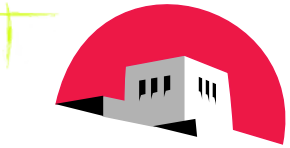

How Many Bootstrap Replicates are Necessary?

Nicholas D. Pattengale(*)[1], Masoud Alipour[2], Olaf R.P. Bininda-Emonds[3], Bernard M.E. Moret[2,4], Alexandros Stamatakis[5]

[1]Department of Computer Science, University of New Mexico, Albuquerque NM, USA

[2]Laboratory for Computational Biology and Bioinformatics, EPFL, Switzerland

[3] AG Systematik und Evolutionsbiologie, Institut für Biologie und Umweltwissenschaften, University of Oldenburg, Germany

[4] Swiss Institute of Bioinformatics, Lausanne, Switzerland

[5]The Exelixis Lab, Department of Computer Science, TU München, Germany

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Main Result/Contribution

Two criteria for *stopping numbers* in phylogenetic bootstrapping

- First empirical assessment of variability in support value, as a function of replicate count, in bootstrapping
- Validate our proposals for stopping criteria

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- Background
  - Phylogeny and Splits
  - The Phylogenetic Bootstrap
  - Stopping Numbers
- Our Technique
  - The Framework
    - Motivation – Permutation Test
    - Frequency Criterion (FC)
    - Weighted Criterion(WC)
  - The Experiment(s)
- Conclusion

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Phylogenetic Reconstruction

Human

Chimpanzee

Gorilla

Orangutan

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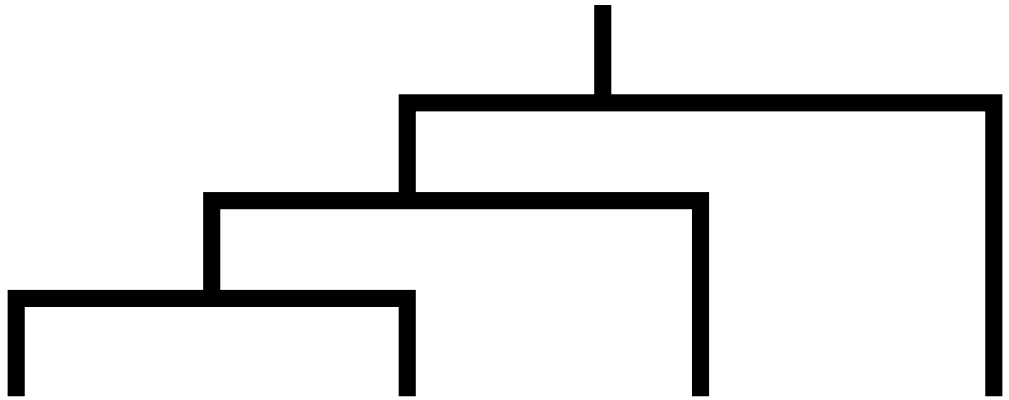
Phylogenetic Reconstruction



Human Chimpanzee Gorilla Orangutan

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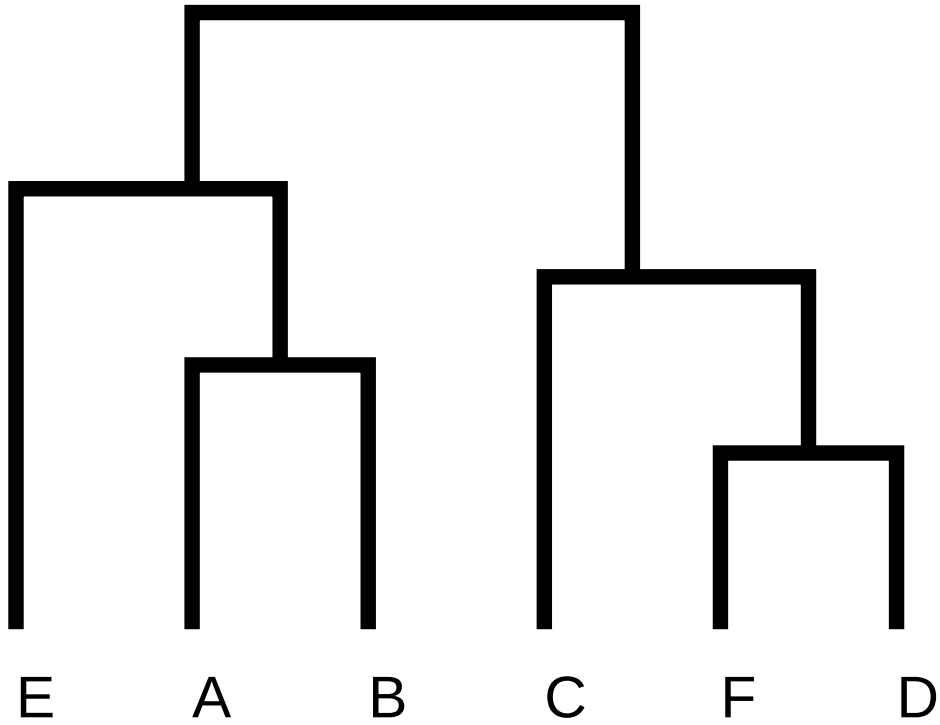
Canonical Representation - Splits



