# Introduction to phylogenetics

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15 December 2017

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# Outline

Phylogenies...

Distance trees

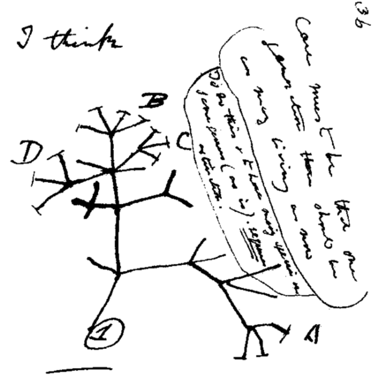
Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

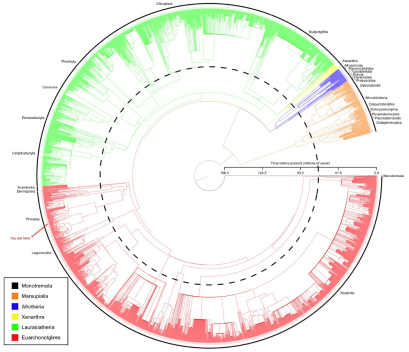
# Phylogenetics: from the origins...



C. Darwin, Notebook, 1837.

*'From the first growth of the tree, many a limb and branch has decayed and dropped off; and these fallen branches of various sizes may represent those whole orders, families, and genera which have now no living representatives, and which are known to us only in a fossil state.'*

# Phylogenetics: ...to the present



Bininda-Emonds *et al.*, 2007, Nature.

- phylogenetic trees are part of the standard toolbox of genetic data analysis
- represent the evolutionary history of a set of (sampled) taxa

# And the main difference is...

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Current trees look better!

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**Current trees look better!**

(and some other minor differences)

# About the minor differences...

- DNA sequencing revolution
- huge data banks freely available (e.g. GenBank)
- easier, cheaper, faster to obtain DNA sequences
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**Different ways to exploit this information.**

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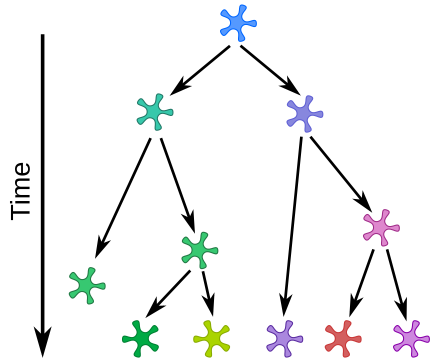
# Genetic changes (substitution) accumulate over time

**Substitution**: replacement of a nucleotide (e.g. a → t)



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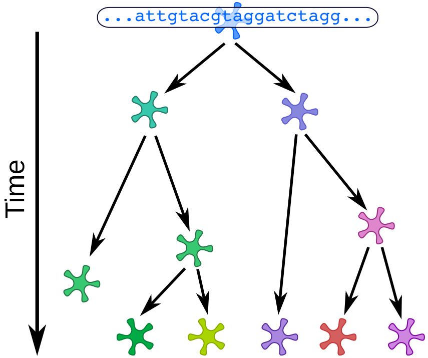

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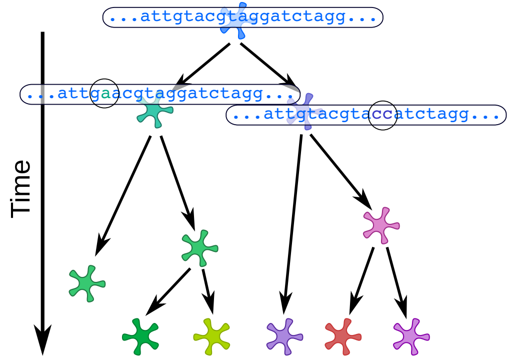

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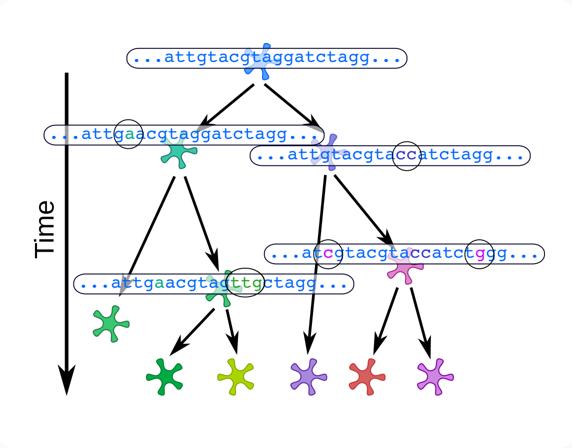

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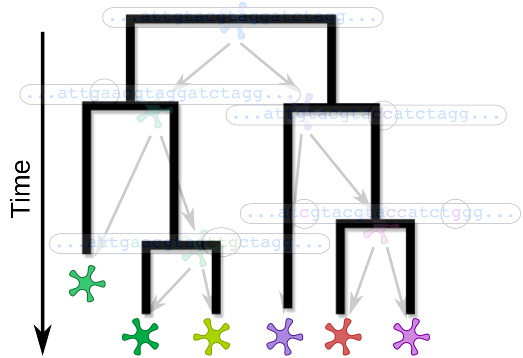

