Four techniques for large-scale multiple sequence alignment and phylogenetic estimation

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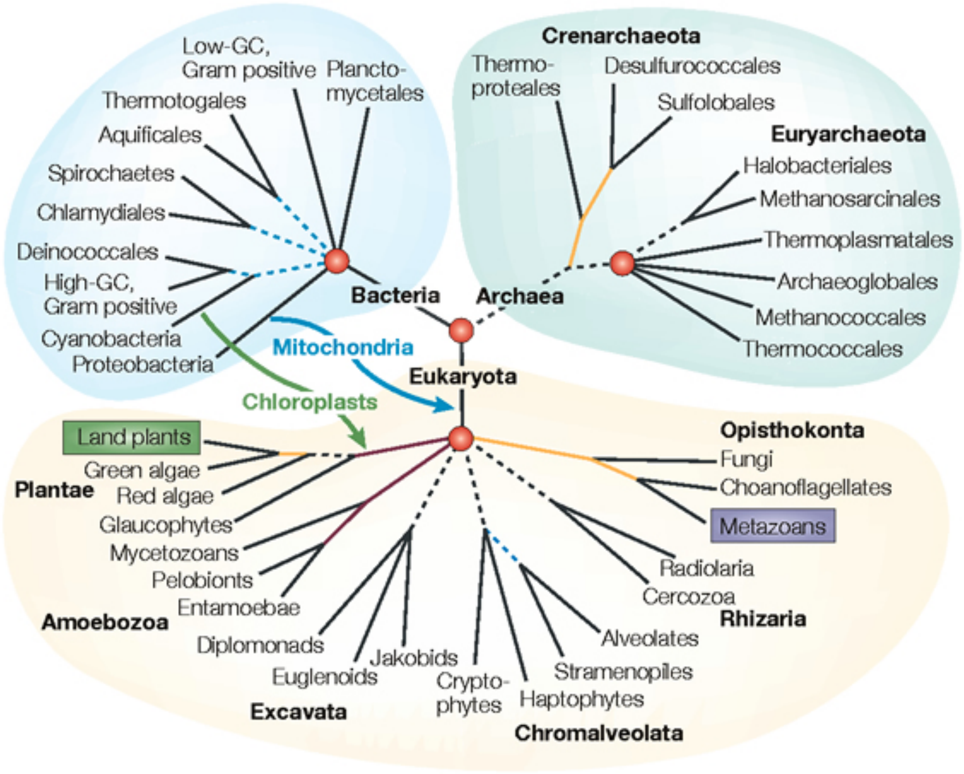
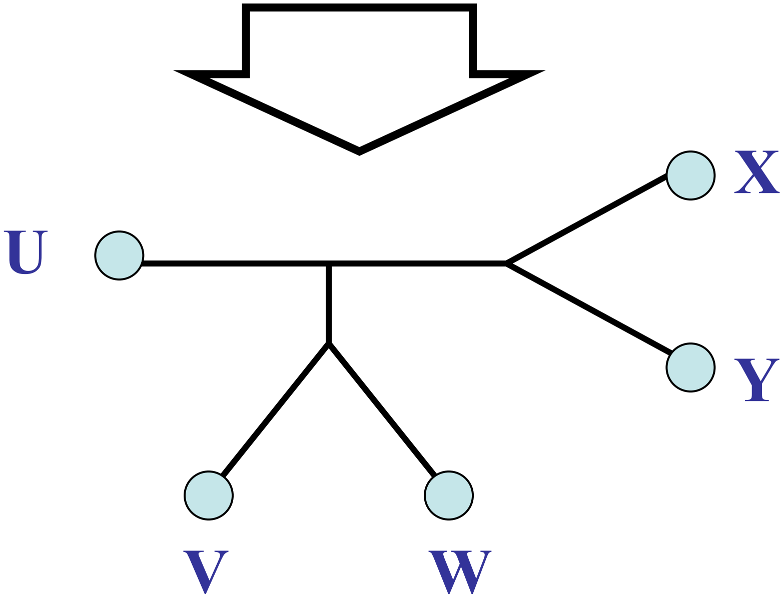
Assembling the Tree of Life

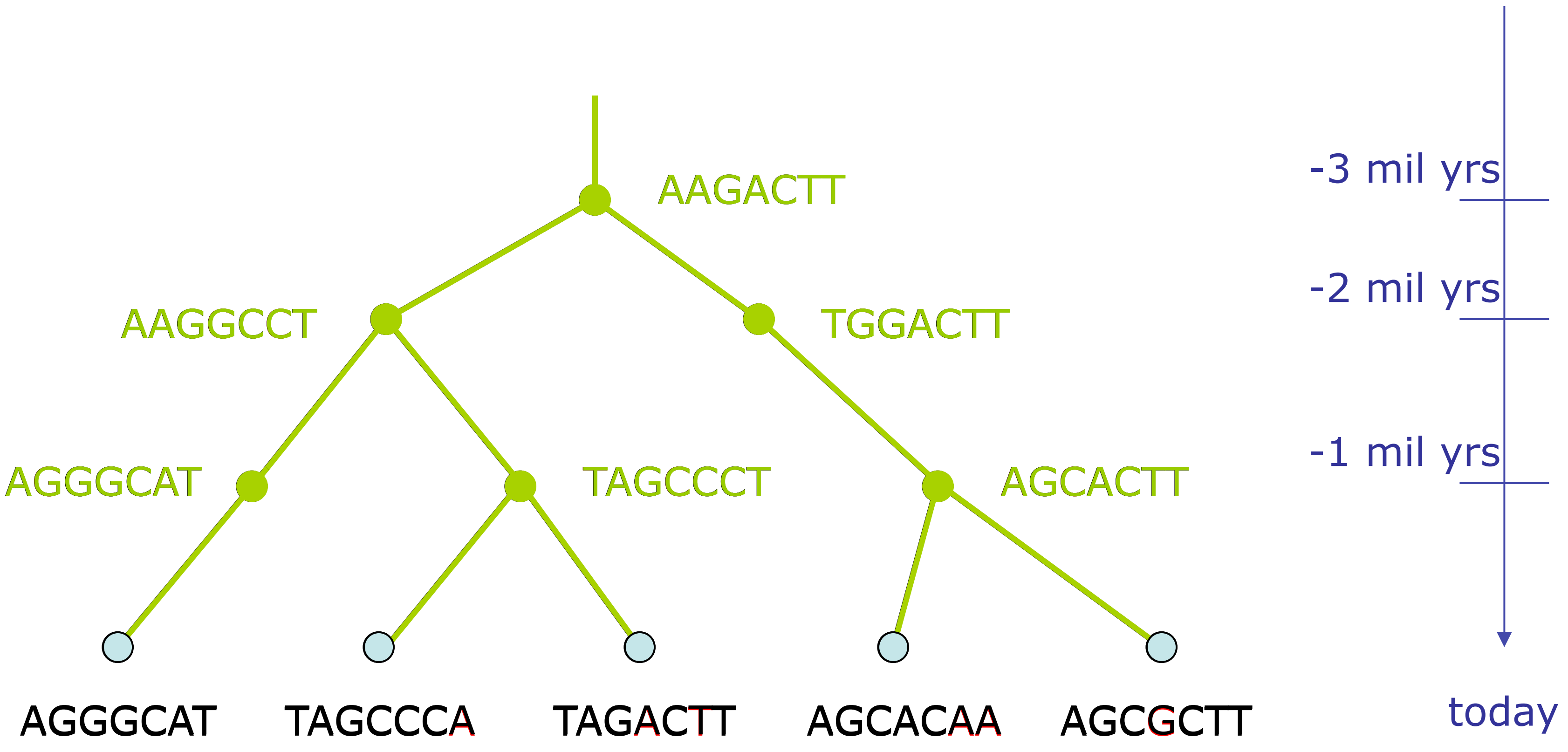


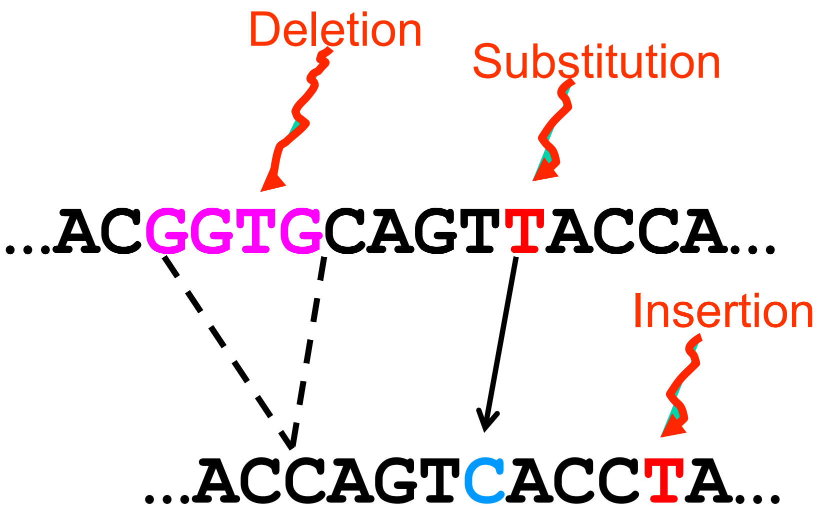
Nature Reviews | Genetics

U	V	W	X	Y
AGGGCATGA	AGAT	TAGACTT	TGCACAA	TGCGCTT



DNA Sequence Evolution





...ACGGTGCAGTTACCA...

...ACCAGTCACCTA...

```
...ACGGTGCAGTTACC-A...
...AC----CAGTCACCTA...
```

The true multiple alignment

- **Reflects historical substitution, insertion, and deletion events**
- **Defined using transitive closure of pairwise alignments computed on edges of the true tree**

Input: unaligned sequences

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

Phase 1: Alignment

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

→

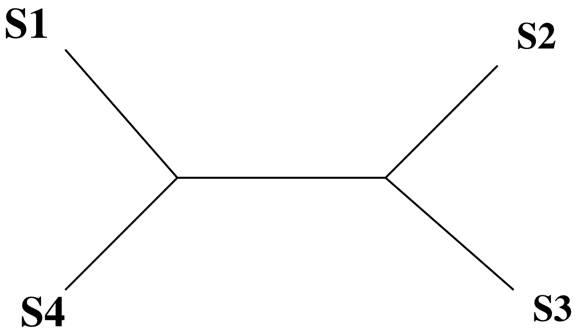
```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```

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
```



Two-phase estimation

Alignment methods

- Clustal
- POY (and POY*)
- Probcons (and Probtree)
- Probalign
- MAFFT
- Muscle
- Di-align
- T-Coffee
- Prank (PNAS 2005, Science 2008)
- Opal (ISMB and Bioinf. 2007)
- *FSA (PLoS Comp. Bio. 2009)*
- *Infernal (Bioinf. 2009)*
- Etc.

Phylogeny methods

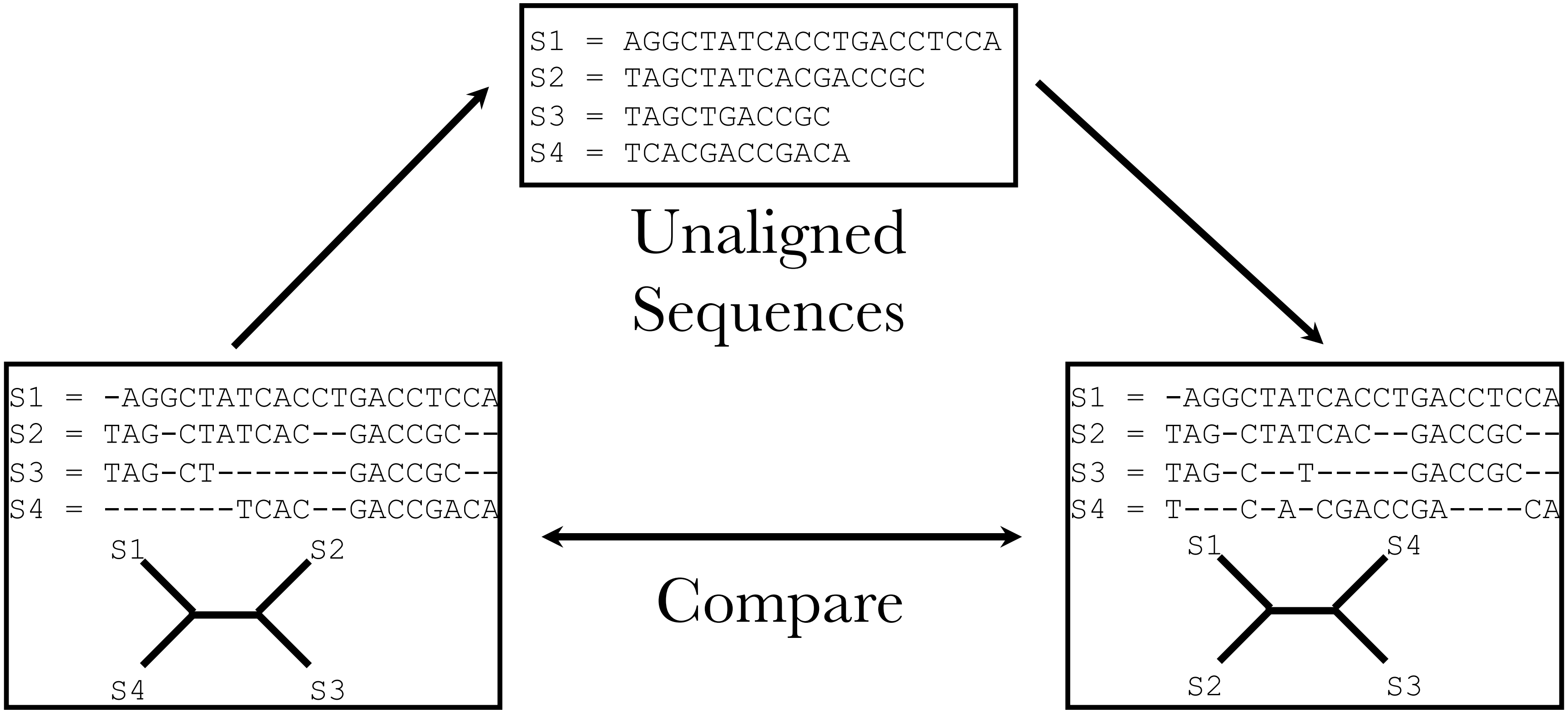
- Bayesian MCMC
- Maximum parsimony
- Maximum likelihood
- Neighbor joining
- FastME
- UPGMA
- Quartet puzzling
- Etc.

