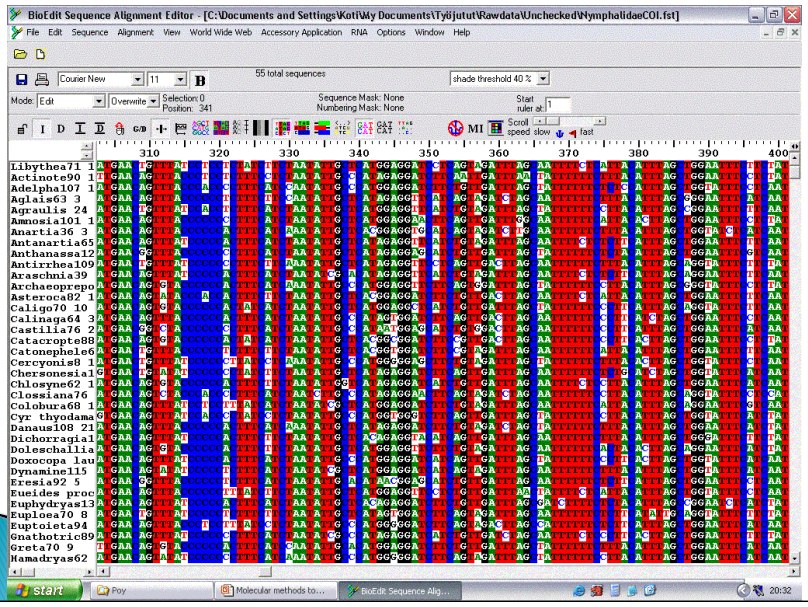
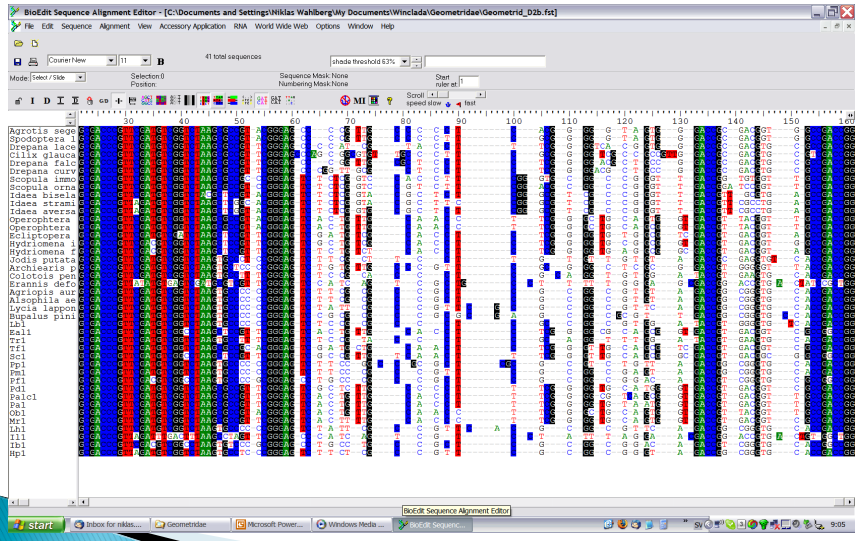
Multiple Sequence Alignment

Alignment can be easy…



… or difficult



Homology: Definition

- Homology: similarity that is the result of inheritance from a common ancestor – identification and analysis of homologies is central to phylogenetic systematics
- An alignment is a hypothesis of positional homology between bases/amino acids

Multiple sequence alignment–goals

- To generate a concise, information-rich summary of sequence data
- Alignments can be treated as models that can be used to test hypotheses
- Does this model of events accurately reflect known biological evidence?

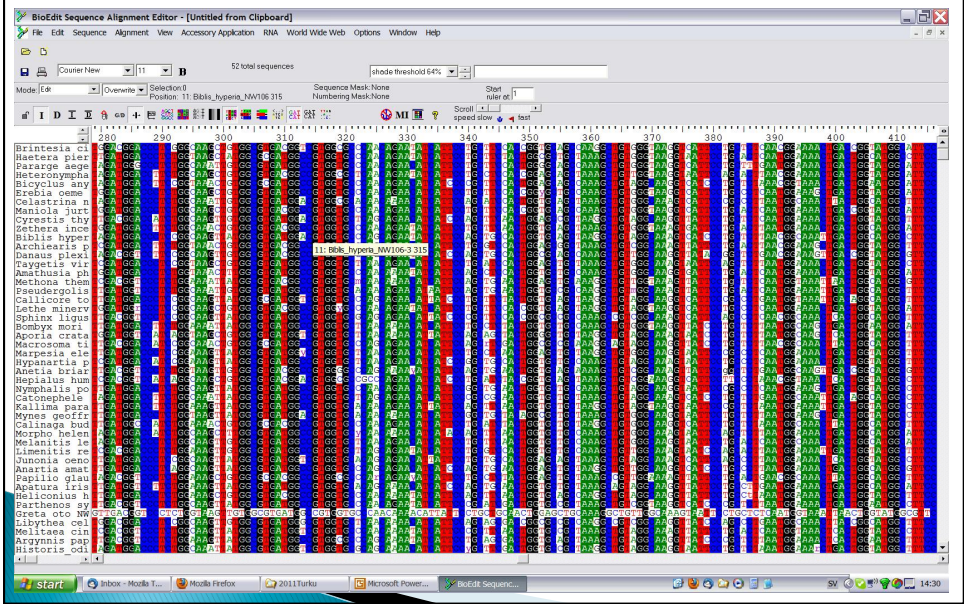
Multiple sequence alignment

- Manual
- Dynamic programming
- Heuristic methods
  - Progressive alignment
  - Consistency-based scoring
  - Iterative refinement methods