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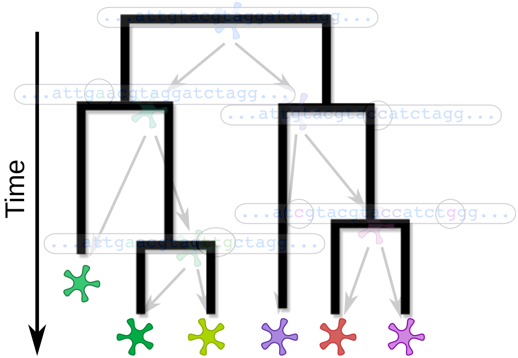
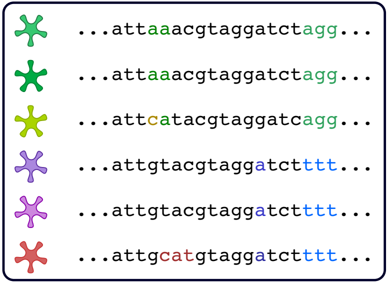


Substitution patterns reflect the evolutionary history

# Using substitution patterns to reconstruct the evolutionary history



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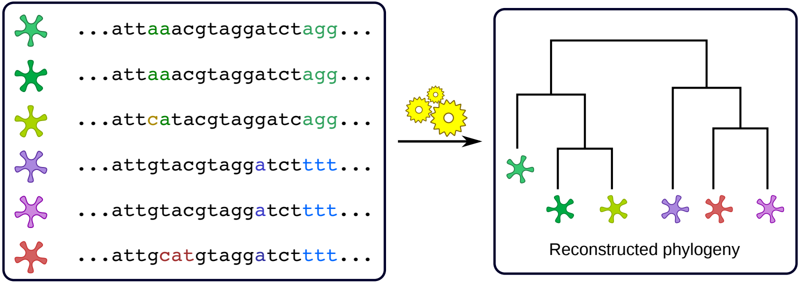

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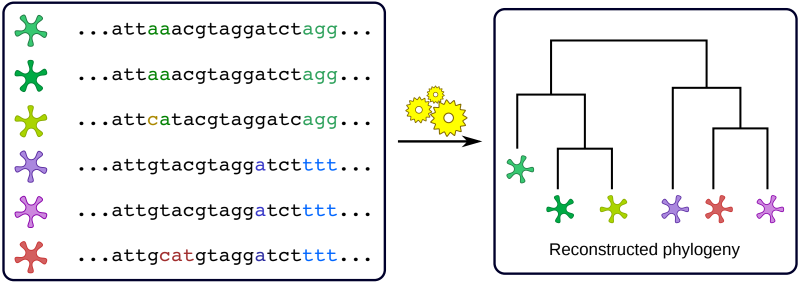
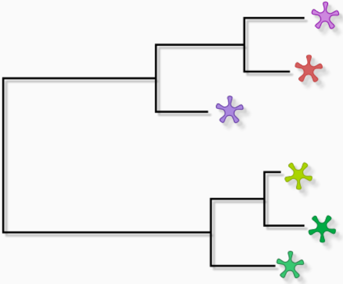


Phylogenetics aim to reconstruct evolutionary trees (*phylogenies*) from genetic sequence data.

# Using trees to represent the evolutionary history



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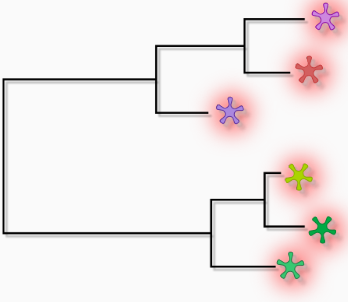
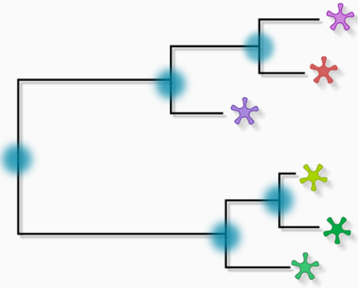


analysed sequences

- *"taxa"*
- *"tips"*
- *"leaves"*
- *"Operational Taxonomic Units (OTUs)"*

# Using trees to represent the evolutionary history



Most Recent Common Ancestors (MRCA)

- *"nodes"*
- *"Hypothetical Taxonomic Units (HTUs)"*

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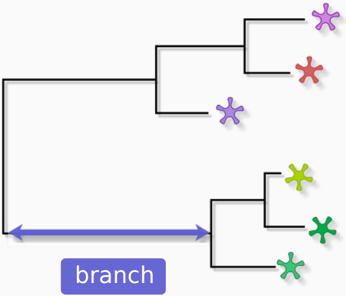
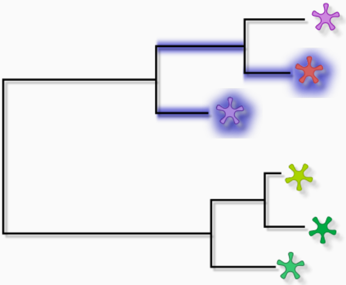


