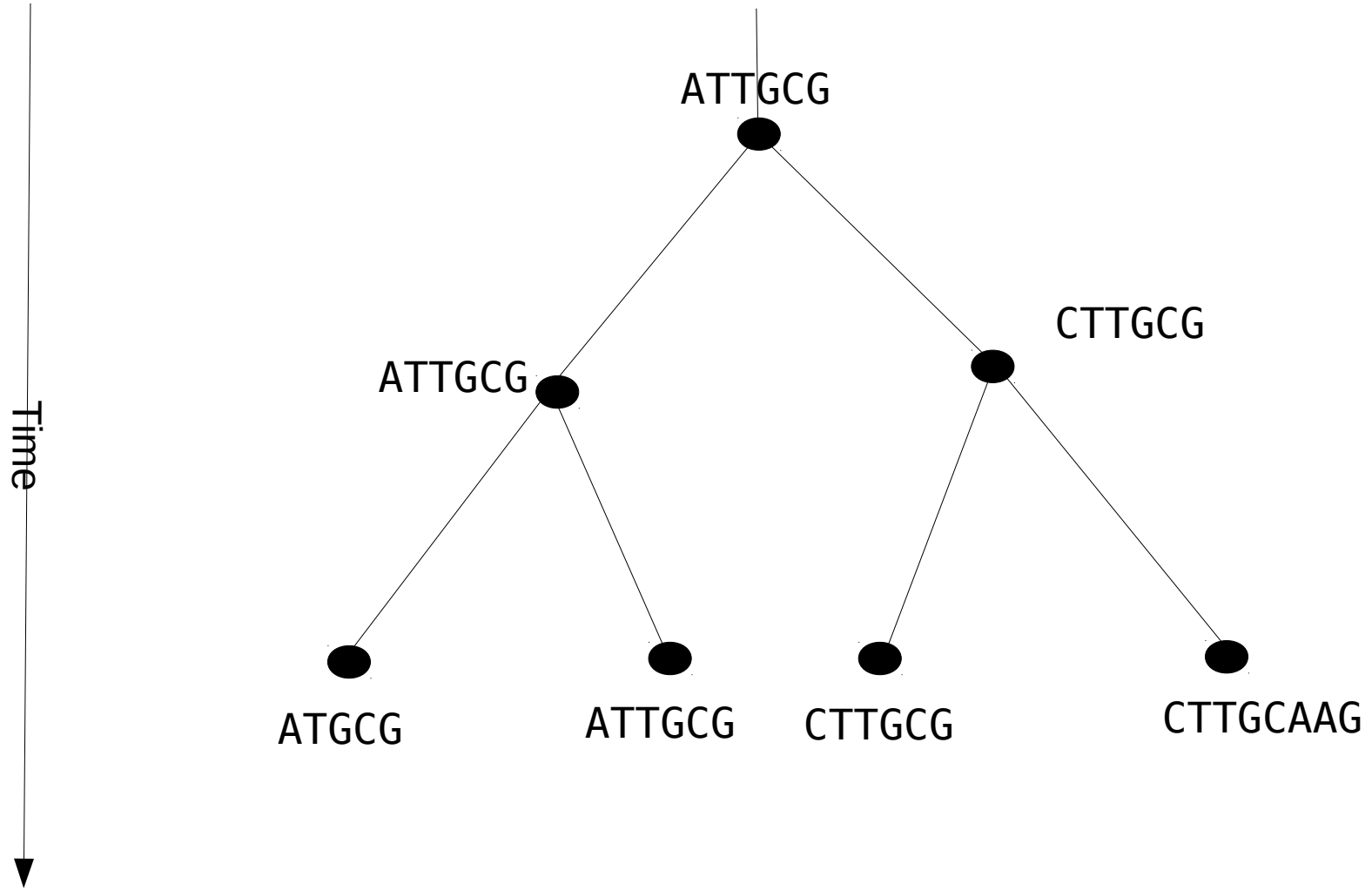
Plan for next lectures

- Today: Multiple Sequence Alignment
- Lecture 6 (Alexis): Introduction to phylogenetics
- Lecture 7 (Alexis): Phylogenetic search algorithms
- Lecture 8 (Alexis): Statistical Models of Evolution I
- Lecture 9 (Alexis): Statistical Models of Evolution II
- Lecture 10 (Pierre): Discrete Operations on Trees

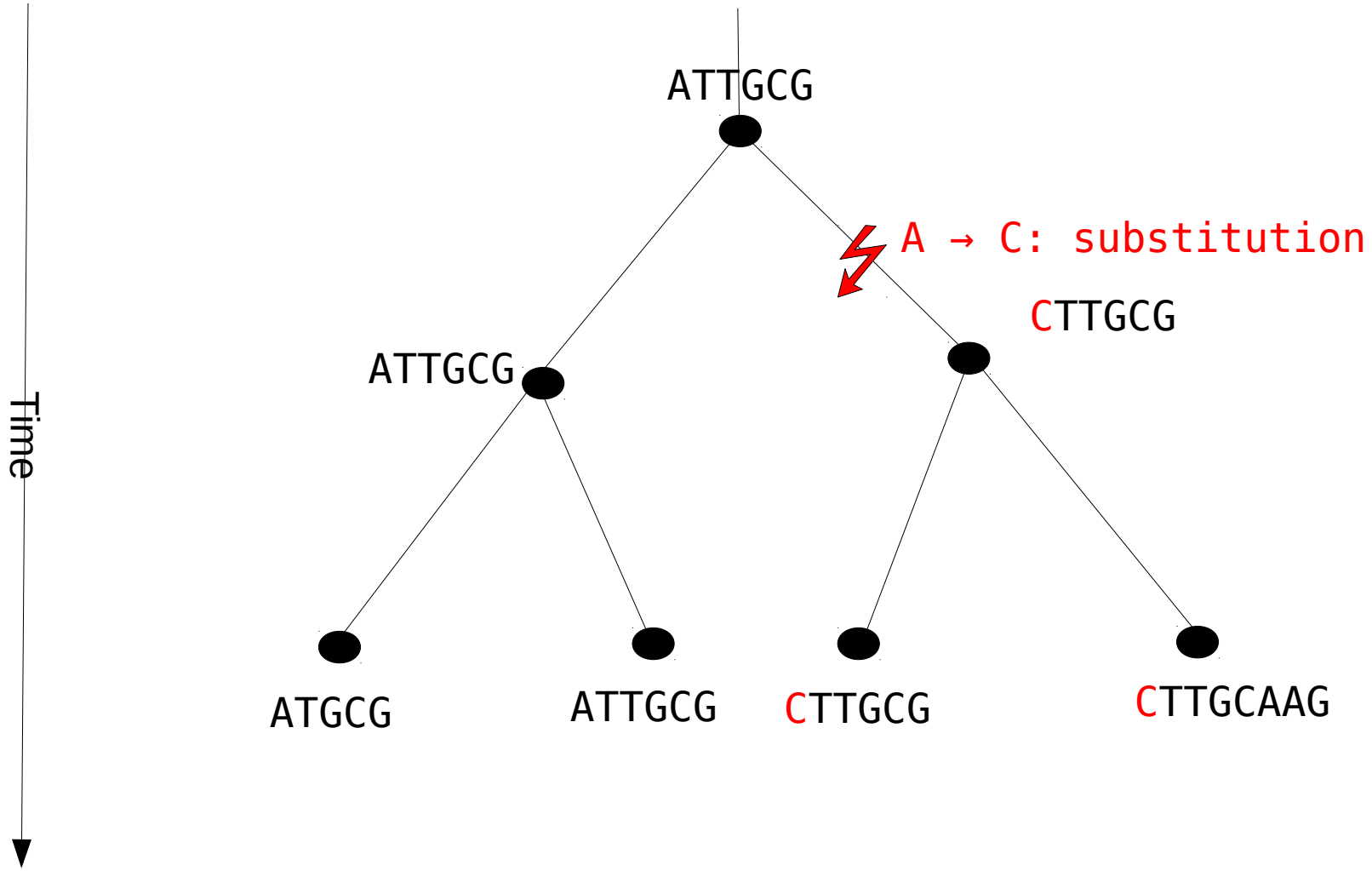
Multiple Sequence Alignment

- What are we trying to reconstruct?

Insertions, Deletions & Substitutions



Insertions, Deletions & Substitutions



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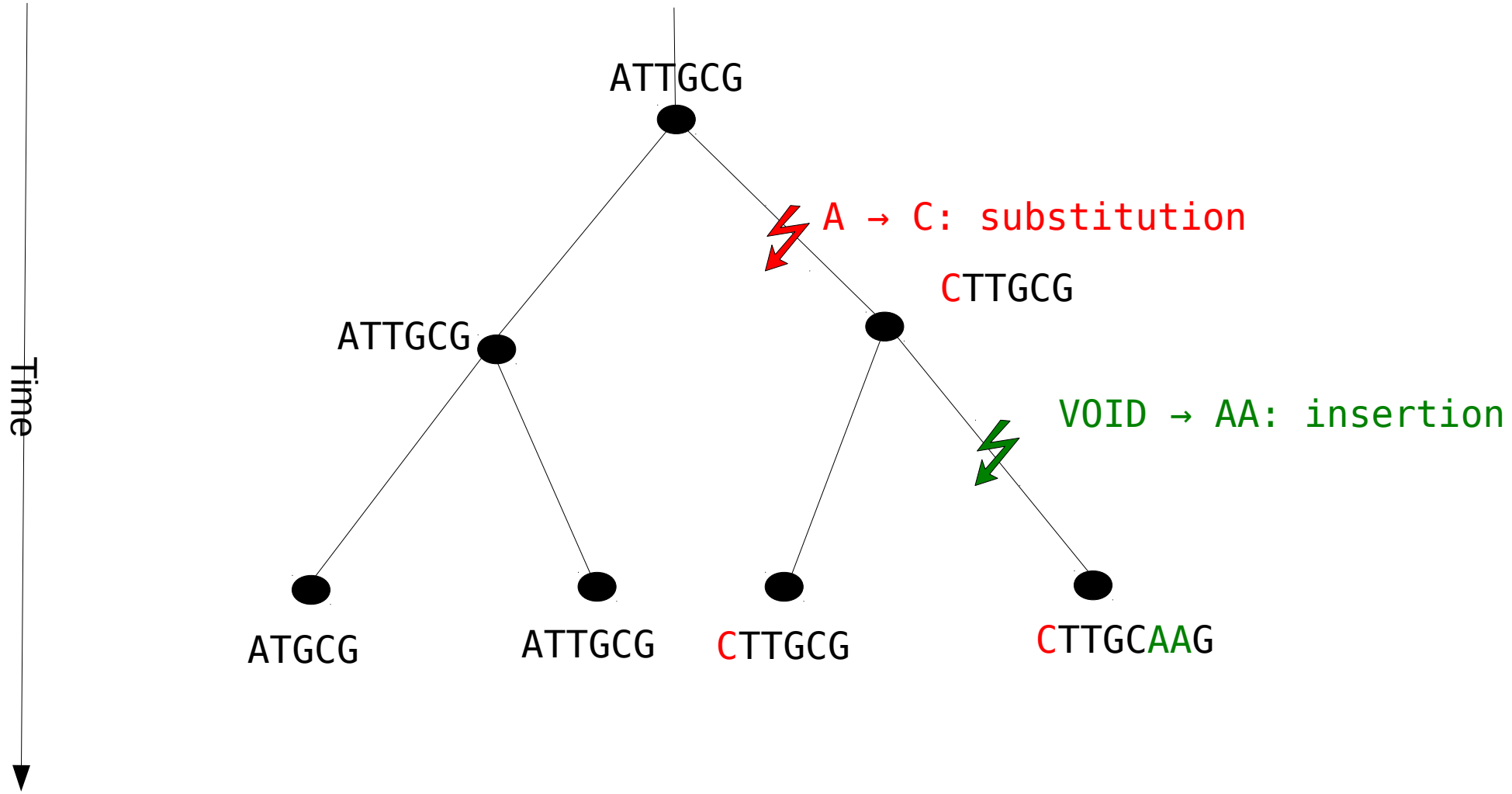

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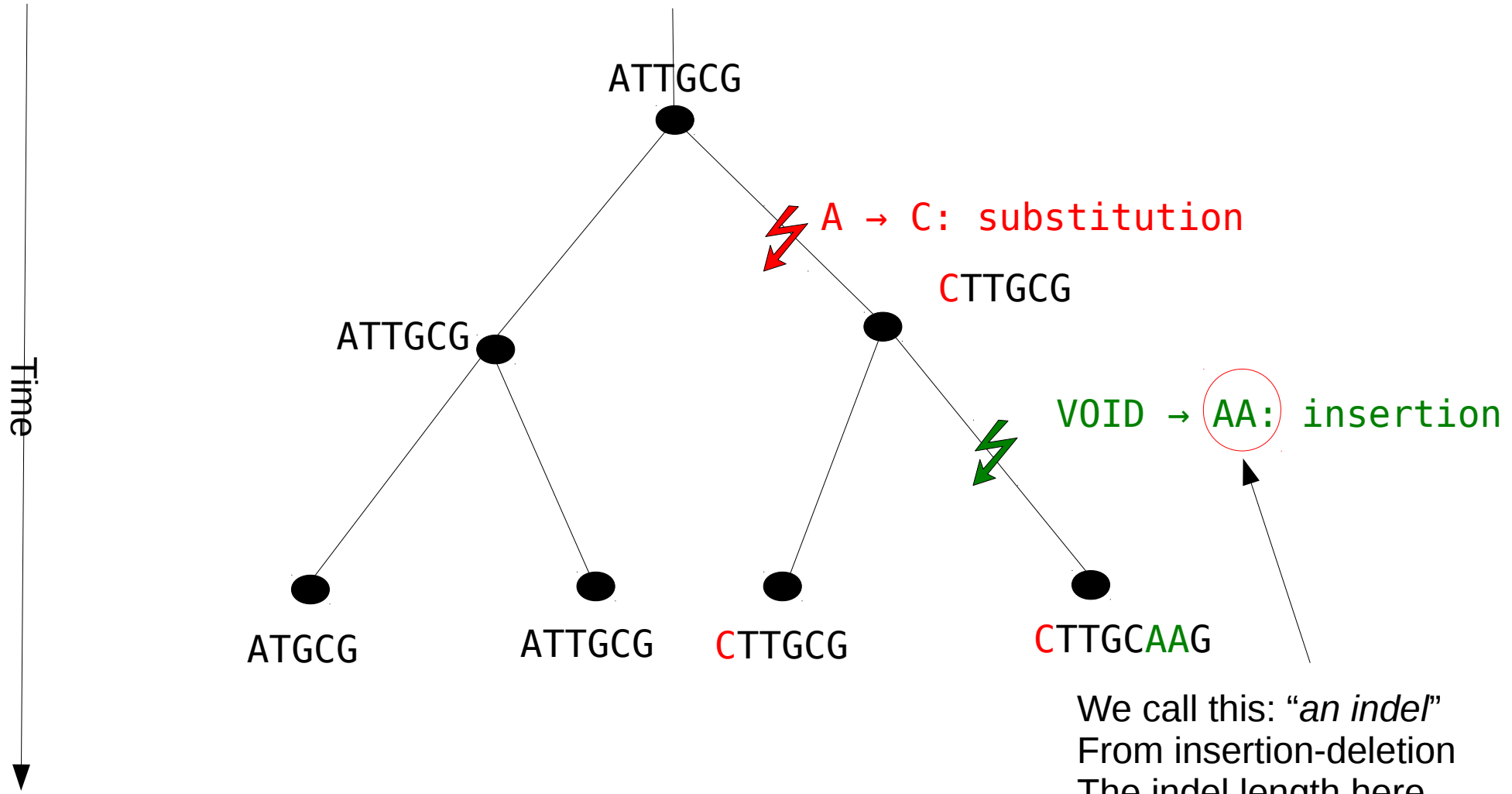
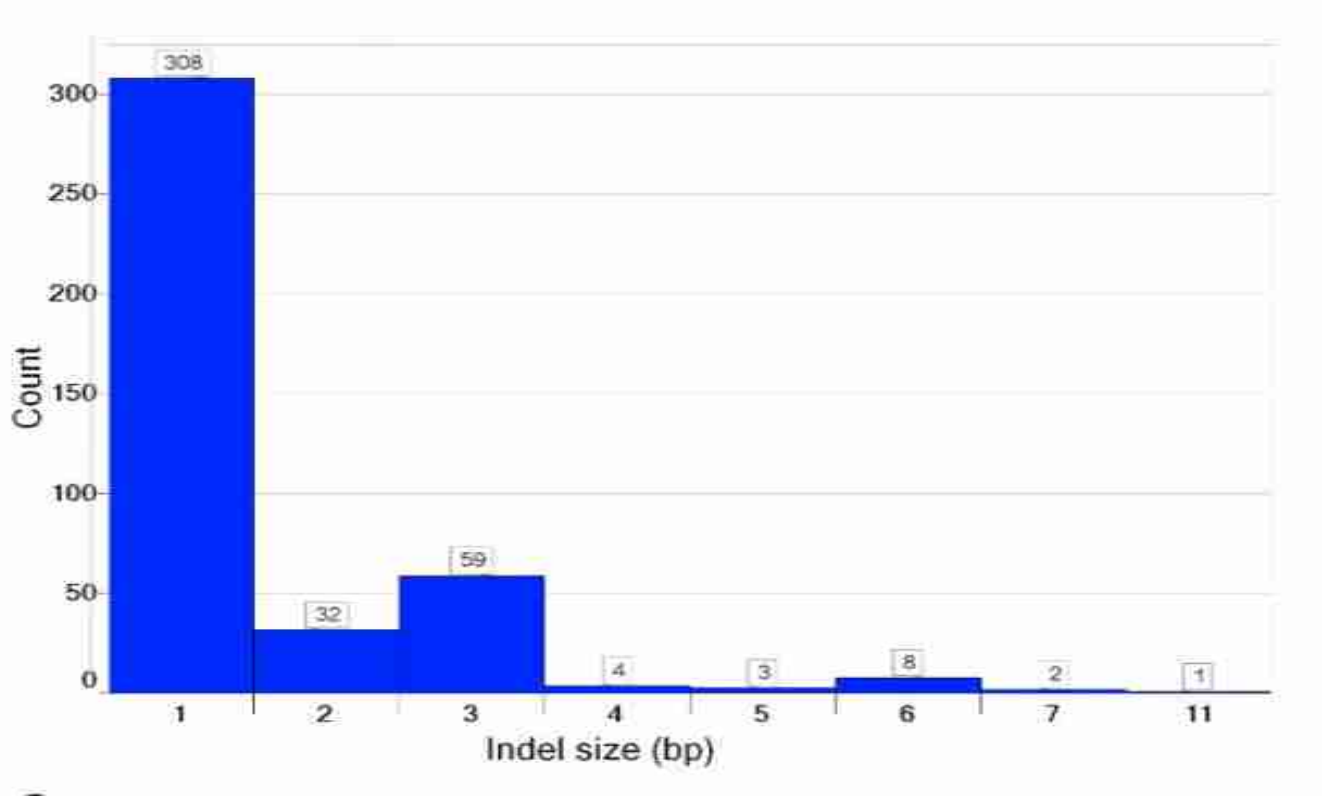


We call this: "*an indel*"
From insertion-deletion
The indel length here
is *2*, longer indels
lengths are not uncommon!