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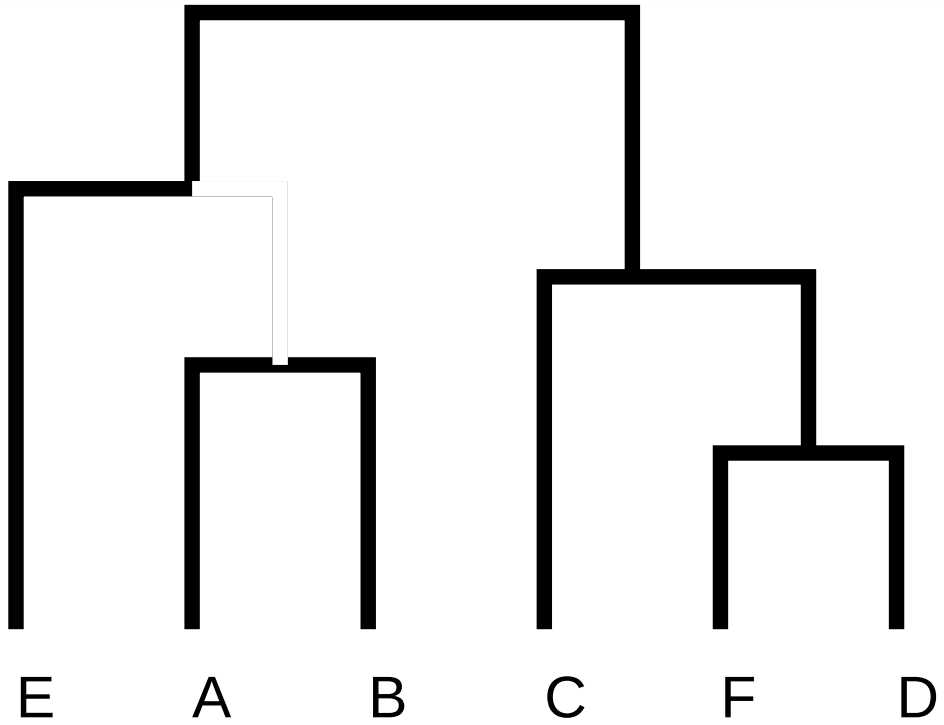
Canonical Representation - Splits

- AB|CDEF



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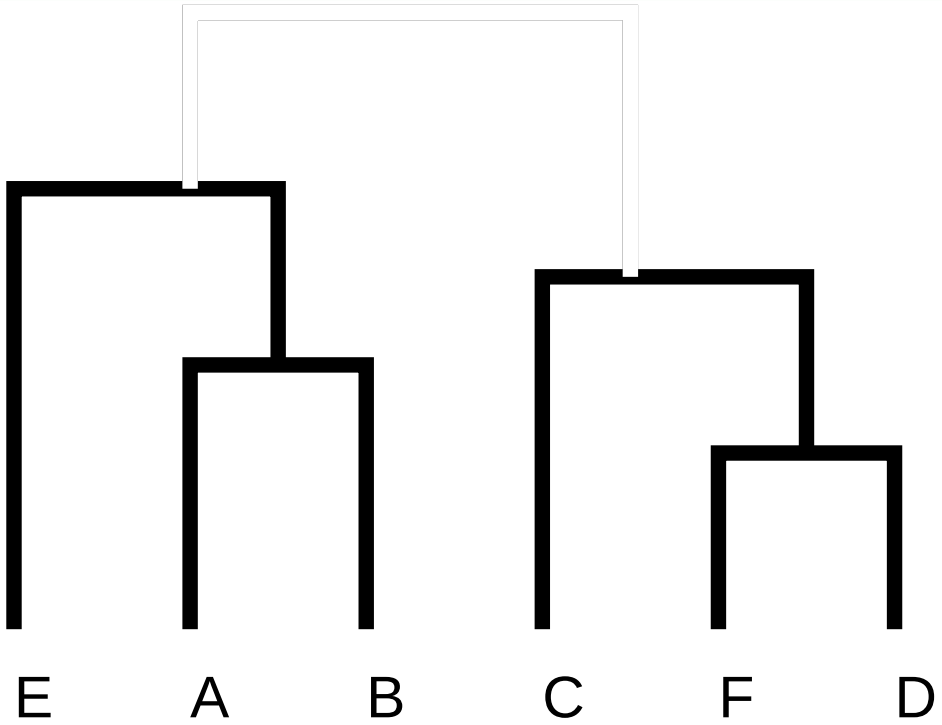
Canonical Representation - Splits

- AB|CDEF
- ABE|CDF



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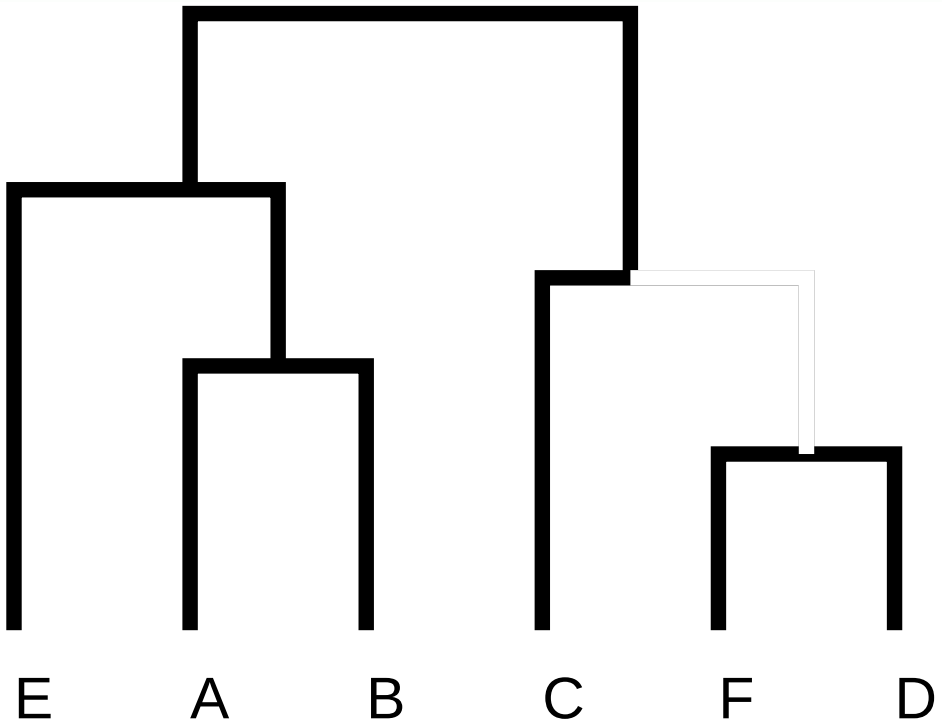
Canonical Representation - Splits