- "*edge*"
- length = amount of evolution (**not time**, as a rule)
- length is optional

# Using trees to represent the evolutionary history



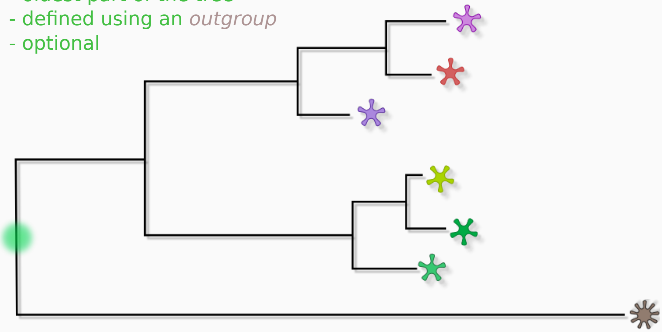
**distances between tips**

- "*patristic*" distance: sum of branch lengths
- other measures of distance/dissimilarity
- vertical axis meaningless

# Using trees to represent the evolutionary history

Root

- oldest part of the tree
- defined using an *outgroup*
- optional



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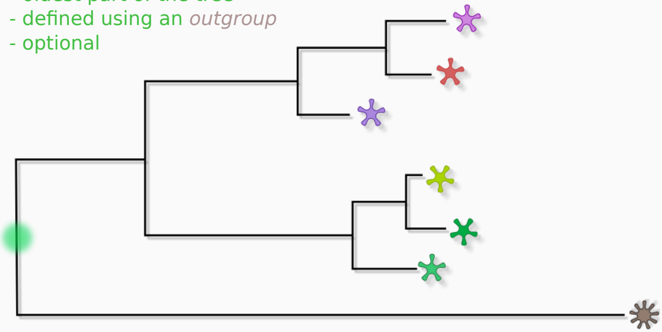

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How to we build them?

# Workflow

## Prepare data

- align sequences: alignment software + manual refinement

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- assess uncertainty
- test phylogenetic signal
- model trait evolution
- ...

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- **distance-based methods**
- **maximum parsimony**
- **likelihood-based methods (ML, Bayesian)**

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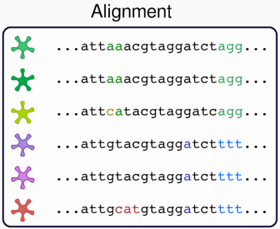
# Distance-based phylogenetic reconstruction

Approaches relying on **agglomerative clustering** algorithms (e.g. Single linkage, UPGMA, Neighbor-Joining)

**Rationale**

1. compute pairwise genetic distances $\mathbf{D}$
2. group closest sequences
3. update $\mathbf{D}$
4. go back to 2) until all sequences are grouped

# Distance-based phylogenetic reconstruction



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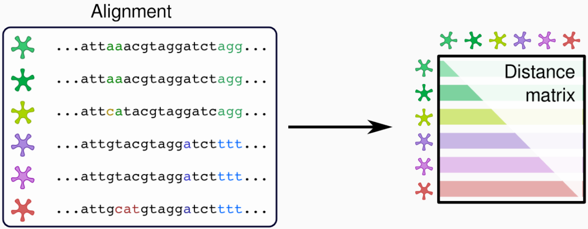

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Distance matrix

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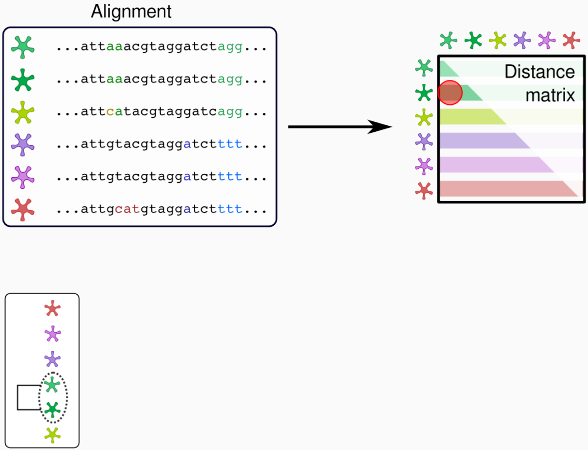

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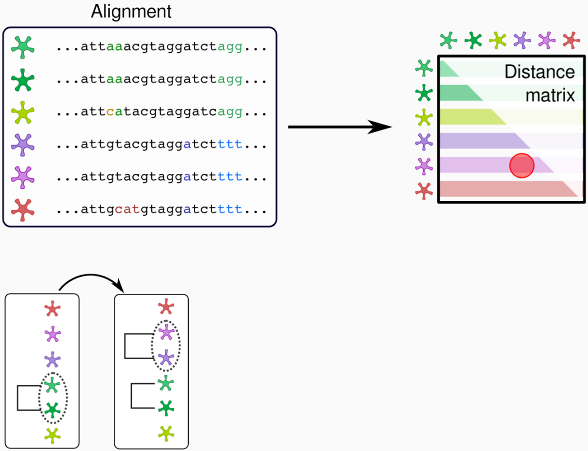

