Fast Phylogenetic Approaches

Xiaofan Zhou
Integrative Microbiology Research Centre
South China Agriculture University

Why is tree inference so diffcult?

- Too many trees to look at
- Too many calculations to do

Too many trees

No. of binary unrooted trees with n tips:

$$= 1 \times 3 \times 5 \cdots \times (2n-5)$$
$$= (2n-3)!/\left(2^{(n-2)}(n-2)!\right)$$

Tips	Binary unrooted trees
5	15
10	2,027,025
20	2.22 x 10^{18}
30	8.69 x 10^{36}
40	1.31 x 10^{55}
50	2.84 x 10^{74}
⋮	⋮

Sunway Taihu-Light
World's fastest and largest supercomputer
Peak Flops: 125.4 x 10^{15}

~3.31 x 10^{21} billion years

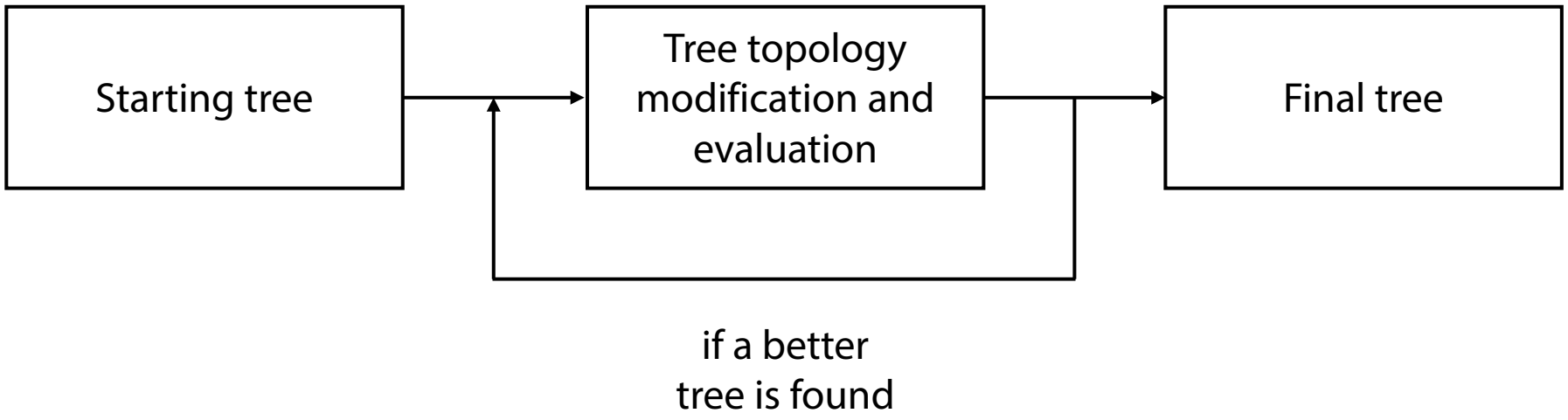
Too many calculations

- Branch length estimation
- Model parameter optimization
- ……

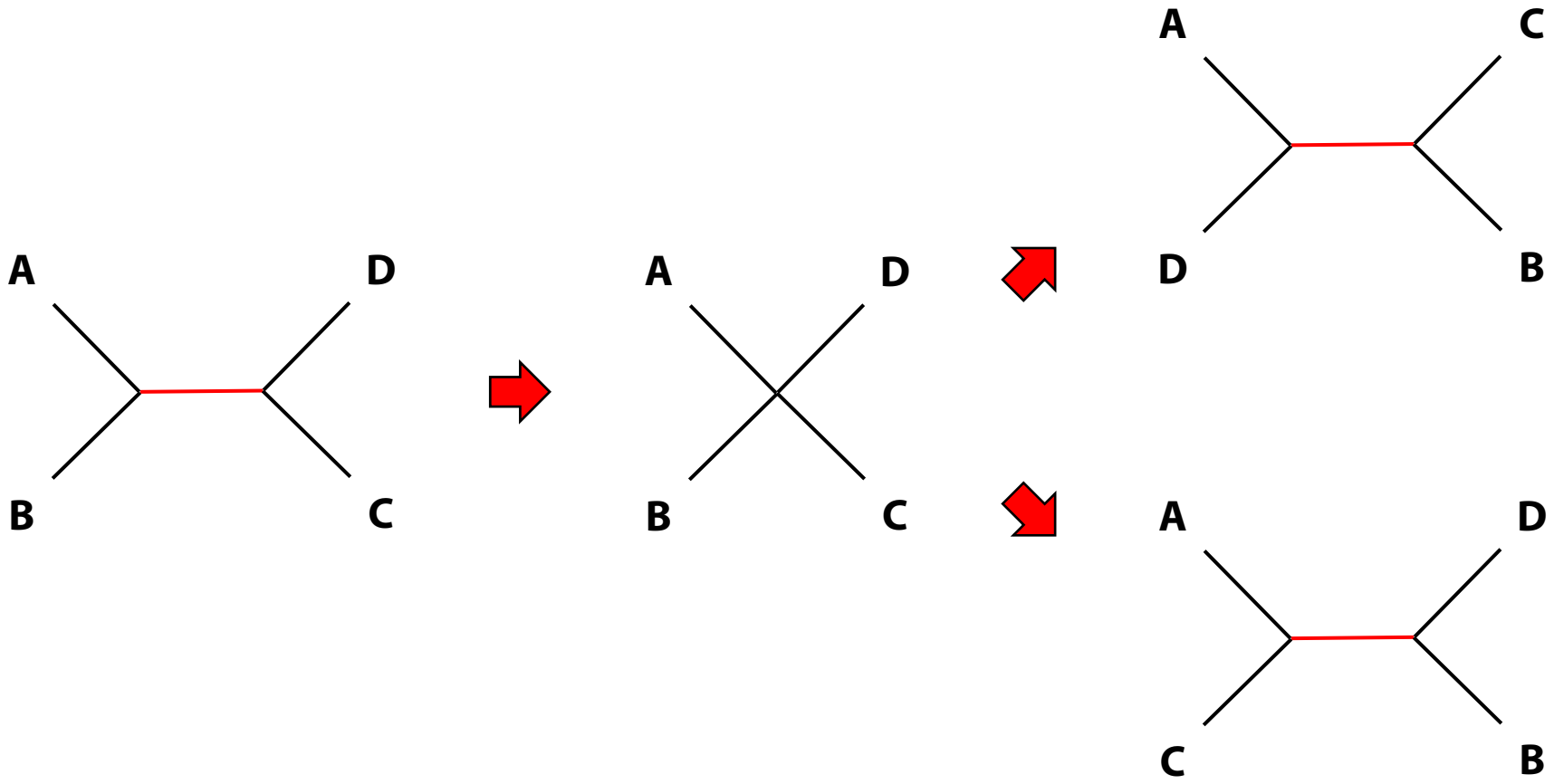
Fast phylogenetic approaches

- Too many trees to look at
  - Heuristic search of the tree space
- Too many calculations to do
  - Approximate estimation of branch length
- Other techniques for fast phylogenetics

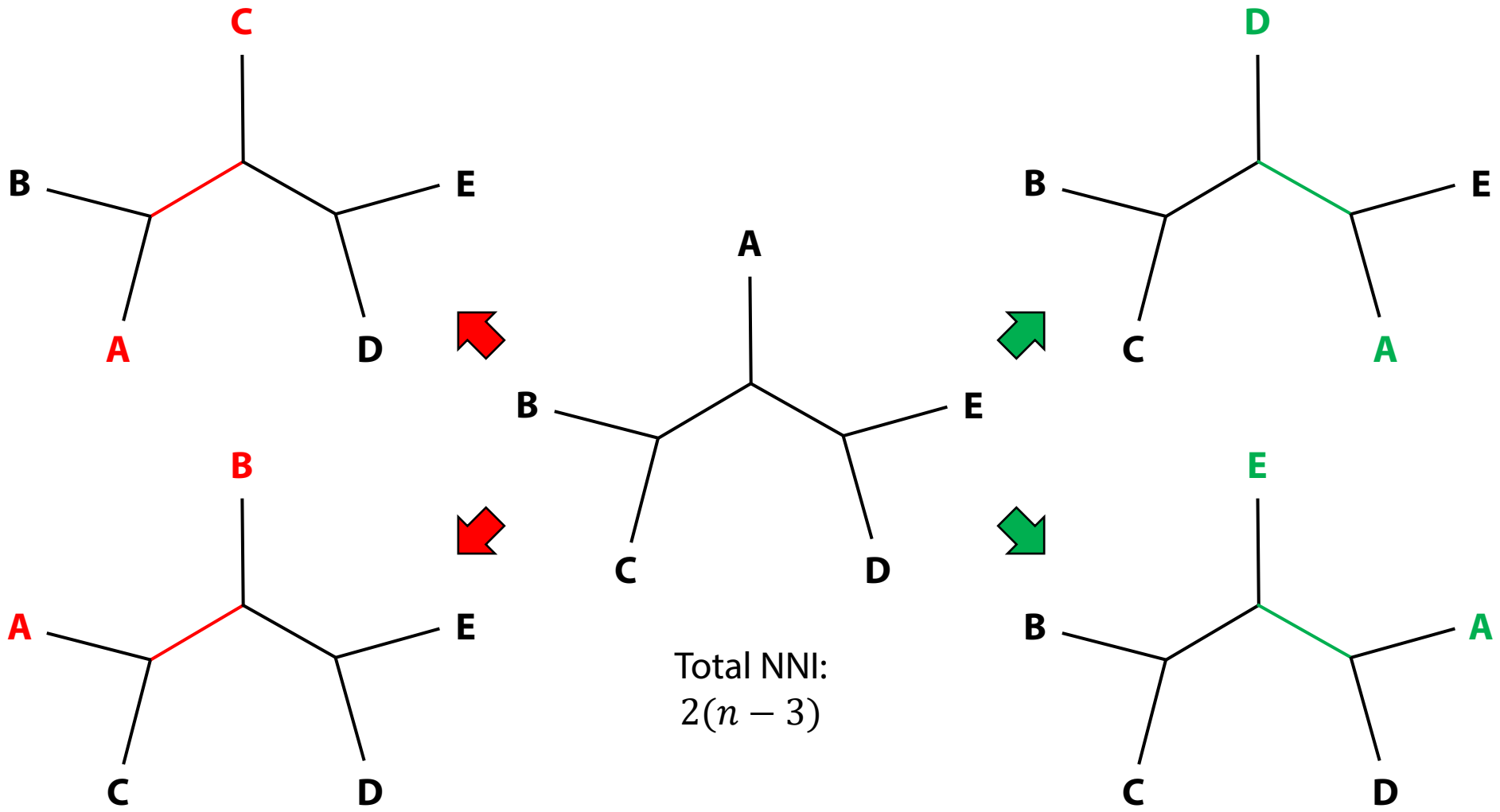
Heuristic tree search



Nearest Neighbor Interchange (NNI)

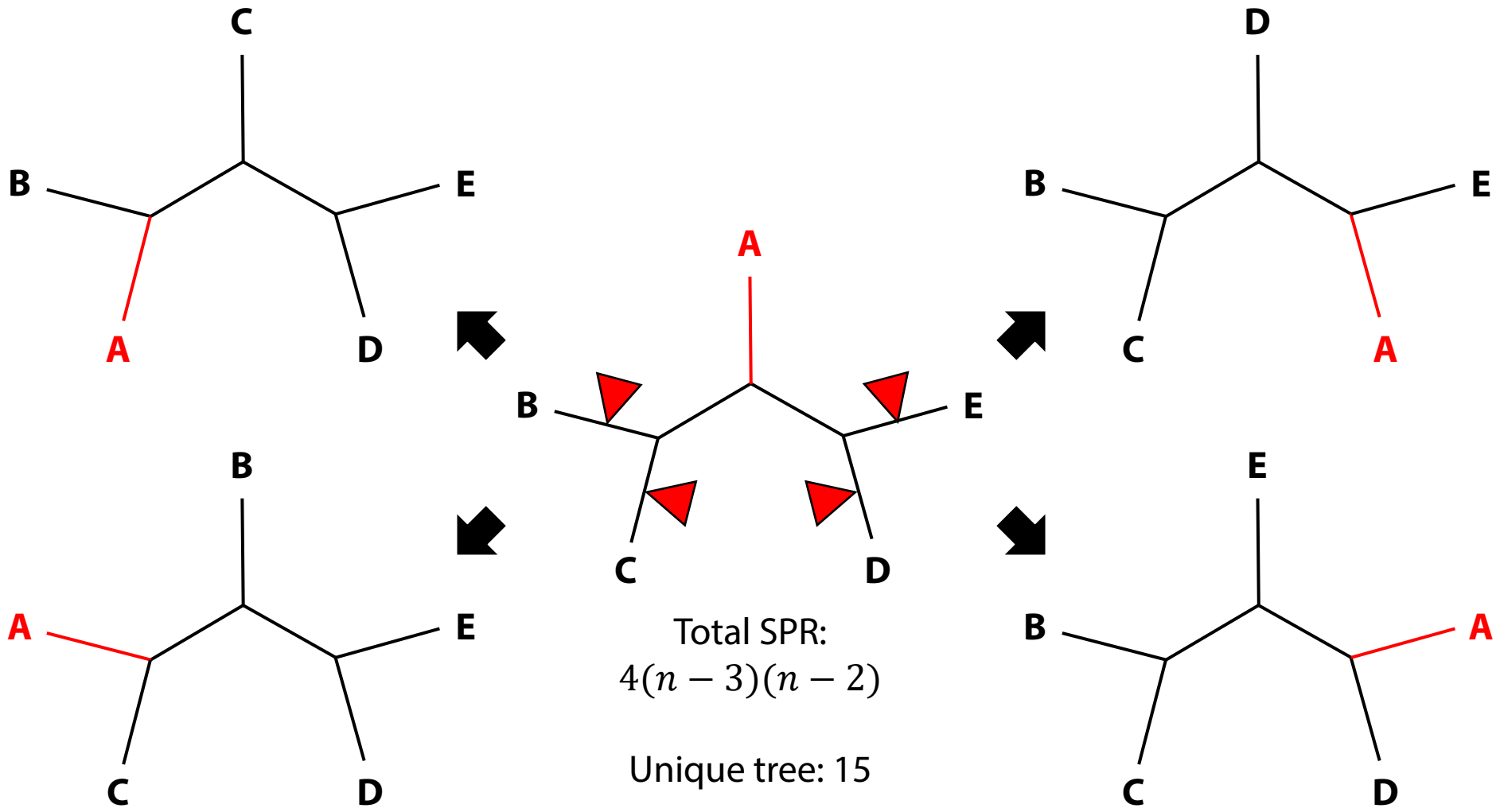


Nearest Neighbor Interchange (NNI)



Total NNI:
$2(n-3)$

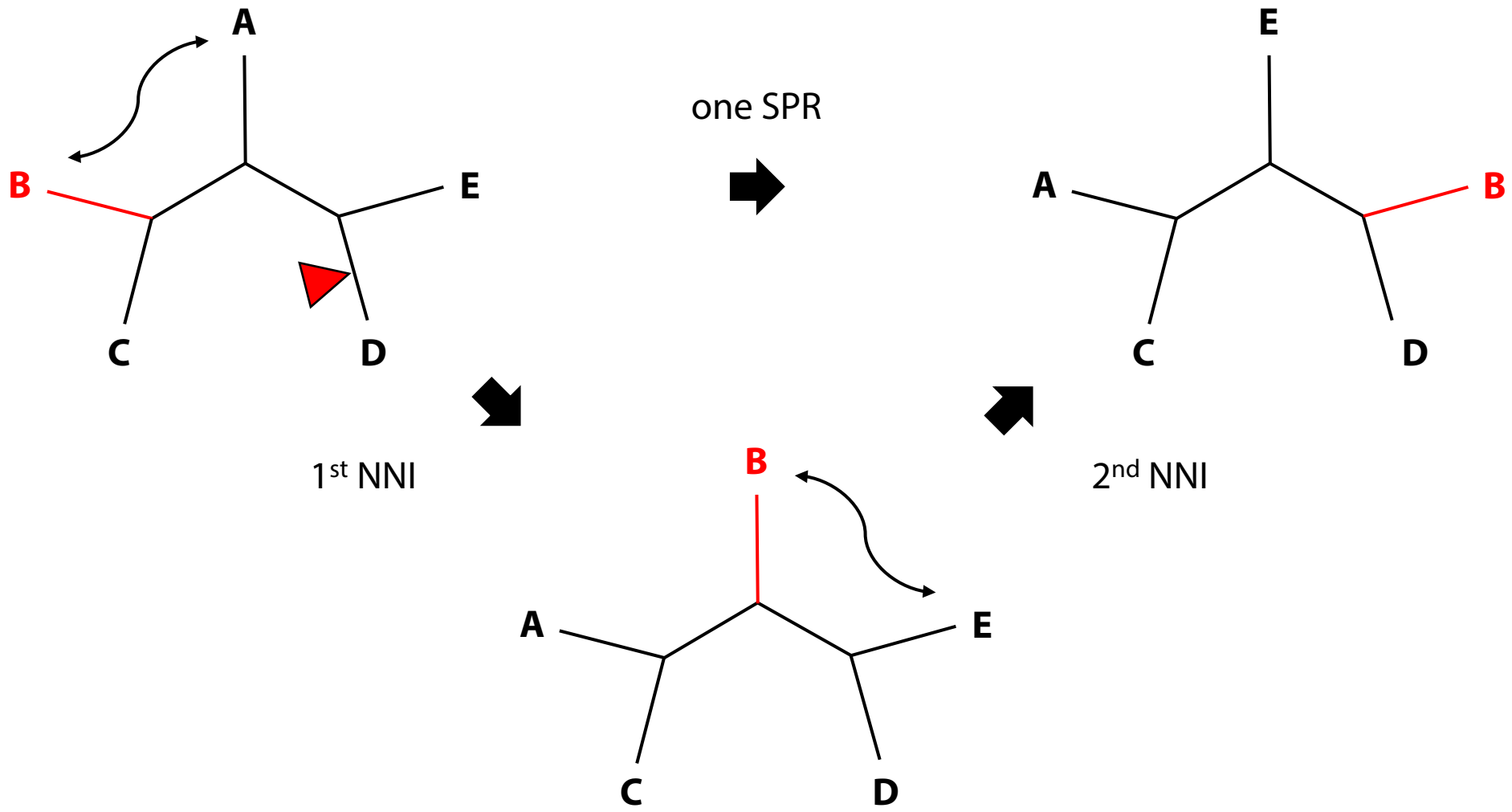
Subtree Pruning and Re-grafting (SPR)



