# Revealing the evolutionary dynamics of influenza

**Summary:** This tutorial provides a step-by-step explanation on how to reconstruct the evolutionary dynamics of influenza based on a set of virus sequences w have been isolated at different points in time ('heterochronous' data) using BEAST. We will focus on influenza A virus evolution, in particular on the emergence c swine-origin pandemic influenza A (H1N1) virus in 2009 (H1N1pdm) and on the seasonal dynamics of H3N2 in the New York State. The H1N1pdm data set is a subset of an analyzed set genomes in a study that provides insights into the origins and evolutionary genomics of this pandemic (Smith et al., 2009). The H3N2 data is a subset of a comprehensive data set spanning several epidemic seasons in the New York state, which has been used to unravel the genomic and epidemiological dynamics of this virus (Rambaut et al., 2008). In the first exercise, the aim is to obtain an estimate of the date of the origin of the epidemic and estimate of the H1N1pdm epidemic growth and basic reproductive number. In the second exercise, we will examine how H3N2 diversity fluctuates through time

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# Introduction

The first step will be to convert a NEXUS file with a DATA or CHARACTERS block into a BEAST XML input file. This is done using the program BEAUti (this stands for Bayesian Evolutionary Analysis Utility). This is a us friendly program for setting the evolutionary model and options for the MCMC analysis. The second step is to actually run BEAST using the input file that contains the data, model and settings. The final step is to exp the output of BEAST in order to diagnose problems and to summarize the results.

To undertake this tutorial, you will need to download three software packages in a format that is compatible with your computer system (all three are available for Mac OS X, Windows and Linux/UNIX operating syster

Ast - this package contains the BEAST (beast) program, BEAUti (beauti) and a couple of utility programs. At the time of writing, the current version is v1.10.4. BEAST releases are available for download (beast) monor beast-dev/beast-momo/releases C.

# EXERCISE 1: The swine-origin influenza A outbreak

📩 The data file is called 'H1N1pdm\_2009. nex' and can be downloaded from here (/tutorials/workshop influenza\_phylodynamics/files/H1N1pdm\_2009.nex).

## Running BEAUti

other files/folders linked further in the tutorial.

Bun BEAUti (beauti) by double clicking on its icon. BEAUti is an interactive graphical application for designing your analysis and generating the control file (a BEAST XML file) which BEAST will use to run the analysis.

To load a NEXUS format alignment, simply select the Import NEXUS... option from the File menu.

Select the file called H1N1pdm\_2009.nex. This file contains an alignment of 50 genomes (all 8 genomic segments concatinated), 13109 nucleotides in length. Once loaded, the new data will be listed under Partition shown in the figure:

	Partitions	Таха	Tips	Traits	Sites	Clocks	Trees	States	Priors	Operators	MCMC		
Unlink Subst. N	Nodels Link	Subst. M	lodels	Un	link Clo	ck Models	Lin	k Clock M	odels	Unlink T	rees	Link Trees	
Partition Name	File Name	Taxa		Site	s	Dat	а Туре	Site	Model	Clock M	odel	Partition Tree	
H1N1pdm_2009	H1N1pdm_2009	50		131	09	nuc	leotide	defa	ault	‡ default		‡ default	;

### Setting the tip dates

To undertake a phylodynamic analysis we need to specify the dates that the individual viruses were collected. In this case, the sequences were sampled from the H1N1 2009 pandemic between March and May 2009 set these dates switch to the Tips panel using the tabs at the top of the window.

Select the box labelled Use tip dates. The actual sampling time in fractional years is encoded in the name of each taxon and we can use the Parse Dates button at the top of the panel to extract these. For th H1N1pdm\_2009 sequences you can keep the default Defined just by its order and select last from the drop-down menu for the order and press 0K. The dates will appear in the appropriate column of 1 main window. You can then check these and edit them manually as required.