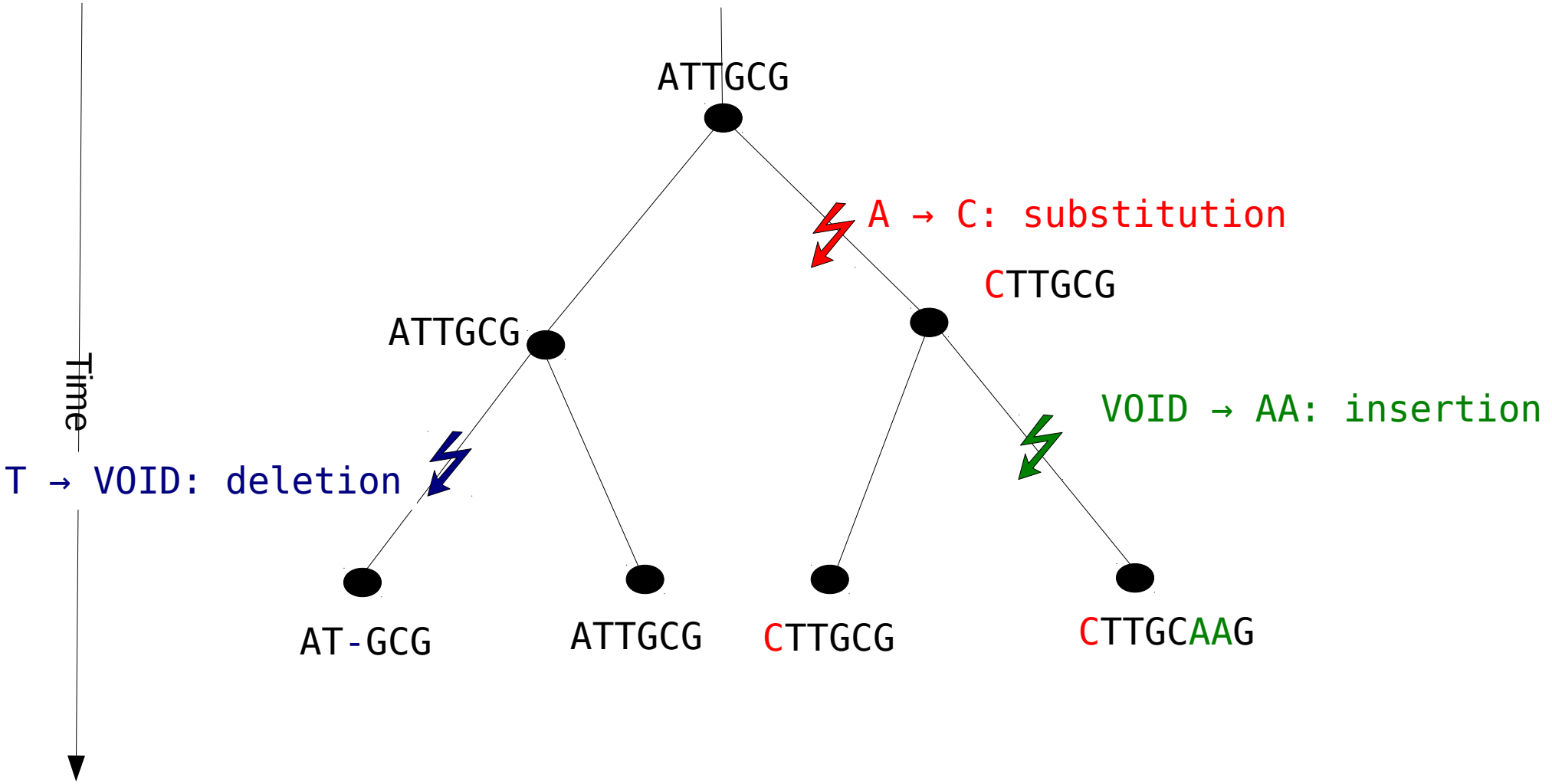
Indel size distribution

- Why are indels of size *3* rather frequent?



Indel size distribution in *coding regions* of *cattle genomes*

Insertions, Deletions & Substitutions



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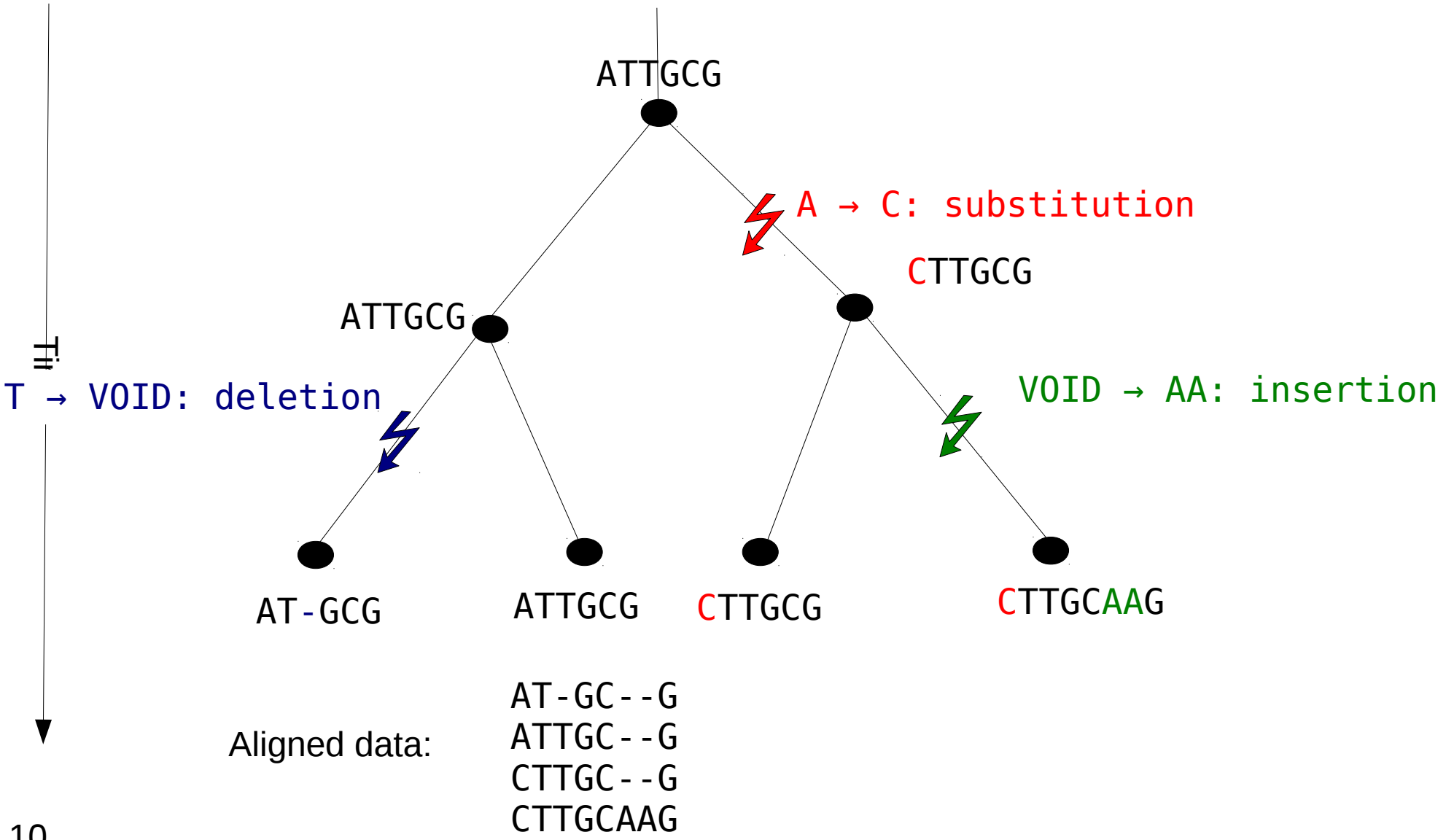

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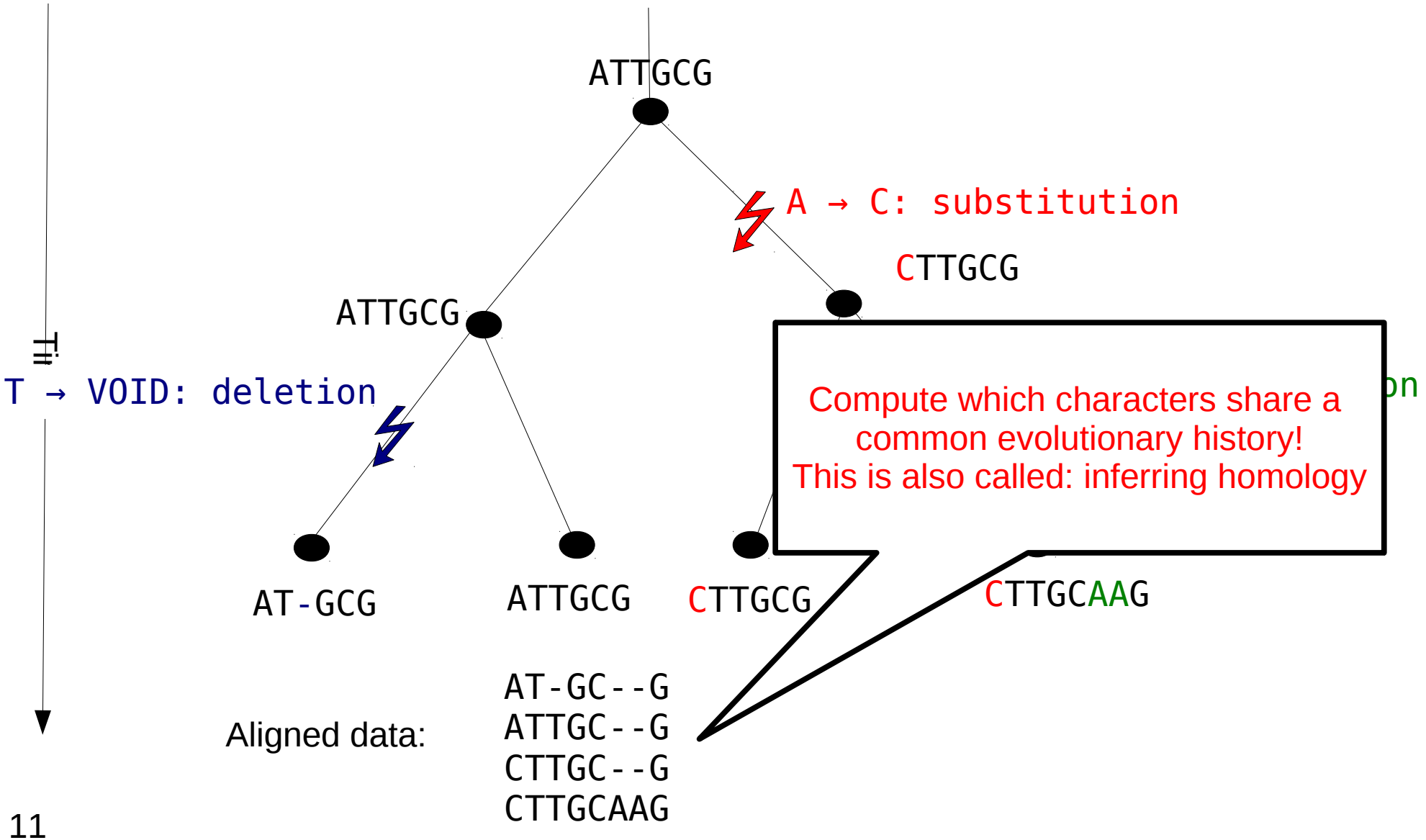


Multiple Sequence Alignment

- So far:
  - Comparing two sequences
  - Mapping a sequence/read to a reference genome
- What do we do when we want to compare more than two sequences at a time?
- Multiple Sequence Alignment (MSA)
- Open question: how do we assess the quality/accuracy of MSA algorithms?

  → nice review paper: “Who watches the watchmen?” http://arxiv.org/abs/1211.2160

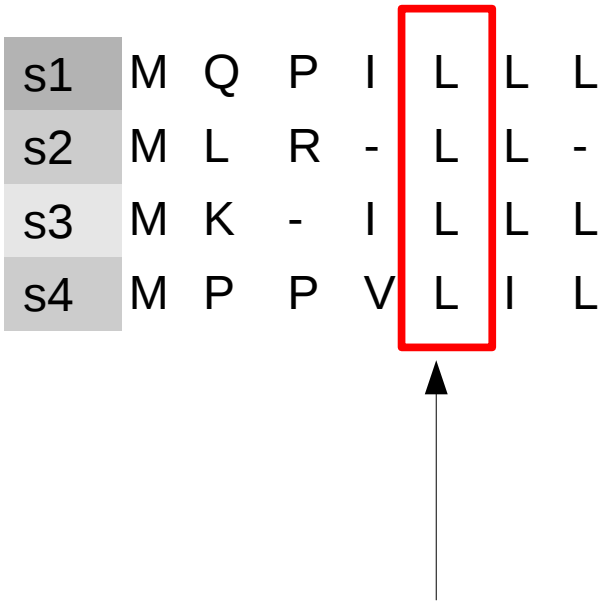
Why do we need MSAs?

- Input for phylogenetic reconstruction
- Discover important (conserved) parts of a *protein family*
- *Protein family* → group of evolutionarily related genes/proteins in different species with similar function/structure
- ***Family*** has a different meaning than in taxonomy!

MSA

- Generalization of pair-wise sequence alignment problem
- Given *n* orthologous sequences $s_1,\ldots,s_n$ of different lengths, insert gaps "-" such that:
  - All sequences have the same length
  - Some criterion is optimized
  - *Corresponding (homologous) characters* in s_i and s_j are aligned to each other (in the same alignment column/site)
  - Columns/sites that entirely consist of gaps are **not** allowed

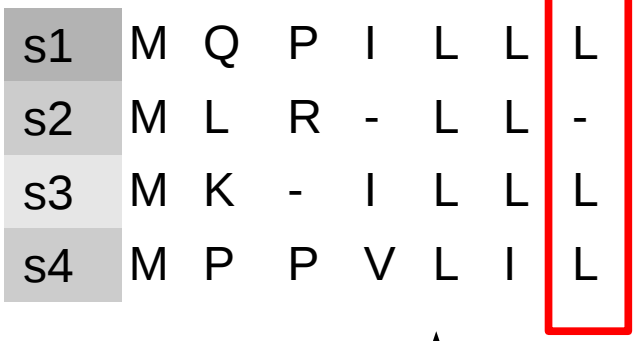
MSA Terminology



Orthologous sequences:
Sequences in different species that have evolved from the same **ancestral** gene

