Maximum Likelihood Tree Searching

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(With thanks to Derrick Zwickl, Mark Holder, Dave Swofford and Paul Lewis for some images and slides)

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- Need to maximize the likelihood for each topology

Enormous numbers of topologies to consider

Taxa	Unrooted binary trees	Rooted binary trees
3	1	3
4	3	15
5	15	105
6	105	945
7	945	10,395
8	10,395	135,135
9	135,135	2,027,025
10	2,027,025	3×10^{7}
15	7×10^{12}	2×10^{14}
20	2×10^{20}	8×10^{21}
50	3×10^{74}	
100	2×10^{182}	
1,000	2×10^{2860}	
10,000	8×10^{38658}	
1,000,000	1×10^{5866723}	

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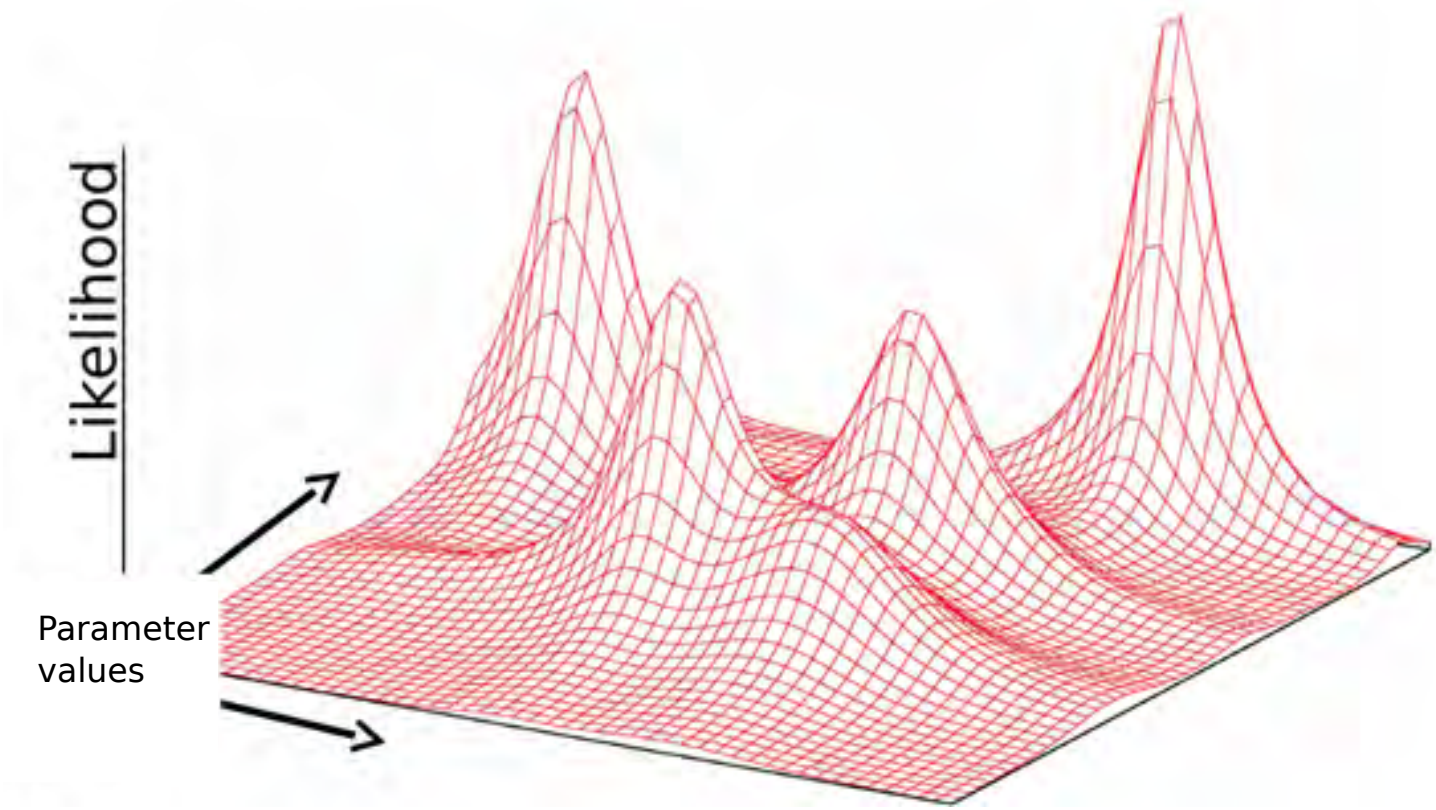
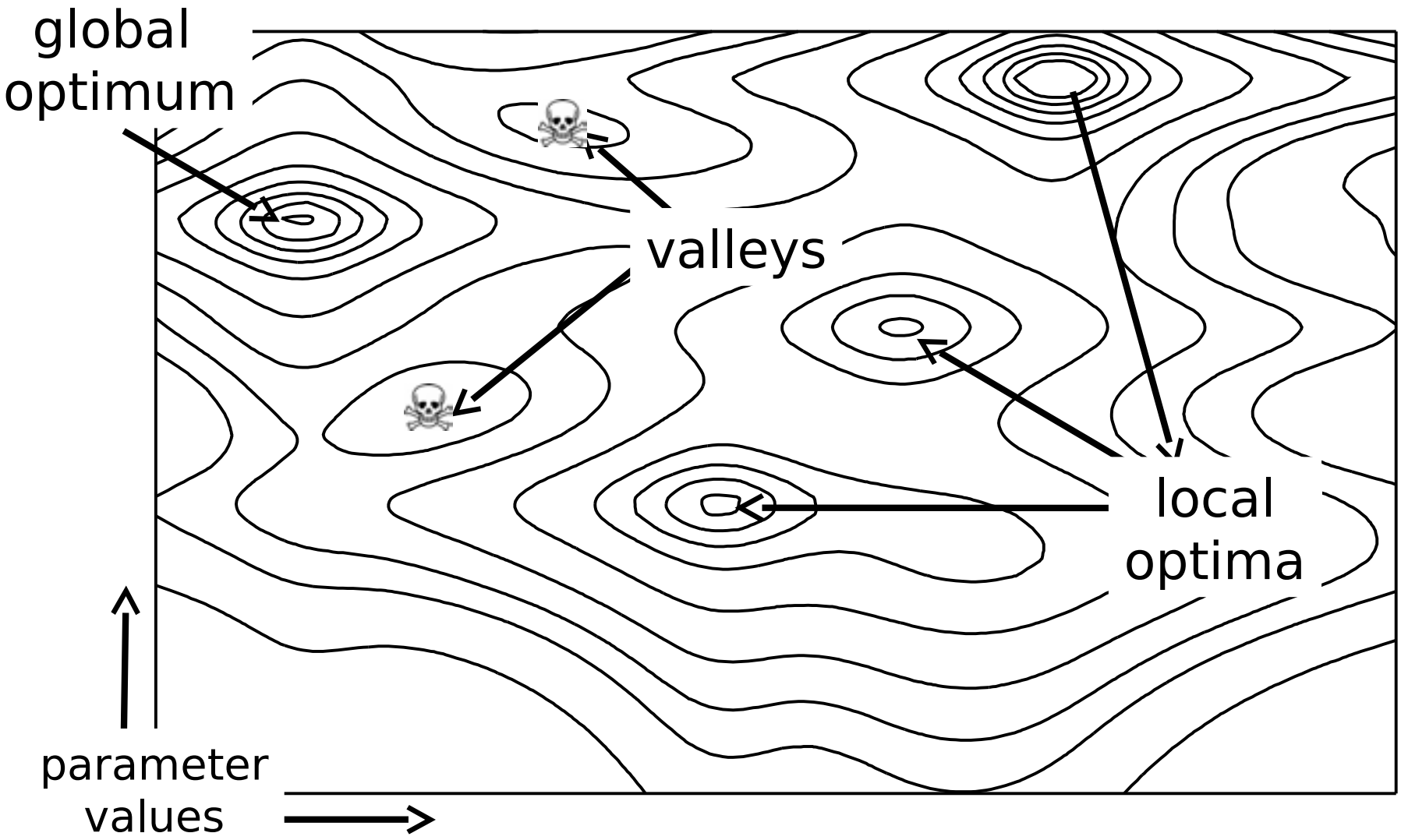
it is estimated that the there are between 10^{78} to 10^{82} atoms in the known, observable universe.

There may be multiple local likelihood optima



(From Zwickl)

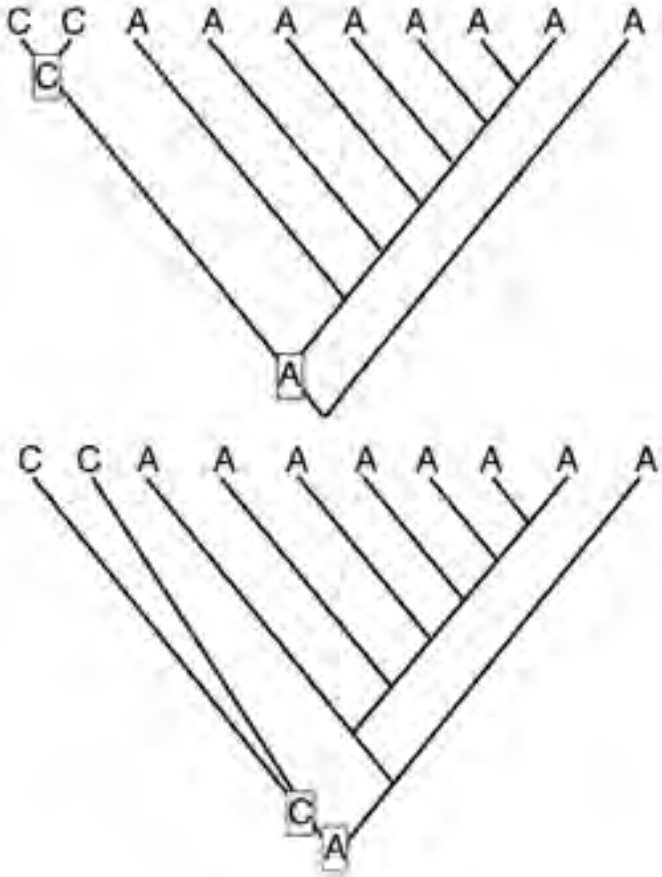
A likelihood surface (from above)



Need to maximize the likelihood for each topology

- Update numerical parameters of the model of sequence evolution
- Branch-length parameters

The Relevance of Branch Lengths



(From Swofford)

Neat widgets created by Mark Holder:

`http://phylo.bio.ku.edu/mephytis/brlen-opt.html`

`http://phylo.bio.ku.edu/mephytis/tree-opt.html`

How do algorithms for Maximum Likelihood phylogenetics estimation solve these problems?

How do you know that you have gotten the ML tree?

How do algorithms for Maximum Likelihood phylogenetics estimation attempt to solve these problems?

How do you know that you have gotten the ML tree? you don't!

How do algorithms for Maximum Likelihood phylogenetics estimation attempt to solve these problems?

How do you know that you have gotten the ML tree? you don't!