Manual alignment – reasons

- Might be carried out because:
  - Alignment is easy
  - There is some extraneous information (structural)
  - Automated alignment methods have encountered a local minimum problem.
  - An automated alignment method can be “improved”

Protein-coding genes can be manually aligned



How to align these sequences:

```
AGGGCTTTAA
AGGCTA
AATGGCTCTAA
GGAGCCCTAA
```

How to align these sequences:

```
A-GGGCTTTAA
A--GGCT--A-
AATGGCTCTAA
GGAG-CCCTAA
```

How to align these sequences:

```
-AGGGCTTTAA
-A-GGC--TA-
AATGGCTCTAA
-GGAGCCCTAA
```

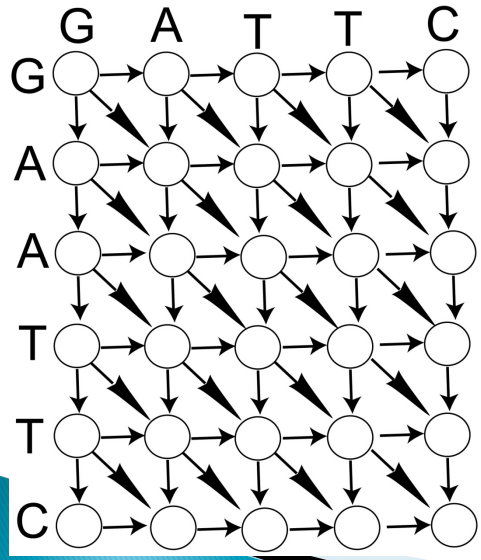
Multiple sequence alignment

- Is not easy! How to be objective?
- Dynamic programming
- Heuristic methods
  - Progressive alignment
  - Consistency-based scoring
  - Iterative refinement methods

Dynamic programming

- For two sequences, the best alignment can be found by scoring all possible pairs of aligned nucleotides and penalizing gaps
- An optimality criterion
- Rapidly becomes unfeasible with more sequences

Path Graph for aligning two sequences

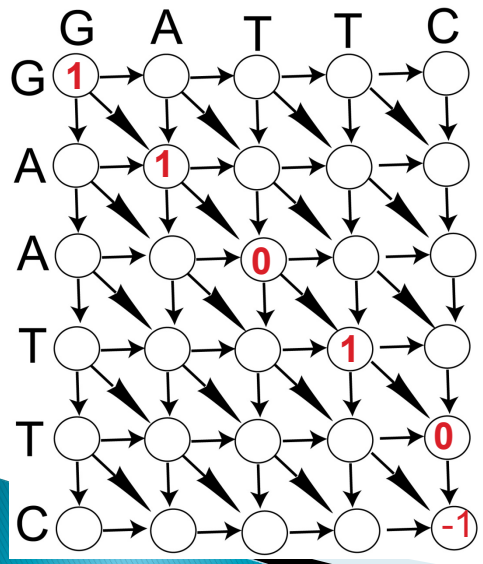


What is the optimal alignment for:

GATTC

GAATTC

Possible alignment

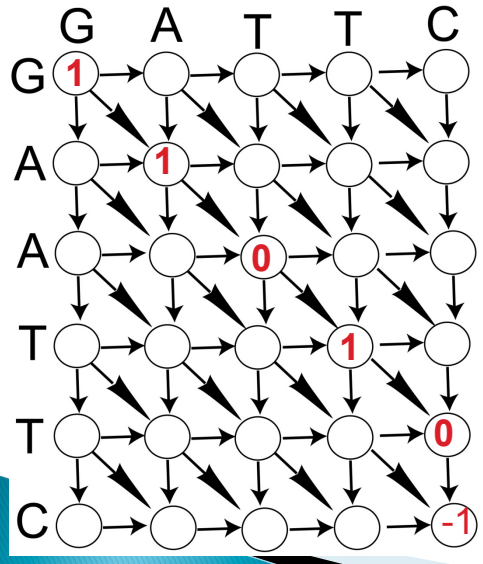


Scoring Scheme:

- **Match: +1**
- **Mismatch: 0**
- **Indel: -1**

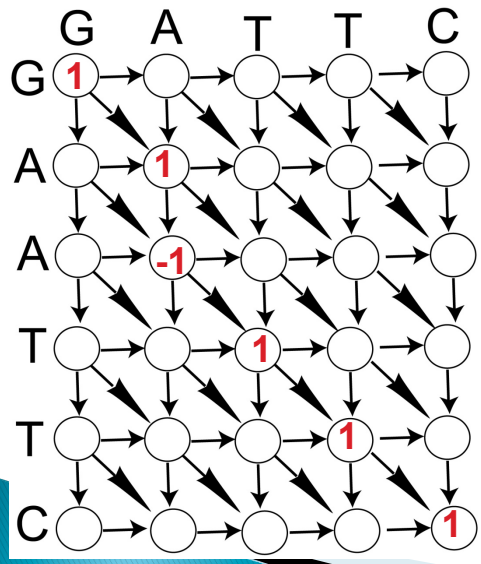
Score for this path= 2

Alignment using this path



GATTC -
GAATTC

Optimal Alignment 1

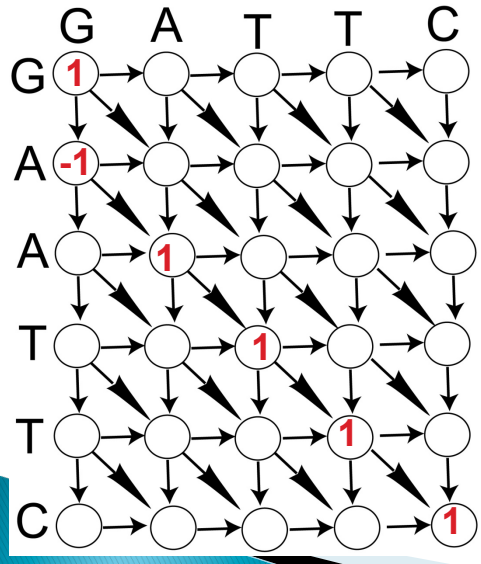


Alignment using this path

GA -TTC
GAATTC

Alignment score: 4

Optimal Alignment 2



Alignment using this path

G -ATTC
GAATTC

Alignment score: 4

Dynamic programming

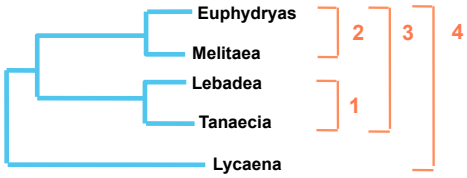
- Time and computer memory needed grows exponentially with number of sequences
- Becomes impossible to align more than 4 sequences of modest length
- Fails to fully exploit phylogeny and does not incorporate an evolutionary model

Heuristics: Progressive alignment

- Devised by Feng and Doolittle in 1987
- A heuristic method and as such is not guaranteed to find the 'optimal' alignment
- Requires $n-1+n-2+n-3...n-n+1$ pairwise alignments as a starting point
- Most successful implementation is Clustal
  - ClustalW
  - ClustalX

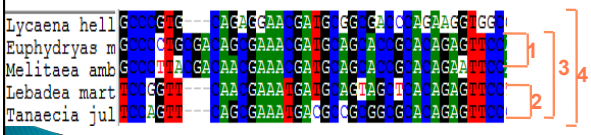
Overview of Clustal procedure

Euphydryas	1	-			
Melitaea	2	.17	-		
Lebadea	3	.59	.60	-	
Tanaecia	4	.59	.59	.13	-
Lycaena	5	.77	.77	.75	.75	-



Quick pairwise alignment: calculate distance matrix

Neighbour-joining tree (guide tree)



Progressive alignment following guide tree

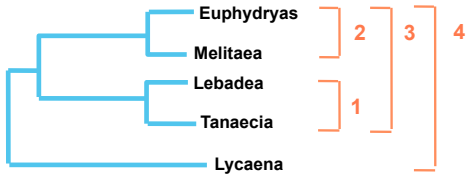
Clustal – pairwise alignments

- First perform all possible pairwise alignments between each pair of sequences
- Calculate the 'distance' between each pair of sequences based on these isolated pairwise alignments
- Generate a distance matrix

Euphydryas	1	-			
Melitaea	2	.17	-		
Lebadea	3	.59	.60	-	
Tanaecia	4	.59	.59	.13	-
Lycaena	5	.77	.77	.75	.75	-

Clustal – guide tree

- Generate a Neighbour-Joining 'guide tree' from these pairwise distances
- This guide tree gives the order in which the progressive alignment will be carried out



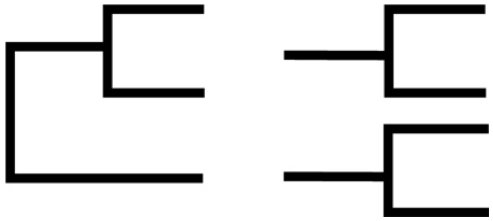
Multiple alignment- first pair

- Align the two most closely-related sequences first
- This alignment is then 'fixed' and will never change
- If a gap is to be introduced subsequently, then it will be introduced in the same place in both sequences, but their relative alignment remains unchanged

Clustal – decision time

- Consult the guide tree to see what alignment is performed next.
  - Align a third sequence to the first two

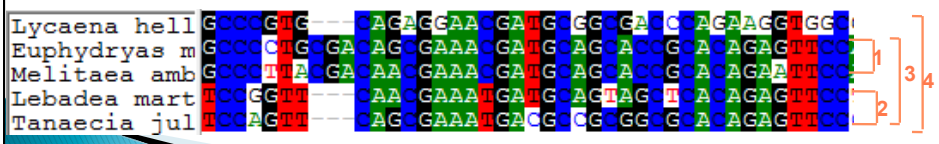
  Or
  - Align two entirely different sequences to each other.