- AB|CDEF
- ABE|CDF
- DF|ABCE



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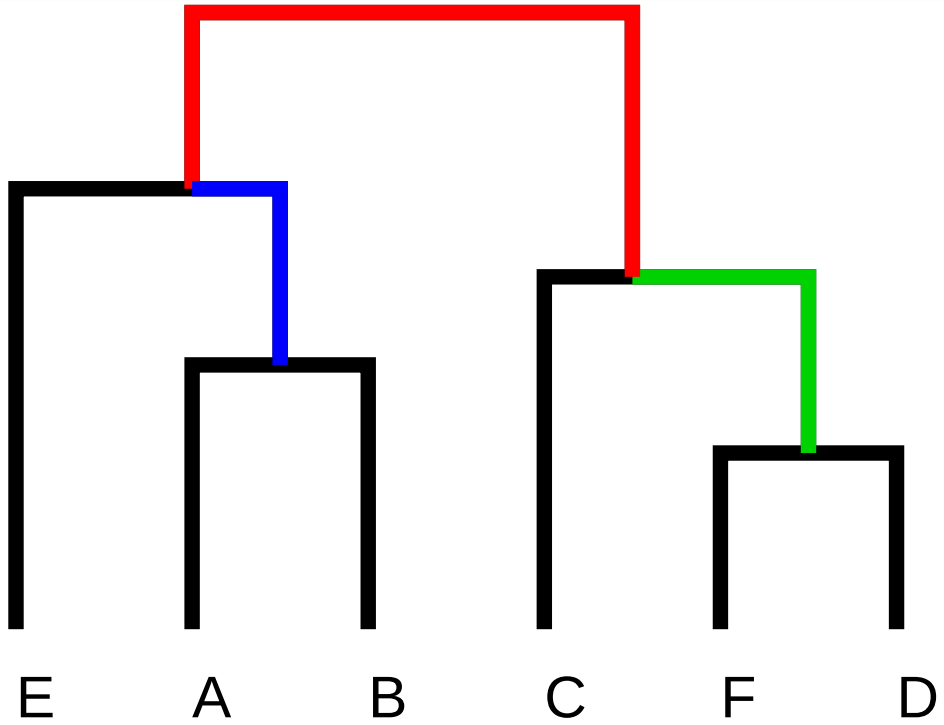

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The Phylogenetic Bootstrap

- So you've reconstructed a tree via MP, or ML...

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The Phylogenetic Bootstrap

- So you've reconstructed a tree via MP, or ML...
- and you'd like to asses how well your data supports your tree

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The Phylogenetic Bootstrap

- So you've reconstructed a tree via MP, or ML...
- and you'd like to asses how well your data supports your tree
- One answer: **the phylogenetic bootstrap**

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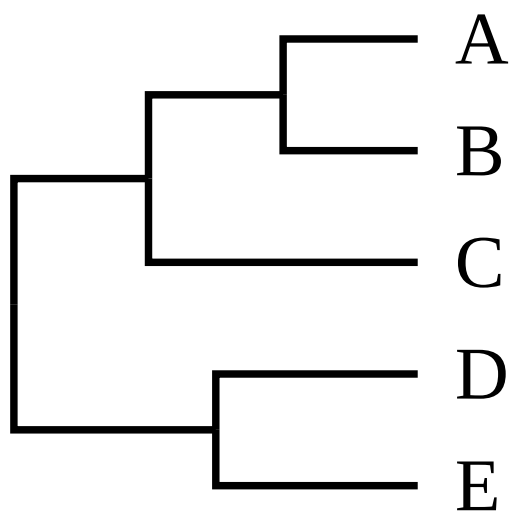
The Phylogenetic Bootstrap

Original Data	0	1	2	3
Species A	C	C	T	C
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C



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The Phylogenetic Bootstrap

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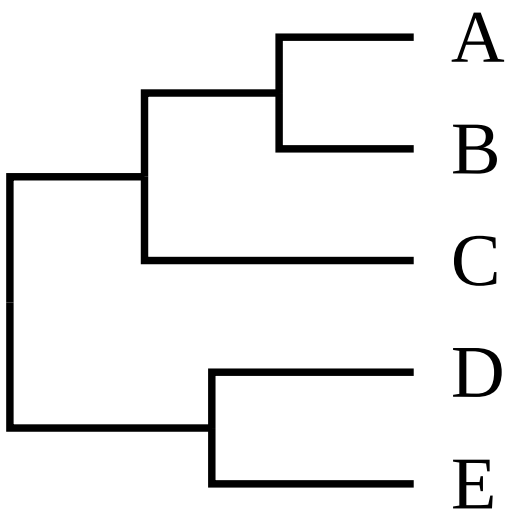
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C

Bootstrap 1	1	3	1	3
Species A	C	C	C	C
Species B	C	G	C	G
Species C	-	G	-	G
Species D	C	C	C	C
Species E	G	C	G	C



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The Phylogenetic Bootstrap

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Species A	C	C	T	C
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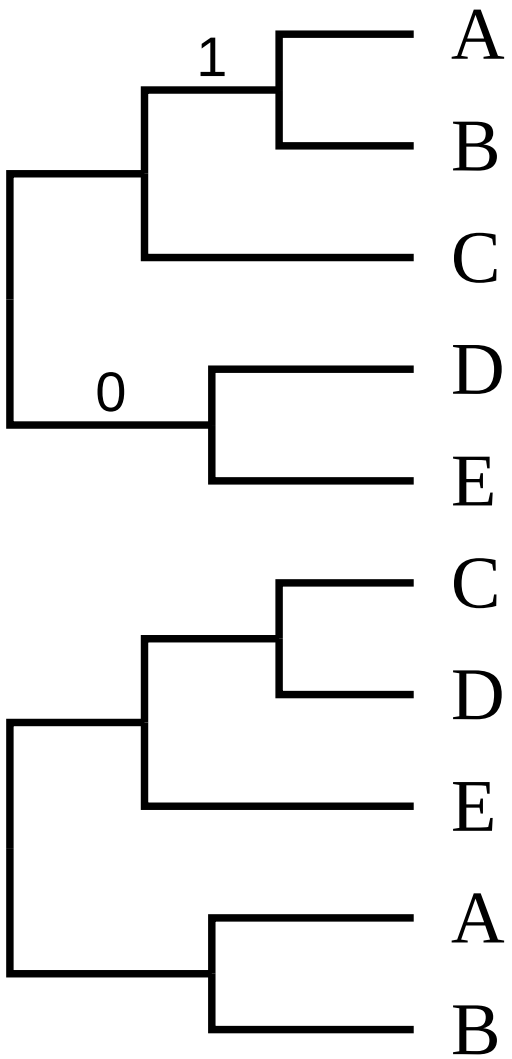
Bootstrap 1	1	3	1	3
Species A	C	C	C	C
Species B	C	G	C	G
Species C	-	G	-	G
Species D	C	C	C	C
Species E	G	C	G	C