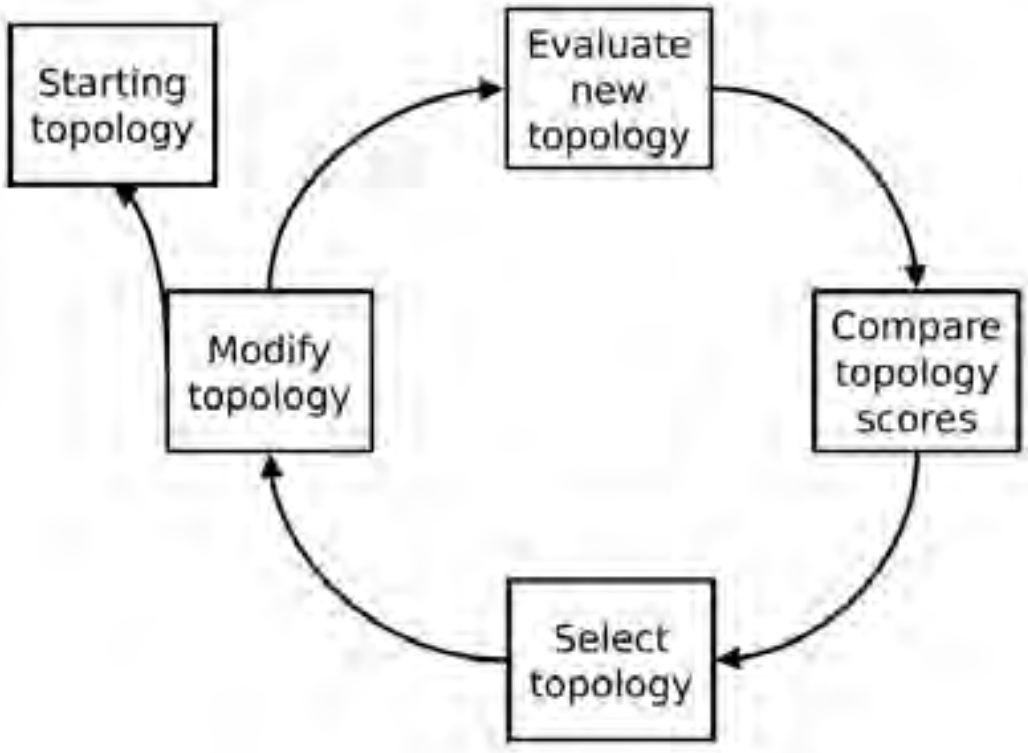
Use a heuristic search to find the best tree you can.

The general concept of heuristic tree search:

1. Start with a tree
2. Calculate the likelihood of that tree given your data (alignment)
3. Look at some trees that are similar
4. Calculate the likelihood for those trees
5. See if you did any better! Return to step 3.

Heuristic runtimes

Inference time = # of topologies to evaluate x time to evaluate each



Both are strongly a function of the # of sequences when calculating maximized likelihood

(From Zwickl)

Questions for a heuristic search:

- Where to start the search?

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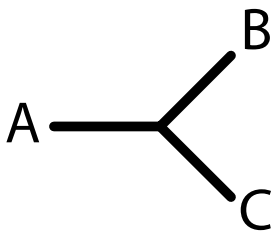
- How do you know if you are done?

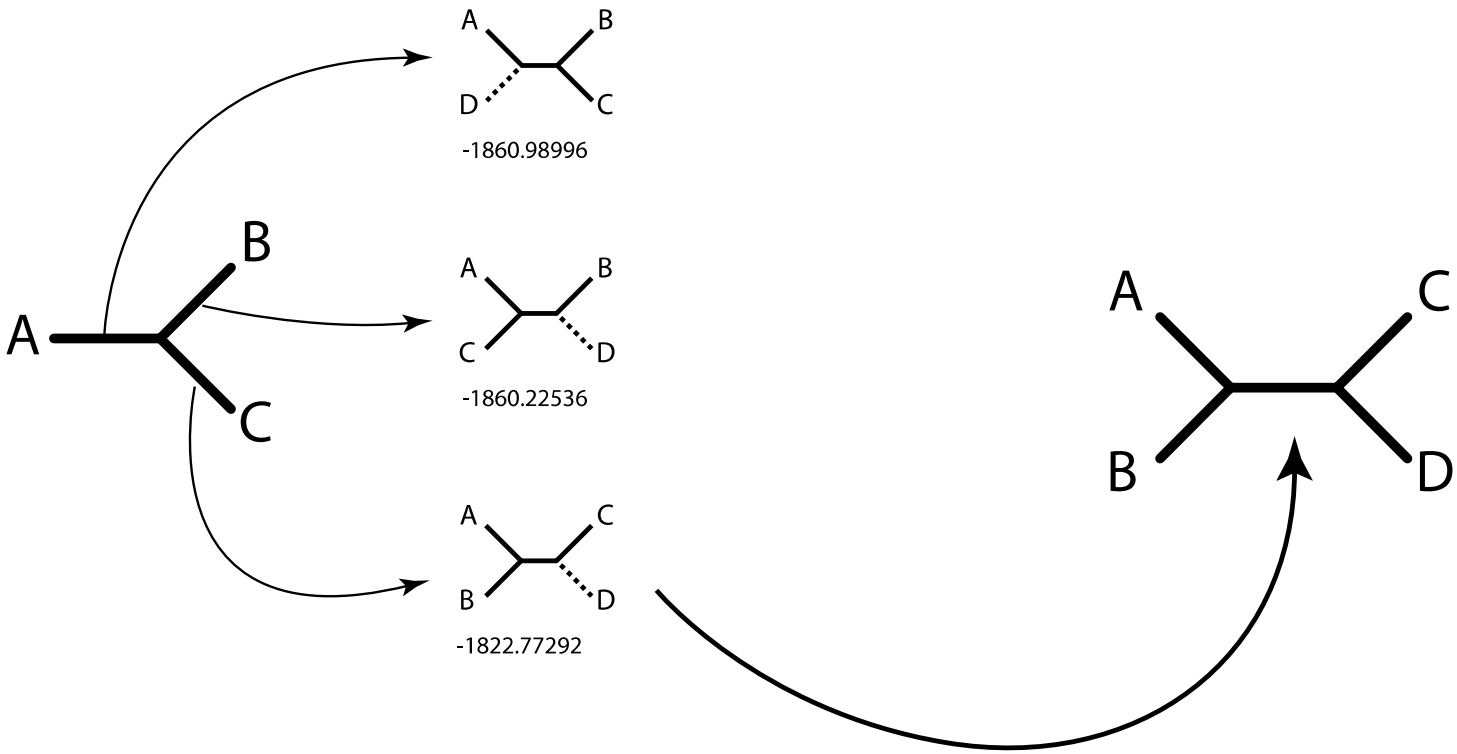
Where to start the search?

- User supplied starting tree
- Star decomposition or Stepwise Addition
- A randomly chosen tree

Stepwise addition

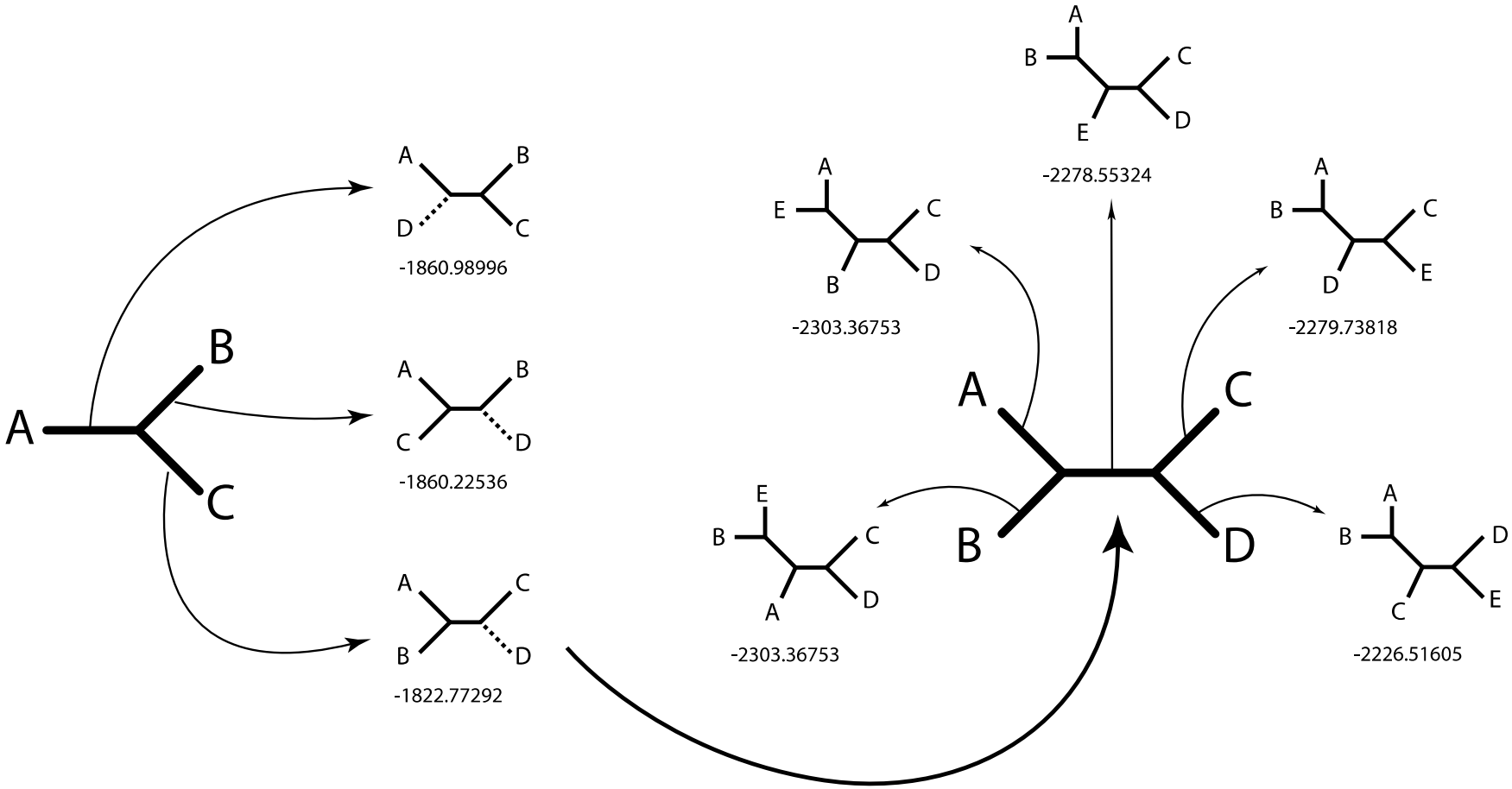


Stepwise addition



(slide from POL)

Stepwise addition



(slide from POL)

