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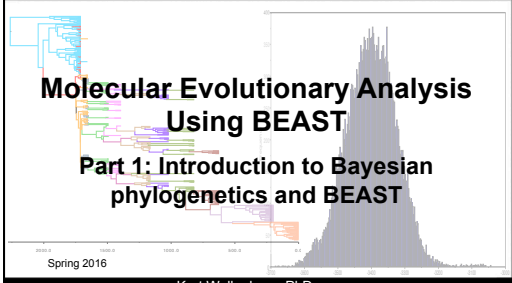

Molecular Evolutionary Analysis Using BEAST

Part 1: Introduction to Bayesian phylogenetics and BEAST

Spring 2016

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Bioinformatics and Computational Biosciences Branch
Office of Cyber Infrastructure and Computational Biology

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Course Organization

- Introduction to Bayesian phylogenetics
- Introduction to BEAST
- Building a Bayesian phylogeny
- Incorporating sample time in the phylogeny
- Estimating demographic parameters
- Estimating species trees from gene trees
- Estimating ancestral trait states (esp. geography)

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Lecture Organization

- Why Bayesian phylogenetics is well-suited to the analysis of pathogen molecular evolution
- A short tour of Bayesian MCMC analysis
- What is BEAST? An overview of the BEAST package
- BEAST Analysis Demo

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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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Why use Bayesian methods on pathogens?

- Coalescent approach is more appropriate
- Can incorporate temporal data
- Can incorporate geographical data
- Can incorporate host 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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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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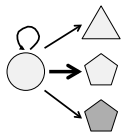
What is MCMC?

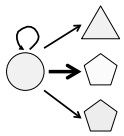
Markov Chain Monte Carlo

Markov chain



One link in the chain

Monte Carlo



Choosing a link

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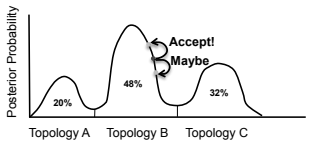
What is MCMC?

Markov Chain Monte Carlo: accept or reject?

Metropolis-Hastings algorithm



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

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What is BEAST?

- **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 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
- Estimated population dynamics (Bayesian skyline/ skyride/skygrid)
- Combined gene and species phylogeny estimate (*BEAST)
- Phylogeographic analysis (time and location data)

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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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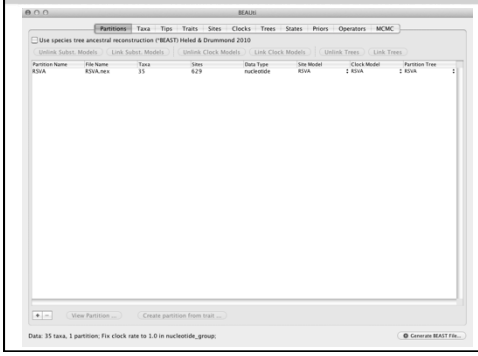
Setting up the analysis: BEAUTi



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Setting up the analysis: BEAUTi

- Import data – Nexus or fasta format
- Incorporate known structure - taxa
- Substitution model parameters
- Strict or relaxed clock?
- Tree prior
- Substitution model priors
- Adjustments from previous runs (operators)
- Setting the chain

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Setting up the analysis: BEAUTi

Import data: Nexus format

```
#NEXUS
[These are comments.
 They are ignored by the program.]

Begin data;
   dimensions ntax=5 nchar=15;
   format datatype=DNA gap=- missing=?;
   matrix
Bug1 ACCTGATTACGGGCA
Bug2 ACCCGAATACGGACA
Bug3 ACCTATTTACGCCCA
BugF ACTATATTACCGGCA
BugBX4W ACCAAA---CGGGCA
;
End;
```

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Setting up the analysis: BEAUTi

Import data: Fasta format

```
>Bug1
ACCTGATTACGGGCA
>Bug2
ACCCGAATACGGACA
>Bug3
ACCTATTTACGCCCA
>BugF
ACTATATTACCGGCA
>BugBX4W
ACCAAA---CGGGCA
```

