National Institute of Allergy and Infectious Diseases

Phylogenetics and Sequence Analysis

Lecture 7: Molecular Evolutionary Analysis of Pathogens Using BEAST

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Fall 2015

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Course Organization

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

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Lecture Organization

- What's so special about pathogens?
- What is BEAST?
- A short tour of Bayesian MCMC analysis
- An overview of the BEAST package
- **BEAST Analysis Demo**
- Odds and ends

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What's so special about pathogens?

- Short generation time
- Rapid evolution
- Genotypes - easy, phenotypes - hard
- Large populations
- Structured populations
- Rigorous temporal sampling of genotypes

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What is BEAST?

- **B**ayesian **E**volutionary **A**nalysis **S**ampling **T**rees
- A collection of programs for performing Bayesian MCMC analysis of molecular sequences
- Can incorporate sample time information
- Can perform a broad range of other evolutionary analyses using sequence data.

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What is Bayesian analysis?

- Calculation of the probability of parameters (tree, substitution model) given the data (sequence alignment)
- $p(\theta|D)$ = (Likelihood x prior)/probability of the data
- $p(\theta|D) = p(D|\theta)p(\theta)/p(D)$

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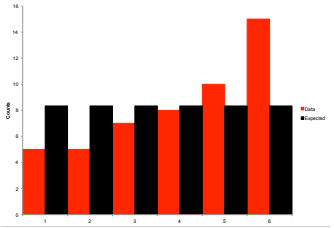
What is Bayesian analysis?

Likelihood that this die is unbiased?



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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

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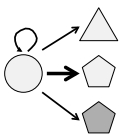
What is MCMC?

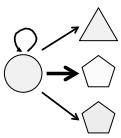
Markov Chain Monte Carlo

Markov chain



One link in the chain

Monte Carlo



Choosing a link

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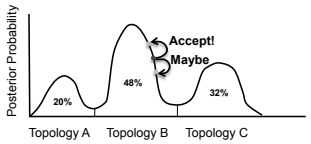
What is MCMC?

Markov Chain Monte Carlo: accept or reject?



$$\Pr(\text{accept}) = \min\left(1, \frac{\Pr(a)}{\Pr(A)} \times \frac{\Pr(X|a)}{\Pr(X|A)} \times \frac{\Pr(A|a)}{\Pr(a|A)}\right)$$

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What is BEAST?

The Programs:

- BEAUti - Creating XML input files
- BEAST - MCMC analysis of molecular sequences
- Tracer - Viewing MCMC output
- LogCombiner - Combining output files
- TreeAnnotator - Generate the consensus tree
- FigTree - Drawing a tree

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Different types of BEAST analyses

- Calculating a Bayesian coalescent phylogeny
- **Calculating a Time-Stamped Bayesian coalescent**
- Phylogeographic analysis (time and location data)
- Estimated population dynamics (Bayesian skyride and skygrid)
- Combined gene and species phylogeny estimate (*BEAST)

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Defining your analysis

- Prior knowledge of tree?
- Calibrating nodes?
- Substitution model?
- Effective population sizes?
- What priors to use?

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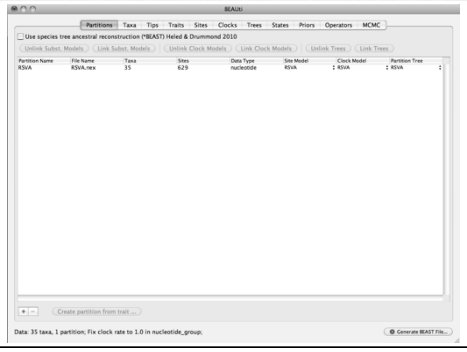
Setting up the analysis: BEAUTi



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Setting up the analysis: BEAUTi

- Incorporate sample times
- Substitution model parameters
- Strict or relaxed clock?
- Tree prior
- Substitution model priors
- Adjustments from previous runs (operators)
- Setting the chain

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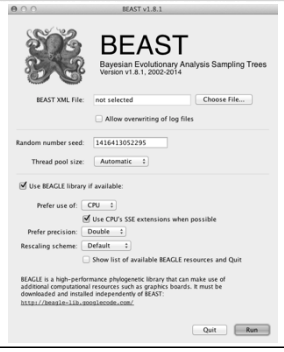
Running the analysis: BEAST