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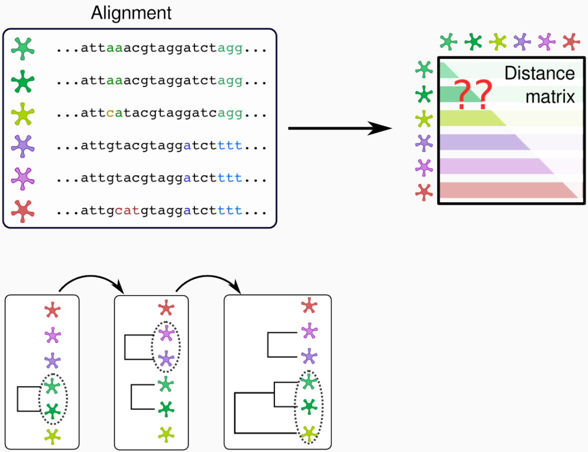

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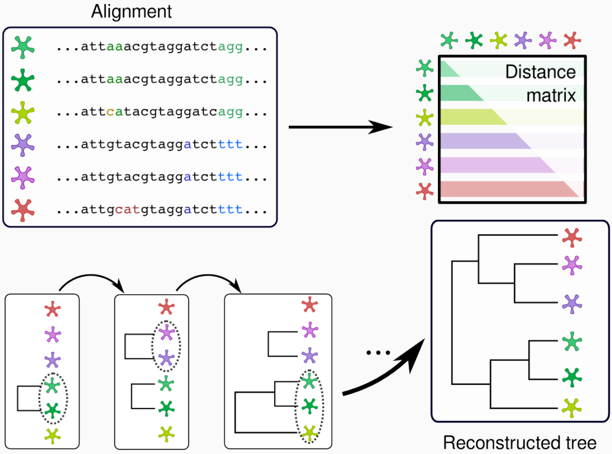

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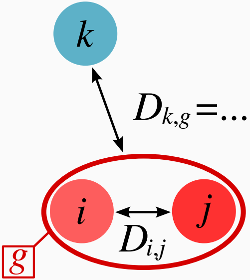
Distance matrix

Reconstructed tree

# What is the distance between a node and tips?

Hierarchical clustering:



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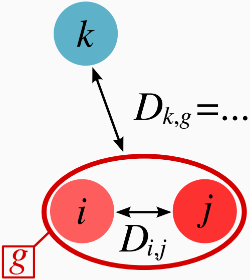

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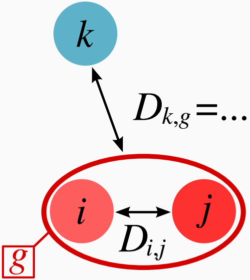

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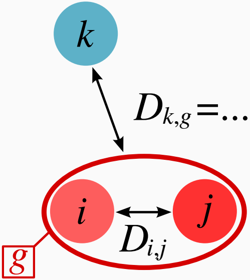

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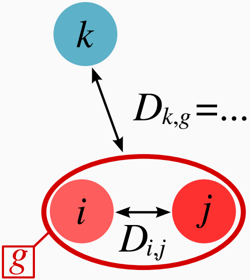

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**Neighbor joining:**

Transforms original distances to account for heterogeneous rates of evolution.

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**Advantages**

- simple
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**Limitations**

- sensitive to distance/clustering chosen
- evolutionary rates are not estimated
- no measure of uncertainty for the tree obtained

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Phylogenies...

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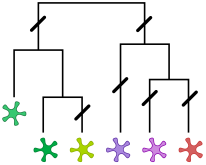
# Maximum parsimony phylogenies

Approaches relying on finding the tree with the **smallest number of character changes (substitutions)**

**Rationale**

1. start from a pre-defined tree
2. compute initial parsimony score
3. permute branches and compute parsimony score
4. accept new tree if the parsimony score is improved
5. go back to 3) until convergence

