



National Institute of Allergy and Infectious Diseases

Phylogenetics and Sequence Analysis

Lecture 4
Tree Building 2



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Course Organization

- Building a clean sequence
- Collecting homologs
- Aligning your sequences
- **Building trees**
- Further Analysis



Tree building, so far

- Generate a distance tree
- Generate maximum parsimony tree(s)
- Calculate bootstrap support



What's next?

Statistical Methods for Calculating Trees

- Maximum Likelihood
- Bayesian Phylogenetics

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Optimality Criterion: Likelihood

Calculating likelihood

The Tree	The Data
	<p>S1: AAG</p> <p>S2: AAA</p> <p>S3: GGA</p> <p>S4: AGA</p>

$L(\text{Tree}) = \text{Prob}(\text{Data}|\text{Tree}) = \prod_i \text{Prob}(\text{Data}^{(i)}|\text{Tree})$

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Optimality Criterion: Likelihood

Calculating likelihood: Setting parameters

$L(\text{Tree}) = \text{Prob}(\text{Data}|\text{Tree}) = \prod_i \text{Prob}(\text{Data}^{(i)}|\text{Tree})$

What values do you use for the substitution model?

Run jModelTest (or ProtTest for protein MSAs)

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Optimality Criterion: Likelihood

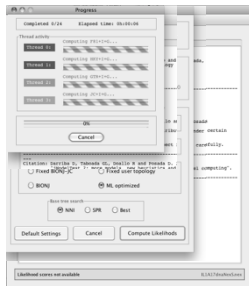
Calculating likelihood: jModelTest



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Optimality Criterion: Likelihood

Calculating likelihood: jModelTest



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Optimality Criterion: Likelihood

Calculating likelihood: jModelTest Results



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Optimality Criterion: Likelihood

Calculating likelihood: jModelTest Results

Substitution models
<http://www.molecularevolution.org/resources/models/nucleotide>

Optimality Criterion: Likelihood

Calculating likelihood: jModelTest Results

Substitution models
<http://www.molecularevolution.org/resources/models/nucleotide>

The Gamma Distribution

$k = 1, \theta = 2.0$
 $k = 2, \theta = 2.0$
 $k = 3, \theta = 2.0$
 $k = 5, \theta = 1.0$
 $k = 9, \theta = 0.5$

Mean = $k\theta$ Shape parameter = θ
 Coefficient of Variation = $1/\sqrt{\theta}$

Calculating likelihood: GARLi

Specifying input parameters:
garli.conf

```
[general]
dataname = /Users/wellenberg/Desktop/11A7AnfA.fasta
compression = none
streamname = stepwise
attachweightspercent = 50
edgefile = 11A7AnfA.cturef
randomseed = 1
maxlikelihood = 512
loggers = 10
memory = 300
refinestart = 1
BLANKLINES
outgroup = 1
BLANKLINES
collapsebranches = 1
bootstraps = 1
[models]
datagen = nucleotide
ratematrix = GTR
stepfractionsize = 0.1
ratemodel = gamma
numstates = 4
invariant sites = estimate
[options]
miniter = 4
haltiter = 1
likelihoodlimity = 0.5
haltoverdensity = 0
stopgap = 500000
stopline = 500000
Other Stuff down here
```

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www.nescent.org/wg_garli/GARLi_Configuration_Settings



Calculating likelihood: GARLi

Running the program

```
wellenberg@181841882 ~ % ls garli
wellenberg@181841882 /Applications/Garli-2.01-IntelOSX/bin $ ls -l
total 5168
-rwxr-xr-x 1 wellenberg NIMDomain Users 2638080 May 21 2012 Garli-2.01
wellenberg@181841882 /Applications/Garli-2.01-IntelOSX/bin $ ./garli
wellenberg@181841882 /Applications/Garli-2.01-IntelOSX/bin $ ./garli-2.01-garlilect4.conf
```