🗸 Use tip dates			
Parse Dates Import Dates	Set Dates	Clear Dates Set Uncertainty Dates	specified as Years 🗘
Specify origin date:		unable to parse date	
Name	Date	Uncertainty	Height
A/Beijing/01/2009_135_2009.37	2009.37	0.0	0.0330000000012915
A/Beijing/3/2009_140_2009.384	2009.384	0.0	0.0190000000005457
A/California/06/2009_106_2009.29	2009.29	0.0	0.113000000005639
A/California/14/2009_115_2009.3	2009.315	0.0	0.0879999999996544
A/Canada-NS/RV1535/2009_114	2009.312	0.0	0.091000000012187
A/Canada-ON/RV1527/2009_114	2009.312	0.0	0.0910000000012187
A/England/207/2009_120_2009.3	2009.329	0.0	0.074000000006912
A/Fujian/1/2009_142_2009.389	2009.389	0.0	0.01400000000123691
A/Guangdong/1/2009_138_2009	2009.378	0.0	0.0250000000009095
A/Guangdong/2/2009_147_2009	2009.403	0.0	0.0
A/Hong_Kong/01/2009_120_2009	2009.329	0.0	0.074000000006912
A/Hyogo/1/2009_137_2009.375	2009.375	0.0	0.0280000000002001
A/Indiana/09/2009_112_2009.307	2009.307	0.0	0.096000000000364
A/Korea/01/2009_122_2009.334	2009.334	0.0	0.0689999999999998
A/Mexico/4486/2009_104_2009	2009.285	0.0	0.1179999999993815
A/Mexico/4604/2009_109_2009	2009.299	0.0	0.1040000000004184
A/Mexico/47N/2009_115_2009.315	2009.315	0.0	0.0879999999996544
A/Mexico/InDRE4487/2009_104_2	2009.285	0.0	0.1179999999993815
A/Michigan/02/2009_116_2009.318	2009.318	0.0	0.085000000003638
A/Moscow/01/2009 141 2009.386	2009.386	0.0	0.017000000005275
A/Narita/1/2009 128 2009.351	2009.351	0.0	0.0519999999990723
A/Netherlands/602/2009_119_20	2009.326	0.0	0.076999999999818
A/New York/10/2009 115 2009	2009.315	0.0	0.0879999999996544
A/New York/1682/2009 117 200	2009.321	0.0	0.0820000000010732
A/New York/23/2009 114 2009	2009.312	0.0	0.0910000000012187
A/New York/3099/2009 119 200	2009.326	0.0	0.0769999999999818
A/New York/3168/2009 116 200	2009.318	0.0	0.085000000003638
A/New York/3172/2009 117 200	2009.321	0.0	0.0820000000010732
A/New_York/3173/2009_118_200	2009.323	0.0	0.0799999999992724
Tip date sampling: Off		♦ Apply to taxon set: All taxa ♦	

Tip: There are many other options for reading and parsing tip dates in different formats. See this page for a more detailed description of these options. (tip\_dates.html)

#### Setting the substitution model

The next thing to do is to click on the Sites tab at the top of the main window to specify the evolutionary model settings for BEAST:

•						BEAU	ti					
	Partitions	Таха	Tips	Traits	Sites	Clocks	Trees	States	Priors	Operator	s MCMC	
Substitution Model	Nucleotid	e Substitu	tion Mode	el – default								
default			S	ubstituti	on Model:	НКҮ						
				Base fre	auencies	Estin	nated ᅌ					
			Site Het	erogene	tv Model:	Gam	ma					
		Num	ber of G	iamma C	ategories:	4	•					
		Parti	tion into	codon	JUSICIONS:	Link/I	Jolink para	meters:		<b>~</b>		
						I U	nlink sub	stitution	rate parar	neters acros	ss codon positions	
						⊡ U	nlink rate	heteroge	neity moo	iel across c	odon positions	
	^					⊻ Ui	nlink bas	e frequen	cies acros	s codon po	sitions	
						Use	Yang96	model				
						Use	SRD06 n	nodel				
Clone Settings												
a: 50 taxa, 1 partition											🕸 Gene	erate BEAST Fil

For this tutorial, keep the default HKY model, the default Estimated base frequencies and select Gamma as Site Heterogeneity Model (with 4 discrete categories) before proceeding to the Clocks tab.

#### Setting the molecular clock model

The Clock panel options allows us to choose between a strict and a relaxed (uncorrelated lognormal or uncorrelated exponential) clock. Because of the low diversity data we analyze here, a relaxed clock would prc be over-parameterization. Hence, we keep a strict clock setting.