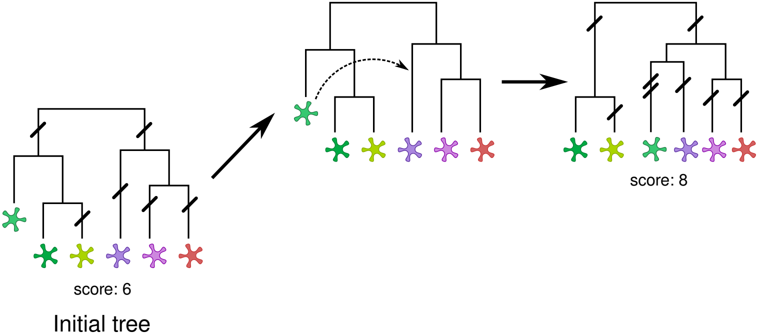
# Maximum parsimony phylogenies



score: 6

Initial tree

# Maximum parsimony phylogenies



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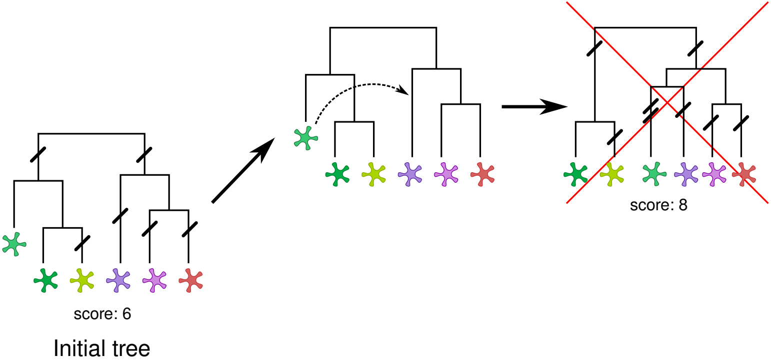

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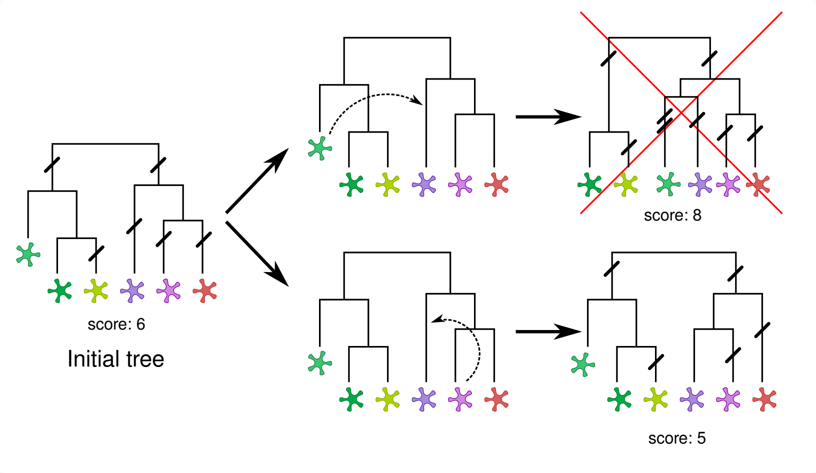

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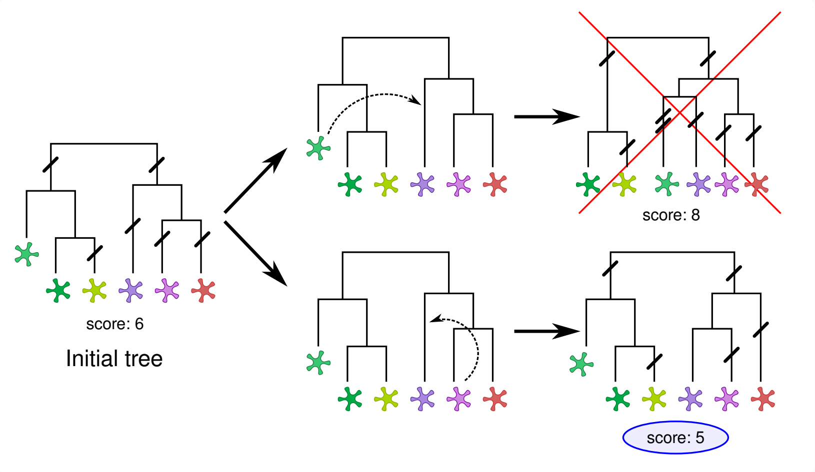

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## Limitations

- evolutionary rates are not estimated
- no measure of uncertainty for the tree obtained
- computer-intensive
- different types of substitutions ignored
- evolution not necessarily parsimonious
- sensitive to heterogeneous rates of evolution (*long branch attraction*)

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Approaches relying on a **model of sequence evolution**:

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2. compute initial likelihood/posterior
3. permute branches, sample new parameters and compute likelihood/posterior
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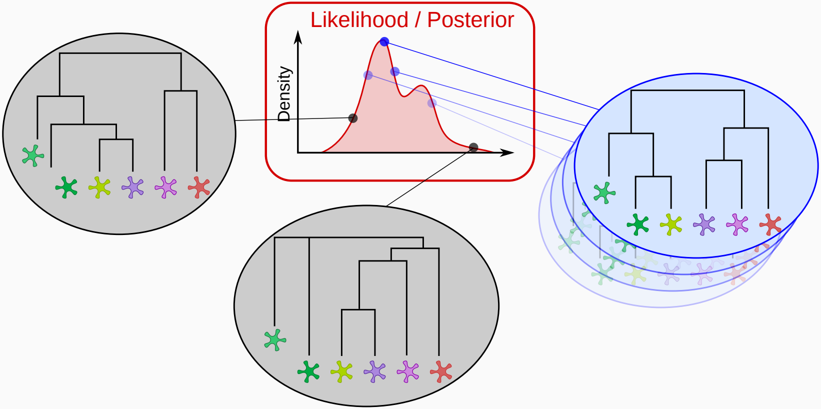