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Setting up the analysis: 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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Setting up the analysis: Models

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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Setting up the analysis: Models

Estimating best-fit models and initial parameters: 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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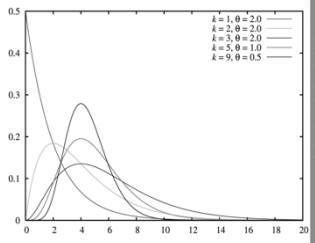
Setting up the analysis: Models

Site heterogeneity models:
The Gamma Distribution

Mean = kθ

Shape parameter = θ

Coefficient of Variation = 1/√θ



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Setting up the analysis: Models

Clock Models

- Strict clock – same rate for all branches
- Relaxed clock – independent rate among branches
  - Exponential or Lognormal distribution of rates
- For contemporaneous data setting a fixed mean substitution rate of 1.0 (uncheck "Estimate") results in node ages as substitutions per site (MrBayes branch lengths)

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Setting up the analysis: Models

Tree Prior

- Coalescent
  - constant size
  - exponential growth
  - GMRF Bayesian Skygrid
- Speciation
  - Yule process
  - Birth-Death
- Epidemiology

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Setting up the analysis: Models

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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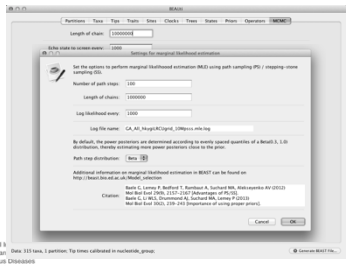
Setting up the analysis: Models

Testing Models and Priors

Path Sampling/Stepping Stone analysis



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Setting up the analysis: Models

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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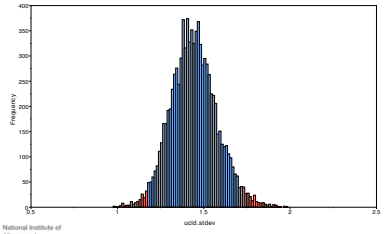
Setting up the analysis: Models

Testing Models and Priors

Does the relaxed clock fit the data?



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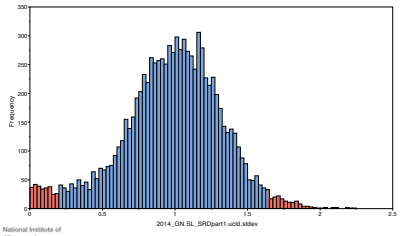
Setting up the analysis: Models

Testing Models and Priors

Does the relaxed clock fit the data?



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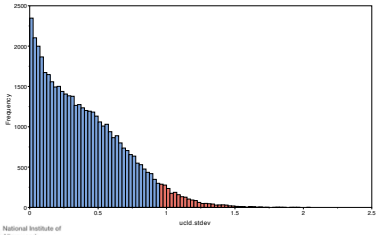
Setting up the analysis: Models

Testing Models and Priors

Does the relaxed clock fit the data?



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Running the analysis: BEAST

- Load your input file
- That's it

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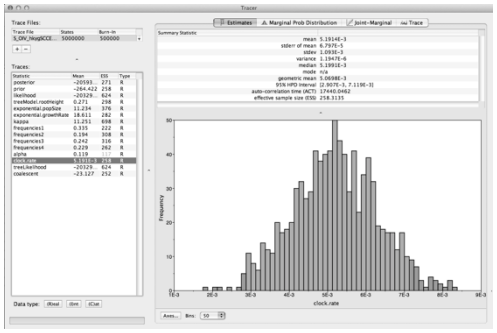
Evaluating the analysis: Tracer



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Evaluating the analysis: Tracer

- Check for convergence
- Evaluating ESS values
- Viewing behavior of parameter estimates
- Examining traces
- Extracting 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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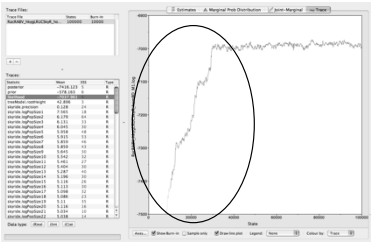
Evaluating the analysis: Tracer

What if my analysis didn't converge?