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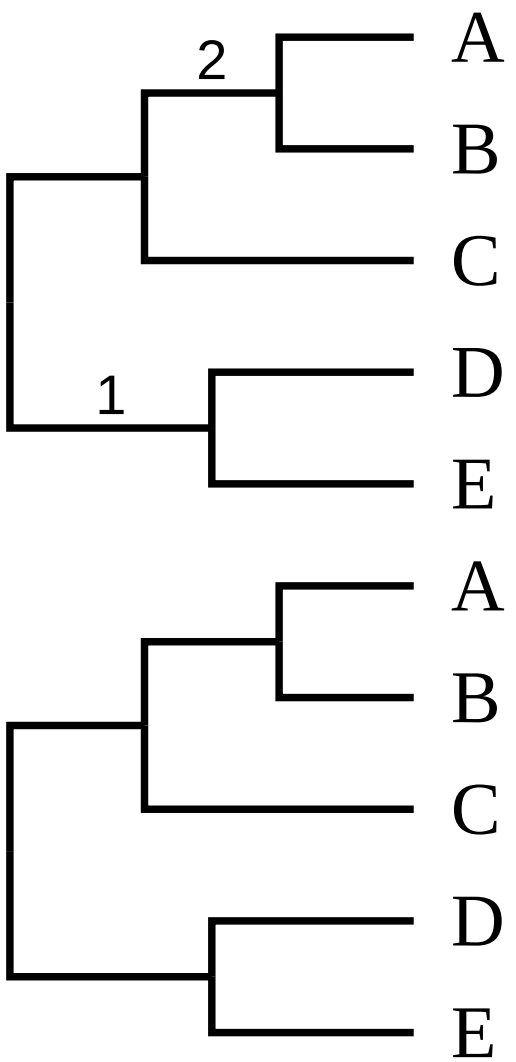
The Phylogenetic Bootstrap

Original Data	0	1	2	3
Species A	C	C	T	C
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C

Bootstrap 2	2	1	0	0
Species A	T	C	C	C
Species B	T	C	A	A
Species C	-	-	C	C
Species D	C	C	A	A
Species E	-	G	A	A



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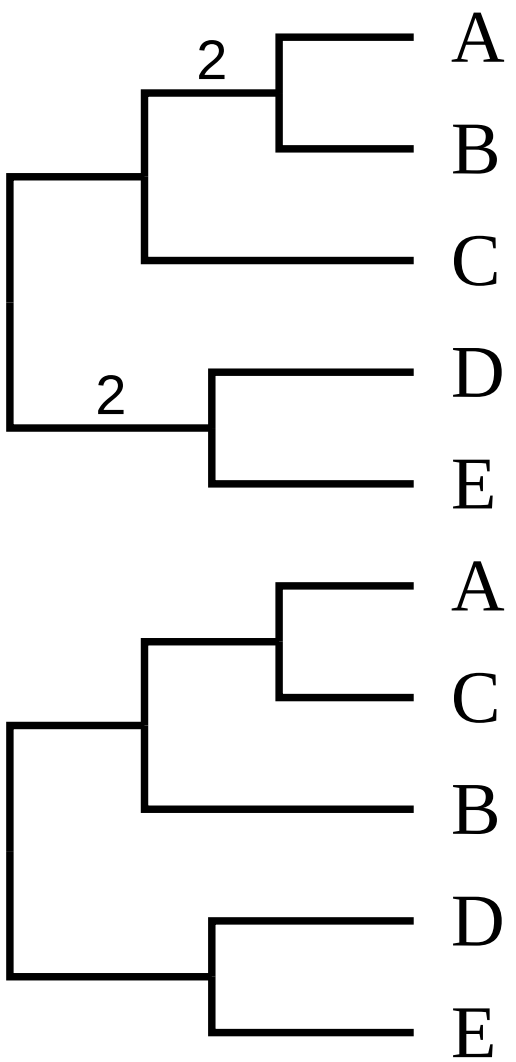
The Phylogenetic Bootstrap

Original Data	0	1	2	3
Species A	C	C	T	C
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C

Bootstrap 3	0	0	3	0
Species A	C	C	C	C
Species B	A	A	G	A
Species C	C	C	G	C
Species D	A	A	C	A
Species E	A	A	C	A