Partitions Taxa Tips	Traits Sites Clocks	Trees States Priors Operato	rs MCMC
Clock Model Clock Model - default			
default	Clock Type:	Strict clock	

#### Now move on to the Trees panel.

#### Setting the tree prior

This panel contains settings about the tree. Firstly the starting tree is specified to be 'randomly generated'. The other main setting here is to specify the 'Tree prior' which describes how the population size is expected change over time for coalescent models. The default tree prior is set to a constant size coalescent prior. The range of different tree priors (coalescent and other models) are described on this page (tree\_priors).

To estimate the epidemic growth rate, we will change this demographic model to an exponential growth coalescent prior, which is intuitively appealing for viral outbreaks. Switch the option for Tree Prior to Coalescent: Exponential Growth :

Trees	Tree prior shared by all tree models											
default	Tree Prior: Coalescent: Exponential Growth											
	Parameterization for growth: Growth Rate											
	Citation: Griffiths RC, Tavare S (1994) Phil Trans R Soc Lond B Biol Sci 344, 403–410 [Parametric Coalescent].											
	Tree Model - default											
	• Random starting tree											
	UPGMA starting tree											
	O User-specified starting tree											
	Select user-specified tree: no tree loaded 🗘											
	Export format for tree: Newick 0											
	Import user-specified starting trees from NEXUS format data files using the "Import Data" menu option. Trees must be rooted and strictly bifurcating (binary).											

#### Setting up the priors

Now switch to the **Priors** tab. This panel has a table showing every parameter of the currently selected model and what the prior distribution is for each. A strong prior allows the user to 'inform' the analysis by sel a particular distribution with a small variance. Alternatively we can select a weak (diffuse) prior to try to minimise the effect on the analysis. Note that a prior distribution must be specified for every parameter and while BEAUti provides default options these are not necessarily tailored to the problem and data being analyzed.



In this case, the default prior for the exponential growth rate (the Laplace distribution) prefers relatively small growth rates because the of the default scale (1.0). However, on this epidemic scale, the growth rate para take could take on relatively large values. Therefore, we will increase the variance of this prior distribution by setting the scale to 100. A useful exercise could be to examine the sensitity of the growth rate estimates to different scale values for this prior distribution (e.g. scale = 1, 10, 100).

The other priors can be left at their default options.

#### Setting up the operators

Each parameter in the model has one or more "operators" (these are variously called moves, proposals or transition kernels by other MCMC software packages such as MrBayes and LAMARC). The operators specify the parameters change as the MCMC runs. The **Operators** tab in BEAUti has a table that lists the parameters, their operators and the tuning settings for these operators:

		Partitions	axa lips iraits	Sites	CIOCKS Tree	s states priors Operators MCMC
🗹 Au	to Optimize	Operator mix:	classic operator mix	2		
In use	Operates on		Туре	Tuning	Weight	Description
	Multiple		adaptiveMultivariate	1.0	30.0	Adaptive Multivariate Normal
<	kappa		scale	0.75	1.0	HKY transition-transversion parameter
	frequencies		deltaExchange	0.01	1.0	frequencies
	alpha		scale	0.75	1.0	gamma shape parameter
<	clock.rate		scale	0.75	3.0	substitution rate
<	Substitution ra	te and heights	upDown	0.75	3.0	Scales substitution rates inversely to node heights of the tree
<b>Z</b>	Tree		subtreeSlide	1.0	30.0	Performs the subtree-slide rearrangement of the tree
<b>Z</b>	Tree		narrowExchange	n/a	30.0	Performs local rearrangements of the tree
2	Tree		wideExchange	n/a	3.0	Performs global rearrangements of the tree
≤	Tree		wilsonBalding	n/a	3.0	Performs the Wilson-Balding rearrangement of the tree
≤	treeModel.roo	tHeight	scale	0.75	3.0	root height of the tree
<b>Z</b>	Internal node	heights	uniform	n/a	30.0	Draws new internal node heights uniformally
U.	Tree		subtreeLeap	1.0	50.0	Performs the subtree-leap rearrangement of the tree
	Tree		subtreeJump	1.0	5.0	Performs the subtree-jump rearrangement of the tree
<u> </u>	exponential.p	opSize	scale	0.75	3.0	coalescent population size parameter

Notice that the coalescent growth rate parameter (exponential.growthrate) has a randomWalk operator. This is appropriate for a parameter that can take both positive and negative values (parameters that an strictly positive can use a scale operator). No changes are required in this table.