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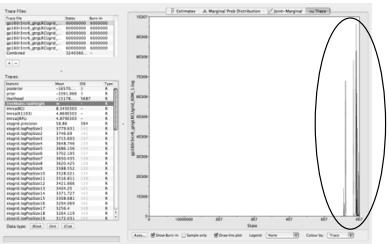
Evaluating the analysis: Tracer

What if my analysis didn't converge?



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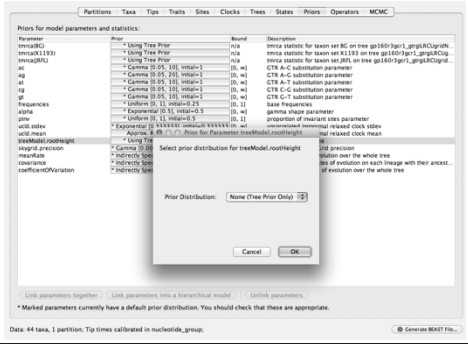
What if my analysis didn't converge?



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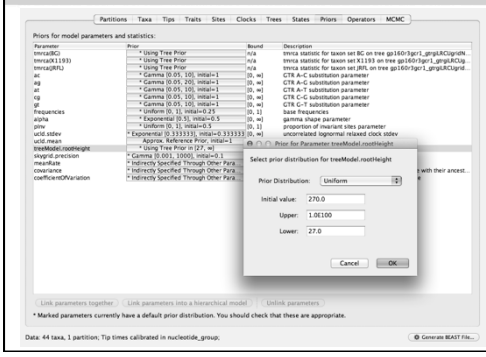
What if my analysis didn't converge?



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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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Merging output files: LogCombiner

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Merging output files: LogCombiner

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

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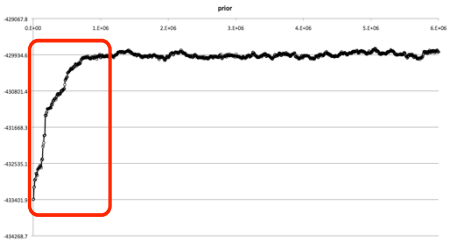
Merging output files: LogCombiner

- Burn in?



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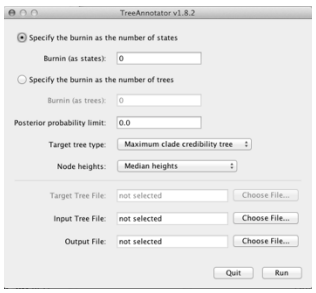
Calculating the tree: TreeAnnotator



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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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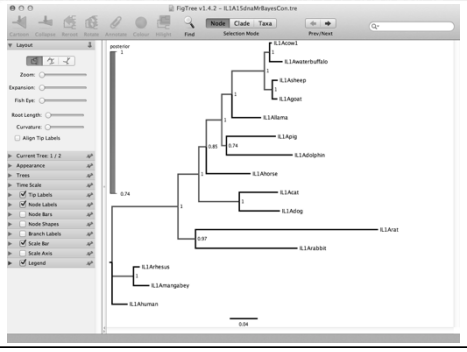
Drawing trees: FigTree



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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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Setting up the analysis: BEAUTi

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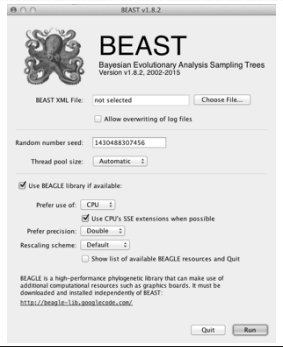
Running the analysis: BEAST