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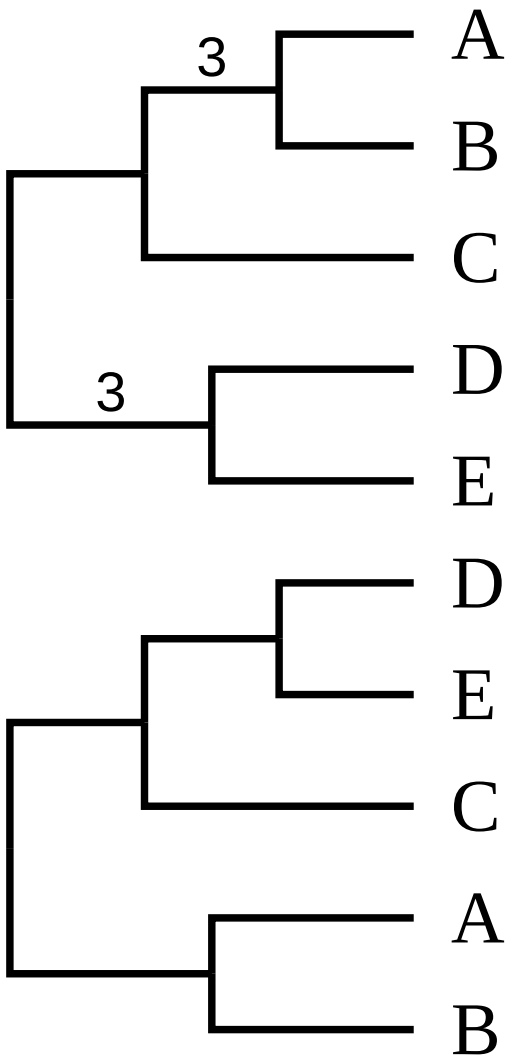
The Phylogenetic Bootstrap

Original Data	0	1	2	3
Species A	C	C	T	C
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C

Bootstrap 4	2	1	0	2
Species A	T	C	C	T
Species B	T	C	A	T
Species C	-	-	C	-
Species D	C	C	A	C
Species E	-	G	A	-



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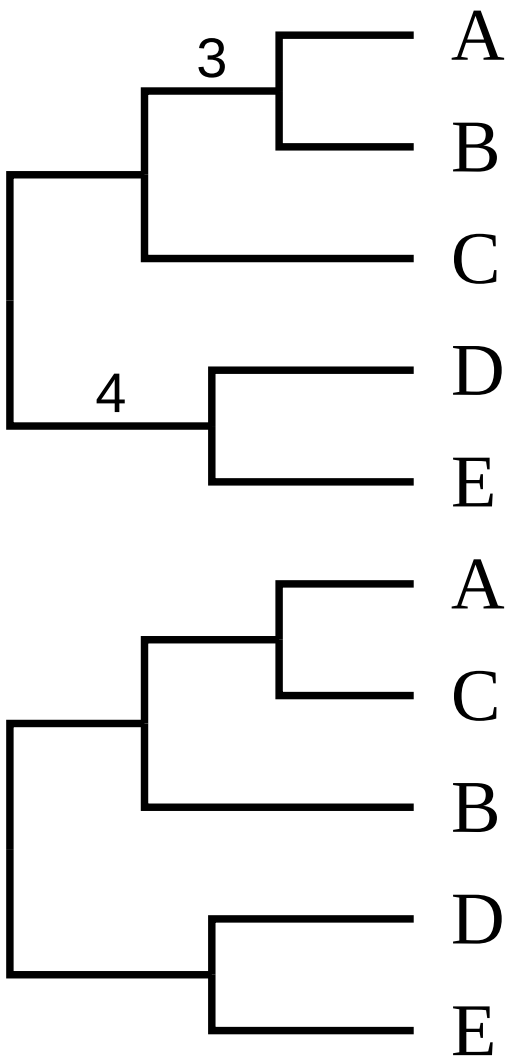
The Phylogenetic Bootstrap

Original Data	0	1	2	3
Species A	C	C	T	C
Species B	A	C	T	G
Species C	C	-	-	G
Species D	A	C	C	C
Species E	A	G	-	C

Bootstrap 5	2	2	1	3
Species A	T	T	C	C
Species B	T	T	C	G
Species C	-	-	-	G
Species D	C	C	C	C
Species E	-	-	G	C



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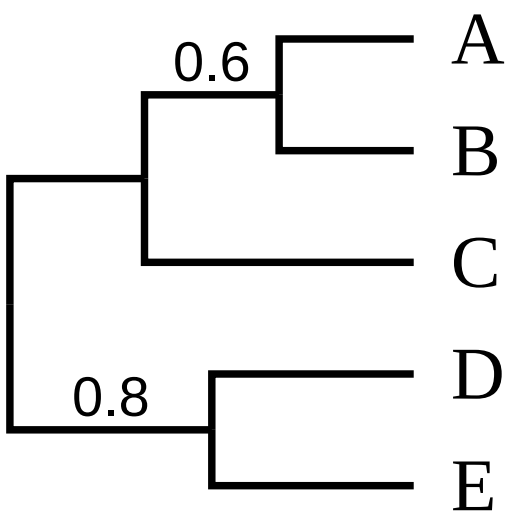

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The Phylogenetic Bootstrap

- Motivated by *resampling* technique from statistics
  - Used to assess the stability of simple summary statistics
- Computationally expensive – days to months

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Stopping Numbers

- Question we address: How many replicates?
- Theory exists for simpler estimators
  - In phylogeny, estimator is not only complex, but number of bipartitions grow
- State of the art in phylogeny: choose arbitrarily
- Hedges chooses *a priori* for a given level of significance
  - but ignores factors which greatly influence the estimator (the tree search algorithm) and hence the stability of BS replicates

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Our Framework

- Major goal: not be biased by current best tree
- Devise an *adaptive* criterion – to be used at run time
- Based on a *Permutation Test*
  - Typically used to reject that two samples arise from same distribution
  - We use to assess when a population subset sufficiently resembles full population

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Our Framework

- Bootstop()
  - With m replicates
    - Repeat $p = 100$ times
      - randomly split into two sets (of size $\frac{m}{2}$)
      - score similarity between two sets
    - Assess –
      - If $\frac{99}{100}$ scores beat threshold – DONE
      - Else – increment m (by, e.g. 50)

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      - Else – increment m (by, e.g. 50)
- Well less than 2^n possible
- Our two approaches differ in their defn. of similarity

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Scoring (Dis)similarity

- Frequency Criterion (FC)
  - Build vectors of edge support for the two subsets
  - Take Pearson's Correlation Coefficient between the two vectors

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  - Take Weighted RF distance between the two trees

