Novel approaches for large-scale multiple sequence alignment and phylogenetic estimation

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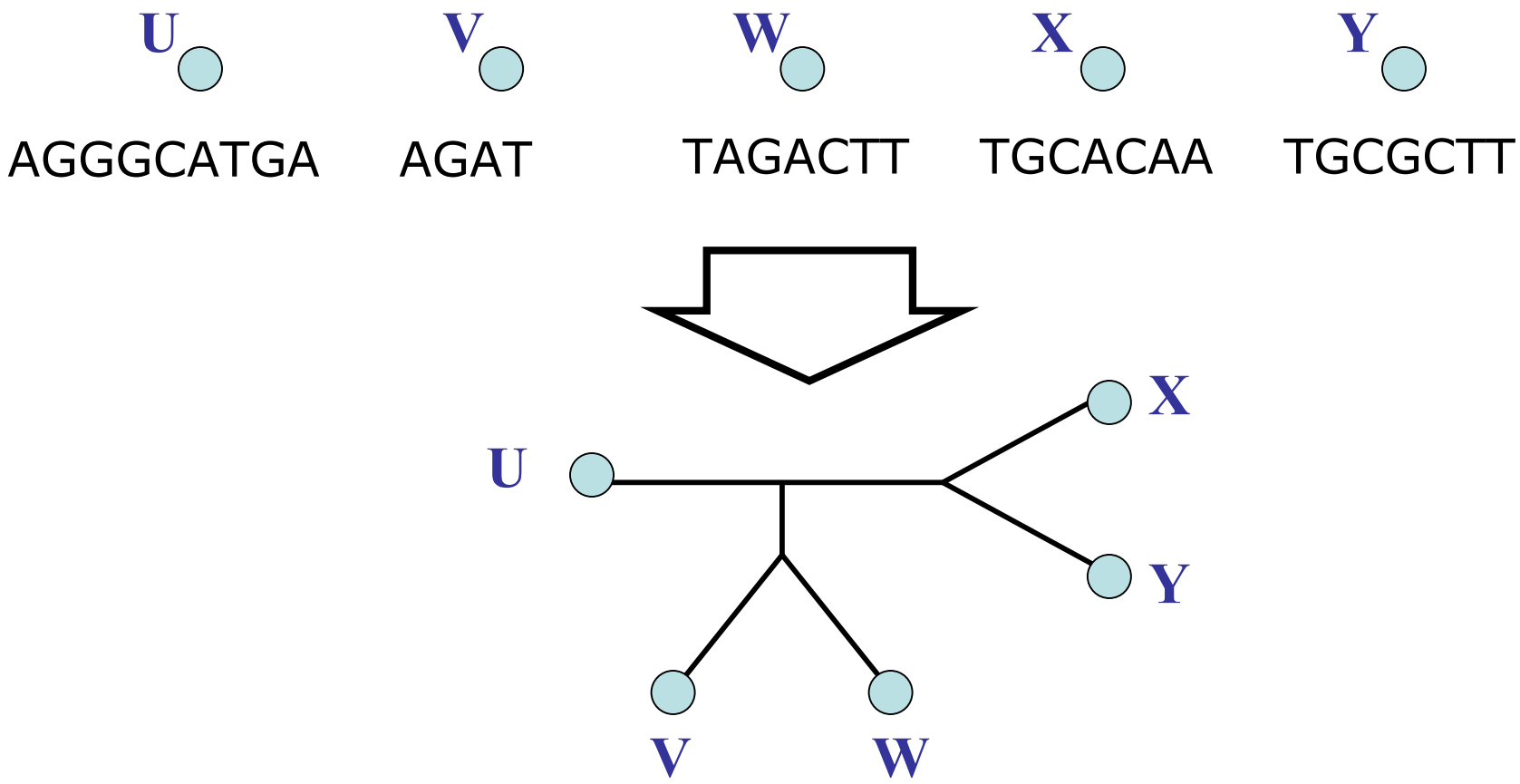

U
V
W
X
Y
AGGGCATGA
AGAT
TAGACTT
TGCACAA
TGCGCTT
X
U
Y
V
W

Input: unaligned sequences

```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

Phase 1: Alignment

```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

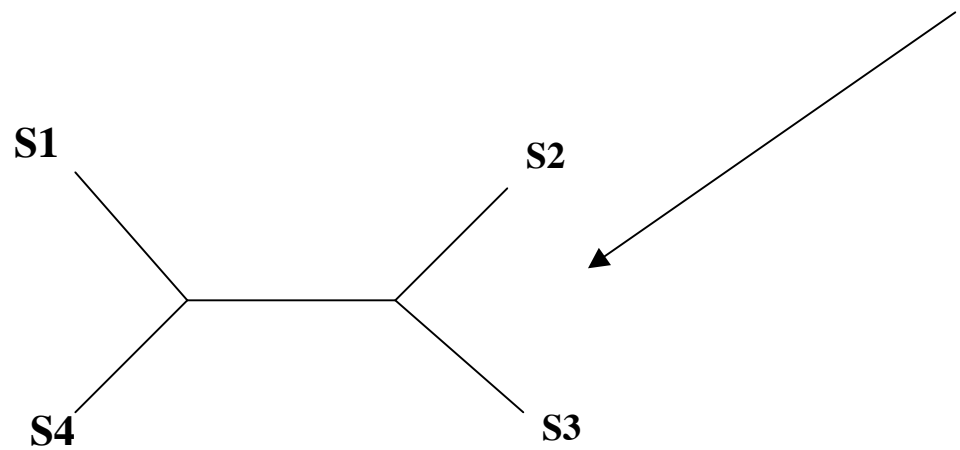
→

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```

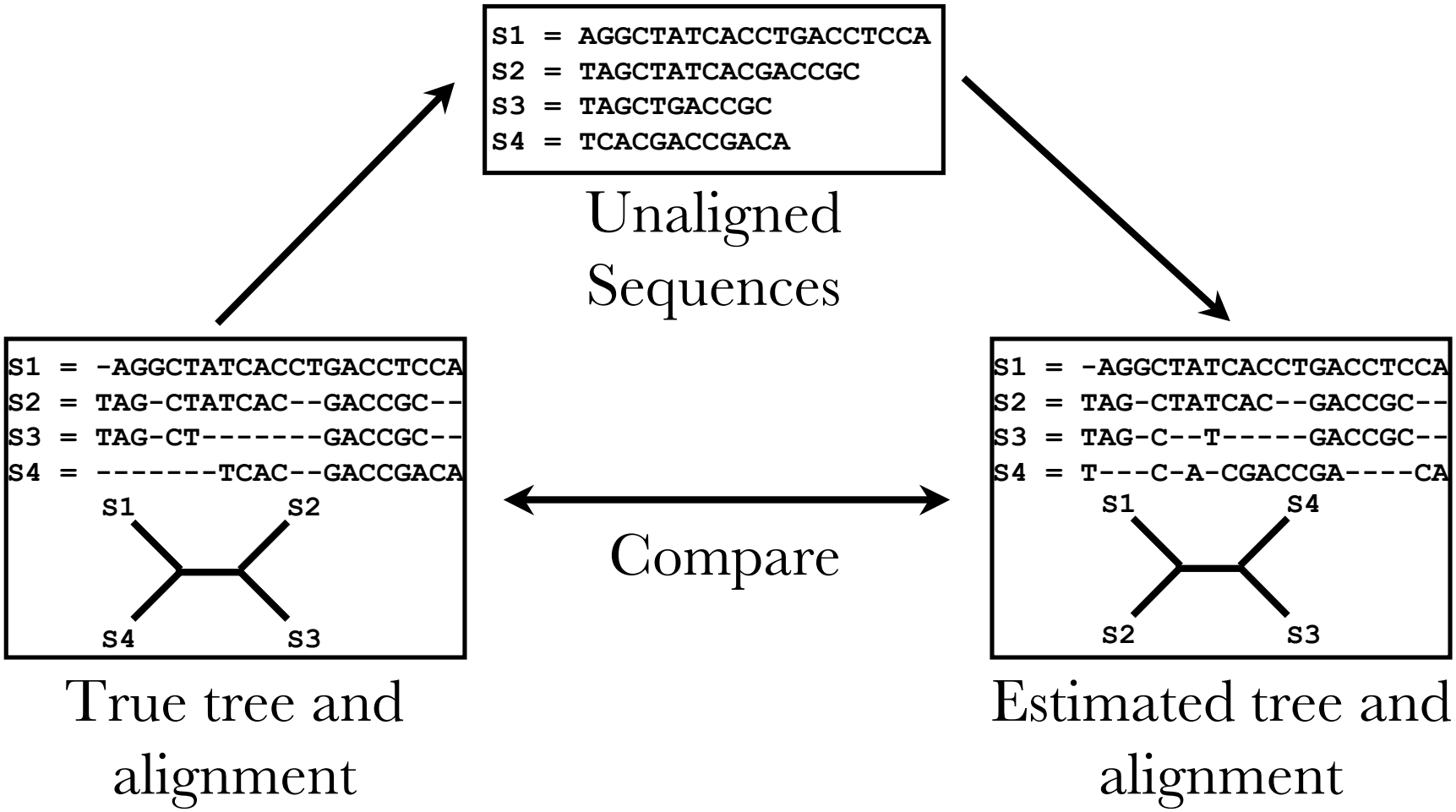
Phase 2: Construct tree

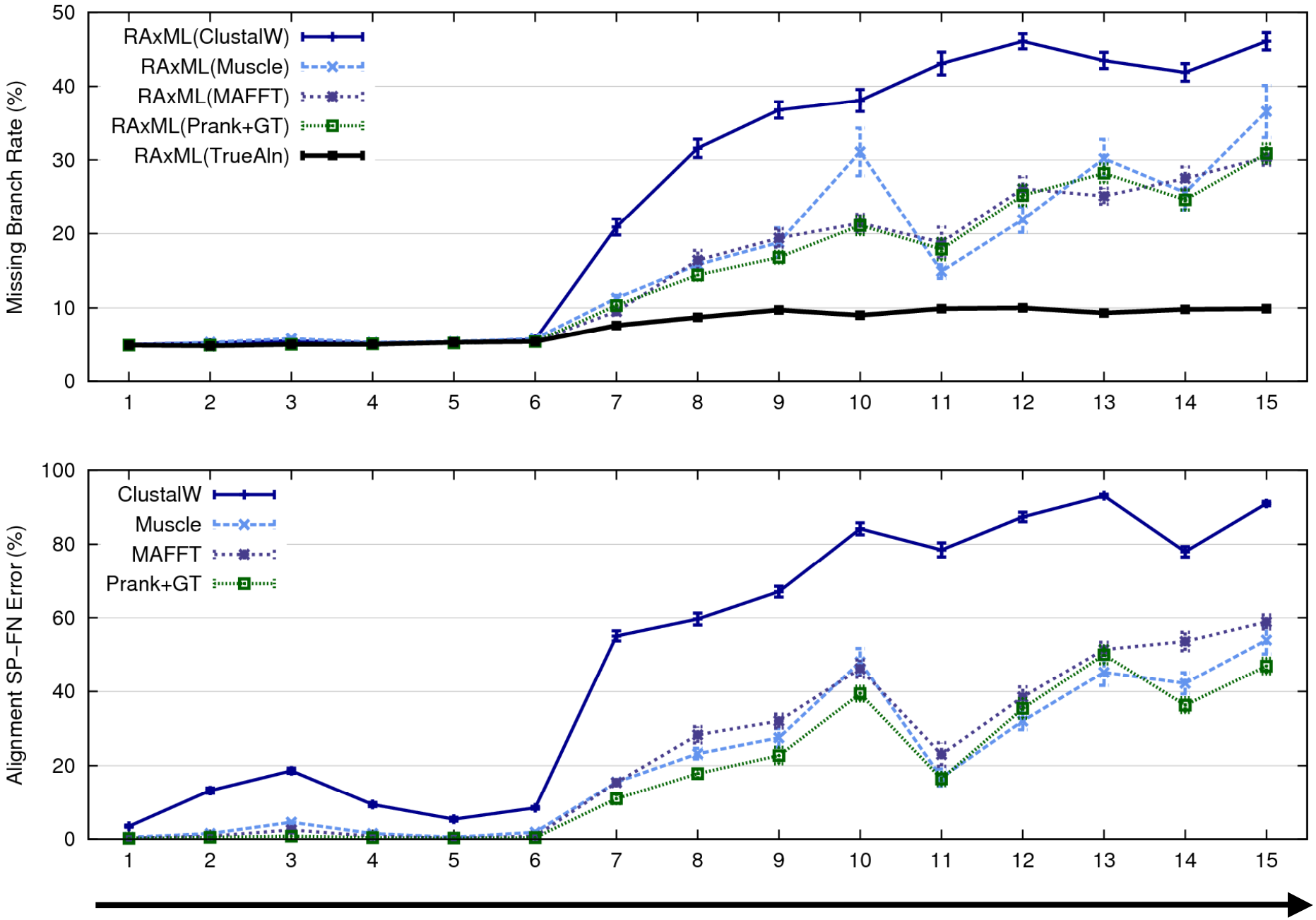
```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```



Simulation Studies





1000 taxon models, ordered by difficulty (Liu et al., 2009)

Major Challenges:
large datasets, fragmentary sequences

- **Phylogenetic analyses:** standard methods have *poor accuracy* on even moderately large datasets, and the most accurate methods are enormously *computationally intensive* (weeks or months, high memory requirements).