→ sequences that share a common evolutionary history

MSA Terminology

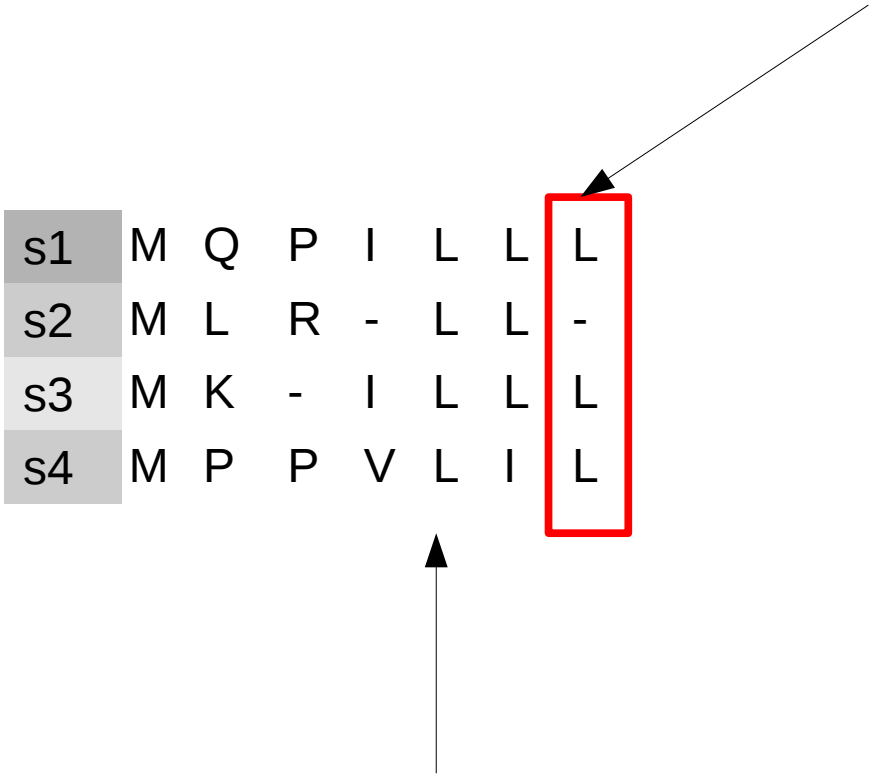


s1	M	Q	P	I	L	L	L
s2	M	L	R	-	L	L	-
s3	M	K	-	I	L	L	L
s4	M	P	P	V	L	I	L

Homologous characters:
Characters that share a common evolutionary history

Alignment site/Alignment column

MSA Terminology

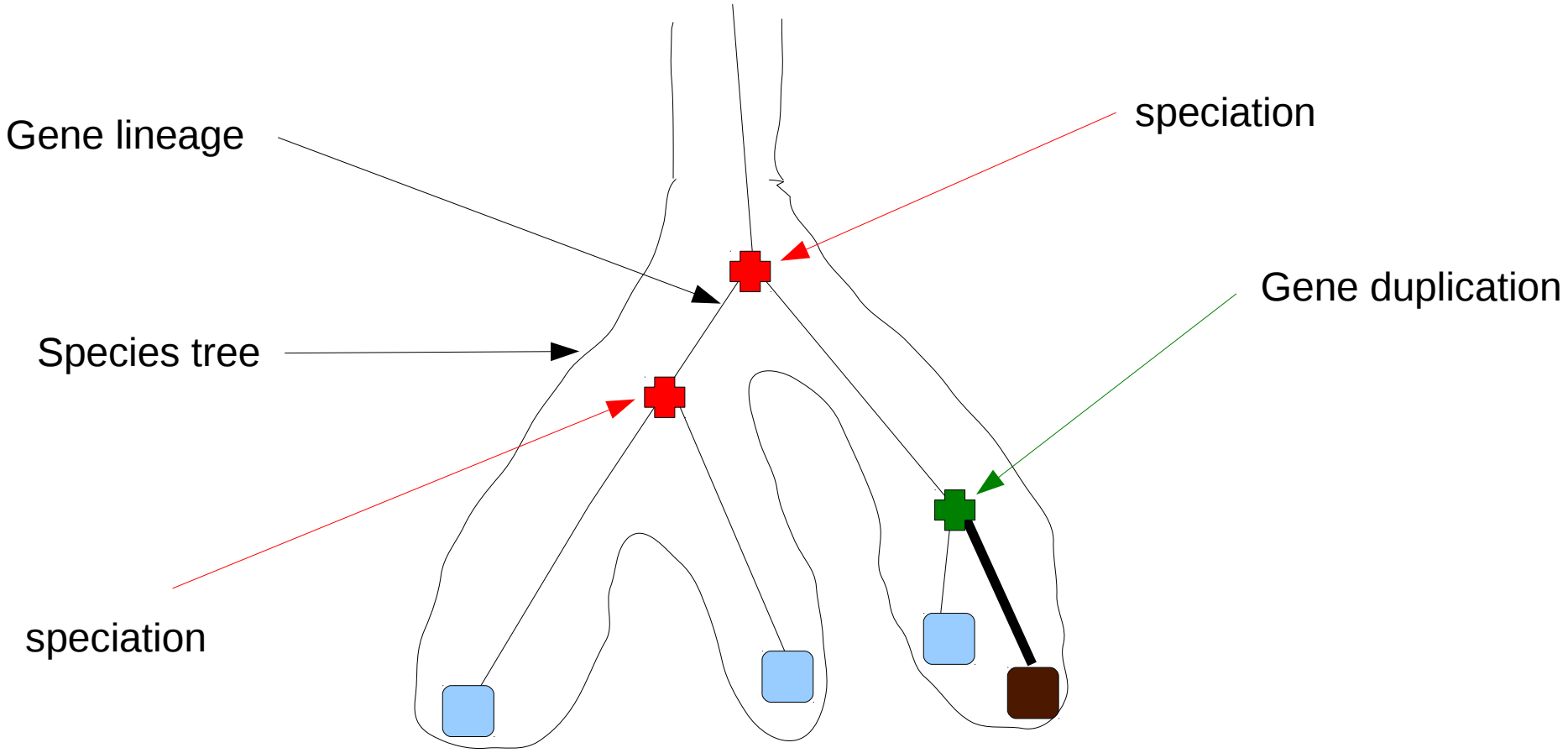


Homologous characters:
Characters that share a common evolutionary history

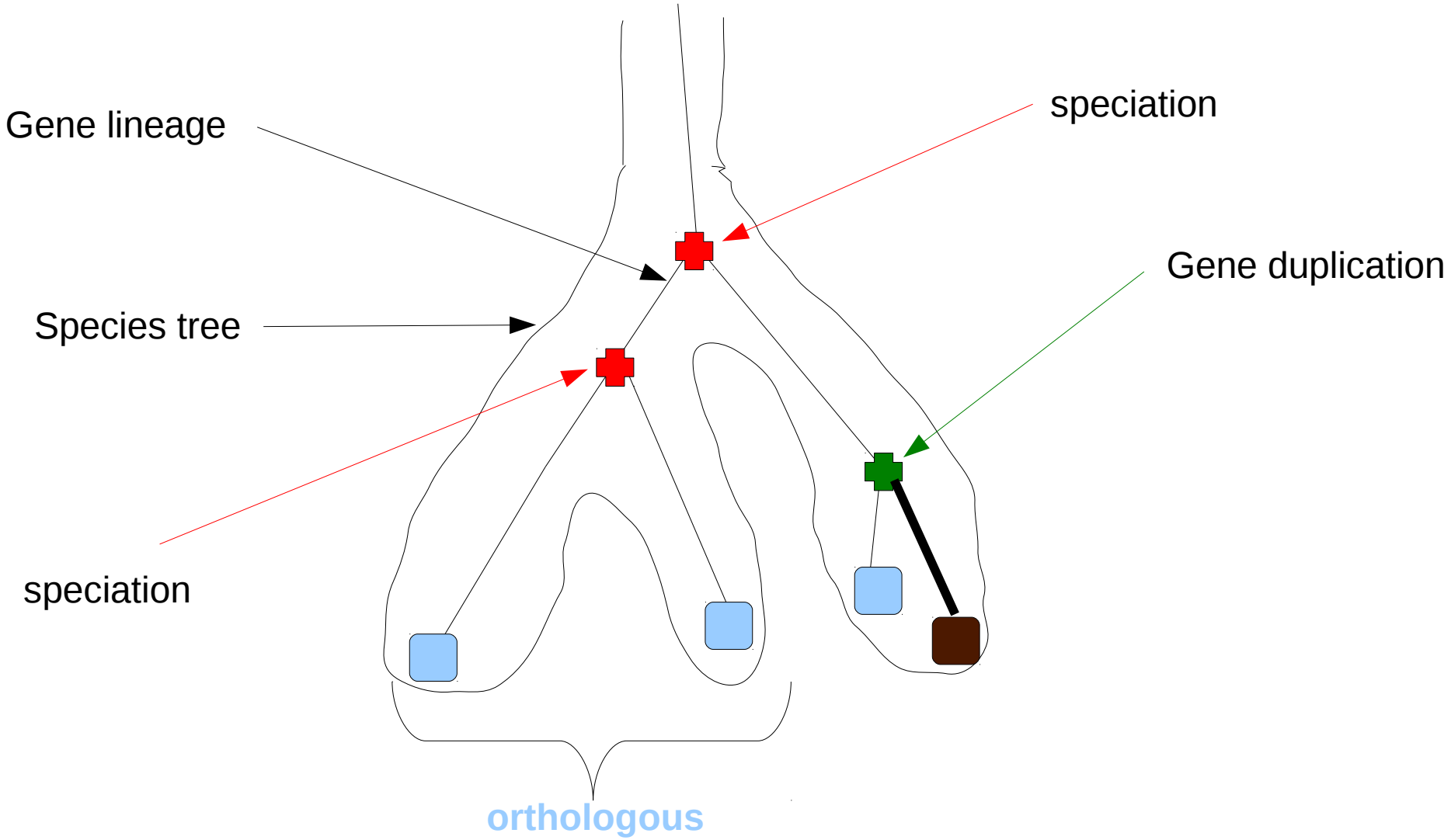
Note that, in this column the characters are similar (*analogous*), but this does not automatically induce homology!

They could be similar by chance or via Convergent evolution (see slides later-on)

Orthology


Gene lineage
speciation
Gene duplication
Species tree
speciation

Orthology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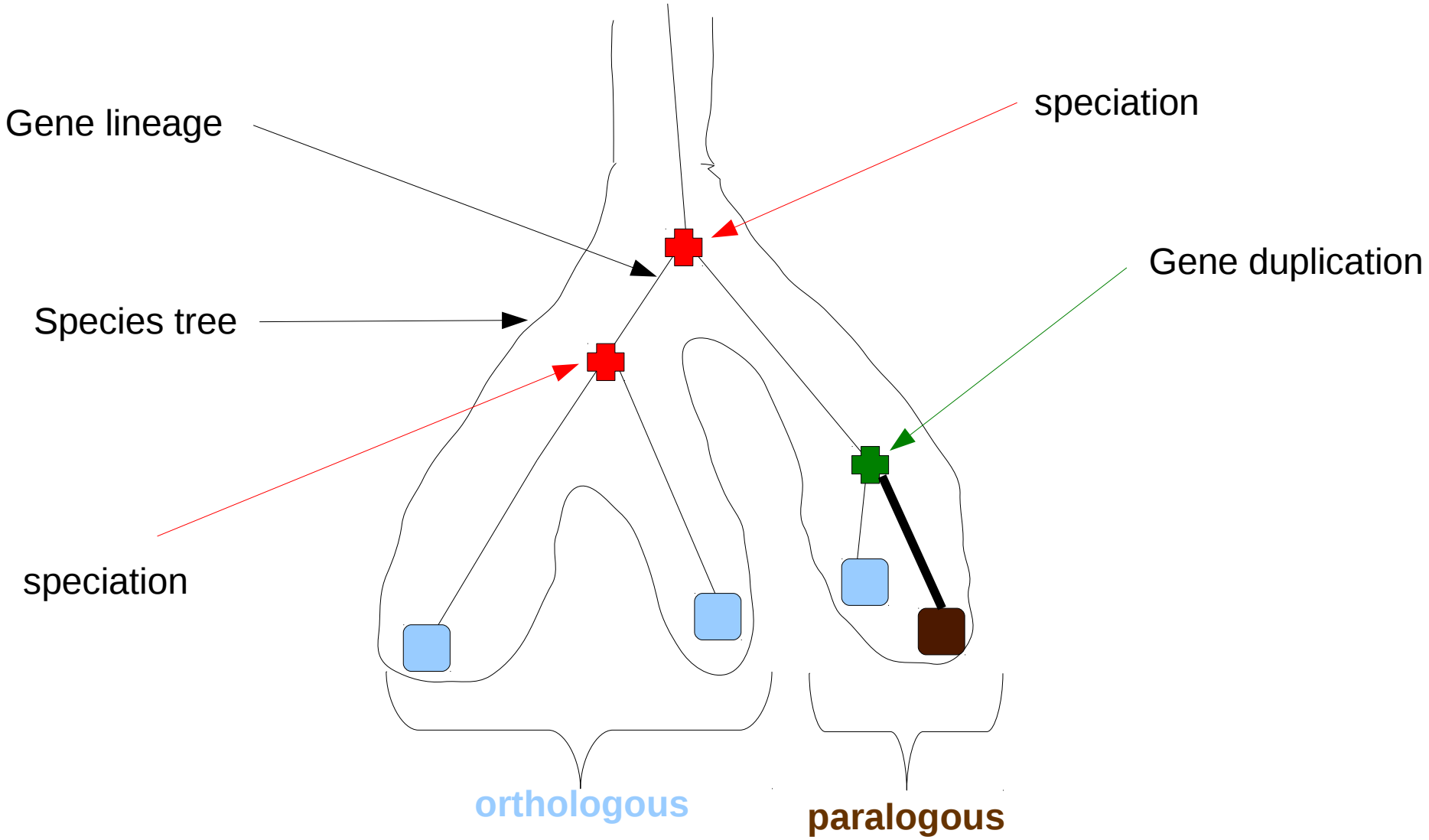
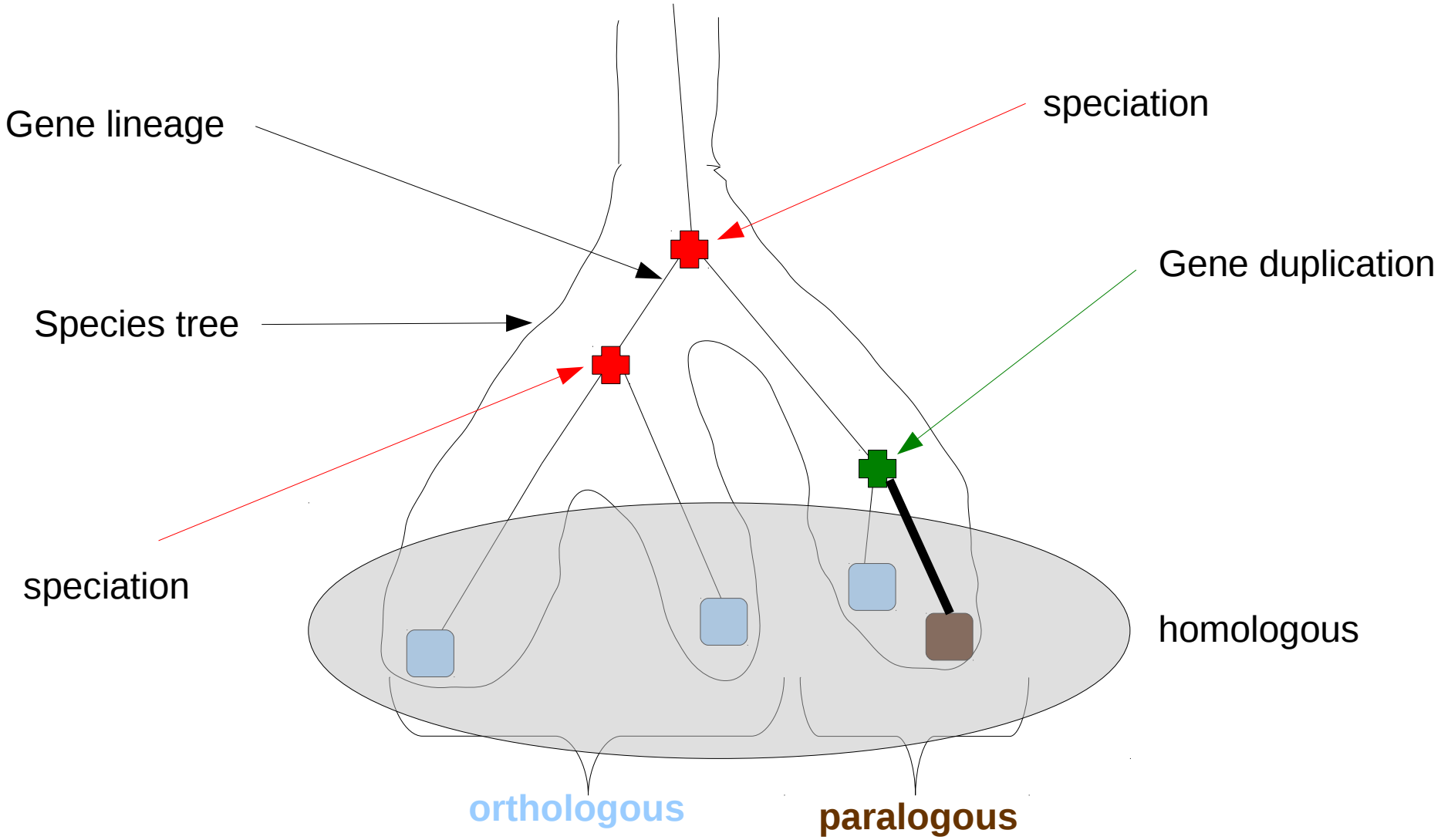
Orthology

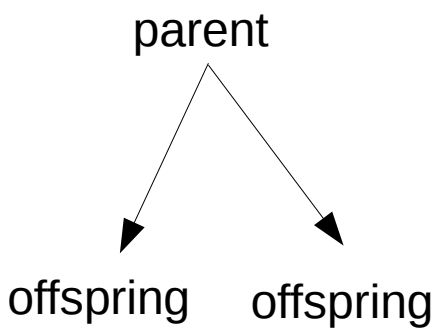

Gene lineage
Species tree
speciation
speciation
Gene duplication
orthologous
paralogous

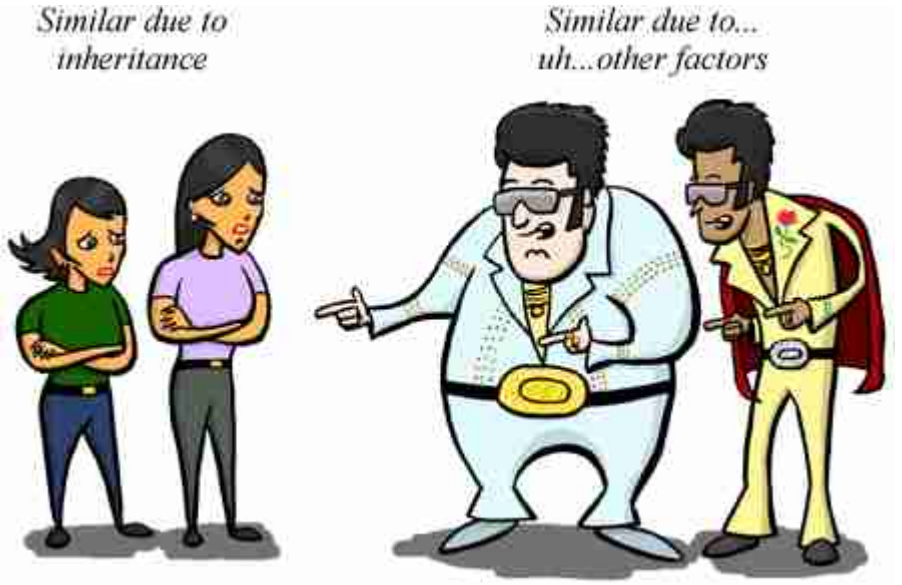
Orthology

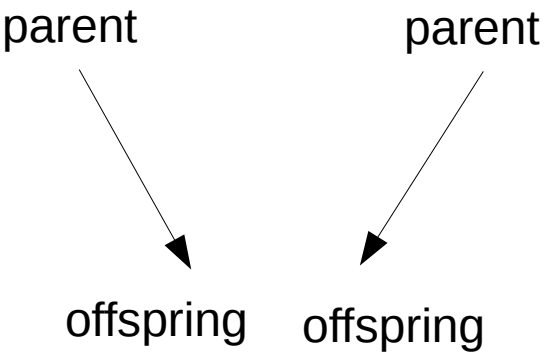

Gene lineage
speciation
Gene duplication
Species tree
speciation
homologous
orthologous
paralogous

Homology

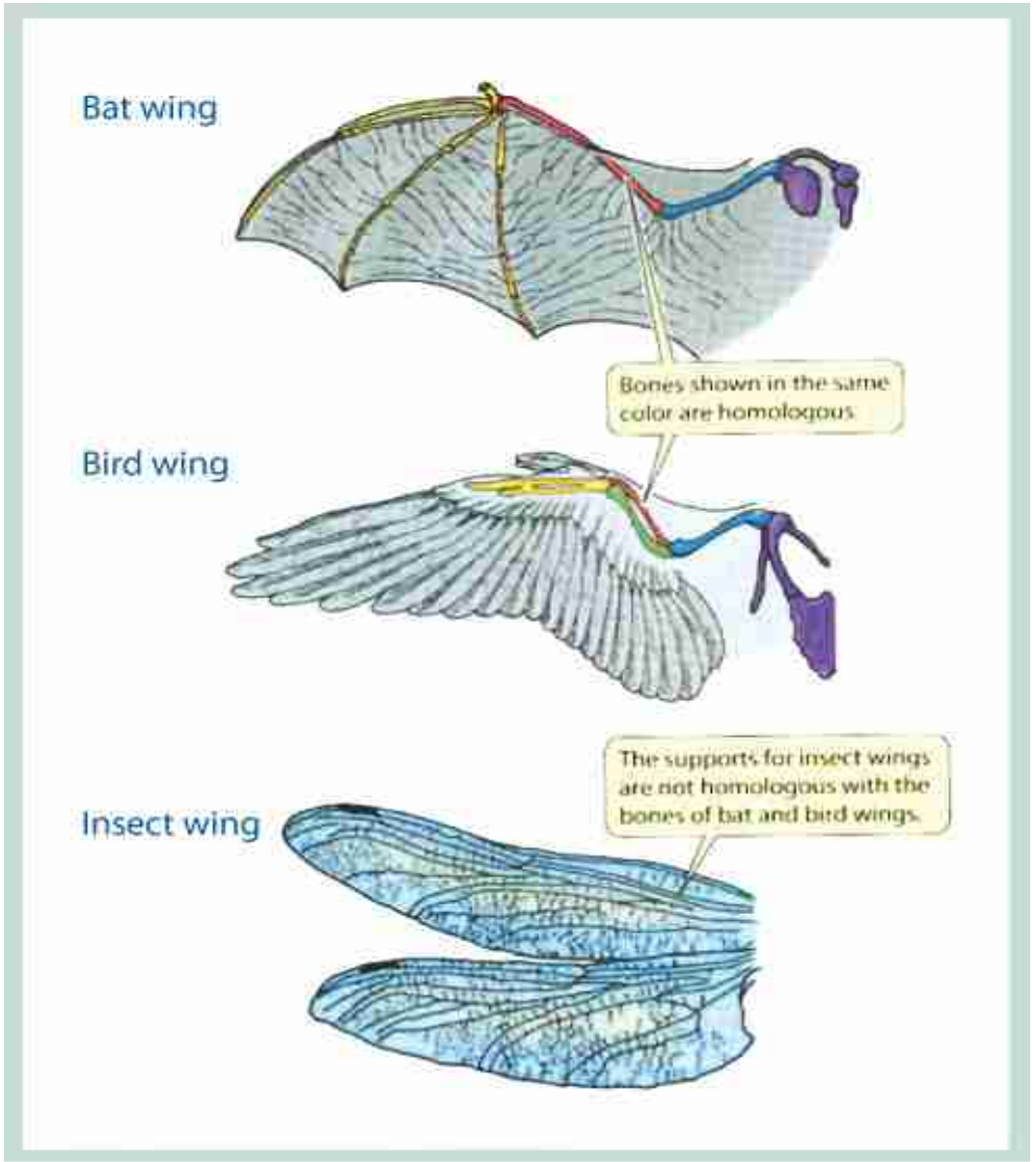
- High sequence similarity does not automatically induce homology
  - Same sequence (gene function) can have evolved independently twice → convergent evolution
  - For short sequences: similar by chance

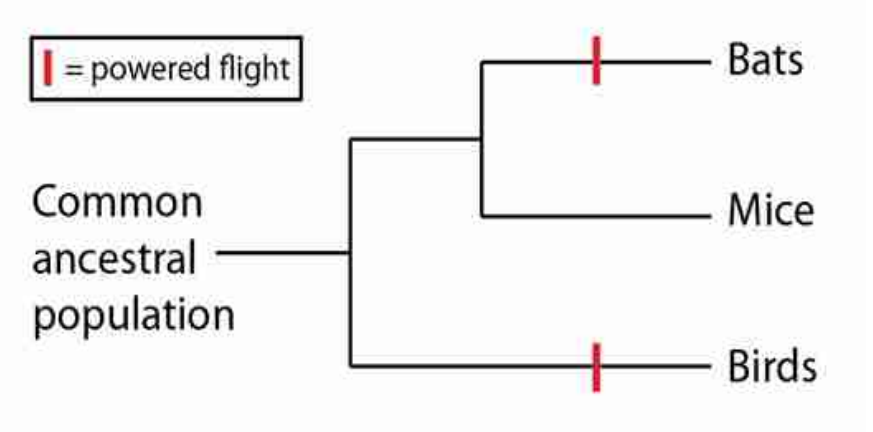






Convergent Evolution





Orthology Assignment

- Numerous methods available
- Will not be covered here → difficult problem
- Let's assume that we have a set of *n* orthologous sequences $s_1,...,s_n$ and see how we can align them

Alignment Criteria

- How do we define alignment quality?
- There are different criteria
  - The SP (sum of pairs) measure
  - Real data benchmarks
  - Curated alignments (based on protein structure)
  - Evolutionary measures
  - Simulations

Alignment Criteria

- How do we define alignment quality?
- There are different criteria
  - **The SP (sum of pairs) measure**
  - Real data benchmarks
  - Curated alignments (based on protein structure)
  - Evolutionary measures
  - Simulations

The SP measure

- **SP**: *sum-of-pairs* score
- Score each MSA site and then add up the scores over all sites
  - Penalize mismatches and gaps
  - Favor matches
  - The per-site score is defined as the sum of all pairwise scores between characters of a site

SP an example

- *SP-score(I, -, I, V)* =

  $p(I,-) + p(I, I) + p(I, V) + p(-, I) + p(-, V) + p(I, V)$

- Where *p()* is the penalty function and *p(-,-) := 0*
- Given a MSA with *n* sequences and *m* sites we can thus compute the overall score as:

```
sp = 0;
for(i = 0; i < m; i++)
  sp += SP-score(sites[i]);
```

An example

s1	A	A	G	A	A	-	A
s2	A	T	-	A	A	T	G
s3	C	T	G	-	G	-	G

Using the the edit distance for *p()* the score is:

2 + 2 + 2 + 2 + 2 + 2 + 2 = 14

Note that, we can also compute this as the sum of pair-wise edit distances between the aligned sequences:

e(s1,s2) + e(s1,s3) + e(s2,s3) = 4 + 5 + 5

Keep in mind that, *p(-,-) := 0*

The SP measure

- Note that, this is only **one way** to quantify the quality of an alignment
- One can build alignment algorithms that optimize the *SP* measure
- However, alignments (MSAs) with larger *SP* scores may better represent the true evolutionary history of the characters!