Option 1 Option 2

Clustal – progression

- The alignment is progressively built up in this way, with each step being treated as a pairwise alignment, sometimes with each member of a 'pair' having more than one sequence



Clustal – good points/bad points

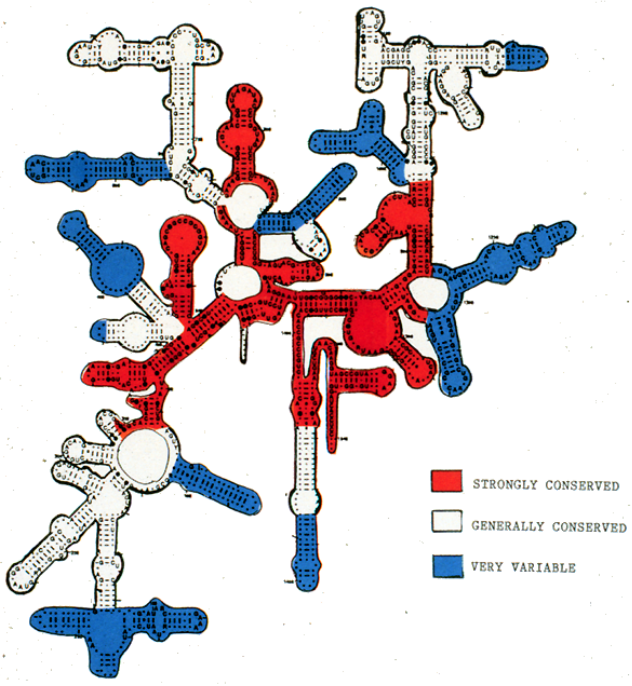
- Advantages:
  - Speed
- Disadvantages:
  - Hierarchic structure introduced that is not necessarily phylogenetic
  - No way of quantifying whether or not the alignment is good
  - No way of knowing if the alignment is 'correct'

Clustal – local minimum

- Potential problems:
  - Local minimum problem. If an error is introduced early in the alignment process, it is impossible to correct this later in the procedure
  - Arbitrary alignment

Increasing the sophistication of the alignment process

- Should we treat all the sequences in the same way?
  - some sequences are closely related and some sequences are distant relatives.
- Should we treat all positions in the sequences as though they were the same?
  - they might have different functions and different locations in the 3-dimensional structure.



Clustal – caveats

- Sequence weighting
- Varying substitution matrices
- Positions in early alignments where gaps have been opened receive locally reduced gap penalties to encourage openings in subsequent alignments

Divergent sequences

- The most divergent sequences (most different, on average from all of the other sequences) are usually the most difficult to align.
- It is sometimes better to delay their aligment until later (when the easier sequences have already been aligned).
- The user has the choice of setting a cutoff (default is 40% identity).
- This will delay the alignment until the others have been aligned.

Advice on progressive alignment

- Progressive alignment is a mathematical process that is completely independent of biological reality.
- Can be a very good estimate
- Can be an impossibly poor estimate.
- Requires user input and skill.
- Treat cautiously
- Can be improved by eye (usually)
- Often helps to have colour-coding.
- Depending on the use, the user should be able to make a judgement on those regions that are reliable or not.
- For phylogeny reconstruction, only use those positions whose hypothesis of positional homology is certain

Consistency-based scoring

- One way to avoid the problems of getting stuck in local minima or fixed gaps
- Based on optimizing a multiple alignment using information from all pairwise alignments
- Identifies those nucleotides that are aligned most consistently across the different alignments
- Used in e.g. T-Coffee

Iterative refinement methods

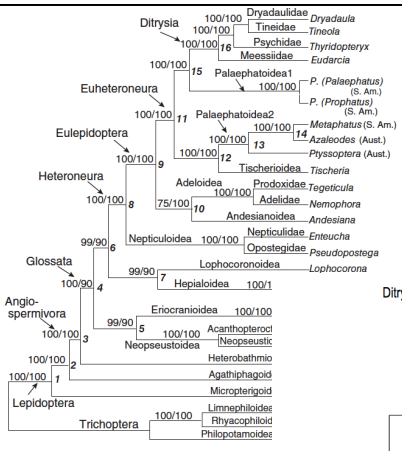
- Initial alignments split into two groups randomly
- Within groups the alignment is kept fixed
- Dynamic programming used to align the two groups to each other
- This is repeated until score converges
- Used in e.g. Muscle and MAFFT

Using models in alignment

- New methods are being developed all the time
- Latest methods include using a Bayesian statistic framework, DNA evolutionary models and alignment concomitantly with estimation of phylogentic relationships
- Still not feasible with a moderately sized dataset