Calculating likelihood: GARLi

Running the program

```
====
SEARCHING FOR THE BEST MODEL
Model 1
Number of states = 4 (nucleotide data)
AC = 1.000, AG = 4.000, AT = 1.000, CG = 1.000, CT = 4.000, GT = 1.000
collapse of rate parameters estimated
AIC2C 0.2000 0.2000 0.2114
Base Heterogeneity Model:
4 discrete gamma distributed rate categories, alpha param estimated
with an invariant (unvariable) site category, proportion estimated
0.1135
substitution rate categories under this model:
rate proportion
0.000 0.1135
0.000 0.2100
0.000 0.2100
2.000 0.2100
Starting with seed=97142
creating likelihood stepwise addition starting tree...
number of base nodes =
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
optimizing parameters... improved 21.790 lnL
log-likelihood (lnL) = -1040.0000... improved 21.790 lnL
lnL = -1040.0000
initial lnL likelihood = -1041.0751
initial lnL likelihood branch lengths, alpha shape, prop_invar, rel rates, eq freq...
pass 1: 10.000 (branch= 2.000 cialen= 0.000 alpha= 0.700 freq= 2.000 rel rates= 10.000 pi=0.000
pass 2: 2.000 (branch= 1.700 cialen= 0.000 alpha= 0.800 freq= 0.000 rel rates= 0.000 pi=0.000
pass 3: 1.000 (branch= 0.000 cialen= 0.000 alpha= 0.000 freq= 0.000 rel rates= 0.000 pi=0.000
pass 4: 0.000 (branch= 0.000 cialen= 0.000 alpha= 0.000 freq= 0.000 rel rates= 0.000 pi=0.000
lnL after optimization = -1039.2222
gen current lnL precision last_tree_imp
0 -1039.2222 0.0000 0
100 -1035.0575 0.0000 0
```



Calculating likelihood: GARLi

Running the program

```

Terminal - bash -- 109x34
>>>Completed Search rep 4 (of 4)<<<
#####
Completed 4 replicate search(es) (of 4):
NOTE: Unless the following output indicates that search replicates found the
same topology, you should assume that they found different topologies.
Results:
Replicate 1 : -4225.8261 (best)
Replicate 2 : -4225.8261 (same topology as 1)
Replicate 3 : -4225.8262 (same topology as 1)
Replicate 4 : -4225.8261 (same topology as 1)

Parameter estimates across search replicates:
r1(C) r1(G) r1(T) r1(C) r1(T) r1(G) r1(T) p1(A) p1(C) p1(G) p1(T) alpha pinv
rep 1: 1.462 9.288 2.85 0.5893 0.434 1 0.244 0.288 0.258 0.218 0.478 0.134
rep 2: 1.462 9.288 2.851 0.5894 0.434 1 0.244 0.288 0.258 0.218 0.478 0.134
rep 3: 1.462 9.289 2.851 0.5894 0.435 1 0.244 0.288 0.258 0.218 0.478 0.134
rep 4: 1.461 9.287 2.85 0.5893 0.434 1 0.244 0.288 0.258 0.218 0.478 0.134

Treelengths:
TL
rep 1: 11.797
rep 2: 11.797
rep 3: 11.805
rep 4: 11.798

Saving final trees from all search reps to RRD_DNA_gtr64_1.best.all.tre
Saving final tree from best search rep (s1) to RRD_DNA_gtr64_1.best.tre
#####
w187@hpcgabin.welltenbergk5
    
```

Calculating likelihood: GARLi

The Tree

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Calculating likelihood: GARLi

Bootstrap results?

GARLi **does not** calculate a bootstrap consensus!
 You must use another piece of software for this.

Dendropy:sumtrees – a very efficient python package for summarizing collections of phylogenetic trees
 Dendropy – Freely distributed from:
<https://pythonhosted.org/DendroPy>
 sumtrees has also been implemented on the NIAID HPC

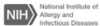

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Bayesian Analysis

Calculating the posterior probability of the evolutionary parameters



$$\Pr(\tau, \nu, \theta | \text{Data}) = \frac{\Pr(D | \tau, \nu, \theta) \times \Pr(\tau, \nu, \theta)}{\Pr(D)}$$

where:
 τ = tree topology
 ν = branch lengths
 θ = substitution parameters

What is Bayesian Analysis?