- **Multiple sequence alignment**: Few methods can run on large datasets, and alignment accuracy is generally poor for large datasets with high rates of evolution.

Both phylogenetic estimation and multiple sequence alignment are also impacted by ***fragmentary data.***

This Talk

- **SATé** - co-estimating trees and alignments
- **DACTAL** - trees almost without alignments
- **SEPP** - phylogenetic placement of **fragmentary** sequence data (e.g., short reads)

Part I: SATé

Simultaneous Alignment and Tree Estimation

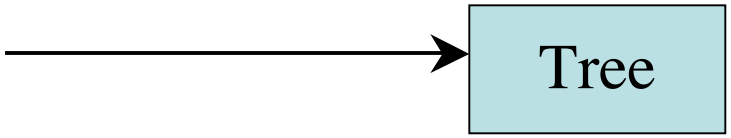
Liu, Nelesen, Raghavan, Linder, and Warnow, *Science*, 19 June 2009, pp. 1561-1564.

Liu et al., Systematic Biology 2012

Public software distribution (open source) through Mark Holder's group at the University of Kansas

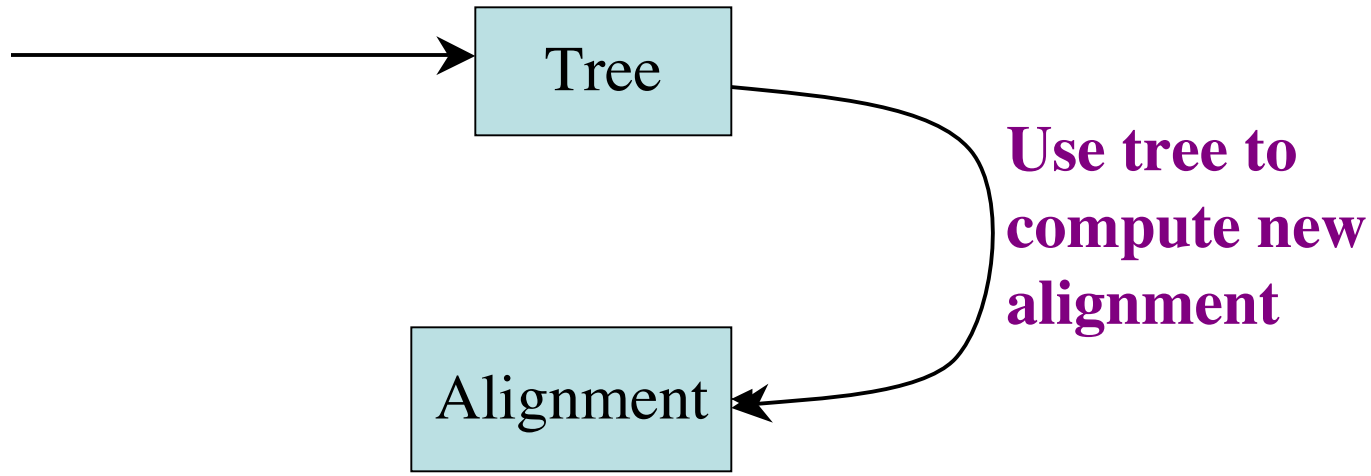
SATé Algorithm

Obtain initial alignment
and estimated ML tree

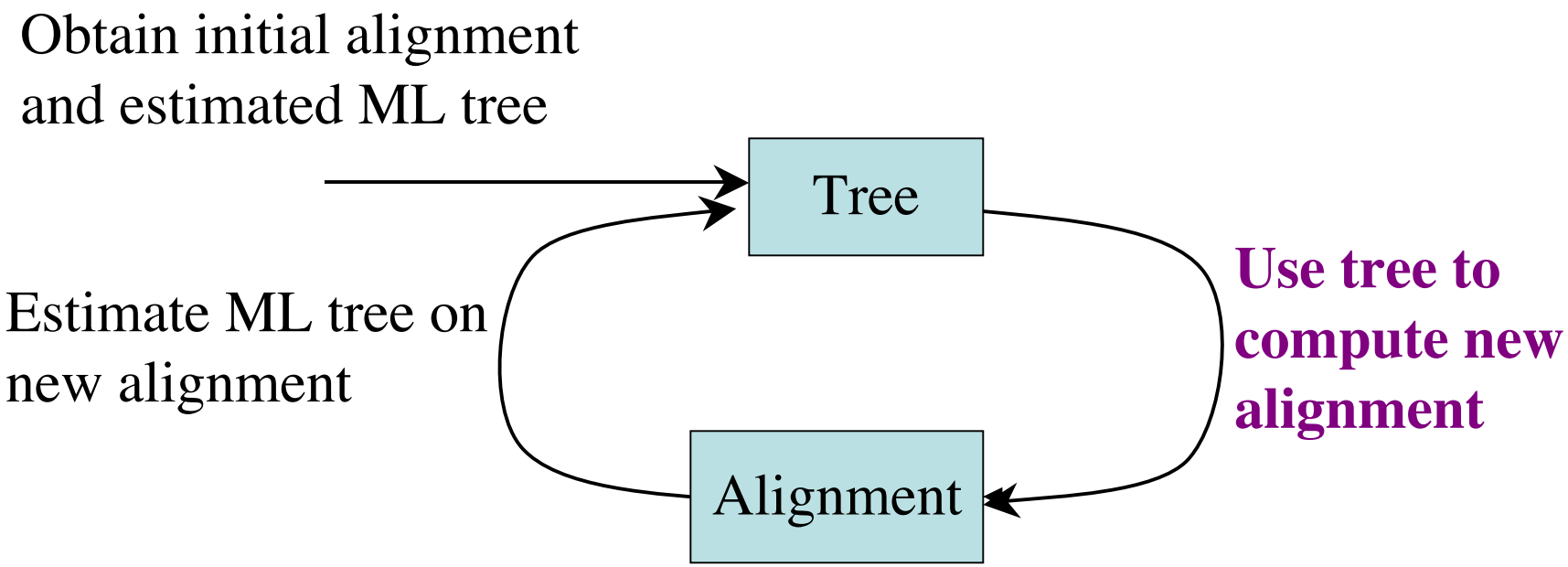


SATé Algorithm

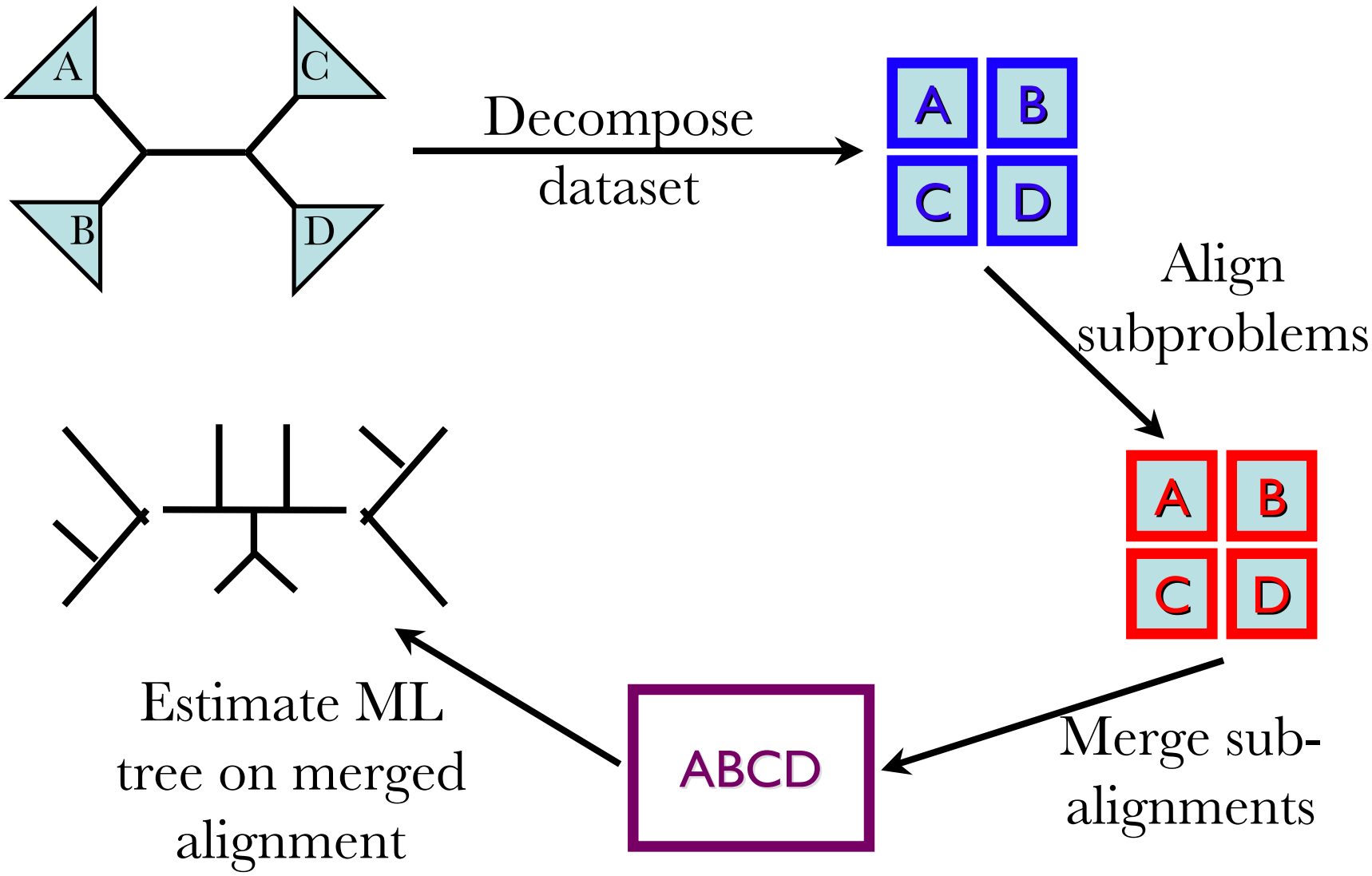
Obtain initial alignment
and estimated ML tree

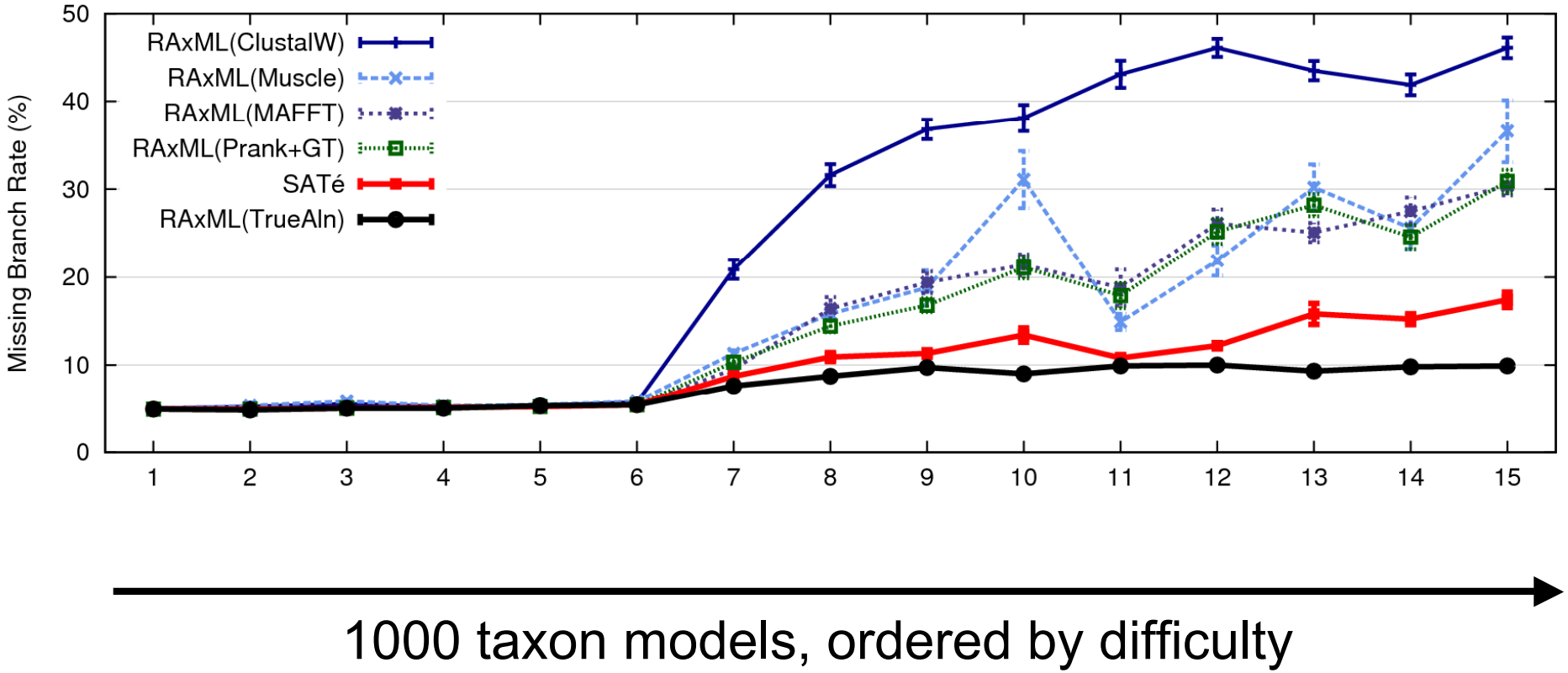


SATé Algorithm



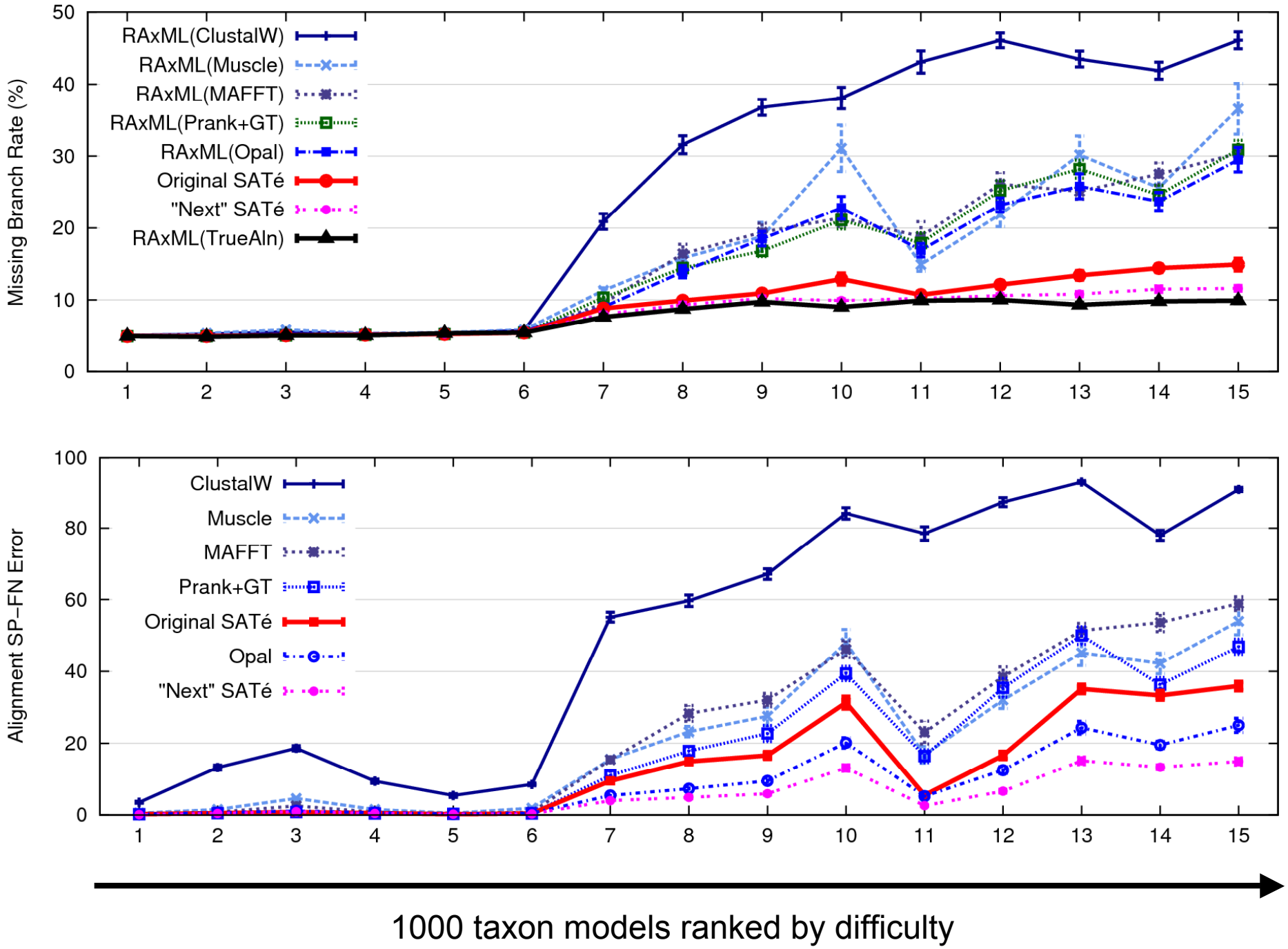
Re-aligning on a tree



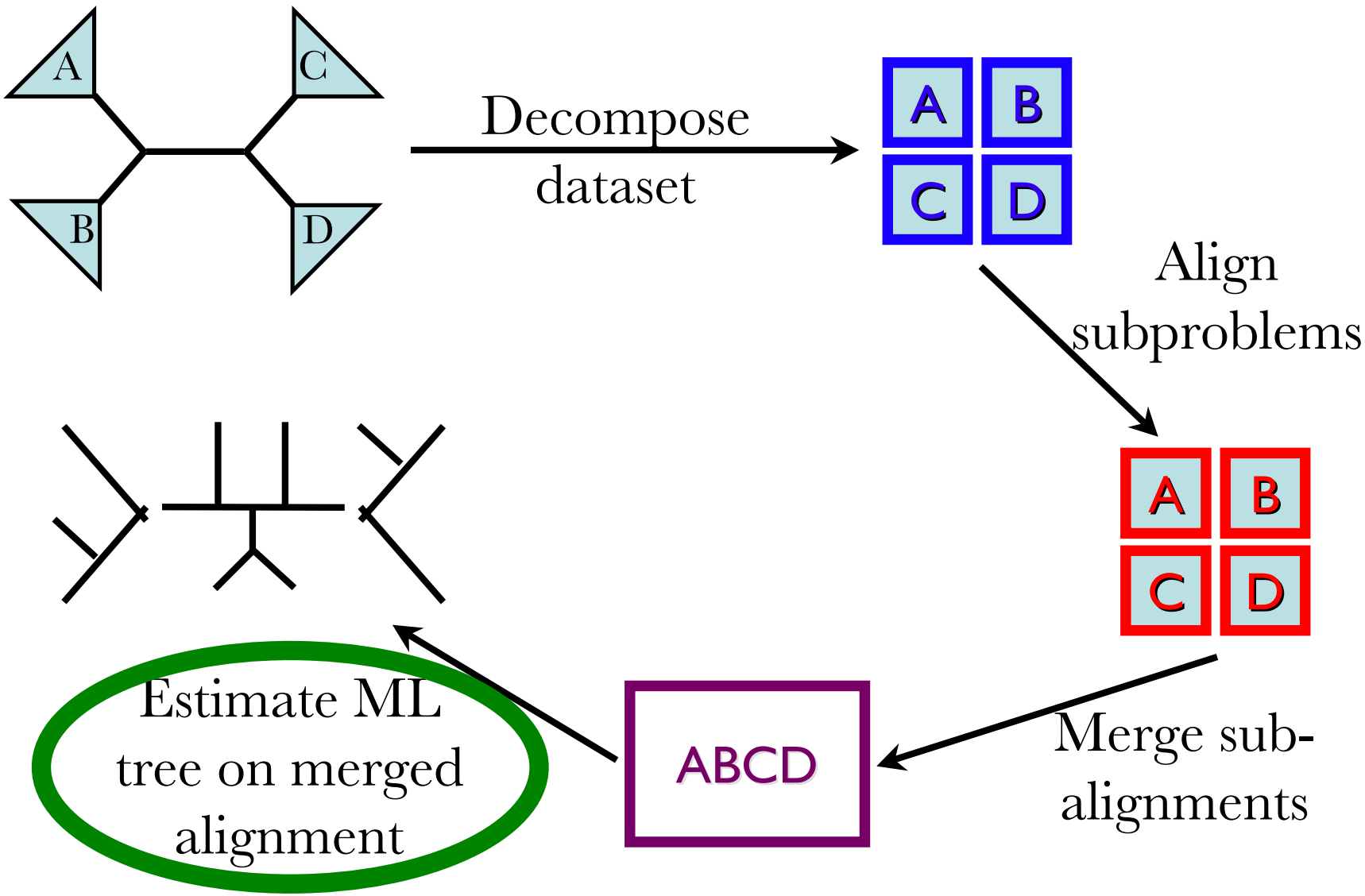


24 hour SATé analysis, on desktop machines

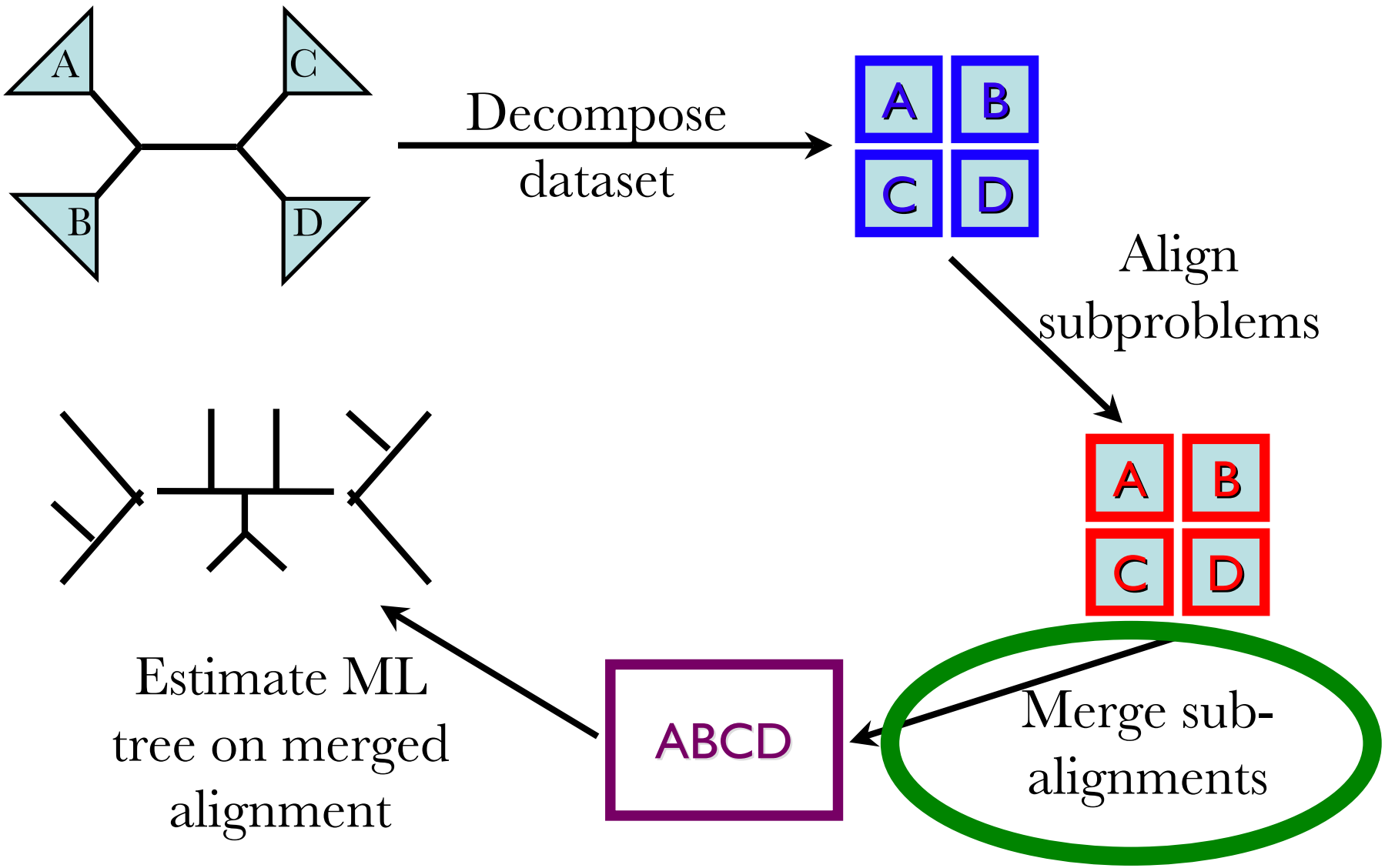
(Similar improvements for biological datasets)


Missing Branch Rate (%)
RAxML(ClustalW)
RAxML(Muscle)
RAxML(MAFFT)
RAxML(Prank+GT)
RAxML(Opal)
Original SATé
"Next" SATé
RAxML(TrueAln)
Alignment SP–FN Error
ClustalW
Muscle
MAFFT
Prank+GT
Original SATé
Opal
"Next" SATé
1000 taxon models ranked by difficulty