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## Limitations

- computer-intensive
- choice of the model of evolution
- (ML) no measure of uncertainty for the tree obtained
- (Bayesian) need to find a consensus tree

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- assess **variability due to sampling the genome** and **conflicting signals**
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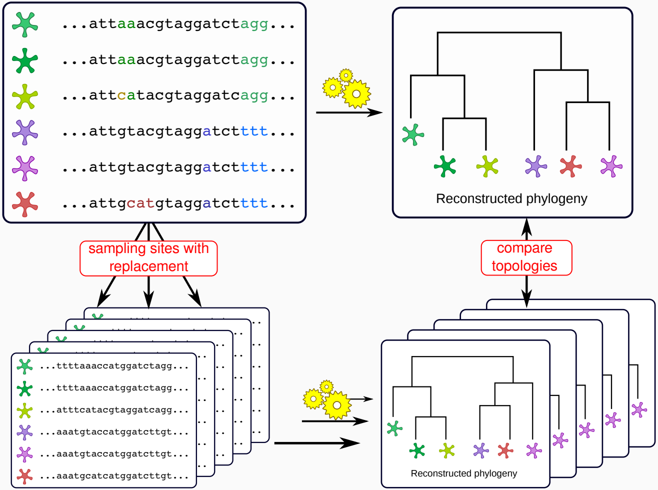
## Rationale

1. obtain a reference tree
2. resample the sites with replacement
3. obtain a tree for the resampled dataset
4. go back to 2) until the desired number of bootstrapped trees is attained
5. compute the frequency of each bifurcation of the reference tree occuring in bootstrapped trees

# Bootstrapping phylogenies



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Reconstructed phylogeny

sampling sites with replacement

compare topologies

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...ttttaaaccatggatctagg...

...atttcatacgtaggatcagg...

...aaatgtaccatggatcttgt...

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Reconstructed phylogeny

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**Limitations**

- possibly computer-intensive
- assumes that the genome has been sampled randomly (often wrong)

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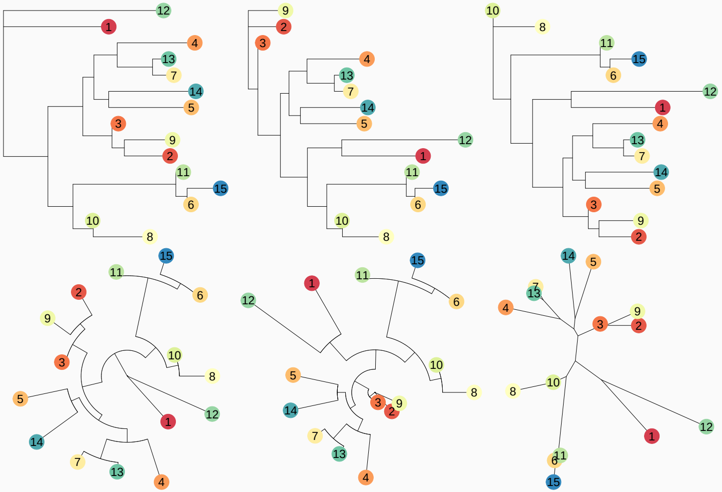
# Plotting trees as rooted



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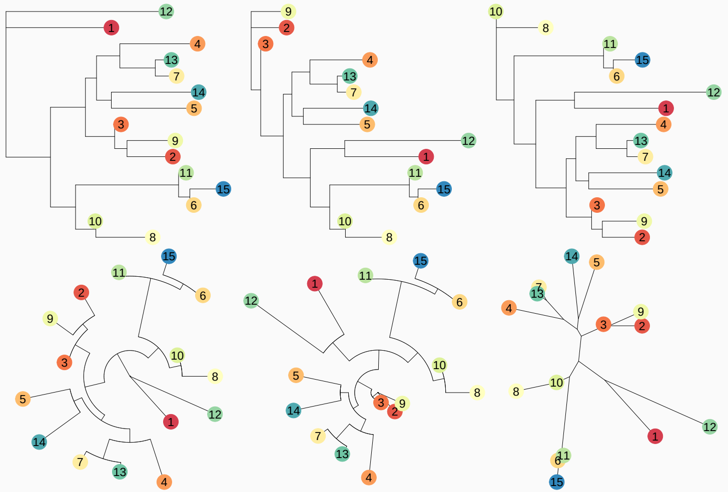
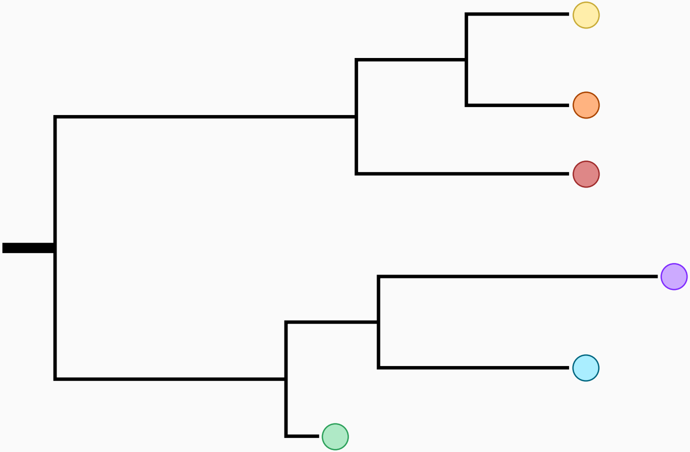


Never plot an unrooted tree as rooted.