How can we extend pair-wise alignment to triple-wise alignment?

- Any ideas?
- What is the time and space complexity?

SP-based optimization

- We can extend the dynamic programming approach for pair-wise sequence alignment to *n* sequences for calculating an *SP-optimal* MSA
- Assume that all *n* sequences have equal length *m*
  - Storing the dynamic programming matrix requires $O(m^n)$ space
  - And the lower bound for time is also $O(m^n)$ because all m^n entries need to be computed → consider an example with *n:= 3*
- As you can imagine, computing the *SP-optimal* MSA is NP-complete

SP-based MSA

- NP-complete
- Not granted that *SP* is the correct (biologically most plausible) criterion!
- Depends on -arbitrary- choice of scoring function *p()*
- We need heuristics or approximation algorithms!
- We will have a look at some basic approaches now …

Star Alignment Approximation

- Pick a center sequence s_c
- Align all remaining sequences to s_c using a pairwise sequence alignment algorithm
- “Once a gap, always a gap” strategy

  → gaps inserted into s_c can not be removed again
- s_c can be picked by computing all $O(n^2)$ [more precisely: $(n^2 / 2) - n]$ optimal pair-wise alignments and selecting *the* sequence that has the largest similarity to all other sequences

Star Alignment

```
s1: ATTGCCATT
s2: ATGGCCATT
s3: ATCCAATTTT
s4: ATCTTCTT
s5: ACTGACC
```

Star Alignment

```
s1: ATTGCCATT ← center sequence
s2: ATGGCCATT
s3: ATCCAATTTT
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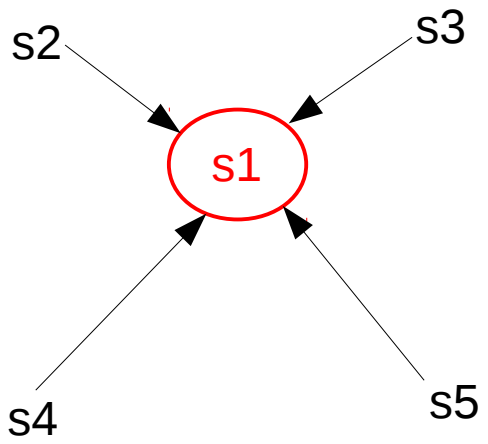
```

Star Alignment



Star Alignment

```
s1: ATTGCCATT
s2: ATGGCCATT

s1: ATTGCCATT--
s3: ATC-CAATTTT

s1: ATTGCCATT
s4: ATCTTC-TT

s1: ATTGCCATT
s5: ACTGACC--
```

Star Alignment

s1: ATTGCCATT
s2: ATGGCCATT

s1: ATTGCCATT--
s3: ATC-CAATTTT

s1: ATTGCCATT--
s4: ATCTTC-TT--

s1: ATTGCCATT--
s5: ACTGACC----

Gaps inserted

“Once a gap, always a gap”

The Star Alignment

```
s1: ATTGCCATT--
s2: ATGGCCATT--
s3: ATC-CAATTTT
s4: ATCTTC-TT--
s5: ACTGACC----
```

Another Example

s1:ATTGCCATT

s2:ATGGCCATT

s3:ATCCAATTTT

s4:ATCTTCTT

s5:ATTGCCGATT

Another Example

s1:ATTGCCATT

s2:ATGGCCATT

s1:ATTGCCATT--

s3:AT-CCAATTTT

s1:ATTGCCATT

s4:ATCTTC-TT

s1:ATTGCC-ATT

s5:ATTGCCGATT

Another Example

s1:ATTGCCATT

s2:ATGGCCATT

s1:ATTGCCATT--

s3:AT-CCAATTTT

s1:ATTGCCATT

s4:ATCTTC-TT

s1:ATTGCC-ATT

s5:ATTGCCGATT

s1:ATTGCCATT

s2:ATGGCCATT

Another Example

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s1:ATTGCCATT
s2:ATGGCCATT

s1:ATTGCCATT--
s2:ATGGCCATT--
s3:AT-CCAATTTT

Another Example

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s2:ATGGCCATT

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s2:ATGGCCATT

s1:ATTGCCATT--
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s3:AT-CCAATTTT

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```

```
s1:ATTGCCATT
s2:ATGGCCATT

s1:ATTGCCATT--
s2:ATGGCCATT--
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s1:ATTGCCATT--
S2:ATGGCCATT--
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s4:ATCTTC-TT--

s1:ATTGCC-ATT--
S2:ATGGCC-ATT--
S3:AT-CCA-ATTTT
s4:ATCTTC--TT--
s5:ATTGCCGATT--
```

Shift right!

Pairwise alignment step

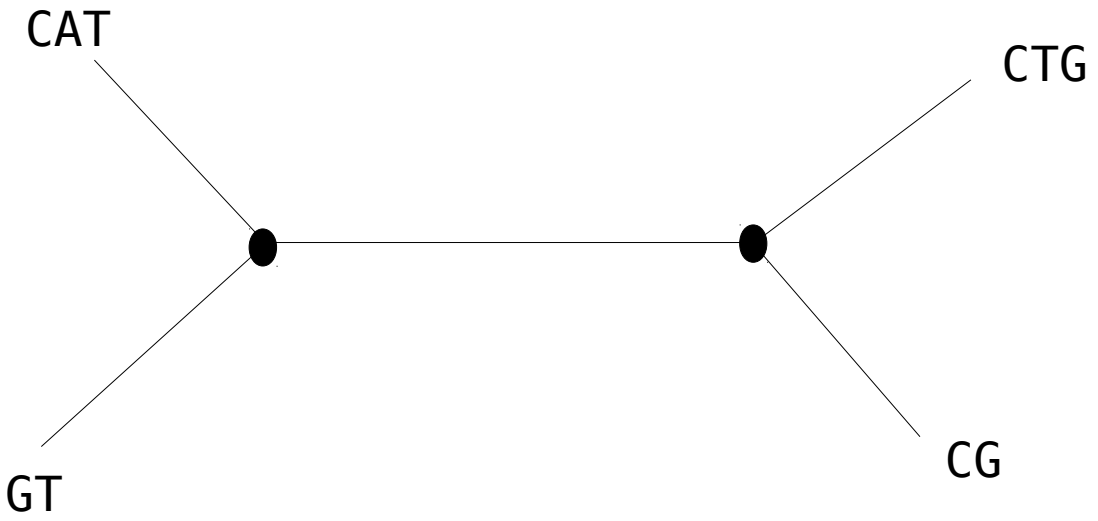
Merging step

Star Alignment Approximation

- Produces an MSA whose *SP* score is < *2 * optimum*
- Proof omitted
- Reference: D. Gusfield “Efficient methods for multiple sequence alignment with guaranteed error bounds”, *Bulletin of Mathematical Biology*, 1993.

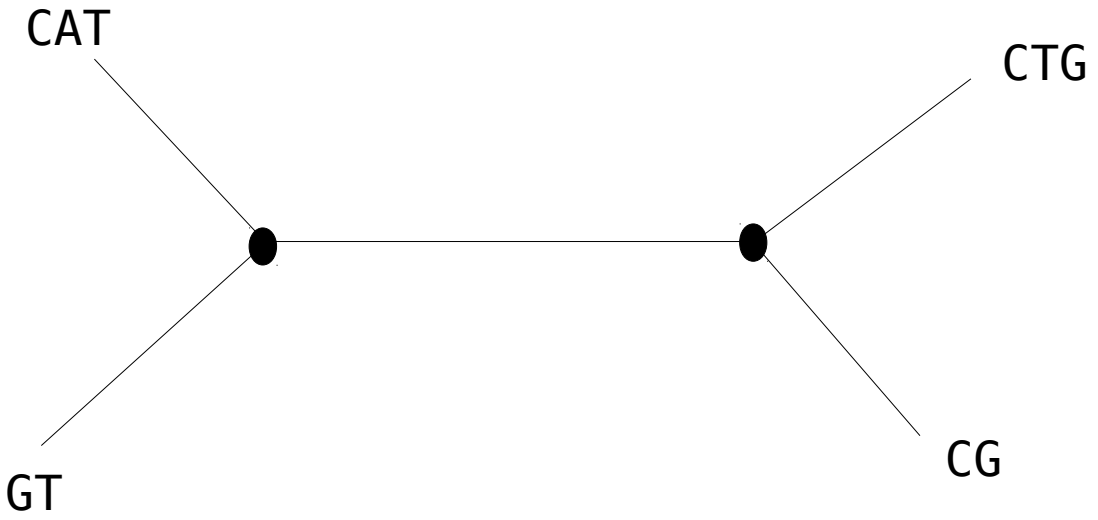
Tree Alignment

- If an evolutionary tree for the sequences is available



Tree Alignment

- Find an assignment of sequences to the inner nodes such that the sum over the similarity scores on all branches is maximized

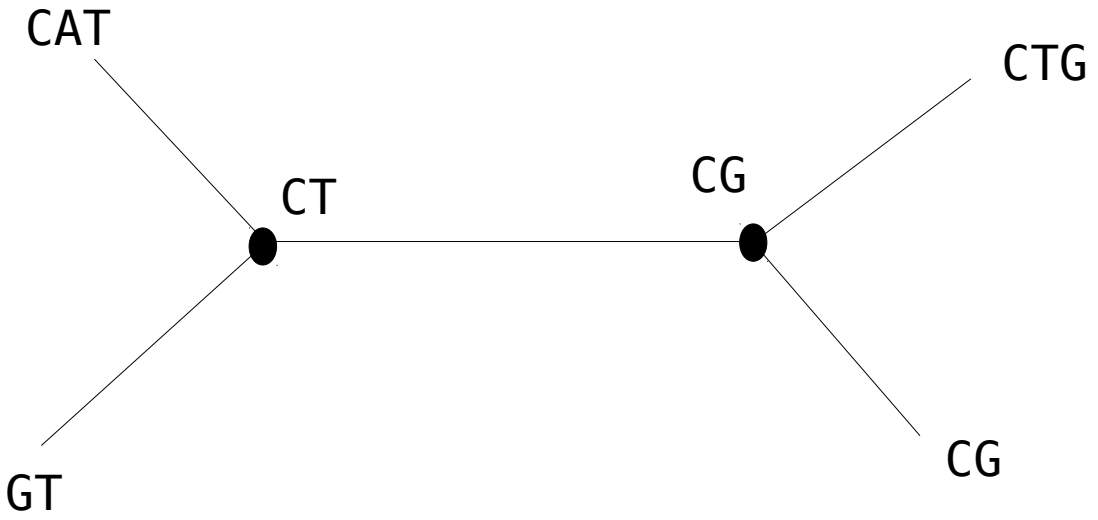


Tree Alignment

p(a,b) := 1 if a = b

p(a,b) := 0 if a ≠b

p(a,-) := -1



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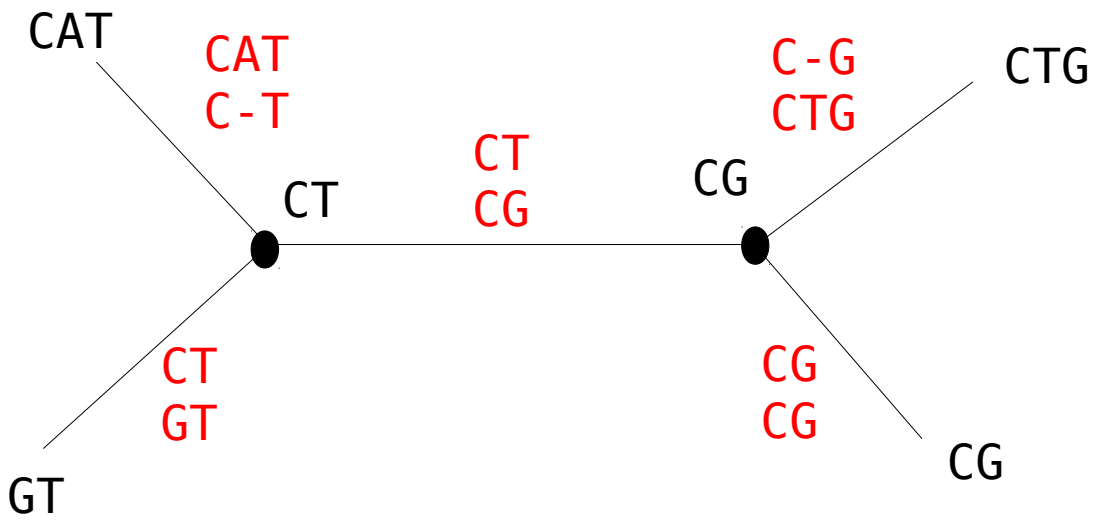

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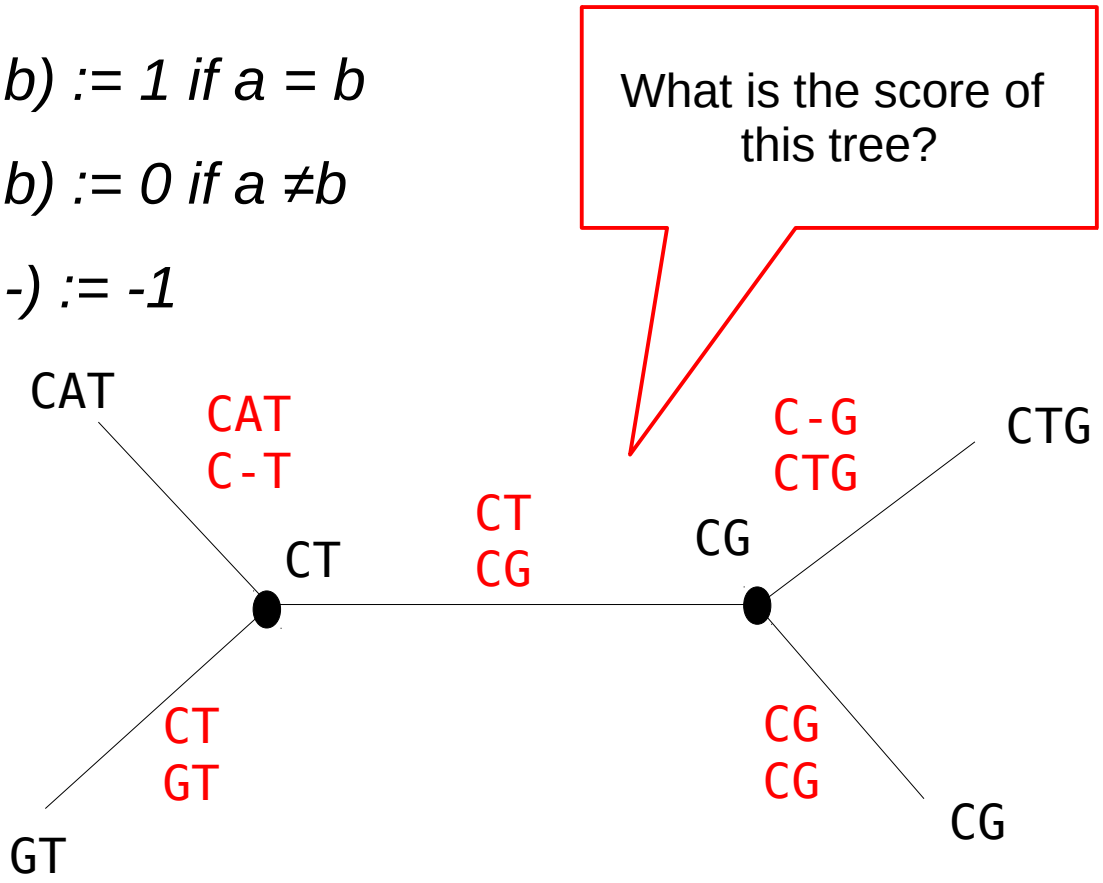

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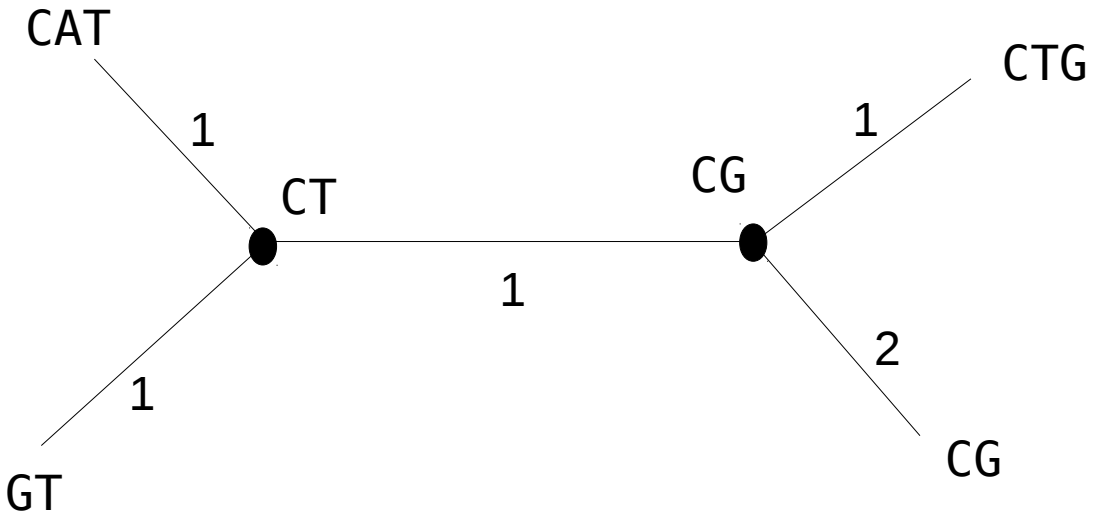