Limitations



Limitations



Part II: DACTAL

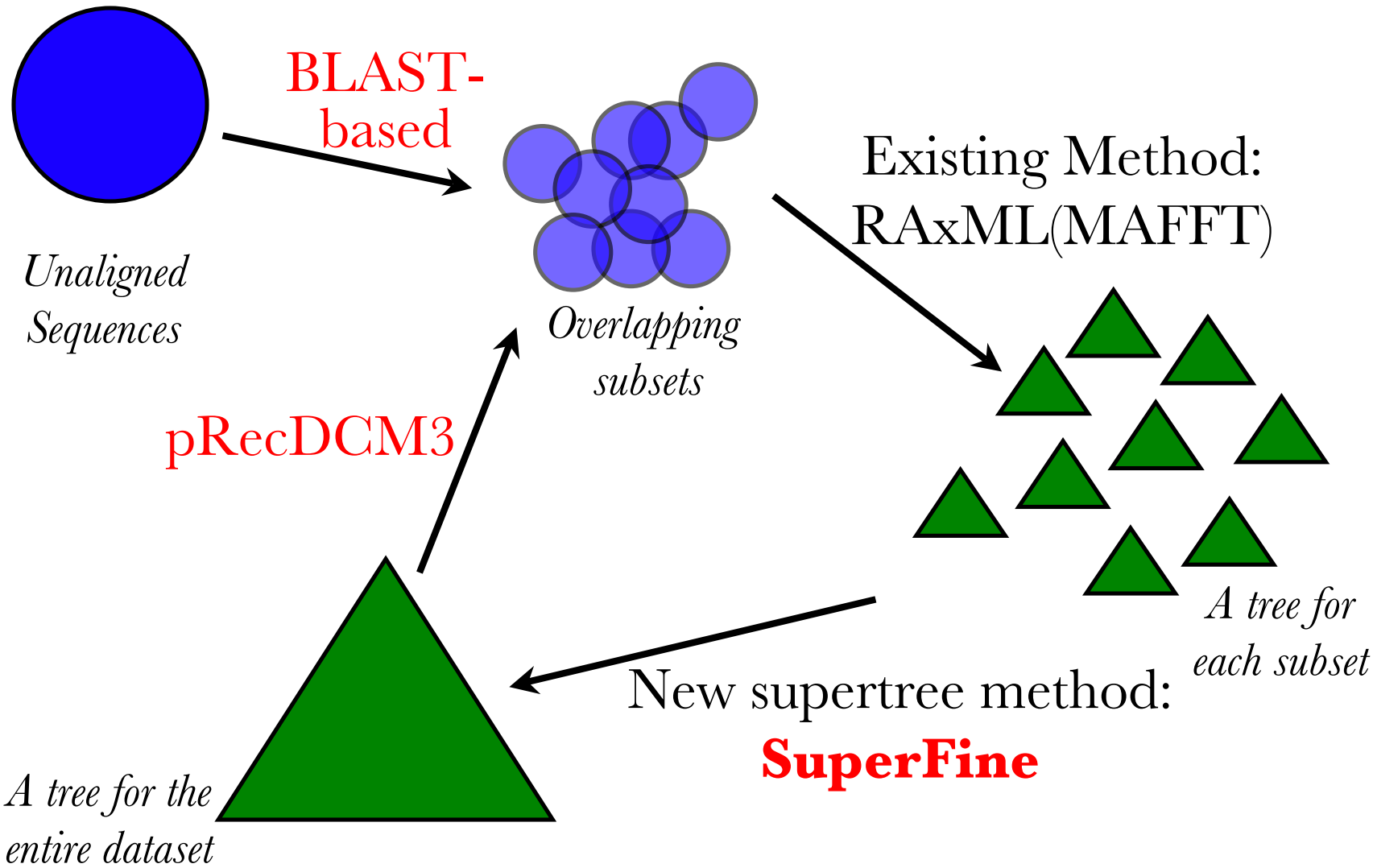
Divide-And-Conquer Trees without Alignments*

- Input: set S of unaligned sequences
- Output: tree on S (but no alignment)

Nelesen, Liu, Wang, Linder, and Warnow, ISMB 2012 and Bioinformatics 2012

***(almost)**

DACTAL


BLAST-based
Unaligned Sequences
Overlapping subsets
Existing Method: RAxML(MAFFT)
A tree for each subset
pRecDCM3
New supertree method: SuperFine
A tree for the entire dataset

Average of 3 Largest CRW Datasets

CRW: Comparative RNA database,

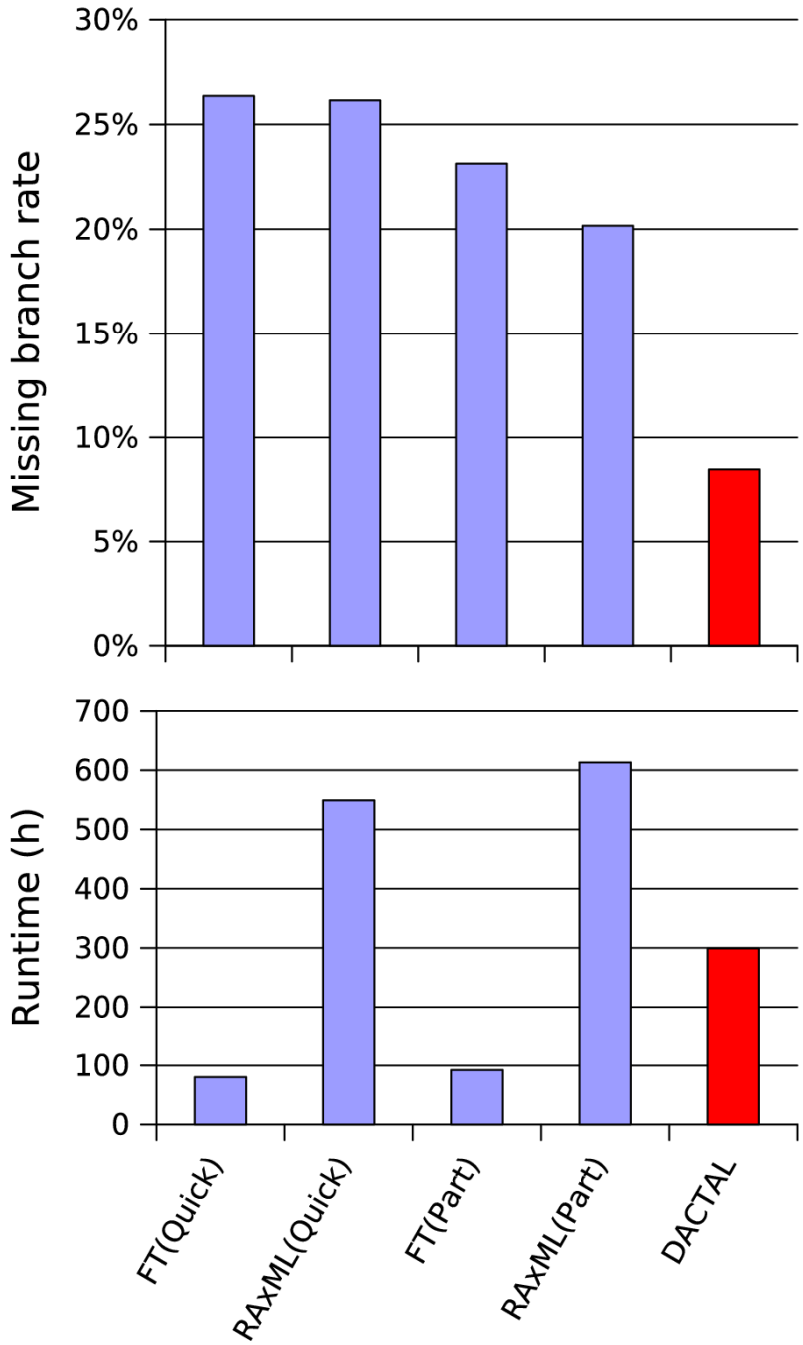
Three 16S datasets with **6,323** to **27,643** sequences

Reference alignments based on secondary structure

Reference trees are 75% RAxML bootstrap trees

DACTAL (shown in red) run for 5 iterations starting from FT(Part)