# Interpreting distances



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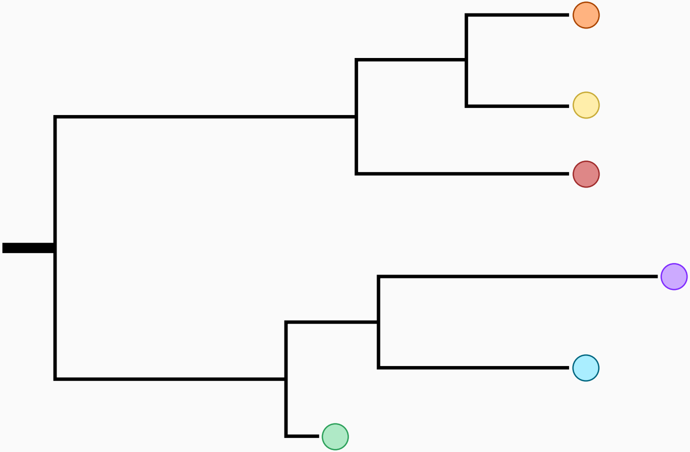

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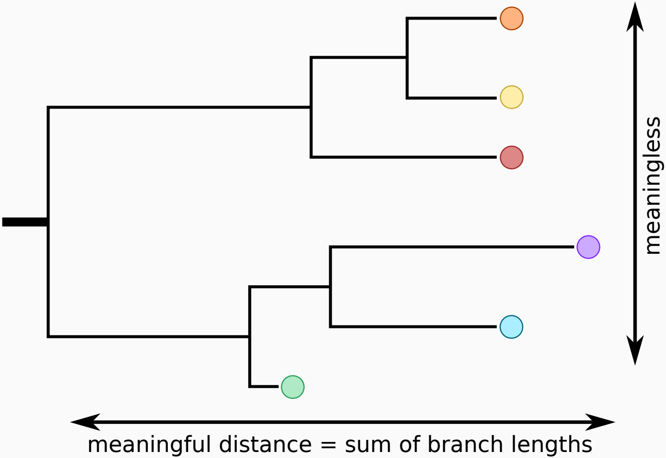
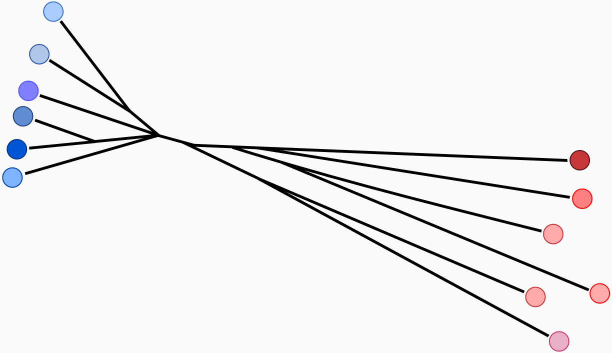


# The paradox of divergent clusters



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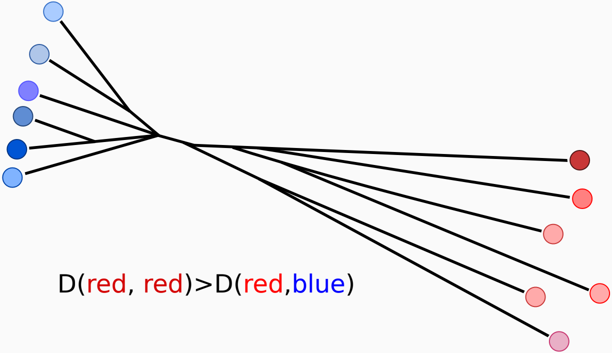
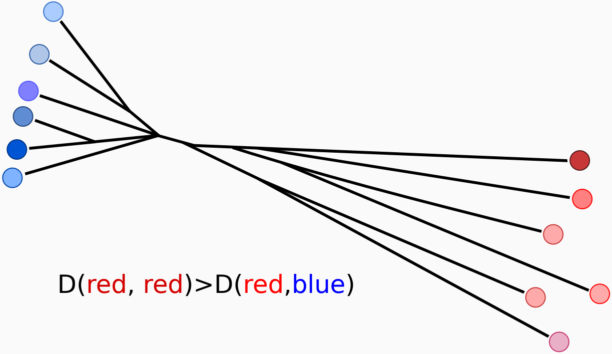


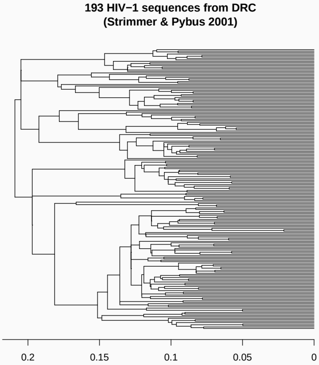
D(red, red)>D(red,blue)

# The paradox of divergent clusters



MRCA and genetic distances may give different information.