#### Setting the MCMC options

The MCMC tab in BEAUti provides settings to control the MCMC chain and the log files that get produced.

•••						BEAU	ti					
	Partitions	Taxa	Tips	Traits	Sites	Clocks	Trees	States	Priors	Operators	мсмс	
			Lengt	th of chai	n: 10	0000						
		Echo sta	te to sc	reen ever	y: 10	0						
		Log	parame	eters ever	y: 10	0						
			File r	name ster	n: H:	LN1pdm_2	009					
			Loc	filo nom	☑	Add .txt si	uffix					
			Trees	s file nam	e: H1	N1pdm_20	09.trees	ι.				
	Su	bstitutio	ns trees	s file nam	e:	Create tree	e log file	with bran	h length:	in substituti	ons:	
						Create ope	rator ana	lysis file:				
	C	Operator a	analysis	s file nam	e:	Sample fro	mprior	nly - cre	ate emoty	alignment		
	Select the stepping– additional	option b stone sa analysis	elow to mpling after th	perform (SS) or ge e standar	margina neralize d MCMC	d likelihoo d stepping C chain has	d estimat J-stone s finished	ion (MLE) ampling (	using pa GSS) whic	th sampling h performs a	(PS) / n	
	Marginal li	ikelihood	d estima	ation (MLI	D: N	one Settings				0		
Data: 50 taxa, 1 partition											the Company of the	

For this dataset let's initially set the chain length to 100,000 and both the sampling frequencies to 100. The File name stem: should already be set to H1N1pdm\_2009 but you can adjust this (perhaps add more indications about the analysis).

We are now ready to create the BEAST XML file. Select Generate XML... from the File menu (or the button at the bottom of the window). BEAUti will ask you to review the prior settings one more time before s the file (and will indicate if any are improper). Continue and choose a name for the file — it will offer the name you gave it in the MOMC panel and we usually end the filename with '.xml' (although on Windows machin may want to give the file the extension '.xml.txt').

🗹 Tip: For convenience, leave the BEAUti window open so that you can change the values and re-generate the BEAST file as required later in this tutorial.

## Running BEAST

Once the BEAST XML file has been created the analysis itself can be performed using BEAST.

Thun BEAST (beast) by double-clicking on the BEAST icon.

Once BEAST has started a dialog box will appear in which you select the XML file:

● ○ ○ B	EAST v1.10.0 Prerelease #VEME2017
SE:	BEAST Bayesian Evolutionary Analysis Sampling Trees Version v1.10.0 Prerelease #VEME2017, 2002-2017 https://github.com/beast-dev/beast-mcmc/commit/VEME2017
BEAST XML File:	H1N1pdm_2009.xml Choose File
	Allow overwriting of log files
Random number seed:	1503680773817
Thread pool size:	Automatic ᅌ
🗹 Use BEAGLE library if	available:
Prefer use of:	PU ᅌ
	Use CPU's SSE extensions when possible
Prefer precision: D	ouble 🗘
Rescaling scheme: D	efault ᅌ
	Show list of available BEAGLE resources and Quit
BEAGLE is a high-perforn additional computational downloaded and installed	nance phylogenetic library that can make use of resources such as graphics boards. It must be I independently of BEAST:
http://github.com/bea	gle-dev/beagle-lib/
	Quit Run

Press the Choose File... button and select the XML file you just created and press Run. The analysis will then be performed with detailed information about the progress of the run being written to the screen. V has finished, the log file and the trees file will have been created in the same location as your XML file.

For more information about the other options in the BEAST dialog box see this page (beast).

# Analyzing the BEAST output

analyze the results of running BEAST we are going to use the program Tracer (tracer). Run Tracer by double clicking on the Tracer icon.

Select the Import Trace File... option from the File menu. Select the log file, H1N1pdm\_2009.log, that you created in the previous section. The file will load and you will be presented with a window simile the one below.



Similarly to the previous tutorial (workshop\_rates\_and\_dates) the effective sample sizes (ESSs) for all the traces are small (ESSs less 100 are highlighted in red respectively by Tracer). In the bottom right of the window frequency plot of the samples, which for the **posterior** trace in the above figure, has multiple peaks.