***RAxML**: heuristic for large-scale ML optimization*

“Big” phylogenetic datasets

- Large numbers of taxa

Simulation Studies



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

Unaligned
Sequences

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

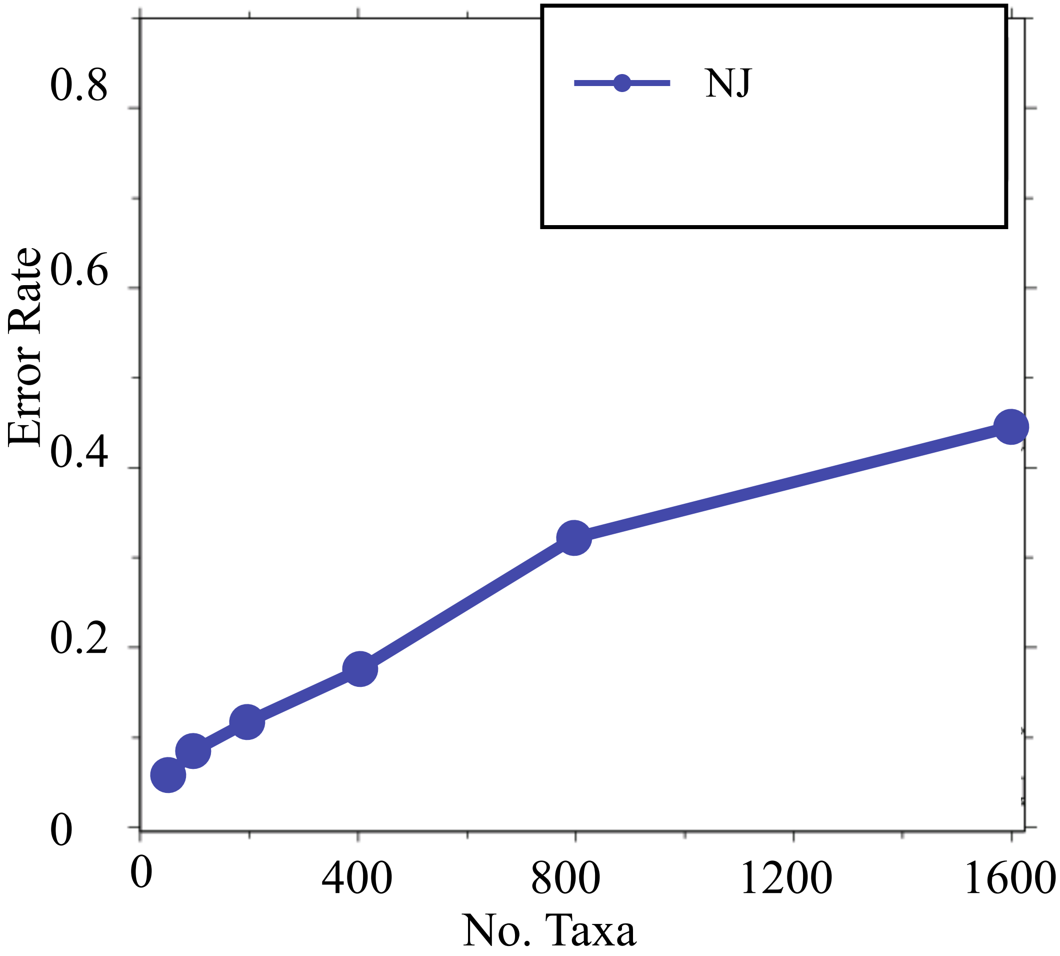
True tree and
alignment

Compare

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-C--T-----GACCGC--
S4 = T---C-A-CGACCGA----CA
```

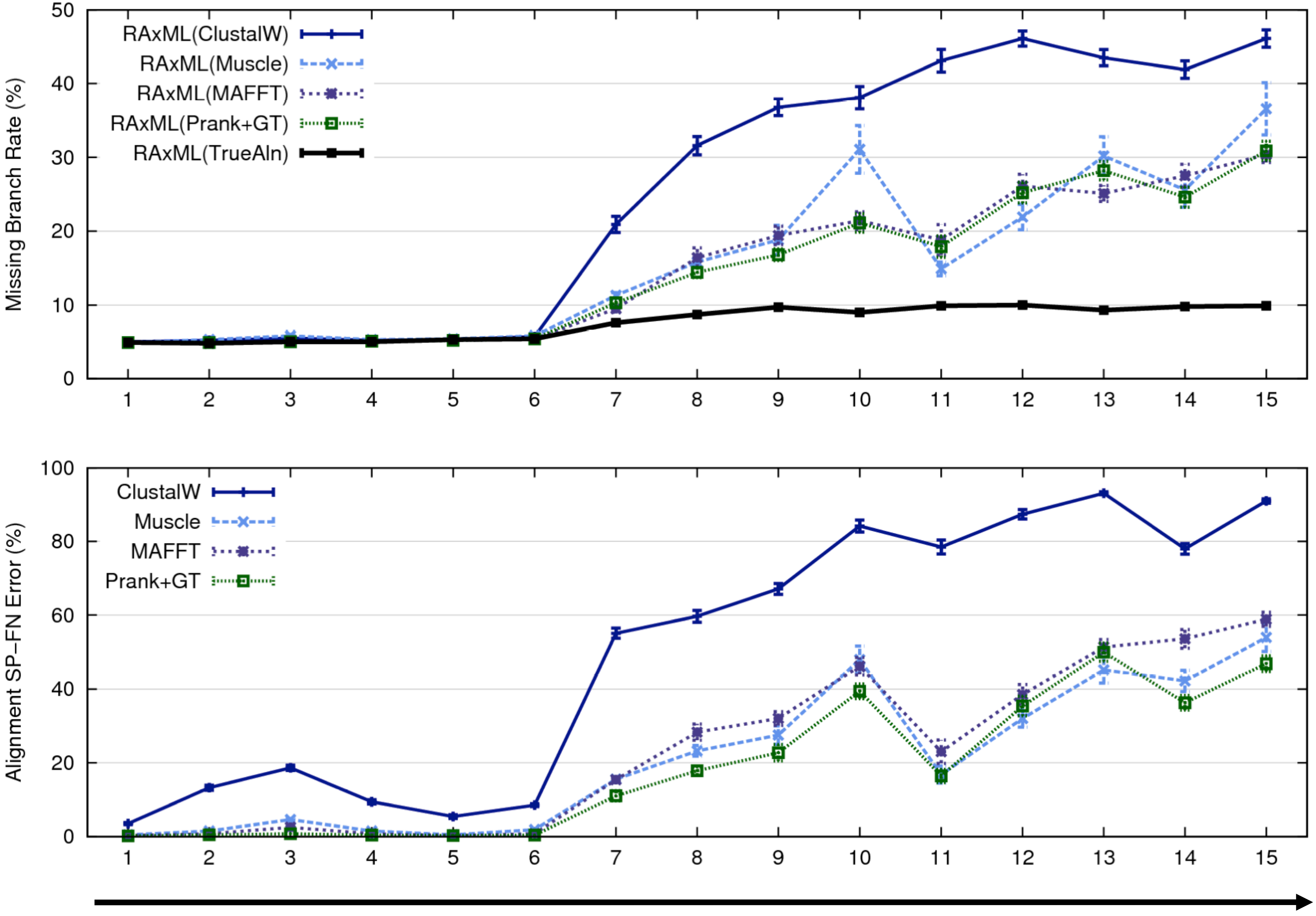
Estimated tree and
alignment

Neighbor joining has poor performance on large diameter trees *[Nakhleh et al. ISMB 2001]*



Theorem (Atteson):

Exponential sequence length requirement for Neighbor Joining!



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

“Big” phylogenetic datasets

- Large numbers of taxa
  - Accurate multiple sequence alignment is challenging, and has a large impact on phylogeny estimation
  - The best phylogeny estimation methods are heuristics for NP-hard problems (standard polynomial time methods can have poor accuracy, even on the true alignment)

“Big” phylogenetic datasets

- Large numbers of genes

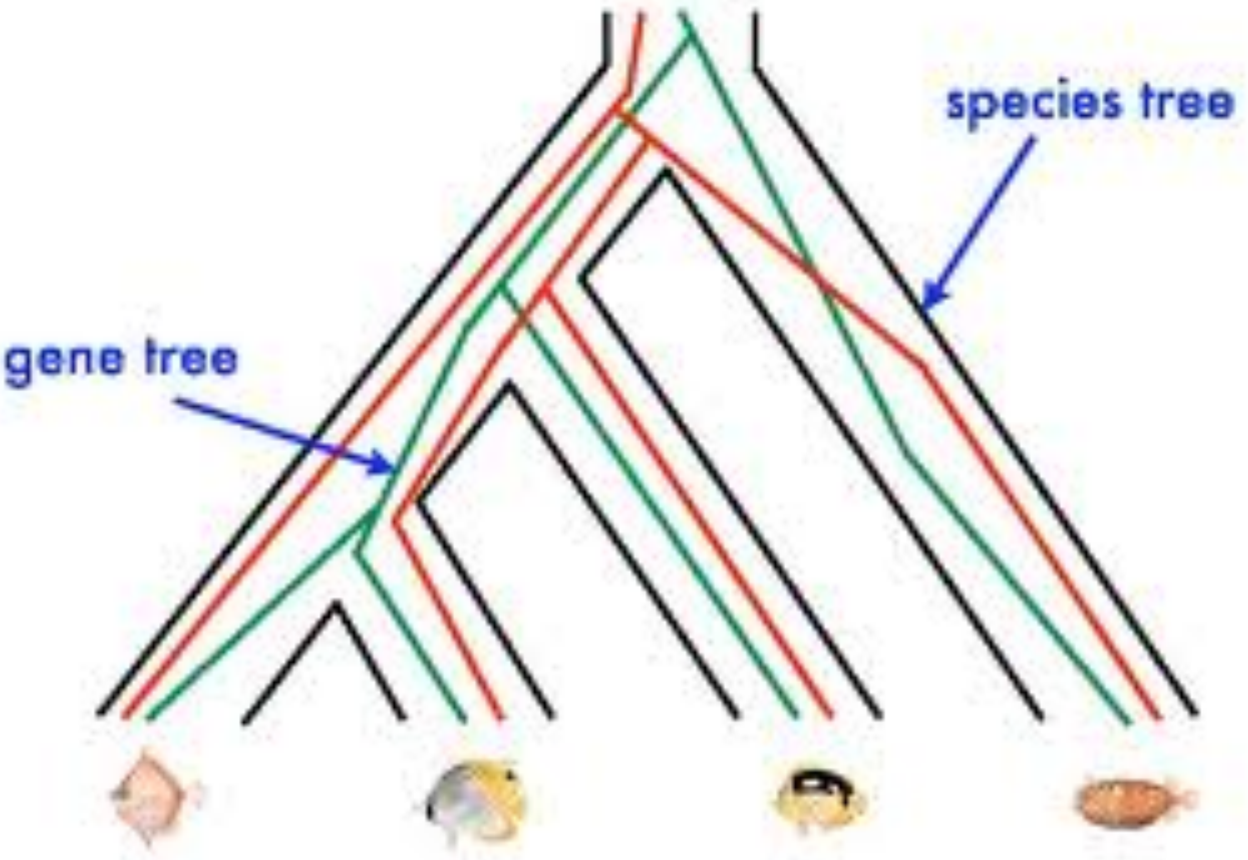
“Big” phylogenetic datasets

- Large numbers of genes
    - “Concatenation” can become computationally infeasible

“Big” phylogenetic datasets

- Large numbers of genes
  - “Concatenation” can become computationally infeasible
  - Gene tree incongruence can make accurate species tree estimation challenging

Red gene tree ≠ species tree (green gene tree okay)



1kp (http://www.onekp.com/)

Gane Ka-Shu
Wong
U Alberta

Jim
Leebens-Mack
U Georgia

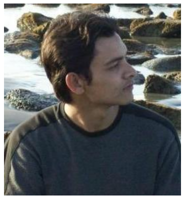
Norm
Wickett
Northwestern

Naim Matasci
iPlant – U Arizona

Tandy Warnow,

Siavash Mirarab,

Nam Nguyen, and
UT-Austin



Md. S. Bayzid

- Transcriptomes of approx. 1200 species
- More than 13,000 gene families (most not single copy)