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Scoring (Dis)similarity

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  - Build vectors of edge support for the two subsets
  - Take Pearson's Correlation Coefficient between the two vectors
- Weighted Criterion(WC)
  - Build (Majority Rules) Consensus trees for the two subsets
  - Take Weighted RF distance between the two trees
- What is the difference?
  - WC takes into account phylogenetically meaningful
  - WC is more conservative, but also sensitive

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Experimental Design

- For 17 diverse, real-world datasets with
  - 125 to 2,554 taxa
  - hudreds to tens of thousands of columns
- we did the following:

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  - Applied our criteria to generate stopping numbers

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- we did the following:
  - Generated $\geq 10,000$ BS replicates (serves $m \rightarrow \infty$)
  - Applied our criteria to generate stopping numbers
  - Assessed quality of our stopping numbers w.r.t. $\geq 10,000$ tree set

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Results

- Stopping numbers
  - FC: 150, 150, 150, 200, 200, 200, 200, 200, 250, 250, 250, 250, 250, 300, 300, 300, 450
  - WC: 50, 200, 300, 350, 400, 400, 400, 400, 450, 450, 500, 550, 600, 600, 650, 700, 1200
- Widely varying, dataset dependent (especially with WC)
- Correlation of support values always exceeds 99.5%
- WRF is smaller than the specified WC threshold value in all cases

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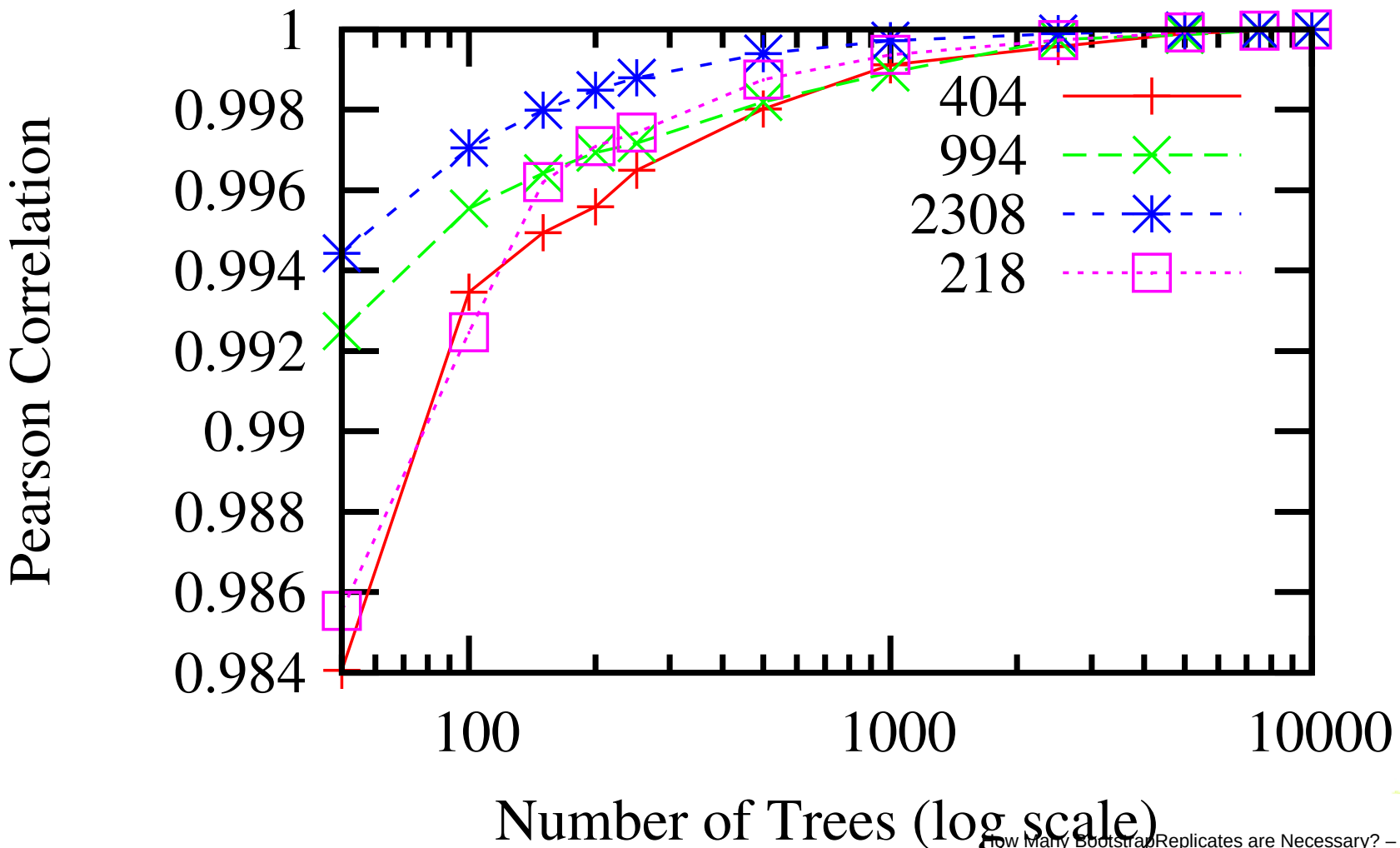
Results