If we select the tab on the right-hand-side labelled Trace we can view the raw trace — the sampled values against the step in the MCMC chain:



Here it is clear default burn-in of 10% of the chain length is inadequate (the posterior values are still increasing over the first part of the chain). Double-click on the Burn-In column in the top left and edit (in the case above, a minimum of 20,000 is needed). However, it is still clear that a chain length of 100,000 was in adequate. Looking at the ESS values (generally in the low double-digits) suggests that a chain length of 10,000,000 would be more appropriate. On a modern computer this would probably only take about 20 minutes but we have provided the output of a run of this length which you can use for the rest of this section

📩 The log and trees files for a longer H1N1 exponential growth rate run <u>can be downloaded from here (/tutorials/workshop\_influenza\_phylodynamics/files/Long\_Run\_H1N1pdm\_Exponential.zip)</u>.

Load the new log file (H1N1pdm\_2009.log) into Tracer (you can leave the old one loaded for comparison). Click on the Trace tab and look at the raw trace plot.

Again we have chosen options that produce 1000 samples and with an ESS of > 300 for the coalescent model parameters there is little auto-correlation between the samples. There are no obvious trends in the plot would suggest that the MCMC has not yet converged, and there are no large-scale fluctuations in the trace which would suggest poor mixing.

As we are satisfied with the behavior of the MCMC we can now move on to one of the parameters of interest: exponential growth rate for the coalescent model we chose as the tree prior. Select exponential.growthRate in the left-hand table. Now choose the density plot by selecting the tab labeled Marginal Prob Distribution. This shows a plot of the posterior probability density of this parameter should see a plot similar to this:



As you can see the posterior probability density is roughly bell-shaped. The default is to show the kernel density estimate (KDE) which is smoothed probability density fitted to the data. Switch the Display: option top to Histogram to see the unsmoothed frequency plot. There is still a lot of noise here but it is a good estimate of the distribution.

The age(root) statistic provides an estimate of the time of the most recent common ancestor of the entire tree. In this case it may be a reasonable estimate of the start of the epidemic, when the virus jumped from pigs into humans. What is the mean estimate and 95% HPDs for the date of the MRCA?

You can visualize the growth estimate using the Demographic Reconstruction... option in the Analysis menu. Select this option and set up the dialog box that appears like this:

000	Demographic Analysis
	Demographic Model: Exponential Growth (Growth Rate) 🗘 Warning! Do not select a model other than that which was specified in BEAST to generate the trace being analysed. Any other model will produce meaningless results.
	Select the traces to use for the arguments:
	Population Size: exponential.popSize
	Growth Rate: exponential.growthRate ᅌ
	Maximum time is the root height's: Upper 95% HPD ᅌ
	Select the trace of the root height: treeModel.rootHeight 🗘
	Number of bins: 100
	✓ Use manual range for bins:
	Minimum time: 2009
	Maximum time: 2009.42
	Age of youngest tip: 2009.403
	You can set the age of sampling of the most recent tip in the tree. If this is set to zero then the plot is shown going backwards in time, otherwise forwards in time.
	Cancel

Select Demographic Model: Exponential Growth (Growth Rate) — note, you must select the tree prior you picked in BEAUti, you can't change this here. Tracer will automatically identify the parameters of t model (exponential.popSize and exponential.growthRate). The option Maximum time is the root height's: pick the Upper 95% HPD. This means it will extend the reconstruction back to the ext the root age credible interval. Set the Age of youngest tip: to 2009.403 (the date of the most recently sampled virus). You can also Use manual range for bins: to make the time-scale a bit cleaner — 1 2009.0 to 2009.42. Then press OK and this window will appear:



This shows the exponential growth line for the median growth rate and the 95% HPD intervals for this growth as a solid area. It is on a log scale so is a straight line. You can play with the axis settings using the Setu button. The dotted vertical lines represent the 95% HPD for the date of the root of the tree.

The exponential growthRate (*r*) provides an estimate of the epidemic growth of H1N1pdm 2009. Given that  $Nt = N_0 e^{-rt}$  (with  $N_0$  being the population size at present), the doubling time for r = 21 is about 0. years or 12 days. Interestingly, it has been shown that the basic reproductive ratio ( $R_0$ ) is related to the growth rate – see this page for details (/estimating\_R0.html). However, the basic reproductive number is dependent not just on an estimate of (*r*), but also a good estimate of the generation time distribution, which reflects the time between successive infections in a chain of transmission. If we assume a generation time distribution that follows the gamma distribution, then  $R_0 = (1 + r/b)^a$ , where *a* and *b* are the parameters of the gamma distribution (and  $a = \mu^2/\sigma^2$ ,  $b = \mu/\sigma^2$ ).