- Multi-institutional project (10+ universities)

Challenges:

Estimating very large gene alignments and trees (100,000+ sequences)

Estimating species trees from incongruent gene trees

Avian Phylogenomics Project

E.Jarvis, HHMI
MTP Gilbert, Copenhagen
G. Zhang, BGI
S. Mirarab, T. Warnow, and Md. S.Bayzid, UT-Austin

- Approx. 50 species, whole genomes
- 8000+ genes, UCEs
- Gene trees and sequence alignments computed using SATé
- Species tree estimated using maximum likelihood (RAxML)
- Multi-national team (20+ investigators)

Biggest challenges:

Estimating species tree from incongruent gene trees,
Poor phylogenetic signal in most genes

Major Challenges:

large datasets, fragmentary sequences

- **Multiple sequence alignment**: Few methods can run on large datasets, and alignment accuracy is generally poor for large datasets with high rates of evolution.
- **Gene Tree Estimation:** 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).
- **Species Tree Estimation**: gene tree incongruence makes accurate estimation of species tree challenging.

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

This Talk

SATé - co-estimating trees and alignments (Science, 2009)

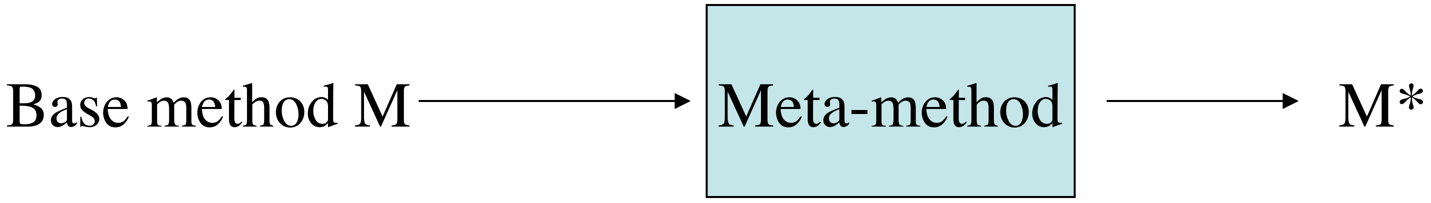
DACTAL – estimating trees (almost) without alignments (ISMB 2012)

SEPP - phylogenetic placement of fragmentary sequence data (e.g., short reads) (PSB 2012)

UPP - Ultra-large alignment using SEPP (<u>unpublished</u>)

Meta-Methods

- Meta-methods “boost” the performance of base methods (phylogeny reconstruction, alignment estimation, etc).



Base method M → Meta-method → M*

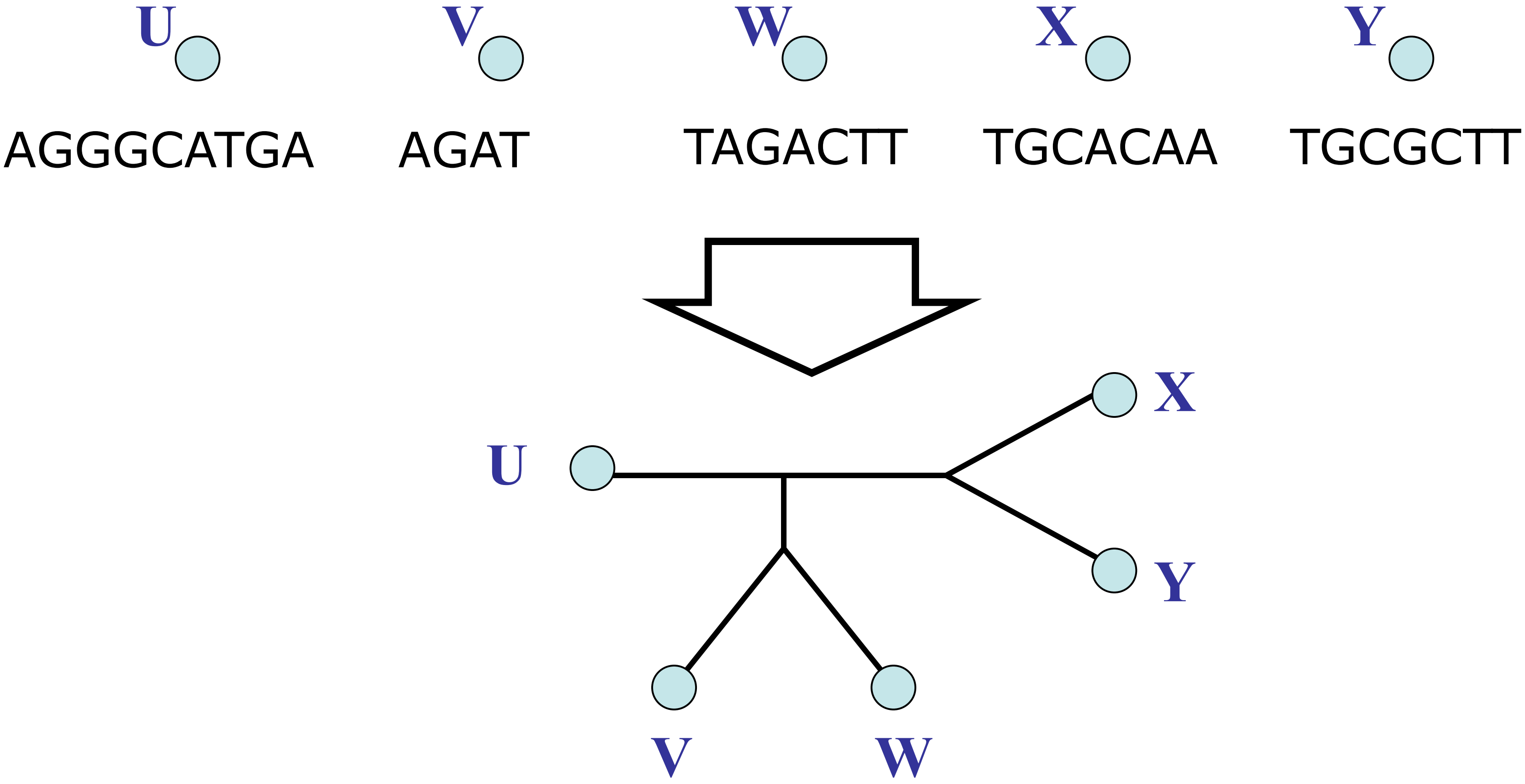
Part I: SATé

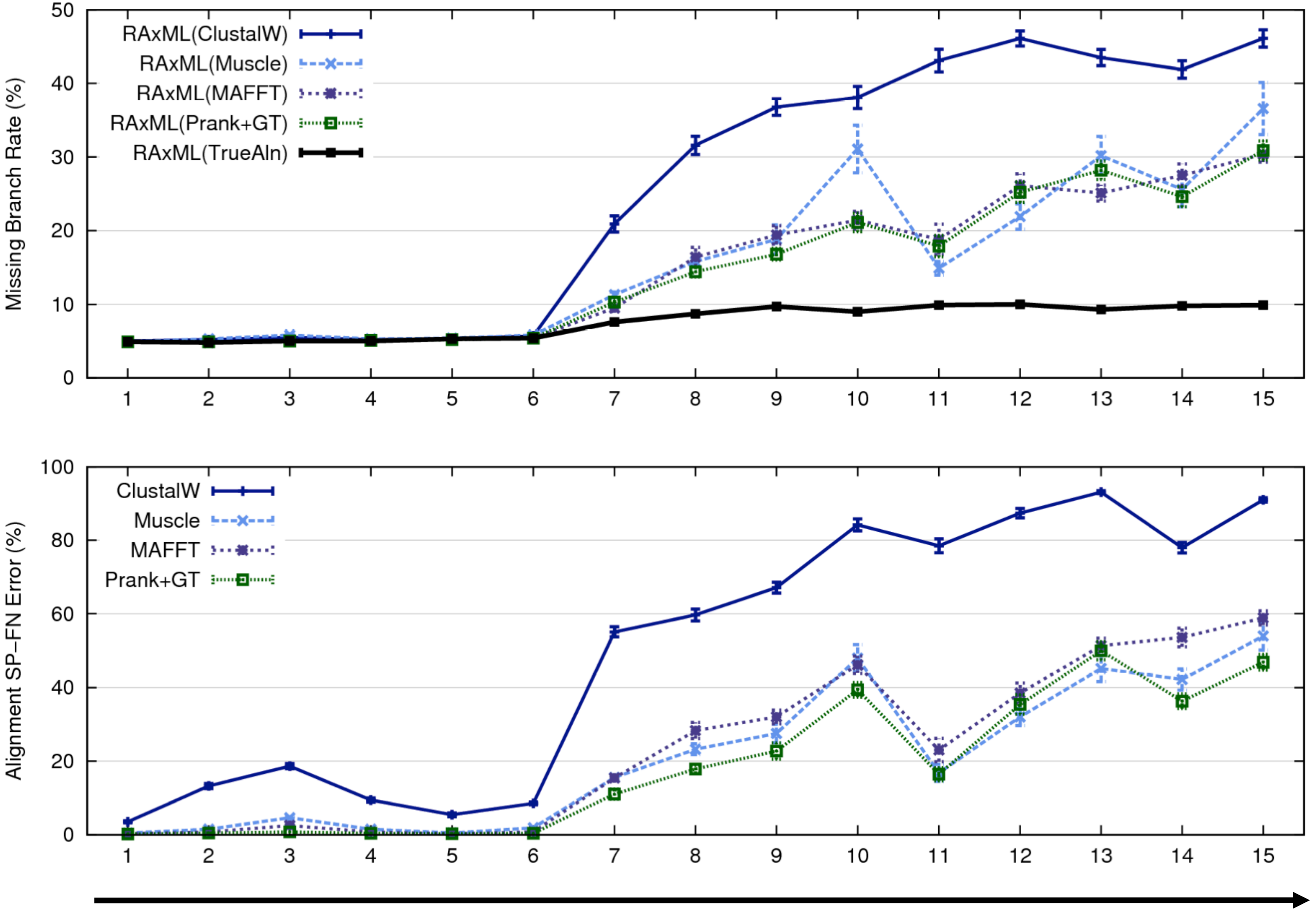
Simultaneous Alignment and Tree Estimation

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

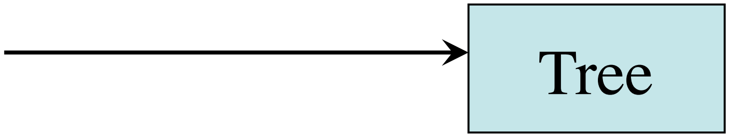

U
V
W
X
Y
AGGGCATGA
AGAT
TAGACTT
TGCACAA