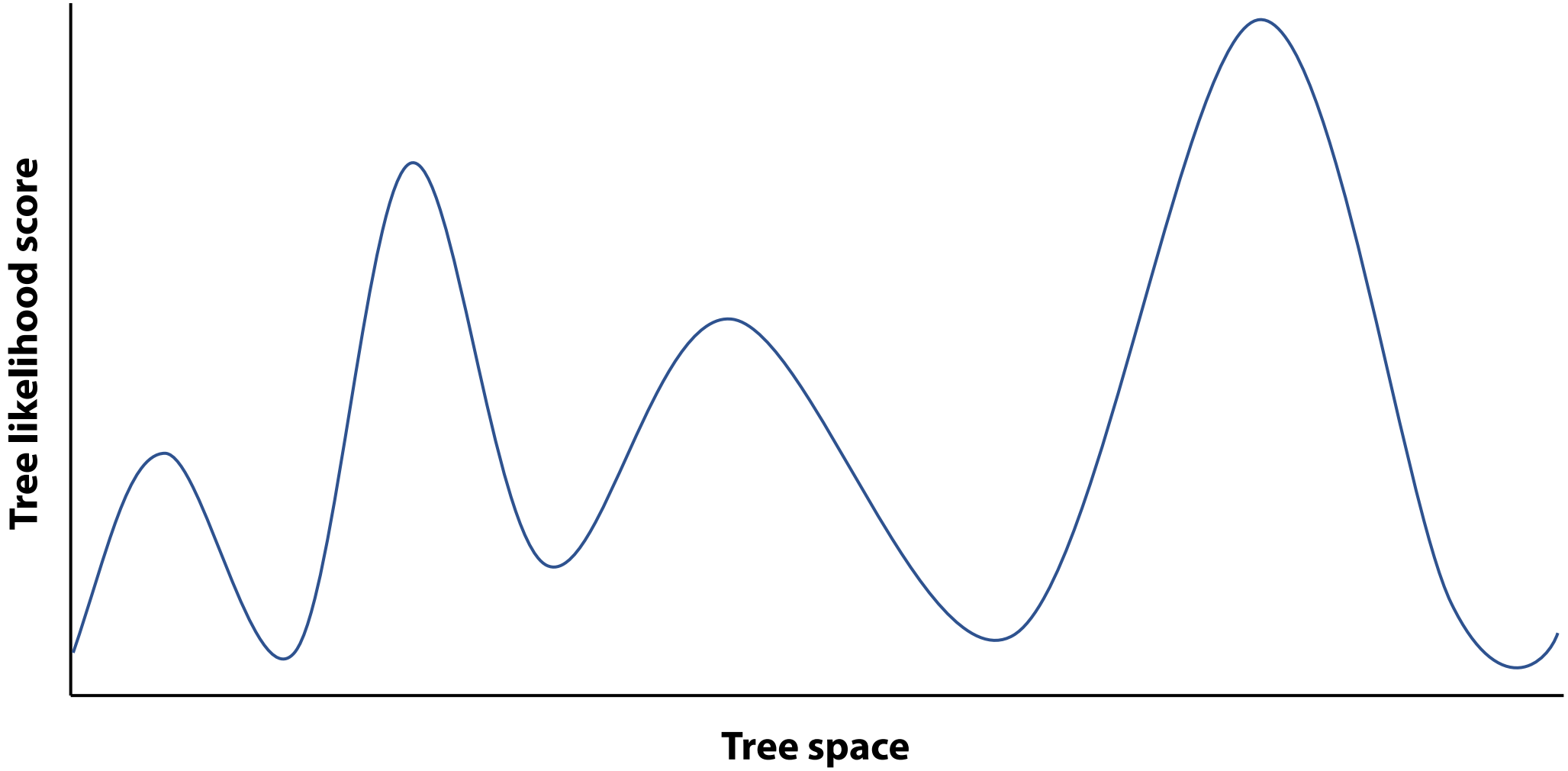
Total SPR:
$4(n-3)(n-2)$

Unique tree: 15

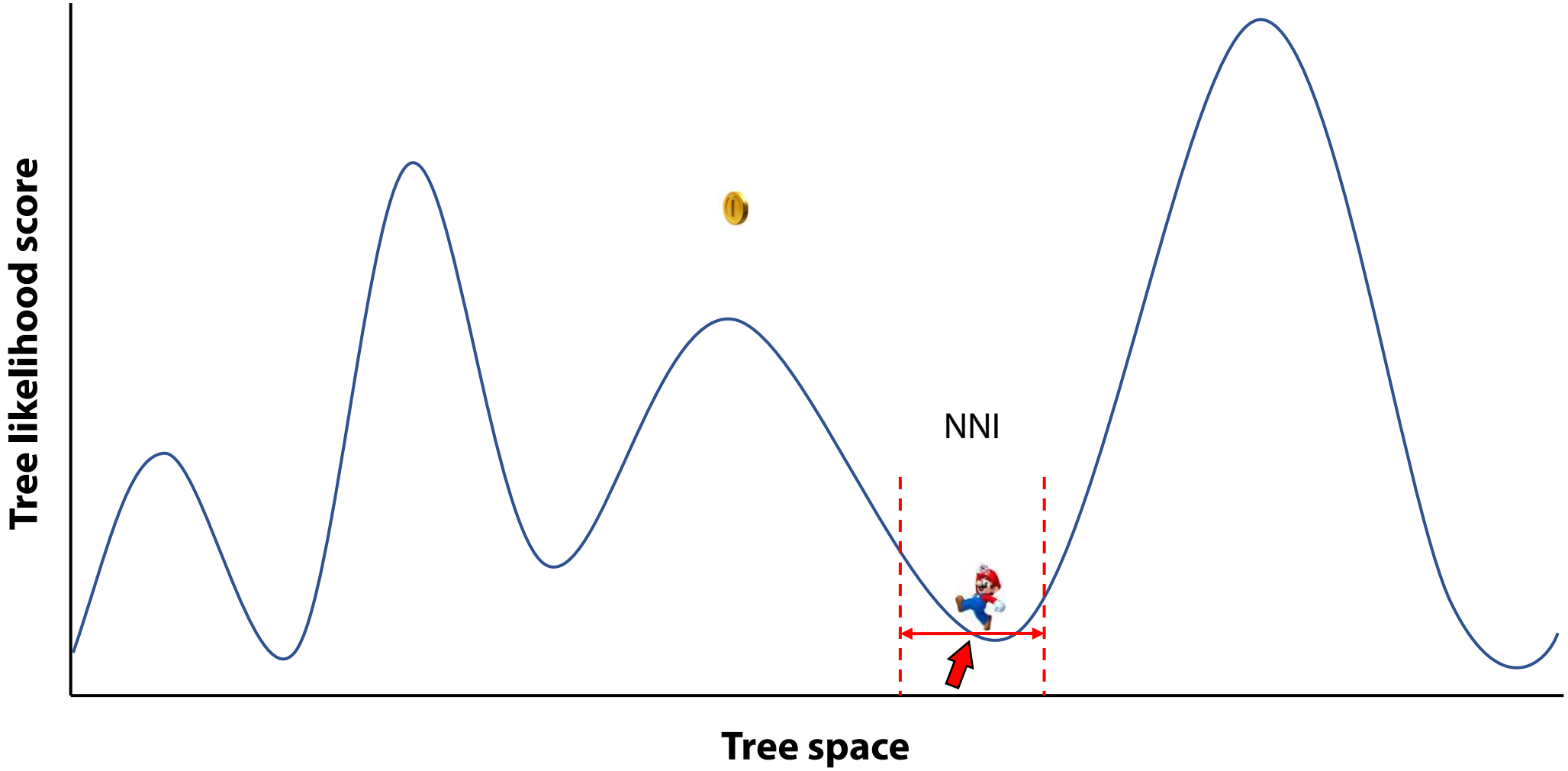
SPR as a chain of NNI



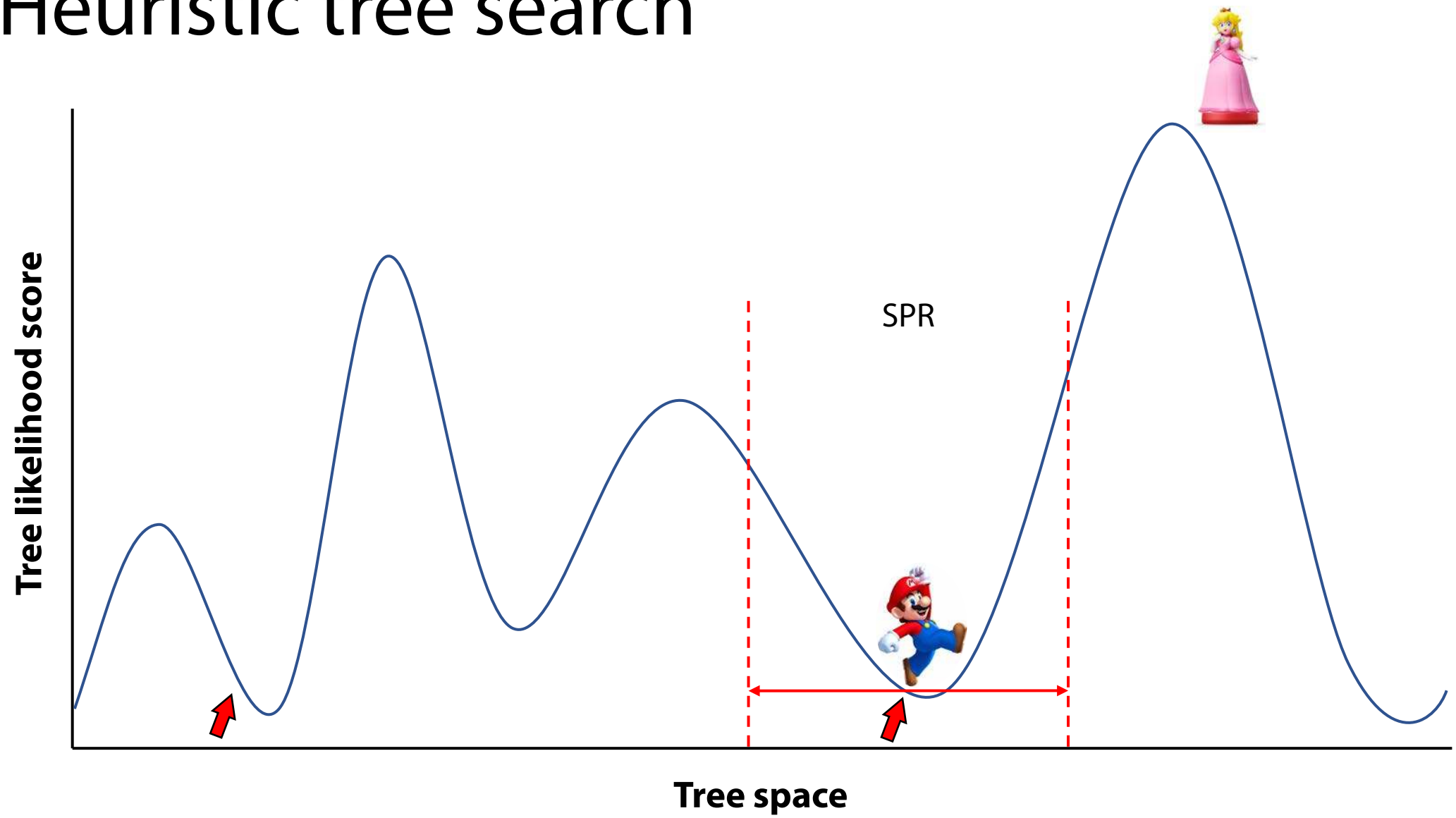
Heuristic search of tree space



Heuristic tree search

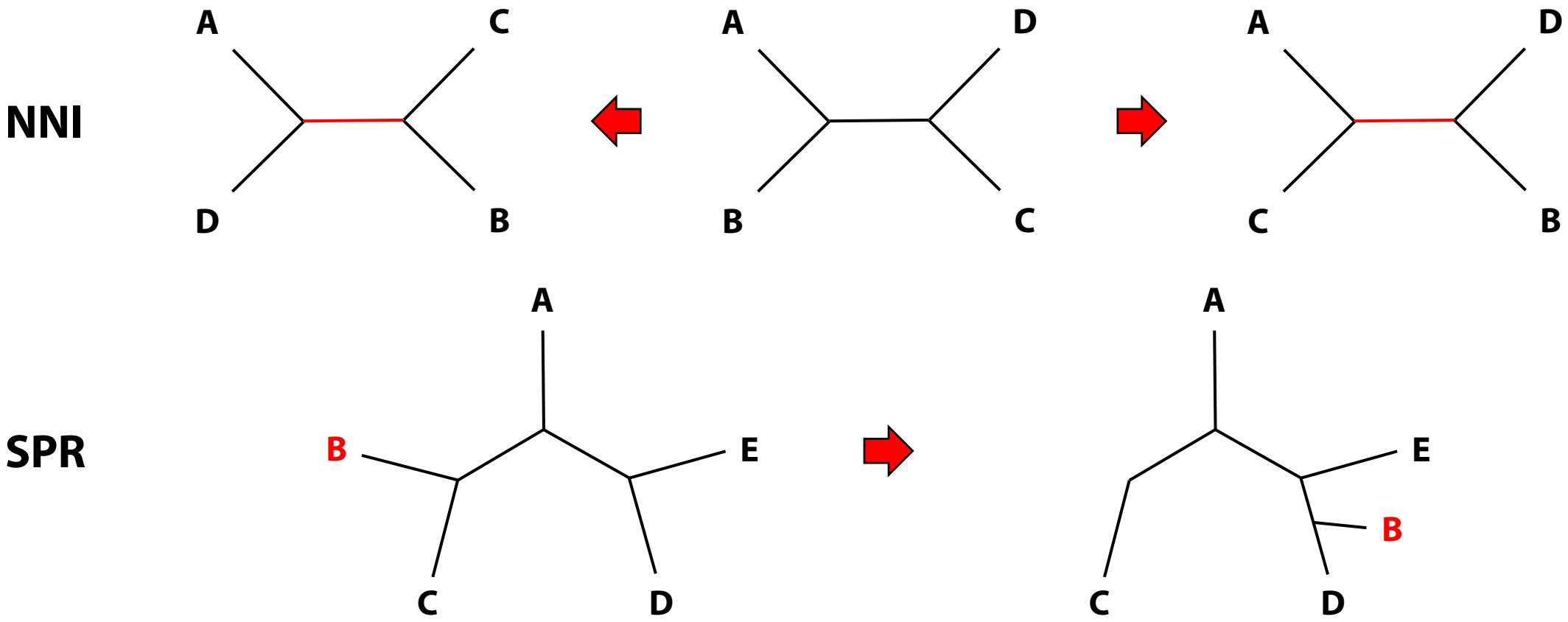


Heuristic tree search



Approximate likelihood calculation

- Global optimization vs. local optimization



Approximate likelihood calculation

- Global optimization vs. local optimization

- Exhaustive optimization vs. approximate optimization
  - Diminished return from extra efforts
  - Subsequent topological changes can invalidate extra efforts

Other techniques for fast phylogenetics

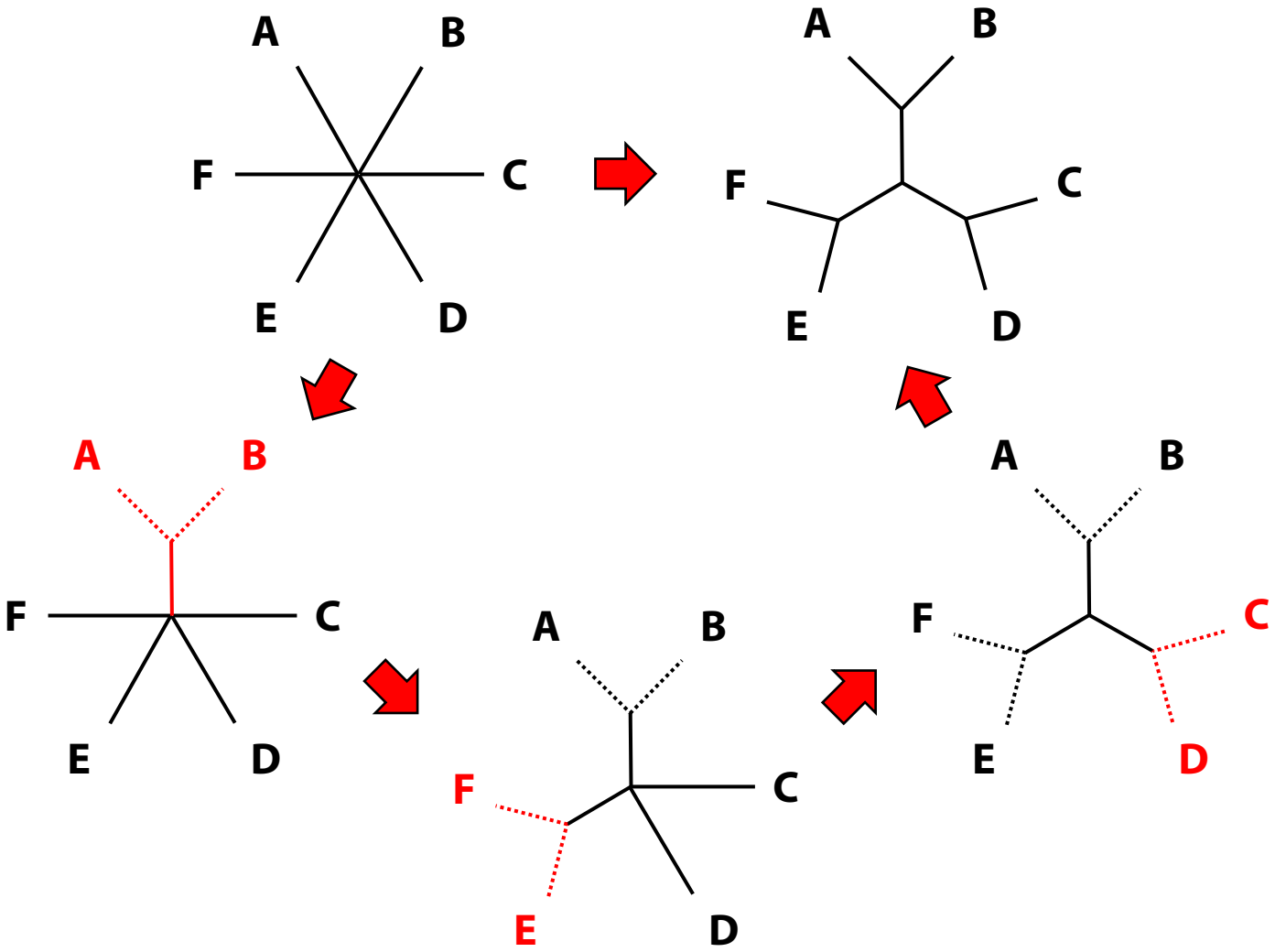
- GAMMA vs CAT
- Fast approaches for node support
- Parallelization
- Memory saving

Outline

- Popular fast phylogenetic programs
  - FastTree, RAxML, PhyML, IQ-TREE
  - Main algorithm and development

- Empirical evaluation using recent phylogenomic datasets

NJ – Neighbor Joining



Pairwise distance matrix: D

For each tip, calculate:

$$u_i = \sum_{k:k\neq i}^{n} D_{ik}/(n-2)$$

Identify the pair of tips i and j that minimize the NJ criterion:

$$Q = Dij - ui - uj$$

Join i and j, replace with a new node (ij), and update D:

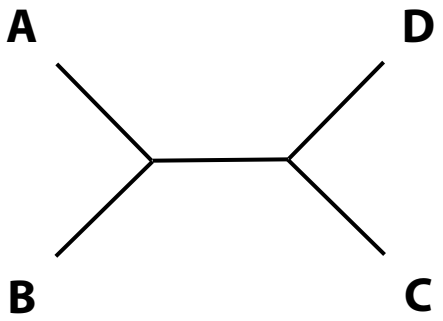
$$D_{(ij),k} = (Dik + Djk - Dij)/2$$

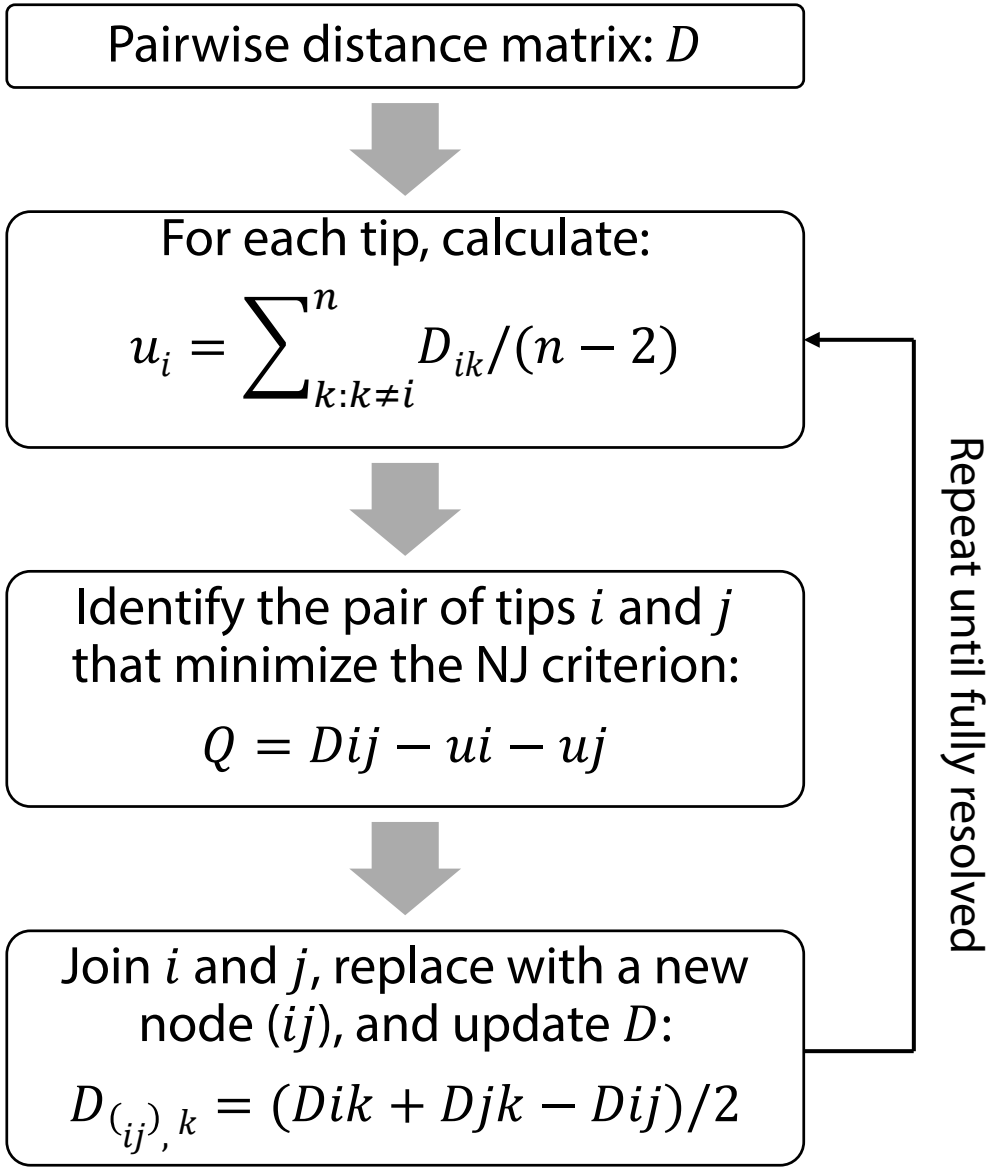
Repeat until fully resolved

What is NJ optimizing?

- NJ is a greedy algorithm optimizing "balance" tree length:

$$L = \sum_{i,j} 2^{1-pij} D_{ij}$$





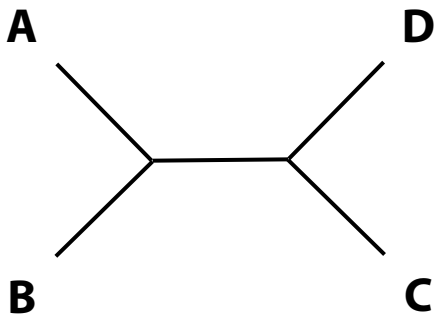
What is NJ optimizing?

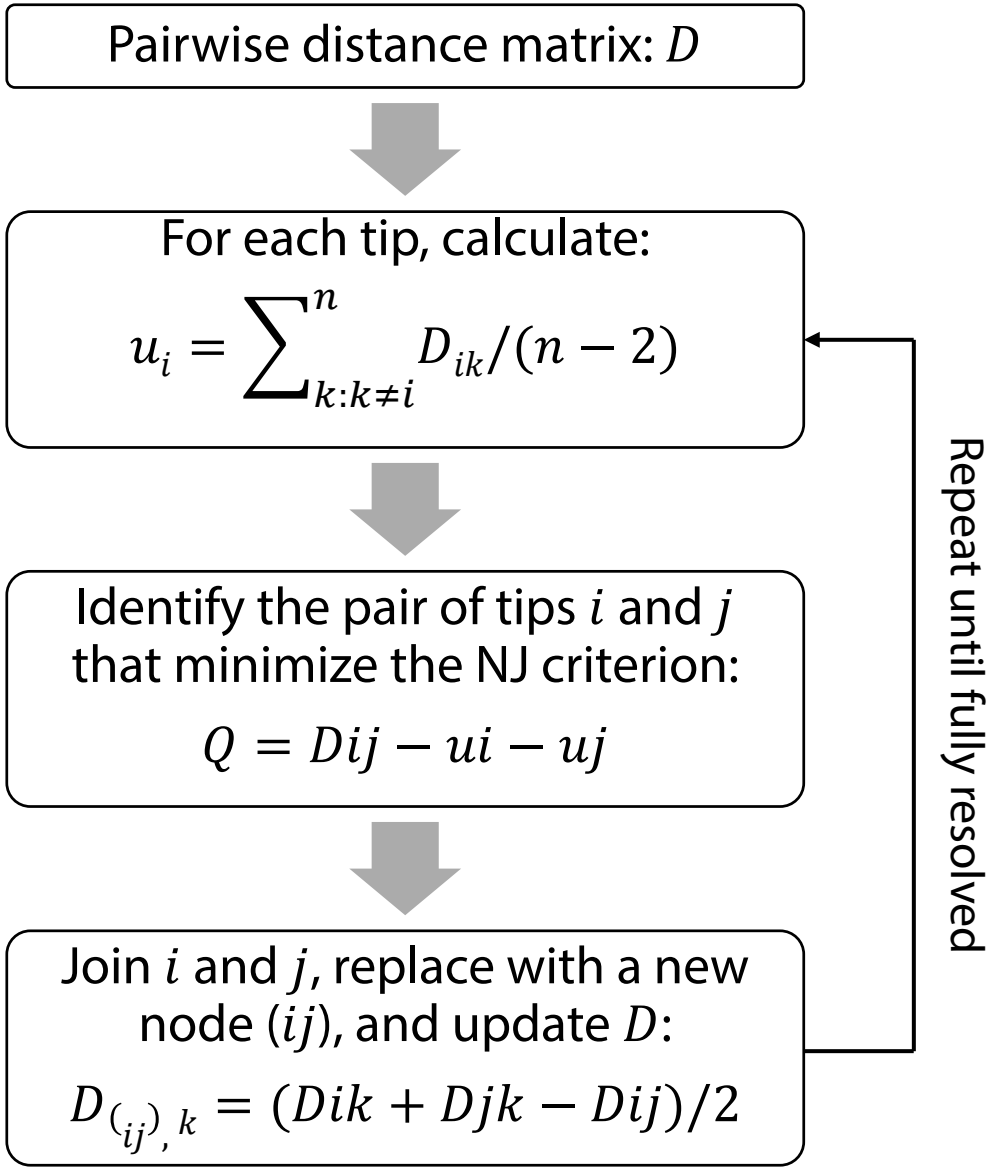
- Balanced Minimum Evolution
  - FastME:

  → stepwise addition starting tree

  → NNI

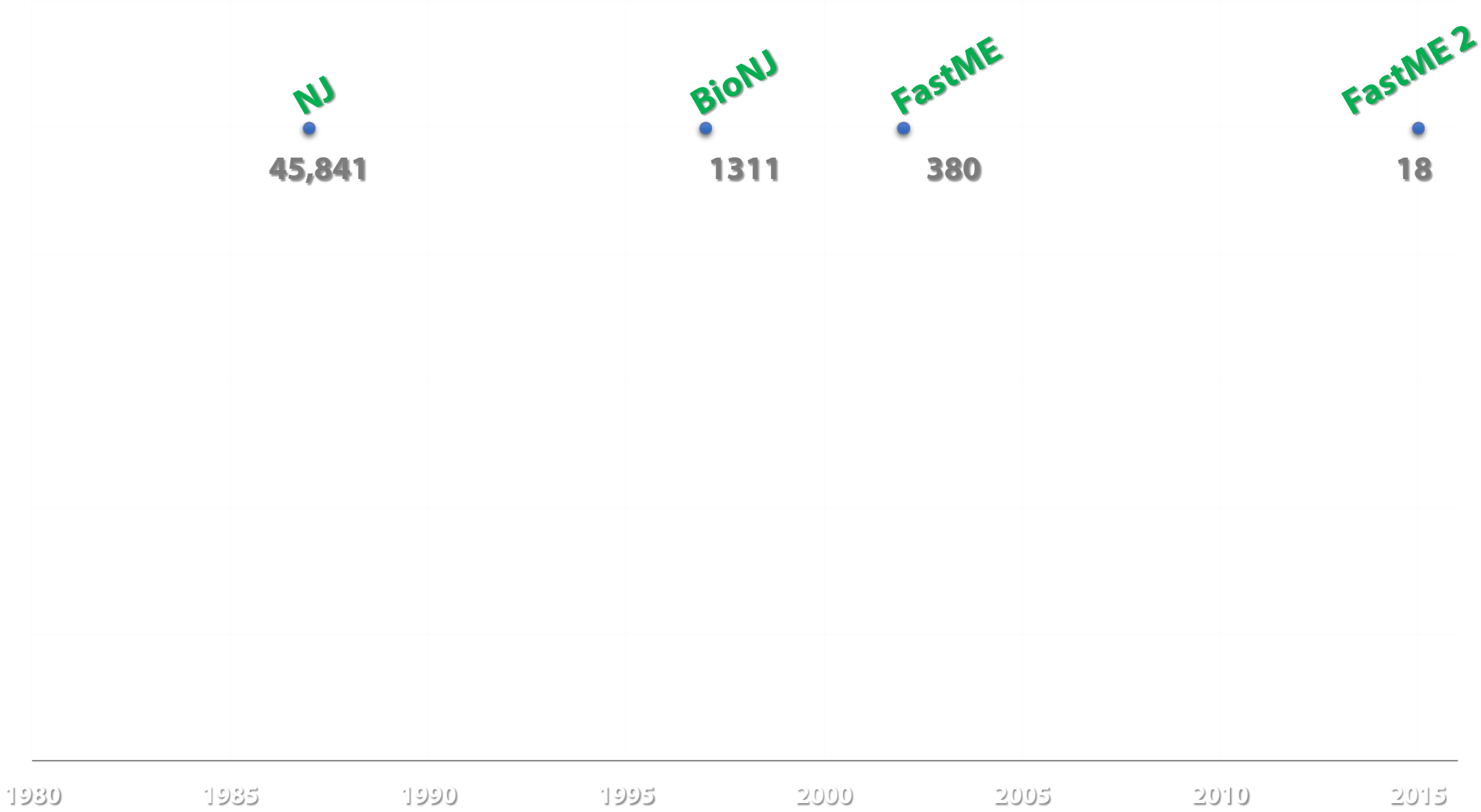
  → SPR (FastME 2)