Taking  $\mu = 3$  days and  $\sigma = 2$  days, what would be the mean estimate of  $R_0$  for the H1N1pdm 2009  $R_0$ ?

## Summarizing the trees

We have seen how we can diagnose our MCMC run using Tracer and produce estimates of the marginal posterior distributions of parameters of our model. Next we can use the TreeAnnotator (treeannotator) tool the provided as part of the BEAST package to summarize the information contained within our sampled trees.

We un TreeAnnotator by double clicking on the icon.

TreeAnnotator takes a single 'target' tree and annotates it with the summarized information from the entire sample of trees. The summarized information includes the average node ages (along with the HPD intervals), posterior support and the average rate of evolution on each branch (for relaxed clock models where this can vary). The program calculates these values for each node or clade observed in the specified 'target' tree.

Use a Burnin (as states): of 1,000,000. This is 10% of the chain and we confirmed that this was adequate in Tracer, above. Use the defaults for the rest of the options - Posterior probability limit Target tree type: Maximum clade credibility tree, and Node heights: Median.

Use the Choose File... button to select an input trees file, H1N1pdm\_2009.trees.

🗹 Tip: In most of the BEAST package, if there is a button to select a file, you can also simply drag the file into this area.

Select a name for the output tree file (e.g., H1N1pdm\_2009.MCC.tre).

Once you have selected all the options, above, press the Run button. TreeAnnotator will analyse the input tree file and write the summary tree to the file you specified. This tree is in standard NEXUS tree file format s be loaded into any tree drawing package that supports this. However, it also contains additional information that can only be displayed using the FigTree (figtree) program.

## Viewing the annotated tree

🤯gTree (figtree) is a user-friendly, graphical program for viewing trees and the associated information provided by BEAST. Double-click on the FigTree icon to run it.

Run FigTree now and select the **Open...** command from the File menu. Select the tree file you created using TreeAnnotator in the previous section. The tree will be displayed in the FigTree window. On the left has side of the window are the options and settings which control how the tree is displayed. In this case we want to display the posterior probabilities of each of the clades present in the tree and estimates of the age of e node. In order to do this you need to change some of the settings.

First, re-order the node order by Increasing Node Order under the Tree menu. Switch on Branch Labels in the control panel on the left and open its section by clicking on the arrow on the left. Now select posterior under the Display: option. Reduce Sig. Digits to 2.

We now want to display bars on the tree to represent the estimated uncertainty in the date for each node. TreeAnnotator will have placed this information in the tree file in the shape of the 95% highest posterior densi (HPD) credible intervals. Switch on Node Bars in the control panel and open this section; select height\_95%\_HPD to display the 95% HPDs of the node heights.

We can also plot a time scale axis for this evolutionary history. Switch on Scale Axis (and switch off Scale bar) and select Reverse Axis in the Scale Axis options (you can also increase the font size a b appropriate scaling, open the Time Scale section of the control panel, set the Offset: to 2009.403 (the date of our most recently sampled virus).

Finally, open the Appearance panel and alter the Line Weight to draw the tree with thicker lines. The resulting tree will look like this:



None of the options actually alter the tree's topology or branch lengths in anyway so feel free to explore the options and settings. You can also save the tree and this will save most of your settings so that when you k into FigTree again it will be displayed almost exactly as you selected. The tree can also be exported to a graphics file (pdf, eps, etc.).

## EXERCISE 2: reconstructing H3N2 epidemic dynamics in the New York state.

In this tutorial, we will reconstruct a Bayesian skygrid (tree\_priors#skygrid) of human influenza A, H3N2 subtype, over three northern hemisphere epidemic seasons. The data set contains 165 Hemagglutinin gene sequences and takes more time to run in BEAST than available during a practical session. Therefore, this tutorial will discuss how to set up this analysis and how to summarize the results based on runs that have alre been performed.