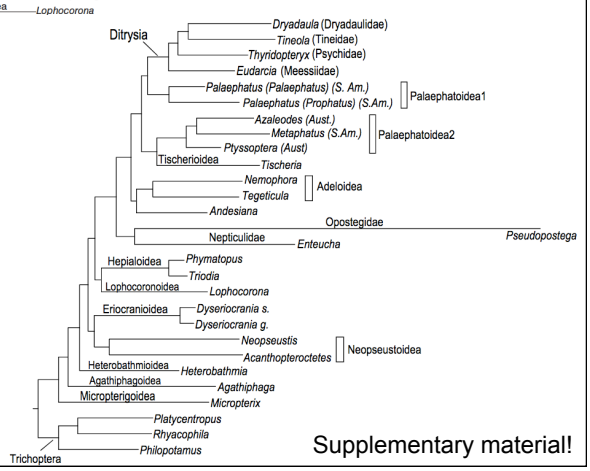
Phylogenomics: Crucial step 1 – orthology!

- We need to know that the genes we are studying are the same (homology)
- Old style PCR primers amplifying orthologous genes
- Genomics relies on bioinformatic methods to determine orthology



Bazinet et al 2017
2212 genes

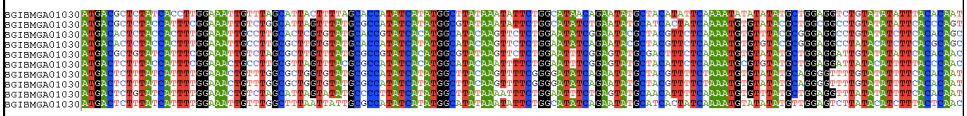
The dirty secrets of bioinformatics



Supplementary material!

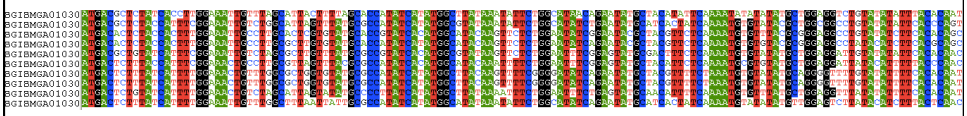
Phylogenomics: Crucial step 2 – alignment!

- Old style manual alignment

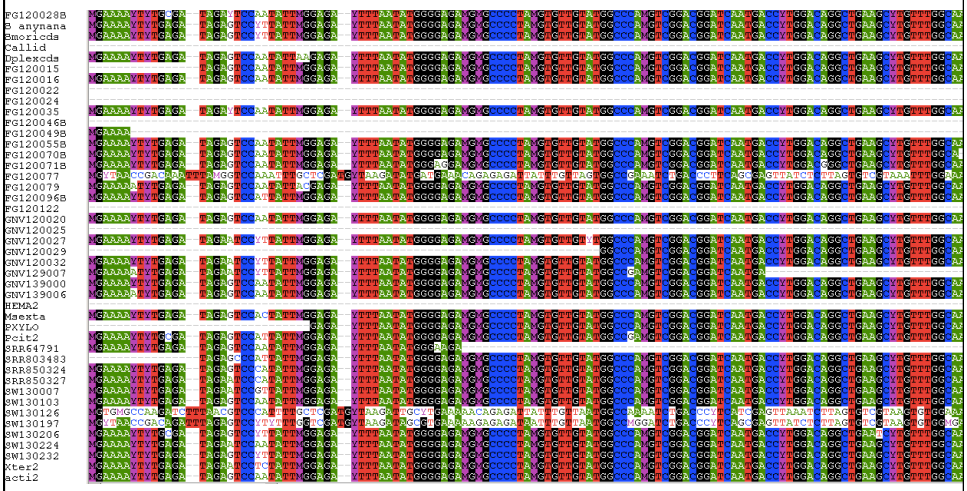


Phylogenomics: Crucial step 2 – alignment!

- Old style manual alignment



- Phylogenomic datasets rely on bioinformatic tools to align



Kawahara & Breinholt 2014

Bottom line

- Alignments are extremely important in phylogenetics
- A bad alignment means many wrong statements of homology, which means pure rubbish as output
- A good alignment can be hard to attain

The Tree

Finding the optimal trees and consensus methods

Numbers of possible trees for N taxa

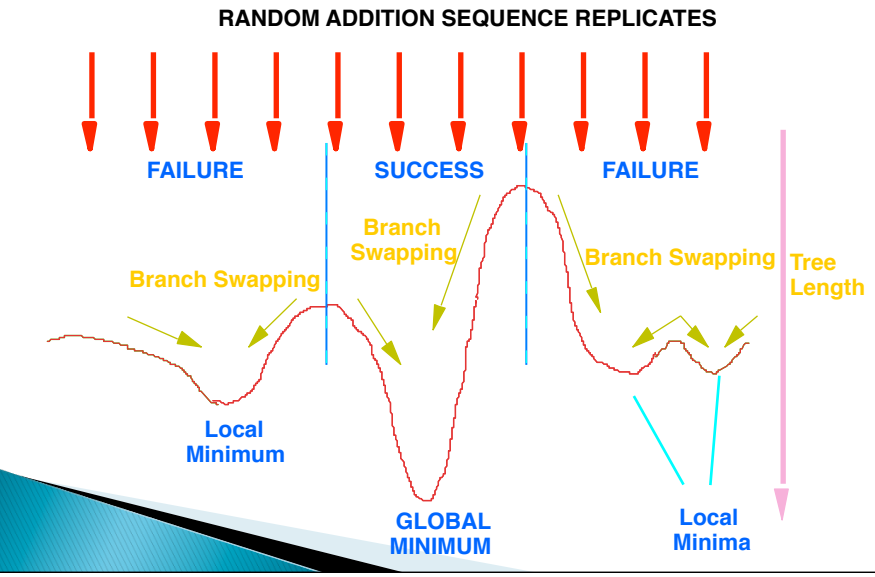
1	1
2	1
3	1
4	3
5	15
6	105
7	945
8	10395
9	135135
10	2027025
11	34459425
12	654729075
13	13749310575
14	316234143225
15	7905853580625
16	213458046676875
17	6190283353629370
18	191898783962510625
19	6332659870762850625
20	221643095476699771875 (2×10^{20})
50	3×10^{74}