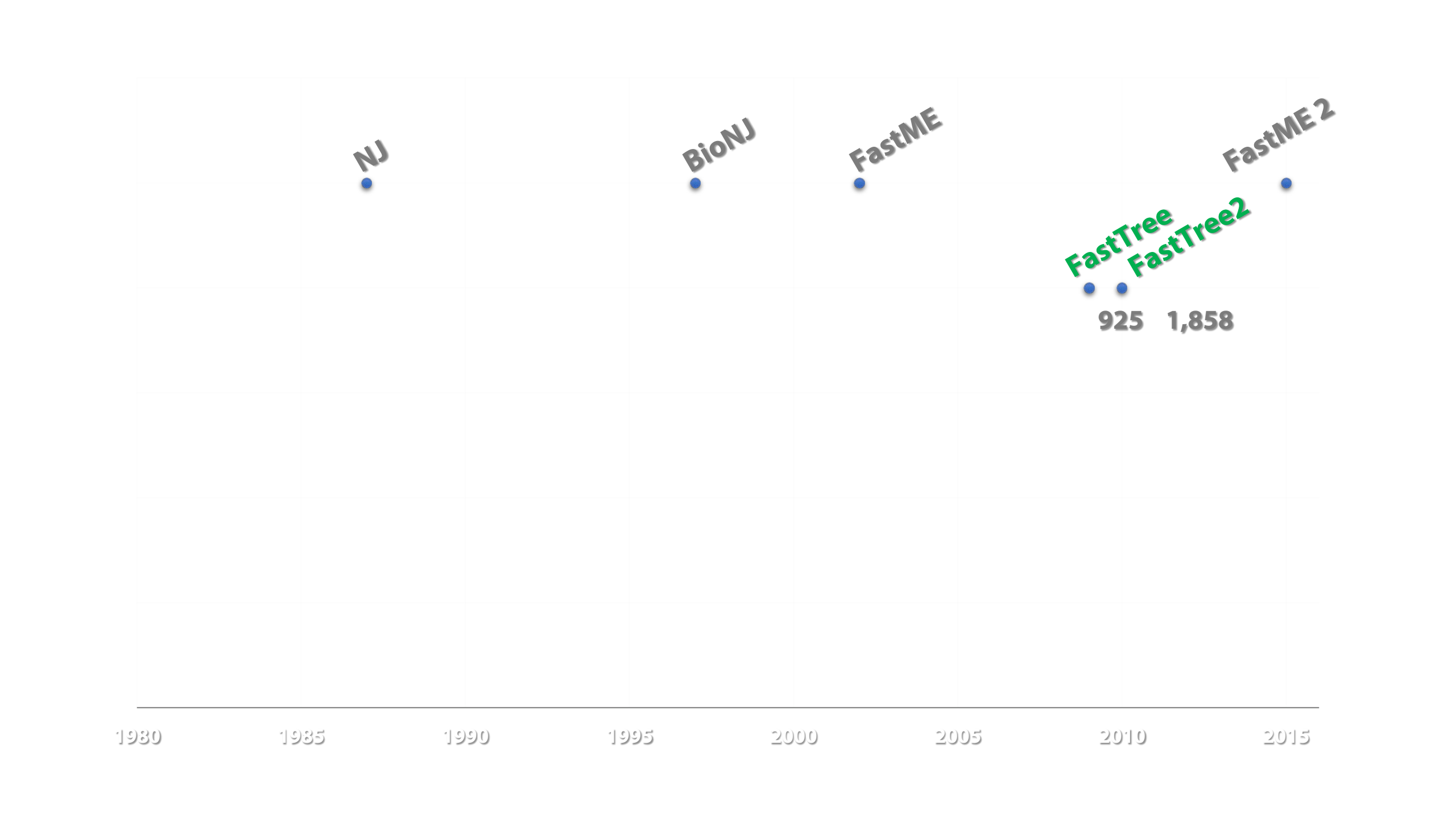




Variants of NJ

- BIONJ/WEIHBOR
  - Take variance/co-variance into consideration
- Relaxed Neighbor Joining
  - Join the pair of neighbors firstly found, instead of the one that minimizing Q
- Fast Neighbor Joining
  - Look for a subset of the pairs


NJ
45,841
BioNJ
1311
FastME
380
FastME 2
18
1980
1985
1990
1995
2000
2005
2010
2015

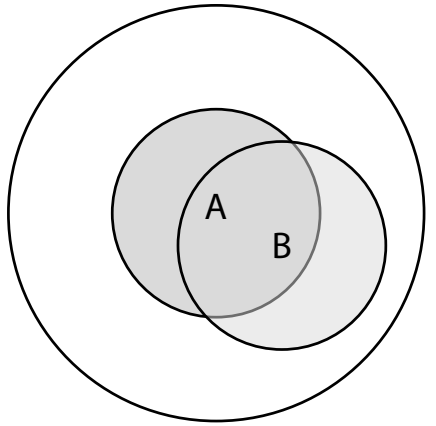

NJ
BioNJ
FastME
FastME 2
FastTree
FastTree2
925
1,858
1980
1985
1990
1995
2000
2005
2010
2015

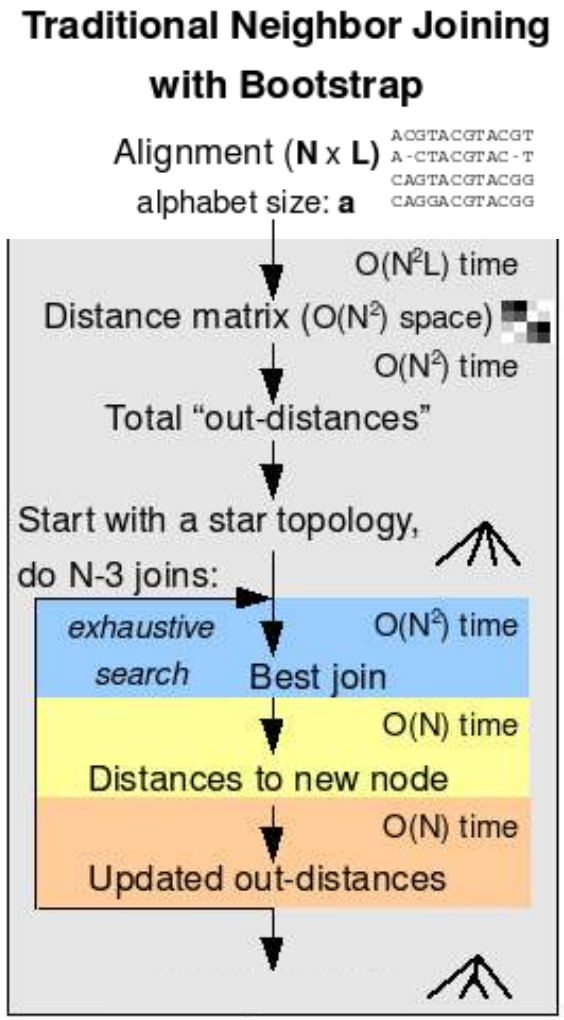
FastTree

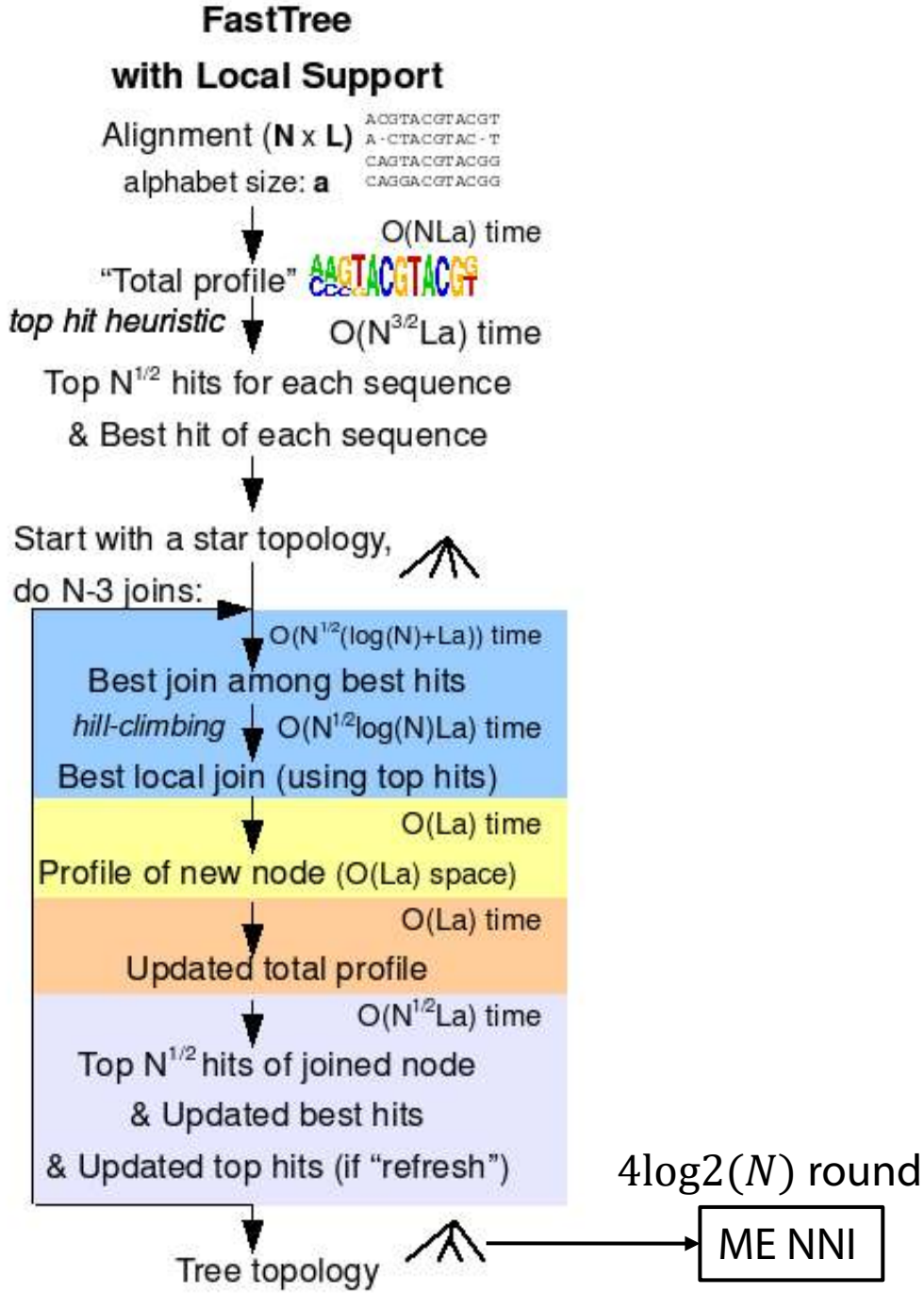
	A	C	G	T	A
	A	–	C	T	A
	C	A	G	T	A
	C	A	G	G	A
A	**0.5**	**0.66**	**0**	**0**	**1**
T	**0**	**0**	**0**	**0.75**	**0**
C	**0.5**	**0.33**	**0.25**	**0**	**0**
G	**0**	**0**	**0.75**	**0.25**	**0**
-	**0**	**0.25**	**0**	**0**	**0**

- Sequence profile instead of distance matrix
  - Reduce memory requirements
  - Use an average profile of all active nodes to calculate the NJ criterion, instead of actually doing all pairwise computing

- Three main heuristics
  - Top-hits: neighbors of neighbors are also likely to be neighbors
  - Fast neighbor joining: remembering the best join candidate for each node
  - Relaxed neighbor joining: hill-climbing search for best join



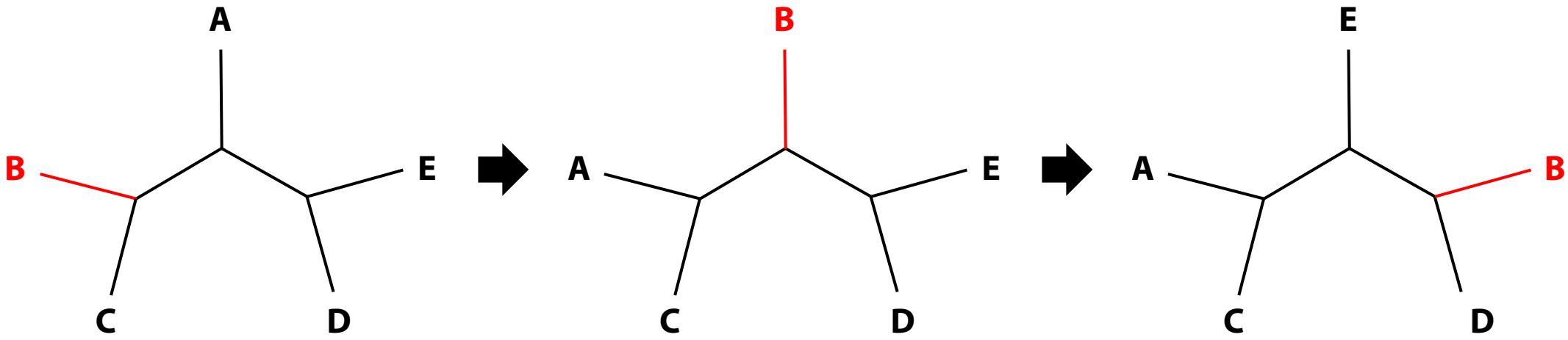




http://www.microbesonline.org/fasttree/FastTreeScheme.png

FastTree2

- ME SPR
  - Extends SPR only along the best path up to length of 10
  - Only two rounds of SPR for each subtree

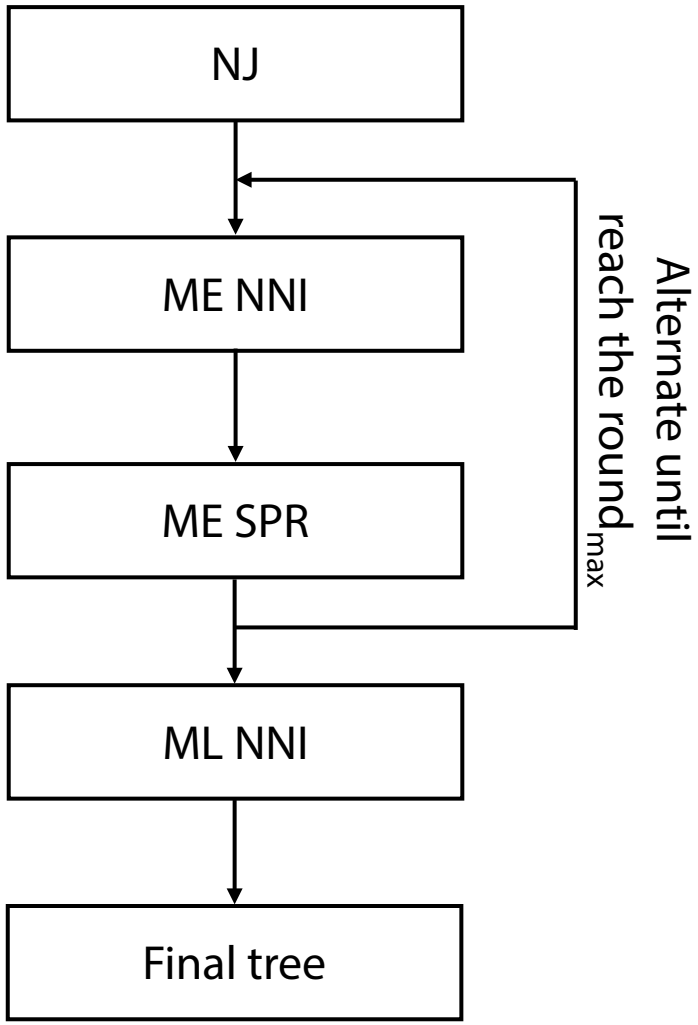


FastTree2

- ME SPR
  - Extends SPR only along the best path up to length of 10
  - Only two rounds of SPR for each subtree

- ML NNI
  - $2\log_2 N$quick rounds + 1 final thorough rounds
  - Heuristics:
    - Branch-length estimation
      - Skip the topology if significantly worse than current tree
    - Star-topology test
      - Pick the winner when one of the quartet tree is significantly better than a star topology
    - Subtree skipping
      - Skip subtrees without significant improvement in recent two rounds

FastTree2


NJ
ME NNI
ME SPR
ML NNI
Final tree
Alternate until reach the round$_{max}$

FastTree: performance

Table 3. Comparison of RAxML and FastTree's log likelihoods, and the agreement of FastTree with RAxML's well-supported splits, for large genuine alignments.

	16S rRNA	16S rRNA	7 COGs
Number of sequences	4,114	6,718	2,500
RAxML 7's Log Likelihood	−325,581	−481,259	−1,238,666
FastTree 2's Log Likelihood	−328,062	−493,841	−1,240,916
Difference	2,481	12,582	2,251
Well-supported RAxML splits (bootstrap ≥ 0.9)			
Total in RAxML tree	851	1,124	–
Found by FastTree	837	1,075	–
Weakly-supported RAxML splits (bootstrap 0.8–0.9)			
Total in RAxML tree	265	419	–
Found by FastTree	250	365	–
Locally-supported RAxML splits (SH ≥ 0.95)			
Total in RAxML tree	1,336	1,927	1,018
Found by FastTree	1,033	1,319	889

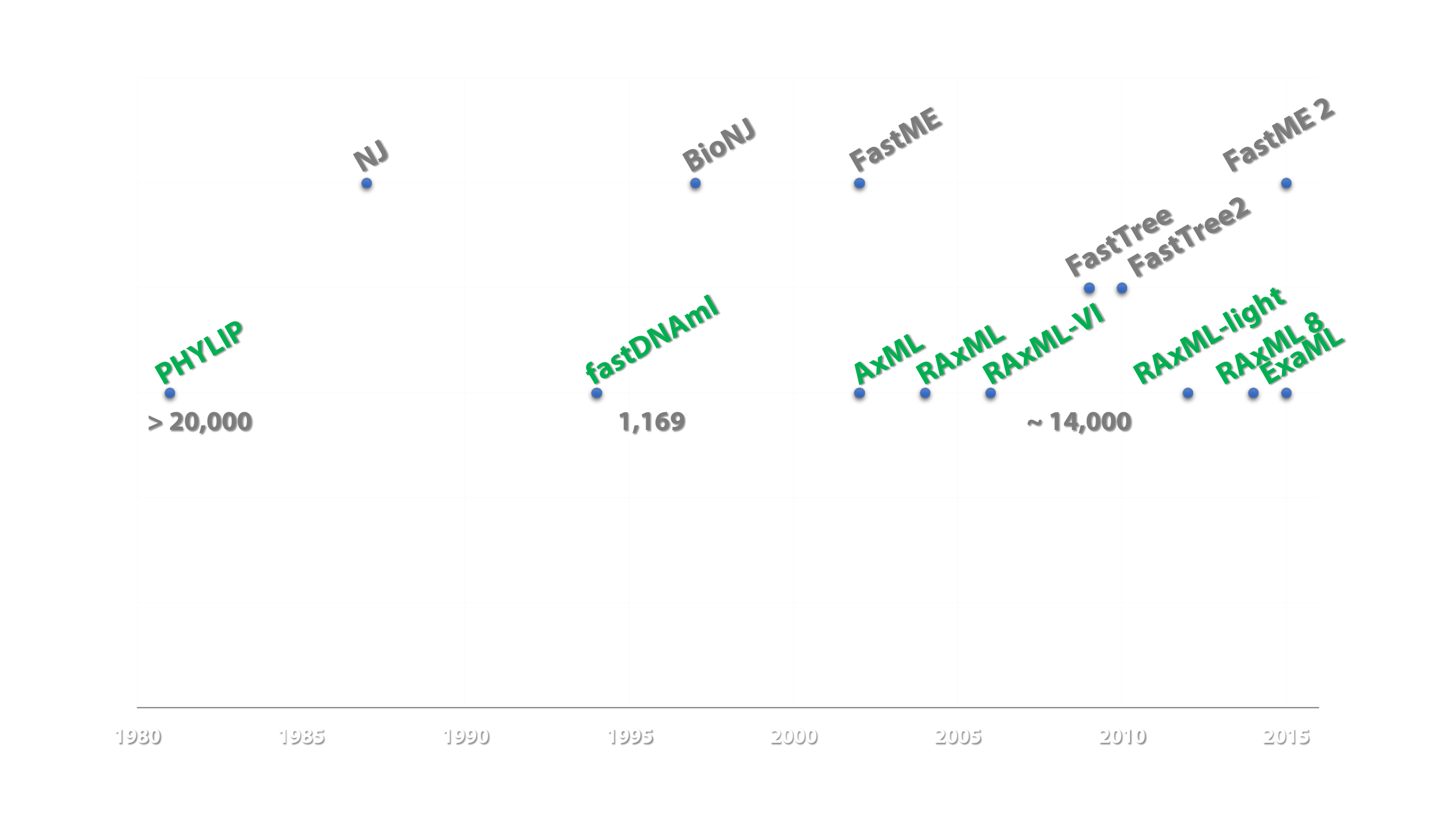
Price et al (2010) PLoS One

FastTree: performance

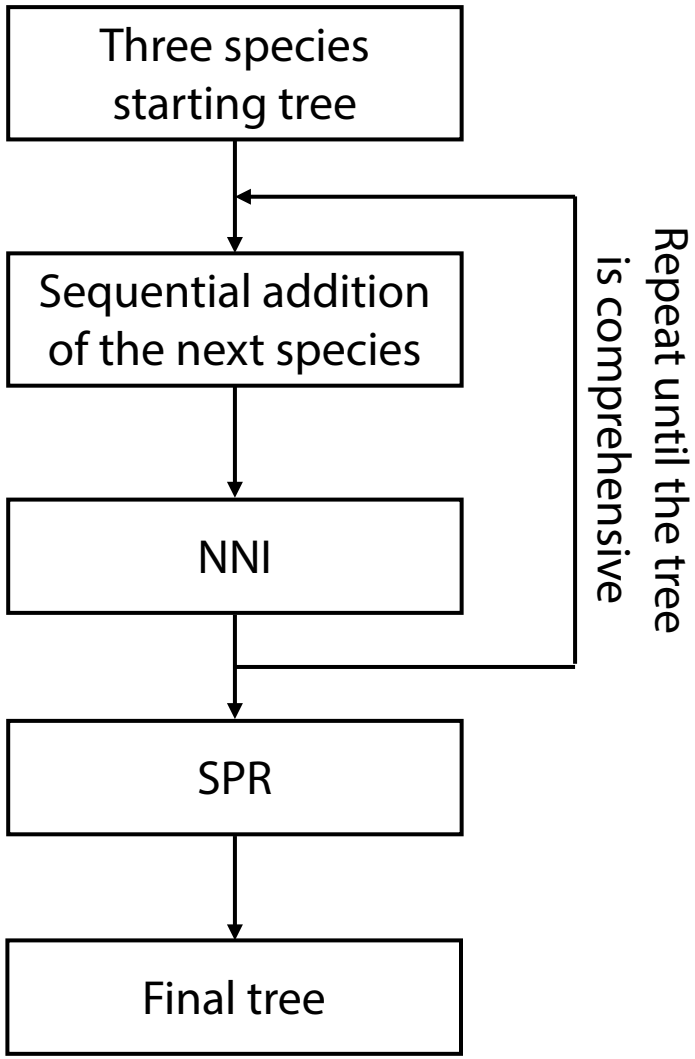
Table 4. Running time and memory usage on genuine alignments.