TGCGCTT
U
X
Y
V
W



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

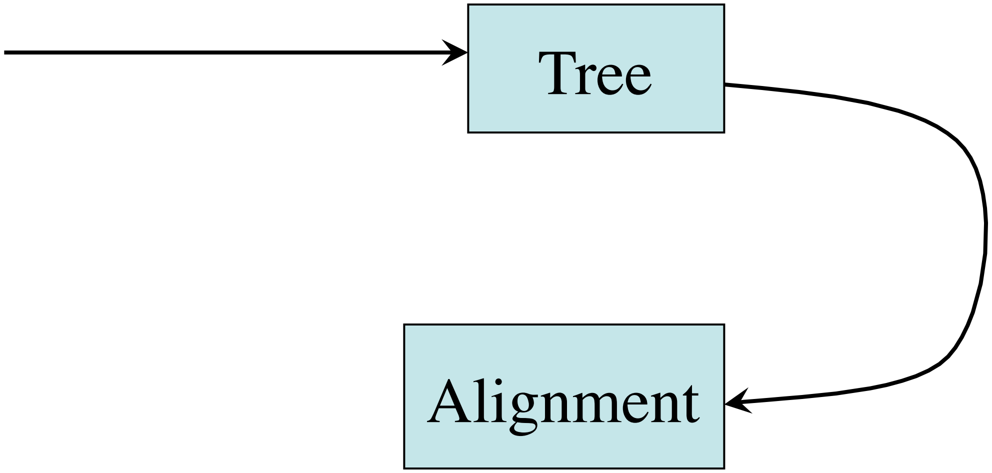
SATé Algorithm

Obtain initial alignment
and estimated ML tree



Tree

SATé Algorithm



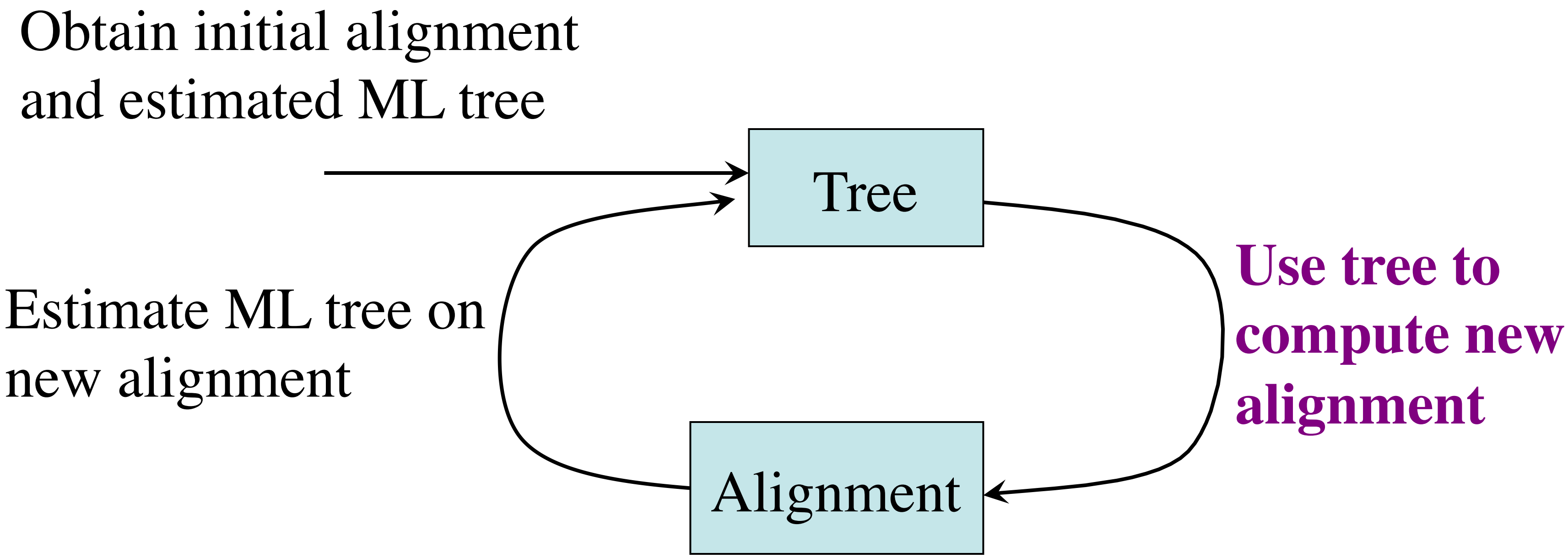
Obtain initial alignment
and estimated ML tree

Tree

Use tree to
compute new
alignment

Alignment

SATé Algorithm



Obtain initial alignment and estimated ML tree

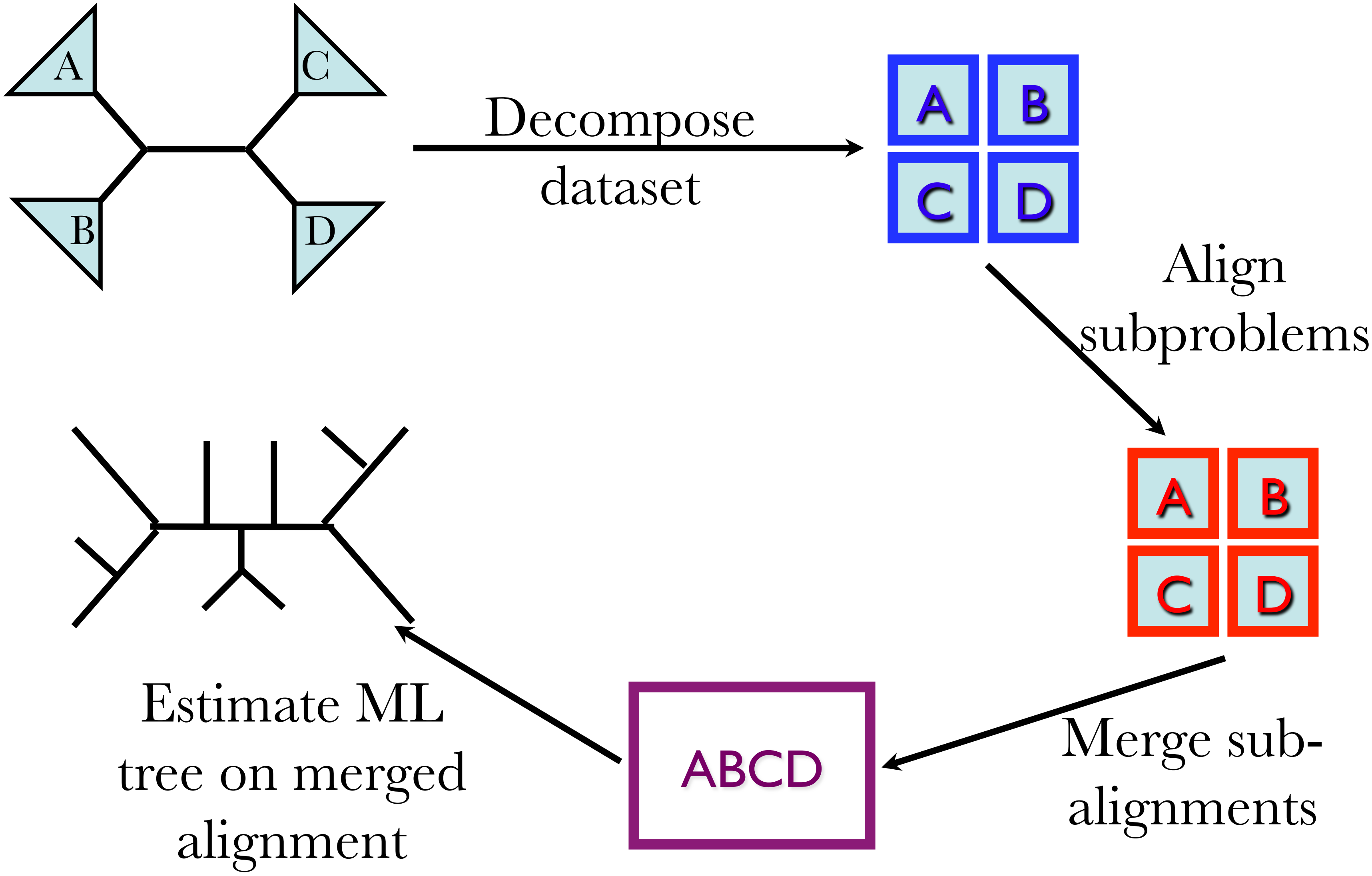
Tree

Use tree to compute new alignment

Alignment

Estimate ML tree on new alignment

Re-aligning on a tree

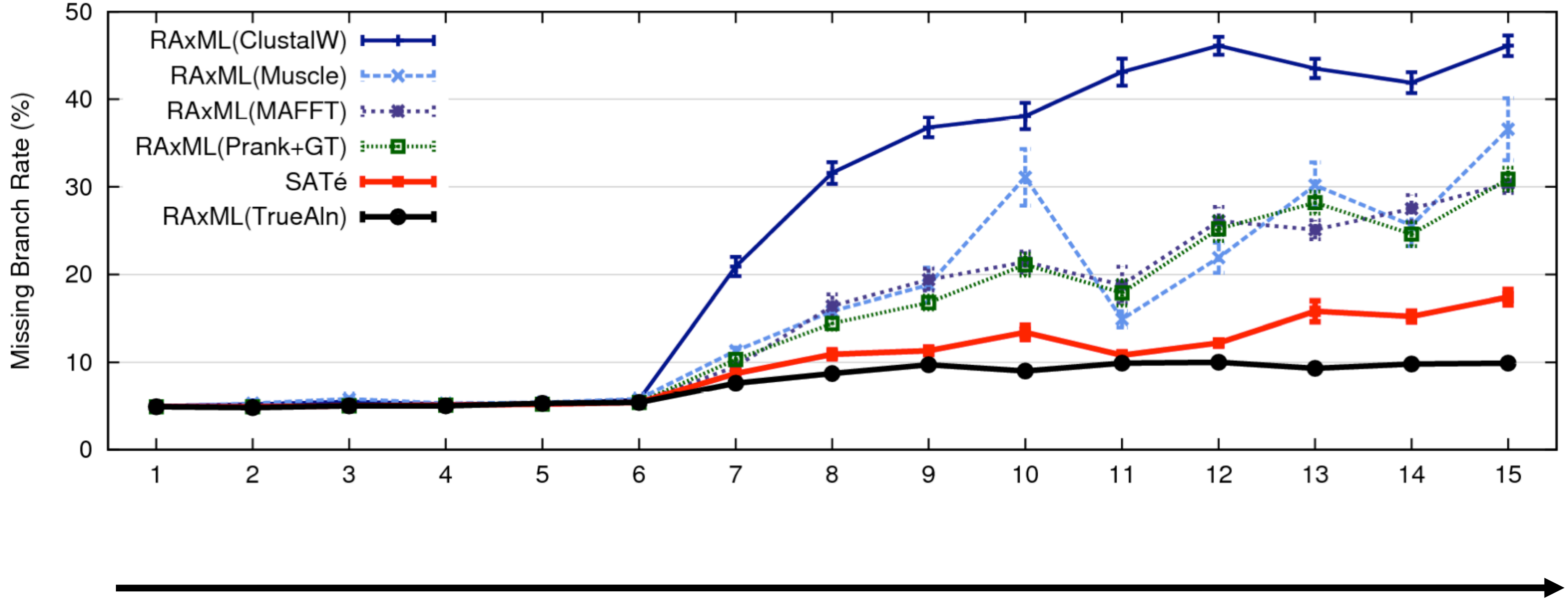


Decompose dataset

Align subproblems

Merge sub-alignments

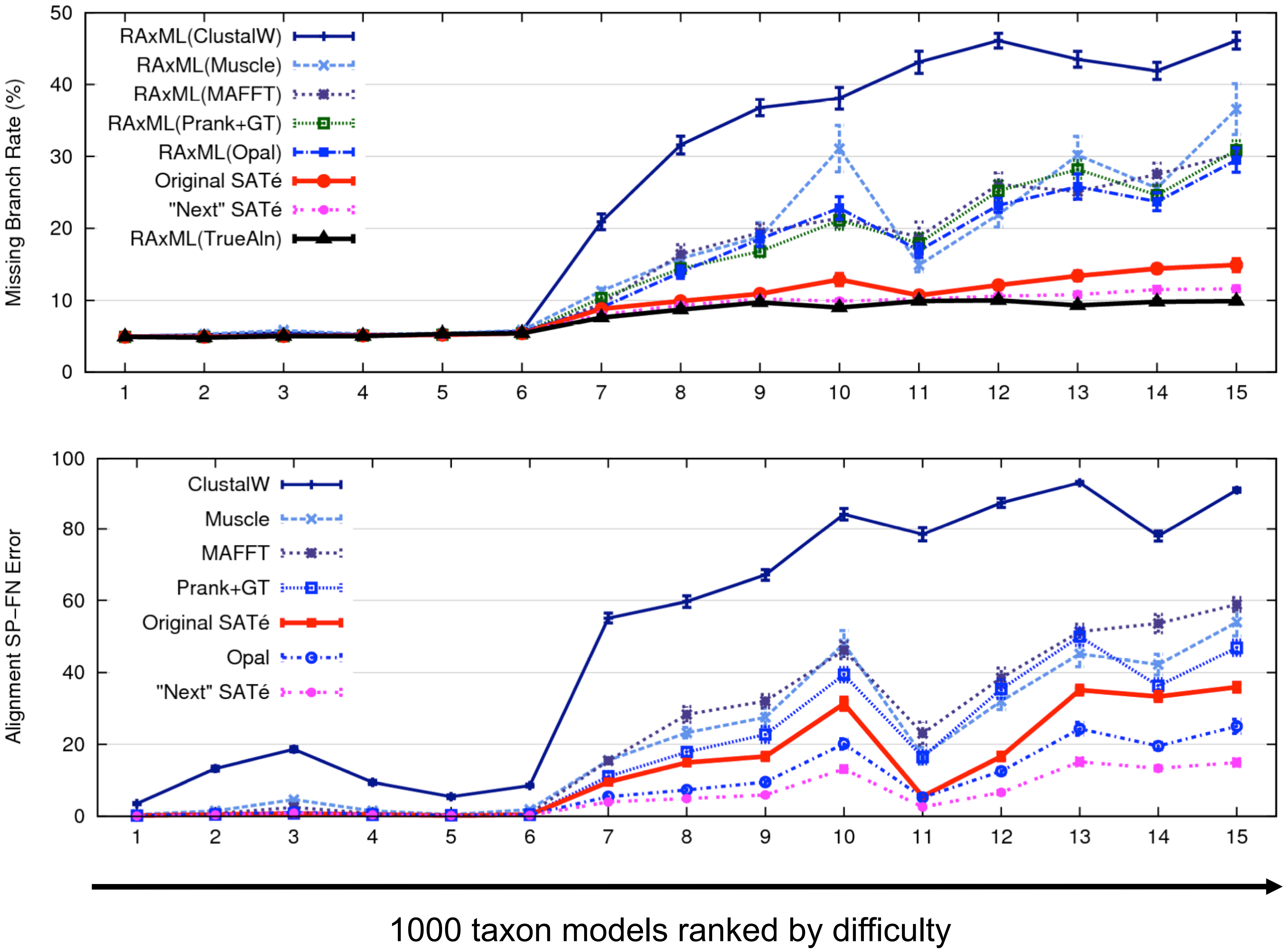
Estimate ML tree on merged alignment



1000 taxon models, ordered by difficulty

24 hour SATé analysis, on desktop machines

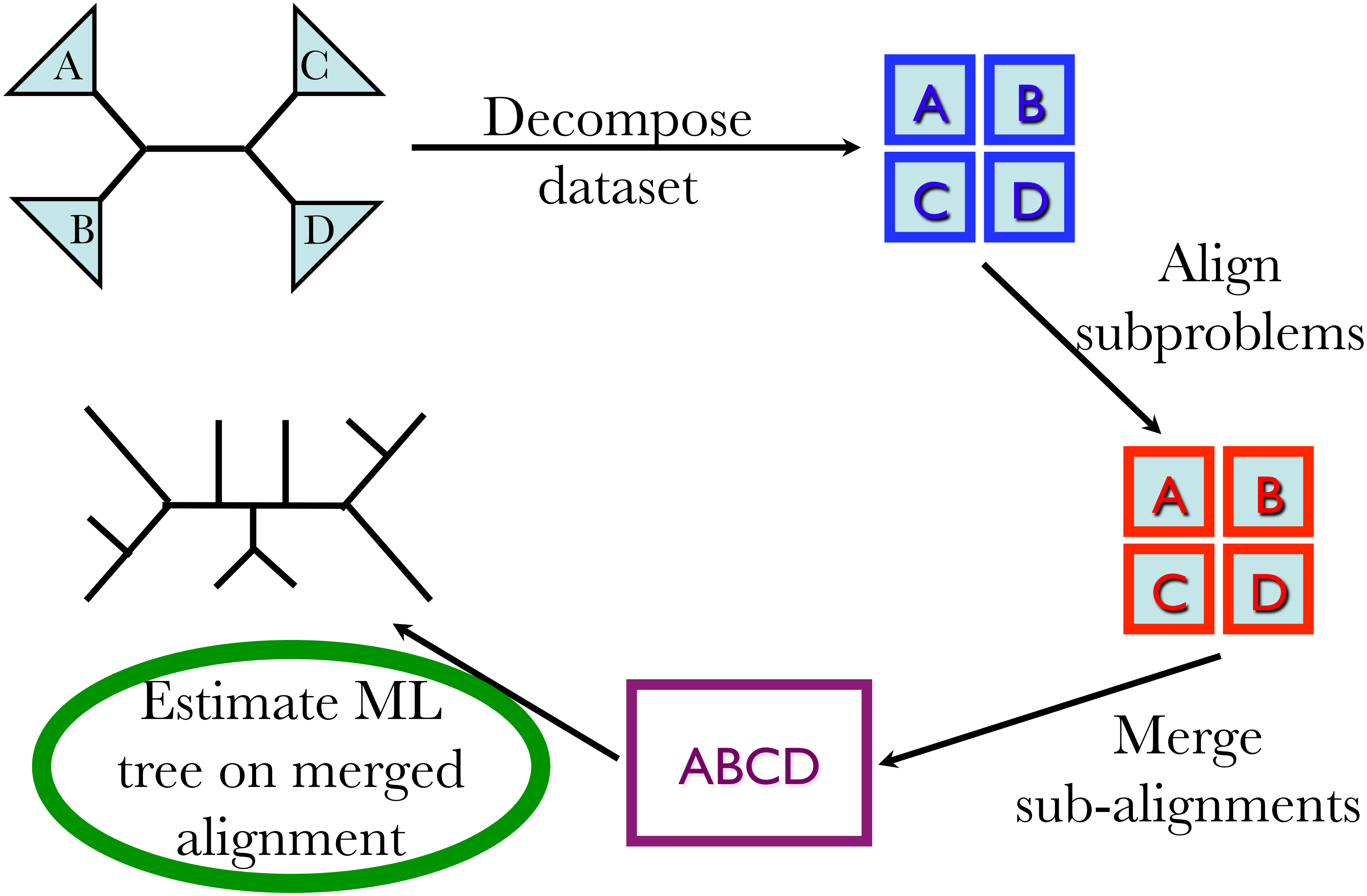
(Similar improvements for biological datasets)


Missing Branch Rate (%)
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
RAxML(ClustalW)
RAxML(Muscle)
RAxML(MAFFT)
RAxML(Prank+GT)
RAxML(Opal)
Original SATé
"Next" SATé
RAxML(TrueAln)
Alignment SP–FN Error
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
ClustalW
Muscle
MAFFT
Prank+GT
Original SATé
Opal
"Next" SATé
1000 taxon models ranked by difficulty

Brief discussion

- **SATé “boosts” the base methods.** Results shown are for SATé used with MAFFT and Muscle. Similar improvements seen for use with Prank, Opal, Muscle, ClustalW, etc.
- **Biological datasets**: Similar results on large benchmark datasets (structurally-based rRNA alignments)
- **No statistical guarantees!!!** In fact, it’s all bad news: ML, treating gaps as missing data (even given the true alignment), can be inconsistent!
- **Performance in practice** results from use of base methods (and ability to use best versions of base methods).