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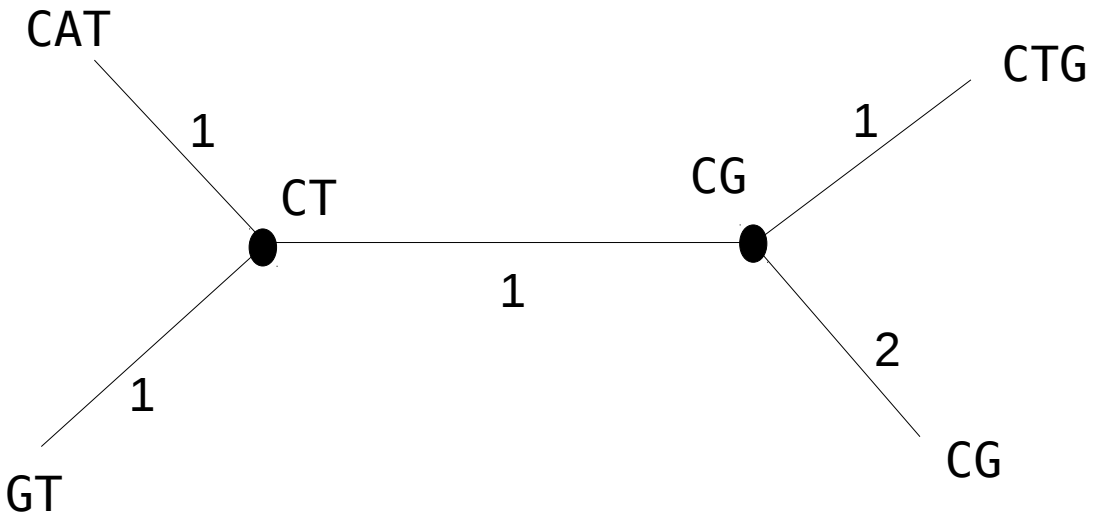

Overall score: 6 → maximize this score

Tree Alignment

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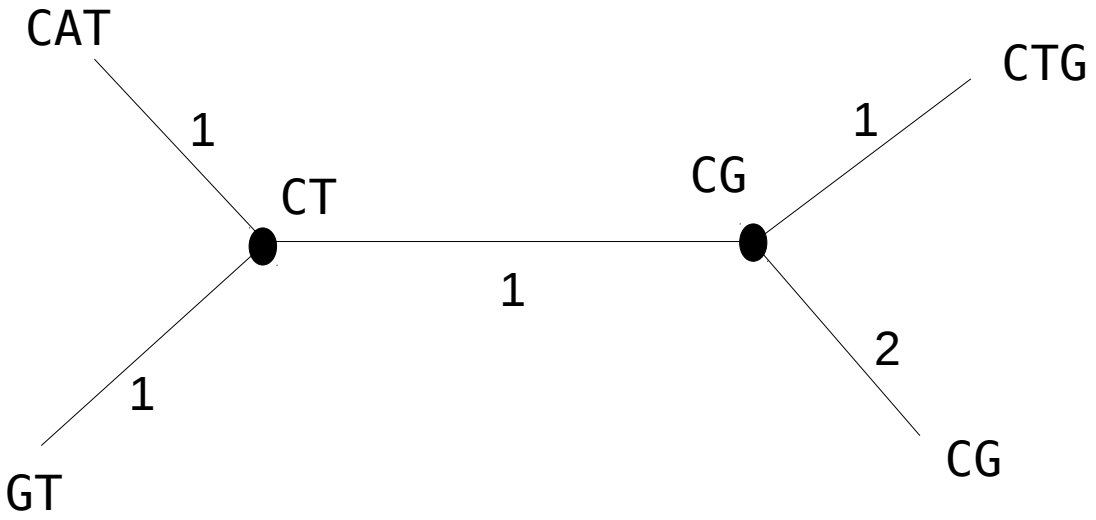


Overall score: 6 → maximize this score

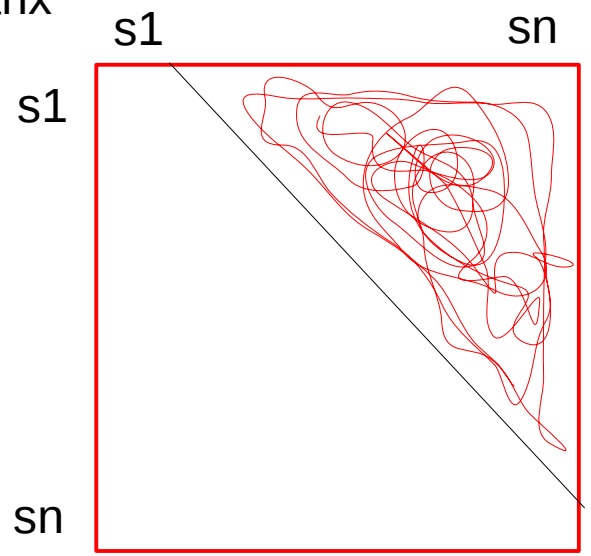
This problem is NP-hard because we don't have the ancestral states

Tree-Based Alignment

- Hen and egg problem
  → we need a MSA to build a tree
  → we need a tree to compute a MSA
  → if the alignment is wrong, the tree might be wrong
  → if the tree is wrong, the MSA might be wrong
- One idea
  → simultaneous inference of tree & alignment
  → very hard problem: trying to solve two generally NP-hard or NP-complete problems simultaneously

Practical approaches

Build a pair-wise distance matrix



Practical approaches

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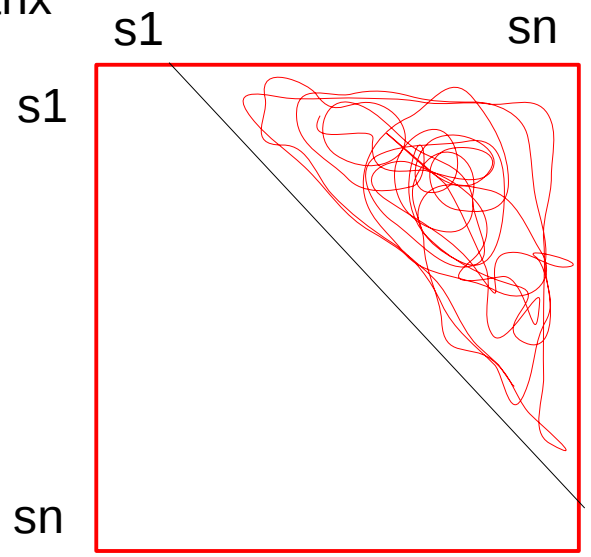
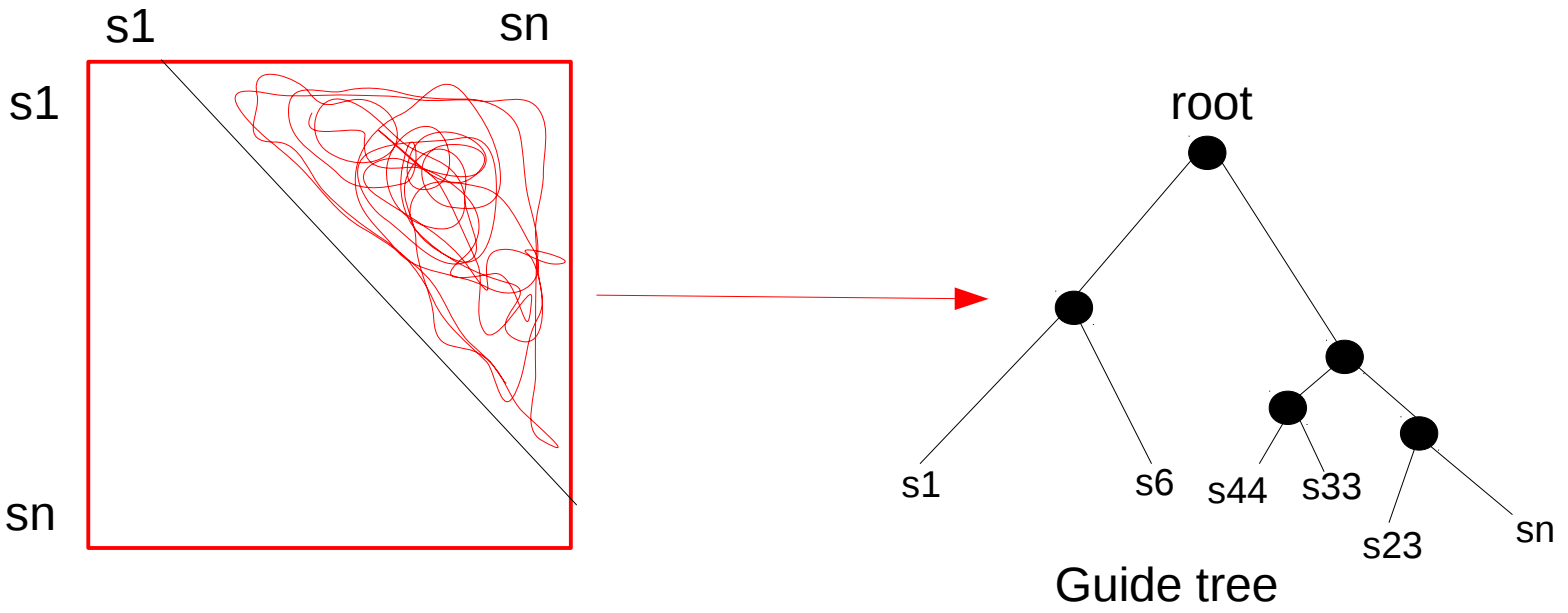
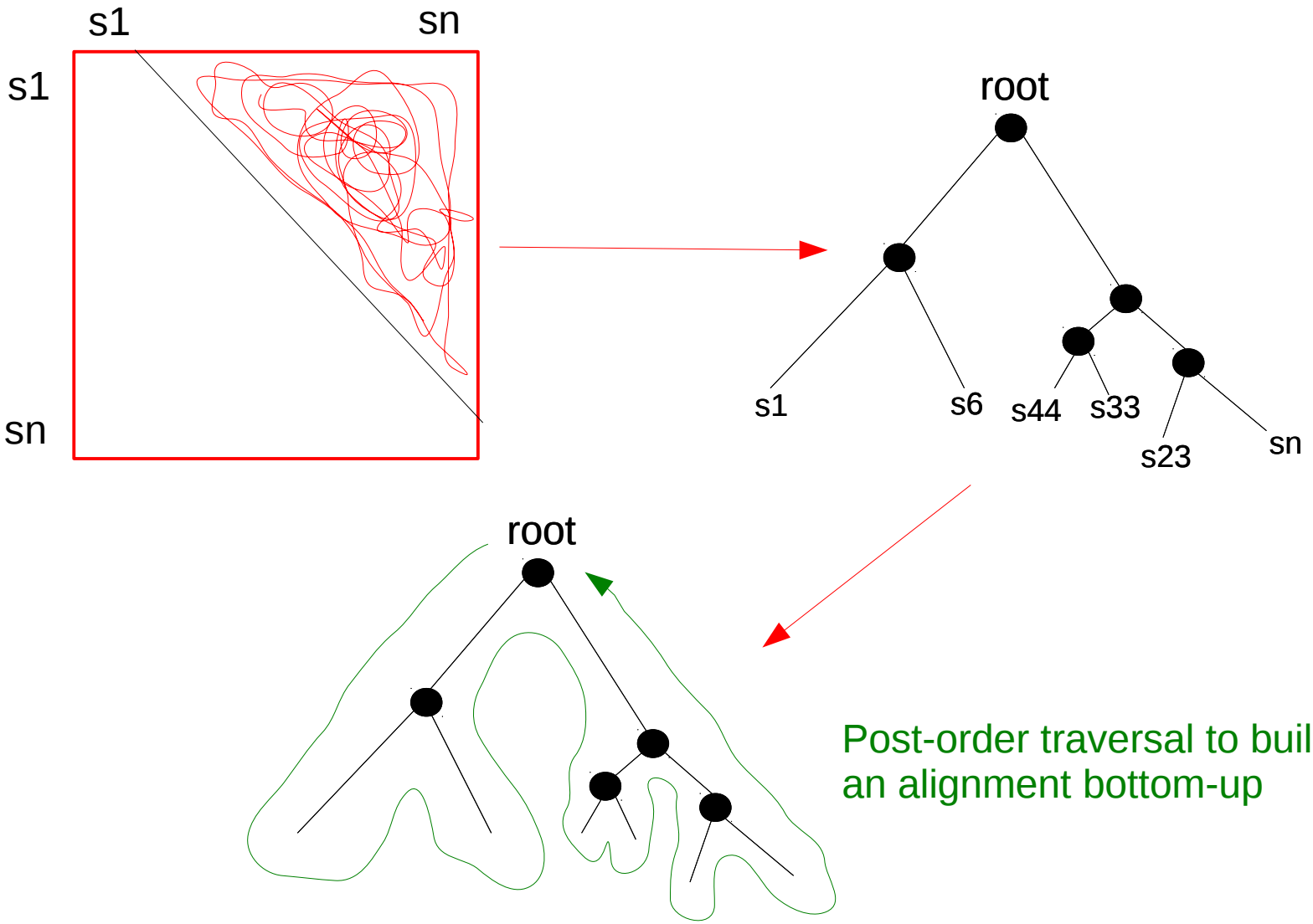


Computation of pair-wise distance matrix
Using pair-wise alignment scores can be time and memory-intensive due to $O(n^2)$ complexity
One may use approximate distance methods based on *k-mers*
(remember last lecture!)

Practical approaches

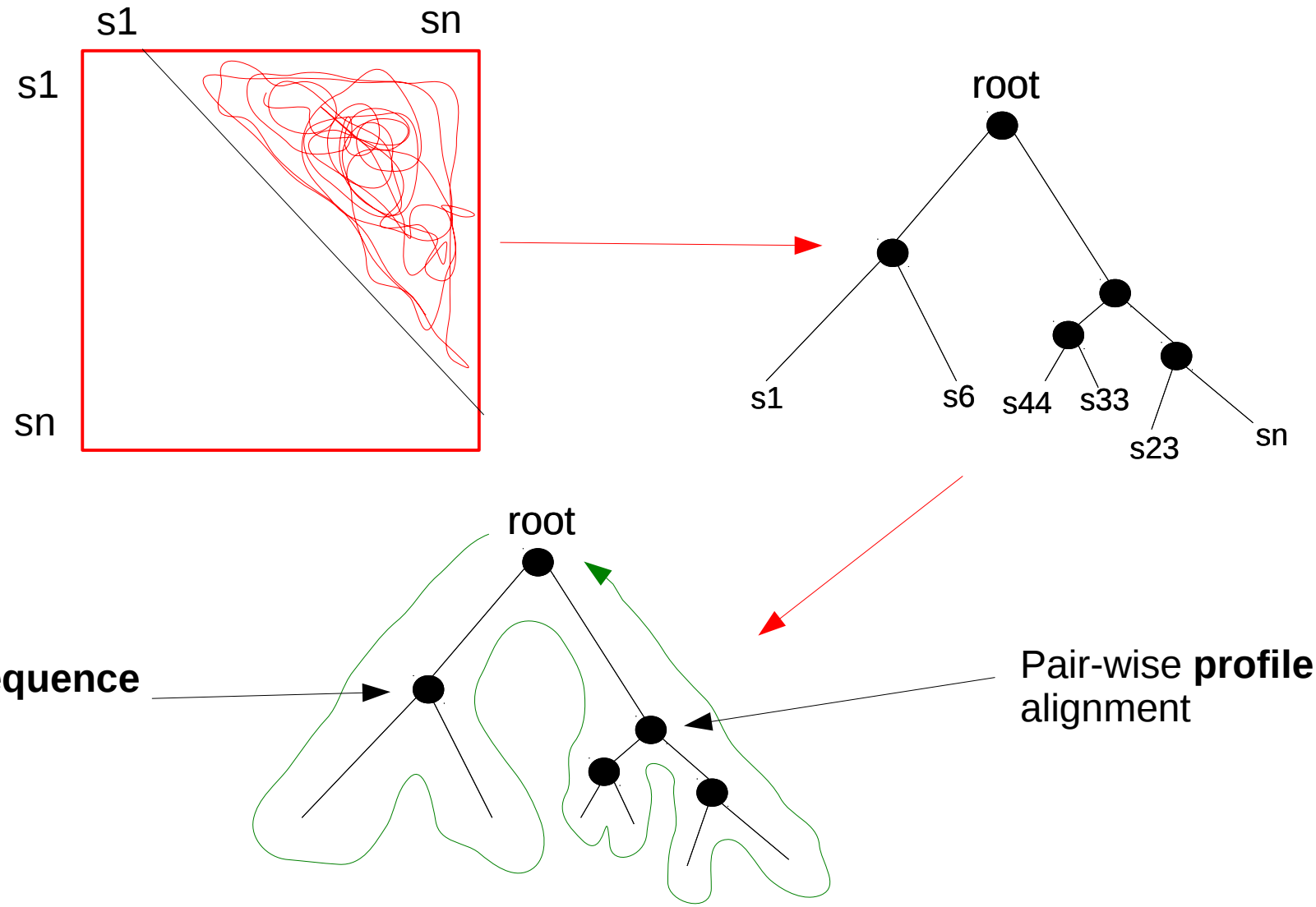


Practical approaches



Post-order traversal to build an alignment bottom-up

Practical approaches



Practical Approaches

- Guide-tree approach
- Compute all $(n^2/2)-n$ pair-wise distances (alignments) between the n sequences
- Use these distances for hierarchical clustering
  - e.g. with the Neighbor Joining (NJ) algorithm → we will see this later-on for tree building
- Use the distance-based tree to calculate pair-wise
  - Sequence-sequence
  - Sequence-profile
  - Profile-profile

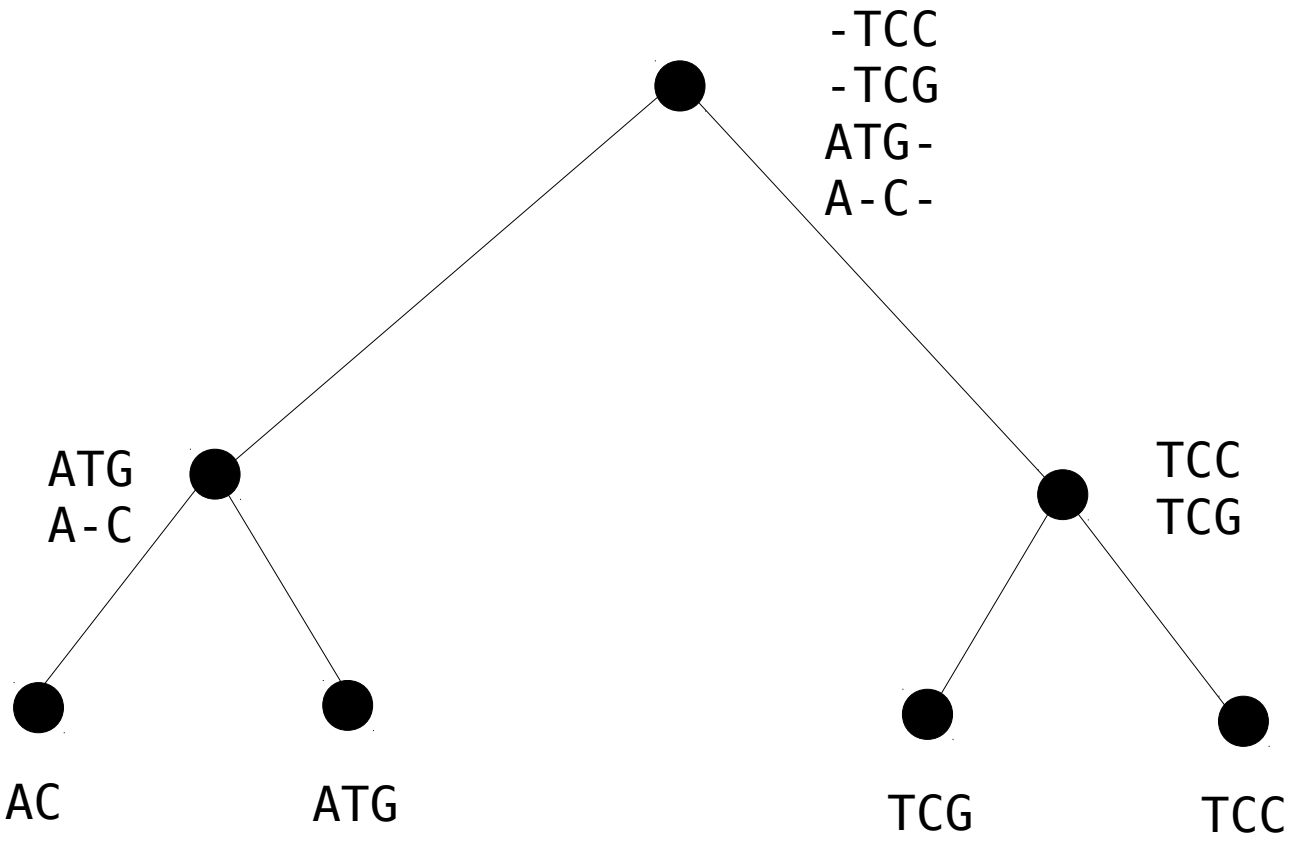
  … alignments bottom up toward the root via a post-order tree traversal
- Many widely-used MSA programs rely on this idea: e.g., **Clustal** family of tools**, T-COFFEE, MUSCLE**