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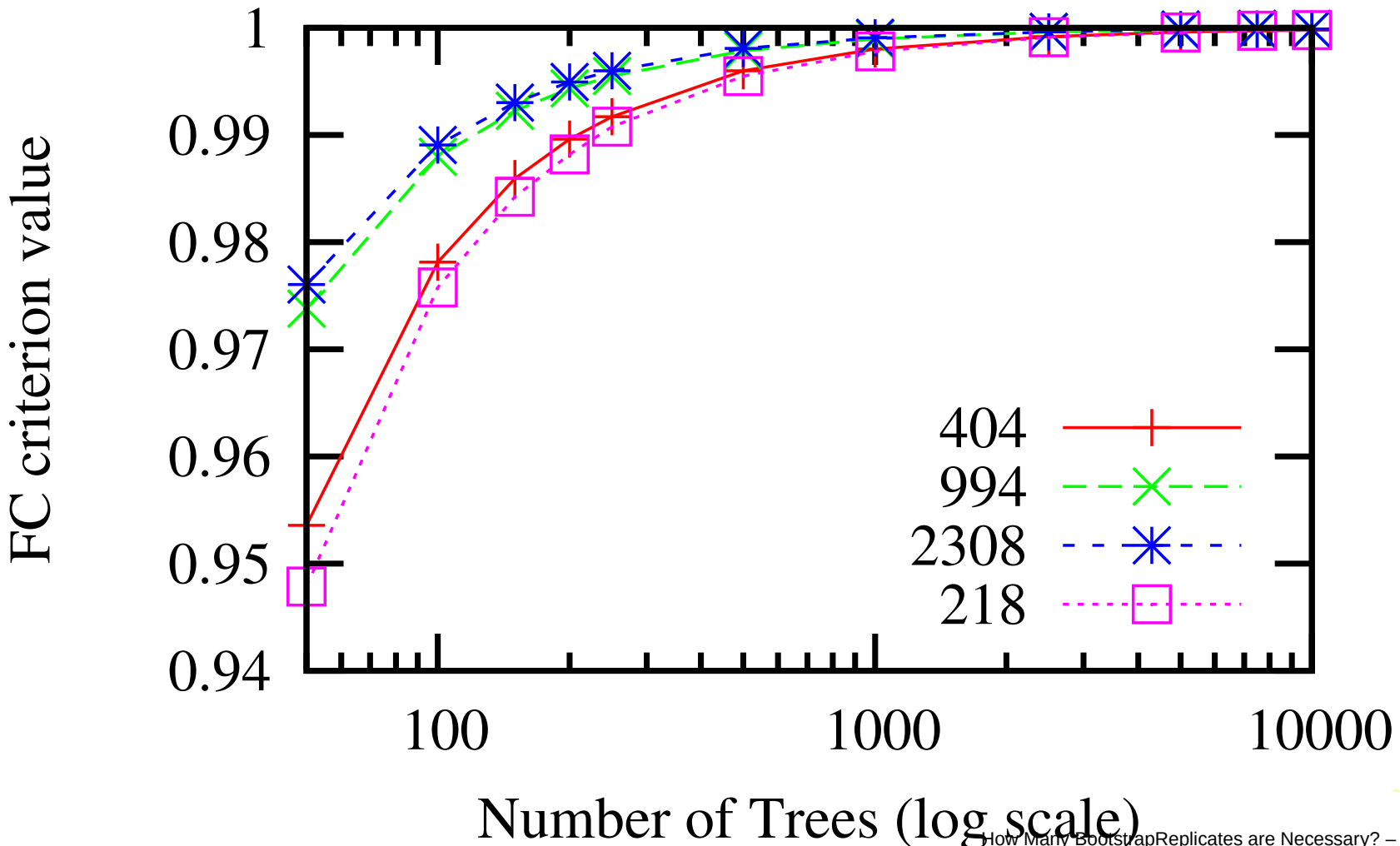
Results



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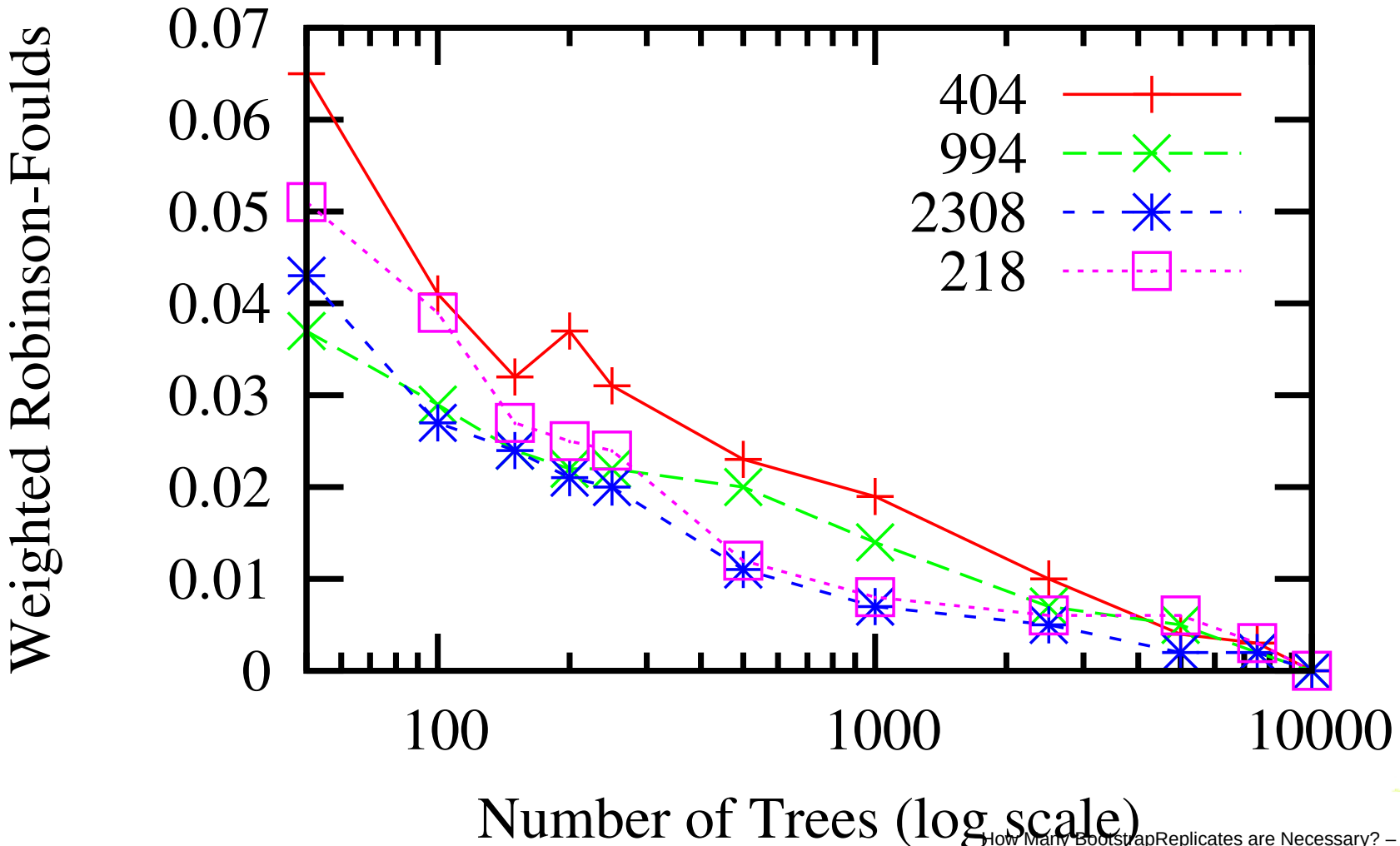
Results