- Load your input file
- Use BEAGLE?
- That's it



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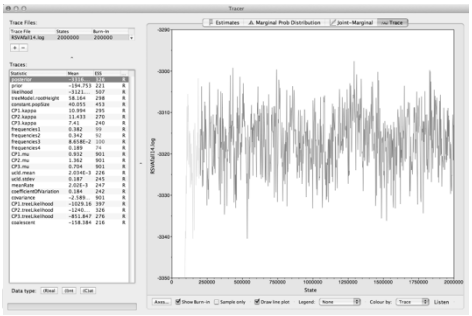
Evaluating the analysis: Tracer



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Evaluating the analysis: Tracer

- Check for convergence
- Viewing parameter estimates from multiple runs
- Extract parameter estimates and statistics

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Evaluating the analysis: Tracer

- What if my analysis didn't converge?
- Can I make multiple simultaneous runs?
  - Swarm on Biowulf

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Merging output files: LogCombiner

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Merging output files: LogCombiner

- Log files vs Tree files
- Selecting files
- Specifying burn-in (number of states)
- Specifying subsampling
- Specifying output file

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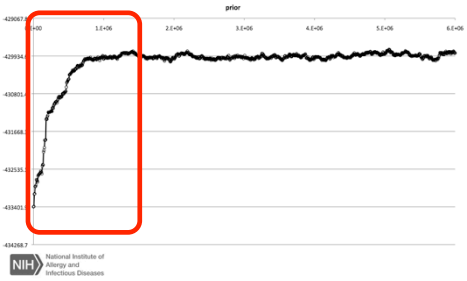
Merging output files: LogCombiner

- Burn in?



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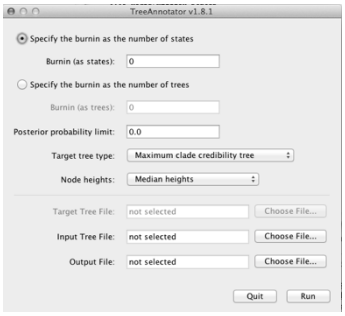
Calculating the tree: TreeAnnotator



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Calculating the tree: TreeAnnotator

- Burn in? Number of trees or the number of steps.
- Tree Type: MCC, Max sum of CC, or target
- Node heights: target, mean, or median
- Specify input and output files

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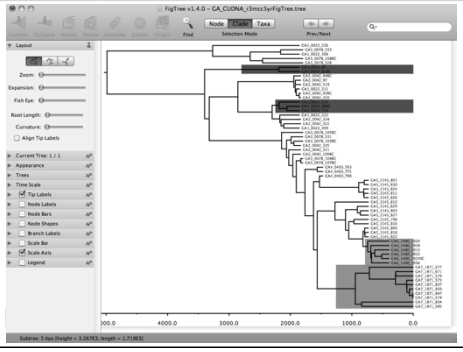
Drawing trees: FigTree



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Drawing trees: FigTree

- Specifying additional values (esp. posterior probabilities)
- Tree appearance
- Ordering branches
- Re-rooting
- Exporting graphics

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Running BEAST

DEMO

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Running BEAST: Demo

- Site Models
- Substitution Models
  - HKY - Unequal base frequencies and transition/transversion rate ratio
    - Must specify prior and initial estimates for transition/transversion rate ratio
  - GTR - Unequal base frequencies and each substitution has its own rate parameter
    - Must specify prior and initial estimates for each substitution rate (relative to C-T rate)

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Running BEAST: Demo

- Site Models
  - Substitution models
  - Site heterogeneity models
- Get estimates from the program jModelTest

```
Model selected: TVM+I+G
 -lnL   =   1676.8109
  K     =   9
  AIC   =   3371.6218

  Base frequencies:
    freqA =              0.2259
    freqC =              0.3199
    freqG =              0.2405
    freqT =              0.2137
  Substitution model:
    Rate matrix
    R(a) [A-C] =         0.2494
    R(b) [A-G] =         4.8655
    R(c) [A-T] =         0.7435
    R(d) [C-G] =         0.3907
    R(e) [C-T] =         4.8655
    R(f) [G-T] =         1.0000
  Among-site rate variation
    Proportion of invariable sites (I) =       0.6508
    Variable sites (G)
     Gamma distribution shape parameter =      0.5913
```