Progressive MSA

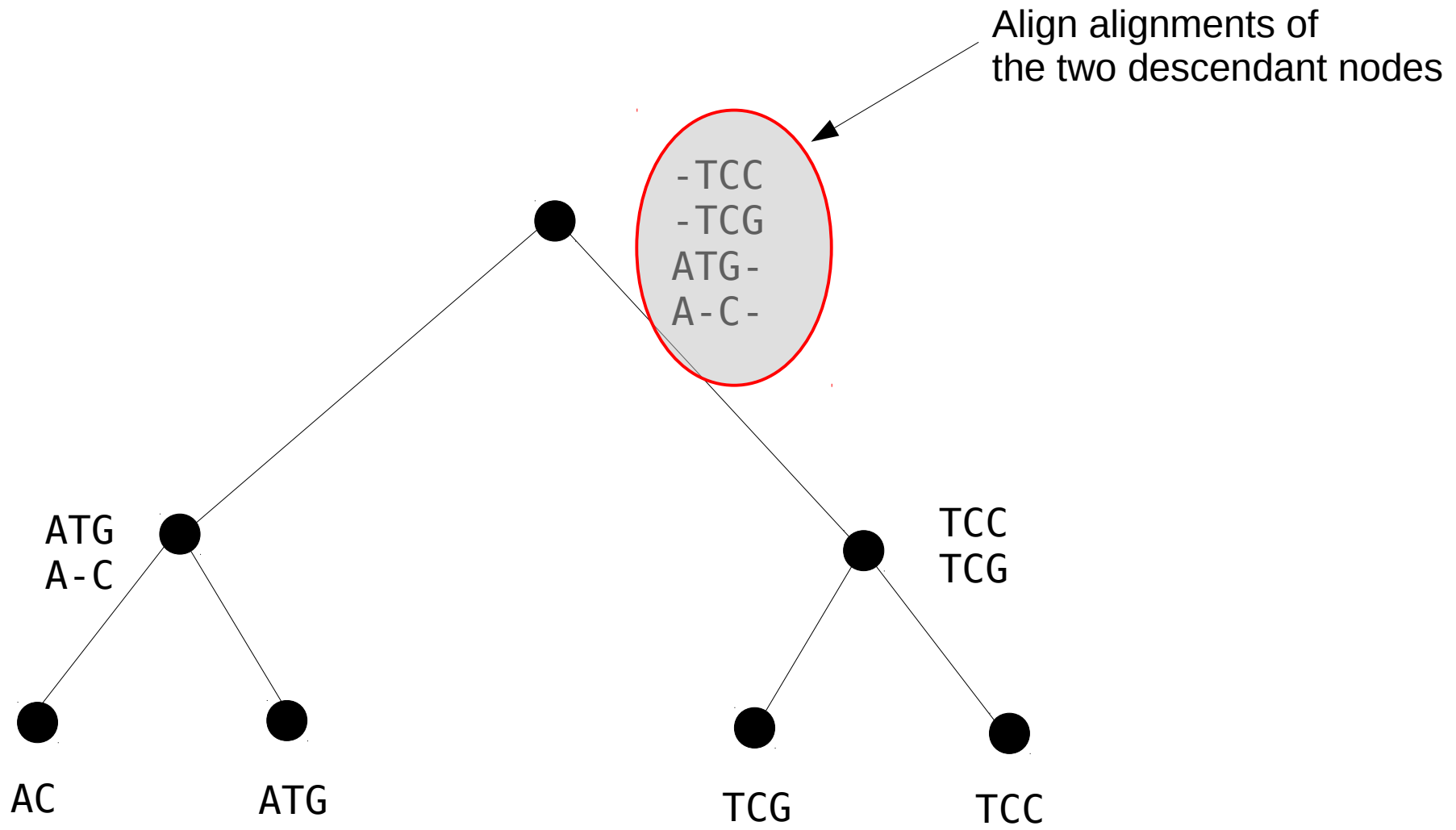
Progressive MSA

Progressive MSA

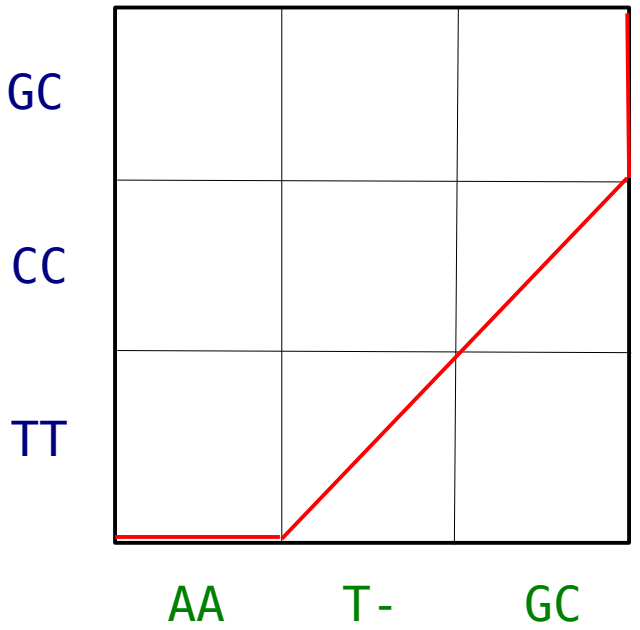
Progressive MSA



Progressive MSA



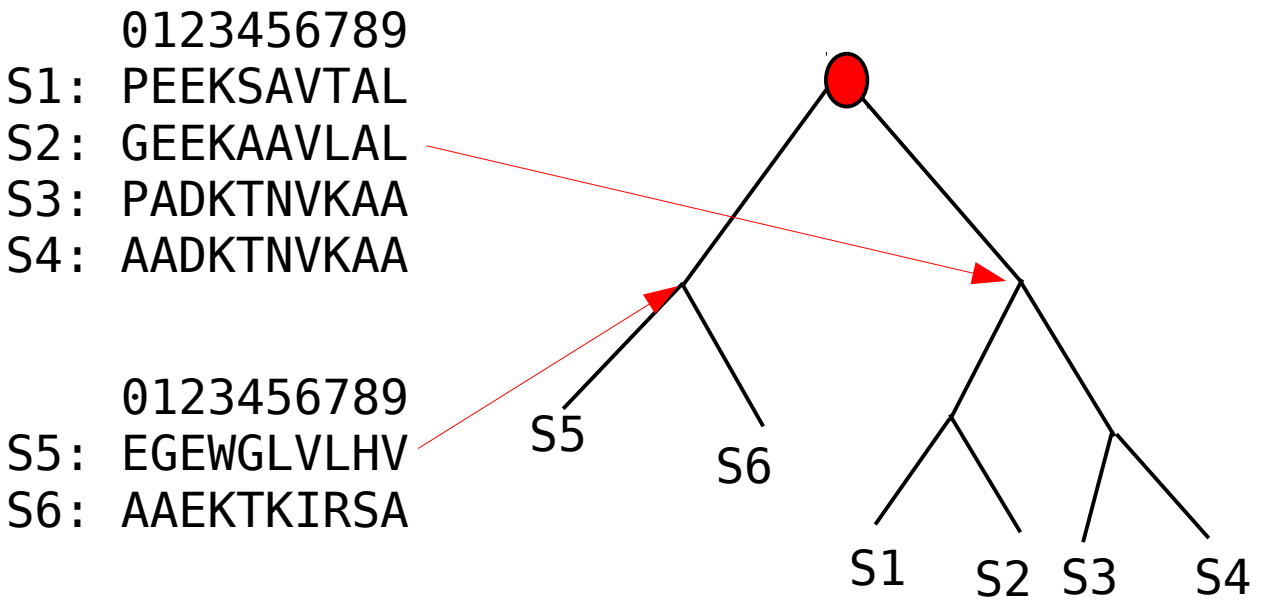
Profile Alignment



-TCC
-TCG
ATG-
A-C-

Profile Alignment

- Generalization of pair-wise sequence alignment to pair-wise profile alignment
- Average over all possibilities

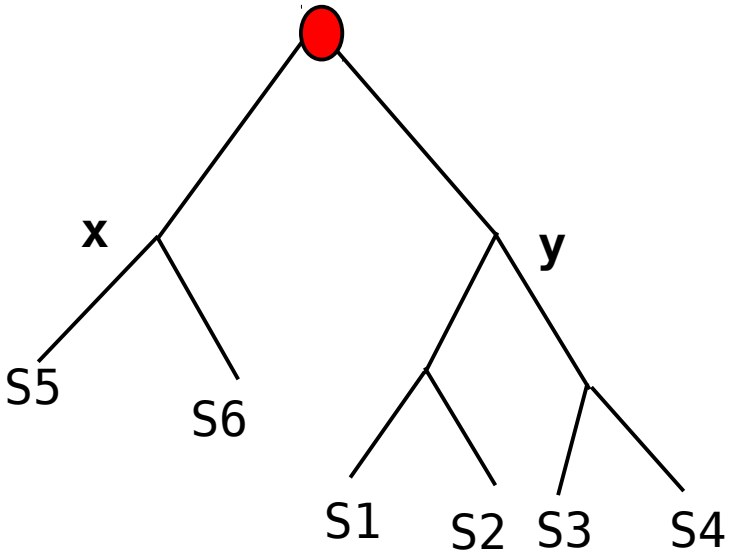


Profile Alignment

- Generalization of pair-wise sequence alignment to pair-wise profile alignment
- Average over all possibilities

```
    0123456789
S1: PEEKSAVTAL
S2: GEEKAAVLAL
S3: PADKTNVKAA
S4: AADKTNVKAA

    0123456789
S5: EGEWGLVLHV
S6: AAEKTKIRSA
```



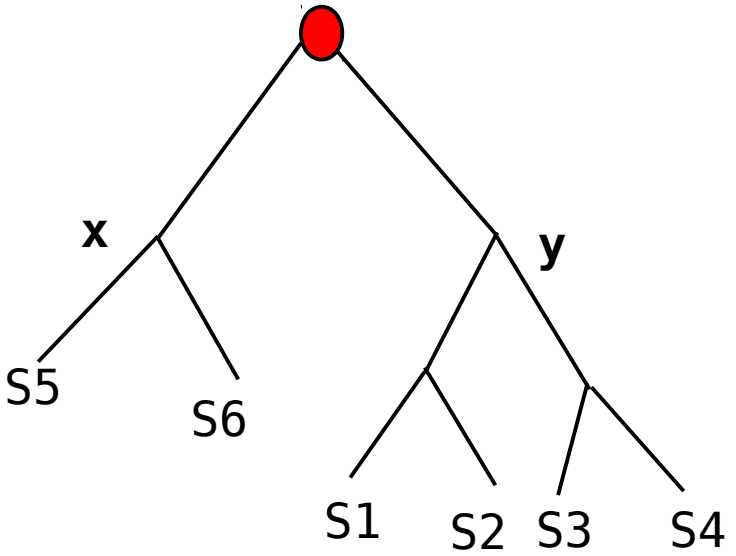
Compute score between position 6 of **x** and position 7 of **y**

Profile Alignment

- Generalization of pair-wise sequence alignment to pair-wise profile alignment
- Average over all possibilities

```
    0123456789
S1: PEEKSAVTAL
S2: GEEKAAVLAL
S3: PADKTNVKAA
S4: AADKTNVKAA

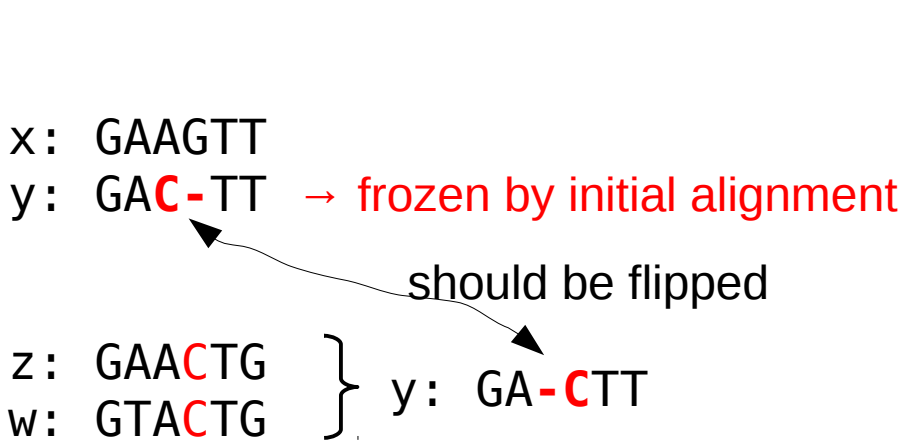
    0123456789
S5: EGEWGLVLHV
S6: AAEKTKIRSA
```

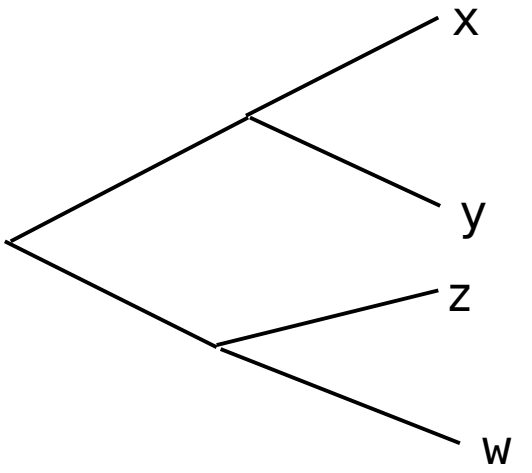


Weighted average over all *8 (2 * 4)* possibilities:
Score: *1/8 * [p(T,V) + p(T,I) + p(L, V) + p(L, I) + p(K,V) + p(K,I) + p(K,V) + p(K,I)]*

Problems with progressive MSA

- Initial pair-wise alignments are “frozen”
- Can't be corrected when new evidence emerges

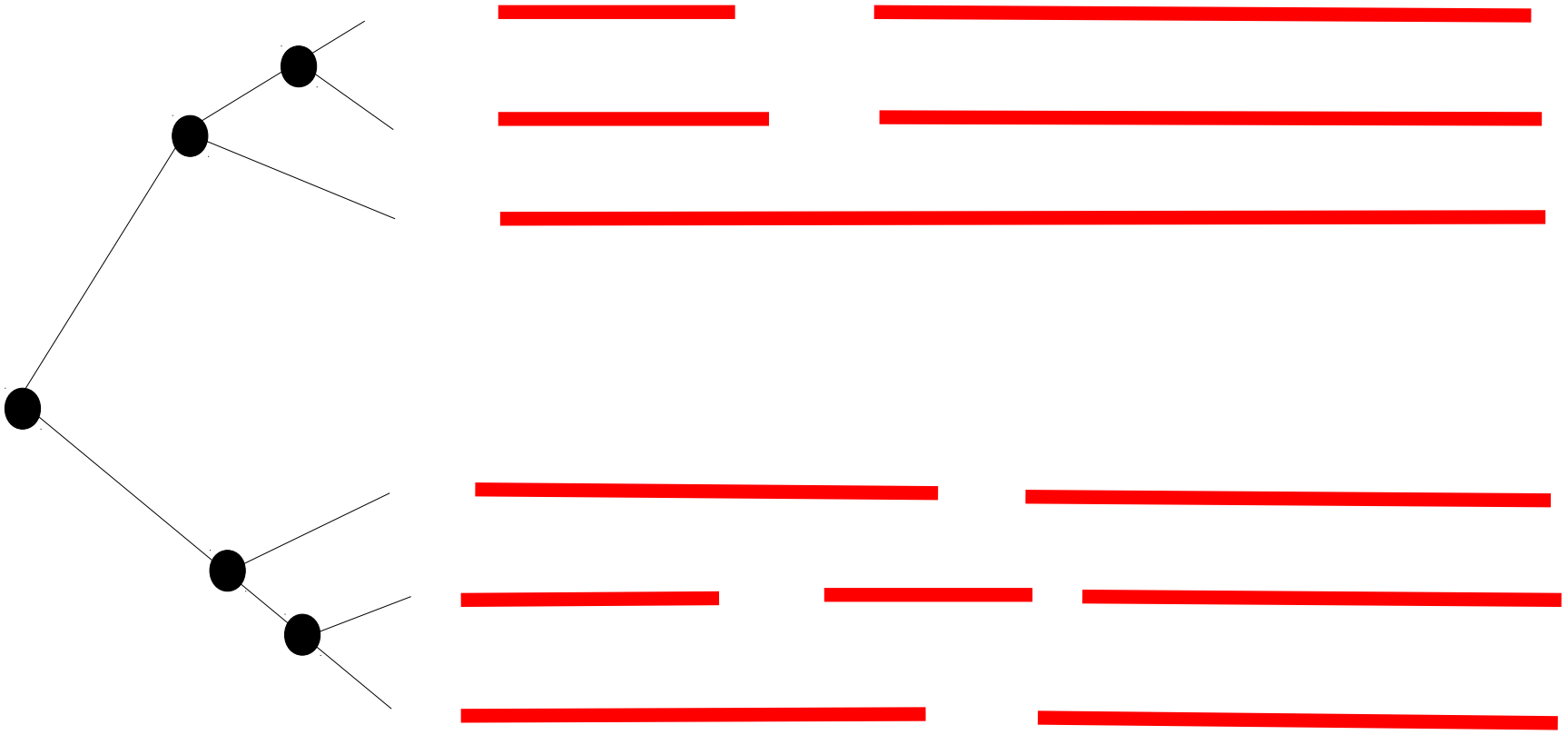




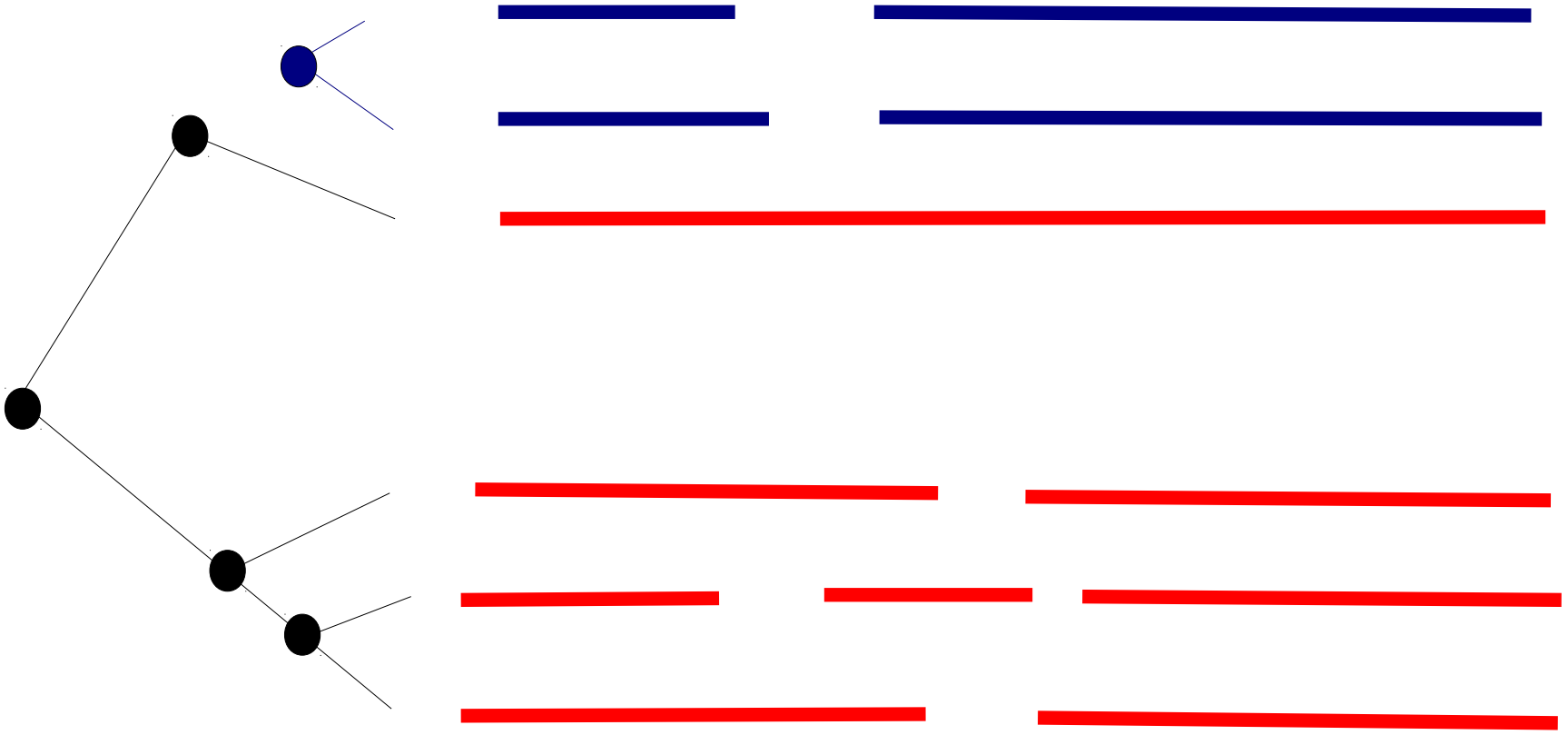
Iterative Progressive MSA

- e.g. MUSCLE, PRRP, MAFFT
- Execute progressive MSA several times to re-fine the alignment