- Calculation of the probability of parameters (tree, substitution model) given the data (sequence alignment)
- $p(\theta | D) = (\text{Likelihood} \times \text{Prior}) / \text{probability of the data}$
- $p(\theta | D) = p(D | \theta)p(\theta) / p(D)$






Bayesian Analysis

Exploring the posterior probability distribution

Posterior probabilities of trees and parameters are approximated using Markov Chain Monte Carlo (MCMC) sampling

Markov Chain: A statement of the probability of moving from one state to another

What is MCMC?

Markov Chain Monte Carlo

Markov chain

One link in the chain

Monte Carlo

Choosing a link

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Bayesian Analysis

Markov Chain example: Jukes-Cantor

$\mu t = 0.25$

	A	C	G	T
A	0.5259	0.158	0.158	0.158
C	0.158	0.5259	0.158	0.158
G	0.158	0.158	0.5259	0.158
T	0.158	0.158	0.158	0.5259

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Bayesian Analysis

Exploring the posterior probability distribution

The **posterior probability** of a specific tree is the number of times the Markov Chain visits that tree

Posterior probability distribution is summarized by the clade probabilities.

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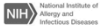
Bayesian Analysis

Using MrBayes

- Input format = Nexus
- Choose a substitution model (jModelTest)
- Check for convergence

Using BEAST



- Input format = XML (made using BEAUTi program)
- Choose a substitution model (jModelTest)
- Check for convergence (using Tracer program)



Bayesian Analysis

Running MrBayes: Model parameters



```
MrBayes> lset nst=6 rates=invgamma
MrBayes> showmodel
MrBayes> mcmc ngen=20000 samplefreq=100
printfreq=100 diagnfreq=100 burninfrac=0.25
```



Bayesian Analysis

Running MrBayes: Setting the Priors

- Generally, the default priors work well
- These are known as “uninformative” priors
- For implementing the Jukes-Cantor model, change statefreqpr to “fixed”

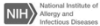



Bayesian Analysis

Running MrBayes: Setting the Priors

Amino acid substitution models



- Poisson - equal rates, equal state frequencies
- Blosum62
- Dayhoff
- Mtrev, Mtmamm - mitochondrial models
- mixed - Let MrBayes choose among the many fixed-rate models

Bayesian Analysis

Running MrBayes: General

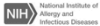

- burnin - initial portion of the run to discard
 - Generally, 25% of the samples
- samplefreq - how often to sample the Markov chain
 - More frequently for small analyses
 - Less frequently for low-complexity data
- printfreq - how often output is sent to the log file(s)

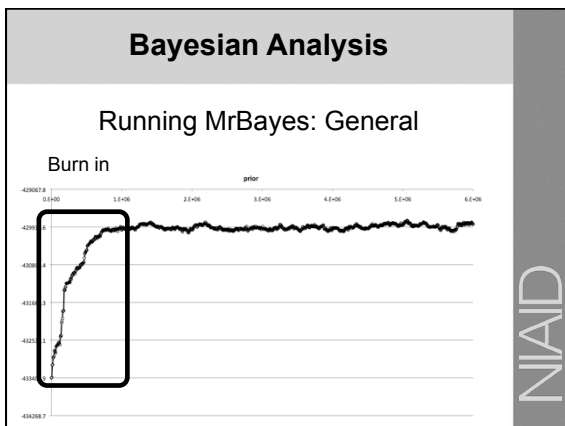



Bayesian Analysis

Running MrBayes: General

```
#NEXUS
begin mrbayes;
  set autoclose=yes nowarn=yes;
  execute /pathtodata/InputData.nex;
  lset nst=6 rates=invgamma;
  mcmc stoprule=yes stopval=0.009;
end;
```



Bayesian Analysis

Running MrBayes: Summarizing results

```
MrBayes> sump (burninfrac=0.25)
MrBayes> sumt (burninfrac=0.25)
```