	Distinct		FastTree 2.0.0			RAxML 7	PhyML 3
Alignment	**Sequences**	**Positions**	**Model**	**Hours**	**GB**	**Hours**	**Hours**
16S rRNA, subsets	500	1,287 nt.	GTR	0.02	–	2.2	2.9
COGs, subsets	500	65–1,009 a.a.	JTT	0.02	–	5.2	7.2
COGs, subsets	2,500	197–384 a.a.	JTT	0.11	–	61	–
Efflux permeases	8,362	394 a.a.	JTT	0.25	0.35	197	>1,200
16S rRNAs, families	15,011	1,287 nt.	GTR	0.66	0.56	64	>2,000
ABC transporters	39,092	214 a.a.	JTT	1.02	0.96	–	–
16S rRNAs, all	237,882	1,287 nt.	JC	21.8	5.8	–	–

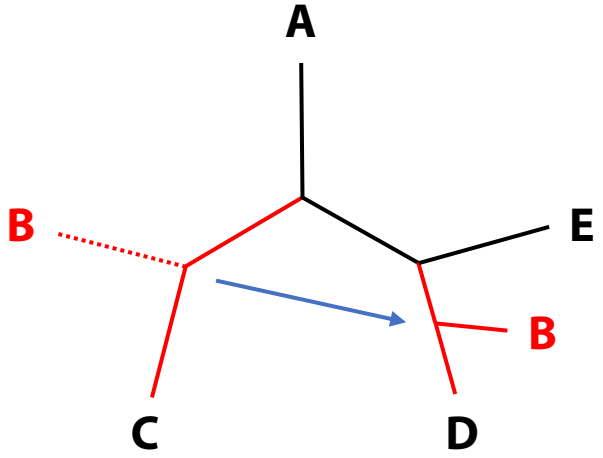
Price et al (2010) PLoS One


NJ
BioNJ
FastME
FastME 2
FastTree
FastTree2
PHYLIP
fastDNAml
AxML
RAxML
RAxML-VI
RAxML-light
RAxML-8
ExaML
> 20,000
1,169
~ 14,000
1980
1985
1990
1995
2000
2005
2010
2015

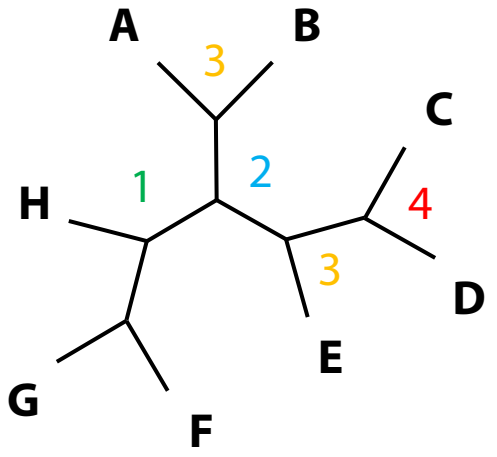
PHYLIP – PHYLogeny Inference Package



fastDNAml



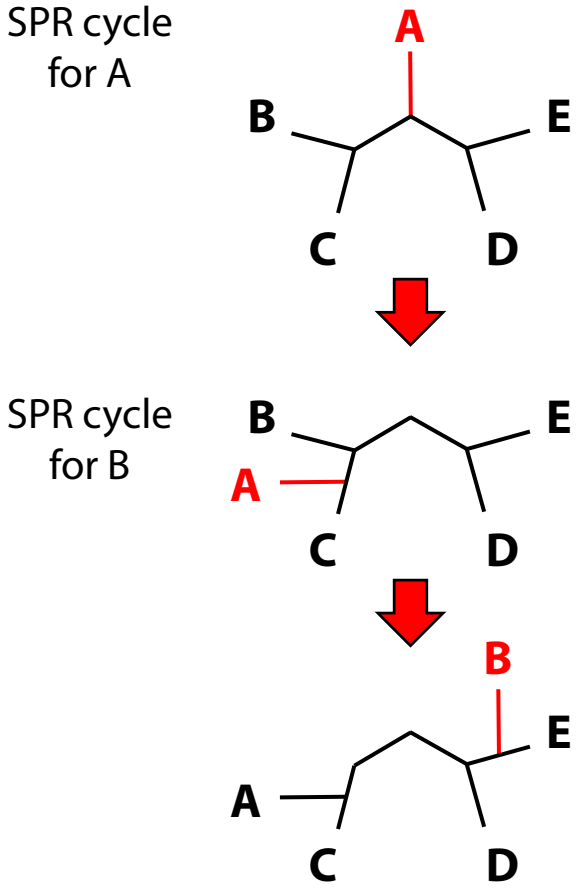
- Algorithm largely the same as PHYLIP
- Less exhaustive branch-length optimization
  - Only optimize the three branches relevant to the sequence addition
- Lazy SPR
  - Only consider re-grafting on branches at most r node away from the pruning position

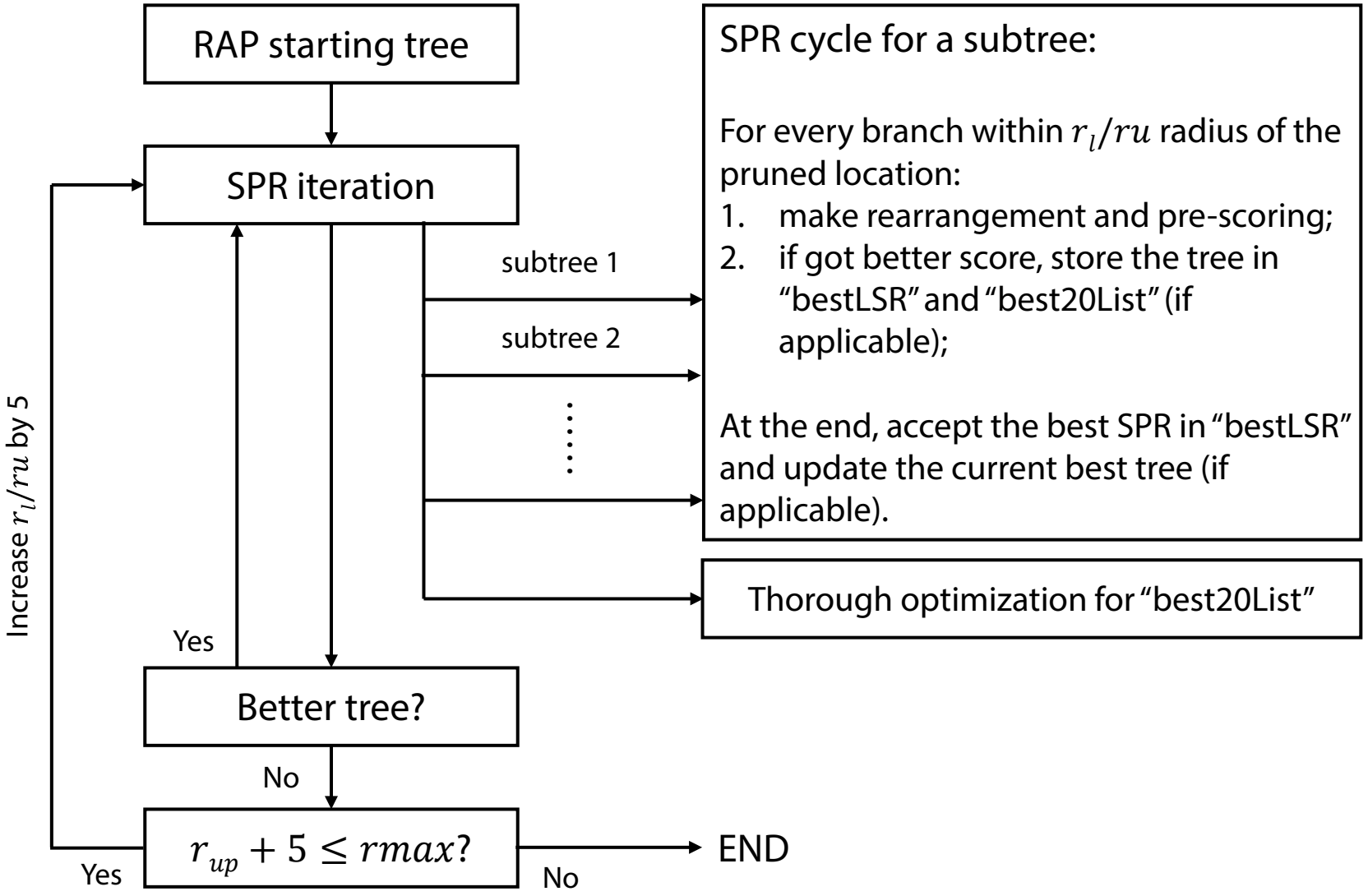


RAxML - Randomized AxML

- Parsimony starting tree:
  - Parsimony is connected with ML
  - Speed and randomization!

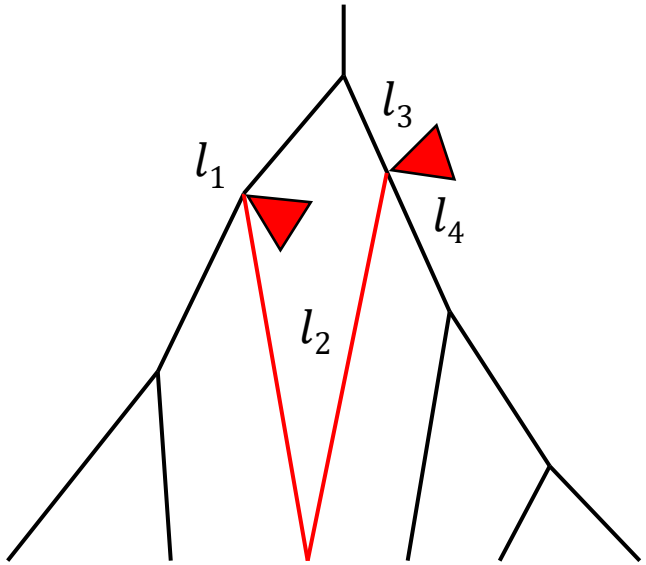
- Lazy SPR
  - Only pre-scoring during one SPR iteration
  - SPRs leading to better scores are immediately implied
  - Dynamic adjustment of Lazy SPR radius




RAP starting tree
SPR iteration
subtree 1
subtree 2
SPR cycle for a subtree:
For every branch within r_l/ru radius of the pruned location:
1. make rearrangement and pre-scoring;
2. if got better score, store the tree in "bestLSR" and "best20List" (if applicable);
At the end, accept the best SPR in "bestLSR" and update the current best tree (if applicable).
Thorough optimization for "best20List"
Yes
Better tree?
No
$r_{up} + 5 \leq rmax$?
Yes
No
END
Increase r_l/ru by 5

RAxML: improvements

- Optimized data structure
  - rearrangement descriptor
  - (pruned position, re-grafting position, l_1, l_2, l_3, l_4)



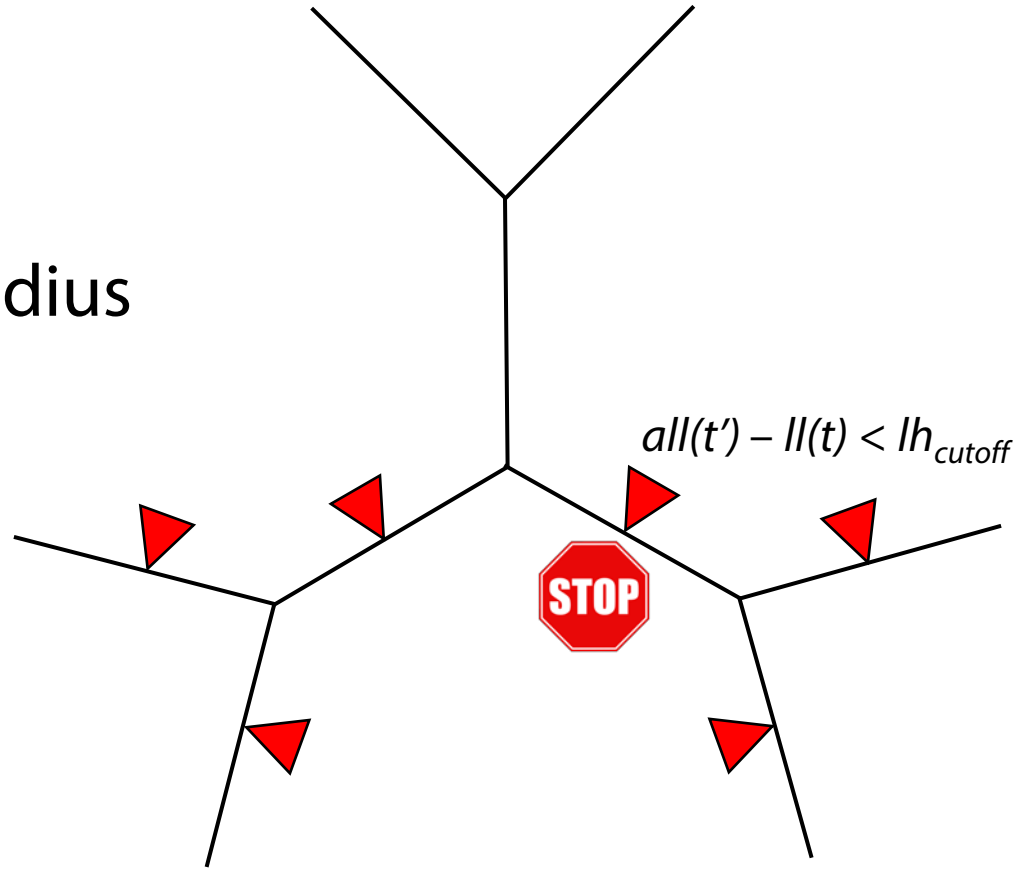
RAxML: improvements

- Optimized data structure

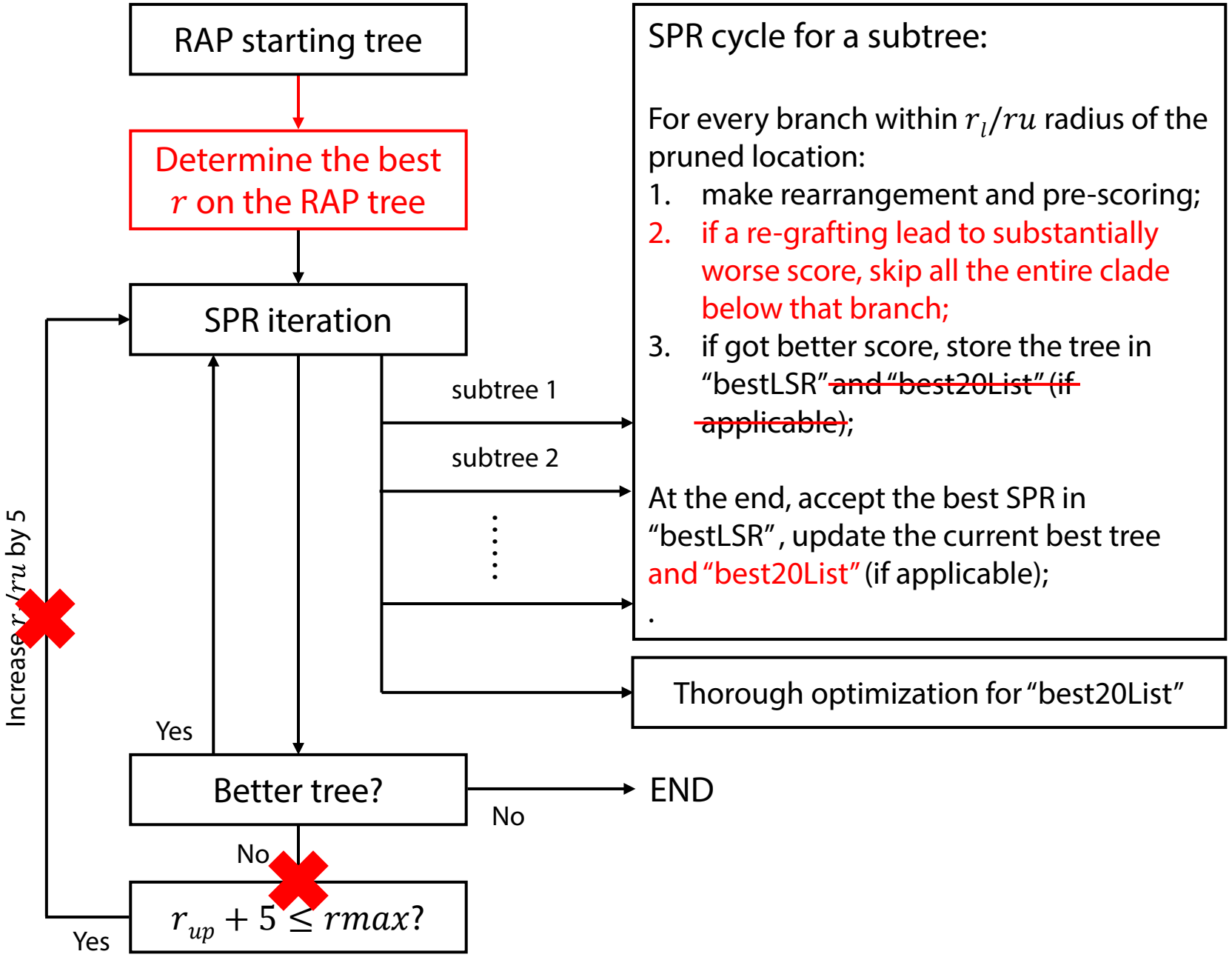
- Adaptive determination of LSR radius
  - SPR on the RAP starting tree with different r value
  - Choose the smallest r giving rise to the best score

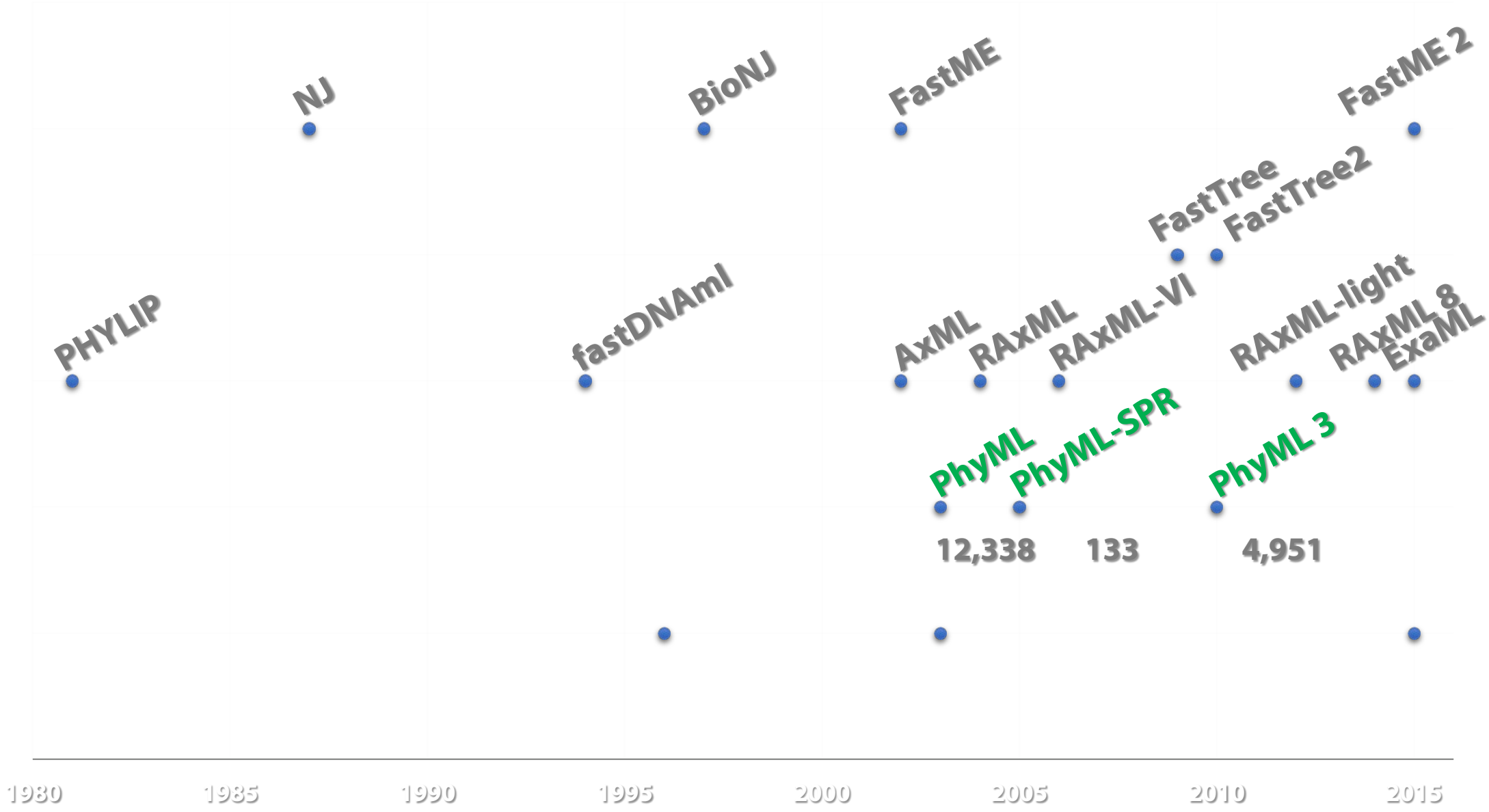
RAxML: improvements

- Optimized data structure
- Adaptive determination of LSR radius
- "Subtree skipping"
  - lh_{cutoff} determined dynamically during SPR cycles



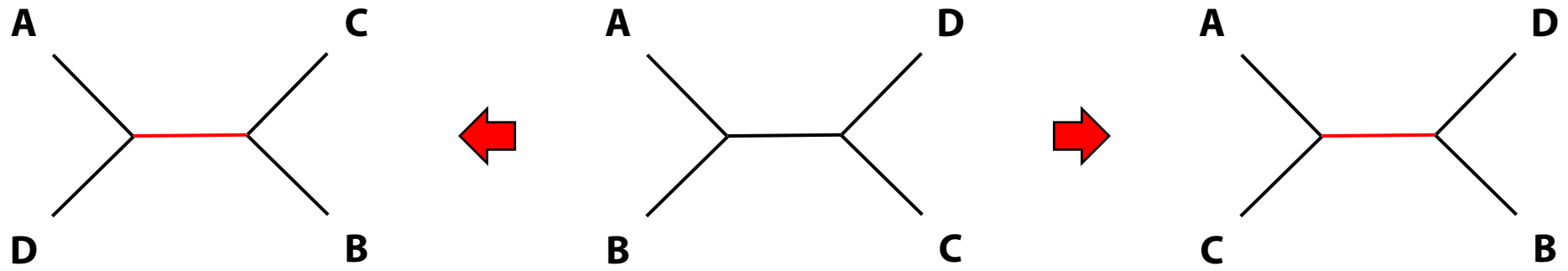
~2.5x speed up


RAP starting tree
Determine the best r on the RAP tree
SPR iteration
subtree 1
subtree 2
SPR cycle for a subtree:
For every branch within r_l/ru radius of the pruned location:
1. make rearrangement and pre-scoring;
2. if a re-grafting lead to substantially worse score, skip all the entire clade below that branch;
3. if got better score, store the tree in "bestLSR" ~~and "best20List" (if applicable)~~;
At the end, accept the best SPR in "bestLSR", update the current best tree and "best20List" (if applicable);
.
Thorough optimization for "best20List"
Increase r_l/ru by 5
Yes
Better tree?
No
END
No
$r_{up} + 5 \leq rmax$?
Yes


PHYLIP
NJ
fastDNAml
BioNJ
FastME
AxML
RAxML
RAxML-VI
FastTree
FastTree2
FastME 2
RAxML-light
RAxML 8
ExaML
PhyML
PhyML-SPR
PhyML 3
12,338
133
4,951
1980
1985
1990
1995
2000
2005
2010
2015