# Taking uncertainty into account



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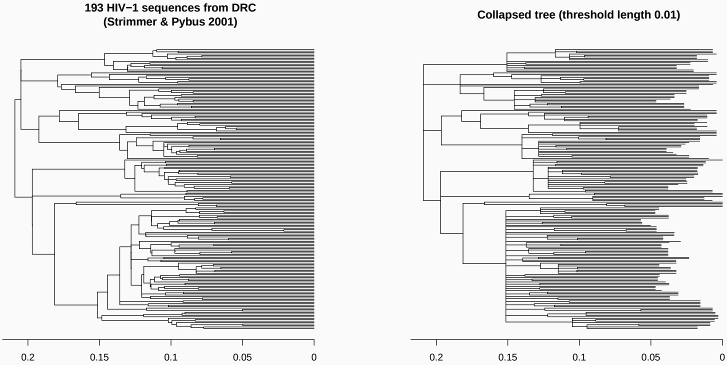

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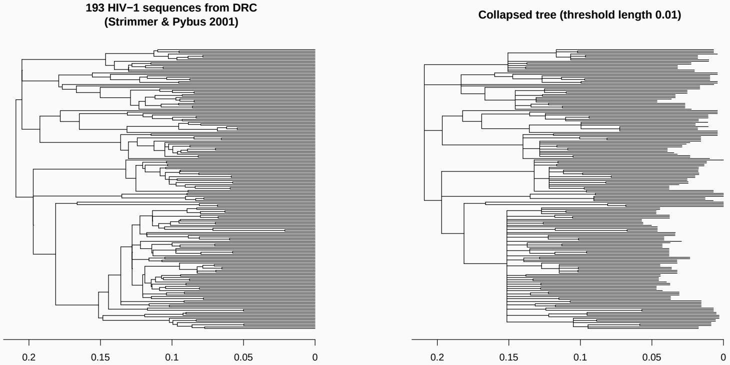
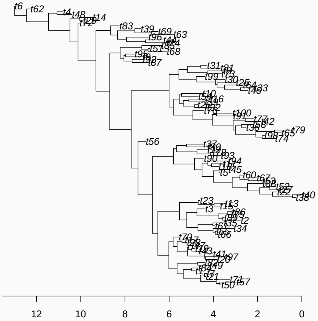


At best, the tree is an estimate of the likely evolutionary history of the taxa studied.

# (Over, Mis)Interpreting temporal trends



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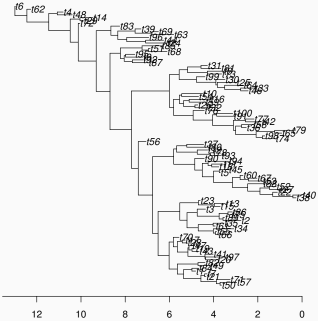

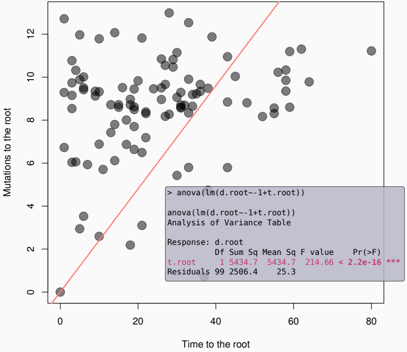

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Analysis of Variance Table

Response: d.root
          Df Sum Sq Mean Sq F value    Pr(>F)
t.root     1 5434.7  5434.7  214.66 < 2.2e-16 ***
Residuals 99 2506.4    25.3
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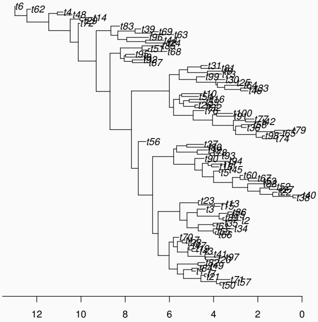

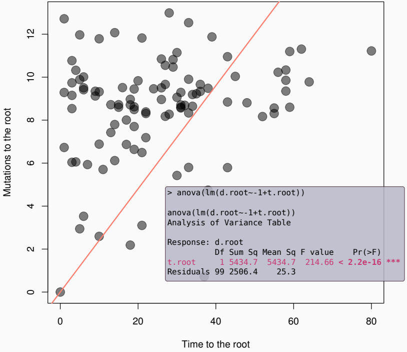

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“Time trees” only make sense under a near-perfect molecular clock.

# Outline

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

# This is only the beginning

Many things can be done with trees

- estimate divergence time

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- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...
- **and also, other approaches than phylogenetics to analyse genetic data**


The End