FastTree (FT) and RAxML are ML methods



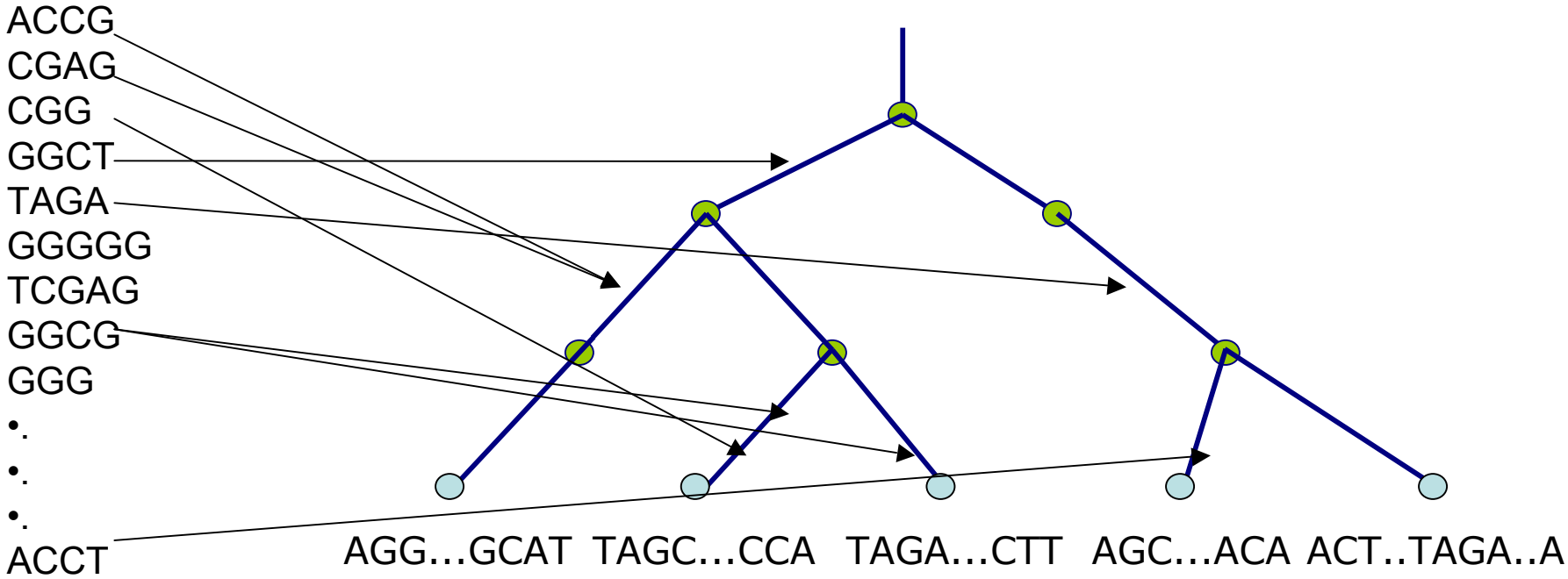
DACTAL and SATé

- DACTAL and SATé estimate large trees from full-length sequences for one or several genes
- DACTAL can be used with other types of data (not just sequences)
- But neither handles fragmentary data (e.g., short reads)

Phylogenetic Placement

Fragmentary Sequences from some gene

Full-length sequences for same gene, and an alignment and a tree



Part III: SEPP

- SEPP: SATé-enabled Phylogenetic Placement, by Mirarab, Nguyen, and Warnow

- Pacific Symposium on Biocomputing, 2012 (special session on the Human Microbiome)

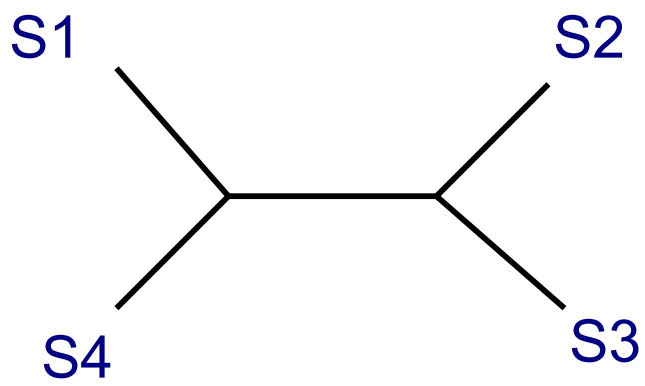
Phylogenetic Placement

Step 1: Align each query sequence to backbone alignment

Step 2: Place each query sequence into backbone tree, using extended alignment