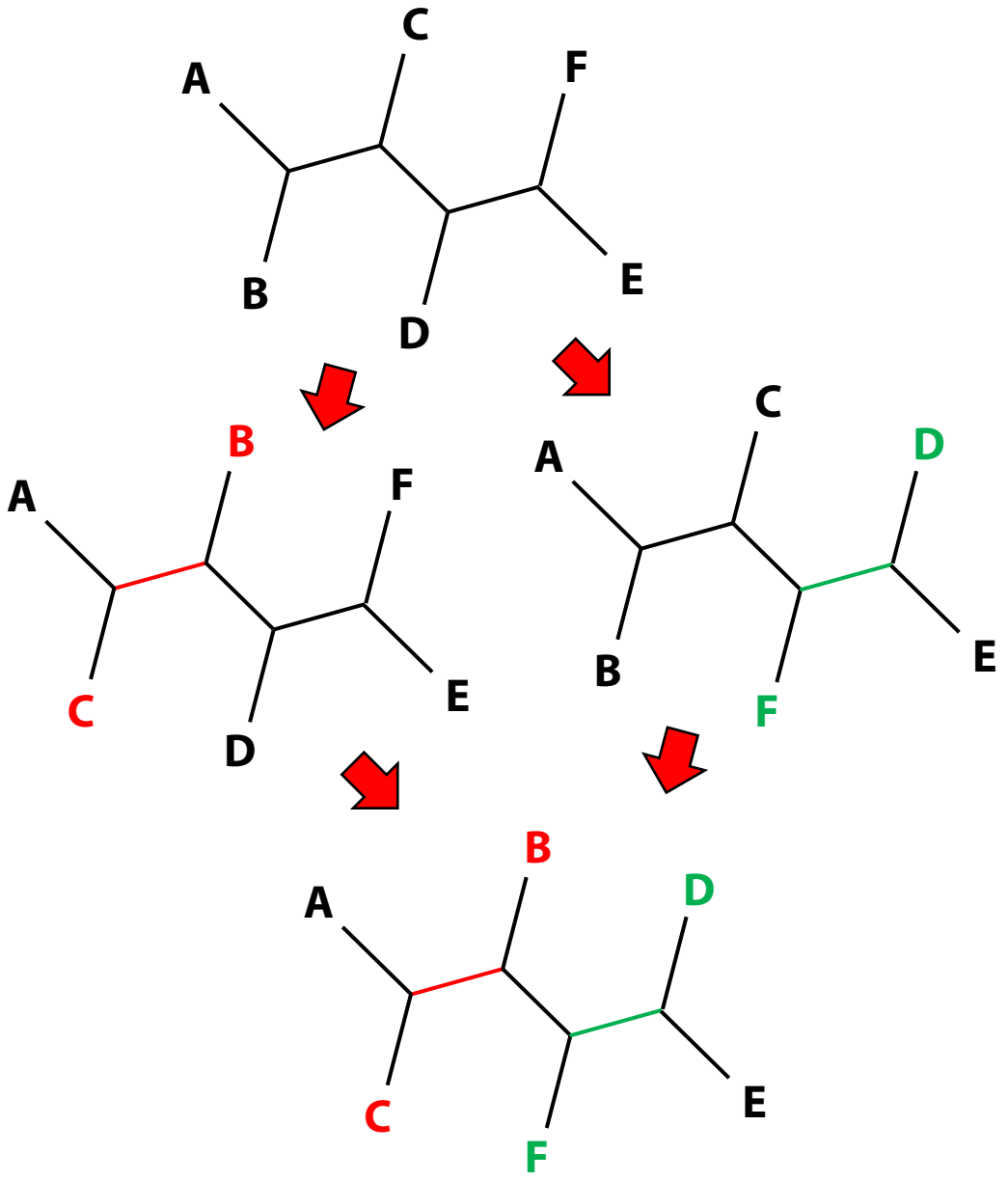
PhyML

- Single internal branch re-optimization

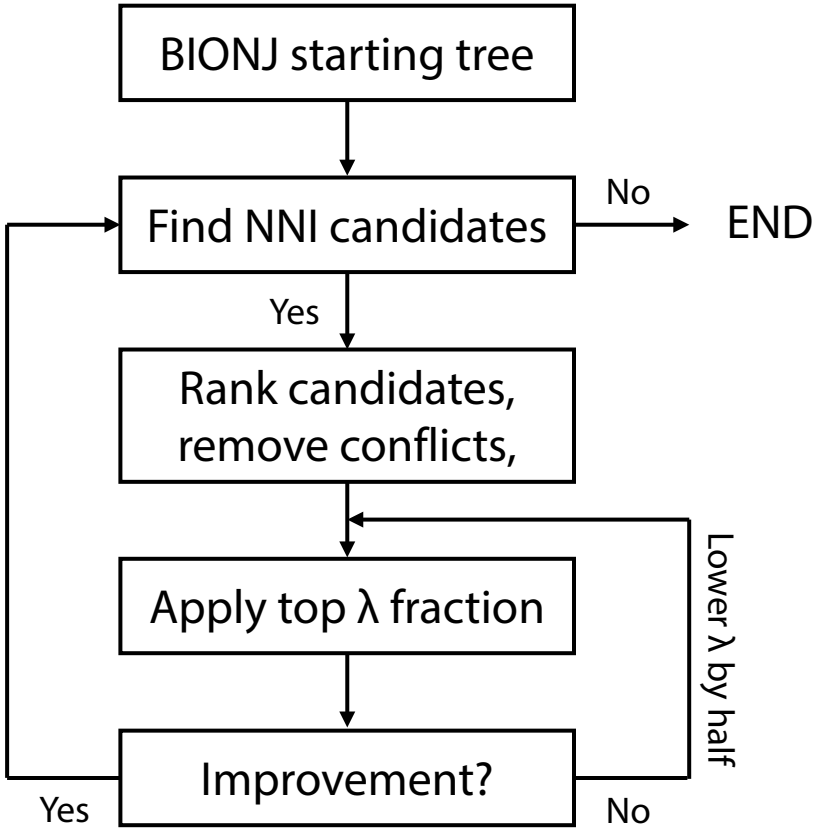


PhyML

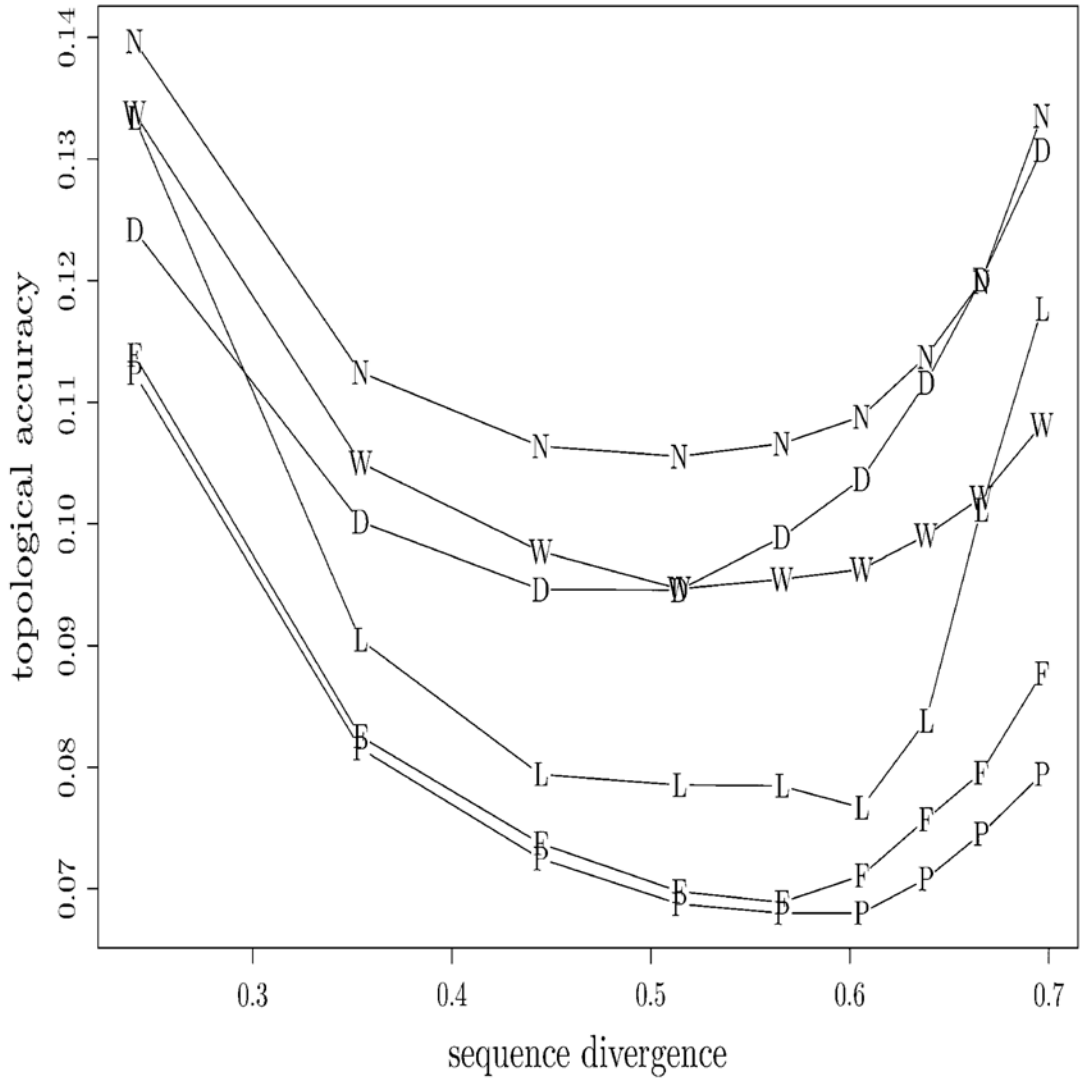
- Single internal branch re-optimization
- Simultaneous topology modifications
  - Rank all NNI candidates by LLS
  - Remove conflicting candidates
  - Apply the top λ fraction simultaneously
  - If get worse score, lower λ by half; keep going until the best one



PhyML


BIONJ starting tree
Find NNI candidates
No
END
Yes
Rank candidates,
remove conflicts,
Apply top λ fraction
Improvement?
Lower λ by half
Yes
No

PhyML: performance



	Real data	
Method	218 taxa (4,182 bp)	500 taxa (1,428 bp)
DNADIST+ NJ/BIONJ	50 sec	2 min, 19 sec
DNADIST+ Weighbor	4 min, 52 sec	58 min, 40 sec
DNAPARS	4 min, 4 sec	13 min, 12 sec
PAUP*		
PAUP*+ NJ	10 hr, 50 min	
MrBayes		
fastDNAml		
NJML		
MetaPIGA	4 hr, 45 min	9 hr, 4 min
MetaPIGA+ NJ	1 hr, 40 min	3 hr
PHYML	8 min, 13 sec (15)	11 min, 59 sec (13)

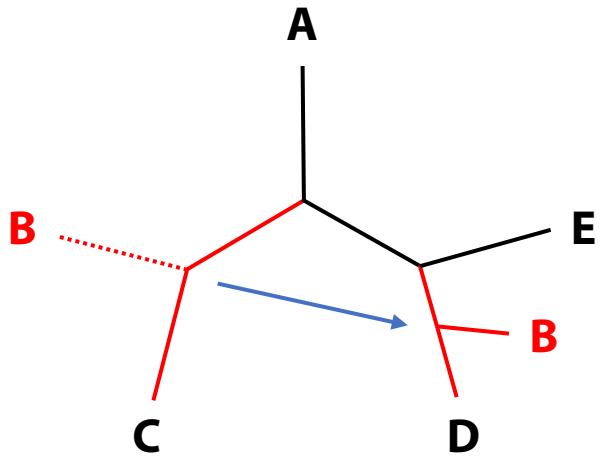
	Simulations	
Method	40 taxa (500 bp)	100 taxa (500 bp)
DNADIST+ NJ/BIONJ	0.3 sec	2.3 sec
DNADIST+ Weighbor	1.5 sec	22 sec
DNAPARS	0.5 sec	6 sec
PAUP*	3 min, 21 sec	1 hr, 4 min
PAUP*+ NJ	1 min, 10 sec	22 min
MrBayes	2 min, 6 sec	32 min, 37 sec
fastDNAml	1 min, 13 sec	26 min, 31 sec
NJML	15 sec	6 min, 4 sec
MetaPIGA	21 sec	3 min, 27 sec
MetaPIGA+ NJ	6 sec	23 sec
PHYML	2.7 sec (6.4)	12 sec (8.3)

Guindon and Gascuel (2003) Syst. Biol.

PhyML-SPR

- Filter candidate SPR moves based on distance criterion

- Quick estimation of branch-length using distance-based approach

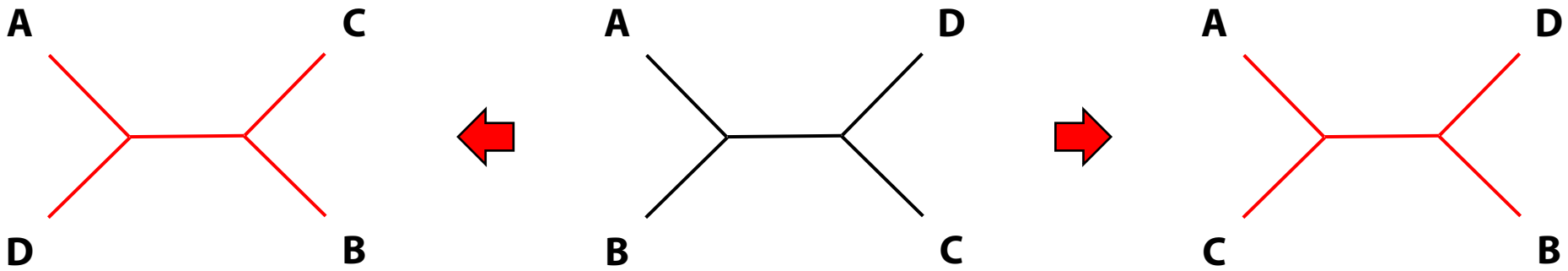


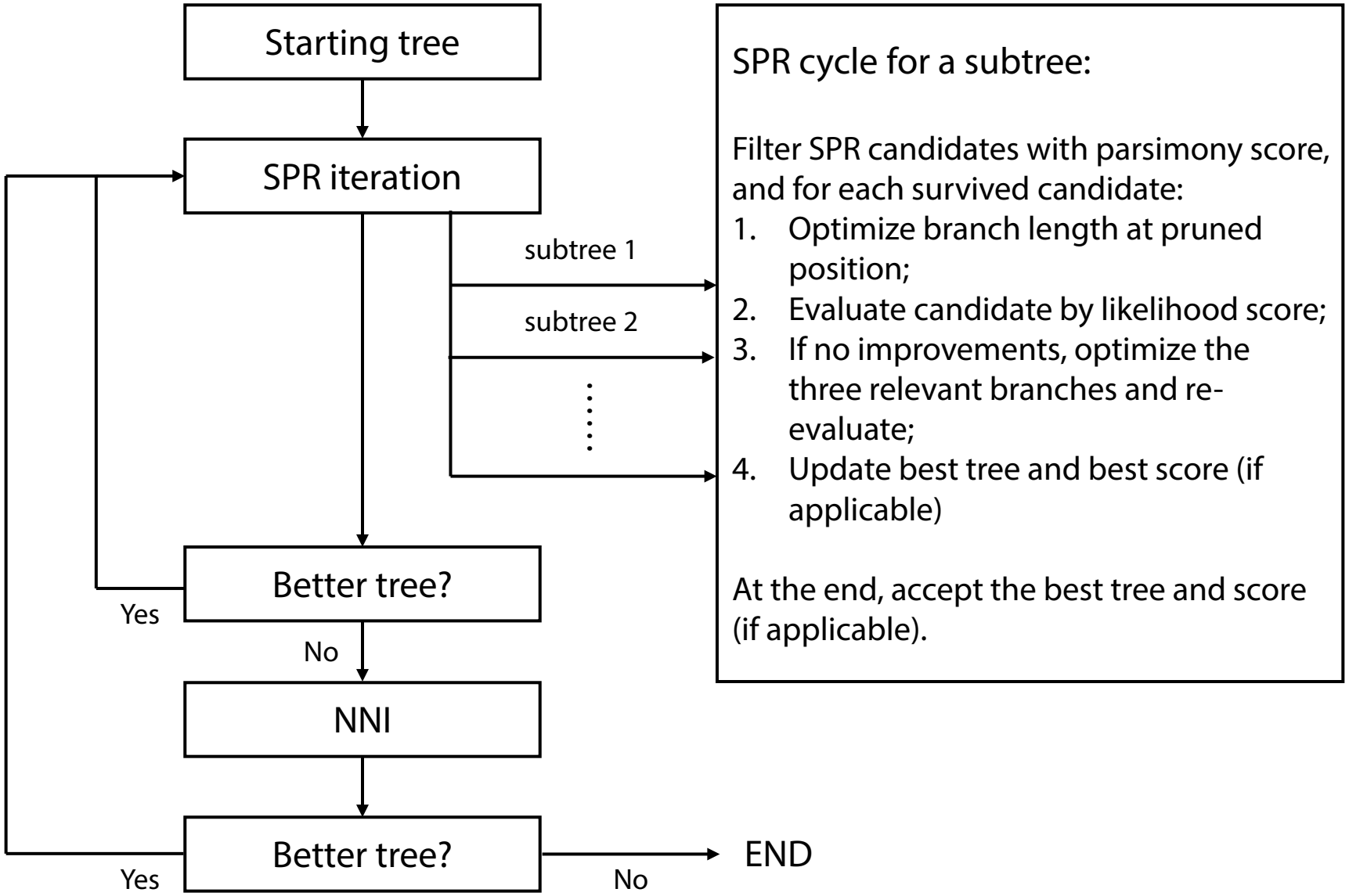
Hordijk and Gascuel (2005) Bioinformatics

PhyML 3

- Use parsimony score instead of distance to filter SPR candidates

- Alternated SPR and NNI searches
  - NNI optimizes all five relevant branches instead of one




Starting tree
SPR iteration
subtree 1
subtree 2
Better tree?
Yes
No
NNI
Better tree?
Yes
No
END
SPR cycle for a subtree:
Filter SPR candidates with parsimony score, and for each survived candidate:
1. Optimize branch length at pruned position;
2. Evaluate candidate by likelihood score;
3. If no improvements, optimize the three relevant branches and re-evaluate;
4. Update best tree and best score (if applicable)
At the end, accept the best tree and score (if applicable).

PhyML 3: performance

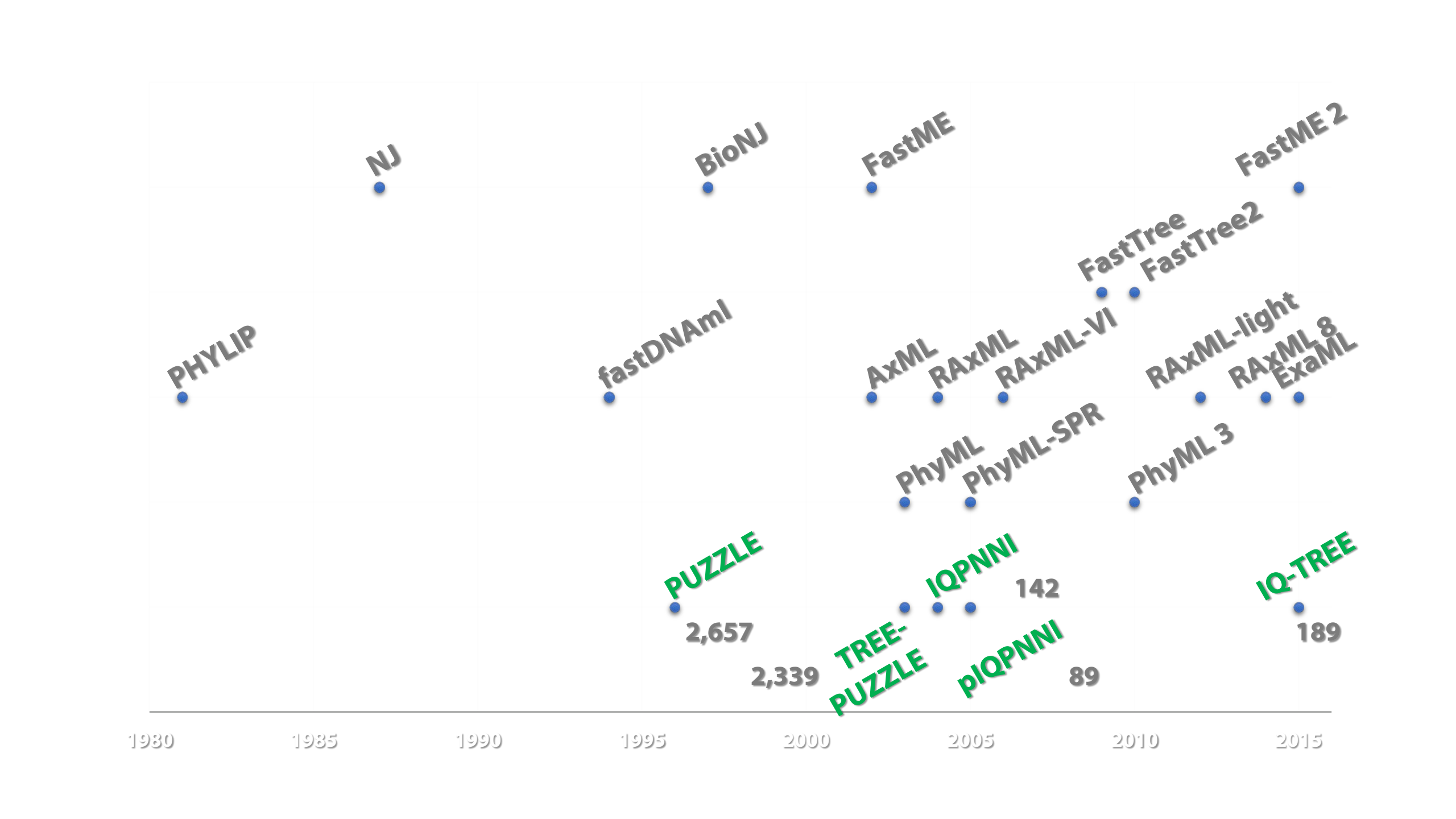
TABLE 3. Comparison of log-likelihoods on 50 DNA and 50 protein medium-size data sets

	Av. LogLk rank	Delta > 5	*P* value <0.05	Av. RF distance
DNA				
PhyML 2.4.5	5.48	34	4	0.30
PhyML 3.0 NNI	5.18	33	5	0.28
PhyML 3.0 SPR	2.78	2	0	0.15
PhyML 3.0 BEST	2.70	2	0	0.15
PhyML 3.0 RAND	1.64	0	0	0.03
RAxML	3.22	3	2	0.20
Protein				
PhyML 2.4.5	5.05	21	1	0.26
PhyML 3.0 NNI	4.33	20	1	0.24
PhyML 3.0 SPR	3.24	5	0	0.14
PhyML 3.0 BEST	3.16	4	0	0.14
PhyML 3.0 RAND	2.35	0	0	0.03
RAxML	2.86	0	0	0.08

TABLE 4. Comparison of log-likelihoods on 10 DNA and 10 protein large data sets

	Av. LogLk rank	Delta > 5	*P* value <0.05	Av. RF distance
DNA				
PhyML 2.4.5	3.50	10	8	0.47
PhyML 3.0 NNI	3.50	10	7	0.46
PhyML 3.0 SPR	1.40	3	0	0.15
RAxML	1.60	5	1	0.23
Protein				
PhyML 2.4.5	3.45	7	3	0.24
PhyML 3.0 NNI	2.65	6	3	0.20
PhyML 3.0 SPR	2.75	7	0	0.18
RAxML	1.14	0	0	0.00

Guindon et al (2010) Syst. Biol.