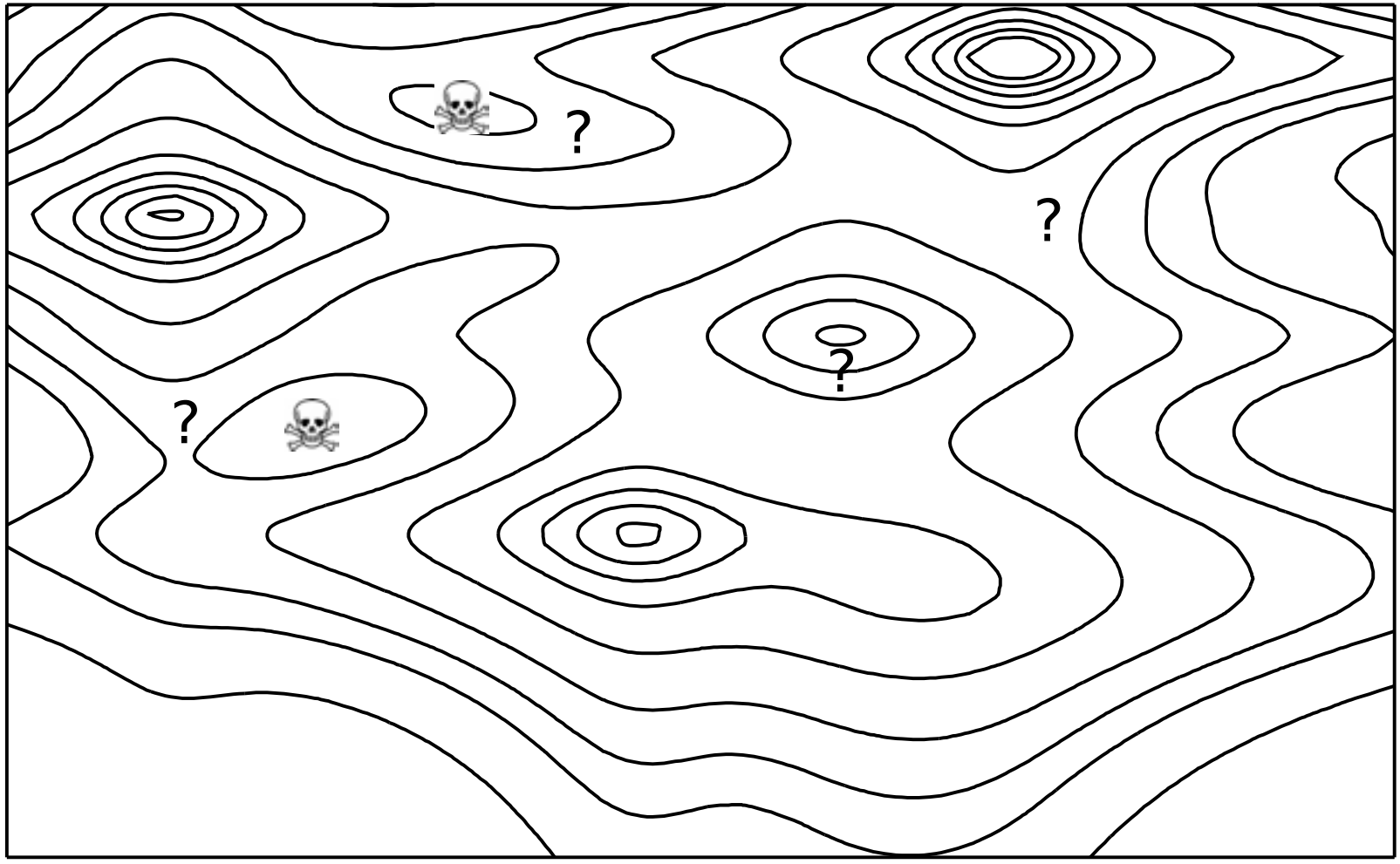
Stepwise addition:

Greedy, but can introduce a new taxon on path between two taxa
Relatively fast way to generate a not-terrible starting tree
Can depend on input order of taxa

Does your starting tree matter?

- Can help escape local optima
- When data is uninformative, bias in starting tree can affect estimate

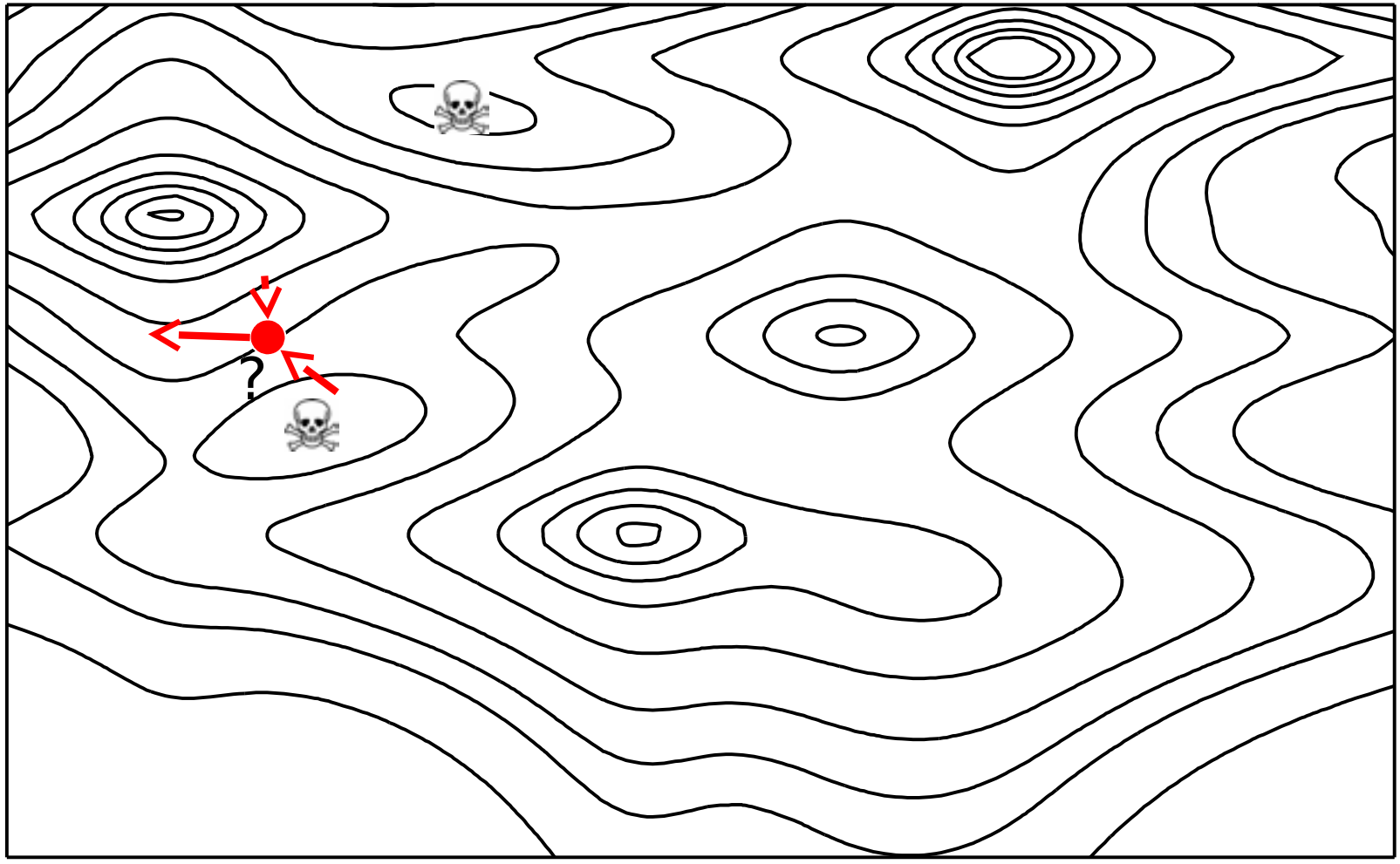
Heuristics: starting point



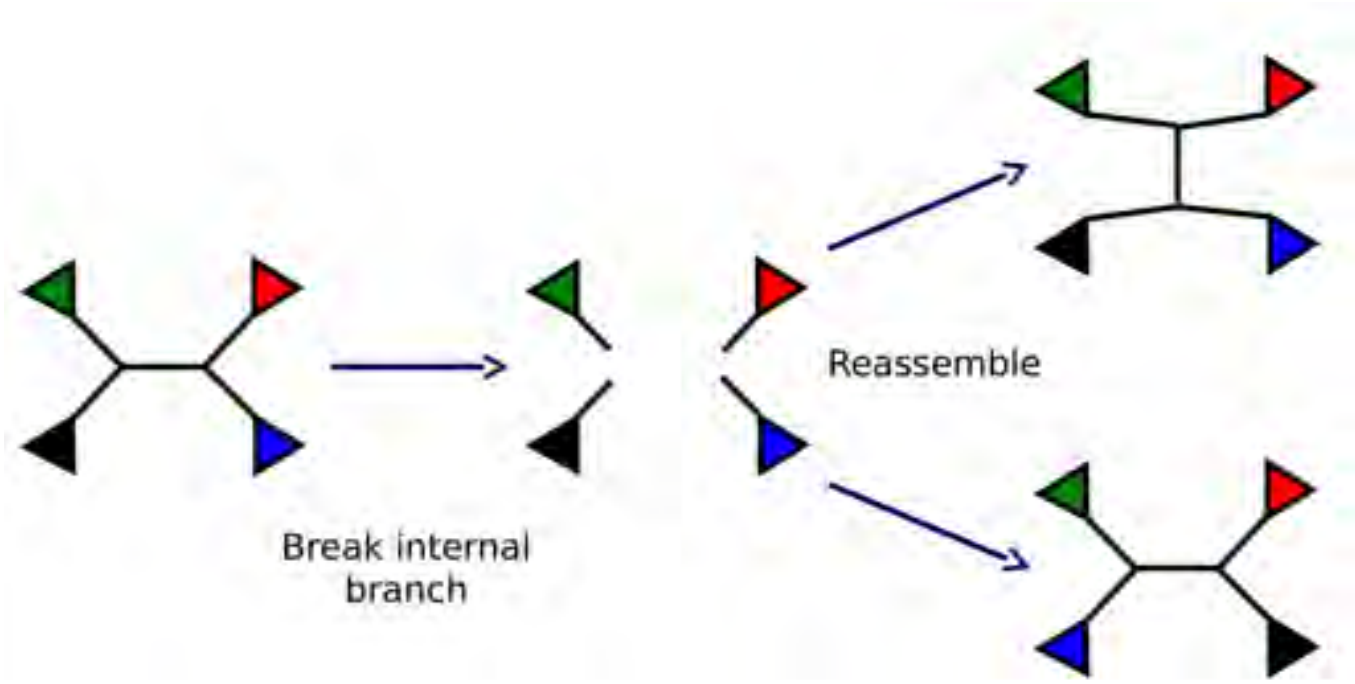
How are new topologies proposed?

- Branch swapping and tree rearrangement

Heuristics: proposing new values

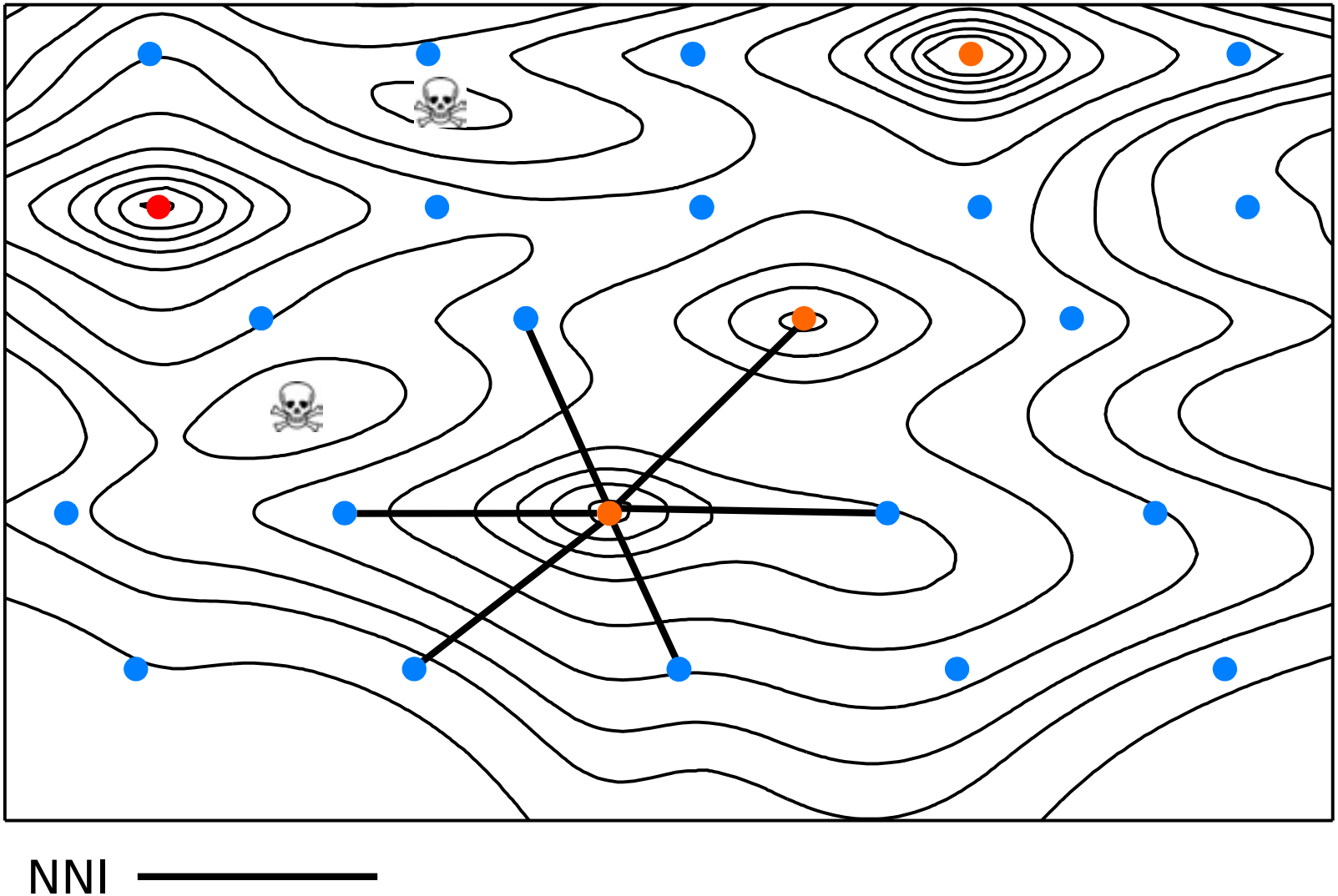


Nearest neighbor interchange (NNI)