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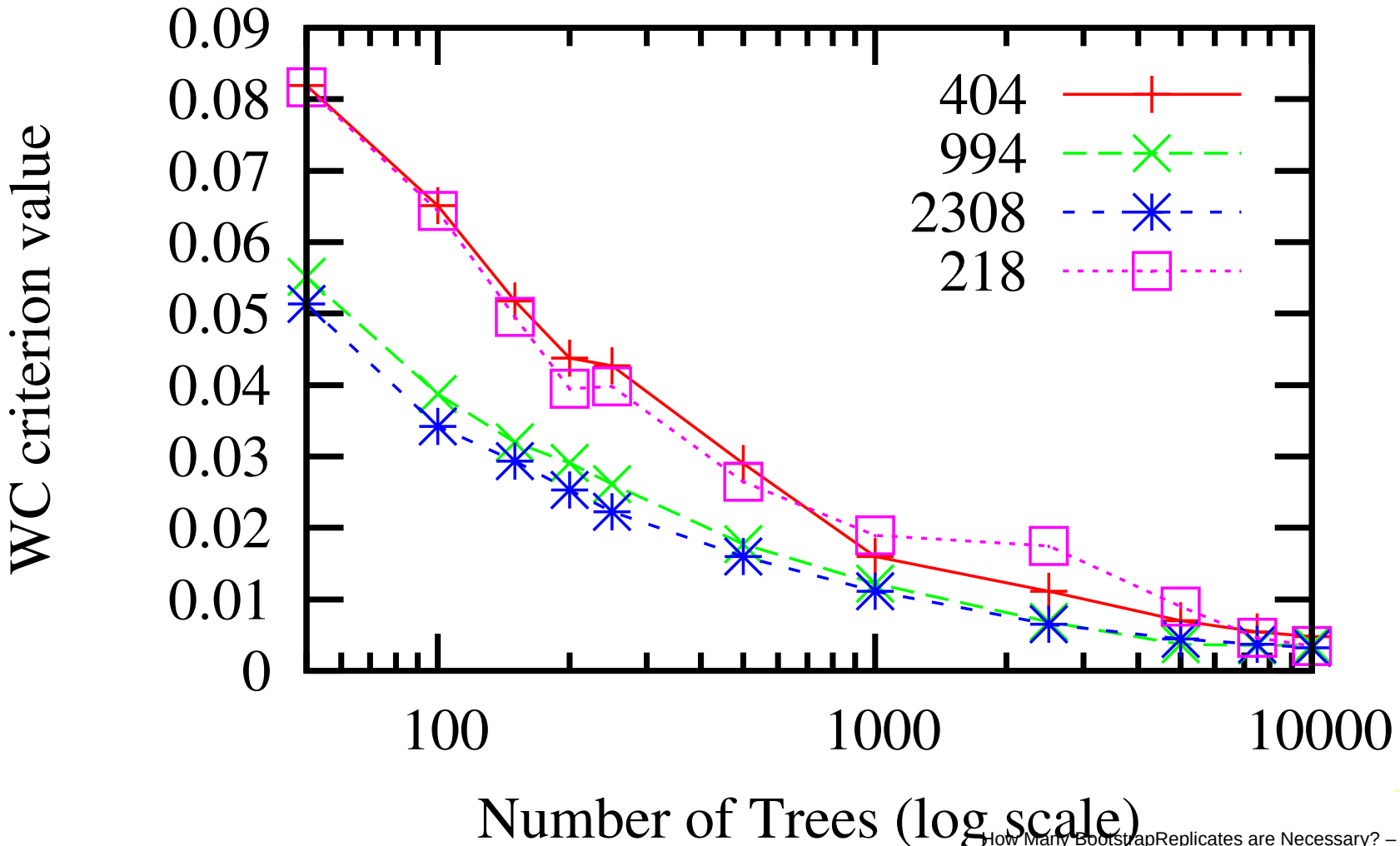

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Conclusion

- First large-scale empirical study of bootstrapping convergence
- Used biological datasets that cover a wide range of input alignment sizes and a broad variety of organisms and genes
- Developed and assessed two bootstopping criteria

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Conclusion

- Two criteria
  - Can be computed at run time
  - Do not rely on externally provided reference trees
  - Designed to capture stopping point providing sufficient accuracy for unambigoous biological interpretation of the resulting consensus trees or best-known ML trees with support values

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Conclusion

- WC criterion yields better performance and higher accuracy than FC
  - Correlates very well with the mean error of support values on the best-scoring tree.
- Advocate the use of WC over FC
  - Takes into account the BS support of “important” bipartitions which are subject to biological interpretation

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Conclusion

- Highly dataset dependent
- Only compute as many trees as needed
- Better methods (and ideally, some supporting theory) may exist

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That's All Folks

- Thanks
  - to my collaborators
  - to the organizers
  - for listening!
- Stopping Criteria are part of RAxML 7.1.0 alpha
  - http://wwwkramer.in.tum.de/exelixis/software.html
- Data for this study is also available
  - http://lcbb.epfl.ch/BS.tar.bz2