PHYLIP
NJ
fastDNAml
BioNJ
AxML
RAxML
RAxML-VI
FastME
FastTree
FastTree2
RAxML-light
RAxML 8
ExaML
FastME 2
PhyML
PhyML-SPR
PhyML 3
PUZZLE
2,657
2,339
TREE-PUZZLE
IQPNNI
142
pIQPNNI
89
IQ-TREE
189
1980
1985
1990
1995
2000
2005
2010
2015

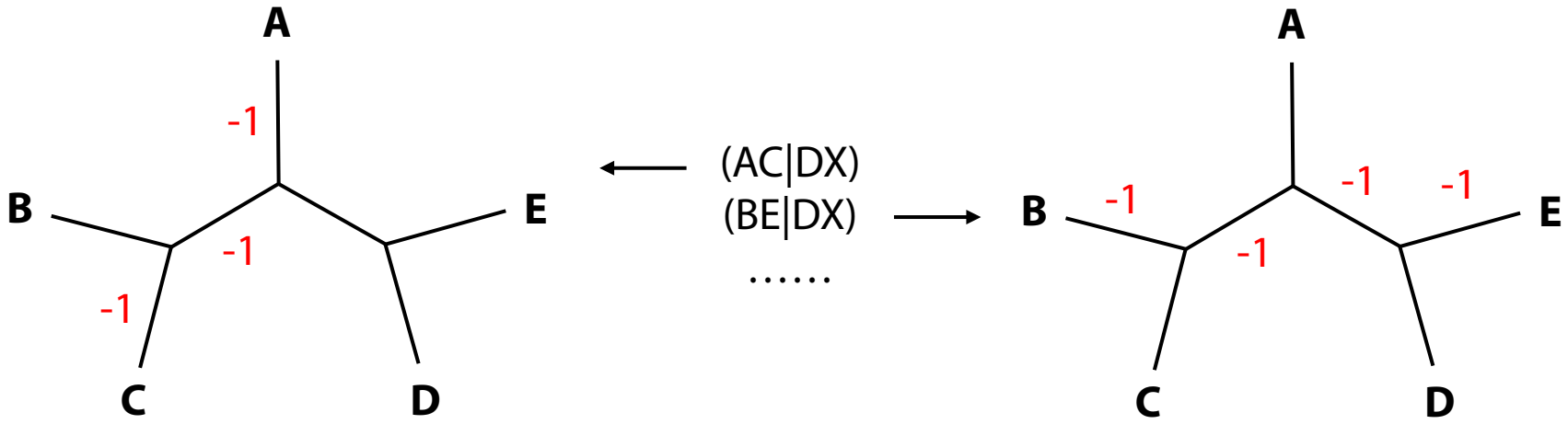
Quartet Puzzling

- Building tree from small pieces
  - 40 sequences

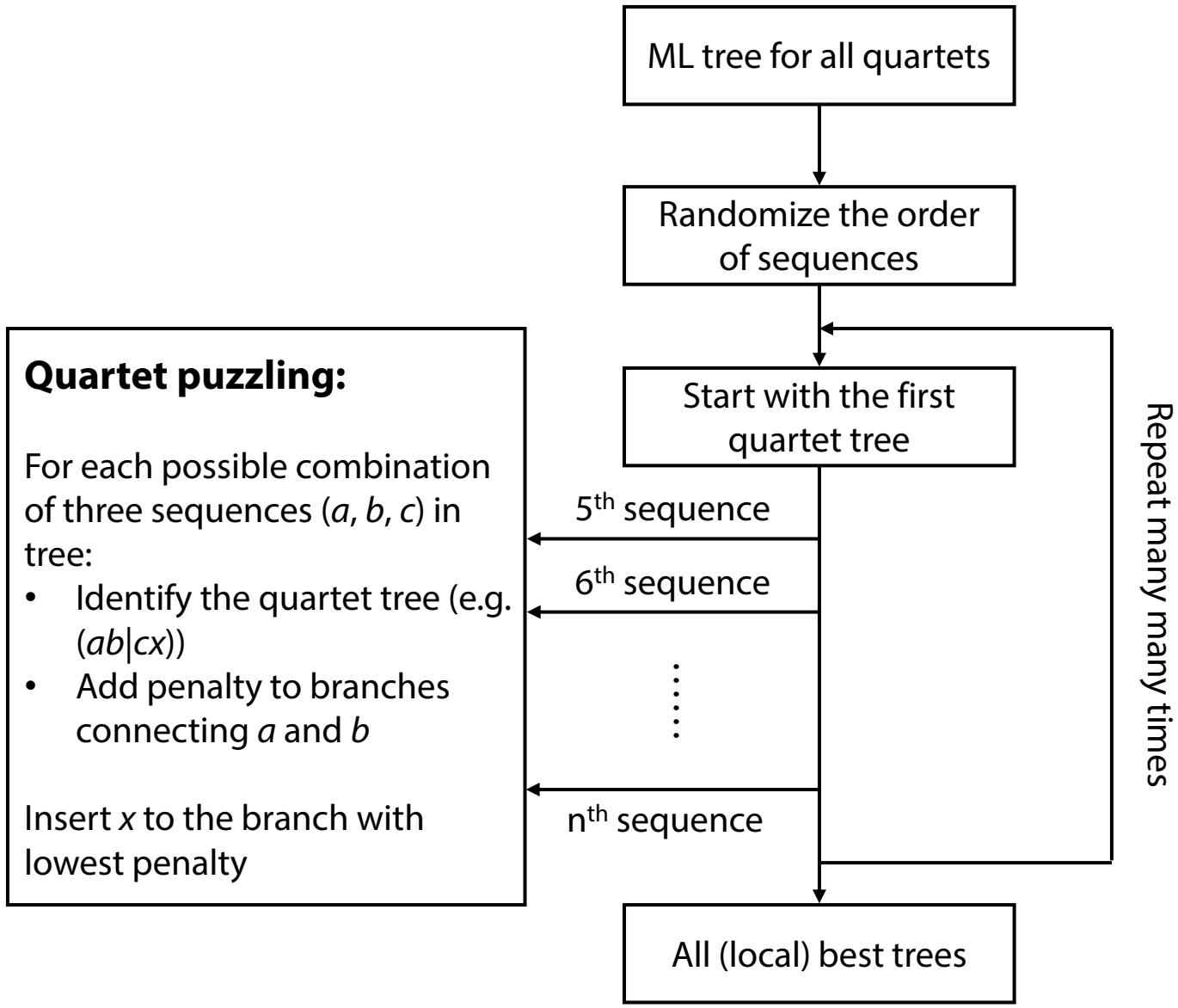
    1.31×10^{51} binary unrooted trees

    931,390 quartets

- Stepwise addition based on quartet trees

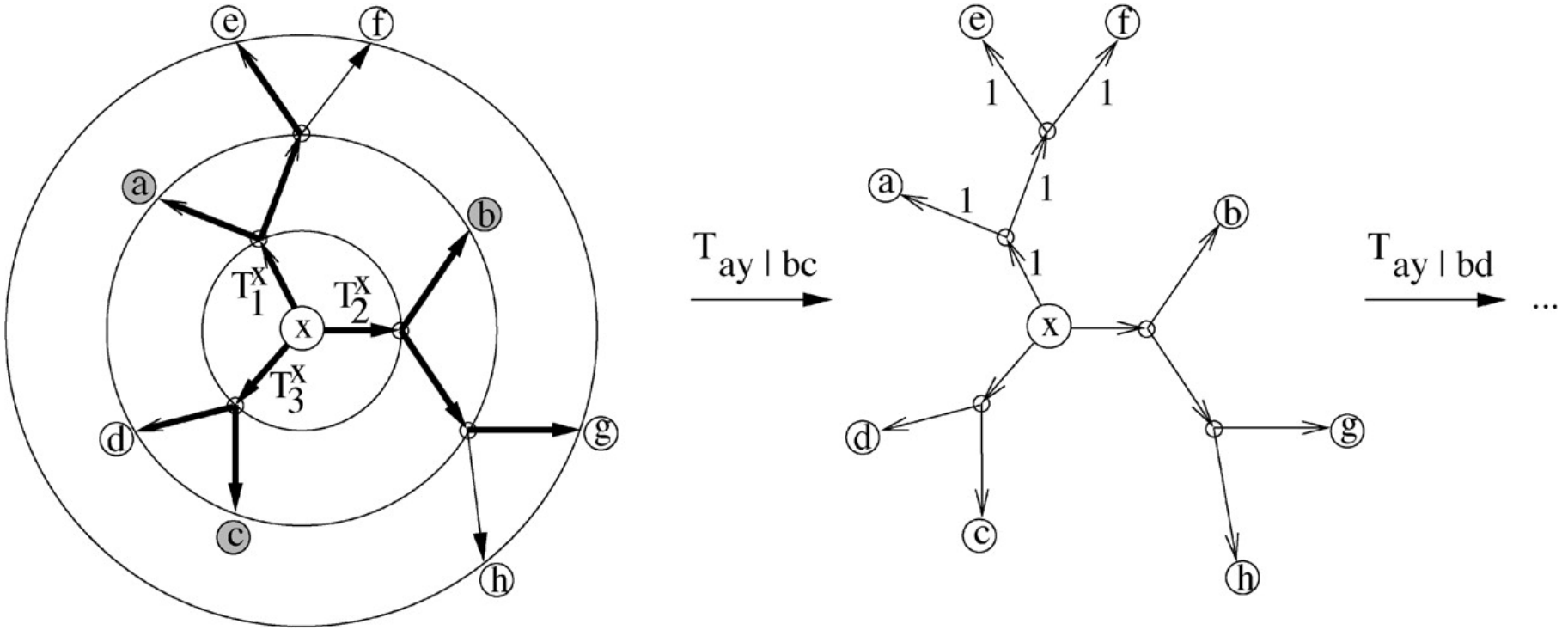


PUZZLE


ML tree for all quartets
Randomize the order of sequences
Start with the first quartet tree
Repeat many many times
5th sequence
6th sequence
nth sequence
All (local) best trees
Quartet puzzling:
For each possible combination of three sequences (a, b, c) in tree:
• Identify the quartet tree (e.g. (ab|cx))
• Add penalty to branches connecting a and b
Insert x to the branch with lowest penalty

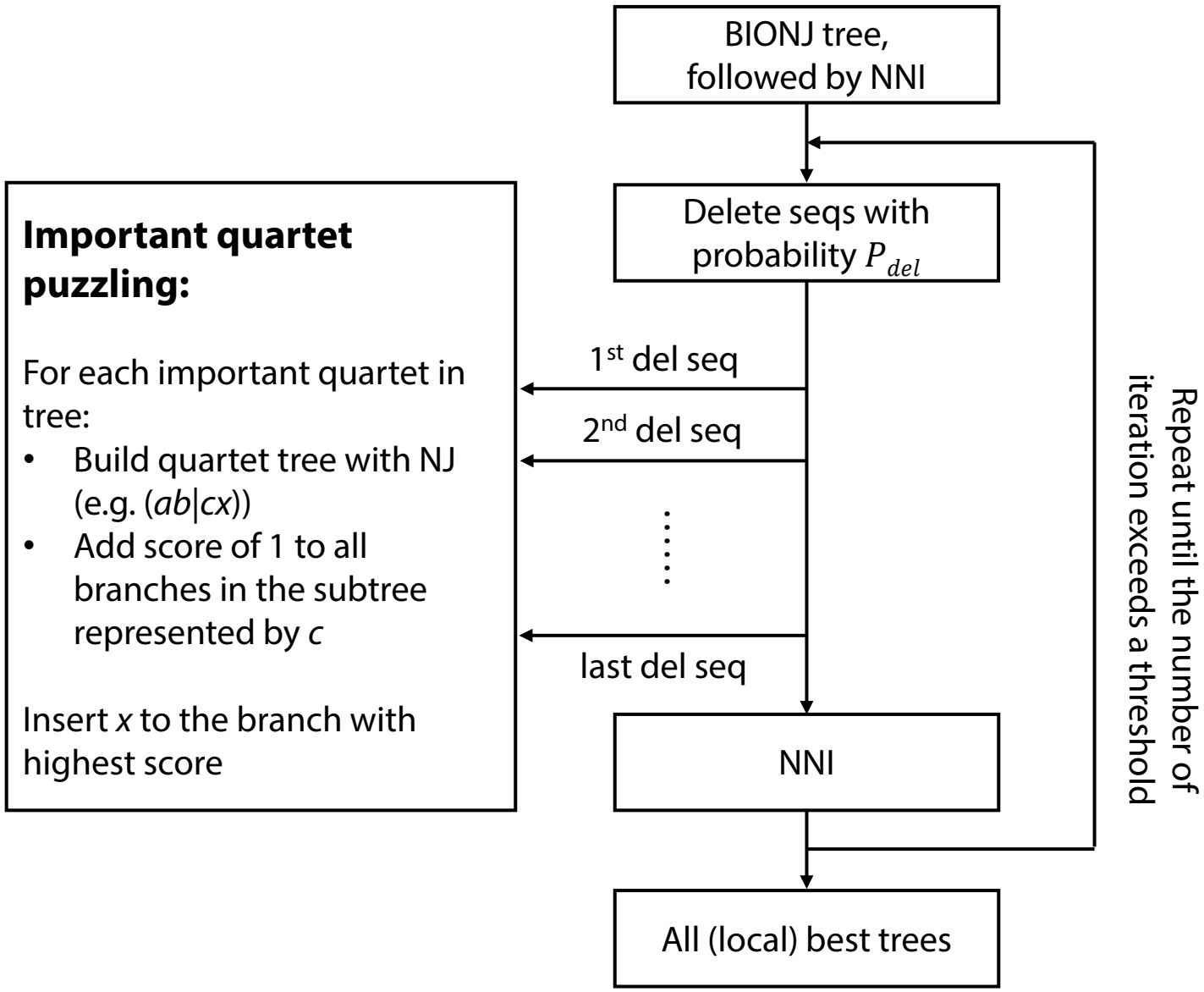
IQPNNI - Important quartet puzzling + NNI

- Important quartet

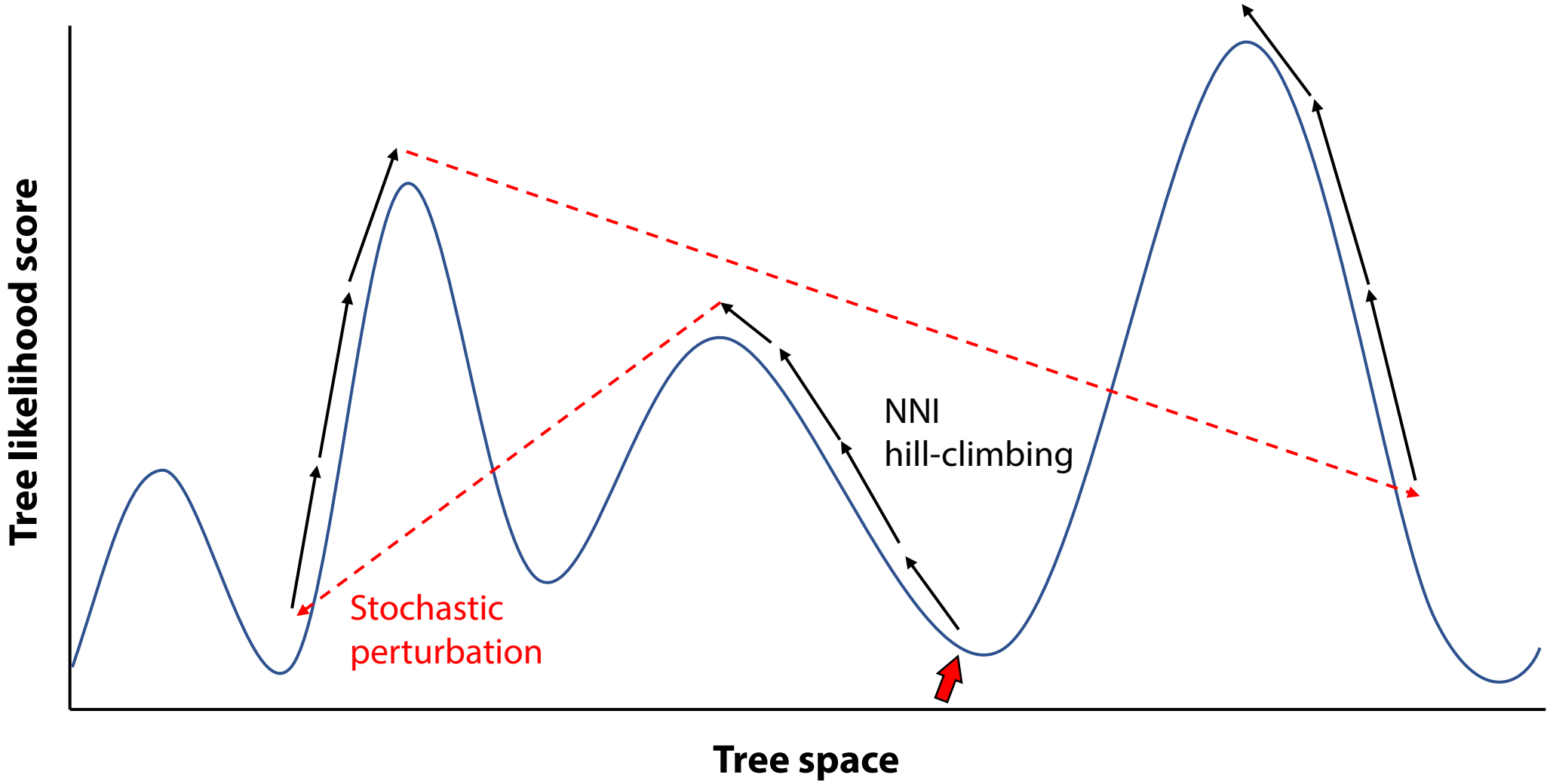


Vinh le and Von Haeseler (2004) Mol. Biol. Evol.

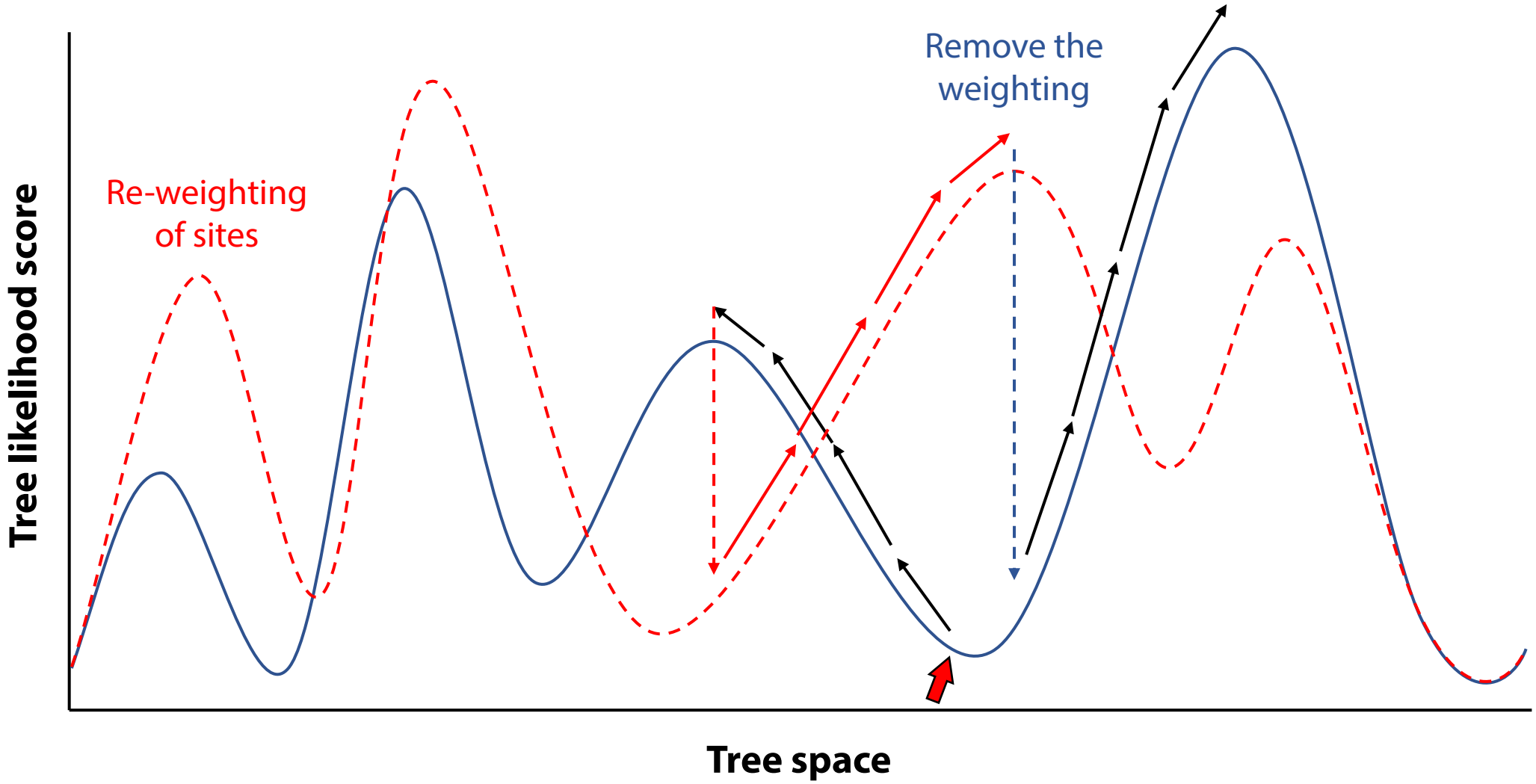
IQPNNI


BIONJ tree,
followed by NNI
Delete seqs with
probability P_{del}
1st del seq
2nd del seq
last del seq
NNI
All (local) best trees
Repeat until the number of
iteration exceeds a threshold
Important quartet
puzzling:
For each important quartet in
tree:
• Build quartet tree with NJ
(e.g. (ab|cx))
• Add score of 1 to all
branches in the subtree
represented by c
Insert x to the branch with
highest score