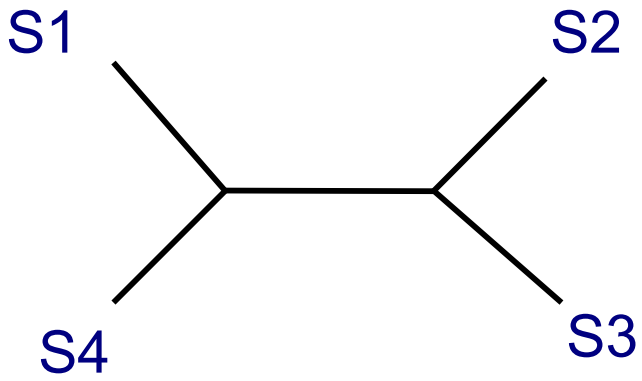
Align Sequence

```
S1  = -AGGCTATCACCTGACCTCCA-AA
S2  = TAG-CTATCAC--GACCGC--GCA
S3  = TAG-CT-------GACCGC--GCT
S4  = TAC----TCAC--GACCGACAGCT
Q1  = TAAAAC
```



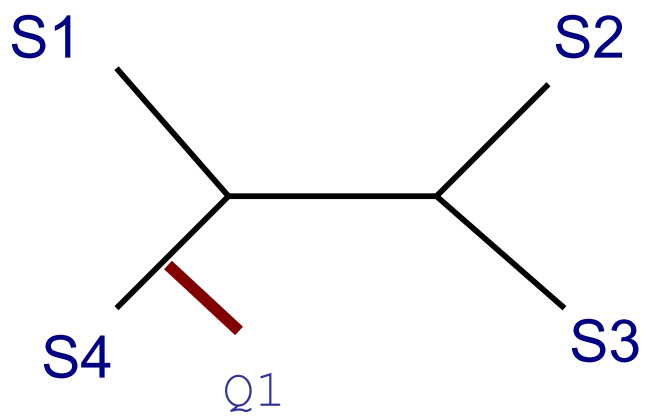
Align Sequence

```
S1  = -AGGCTATCACCTGACCTCCA-AA
S2  = TAG-CTATCAC--GACCGC--GCA
S3  = TAG-CT-------GACCGC--GCT
S4  = TAC----TCAC--GACCGACAGCT
Q1  = -------T-A--AAAC--------
```



Place Sequence

```
S1  = -AGGCTATCACCTGACCTCCA-AA
S2  = TAG-CTATCAC--GACCGC--GCA
S3  = TAG-CT-------GACCGC--GCT
S4  = TAC----TCAC--GACCGACAGCT
Q1  = -------T-A--AAAC--------
```

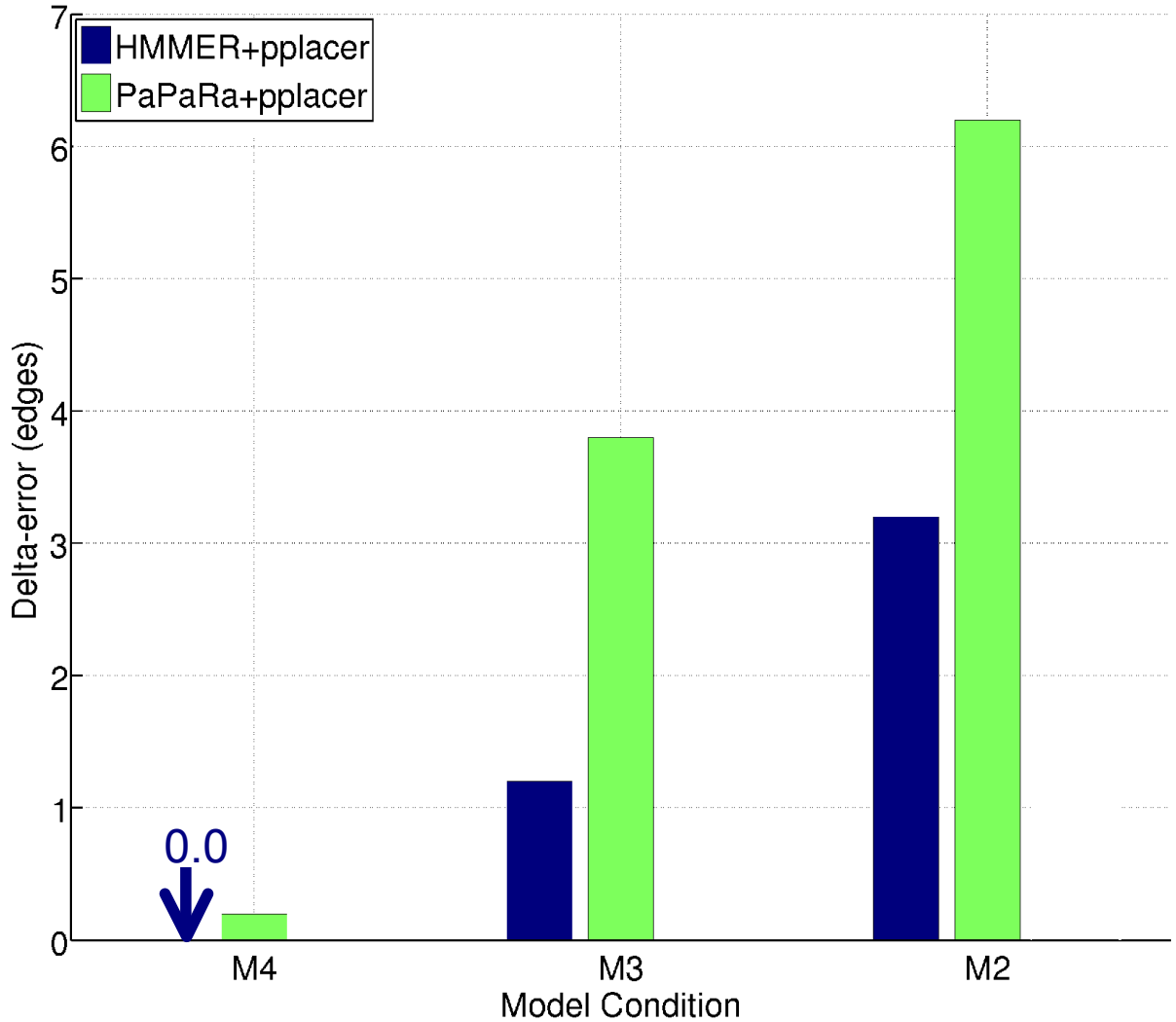


Phylogenetic Placement

- Align each query sequence to backbone alignment
  - HMMALIGN (Eddy, Bioinformatics 1998)