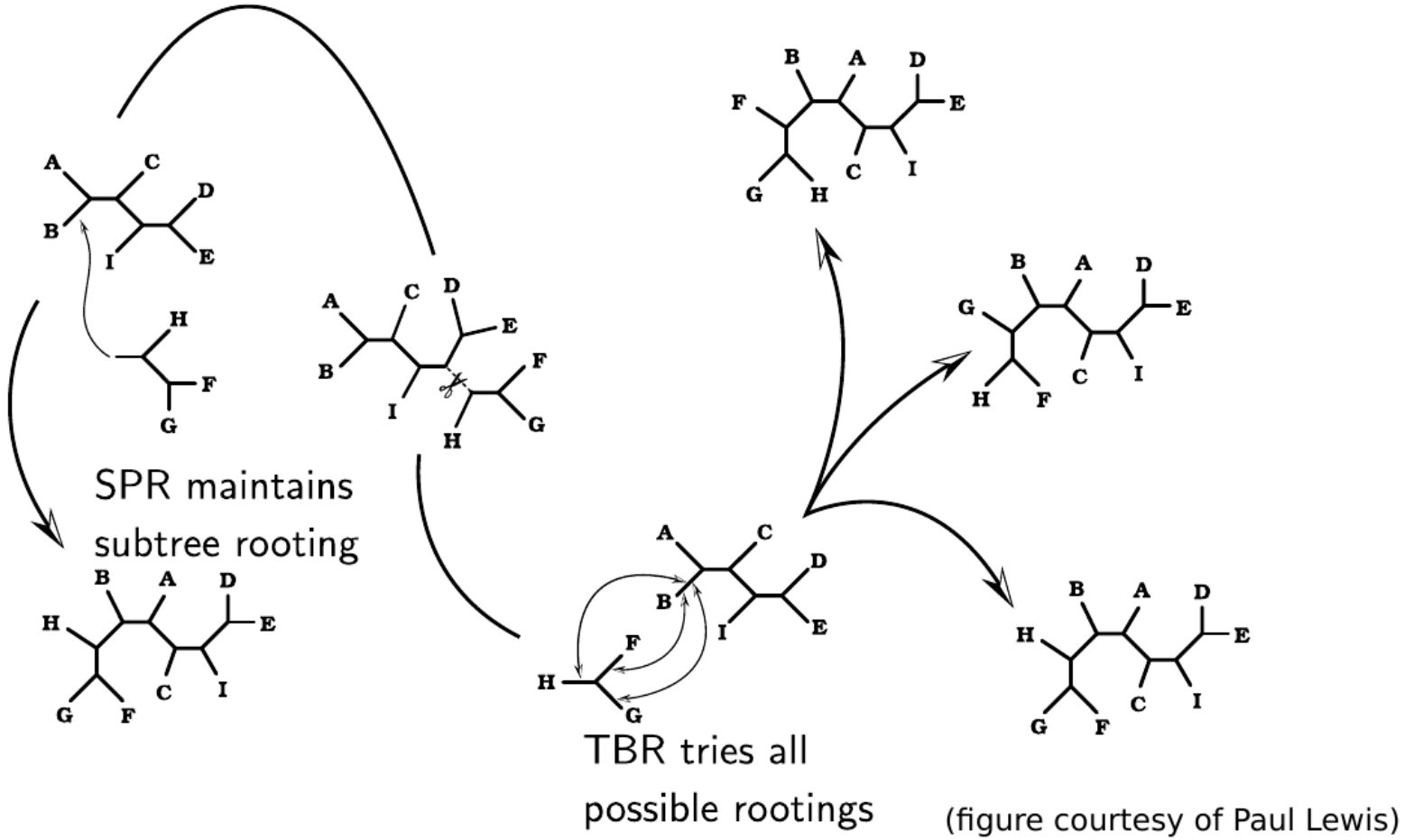


(From Zwickl)

NNI Treespace

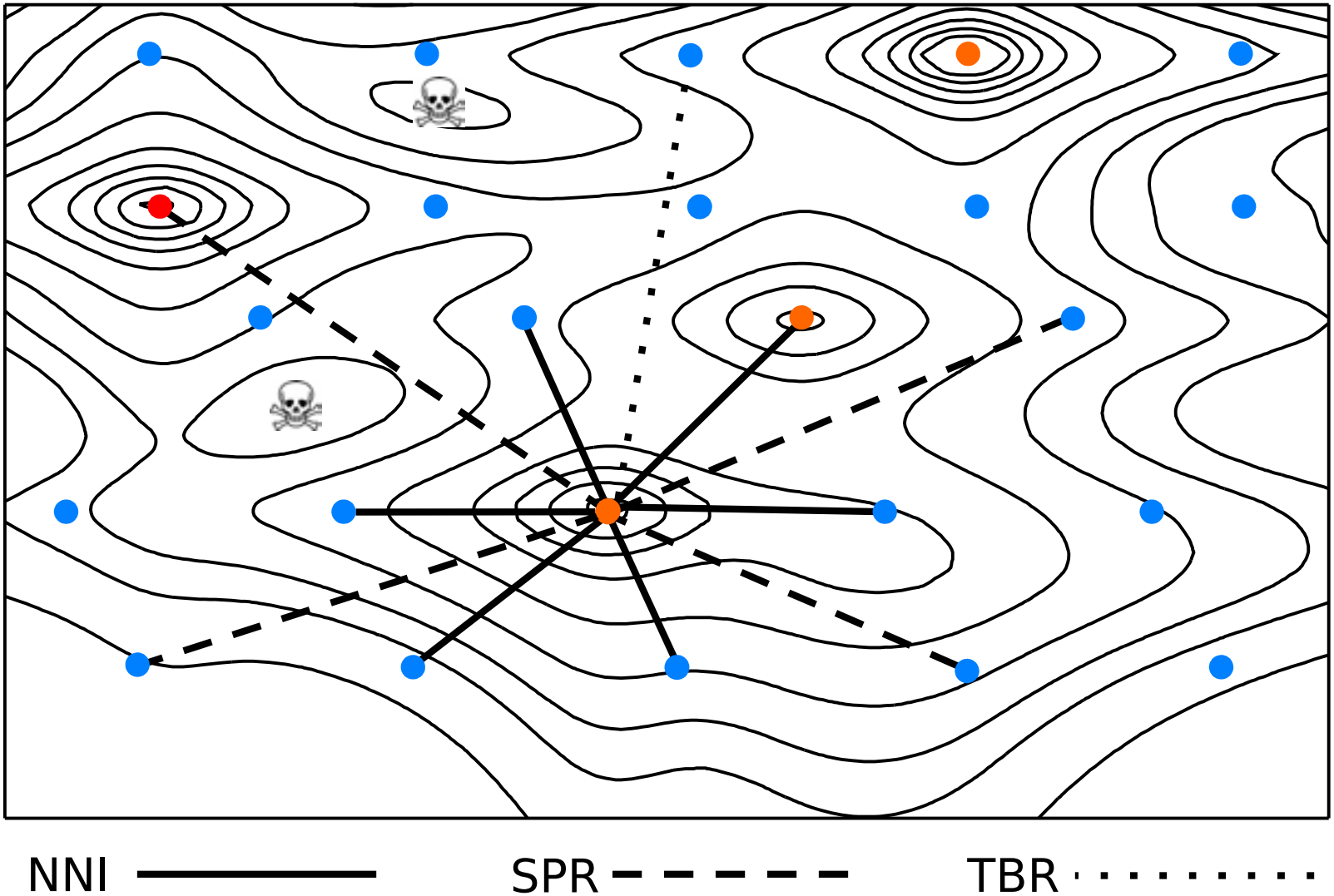


Subtree Pruning Regrafting (SPR) Tree Bisection Reconnection (TBR)



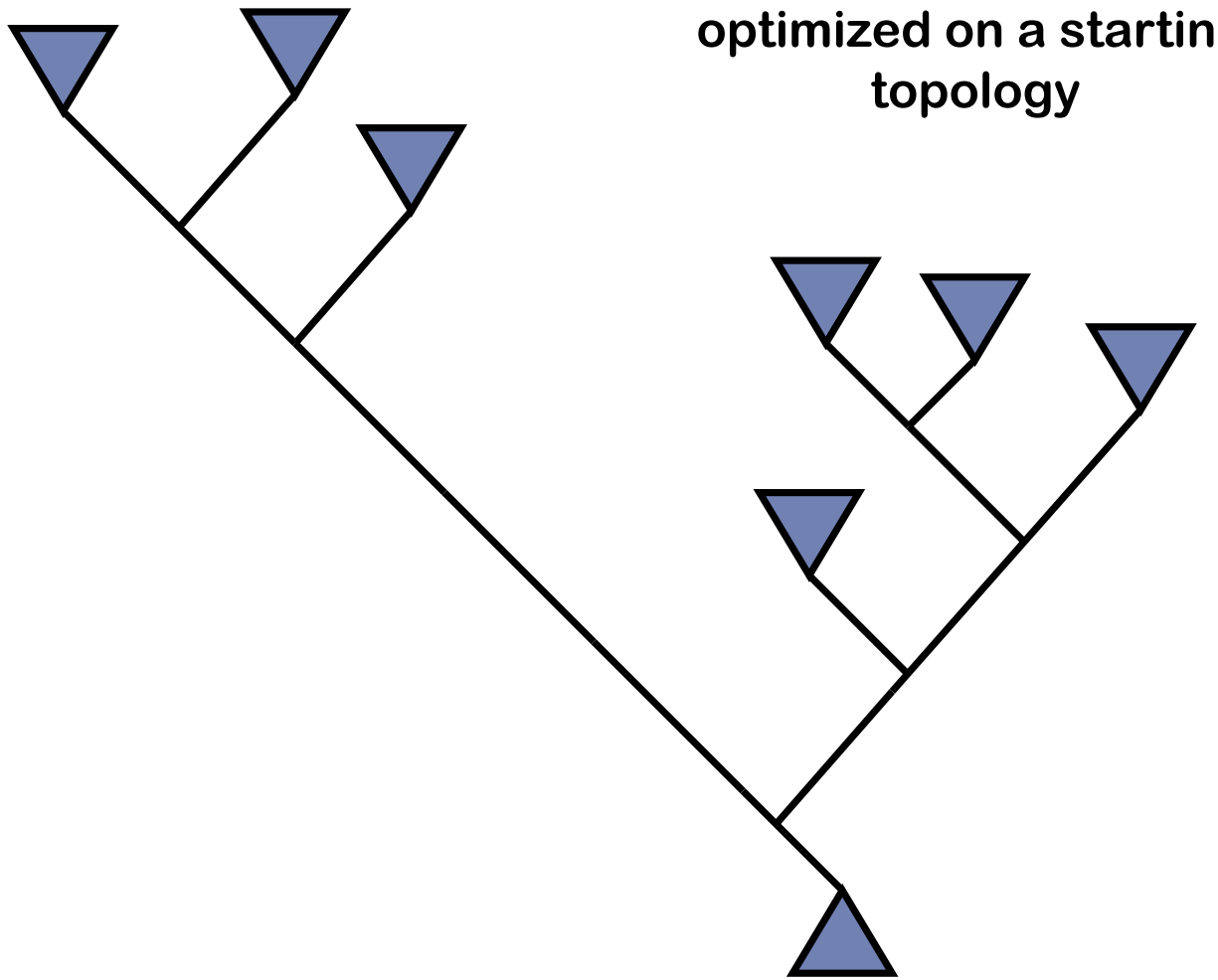
(figure courtesy of Paul Lewis)