- ***Alignment of genome-scale sequences is a different problem.***
- ***SATé is designed for full-length sequences, not fragmentary datasets***

Limitations



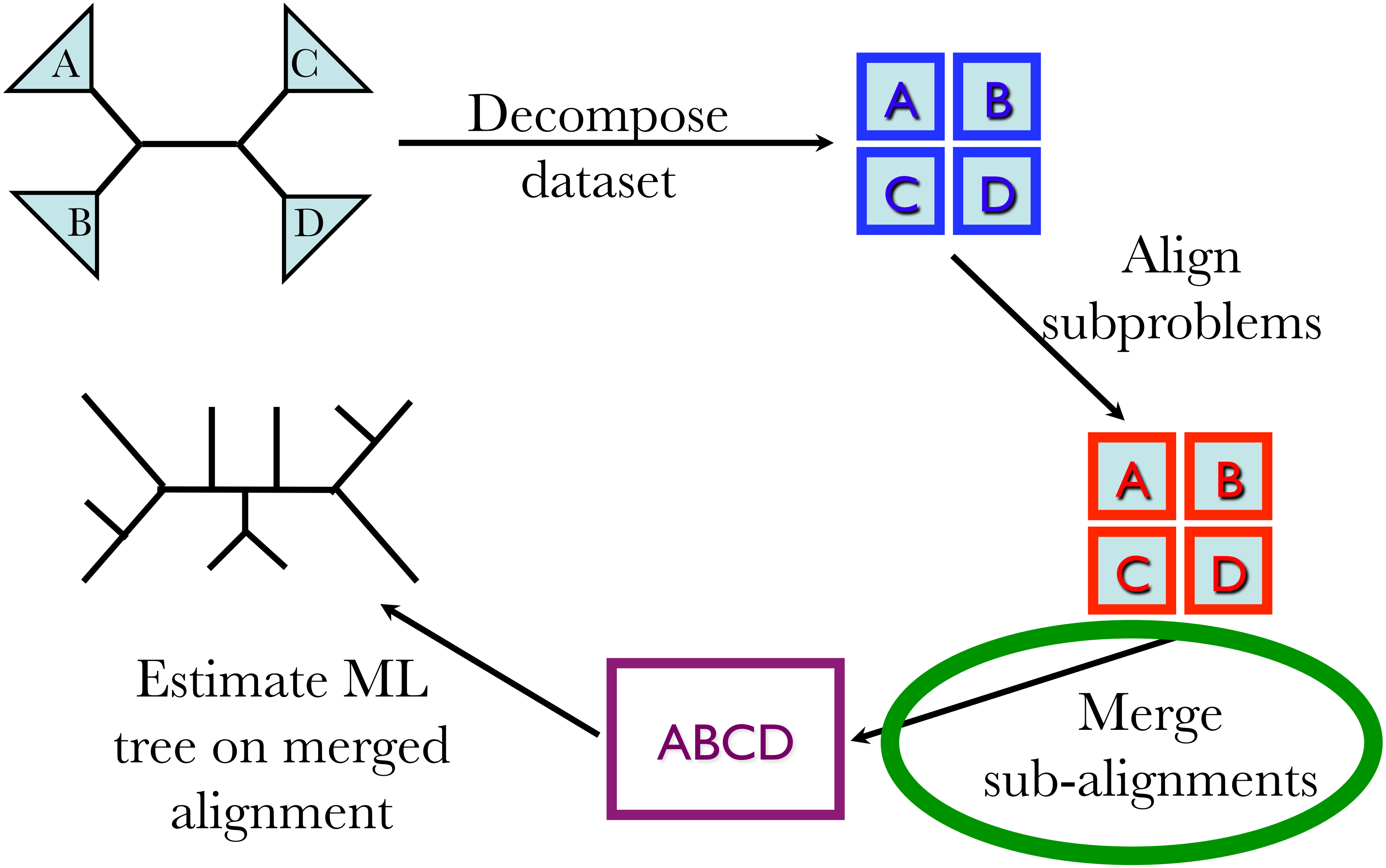
Decompose dataset

Align subproblems

Merge sub-alignments

Estimate ML tree on merged alignment

Limitations



Decompose dataset

Align subproblems

Merge sub-alignments

Estimate ML tree on merged alignment

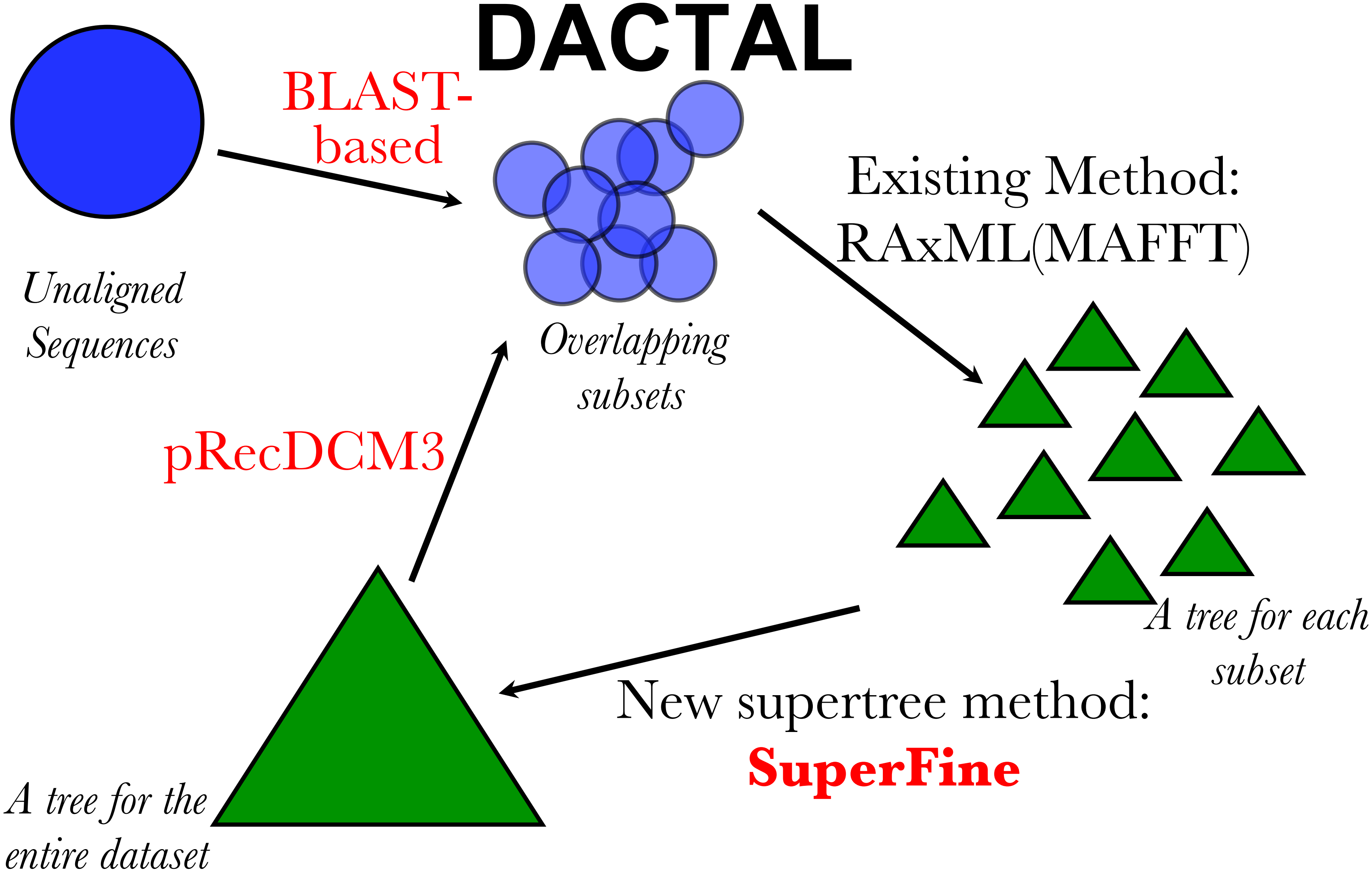
Part II: DACTAL

Divide-And-Conquer Trees
(Almost) 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

DACTAL



Unaligned Sequences

BLAST-based

Overlapping subsets

Existing Method: RAxML(MAFFT)

A tree for each subset

New supertree method: **SuperFine**

A tree for the entire dataset

pRecDCM3

Average of 3 Largest CRW Datasets

CRW: Comparative RNA database,

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

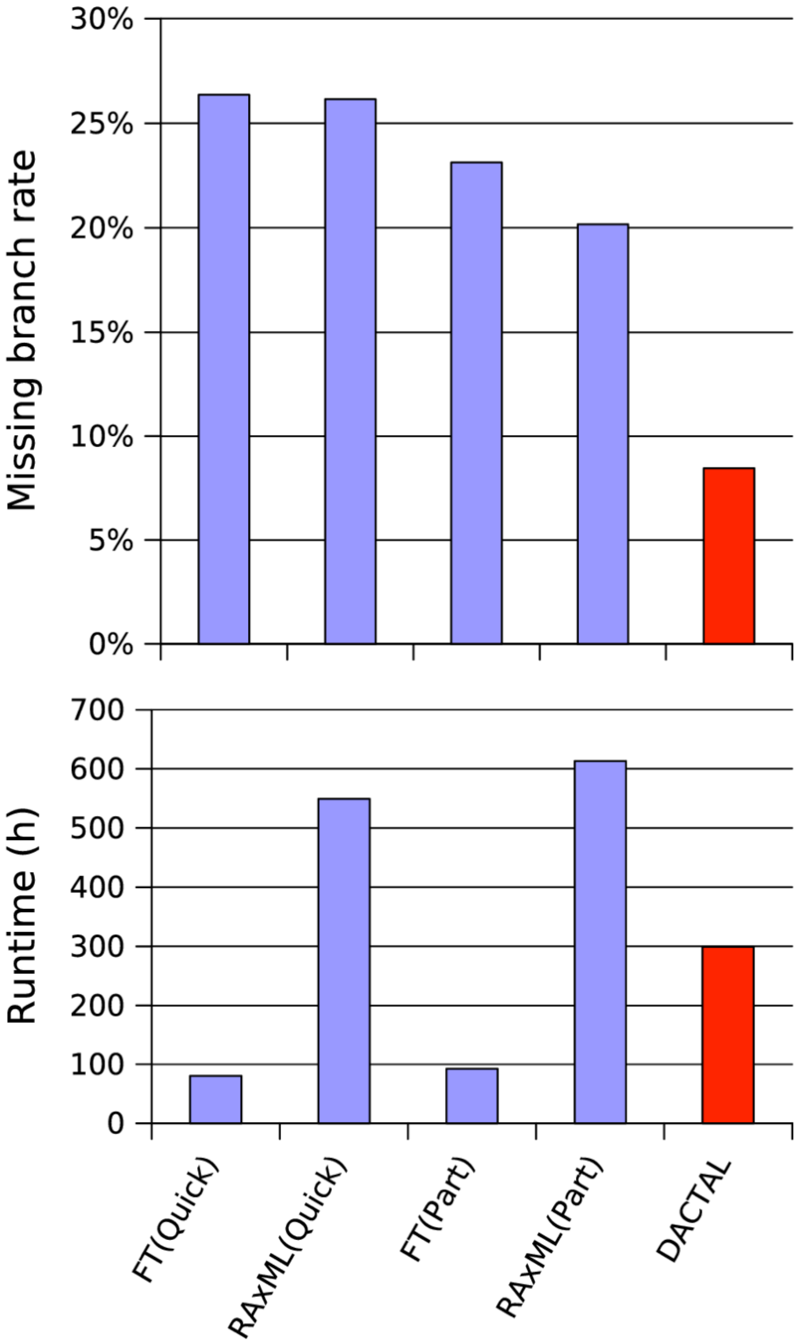
Reference alignments based on secondary structure

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 SATe have comparable accuracy



Part III: SEPP

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

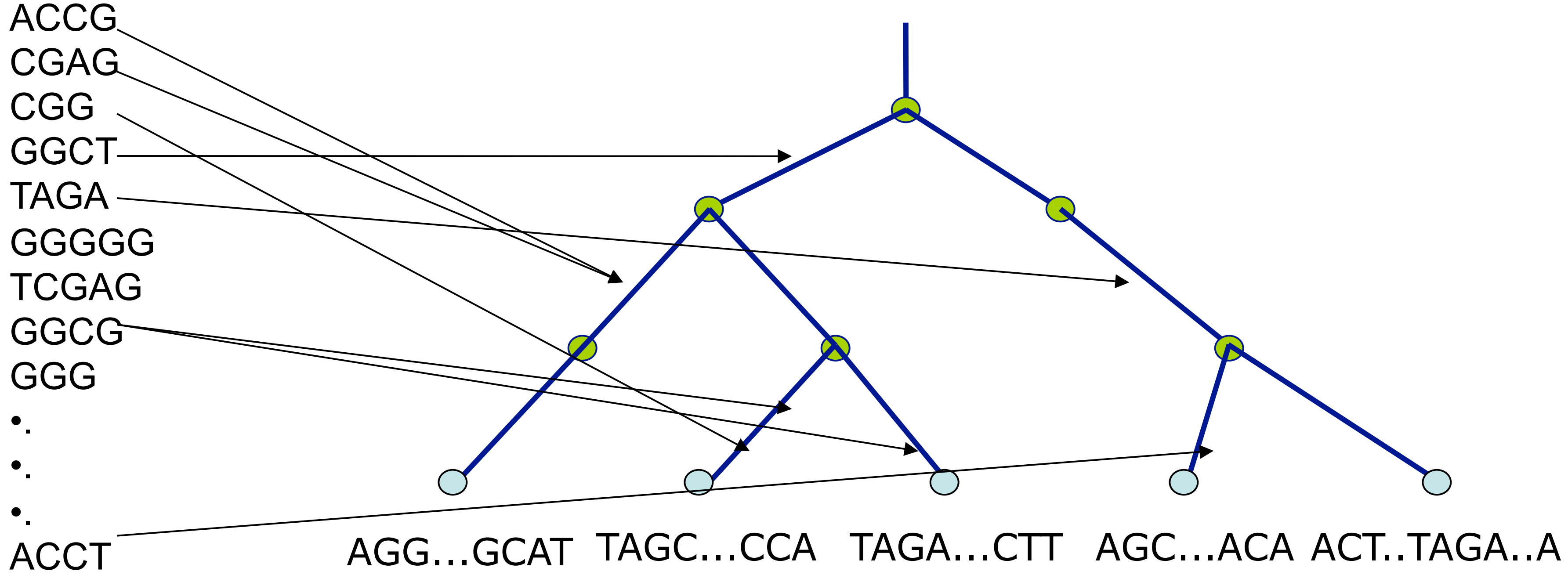
- Pacific Symposium on Biocomputing, 2012 (special session on the Human Microbiome)

- Objective: phylogenetic analysis of single-gene datasets with fragmentary sequences

Phylogenetic Placement

Fragmentary sequences from some gene

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



ACCG
CGAG
CGG
GGCT
TAGA
GGGGG
TCGAG
GGCG
GGG
•.
•.
•.
ACCT

AGG…GCAT TAGC…CCA TAGA…CTT AGC…ACA ACT..TAGA..A

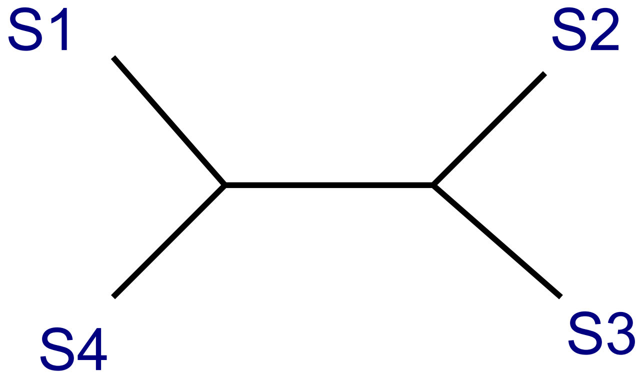
Phylogenetic Placement

Step 1: Align each query sequence to backbone alignment

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

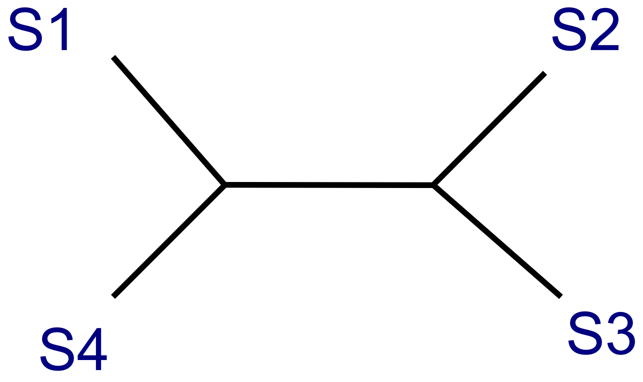
Align Sequence

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



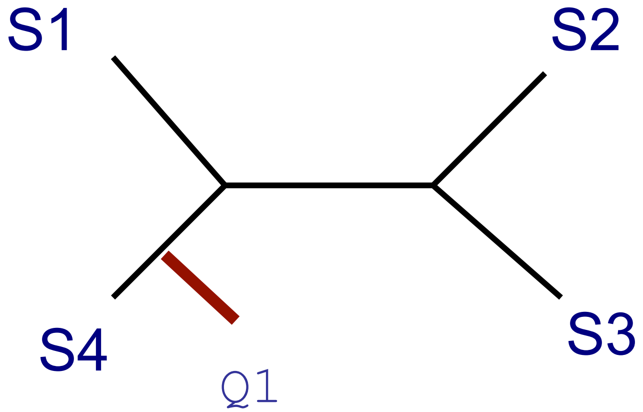
Align Sequence