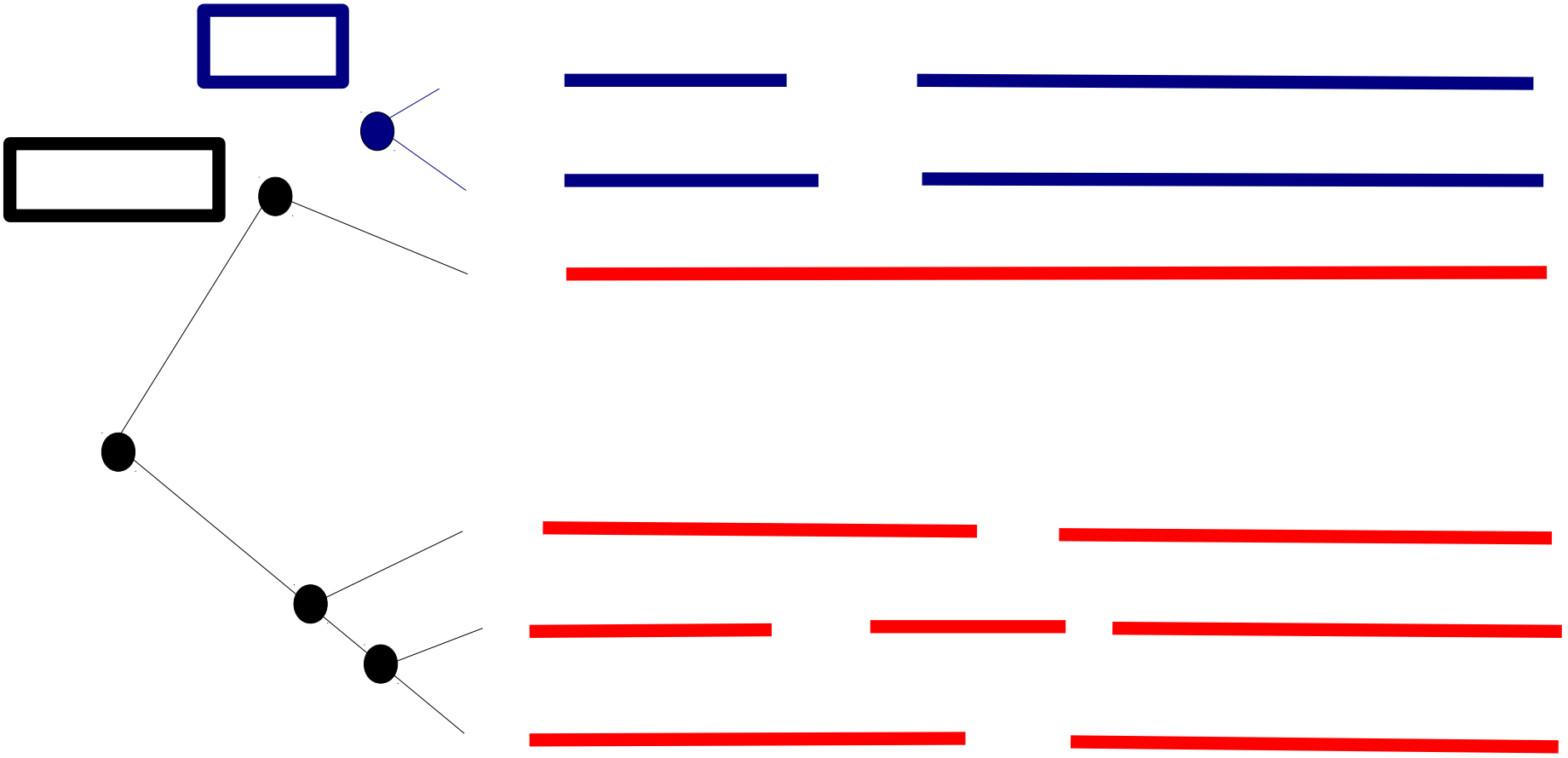
MUSCLE Re-Finement



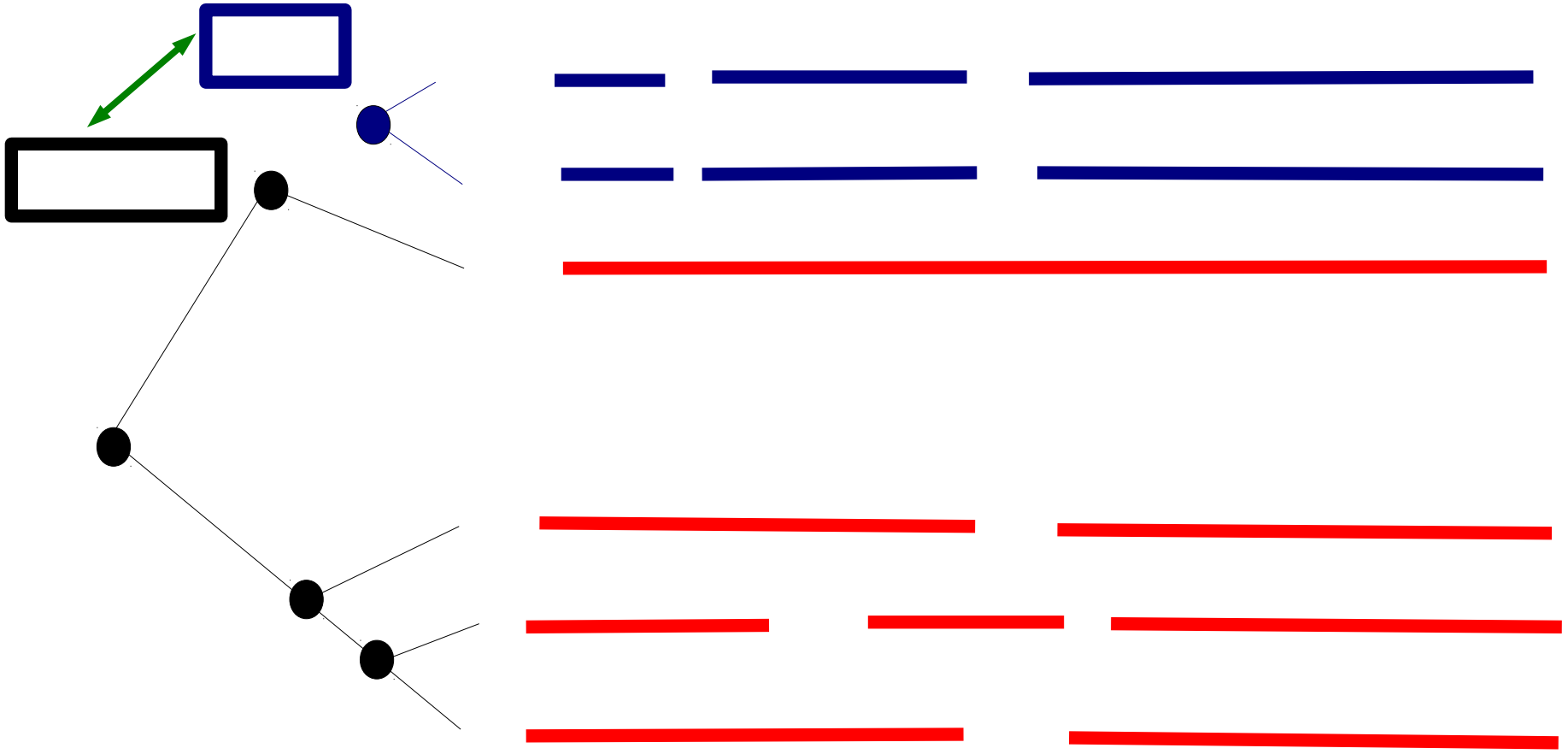
MUSCLE Re-Finement



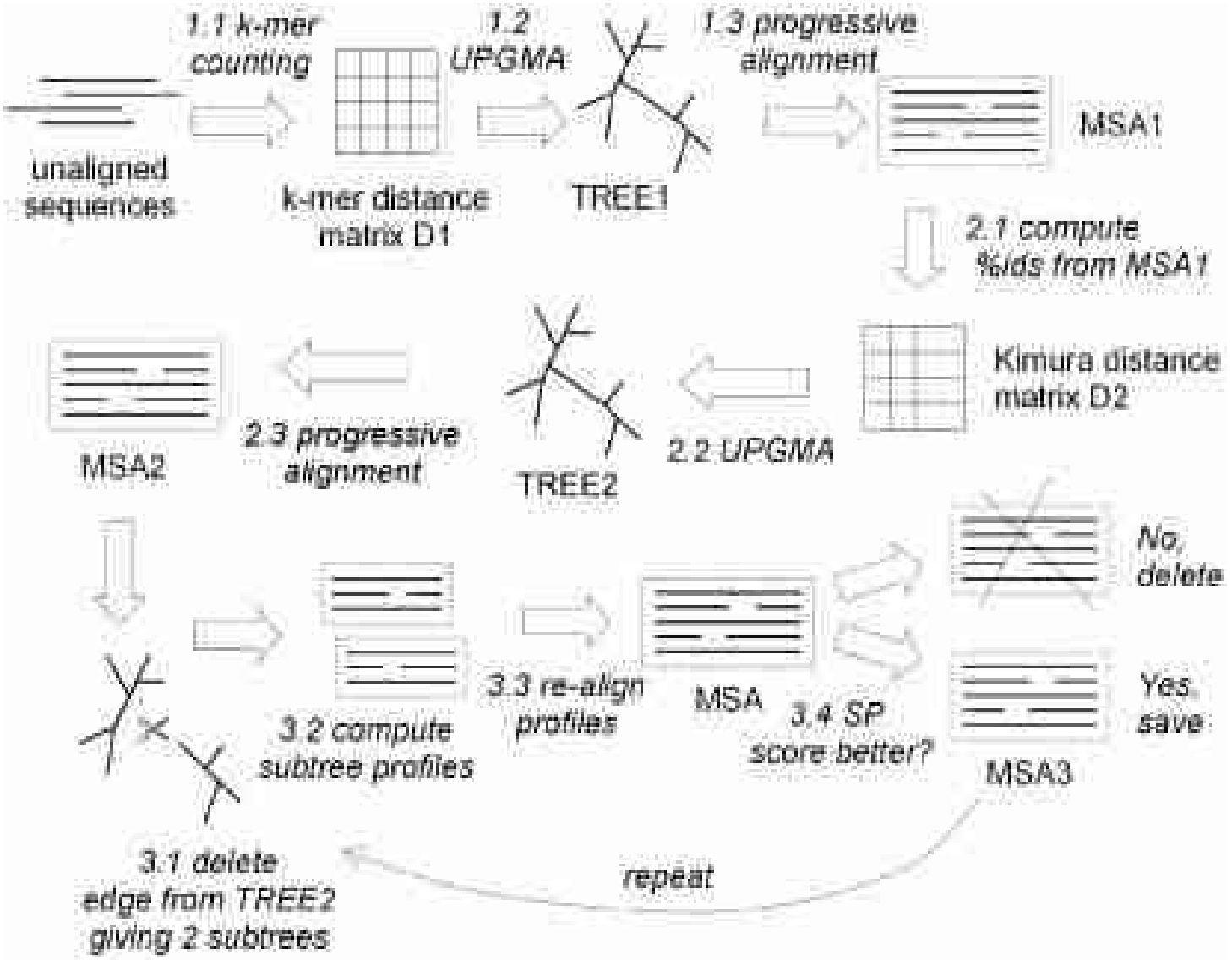
MUSCLE Re-Finement



MUSCLE Re-Finement



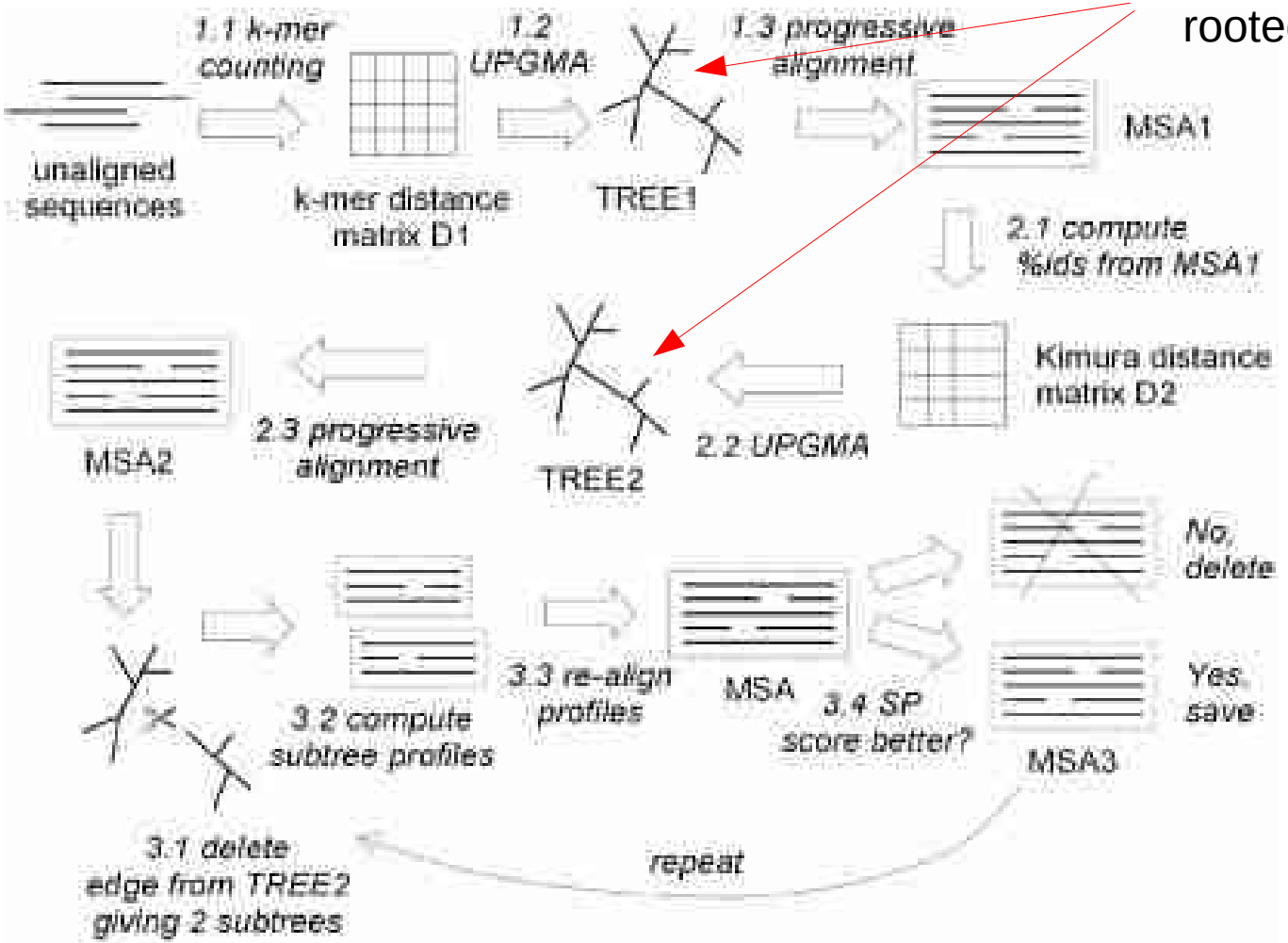
MUSCLE Details



From: “MUSCLE: multiple sequence alignment with high accuracy and high throughput”

MUSCLE Details

These trees are rooted!



From: “MUSCLE: multiple sequence alignment with high accuracy and high throughput”

MUSCLE Refinement

1. TREE2 is divided into two subtrees by deleting the edge. The profile of the multiple alignment in each subtree is computed.
2. A new multiple alignment is produced by re-aligning the two profiles.
3. An edge/branch is chosen from *TREE2* (edges are visited in order of decreasing distance from the root)
4. If the *SP* score is improved, the new alignment is kept, otherwise it is discarded.
5. Steps 1. - 4. are repeated until convergence or until a user-defined limit is reached.