SPR/TBR moves in NNI treespace

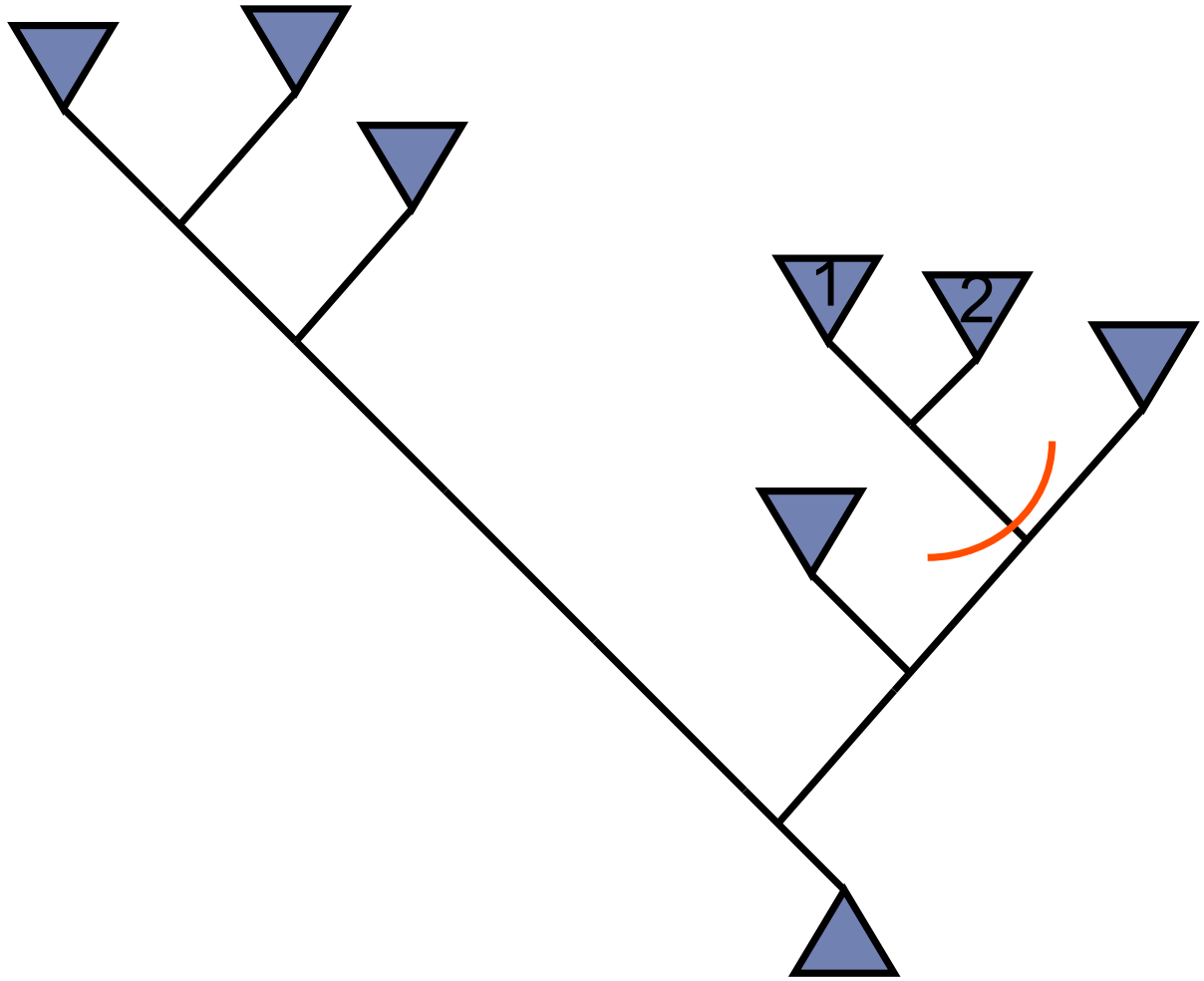


Re-arranging your tree requires updating branch lengths and evolutionary model parameters.

Searching with approximate likelihoods



Altering the tree: subtree pruning-regrafting (SPR)



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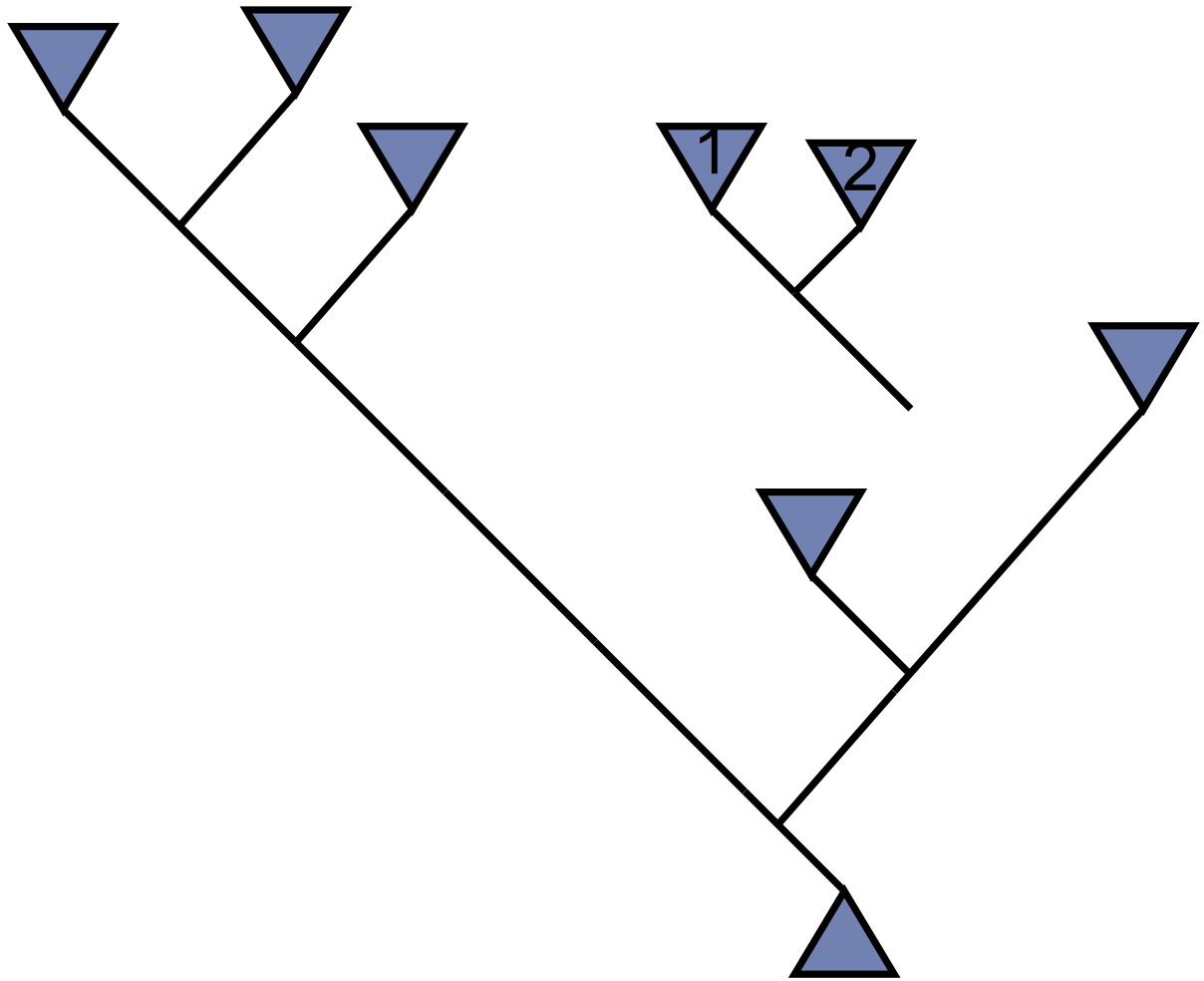