How can we find the most optimal tree?

Criteria for the best tree?

- Optimum tree is the best with today's methods
- Optimality criteria
  - Parsimony: minimizing tree length
  - Modeling methods: maximizing likelihood

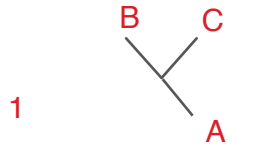
Tree space may be populated by local optima and islands of optimal trees



Finding optimal trees – exact solutions

- Exact solutions can only be used for small numbers of taxa
- Exhaustive search examines all possible trees
- Branch and bound does not examine all trees, but will find optimal tree(s)
- Typically used for problems with 10 –20 taxa

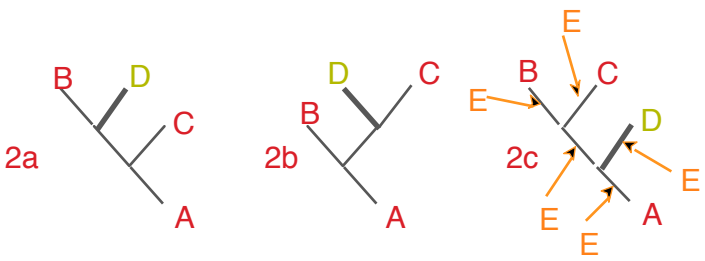
Finding optimal trees – exhaustive search



1

Starting tree, any 3 taxa

Add fourth taxon (D) in each of three possible positions -> three trees



Add fifth taxon (E) in each of the five possible positions on each of the three trees -> 15 trees, and so on ....

Finding optimal trees – exact solutions

- Branch and bound saves time by discarding families of trees during tree construction that cannot be shorter than the shortest tree found so far
- Can be enhanced by specifying an initial upper bound for tree length
- Typically used only for problems with less than 20 taxa

Finding optimal trees – heuristics

- The number of possible trees increases faster than exponentially with the number of taxa making exhaustive searches impractical for many data sets (an NP complete problem)
- Heuristic methods are used to search tree space for optimal trees by building or selecting an initial tree and swapping branches to search for better ones
- The trees found are not guaranteed to be optimal – they are best guesses

Finding optimal trees – heuristics

- Stepwise addition

  Asis – the order in the data matrix

  Closest –starts with shortest 3-taxon tree adds taxa in order that produces the least increase in tree length (greedy heuristic)

  Simple – the first taxon in the matrix is taken as a reference – taxa are added to it in the order of their decreasing similarity to the reference

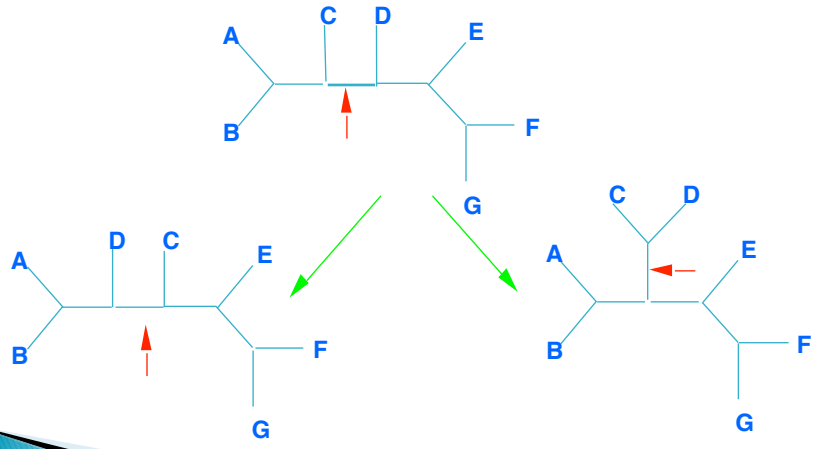
  Random – taxa are added in a random sequence, many different sequences can be used

Finding optimal trees – branch swapping

- Nearest neighbor interchange (NNI)
- Subtree pruning and regrafting (SPR)
- Tree bisection and reconnection (TBR)