```
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--------
```

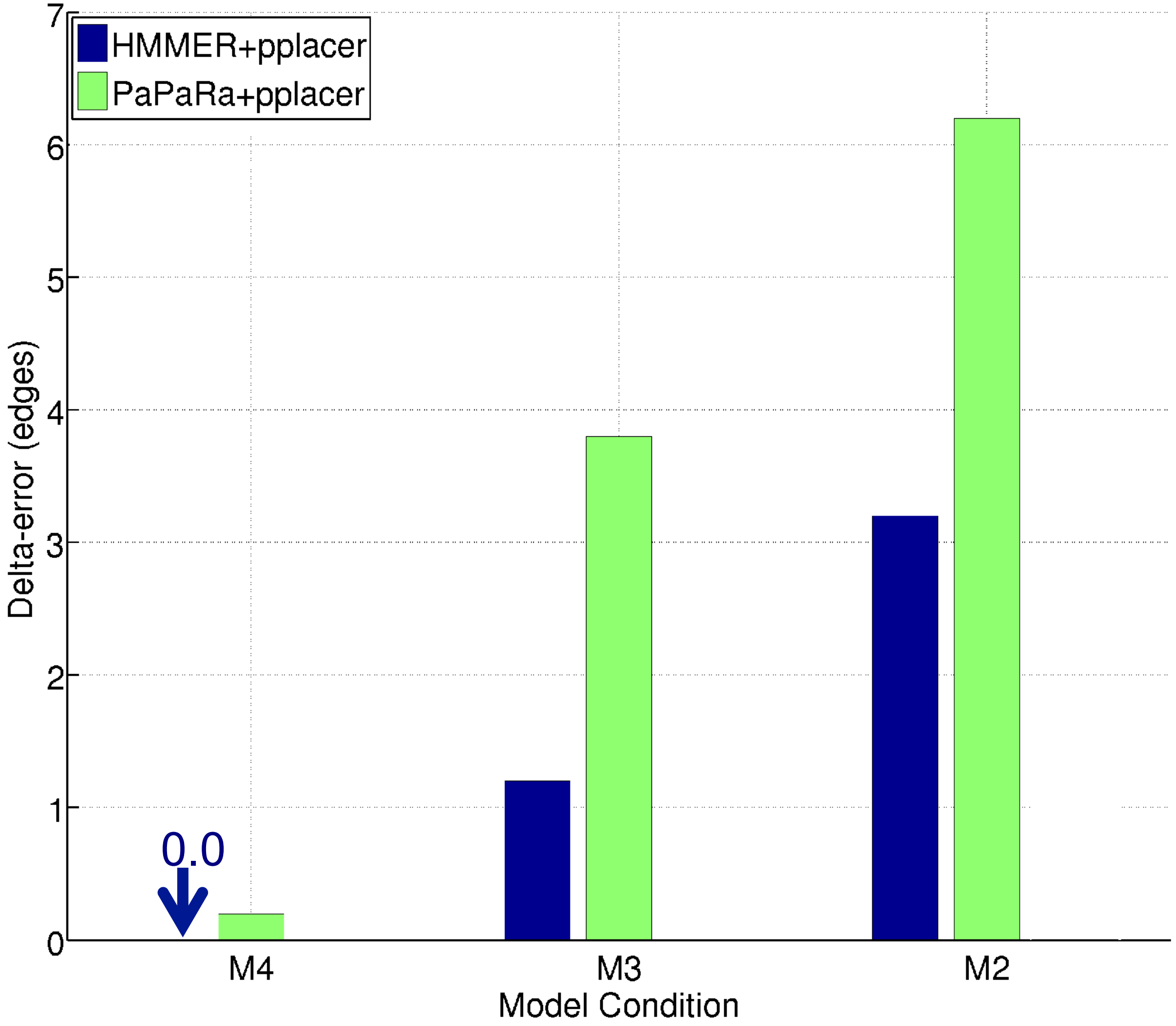


Phylogenetic Placement

- 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.

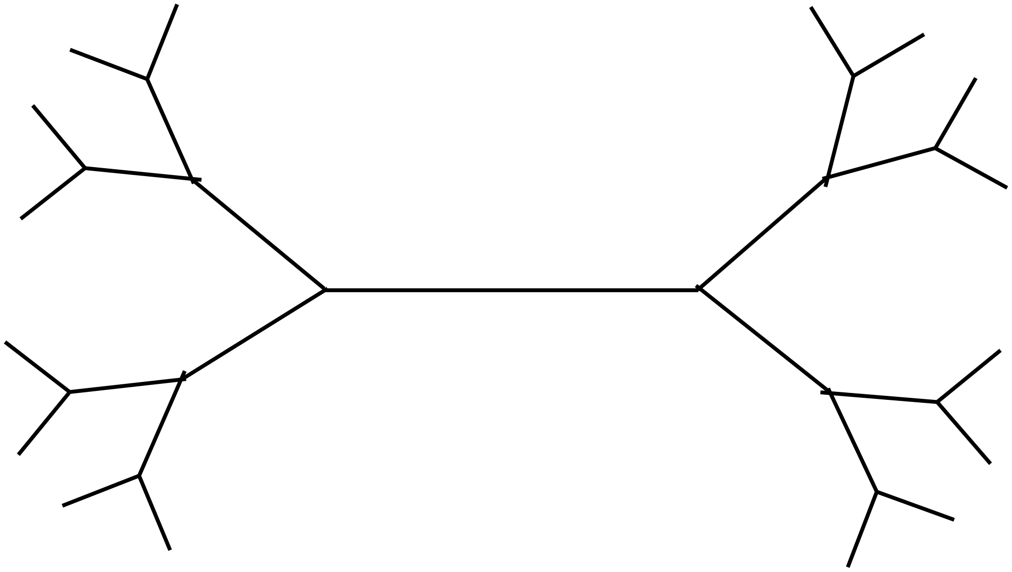
HMMER vs. PaPaRa



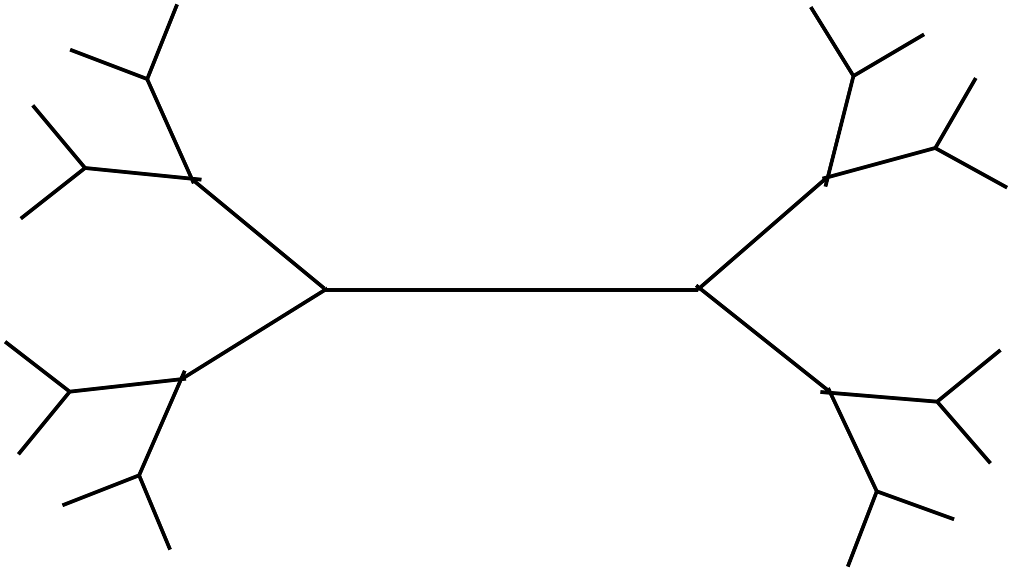
Increasing rate of evolution

HMMER+pplacer:

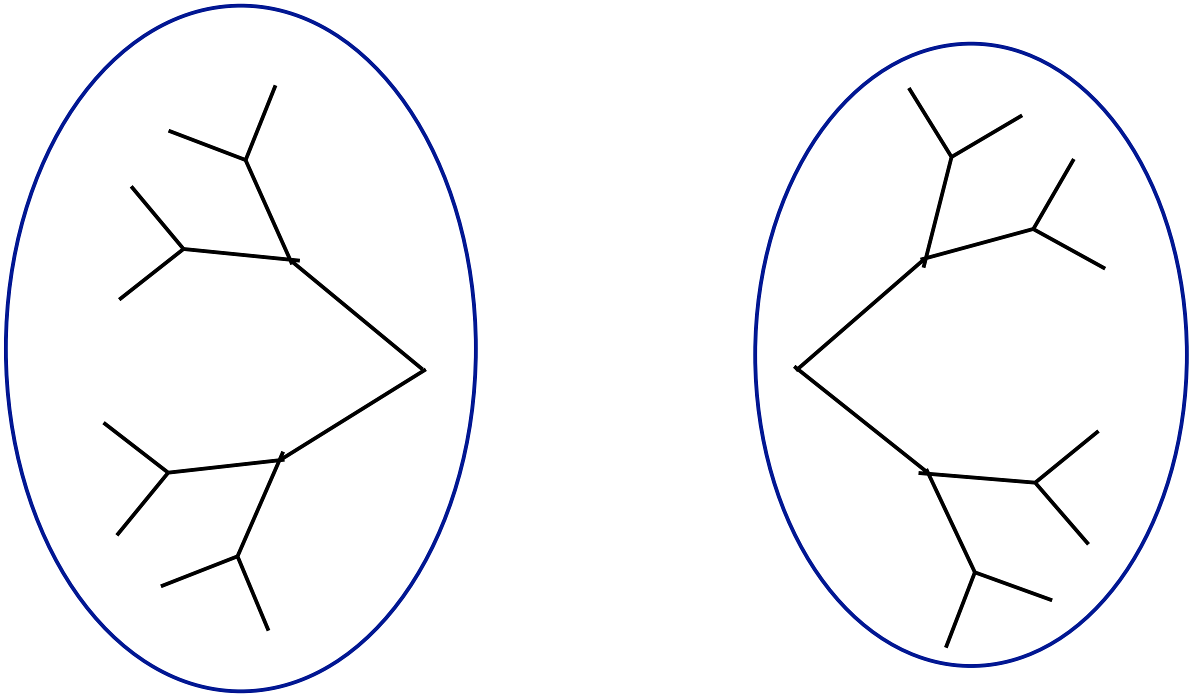
1) build one HMM for the entire alignment
2) Align fragment to the HMM, and insert into alignment
3) Insert fragment into tree to optimize likelihood



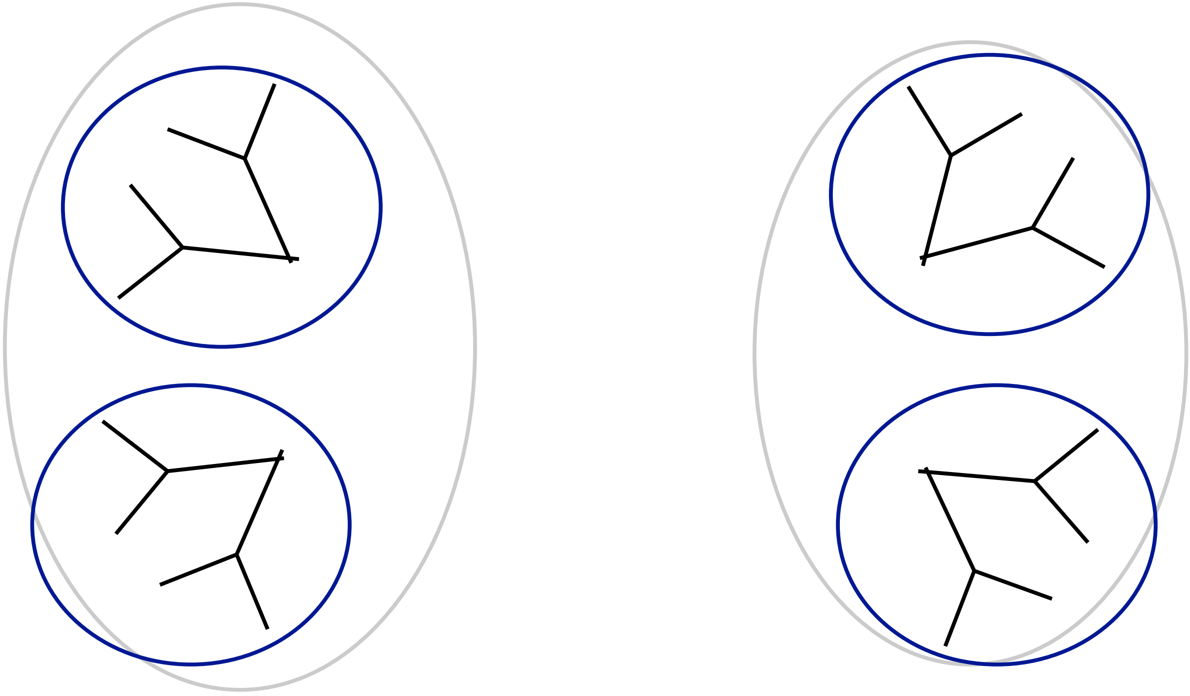
One Hidden Markov Model for the entire alignment?



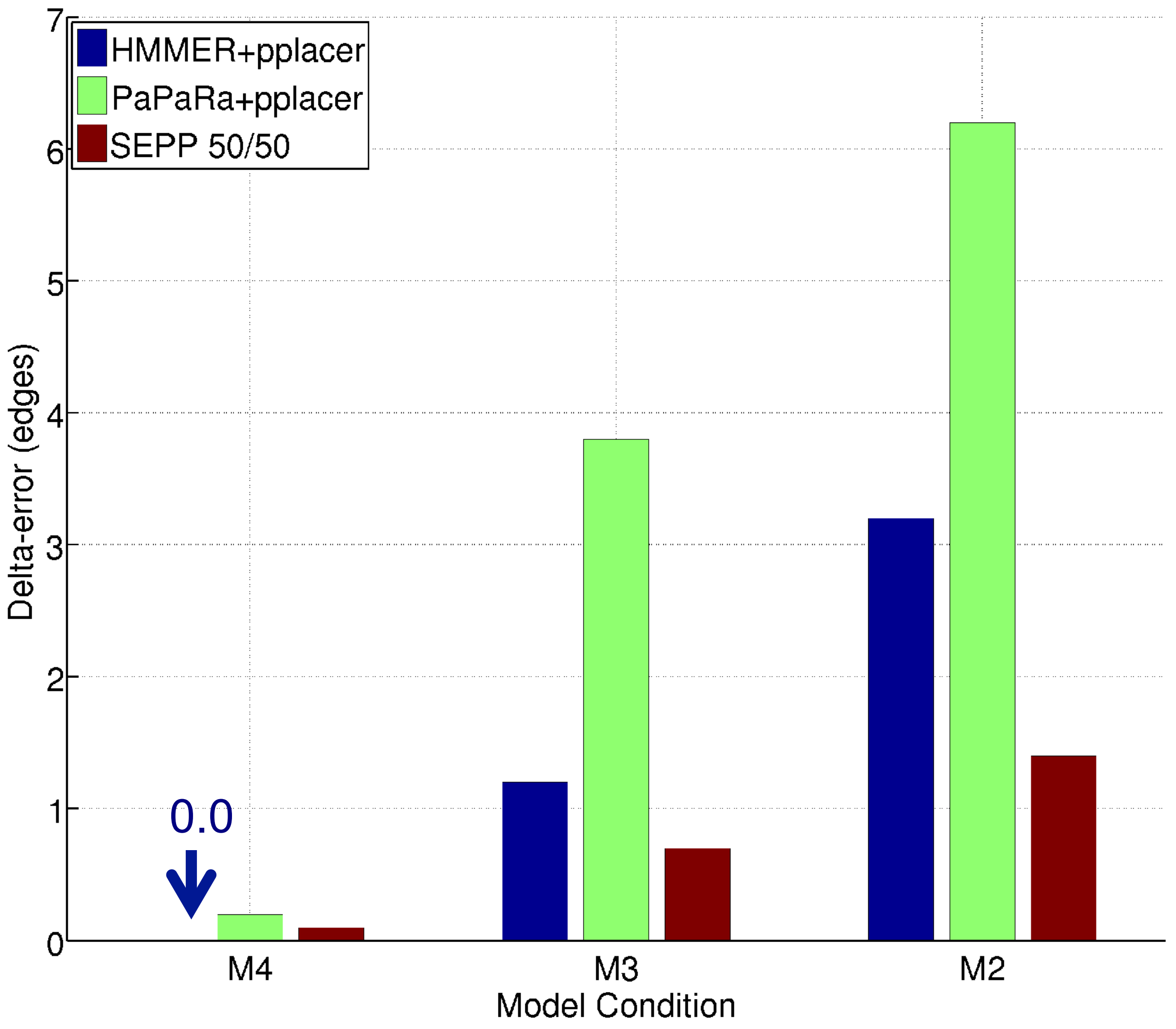
Or 2 HMMs?



Or 4 HMMs?

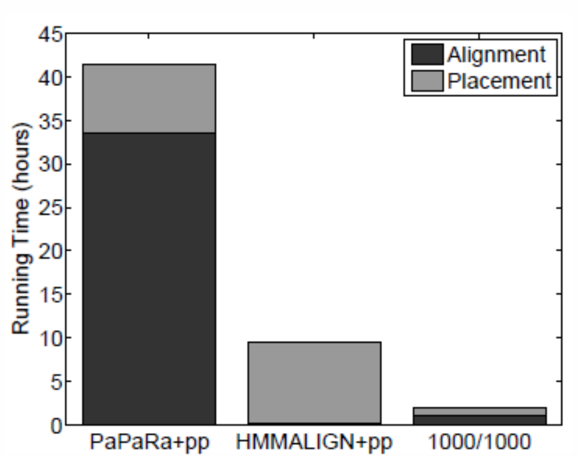


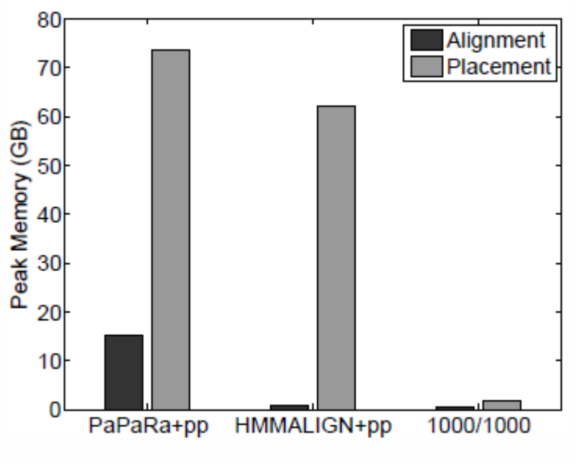
SEPP(10%), based on ~10 HMMs

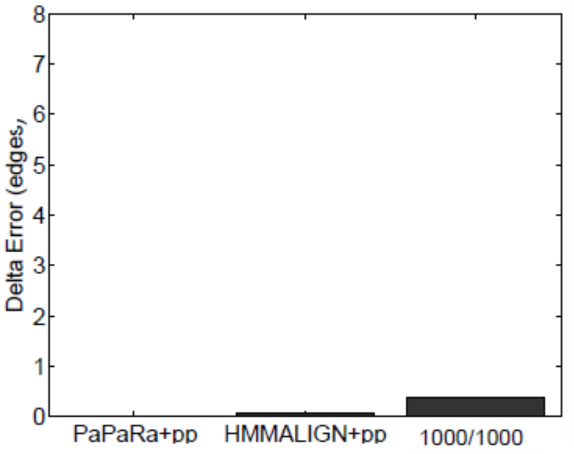


Increasing rate of evolution

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

Applications of SEPP (unpublished)

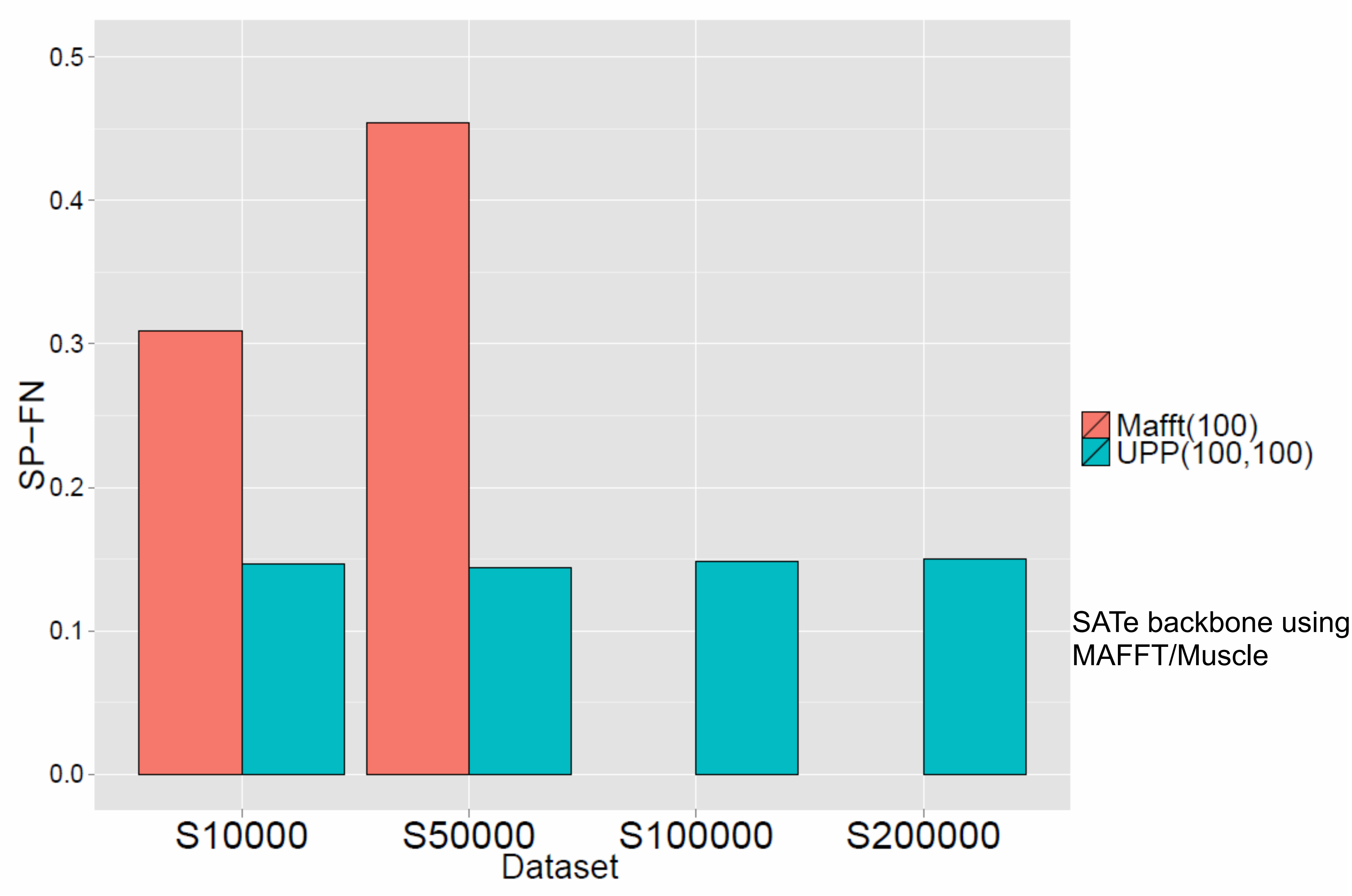
- UPP: Ultra-large alignment using SEPP
- TIPP: taxon identification of fragmentary data (for metagenomic analysis)

Part IV: UPP: Ultra-large alignment using SEPP

Input: set S of unaligned sequences
Output: alignment and tree on S

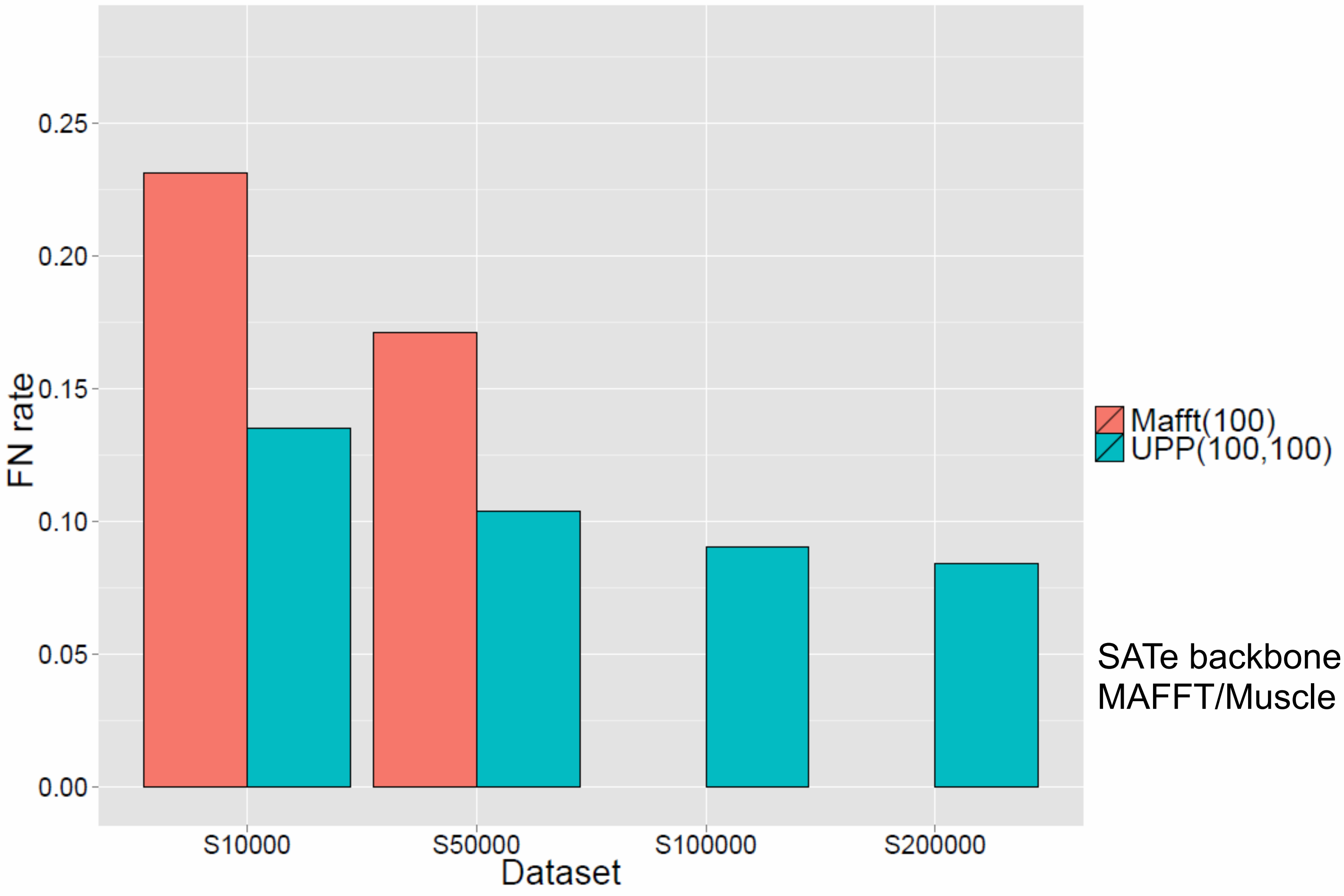
- Select random subset X of sequences
- Estimate alignment and tree on X