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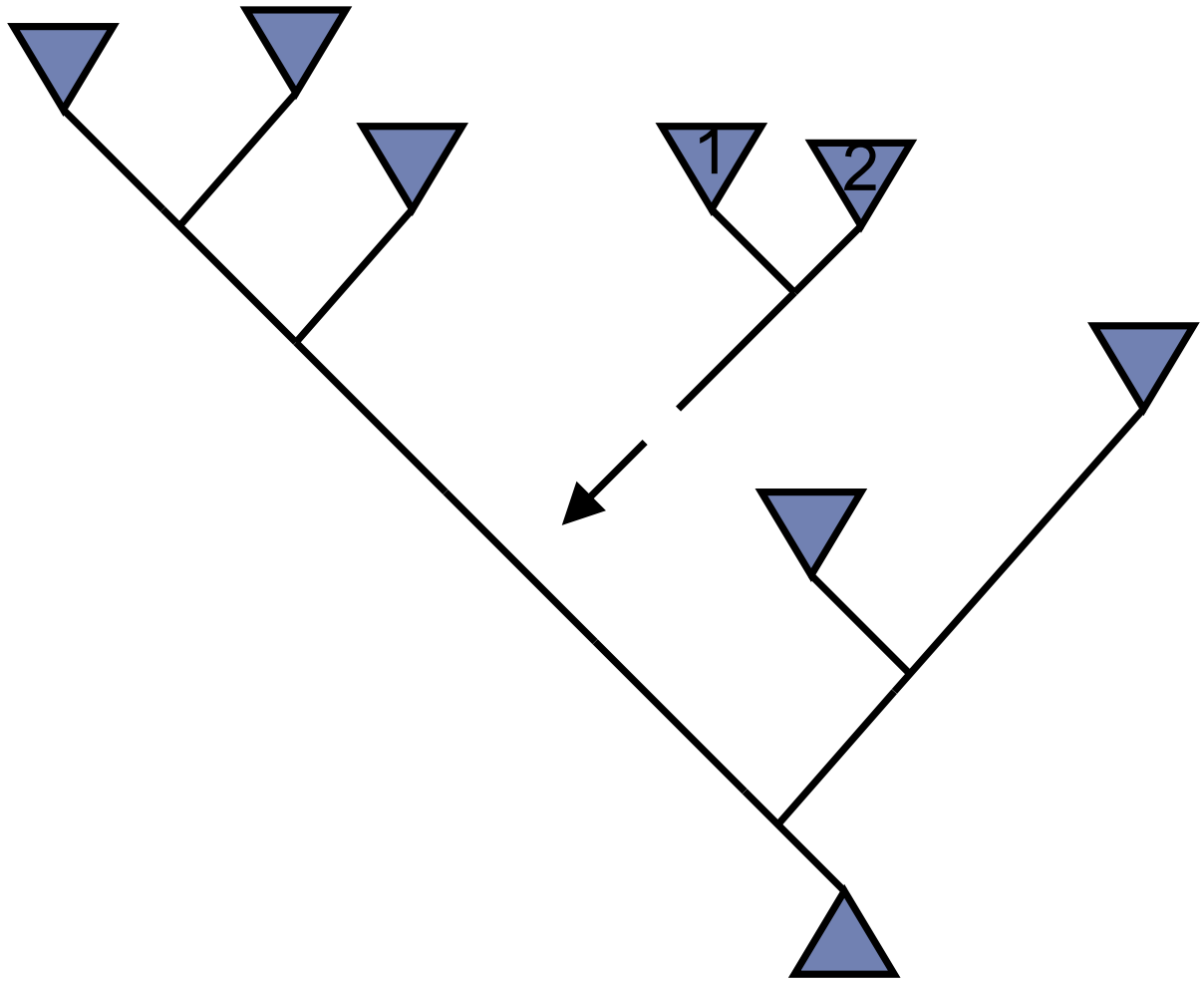
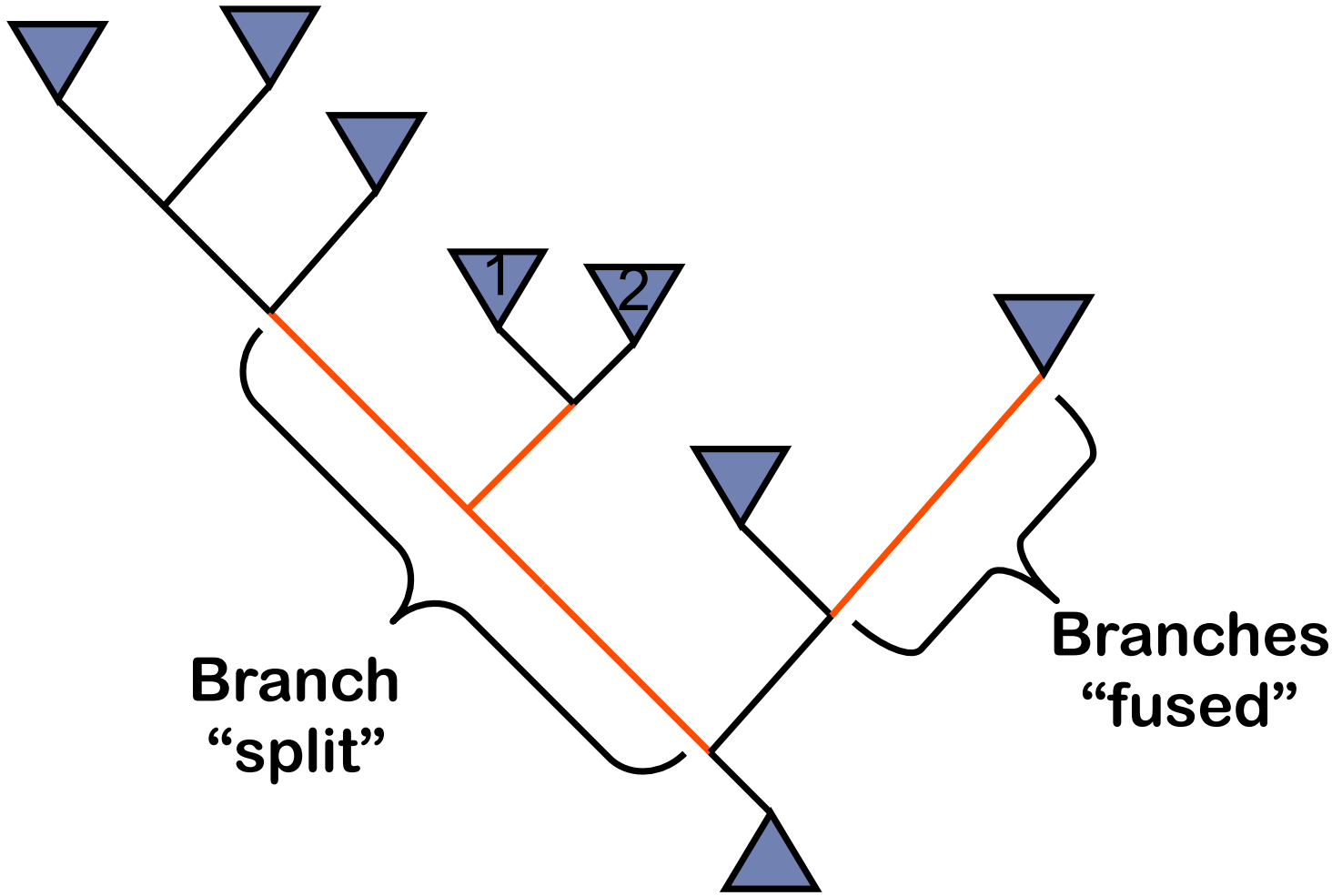


Scoring and optimizing the new topology



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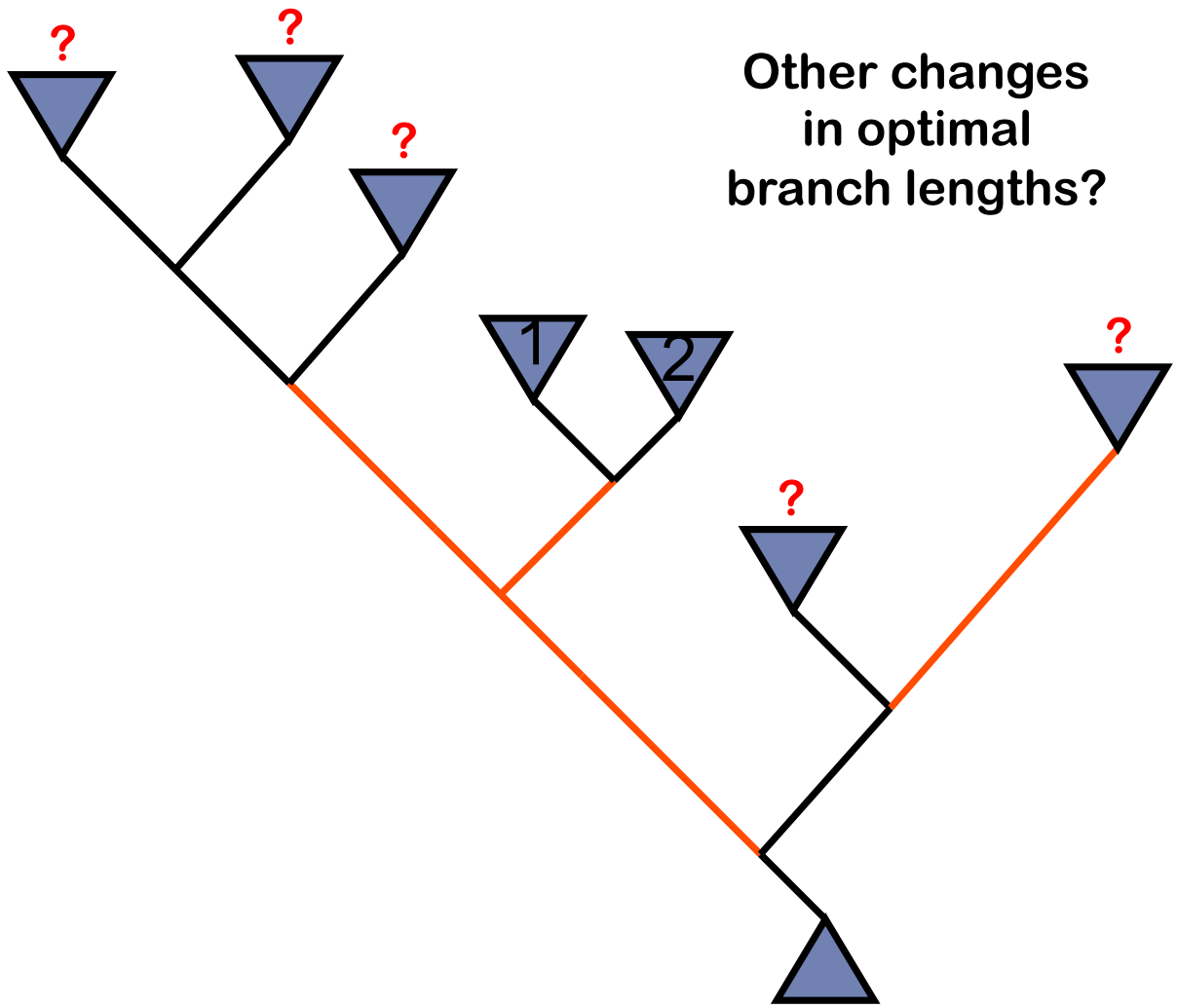

Localizing branch length optimization important for speed of analysis

How do you decide if you should accept a new tree?

- Hill climbing: likelihood score is better (RAxML)
- Computational analog of evolution by natural selection (Garli)

How do you know if you are done?

- Stop tree search when likelihood stops improving.

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- Continue searching until you run at least one additional search that finds the same topology as the best overall result.

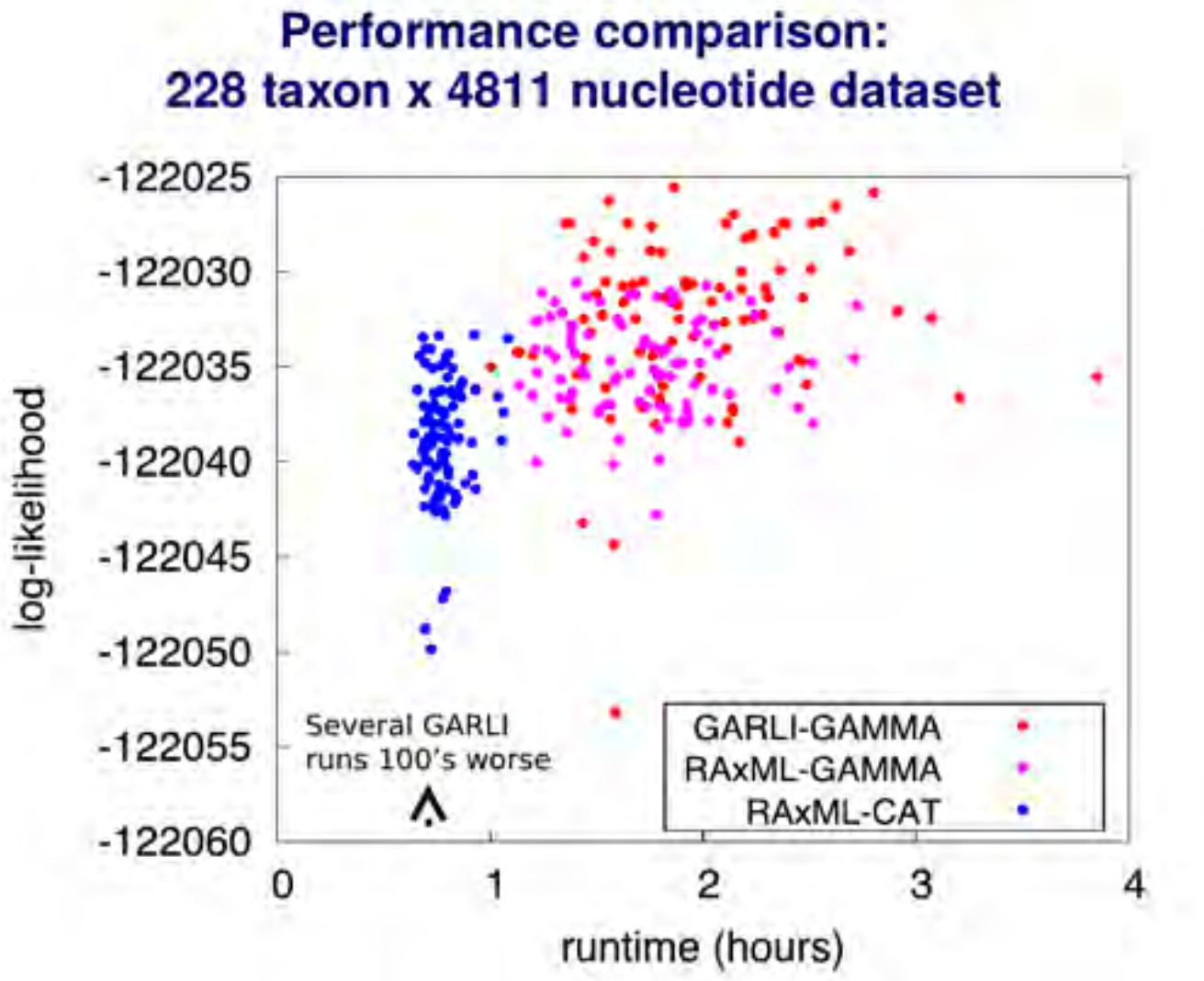
In lab today we will discuss and apply two software packages that estimate ML trees