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Running BEAST: Demo

Site Models

- Site heterogeneity models
  - Gamma
    - Modeling rate of change using a discrete gamma distribution
  - Invariant
    - Percent of non-variable sites in the data

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Running BEAST: Demo

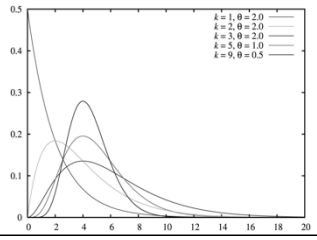
Site Models

- Site heterogeneity models
  - Gamma

Mean = kθ

Shape parameter = θ

Coefficient of Variation = 1/√θ



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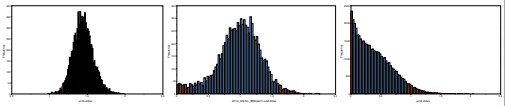
Setting up the analysis: Models

Testing Models and Priors

Does the relaxed clock fit the data?



Examining the ucld.stdev distribution

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Running BEAST

Testing Models and Priors

Path Sampling/Stepping Stone analysis

- Estimation of marginal likelihoods under different analysis parameters.
- Invoke on MCMC tab in BEAUti.
- Separate runs necessary for each changed parameter.
- Runs a complete MCMC analysis, then the *X* PS/SS iterations.

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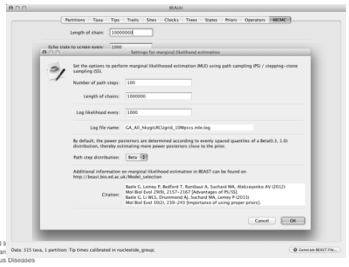
Running BEAST

Testing Models and Priors

Path Sampling/Stepping Stone analysis



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Running BEAST

Testing Models and Priors

Path Sampling/Stepping Stone analysis
log marginal likelihoods

	Path Sampling	Stepping Stone
HKY/strict clock	-4725.85	-4728.68
HKY+gi/strict	-4515.99	-4518.05
HKY+gi/LN relaxed	-4436.10	-4438.75
GTR/strict clock	-4746.62	-4749.14
GTR+gi/strict	-4526.87	-4529.05
GTR+gi/LN relaxed	-4548.39	-4551.22

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Running BEAST: swarm on Biowulf

- Requires a .swarm file
  - A text file containing

```
beast beastJob_1.xml > beastJob_1out.txt
sleep 2; beast beastJob_2.xml > beastJob_2out.txt
sleep 4; beast beastJob_3.xml > beastJob_3out.txt
sleep 6; beast beastJob_4.xml > beastJob_4out.txt
sleep 8; beast beastJob_5.xml > beastJob_5out.txt
```

- Run in command line

```
[username]$ swarm -f beastInput.swarm –module BEAST
```

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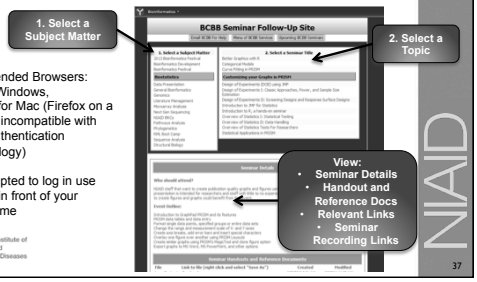
Seminar Follow-Up Site

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



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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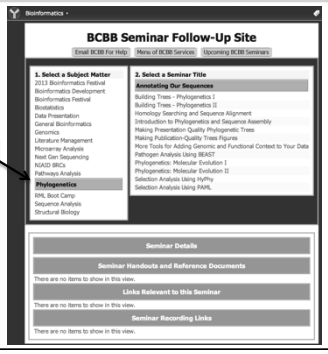
Retrieving Slides/Handouts



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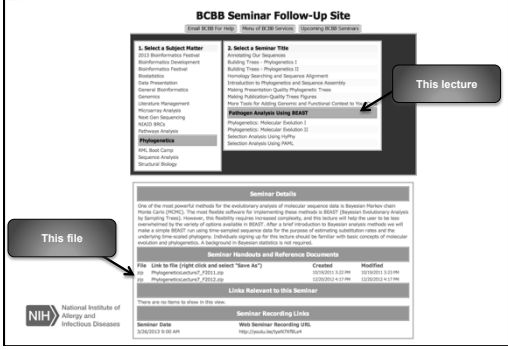
Retrieving Slides/Handouts



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