Escape from local optima

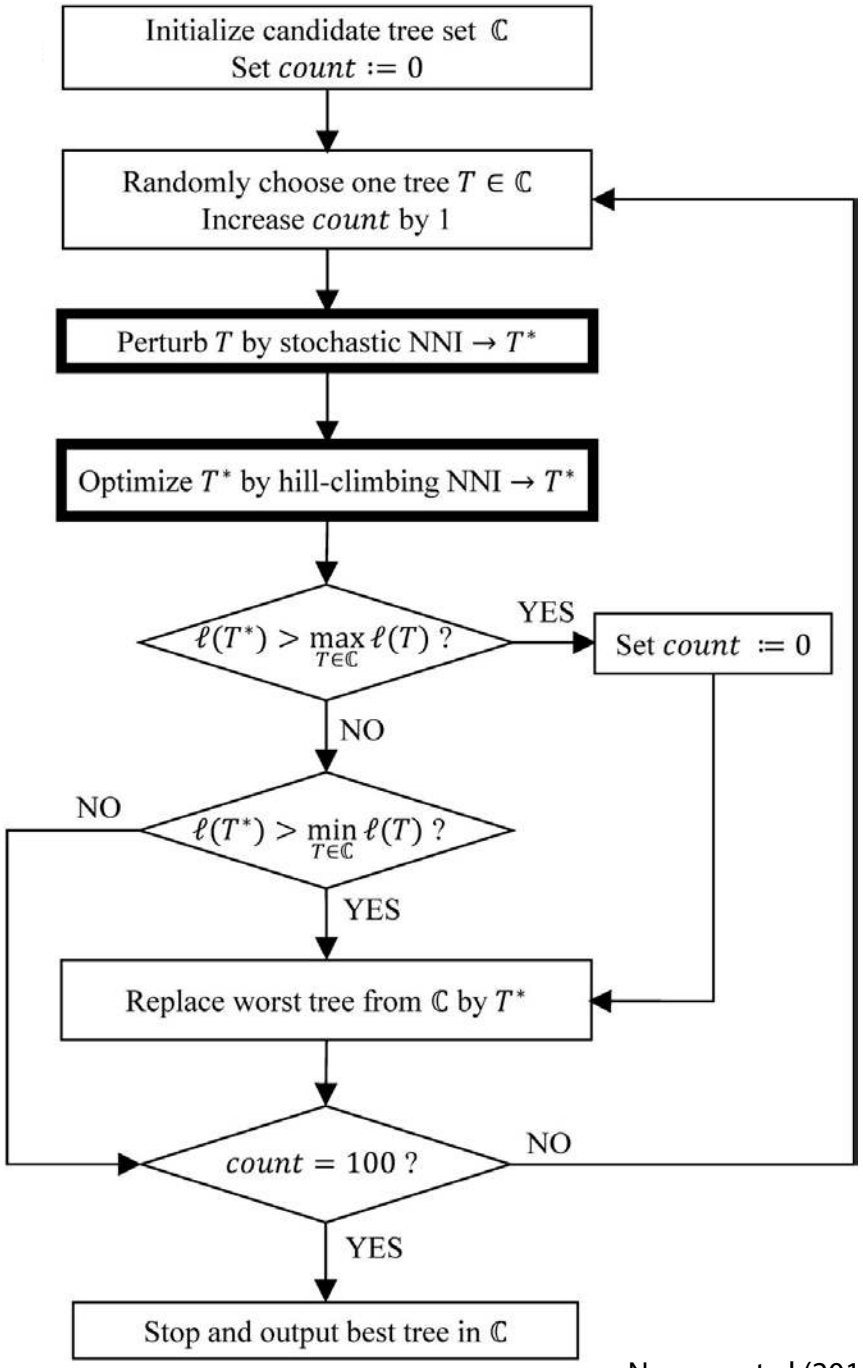


Ratchet


Re-weighting
of sites
Remove the
weighting
Tree likelihood score
Tree space

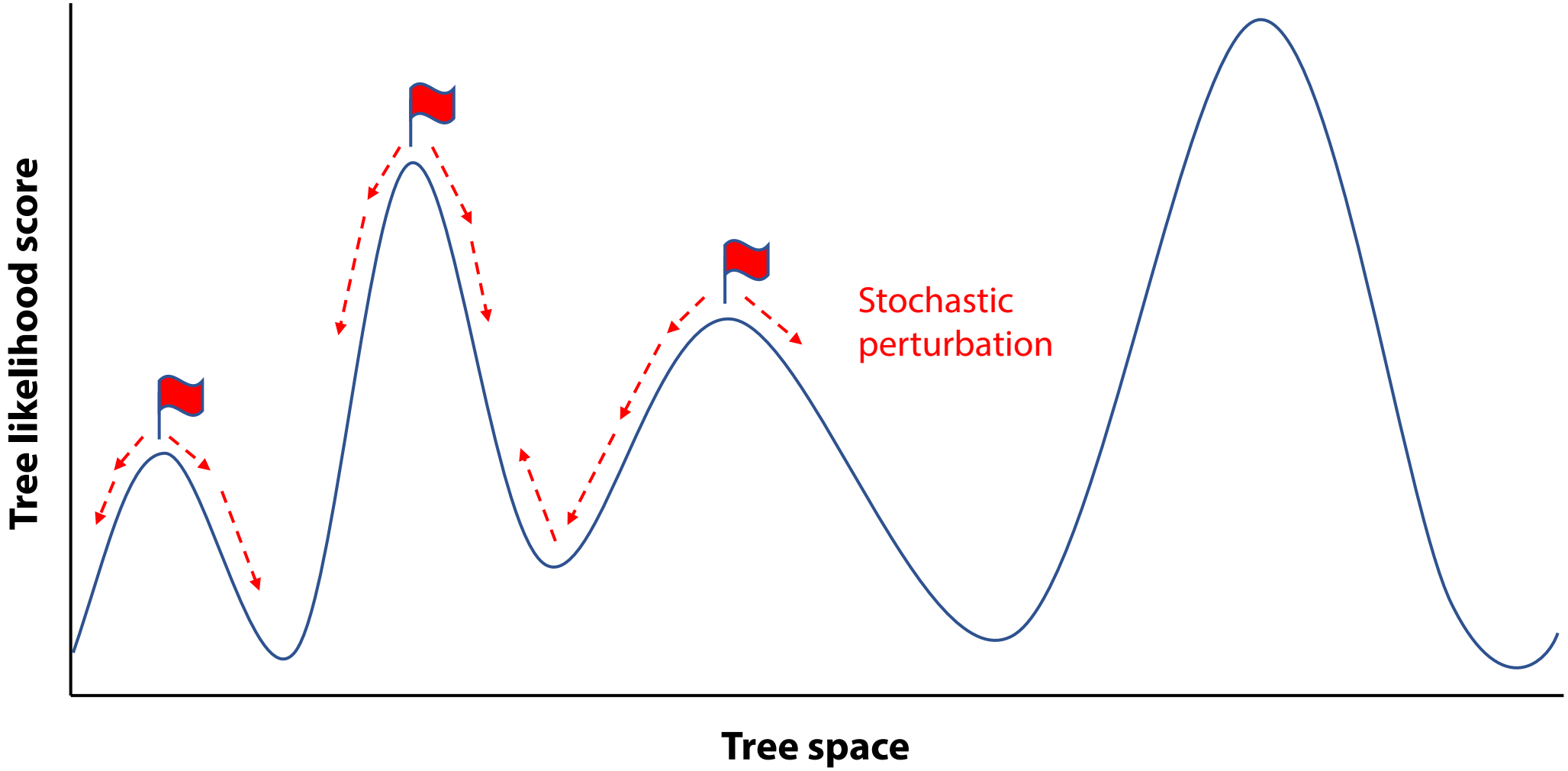
IQ-TREE

- A pool of starting trees
- A pool of candidate trees
- NNI- instead of IQP-based perturbation
- Simultaneous NNI modifications
  - Reduced NNI neighborhood

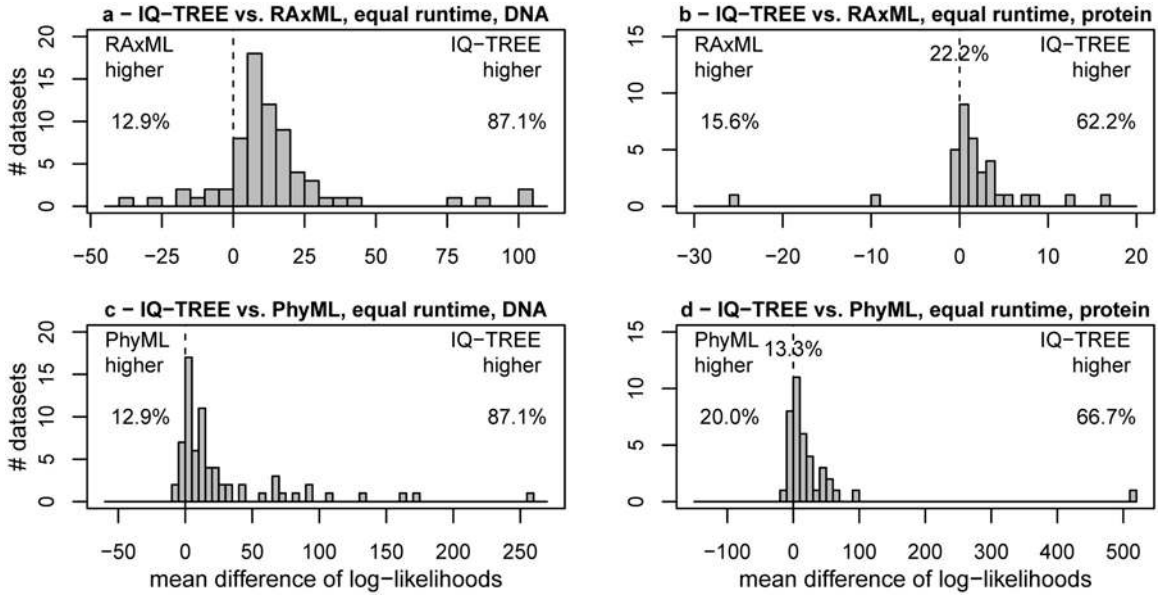


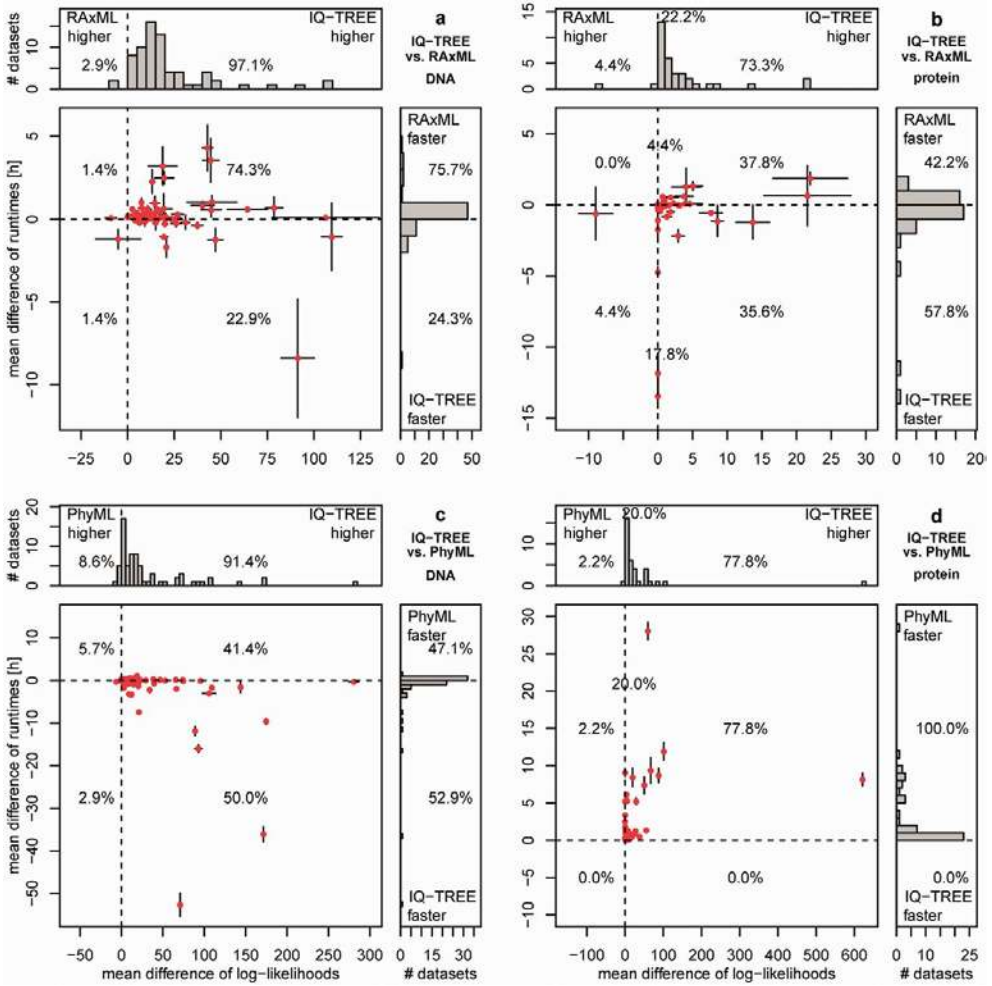
Nguyen et al (2015) Mol. Biol. Evol.

Escape from local optima: IQ-TREE



IQ-TREE: performance





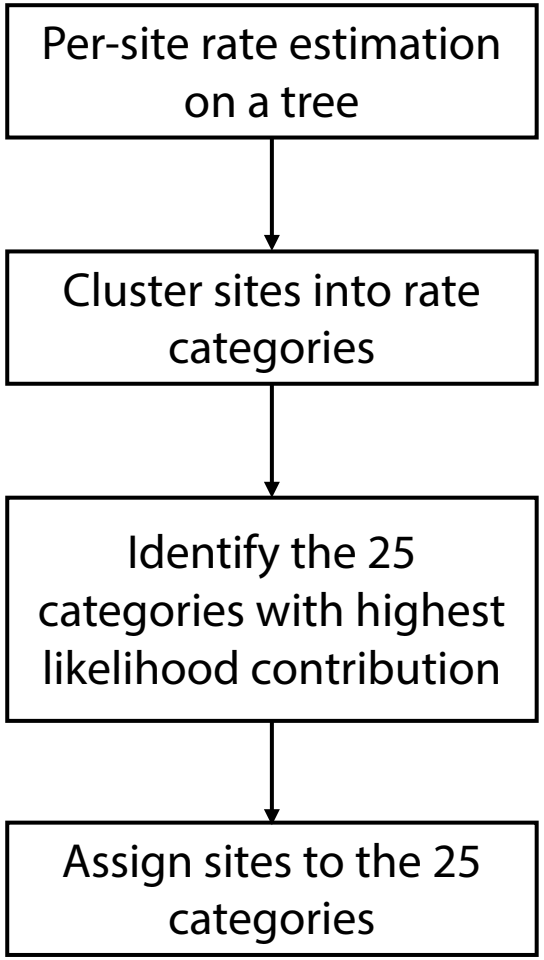
Nguyen et al (2015) Mol. Biol. Evol.

Other techniques for fast phylogenetics

- GAMMA vs CAT

GAMMA vs CAT

- GAMMA
  - model rate heterogeneity among sites using the gamma distribution
  - each site has certain probability belonging to each rate category
- CAT
  - assign sites into fixed number of rate categories
  - each site belongs to a specific rate category



GAMMA vs CAT

Dataset	$T(\Gamma)/T(CAT)$	$T(\Gamma)/T(CAT+R_\Gamma)$	$l_\Gamma(\Gamma)/l_\Gamma(CAT)$	$l_\Gamma(\Gamma)/l_\Gamma(CAT+R_\Gamma)$	$RF(\Gamma,CAT)$	$RF(\Gamma,CAT+R_\Gamma)$	α	# pat
73_OLAF	4.177018	2.779953	0.999959	0.999997	0.008392	0.005594	1.180	1,196
74_OLAF	3.456038	2.429559	0.999963	0.999963	0.029371	0.029371	0.575	578
104_OLAF	2.971896	1.465592	0.999616	1.000293	0.113659	0.098049	0.329	581
128_OLAF	8.728934	4.362863	1.000026	1.000268	0.016996	0.016996	3.166	2,985
144_OLAF	4.353371	2.233404	0.999983	1.000107	0.055789	0.055088	0.825	1,254
178_OLAF	4.742052	2.397997	0.999998	1.000183	0.026346	0.026062	0.634	1,150
180_OLAF	3.261044	2.300603	0.999608	1.000112	0.048179	0.046499	0.454	924
101_SC	8.607863	4.081393	0.999791	0.999873	0.098492	0.084925	0.417	1,630
150_SC	4.212270	2.630621	0.999955	1.000037	0.040404	0.032323	0.433	1,130
150_ARB	6.935958	4.125580	1.000019	1.000032	0.013805	0.014478	0.562	2,137
193_VINH	2.541966	1.822700	0.999929	1.000007	0.117755	0.112272	1.313	459
200_ARB	7.359741	3.981281	1.000068	1.000089	0.036272	0.034257	0.534	2,253
218_RDPII	5.890610	2.320172	0.999824	1.000018	0.120092	0.103695	0.545	1,847
250_ARB	7.076141	3.817160	1.000027	1.000076	0.032394	0.028974	0.580	2,330
500_ARB	7.378079	3.243040	1.000112	1.000207	0.057573	0.050351	0.579	2,751
500_ZILLA	4.156063	3.014160	1.000160	1.000203	0.054162	0.048947	0.494	1,193
715_CHUCK	4.663363	2.297917	0.999991	1.000146	0.043868	0.039804	0.842	1,231
1000_ARB	8.151405	2.894259	1.001454	1.001549	0.051377	0.048072	0.552	3,364
1663_ARB	4.897310	1.827990	1.000221	1.000320	0.087571	0.084487	0.621	1,576
Averages	5.450585	2.843487	1.000037	1.000183	0.055395	0.050539	0.770	1,609

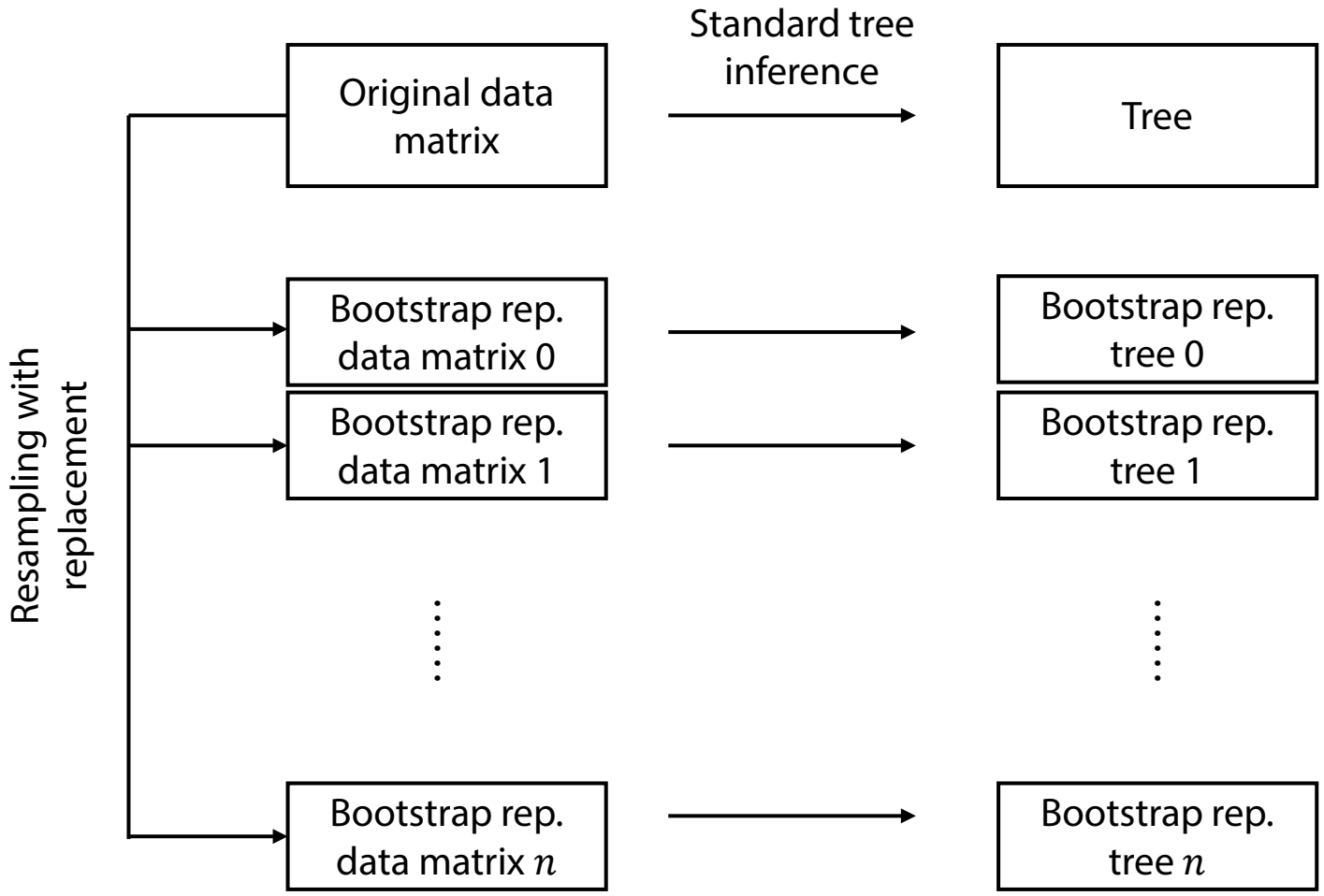
Stamatakis (2006) Proceedings 20th IEEE International Parallel & Distributed Processing Symposium

Other techniques for fast phylogenetics

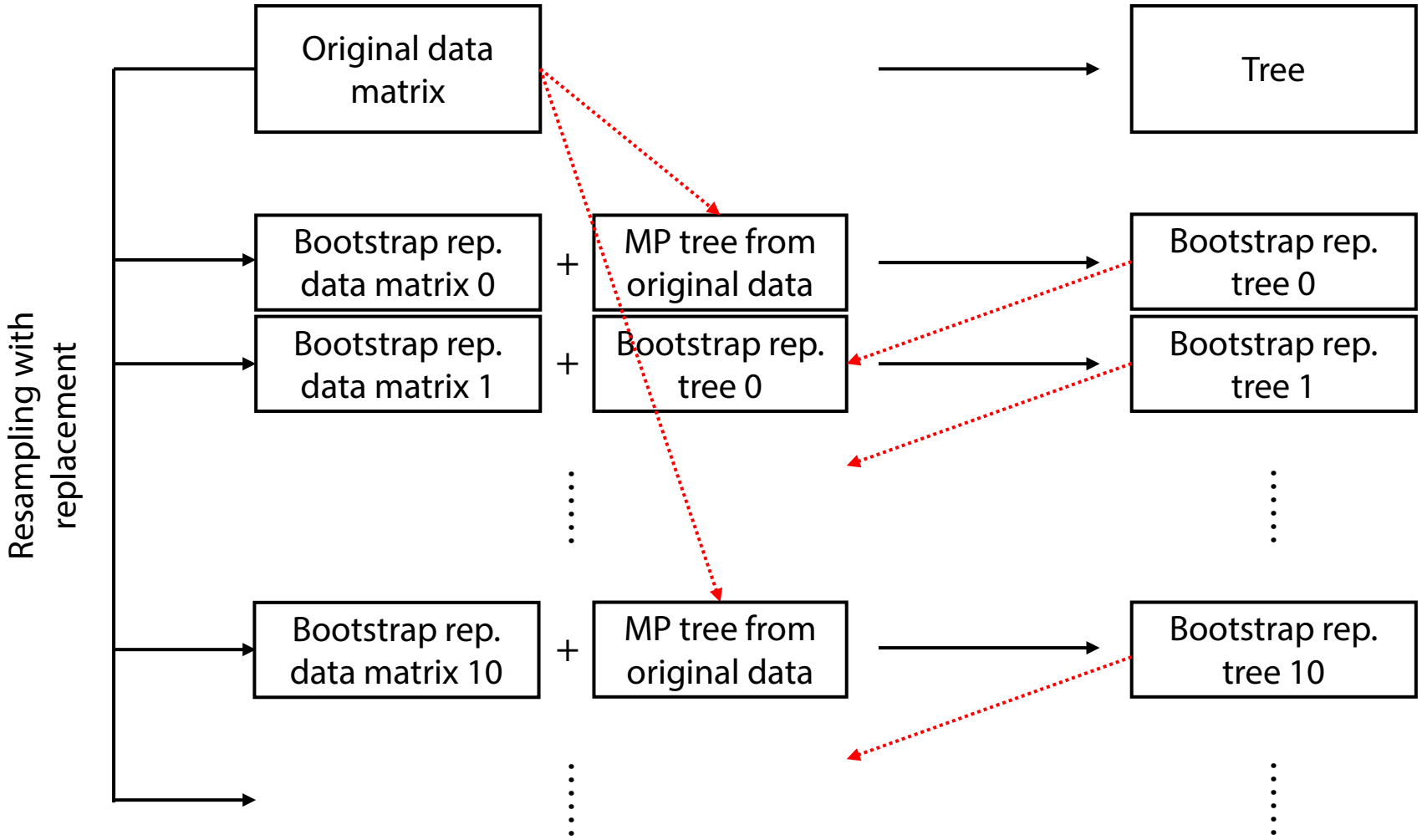
- GAMMA vs CAT

- Fast approaches for node support

Standard Bootstrap



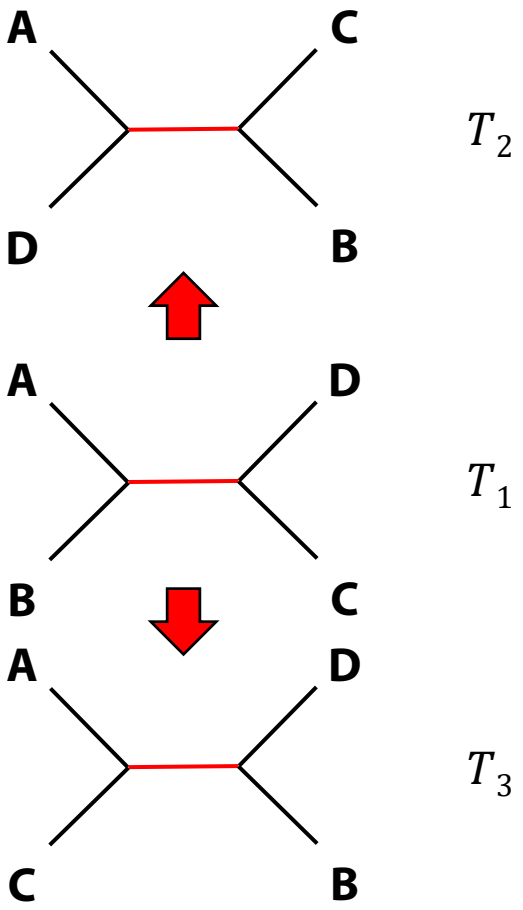
Rapid bootstrap (RAxML)



Additional shortcuts:

- LSR radius randomly chosen between 5 and 15;
- 2 iterations of LSR;
- More aggressive subtree skipping;
- Thorough optimization for best 5 instead of 20;