  - PaPaRa (Berger and Stamatakis, Bioinformatics 2011)
- Place each query sequence into backbone tree
  - Pplacer (Matsen et al., BMC Bioinformatics, 2011)
  - EPA (Berger and Stamatakis, Systematic Biology 2011)

Note: pplacer and EPA use maximum likelihood, and are reported to have the same accuracy.

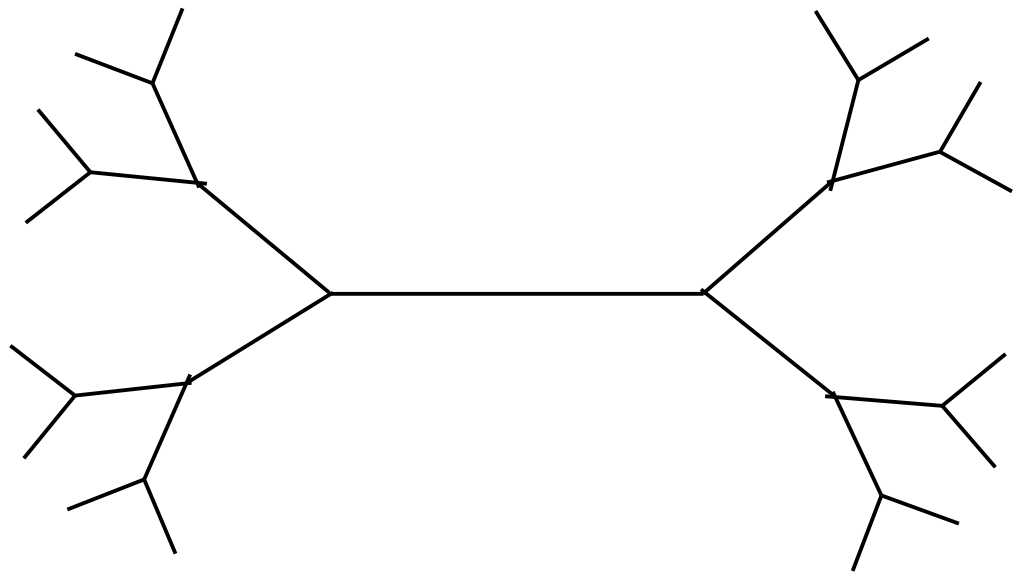
HMMER vs. PaPaRa



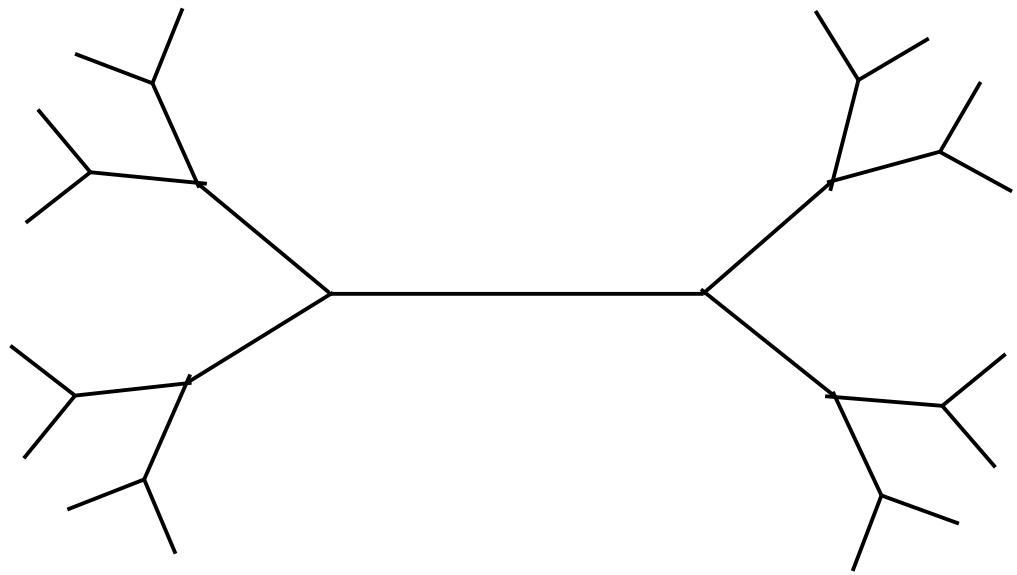
Increasing rate of evolution

HMMER+pplacer:

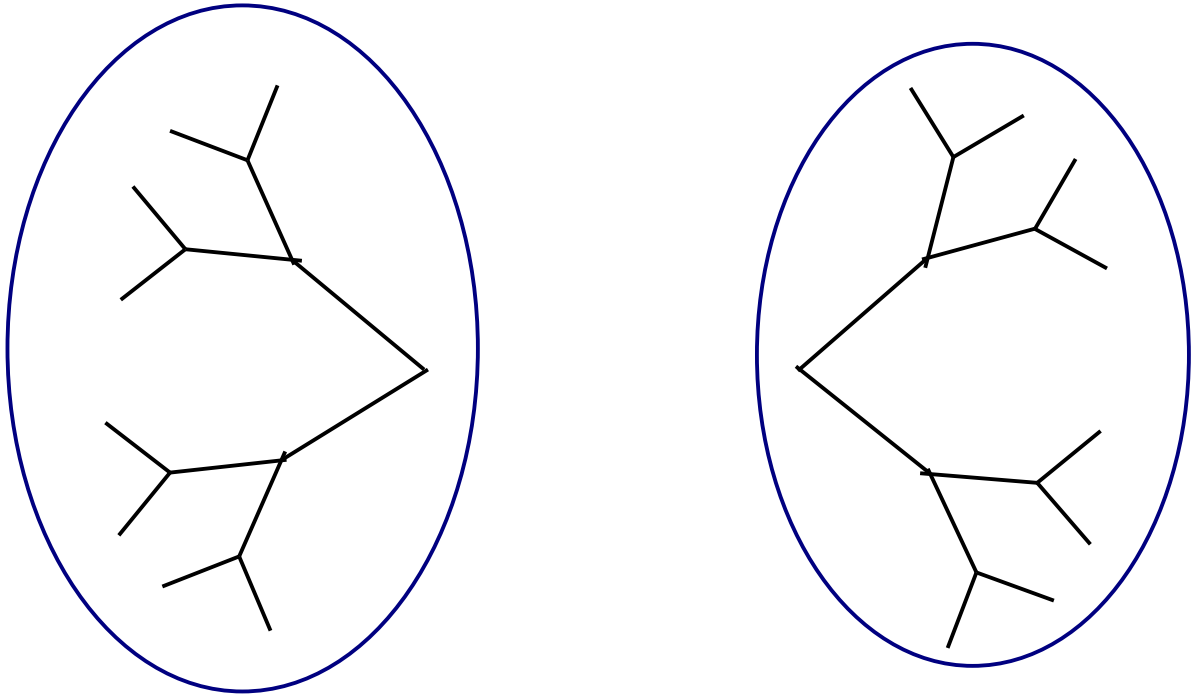
1) build one HMM for the entire alignment
2) Align fragment to the HMM, and insert into alignment
3) Insert fragment into tree to optimize likelihood



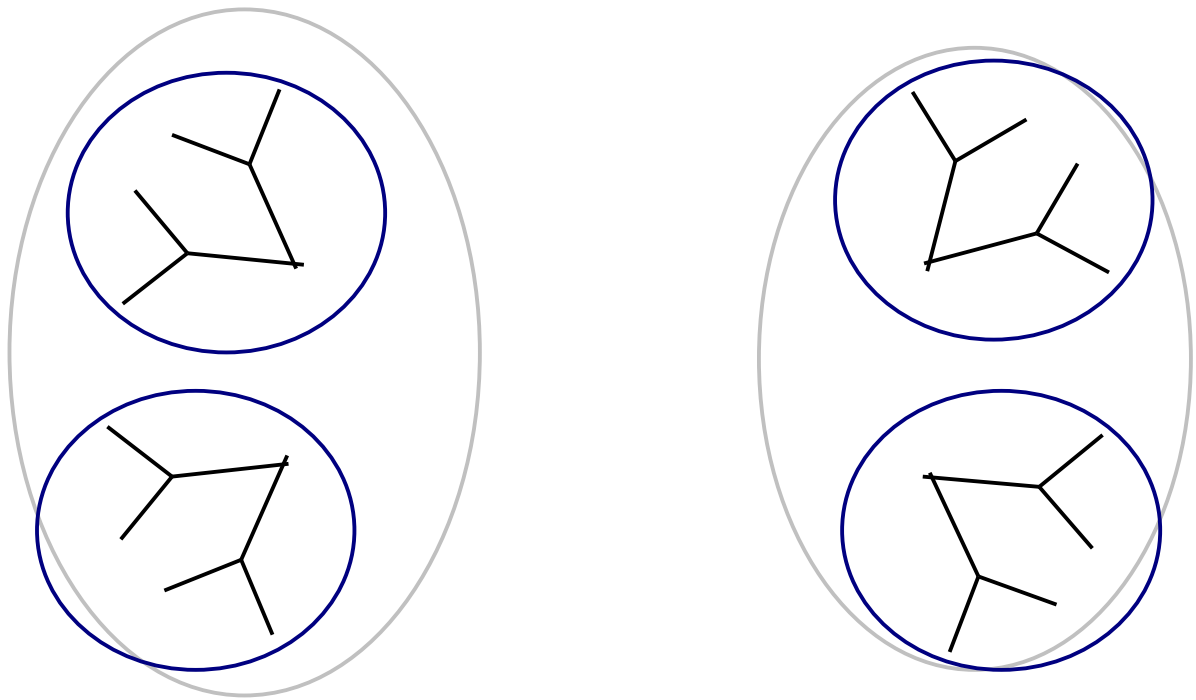
One Hidden Markov Model for the entire alignment?



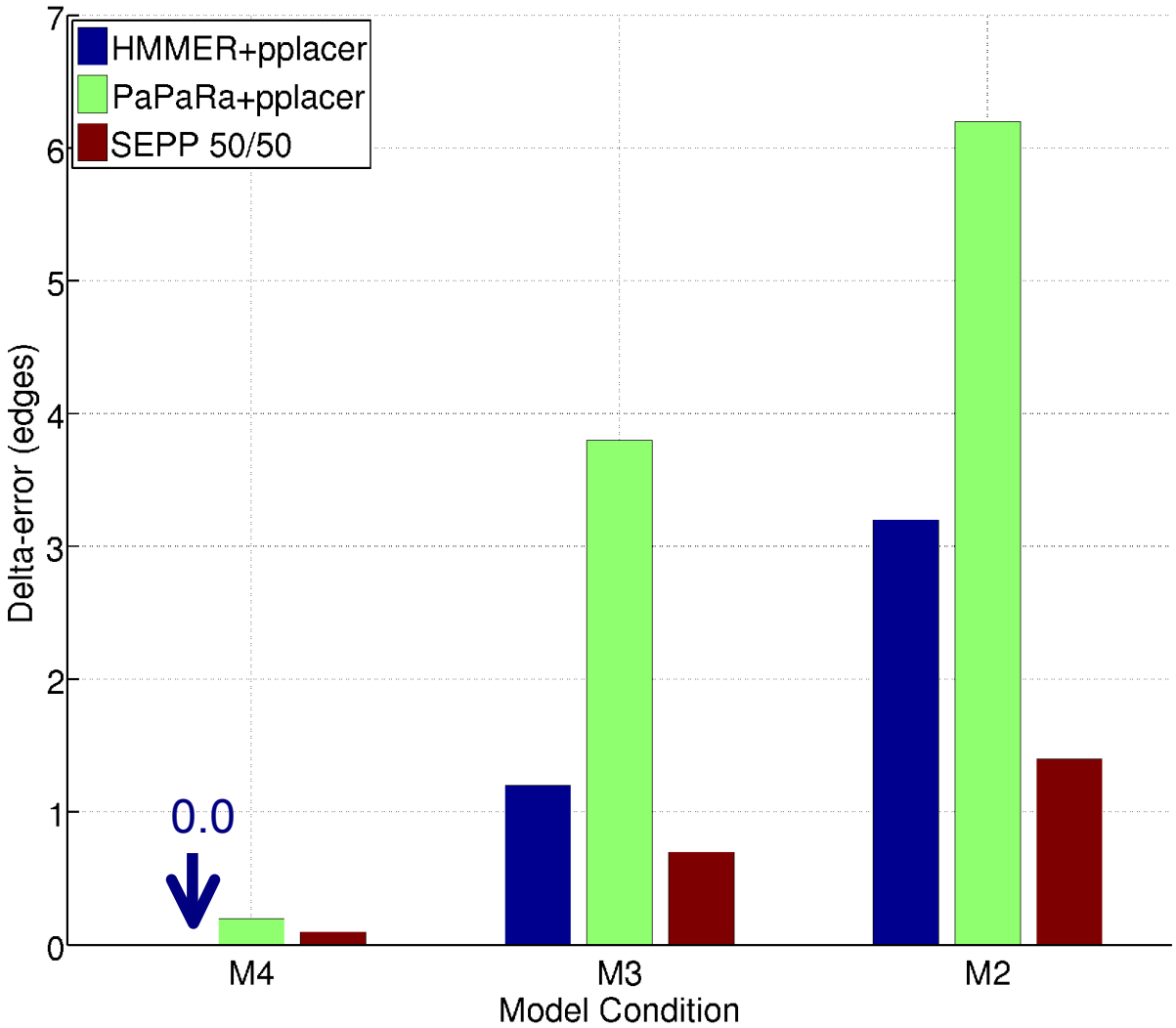
Or 2 HMMs?



Or 4 HMMs?