Motif-based approaches

- Find a small motif (substring) common to all sequences
- Called: anchor, block, region, q-gram *etc*
- If motif is found → shift sequences such that the motifs are “in alignment”
- Then, align regions around these motifs using for instance progressive alignment

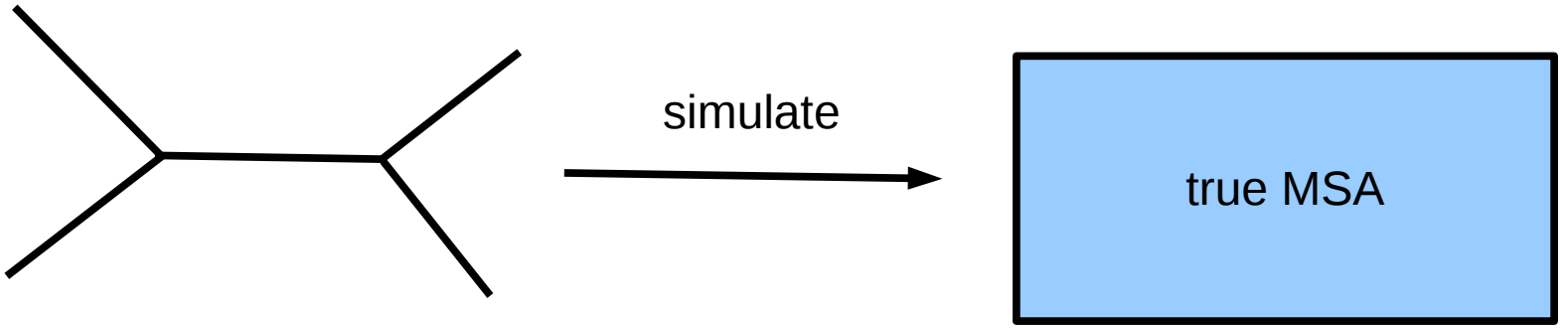
Benchmarking MSAs

- MSA benchmarks → mostly structural protein data that has been manually aligned to reflect the protein structure
  - Databases: BALiBASE 2.0, OXBench, PREFAB, etc
- Simulation

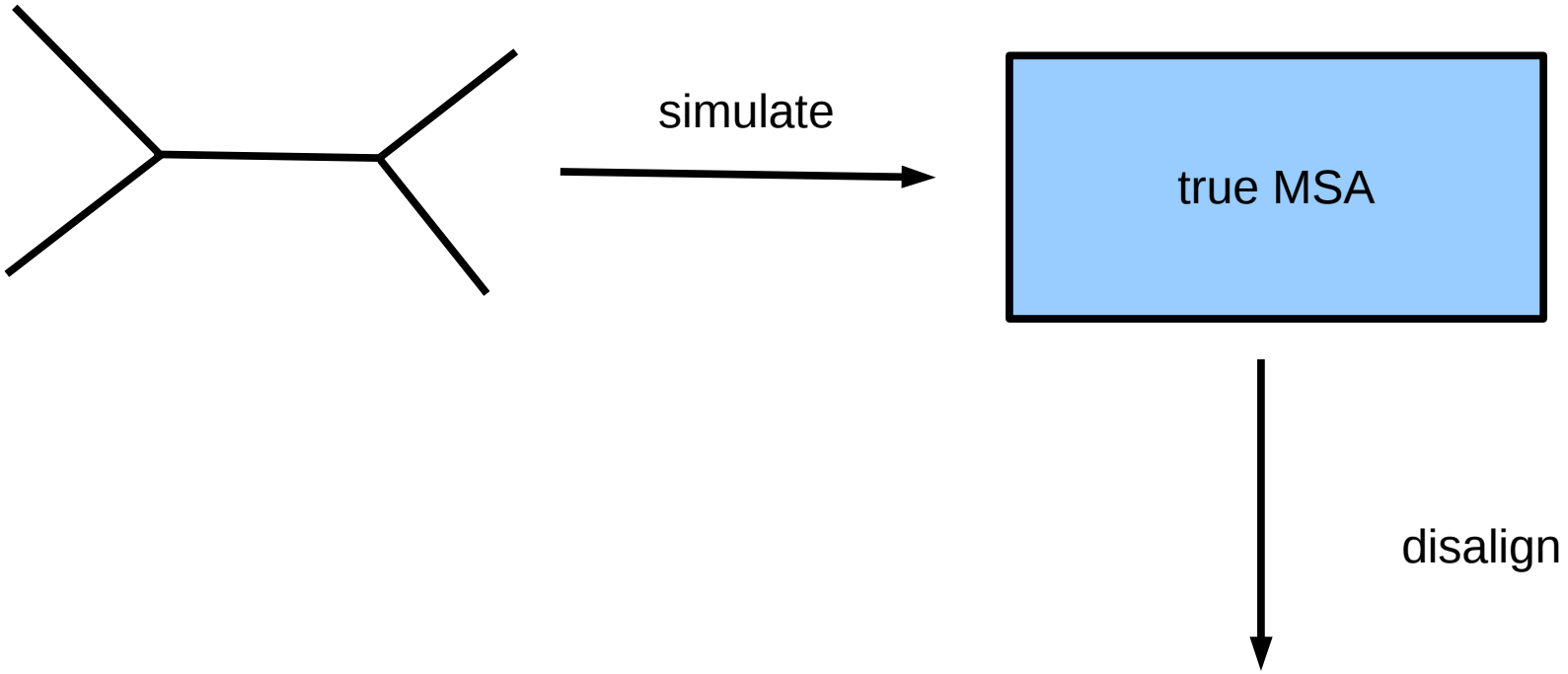
  → focus on alignment

  → focus on phylogeny

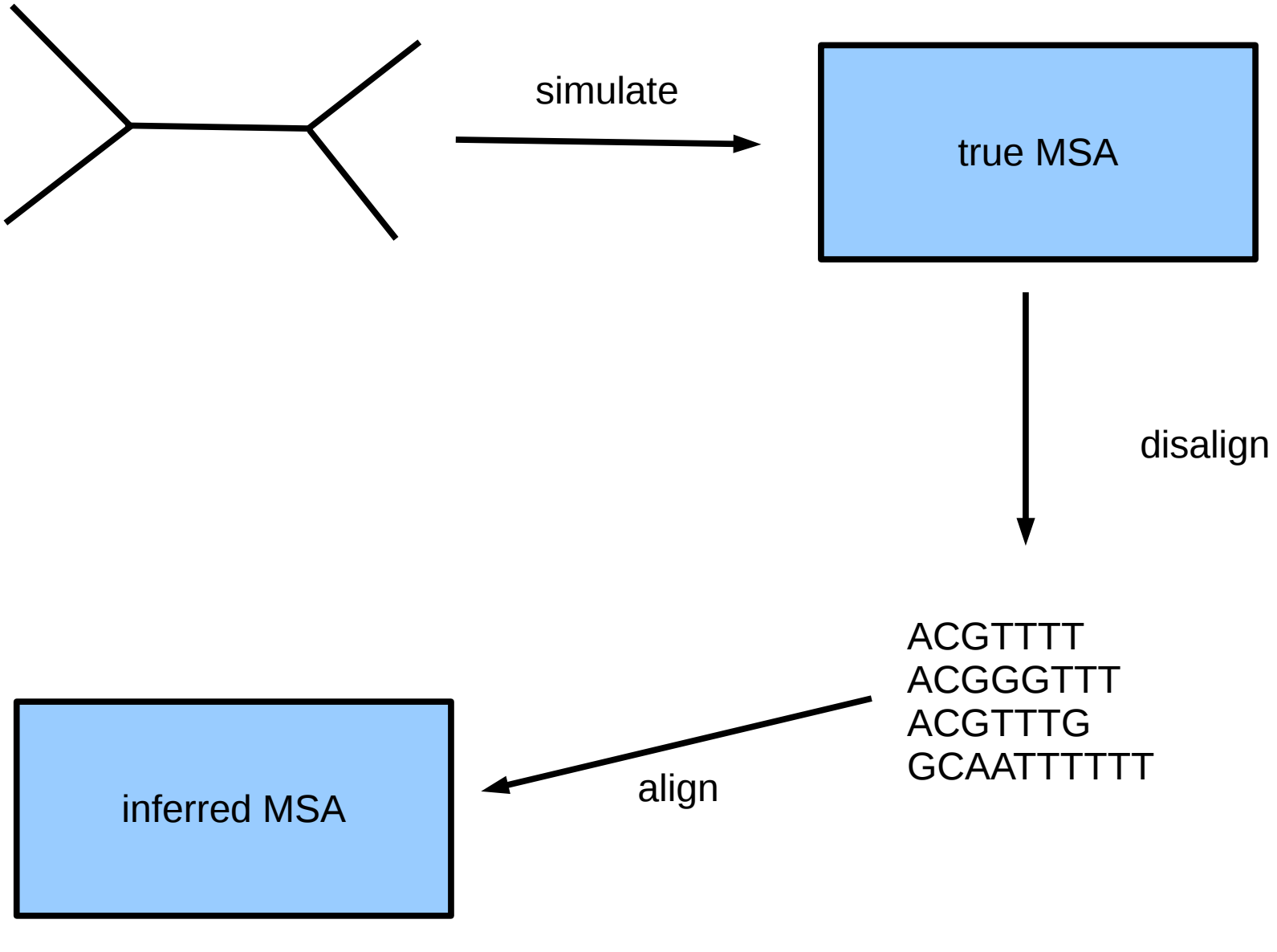
Simulation



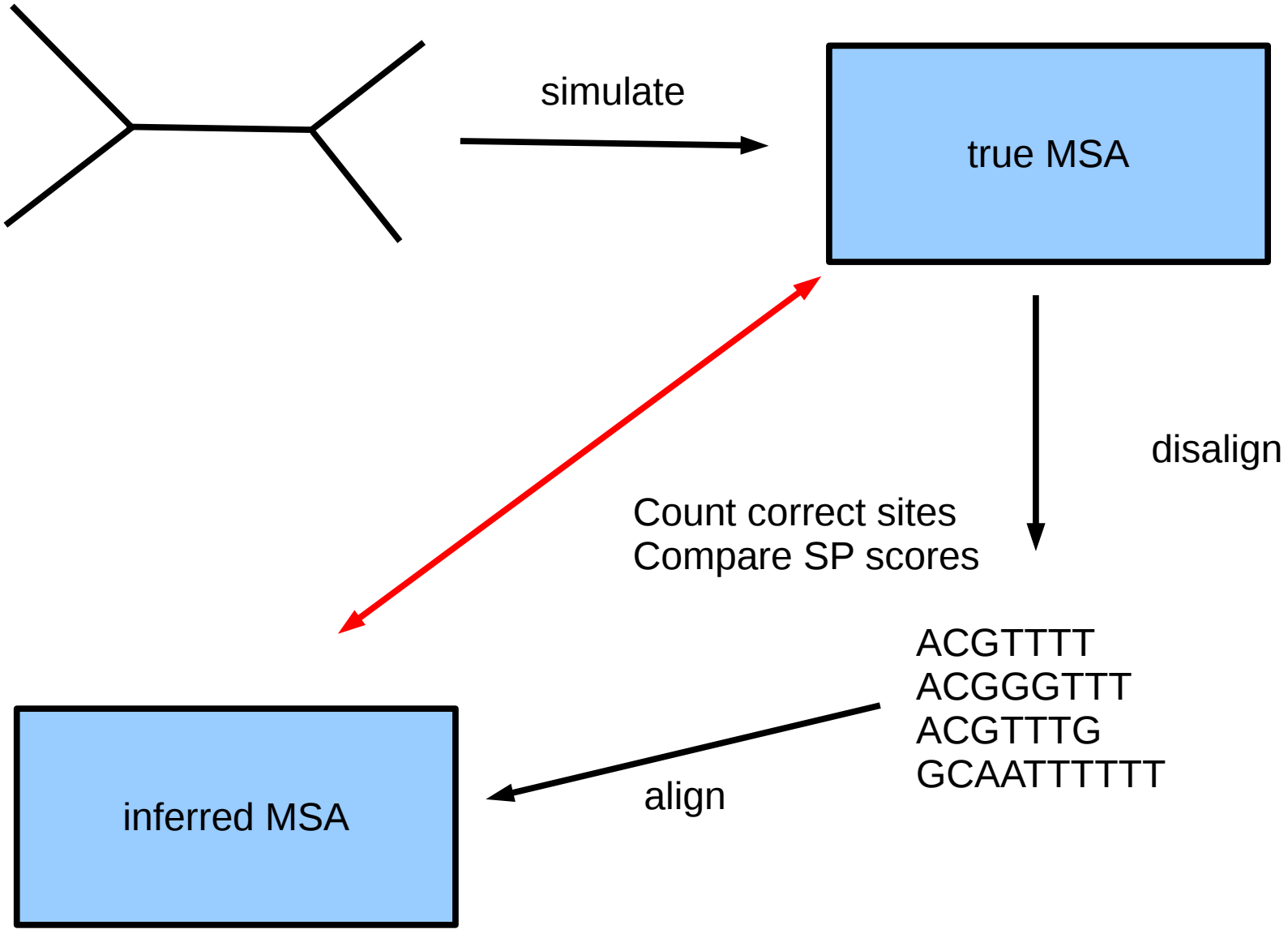
Simulation


simulate
true MSA
disalign
ACGTTTT
ACGGGTTT
ACGTTTG
GCAATTTTTT

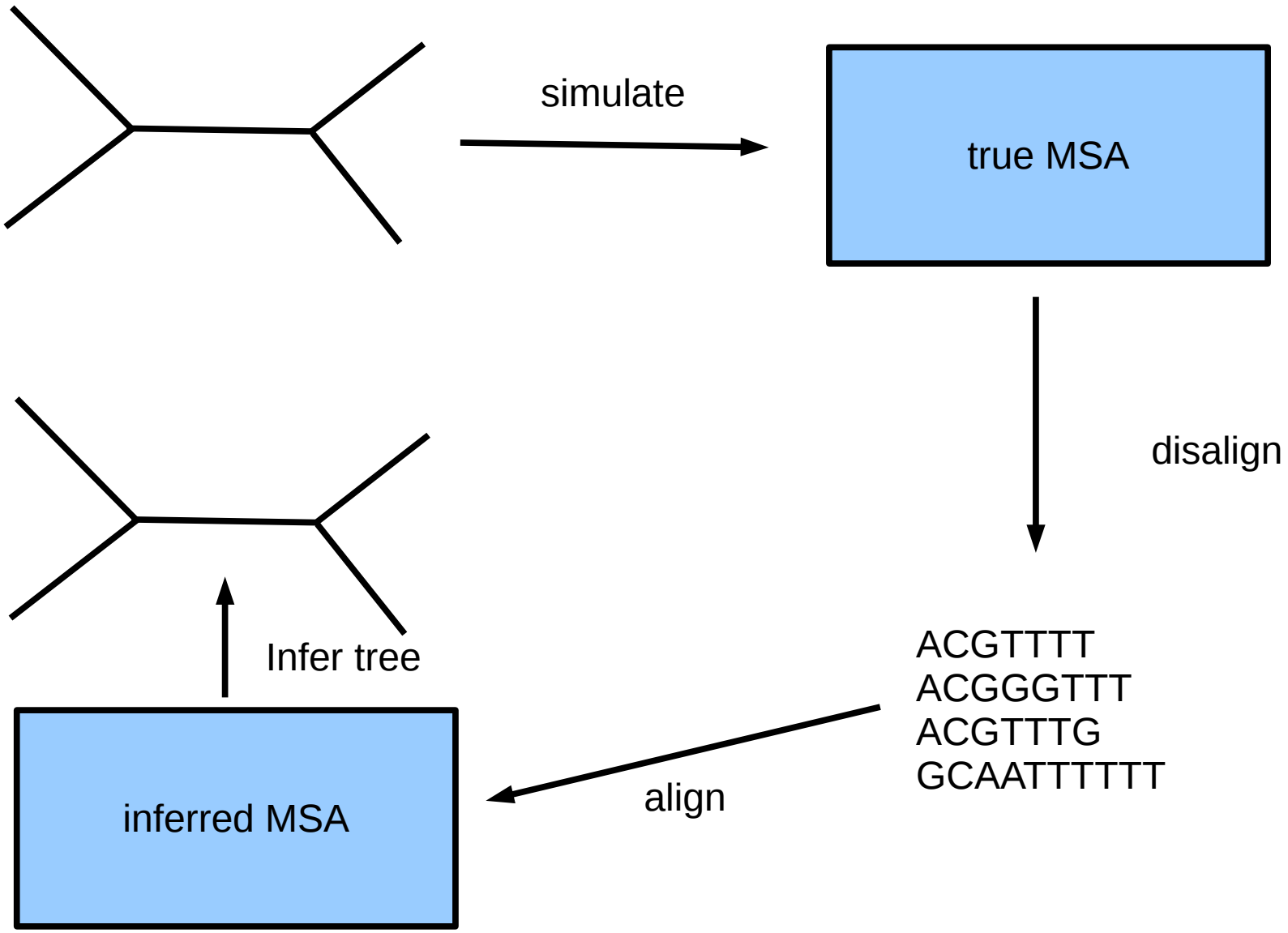
Simulation


simulate
true MSA
disalign
ACGTTTT
ACGGGTTT
ACGTTTG
GCAATTTTTT
align
inferred MSA

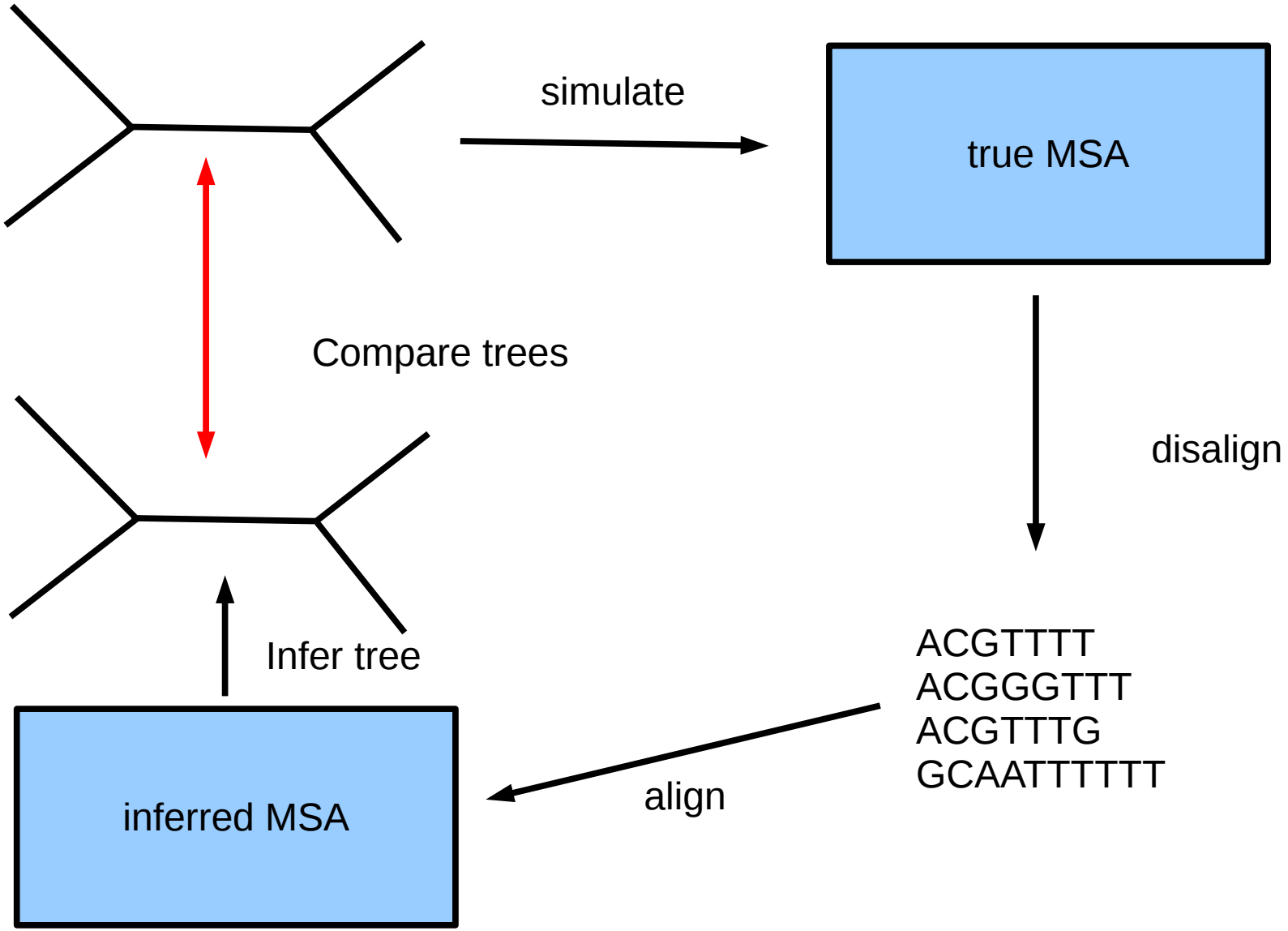
Simulation


simulate
true MSA
disalign
Count correct sites
Compare SP scores
ACGTTTT
ACGGGTTT
ACGTTTG
GCAATTTTTT
align
inferred MSA

Simulation


simulate
true MSA
disalign
ACGTTTT
ACGGGTTT
ACGTTTG
GCAATTTTTT
align
inferred MSA
Infer tree

Simulation


simulate
true MSA
Compare trees
disalign
Infer tree
ACGTTTT
ACGGGTTT
ACGTTTG
GCAATTTTTT
inferred MSA
align

Summary

- MSA is generally difficult due to lack of objective criteria
- MSA as defined per SP score is NP-complete
- Tree-alignment MSA is also NP-complete
- There exist approximation algorithms with performance guarantees
- However, practical approaches use ad hoc heuristics that typically perform better
- Classes of algorithms
  - Progressive MSA
  - Progressive iterative MSA
  - Motif-based approaches
  - Statistical MSA (not covered)
  - Phylogeny-aware MSA (not covered)
  - Simultaneous MSA & tree inference (not covered)