- Garli (Zwickl, 2006)
  - Stochastic, genetic algorithm-like approach
  - Computational analog of evolution by natural selection.
- RAxML (Stamatakis, 2006)
  - Hill-climbing algorithm
  - GTR+CAT approximation provides speedup over GTR+G
    - For modeling rate heterogeneity across very large trees (e.g., hundreds of taxa), and is not recommended for smaller trees.
    - Different than Lartillot CAT model using empirical amino acid profiles (named independently around same time)

ML tree inference software:
For small datasets (< 50 taxa), all of the ML tree inference programs perform well
For large datasets (hundreds of sequences):

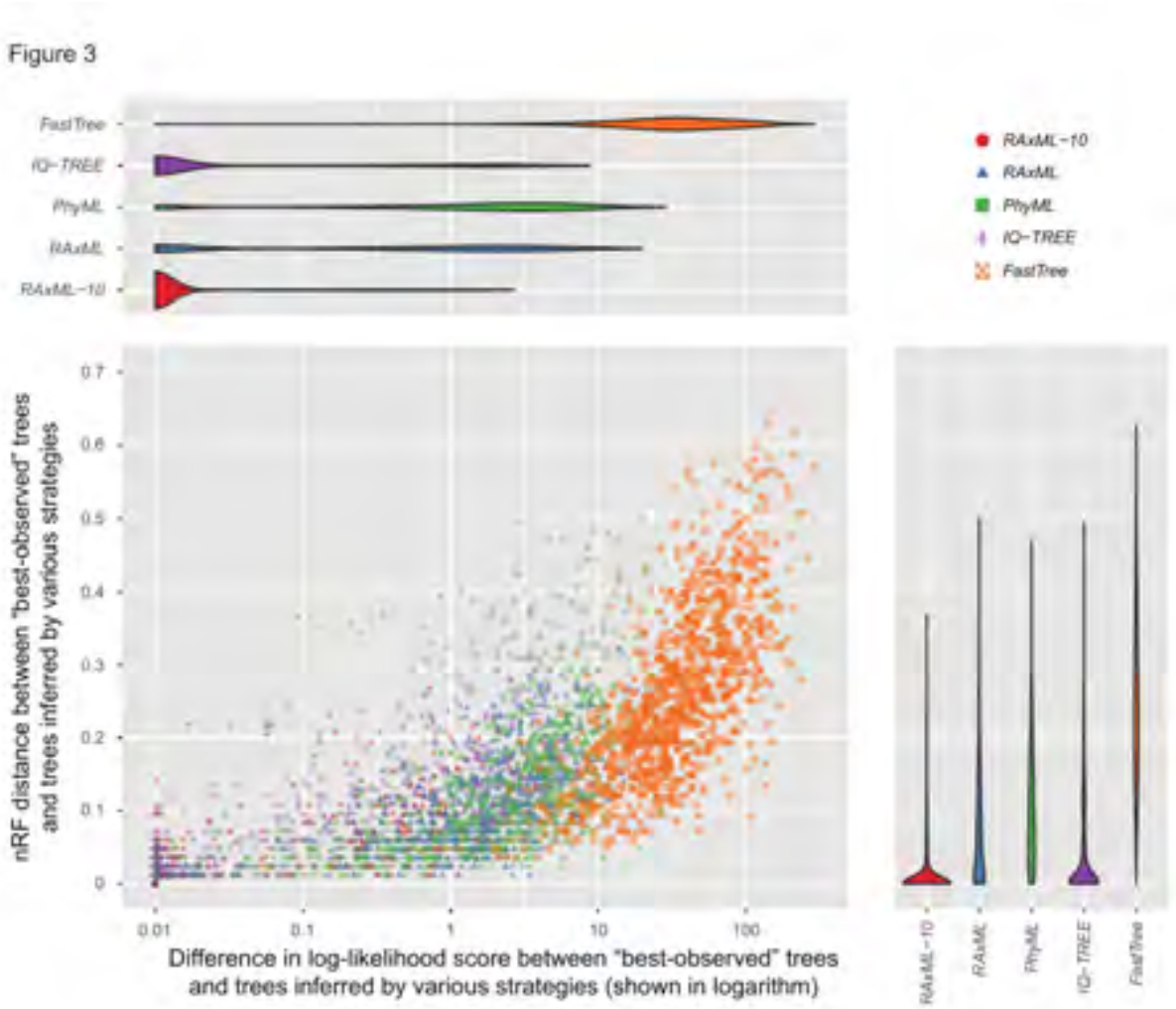
- PAUP* is very rigorous, but slower
- RAxML is generally the fastest
- GARLI often has a slight edge over RAxML in optimality (although often more variability)

Simulations by Zwickl (Garli)



ML tree inference software:
For VERY large datasets (1000+sequences):

- RAxML/EXaML (Kozlov et al., 2015) is very efficient, especially with multiple runs
- IQ-TREE (Nguyen et al., 2015) also fast and accurate (will be presented Tuesday by Bui Quang Minh)
- FASTTREE(Price et al., 2009) is very fast, but (excessive) tradeoffs with accuracy (per Zhou et al. (2017))



Log-likelihood score differences between inferred trees and "best-observed" trees plotted against topological distances.(Zhou et al., 2017)

Your maximum likelihood tree is a best estimate of the relationships, given your data (alignment) and model.

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How much does the differences between trees with similar likelihoods matter?
This can be a challenging statistical question - in depth discussion in chapter posted at
`https://molevol.mbl.edu/images/b/bb/Tree-hyp-testing.pdf`
(Holder and McTavish, 2016)

Measuring differences between trees:

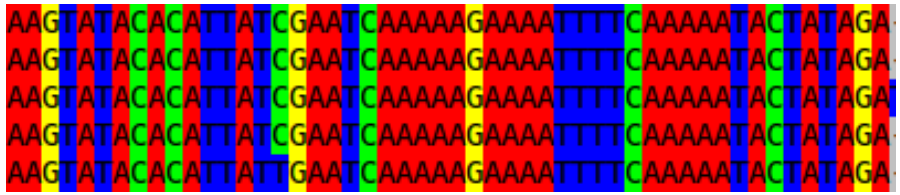
- Robinson–Foulds (RF) distance: (A + B) where A is the number of splits in the first tree but not the second tree and B is the number of splits second tree but not the first tree.
- Weighted RF distance: RF distance weighted by edge lengths. Missing split is counted as length 0.

It is important to consider and attempt to correct for biases in your tree estimation.
Some examples:

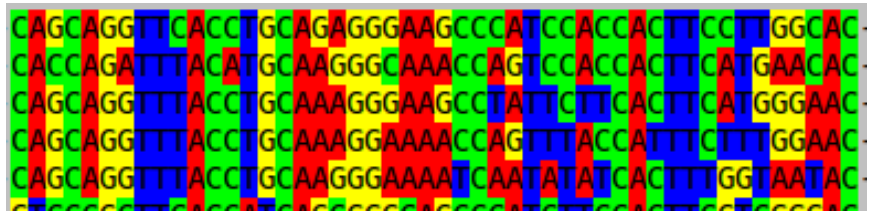
- bias in data collection (ascertainment bias)
- model adequacy
- confirmation bias

How surprised are you?

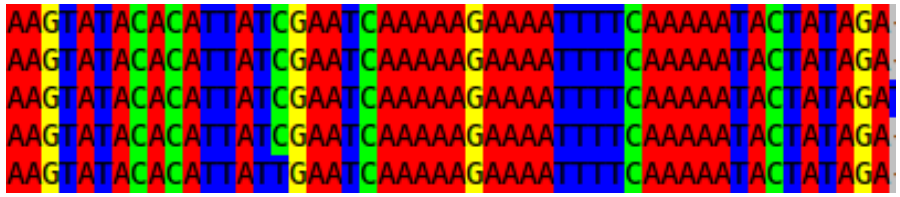
Aln 1



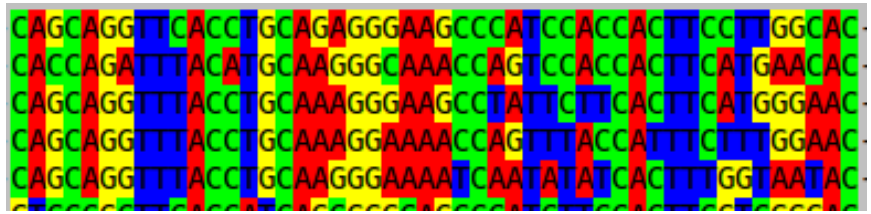
Aln 2



Short Tree



Long Tree



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Some data processing generates alignments of exclusively variable sites.
Extension of Lewis (2001) model to correct for this is implemented in RAxML

Ascertainment bias

A bias in parameter estimation or testing caused by non-random sampling of the data.

'Ascertainment bias' in a broad sense, covers 'selection bias' or 'acquisition bias', all three terms have been used for overlapping issues.

"it is possible in many cases to correct the ascertainment bias relatively easily, if reliable information is available regarding the details of the ascertainment scheme." Nielsen (2004)

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this information is not always available

Despite the large volume of data in genomic studies, ascertainment bias is still an issue

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“ Increasing data set size may reduce stochastic error, but it can also exacerbate systematic error and lead to high confidence in erroneous phylogenies”
(Richards et al., 2018)

Model adequacy

No matter how thorough your tree search is, or how big you data set is, if your model is inadequate, you can get the wrong tree with very high confidence.

Selection of models in phylogenetics often relies on comparison among models

Selection of models in phylogenetics often relies on comparison among models
but the best fitting model from a set of models can still be inadequate!

'the true model is always more complex than any model in our set of models' - Swofford

Several papers published already this year on evaluating model adequacy.
(Brown and Thomson, 2018; Richards et al., 2018; Duchêne et al., 2018)

More on model adequacy tomorrow evening with Michael Landis.