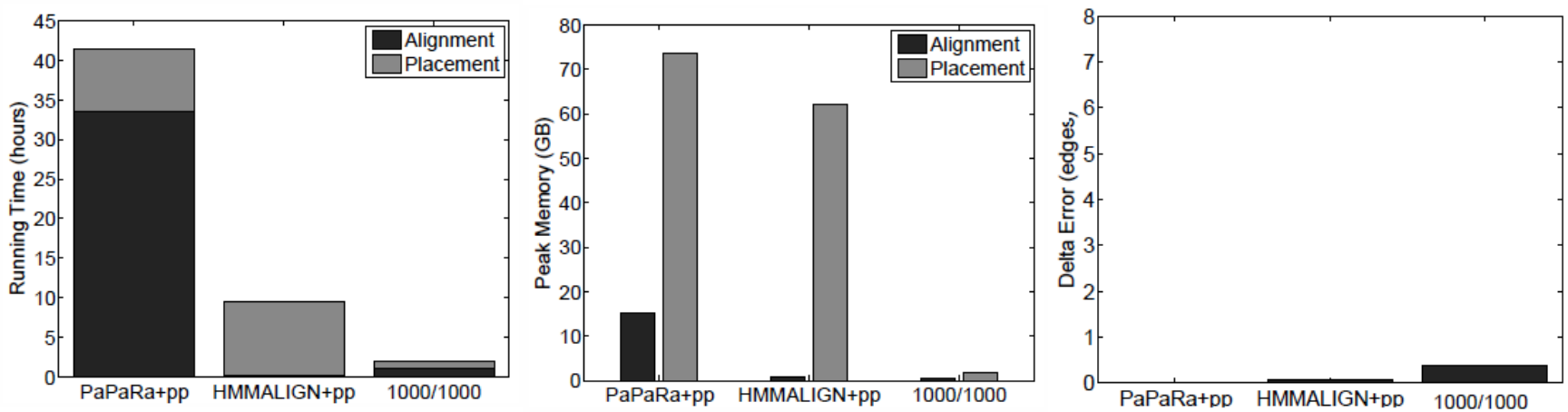


SEPP(10%), based on ~10 HMMs



Increasing rate of evolution

SEPP (10%) on Biological Data



16S.B.ALL dataset, 13k curated backbone tree, 13k total fragments

For 1 million fragments:

PaPaRa+pplacer: ~133 days

HMMALIGN+pplacer: ~30 days

SEPP 1000/1000: ~6 days

Three “Boosters”

- SATé: co-estimation of alignments and trees
- DACTAL: large trees without full alignments
- SEPP: phylogenetic placement of short reads

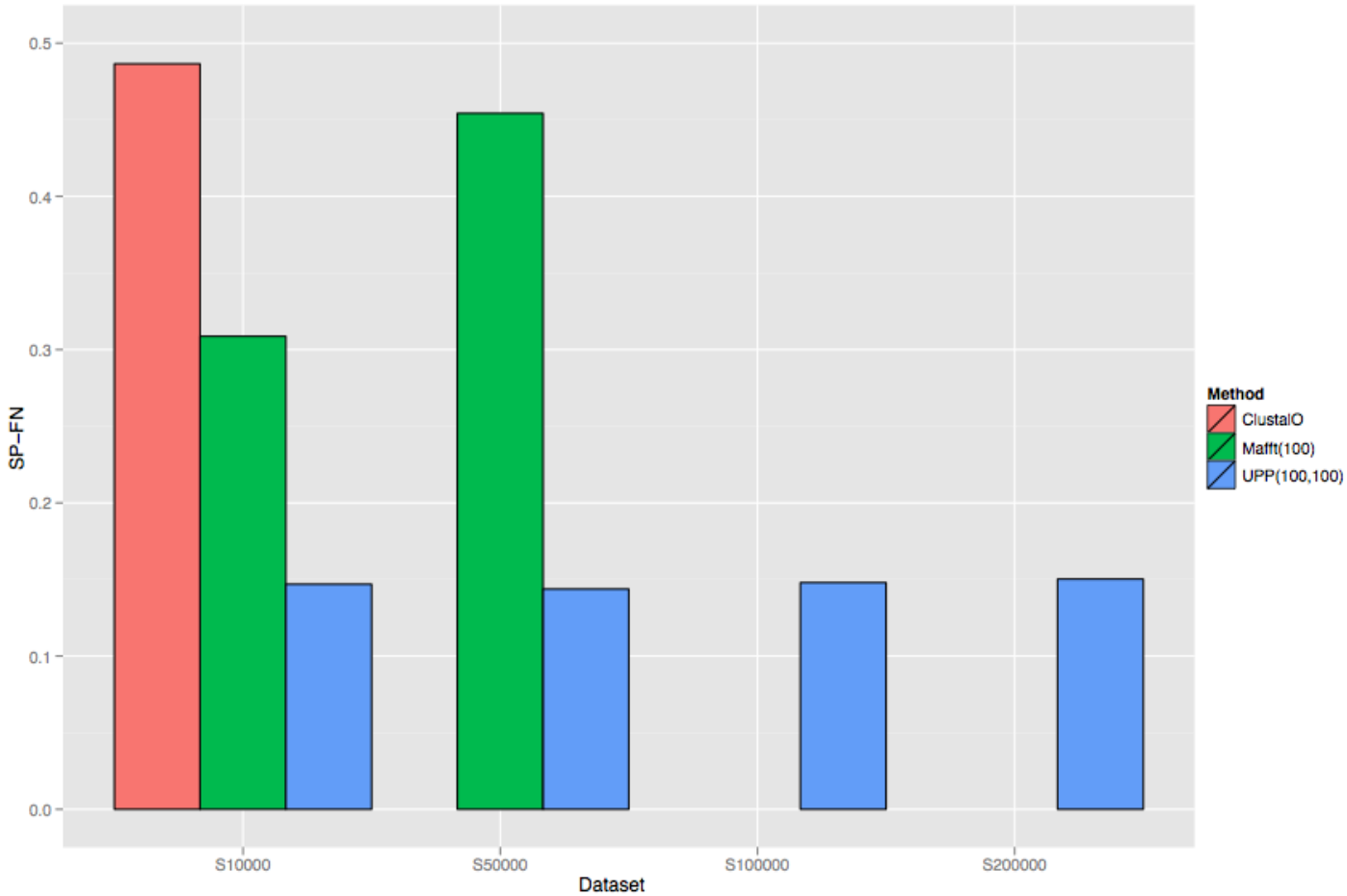
Algorithmic strategies: divide-and-conquer and iteration to improve the accuracy and scalability of a *base method*

Applications of SEPP

- UPP: Ultra-large alignment using SEPP

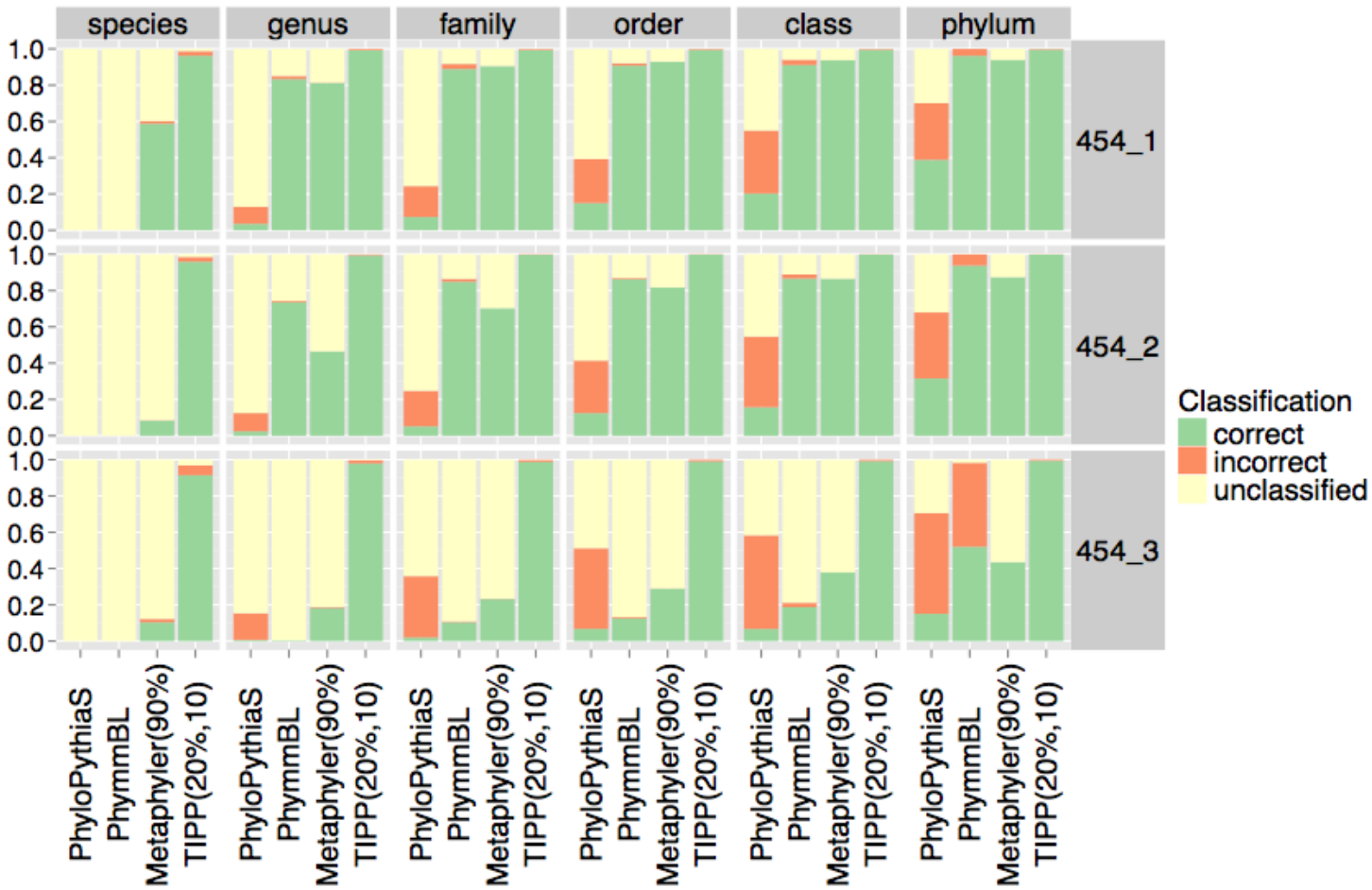
- TIPP: taxon identification of fragmentary data

UPP: Ultra-large alignments using SEPP



Compared to Clustal-Omega and MAFFT on simulated datasets with 10,000 to 200,000 sequences

TIPP: Taxon Identification using SEPP (highly robust to sequencing error)



Using these methods

- SATé is being used in several large-scale projects (e.g., Avian and 1KP)
- SEPP, SuperFine, and DACTAL are available as command line
- UPP and TIPP are under development

We would be very happy to discuss potential collaborations!

Contact me by email, tandy@cs.utexas.edu

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- Funding: Guggenheim Foundation Fellowship, NSF: ATOL, ITR, and IGERT grants, and David Bruton Jr. Professorship

- Collaborators:
  - SATé: Kevin Liu, Serita Nelesen, Sindhu Raghavan, and Randy Linder (and Mark Holder's lab at Kansas for public distribution)
  - DACTAL: Serita Nelesen, Kevin Liu, Li-San Wang, and Randy Linder
  - SEPP and UPP: Siavash Mirarab and Nam Nguyen
  - TIPP: Nam Nguyen, Siavash Mirarab, Mihai Pop, and Bo Liu

- See http://www.cs.utexas.edu/users/ATOL-MSA.html for publications and downloadable software