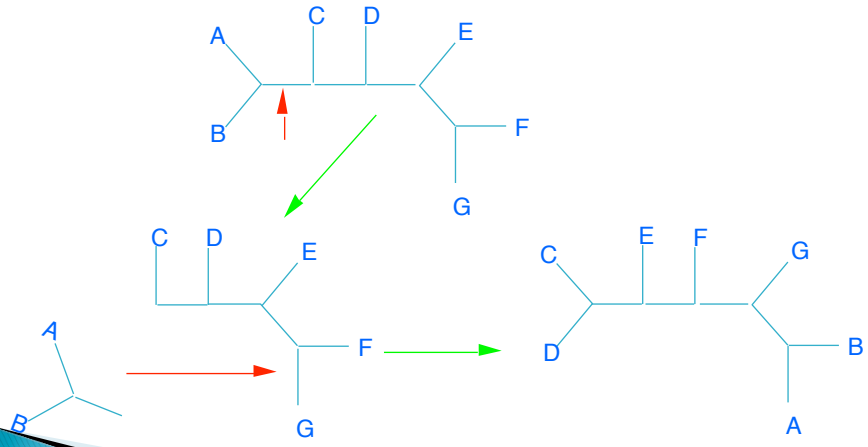
Finding optimal trees – heuristics

Nearest neighbor interchange (NNI)



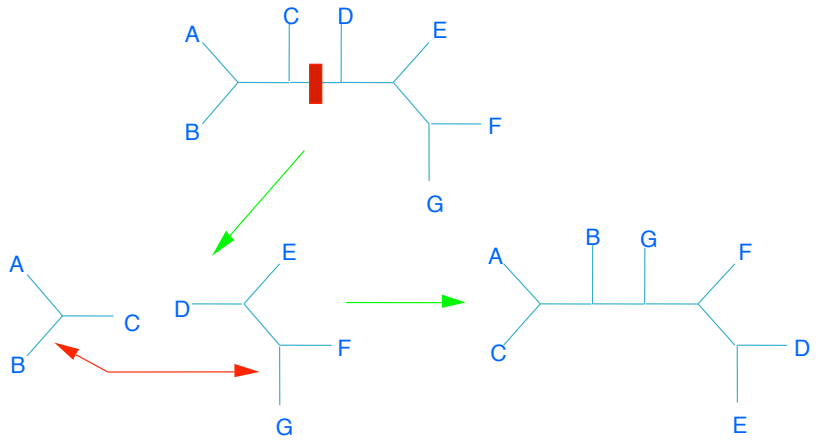
Finding optimal trees – heuristics

Subtree pruning and regrafting (SPR)



Finding optimal trees – heuristics

Tree bisection and reconnection (TBR)

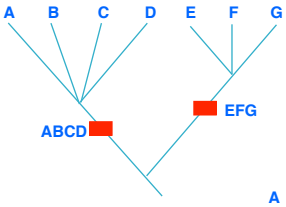


Searching with topological constraints

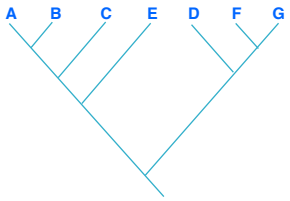
- Topological constraints are user-defined phylogenetic hypotheses
- Can be used to find optimal trees that either:

  1. include a specified clade or set of relationships
  2. exclude a specified clade or set of relationships (reverse constraint)

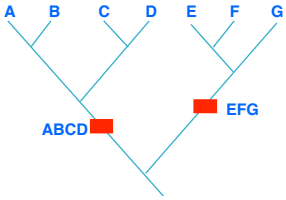
Searching with topological constraints



CONSTRAINT TREE
((A,B,C,D)(E,F,G))



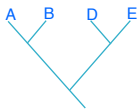
Compatible with reverse constraint tree
Incompatible with constraint tree



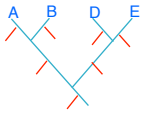
Compatible with constraint tree
Incompatible with reverse constraint tree

Searching with topological constraints backbone constraints

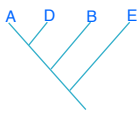
- Backbone constraints specify relationships among a subset of the taxa



BACKBONE CONSTRAINT
((A,B)(D,E))
relationships of taxon C are not specified



/ possible positions of taxon C
Compatible with backbone constraint
Incompatible with reverse constraint



Incompatible with backbone constraint
Compatible with reverse constraint

Consensus methods

Multiple optimal trees

- Many methods can yield multiple equally optimal trees
- We can further select among these trees with additional criteria, but
- Typically, relationships common to all the optimal trees are summarised with *consensus trees*

Consensus methods

- A consensus tree is a summary of the agreement among a set of fundamental trees
- There are many consensus methods that differ in:
  1. the kind of agreement
  2. the level of agreement
- Consensus methods can be used with multiple trees from a single analysis or from multiple analyses

Strict consensus methods

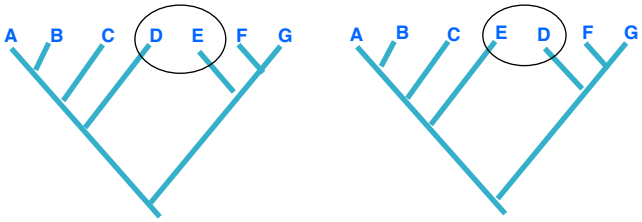
- Strict consensus methods require agreement across all the fundamental trees
- They show only those relationships that are unambiguously supported by the parsimonious interpretation of the data