Confirmation bias:

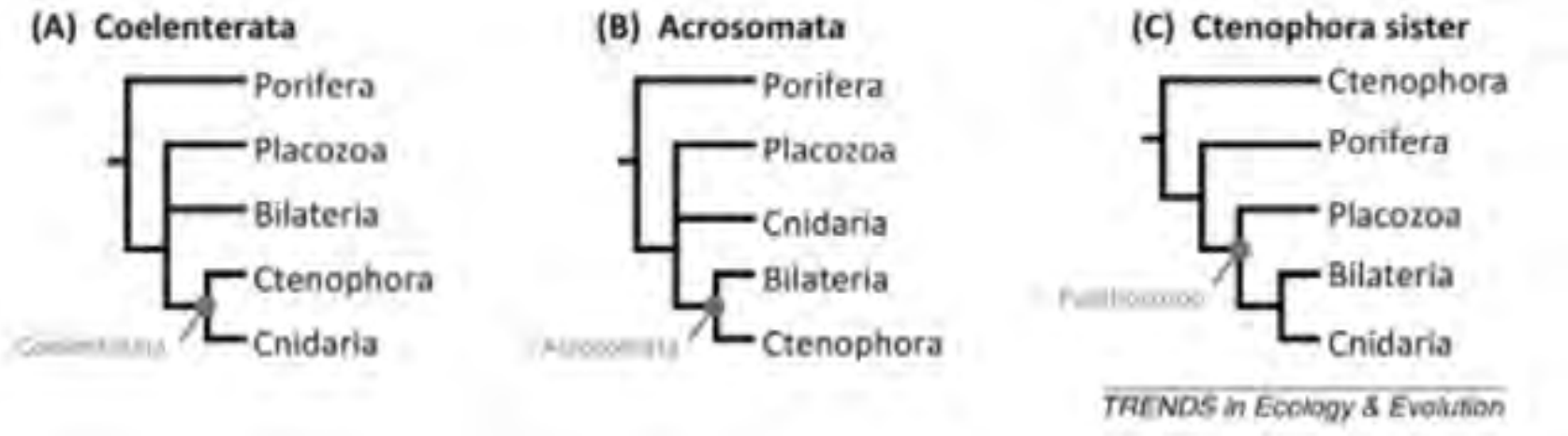
Often datasets, methods, and models are only re-checked if your conclusions don't match your expectations.

Confirmation bias:

Often datasets, methods, and models are only re-checked if your conclusions don't match your expectations.

This means wrong results that fit your assumptions, or wrong results with exciting interpretations,
are more likely to be published!

Confirmation bias:



Dunn et al. (2015)

'Phylotocol'

- New approach to decreasing confirmation bias and increasing reproducibility
- Document in advance all the planned methods and analyses
- Publish edits to strategy and motivation for changes
- Modeled after the NIH clinical trial template

(DeBiasse and Ryan, 2018)

Summary:

- For >15 sequences, an unfathomably large number of trees are possible.
- We have to rely on heuristics that are not guaranteed to find the actual ("global") optimal solution.
- We have control on how thorough our searches are
- Conduct multiple searches to look for evidence that you are not finding trees which are local optima.
- Consider and address potential biases in your analyses

Questions?

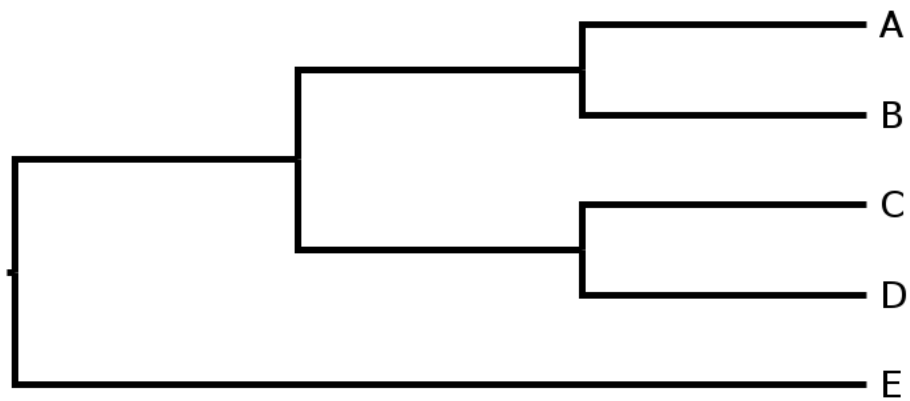
Computer lab:

- Perform ML phylogenetics search
- Compare searches and trees
- Work with variety of phylogenetic software and file formats
- Bootstrapping
- Consequences of model misspecification
- Analyzing data on shared cluster

a brief digression into file formats

Newick

- Parenthetical tree format
- Rooted vs. unrooted trees are not differentiated
- Some programs interpret polytomy at root as ‘unrooted
- Branches and nodes not well differentiated
- A name can contain and characters except blanks, colons, semicolons, parentheses, and square brackets




```
(((A,B),(C,D)),E);
```

Nexus

- Starts with #nexus
- Can contain blocks of alignments, trees, commands, and more!
- Blocks between ‘begin’ and ‘end’
- Trees in Newick format, prepended with [&U] unrooted or [&R] rooted

Nexus

```
#nexus
...
begin taxa;
  dimensions ntax=5;
  taxlabels
    Giardia
    Thermus
    Deinococcus
    Sulfolobus
    Haobacterium
  ;
end;
```

```
#nexus
...
begin data;
  dimensions ntax=5 nchar=54;
  format datatype=dna missing=? gap=-;
  matrix
    Ephedra       TTAAGCCATGCATGTCTAAGTATGAACTAATTCCAAACGGTGAAACTGCGGATG
    Gnetum        TTAAGCCATGCATGTCTATGTACGAACTAATC-AGAACGGTGAAACTGCGGATG
    Welwitschia   TTAAGCCATGCACGTGTAAGTATGAACTAGTC-GAAACGGTGAAACTGCGGATG
    Ginkgo        TTAAGCCATGCATGTGTAAGTATGAACTCTTTACAGACTGTGAAACTGCGAATG
    Pinus         TTAAGCCATGCATGTCTAAGTATGAACTAATTGCAGACTGTGAAACTGCGGATG
                 [----+--10|----+--20|----+--30|----+--40|----+--50|----]
  ;
end;
```

http://hydrodictyon.eeb.uconn.edu/eebedia/index.php/Phylogenetics:_NEXUS_Format

NeXML

- Phylogenetic data as XML
- Can capture all information from Nexus
- Full semantic annotation
- Easily extensible

NeXML

Computer readable, but not very human readable

```
 <otu about="#otu99" id="otu99" label="Parupeneus barberinoides">
  <meta datatype="xsd:string" property="ot:originalLabel" xsi:type="nex:LiteralMeta">Parupeneus
  <meta datatype="xsd:int" property="ot:ottId" xsi:type="nex:LiteralMeta">758968</meta>
  <meta datatype="xsd:string" property="ot:ottTaxonName" xsi:type="nex:LiteralMeta">Parupeneus b
 </otu>
</otus>
<trees about="#trees1" id="trees1" otus="otus1">
 <tree about="#tree1" id="tree1" label="Untitled (tree1)" xsi:type="nex:FloatTree">
  <meta datatype="xsd:string" property="ot:branchLengthDescription" xsi:type="nex:LiteralMeta"/>
  <meta datatype="xsd:string" property="ot:branchLengthMode" xsi:type="nex:LiteralMeta">ot:undef
  <meta datatype="xsd:string" property="ot:curatedType" xsi:type="nex:LiteralMeta">Bayesian infe
  <meta datatype="xsd:string" property="ot:inGroupClade" xsi:type="nex:LiteralMeta">node2</meta>
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  <meta datatype="xsd:string" property="ot:nodeLabelTimeUnit" xsi:type="nex:LiteralMeta"/>
  <meta datatype="xsd:string" property="ot:outGroupEdge" xsi:type="nex:LiteralMeta"/>
  <meta datatype="xsd:string" property="ot:specifiedRoot" xsi:type="nex:LiteralMeta">node1</meta
  <meta datatype="xsd:boolean" property="ot:unrootedTree" xsi:type="nex:LiteralMeta">false</meta
  <node about="#node1" id="node1" root="true"/>
  <node about="#node2" id="node2"/>
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  <node about="#node153" id="node153"/>
  <node about="#node154" id="node154"/>
  <node about="#node155" id="node155" otu="otu72">
   <meta datatype="xsd:boolean" property="ot:isLeaf" xsi:type="nex:LiteralMeta">true</meta>
  </node>
  <node about="#node156" id="node156" otu="otu73">
   <meta datatype="xsd:boolean" property="ot:isLeaf" xsi:type="nex:LiteralMeta">true</meta>
  </node>
  <node about="#node157" id="node157" otu="otu74">
   <meta datatype="xsd:boolean" property="ot:isLeaf" xsi:type="nex:LiteralMeta">true</meta>
  </node>
  <node about="#node158" id="node158"/>
  <node about="#node159" id="node159" otu="otu75">
   <meta datatype="xsd:boolean" property="ot:isLeaf" xsi:type="nex:LiteralMeta">true</meta>
  </node>
```

Phylip (sequence data format)

- First line must be two integers: <number of taxa> <number of sites>
- Sequence ID followed by spaces up to 10 char. (will trunctate names, often resulting in idential names)
- No duplicate names
- Relaxed phylip up to 250 characters followed by a space

```
      5    42
Turkey    AAGCTNGGGC ATTTCAGGGT GAGCCCGGGC AATACAGGGT AT
Salmo gairAAGCCTTGGC AGTGCAGGGT GAGCCGTGGC CGGGCACGGT AT
H. SapiensACCGGTTGGC CGTTCAGGGT ACAGGTTGGC CGTTCAGGGT AA
Chimp     AAACCCTTGC CGTTACGCTT AAACCGAGGC CGGGACACTC AT
Gorilla   AAACCCTTGC CGGTACGCTT AAACCATTGC CGGTACGCTT AA
```

Phylip interleaved

```
  5    42
Turkey    AAGCTNGGGC ATTTCAGGGT
Salmo gairAAGCCTTGGC AGTGCAGGGT
H. SapiensACCGGTTGGC CGTTCAGGGT
Chimp     AAACCCTTGC CGTTACGCTT
Gorilla   AAACCCTTGC CGGTACGCTT

GAGCCCGGGC AATACAGGGT AT
GAGCCGTGGC CGGGCACGGT AT
ACAGGTTGGC CGTTCAGGGT AA
AAACCGAGGC CGGGACACTC AT
AAACCATTGC CGGTACGCTT AA
```

Phylip sequential

```
  5    42
Turkey    AAGCTNGGGC ATTTCAGGGT
GAGCCCGGGC AATACAGGGT AT
Salmo gairAAGCCTTGGC AGTGCAGGGT
GAGCCGTGGC CGGGCACGGT AT
H. SapiensACCGGTTGGC CGTTCAGGGT
ACAGGTTGGC CGTTCAGGGT AA
Chimp     AAACCCTTGC CGTTACGCTT
AAACCGAGGC CGGGACACTC AT
Gorilla   AAACCCTTGC CGGTACGCTT
AAACCATTGC CGGTACGCTT AA
```

Fasta (sequence data format)

- Description line before each sequence starts with (">") symbol in the first column

```
>AB000263 |acc=AB000263|descr=Homo sapiens mRNA for prepro cortistatin like peptide, complete cds.|len
ACAAGATGCCATTGTCCCCCGGCCTCCTGCTGCTGCTGCTCTCCGGGGCCACGGCCACCGCTGCCCTGCC
CCTGGAGGGTGGCCCCACCGGCCGAGACAGCGAGCATATGCAGGAAGCGGCAGGAATAAGGAAAAGCAGC
CTCCTGACTTTCCTCGCTTGGTGGTTTGAGTGGACCTCCCAGGCCAGTGCCGGGCCCCTCATAGGAGAGG
AAGCTCGGGAGGTGGCCAGGCGGCAGGAAGGCGCACCCCCCCAGCAATCCGCGCGCCGGGACAGAATGCC
CTGCAGGAACTTCTTCTGGAAGACCTTCTCCTCCTGCAAATAAAACCTCACCCATGAATGCTCACGCAAG
TTTAATTACAGACCTGAA
```

To translate between data file formats in paup:

```
paup> execute exampledata.nex
paup> export file=example.fas format=fasta
```

Format options include fasta, phylip, nexus among others

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