- Run SEPP to align remaining sequences
- Run favorite tree estimation method on alignment
- UPP(x,y) refers to UPP using backbones of size y and alignment subsets of size x

RNASim: SP-FN Score



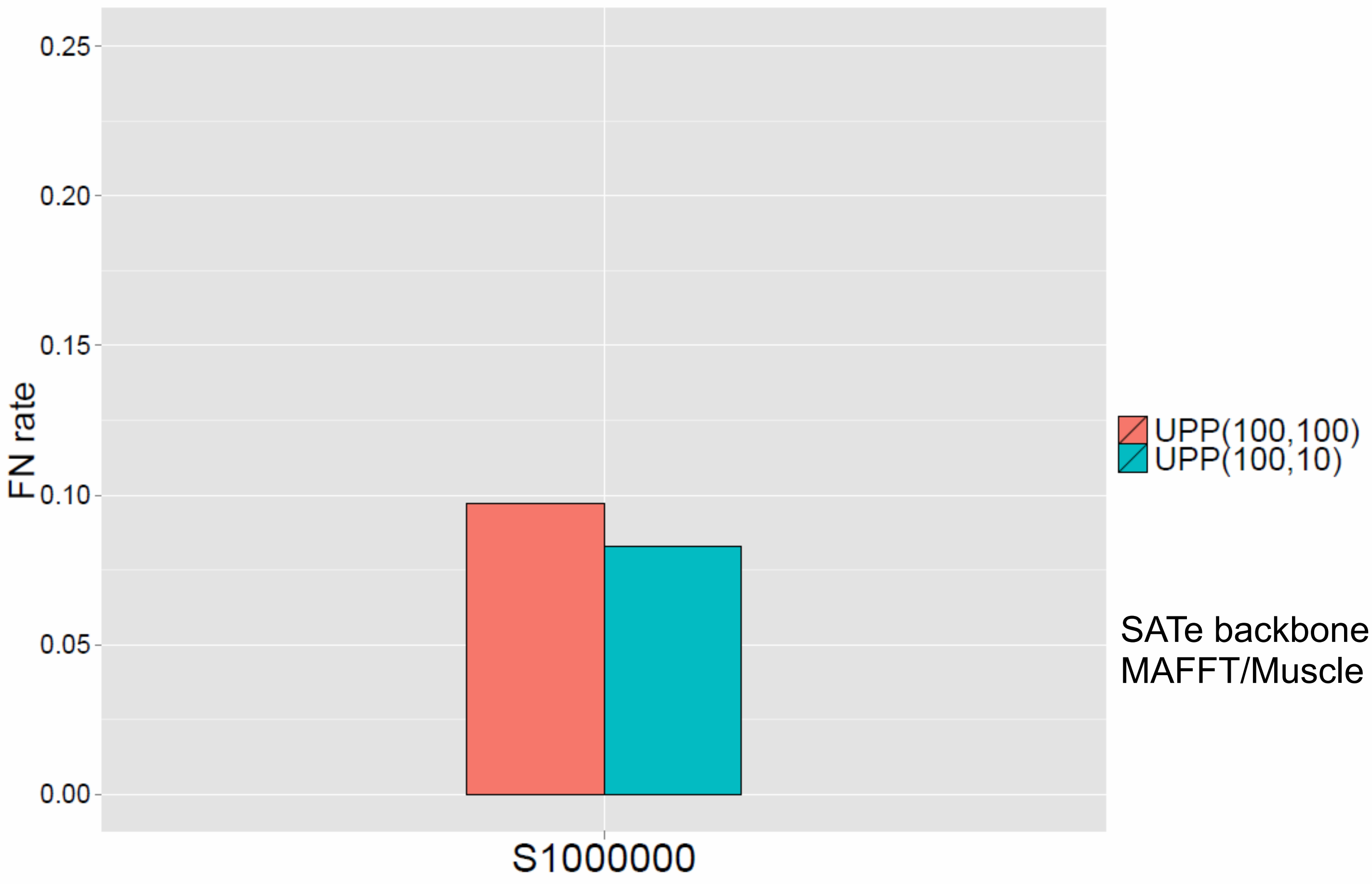
SATe backbone using
MAFFT/Muscle

UPP vs. MAFFT
Tree error on 10K-200K sequences



SATe backbone using
MAFFT/Muscle

UPP(100,100) vs. UPP(100,10)
One Million Taxa: Tree Error



SATe backbone using
MAFFT/Muscle

Note improvement obtained by using SEPP decomposition

Four “Boosters”

SATé: co-estimation of alignments and trees

DACTAL: tree estimation (almost) without alignments

SEPP: phylogenetic placement of short reads

UPP: ultra-large multiple sequence alignment

Phylogenetic “boosters” (meta-methods)

Goal: improve accuracy, speed, robustness, or theoretical guarantees of base methods

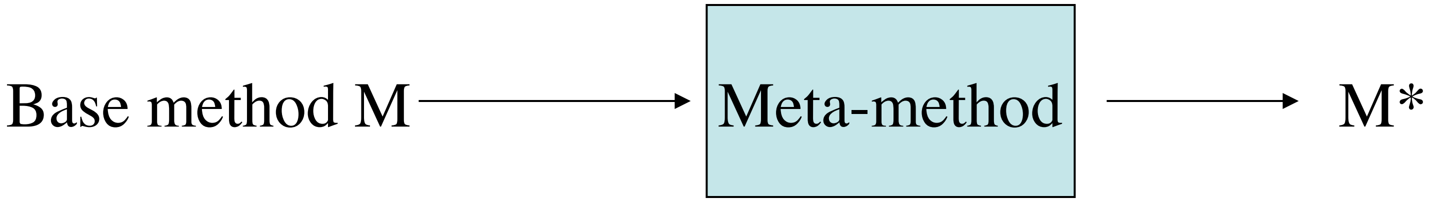
Techniques: divide-and-conquer, iteration, and “bin-and-conquer”

Examples:

- DCM-boosting for distance-based methods (1999)
- DCM-boosting for heuristics for NP-hard problems (1999)
- SATé-boosting for alignment methods (2009)
- SuperFine-boosting for supertree methods (2011)
- DACTAL-boosting: almost alignment-free phylogeny estimation methods (2011)
- SEPP-boosting for phylogenetic placement of short sequences (2012)
- UPP-boosting for alignment methods (unpublished)