L The data file is called 'NewYork.HA.2000–2003.nex' and can be downloaded from here (/tutorials/workshop influenza phylodynamics/files/NewYork.HA.2000-2003.nex).

## Running BEAUti

Run BEAUti, load the nexus file (NewYork.HA.2000–2003.nex) and set the Tips panel's Parse Dates to the last numerical field in the sequence names as previously. Set the same evolutionary model (includi gamma distributed rate variation) and clock model as in the previous exercise. In the Trees tab, select a Coalescent: Bayesian SkyGrid as the Tree Prior. We will construct a grid of 50 intervals over 5 ye (Time at last point: 5) before the most recent sampling date (2003.98 in our case, so going back to about 1999) thus estimating 10 population sizes per year. The panel should look like this:

Trees	Tree prior shared by all tree models									
default	Tree Prior:  Coalescent: Bayesian SkyGrid    Number of parameters:  50    Time at last transition point:  5    Gill MS, Lemey P, Faria NR, Rambaut A, Shapiro B, Suchard MA (2013) Mol Biol Evol 30, 713–724 (SkyGrid Coalescent): Drummond AJ, Nicholis GK, Rodrigo AG, Solomon W (2002) Genetics 161, 1307–1320 (Berally Sampled Data)									
	Tree Model – default									
	• Random starting tree									
	UPGMA starting tree									
	Select user-specified tree: no tree loaded 0									
	Export format for tree: Newick 🗘									
	Import user-specified starting trees from NEXUS format data files using the "Import Data menu option. Trees must be rooted and strictly bifurcating (binary).									

At this point we would usually generate the BEAST XML file, load it into BEAST and run it. However, this data set is a bit bigger than before and the model is a bit more computationally intensive so rather than waiting around for it to finish, you can go straight on and analyse some results files that have already been run.

📩 The log and trees files for a longer H3N2 SkyGrid run <u>can be downloaded from here (/tutorials/workshop\_influenza\_phylodynamics/files/Long\_Run\_H3N2\_SkyGrid.zip)</u>.

## Analyzing the BEAST output

Using Tracer, we can analyze the run based on the output files provided (load the file called NewYork.HA.2000–2003.log). This has been run with a chain length of 50,000,000 sampling every 5,000 steps so a to 10,000 samples:



To reconstruct the Bayesian skygrid plot, select SkyGrid reconstruction... under the Analysis menu. The following window should appear:

	Warning This analysis should only be run on traces where									
	the Sky Grid model was specified as the demographic in BEAST. Any other model will produce meaningless results.									
	Select the traces to use for the arguments:									
	Population Size: skygrid.logPopSize	\$								
	Grid Height: skygrid.cutOff	¢								
	Maximum time is the root height's: Upper 95% HPD ᅌ									
	Select the trace of the root height: treeModel.rootHeight	nt 🗘								
	✓ Use manual range for bins:									
	Minimum time: 1999									
	Maximum time: 2004									
	Age of youngest tip: 2003.98									
	You can set the age of sampling of the most recent tip in the tree. If this is set to zero then the plot is shown going backwards in time, otherwise forwards in time.									
	Cancel	K								

Set the manual bin range from 1999 to 2004 and specify 2003.98 as the Age of the youngest tip at the bottom. Press 0K and after some time, the following Bayesian skyGrid reconstruction should appea solid interval selected):



By default the y-axis is in a log scale. Press the Axes button and turn off Log axis for the Y axis. You will also need to set the Manual range because the upper HPD bounds will be very large. Set this to 0 and you get the following:



Here you can see that the seasonal peaks very strong (but that the uncertainty denoted by the credible intervale is also very large).

## Some Questions

What type of dynamics does the H3N2 skyride plot suggest? Would you expect to see the similar dynamics for H3N2 sampled in a southern hemisphere location?

What happened in 2001?

Is the H1N1pdm 2009 evolutionary rate similar to the seasonal H3N2 evolutionary rate? If not, what could explain their differences?

Based on the H1N1pdm 2009 tree inferred from a limited sampling, how many H1N1pdm introductions in New York would you conclude for this sample?

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## Help and documentation

The BEAST website: http://beast.community

Tutorials: http://beast.community/tutorials

Frequently asked questions: http://beast.community/faq

Tags: tutorial (tag\_tutorial.html)

workshop (tag\_workshop.html)

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