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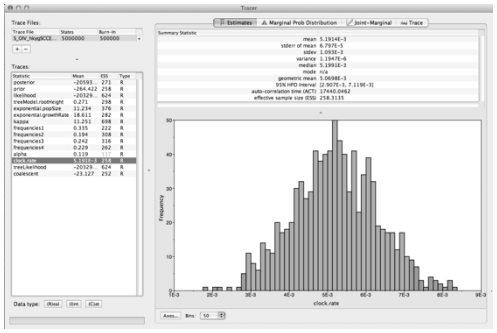
Evaluating the analysis: Tracer



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Merging output files: LogCombiner

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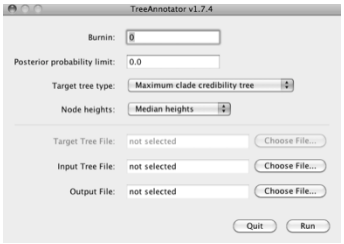
Calculating the tree: TreeAnnotator



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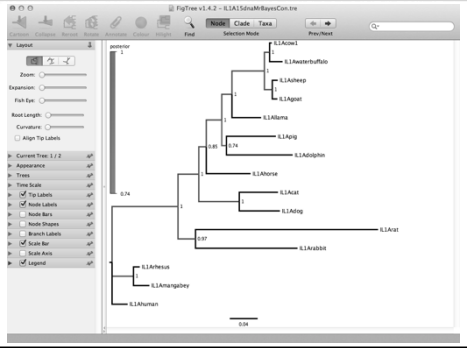
Drawing trees: FigTree



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BEAST2

- Still … **B**ayesian **E**volutionary **A**nalysis **S**ampling **T**rees
- Modular rewrite of the BEAST software
- Various evolutionary analyses performed through a system of independent software packages.
- Access software, documentation, etc., through the website beast2.org
- Still a few bugs in the system…

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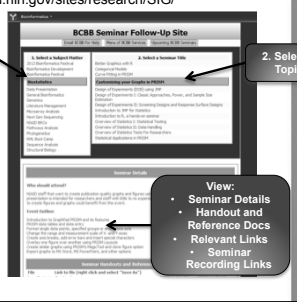
Seminar Follow-Up Site

- 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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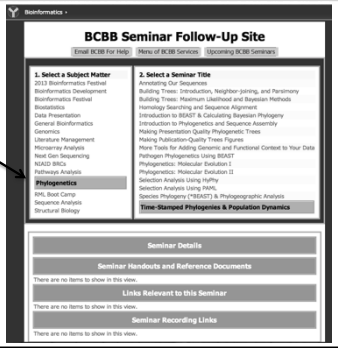
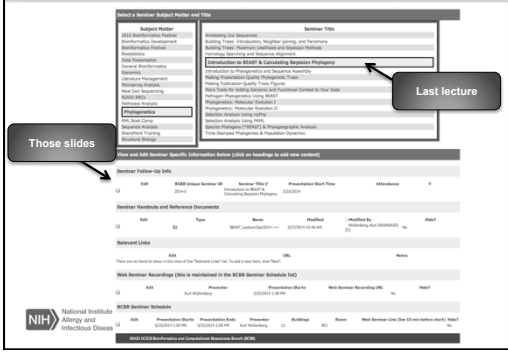
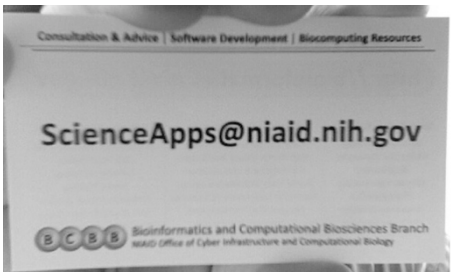
Retrieving Slides/Handouts



Retrieving Slides/Handouts



Questions?



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Next?

- Time-structured phylogenies
- Estimating demographic parameters
  - GMRF skyride analysis

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