- TIPP-boosting for metagenomic taxon identification (unpublished)
- Binning to improve coalescent-based species tree estimation methods (2013)

Meta-Methods

- Meta-methods “boost” the performance of base methods (phylogeny reconstruction, alignment estimation, etc).


Base method M → Meta-method → M*

Algorithmic Strategies

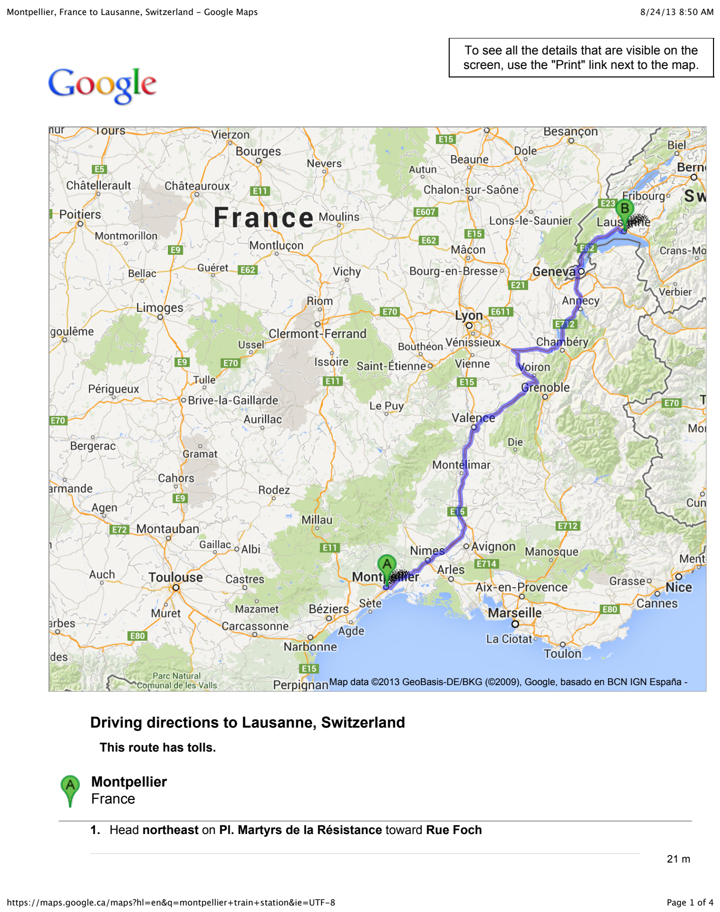
- Divide-and-conquer
- Iteration
- Multiple HMMs instead of one (for classification problems)
- Bin-and-conquer

Acknowledgments

- Guggenheim Foundation Fellowship
- Packard Fellowship
- NSF: ATOL, ITR, and IGERT grants
- David Bruton Jr. Professorship
- HHMI
- Microsoft Research
- Texas Advanced Computing Center (TACC)

- 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, and Randy Linder
  - SEPP and UPP: Siavash Mirarab and Nam Nguyen

2008: David and I deal with a train strike in France



Montpellier, France to Lausanne, Switzerland - Google Maps 8/24/13 8:50 AM

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Driving directions to Lausanne, Switzerland

This route has tolls.

Montpellier
France

1. Head **northeast** on **Pl. Martyrs de la Résistance** toward **Rue Foch**

21 m

https://maps.google.ca/maps?hl=en&q=montpellier+train+station&ie=UTF-8 Page 1 of 4

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Montpellier, France to Lausanne, Switzerland

Thank you David!