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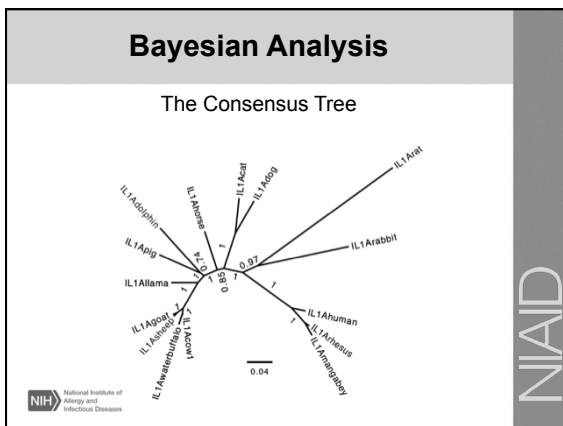
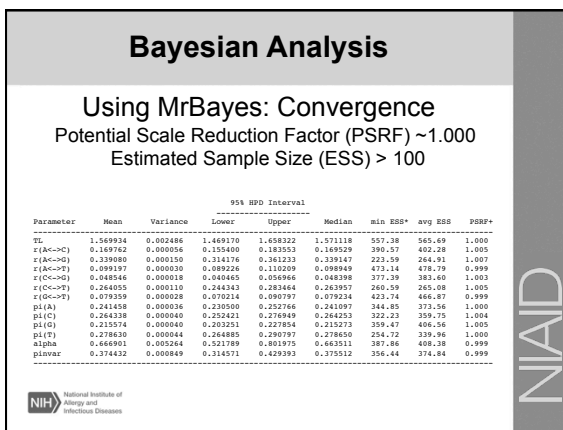
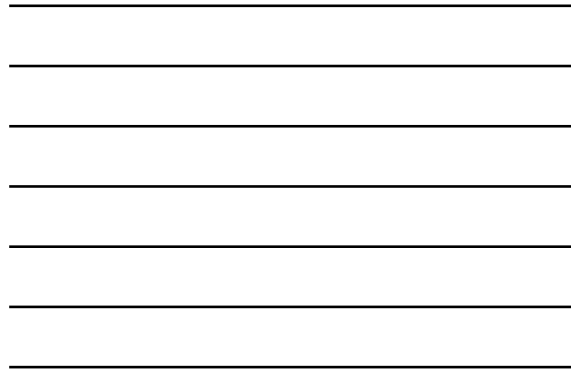
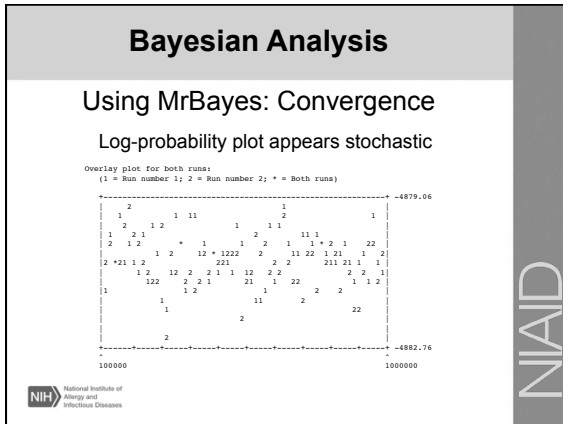
Bayesian Analysis

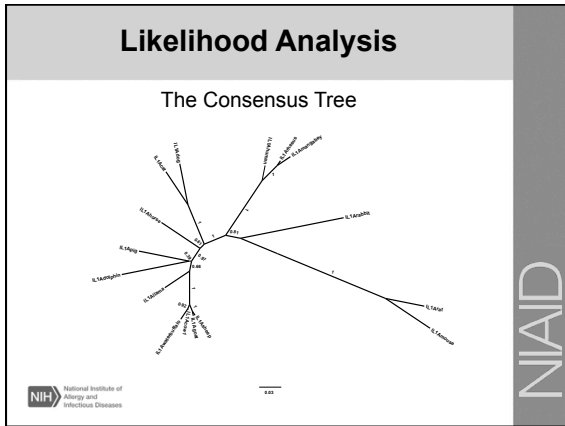
Using MrBayes: Convergence

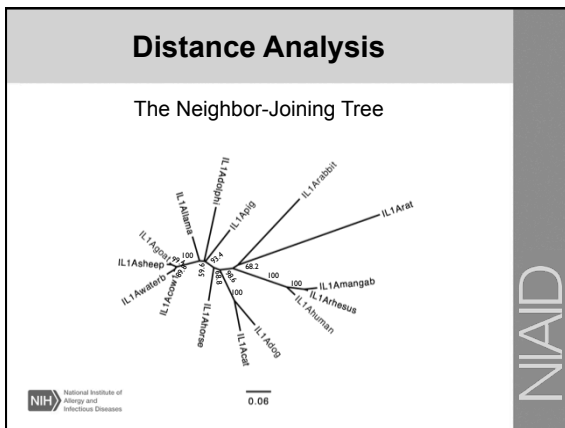
Chain results:

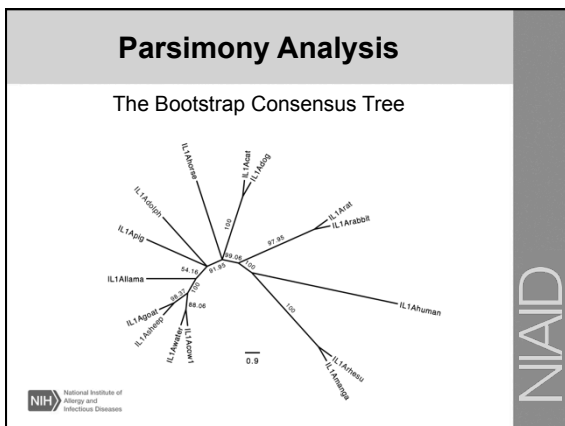
```
1 -- [-5762.003] (-5753.828) [...6 remote chains...]
1000 -- (-4832.654) (-4844.806) [...6 remote chains...] -- 0:16:39
Average standard deviation of split frequencies: 0.143471
2000 -- (-4748.109) (-4762.679) [...6 remote chains...] -- 0:24:57
***** [SNIP] *****
999000 -- (-4886.847) [-4876.966] [...6 remote chains...] -- 0:00:06
Average standard deviation of split frequencies: 0.002371
1000000 -- (-4885.621) [-4889.536] [...6 remote chains...] -- 0:00:00
Average standard deviation of split frequencies: 0.002413
```

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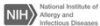




Tree Building, in conclusion



Where have we been? What have we done?

- How to generate trees using distance, parsimony, and likelihood
 - How to calculate bootstrap support
- Bayesian exploration of phylogeny posterior distribution
- Always use more than one tree generation algorithm
- Look for consensus and investigate disagreement




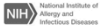
Visualizing Trees

- FigTree
- Dendroscope



REFERENCES

- * Inferring Phylogenies. J. Felsenstein. 2004
A good general reference written in Professor Felsenstein's unique style.
- * The Phylogenetic Handbook. Edited by P. Lemey, et al. 2009
A very thorough exploration of theory and practice.
- * Biological Sequence Analysis. R. Durbin, et al. 1998.
A good introduction to maximum likelihood and hidden Markov models (HMM).
- * Phylogenetic Trees Made Easy. B. Hall
1st, 2nd Edition - A PAUP and PHYLIP manual, cookbook style.
3rd Edition - A MEGA4 manual, among other things, cookbook style.
4th Edition - MEGA5 and MrBayes 3.2 manuals, cookbook style.

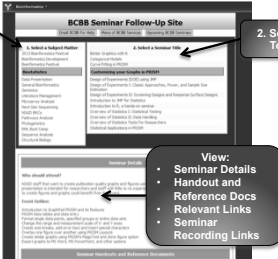


Seminar Follow-Up Site

▪ For access to past recordings, handouts, slides visit this site [from the NIH network: http://collab.niaid.nih.gov/sites/research/SIG/Bioinformatics/](http://collab.niaid.nih.gov/sites/research/SIG/Bioinformatics/)

1. Select a Subject Matter

2. Select a Topic




View:
 • Seminar Details
 • Handout and Reference Docs
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 • Seminar Recording Links

Recommended Browsers:

- IE for Windows.
- Safari for Mac (Firefox on a Mac is incompatible with NIH Authentication technology)

Login

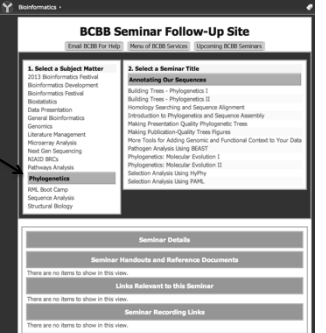
- If prompted to log in use "NIH" in front of your username



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This lecture series

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This lecture

This file

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