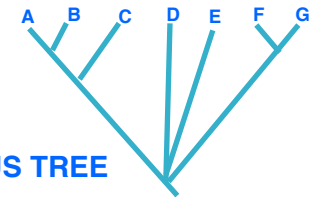
Strict consensus methods

- The commonest method (*strict component consensus*) focuses on clades/components/full splits
- This method produces a consensus tree that includes all and only those full splits found in all the fundamental trees
- Other relationships (those in which the fundamental trees disagree) are shown as unresolved polytomies

Strict consensus methods

TWO FUNDAMENTAL TREES





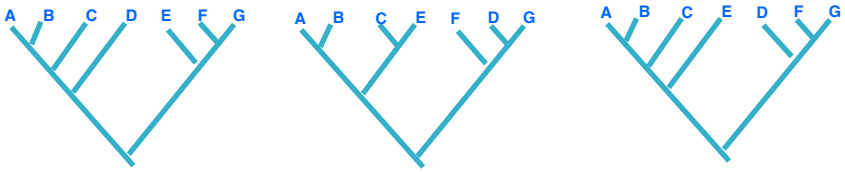
STRICT CONSENSUS TREE

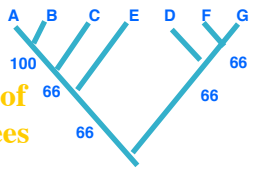
Majority-rule consensus methods

- Majority-rule consensus methods require agreement across a majority of the fundamental trees
- May include relationships that are not supported by the most parsimonious interpretation of the data
- The commonest method focuses on clades/components/full splits
- This method produces a consensus tree that includes all and only those full splits found in a majority (>50%) of the fundamental trees
- Other relationships are shown as unresolved polytomies
- Of particular use in bootstrapping

Majority rule consensus

THREE FUNDAMENTAL TREES





Numbers indicate frequency of clades in the fundamental trees

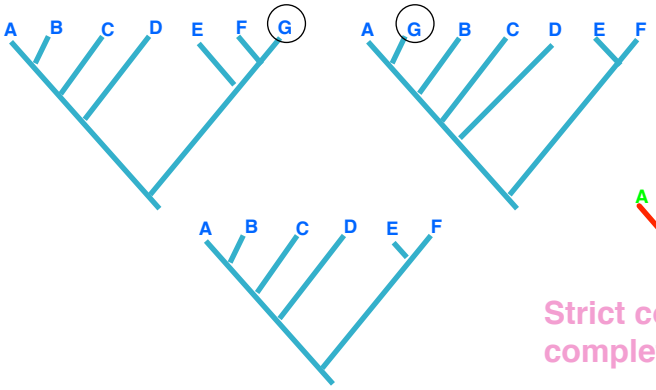
MAJORITY-RULE CONSENSUS TREE

Reduced consensus methods

- Focuses upon any relationships (not just full splits)
- Reduced consensus methods occur in strict and majority-rule varieties
- Other relationships are shown as unresolved polytomies
- May be more sensitive than methods focusing only on clades/components/full splits

Reduced consensus methods

TWO FUNDAMENTAL TREES

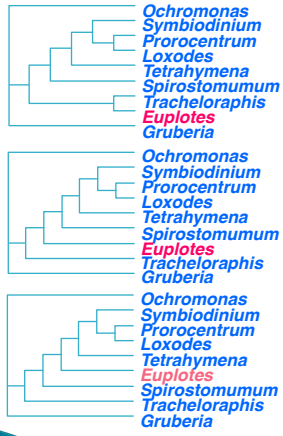


Strict consensus completely unresolved

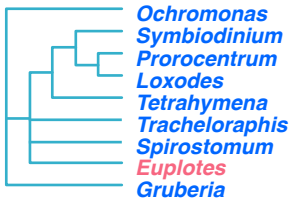
STRICT REDUCED CONSENSUS TREE
Taxon G is excluded

Consensus methods

Three fundamental trees

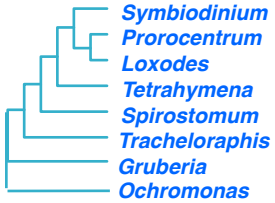


Strict (component)

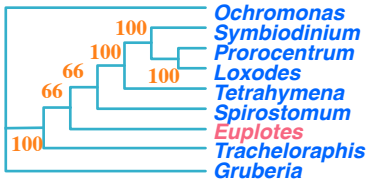


Strict reduced cladistic

Euplotes excluded



Majority-rule



Consensus methods

- Currently majority-rule methods mainly used
  - bootstrapping
  - Bayesian methods
- Reduced methods can be useful to identify problem taxa
- Strict methods mainly used in parsimony analyses
  - rarely used with molecular data

Take home messages from today

- Statements of homology are the basis of phylogenetics
- Alignments of molecular sequences are very strong statements of positional homology
- Finding an optimal tree is not a trivial task