Local branch support

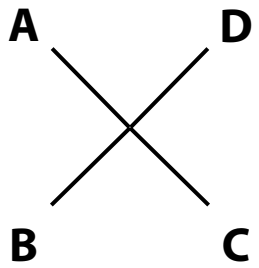


T_2

T_1

T_3

1. Approximate likelihood-ratio test (aLRT):
   - $aLRT \leftarrow 2(l_1 - \max(l_2, l_3)) < 2(l_1 - l_0)$

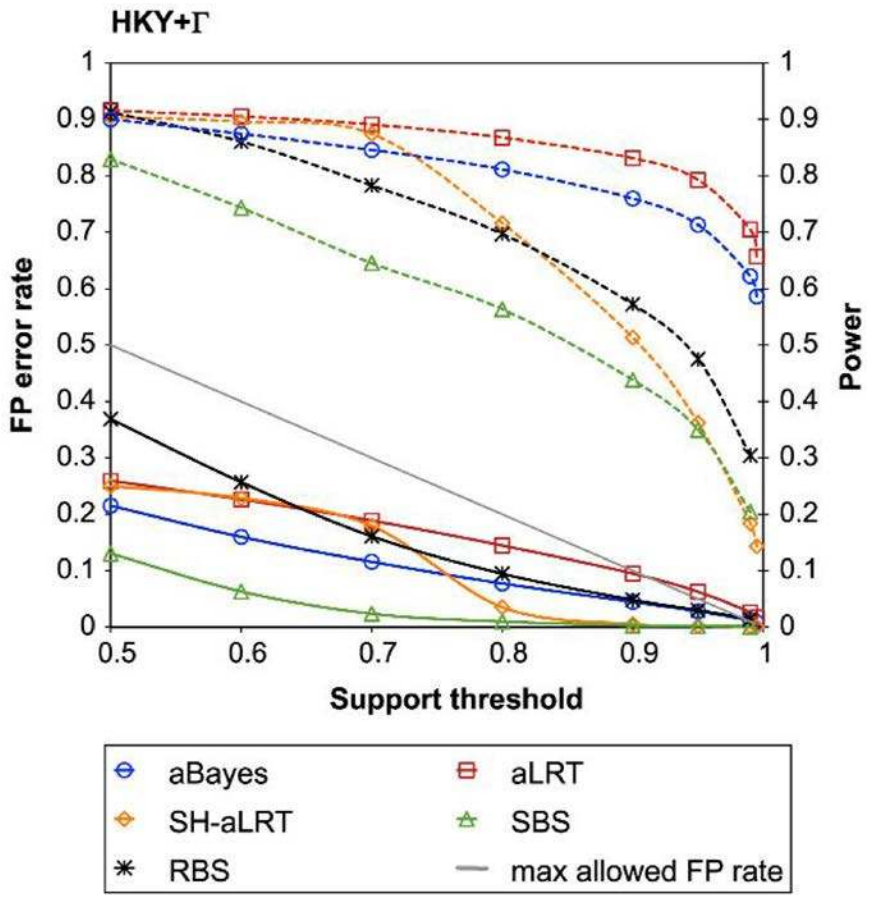


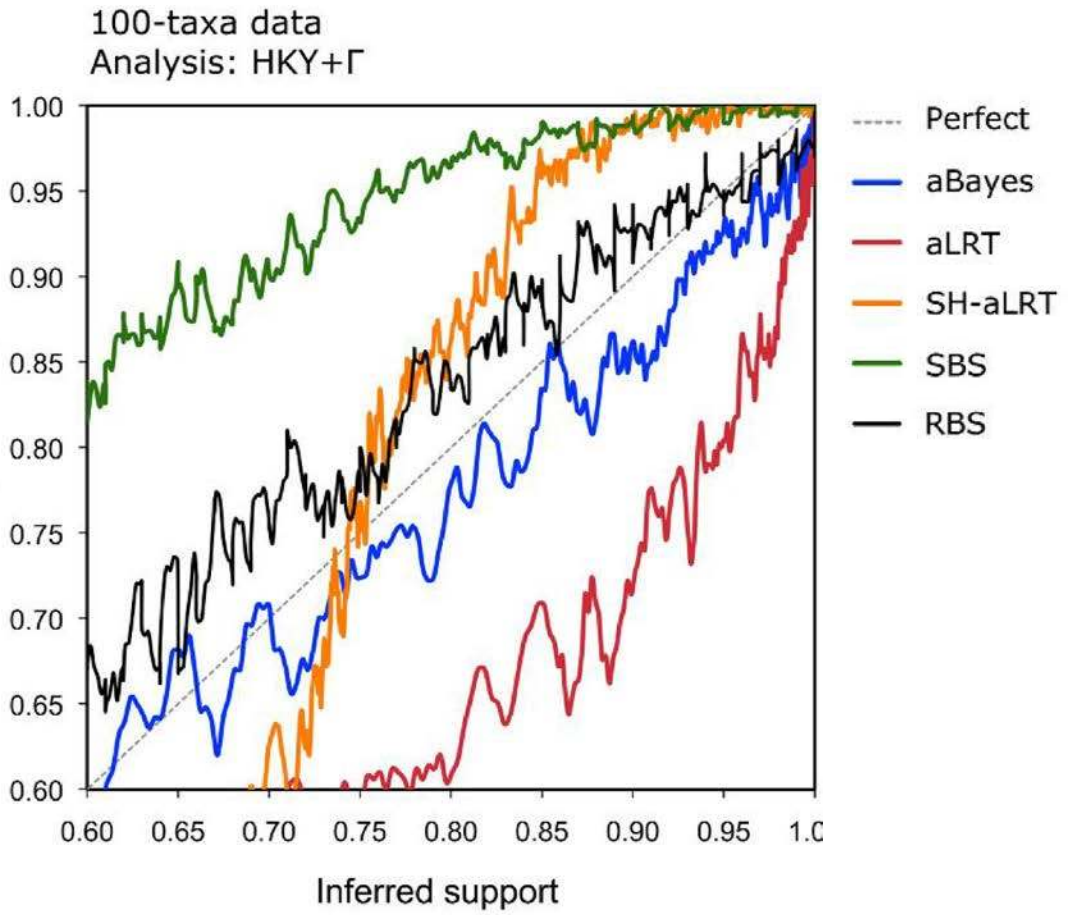
T_0

2. SH-aLRT:
   - aLRT with RELL bootstrap re-sampling
   - Support value = $count(aLRT > aLRT^*)$

3. Approximate Bayes (aBayes):
   - $\Pr(T_1|D) = \Pr(D|T_1)\Pr(T_1)/\sum_{i=1}^{3}\Pr(D|T_i)\Pr(Ti)$

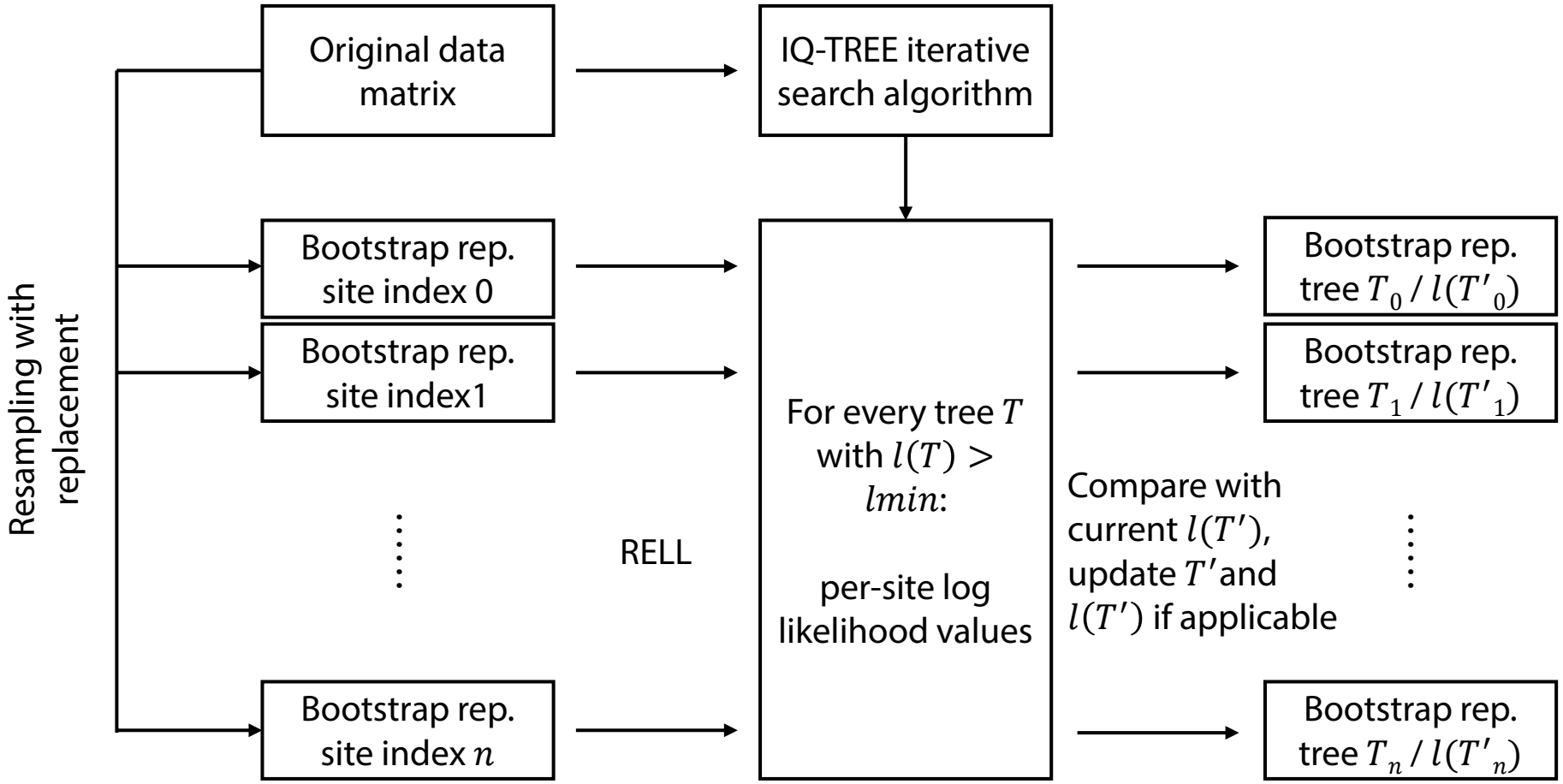
Local branch support: performance



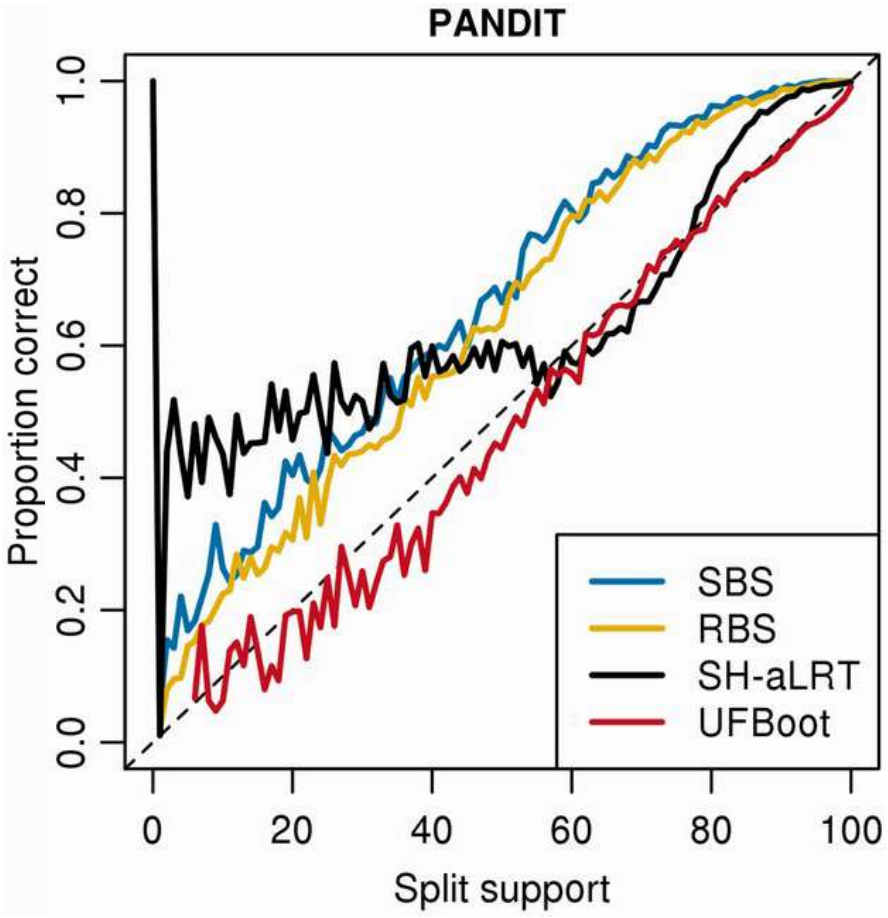


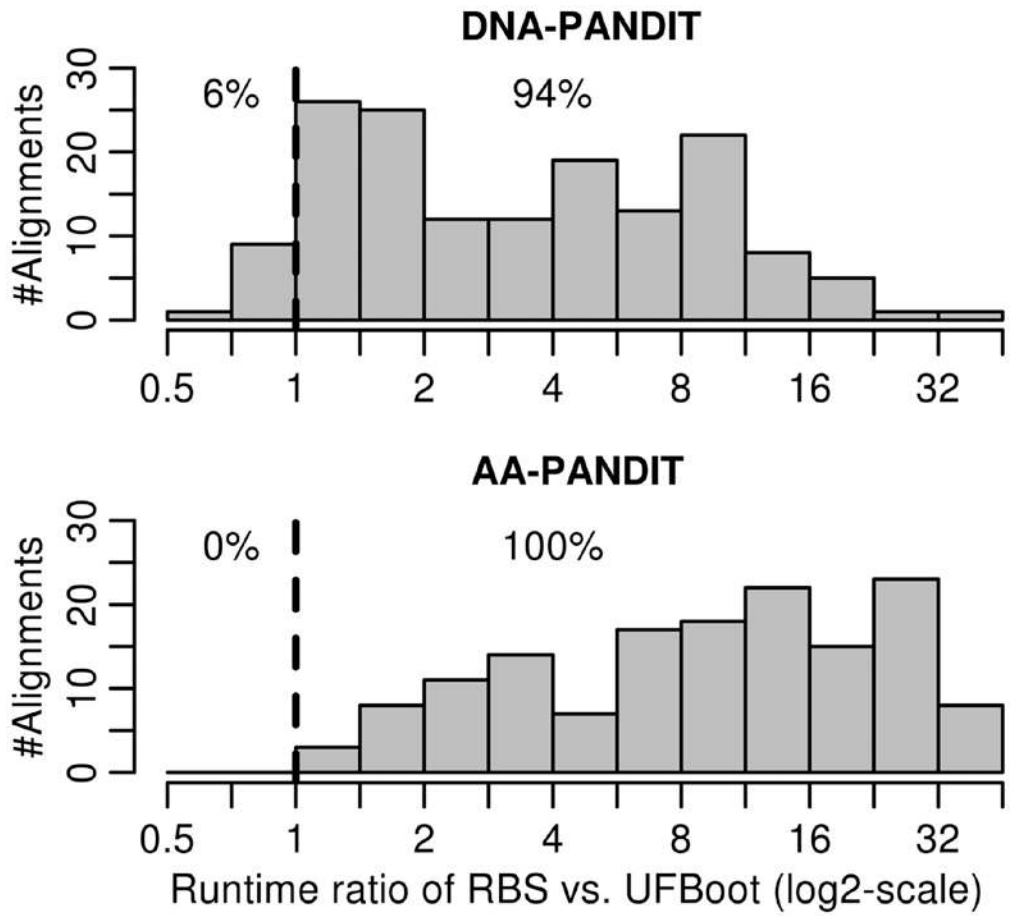
Anisimova et al (2011) Syst. Biol.

Ultra-fast bootstrap (IQ-TREE)



UFBS: performance





Minh et al (2013) Mol. Biol. Evol.

Other techniques for fast phylogenetics

- GAMMA vs CAT
- Fast approaches for node support
- Parallelization

Parallelization

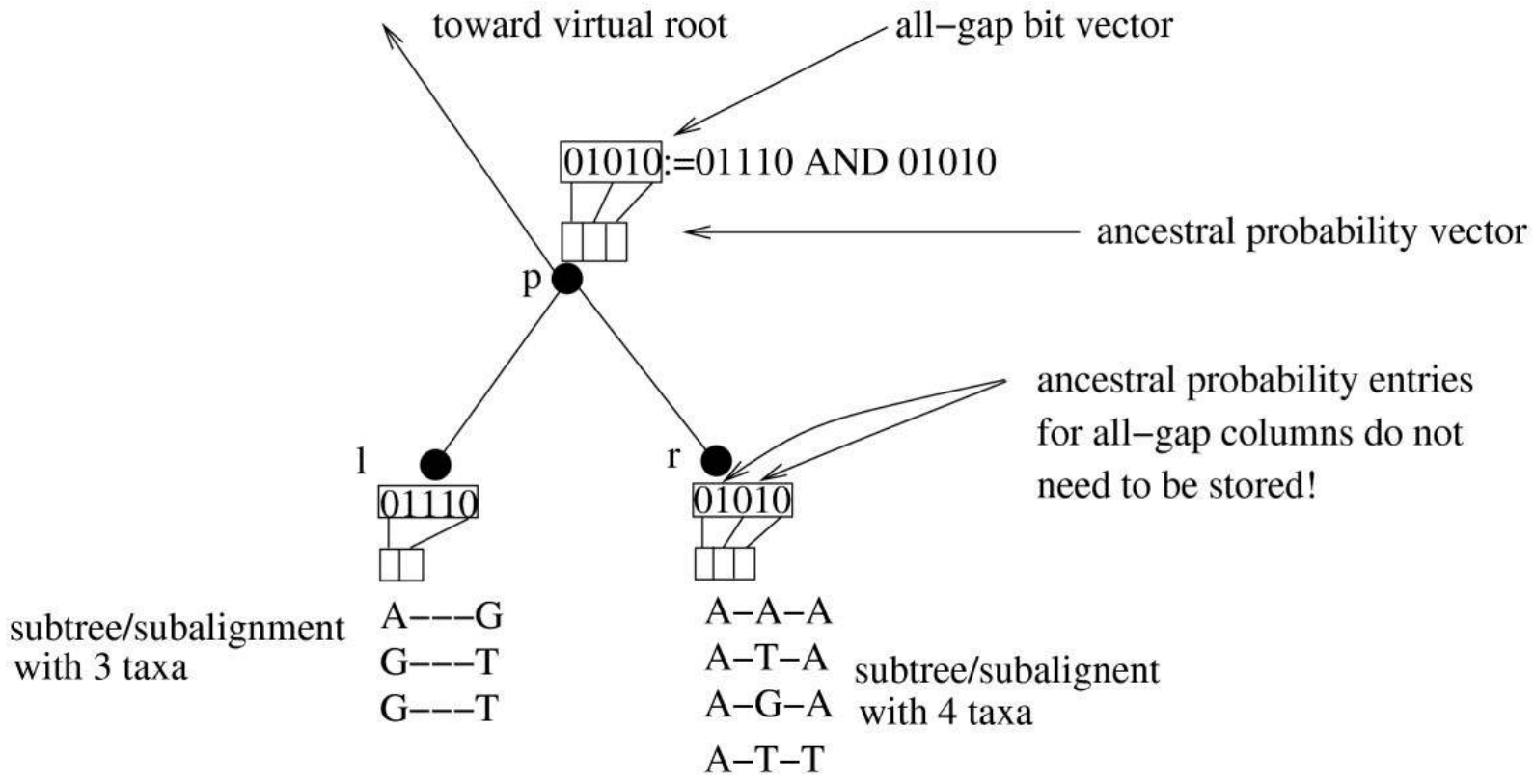
- Multi-threading and MPI
- Parallel tree searches:
  - MPI: RAxML, PhyML, TREE-PUZZLE/pIQPNNI/IQ-TREE
- Likelihood calculation
  - RAxML/IQ-TREE

Other techniques for fast phylogenetics

- GAMMA vs CAT
- Fast approaches for node support
- Parallelization
- Memory saving

Memory saving

- Subtree equality vector (SEV) for gappy data set



Izquierdo-Carrasco et al (2011) BMC Bioinformatics

Recommendations:

- Multiple searches using distinct starting trees

- More thorough searches by tuning key parameters
  - FastTree: more thorough NNI (“-mlacc”, “-slownni”) / no. of ME SPR (“-spr”)
  - RAxML: Lazy SPR radius (“-c”) / old hill-climbing strategy (“-f o”)
  - IQ-TREE: length of search (“-nstop”) / perturbation strength (“-pers”)

- More than one phylogenetic approaches

NJ

Saitou, N. and M. Nei (1987). "The neighbor-joining method: a new method for reconstructing phylogenetic trees." Mol Biol Evol **4**(4): 406-425.

Gascuel, O. and M. Steel (2006). "Neighbor-joining revealed." Mol Biol Evol **23**(11): 1997-2000.

Mihaescu, R., et al. (2009). "Why Neighbor-Joining Works." Algorithmica **54**(1): 1-24.

BIONJ

Gascuel, O. (1997). "BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data." Mol Biol Evol **14**(7): 685-695.

FastME

Desper, R. and O. Gascuel (2002). "Fast and accurate phylogeny reconstruction algorithms based on the minimum-evolution principle." J Comput Biol **9**(5): 687-705.

Lefort, V., et al. (2015). "FastME 2.0: A Comprehensive, Accurate, and Fast Distance-Based Phylogeny Inference Program." Mol Biol Evol **32**(10): 2798-2800.

FastTree

Price, M. N., et al. (2009). "FastTree: computing large minimum evolution trees with profiles instead of a distance matrix." Mol Biol Evol **26**(7): 1641-1650.

Price, M. N., et al. (2010). "FastTree 2--approximately maximum-likelihood trees for large alignments." PLoS One **5**(3): e9490.

RAxML/ExaML

Stamatakis, A. P., et al. (2002). "AxML: a fast program for sequential and parallel phylogenetic tree calculations based on the maximum likelihood method." Proc IEEE Comput Soc Bioinform Conf **1**: 21-28.

Stamatakis, A., et al. (2005). "RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees." Bioinformatics **21**(4): 456-463.

Stamatakis, A. (2006). "RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models." Bioinformatics **22**(21): 2688-2690.

Stamatakis, A. (2014). "RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies." Bioinformatics **30**(9): 1312-1313.

Stamatakis, A., et al. (2012). "RAxML-Light: a tool for computing terabyte phylogenies." Bioinformatics **28**(15): 2064-2066.

Kozlov, A. M., et al. (2015). "ExaML version 3: a tool for phylogenomic analyses on supercomputers." Bioinformatics **31**(15): 2577-2579.

PhyML

Guindon, S. and O. Gascuel (2003). "A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood." Syst Biol **52**(5): 696-704.

Hordijk, W. and O. Gascuel (2005). "Improving the efficiency of SPR moves in phylogenetic tree search methods based on maximum likelihood." Bioinformatics **21**(24): 4338-4347.

Guindon, S., et al. (2010). "New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0." Syst Biol **59**(3): 307-321.

TREE-PUZZLE/IQPNNI/IQ-TREE

Schmidt, H. A., et al. (2002). "TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing." Bioinformatics **18**(3): 502-504.

Vinh le, S. and A. Von Haeseler (2004). "IQPNNI: moving fast through tree space and stopping in time." Mol Biol Evol **21**(8): 1565-1571.

Minh, B. Q., et al. (2005). "pIQPNNI: parallel reconstruction of large maximum likelihood phylogenies." Bioinformatics **21**(19): 3794-3796.

Nguyen, L. T., et al. (2015). "IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies." Mol Biol Evol **32**(1): 268-274.

CAT/FreeRates model

Stamatakis, A. (2006). Phylogenetic models of rate heterogeneity: a high performance computing perspective. Proceedings 20th IEEE International Parallel & Distributed Processing Symposium.

Meyer, S. and A. von Haeseler (2003). "Identifying site-specific substitution rates." Mol Biol Evol **20**(2): 182-189.

Fast approaches for support values

Stamatakis, A., et al. (2008). "A rapid bootstrap algorithm for the RAxML Web servers." Syst Biol **57**(5): 758-771.

Minh, B. Q., et al. (2013). "Ultrafast approximation for phylogenetic bootstrap." Mol Biol Evol **30**(5): 1188-1195.

Anisimova, M. and O. Gascuel (2006). "Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative." Syst Biol **55**(4): 539-552.

Guindon, S., et al. (2010). "New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0." Syst Biol **59**(3): 307-321.

Anisimova, M., et al. (2011). "Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes." Syst Biol **60**(5): 685-699.

Thanks!