

## Analysis 2: Adding in a local clock

In this analysis, we will be exploring different clock rates, and adding in a fixed local clock to the analysis. When viruses jump to new host species, they often require adaptation to that new host. The HA gene encodes the influenza receptor binding protein, which is a common target for host-specific selection. It is a reasonable hypothesis that transmission from horses to dogs could impose selection on the HA gene, resulting in faster evolution and accumulation of amino acid changes. In this analysis, we will test that hypothesis by adding in a fixed local clock. This model allows the clock rate to vary among monophyletic groups in the tree. In our case, we will define two different host partitions in the data, canine and equine, and use a fixed local clock model to estimate the TMRCA for each host and the clock rate within each host species. We can then compare the estimates to determine whether there is evidence of increased adaptation in dogs following transmission from horses.

### Part 1: Setting up the analysis

1. We will be using most of the same parameters for this analysis that we did in Analysis 1. So, starting with the Beasti input from Analysis 1, now go back to the 2nd tab, labeled "Taxa". In order to assign different hosts different clock rates, we need to specify taxon groups, which we will do in this tab. In the bottom on this panel, click on the plus sign, which should add a new taxon group. Label the first one "equine\_taxa" and select the equine sequences to include by selecting them in the left box and clicking on the right arrow to include them in the equine taxon set. Do the same for canine. For both, select "mono". This enforces these groups to be monophyletic, which we know that they are. The results should look like below:

BEASTI

Partitions Taxa Tips Traits Sites Clocks Trees States Priors Operators MCMC

Import Taxon Sets

Taxon Set	Mono?	Stem?	Tree	Age
canine_taxa	<input checked="" type="checkbox"/>	<input type="checkbox"/>	default	
equine_taxa	<input checked="" type="checkbox"/>	<input type="checkbox"/>	default	

Taxon set: equine\_taxa

Excluded Taxa

- A/canine/New\_York/147926-5/2006/2006-12-18/CY...
- A/canine/New\_York/158402-1/2008/2008-12-31/CY...
- A/canine/New\_York/159903/2012/2012-12-17/H...
- A/canine/New\_York/1623.1/2010/2010-10-10/H...
- A/canine/New\_York/3699/2010/2010-10-10/H3N8/...
- A/canine/New\_York/3821631/2011/2011-07-06/JX2...
- A/canine/New\_York/4986-2/2006/2006-01-16/CY06...
- A/canine/New\_York/5183-6/2006/2006-01-16/CY06...
- A/canine/New\_York/51854/2008/2008-04-24/CY06...
- A/canine/New\_York/6977983/2010/2010-09-05/JX2...
- A/canine/PA/111788.2/2009/2009-10-10/KM359813/H3N8/...
- A/canine/PA/27637.3/2010/2010-10-10/KM359806/H3N8/...
- A/canine/PA/33225.4/2010/2010-10-10/KM359804/H3N8/...
- A/canine/PA/96978/2009/2009-10-10/KM359808/H3N8/...
- A/canine/Pennsylvania/10909/2007/2007-01-30/CY0...
- A/canine/Pennsylvania/137154/2008/2008-10-28/CY0...
- A/canine/Pennsylvania/16699/2007/2007-02-15/CY0...
- A/canine/Pennsylvania/94930-3/2007/2007-07-21/C...
- A/canine/Pennsylvania/96978/2009/2009-10-10/H3N8/...
- A/canine/South\_Carolina/89215/2009/2009-12-05/JX...
- A/canine/Sydney/6525/2007/2007-10-14/GU045762...
- A/canine/Sydney/6692/2007/2007-10-15/GU045761...
- A/canine/Texas/1/2004/2004-10-10/H3N8/Usa/...
- A/canine/VT/11039/2013/2013-10-10/KM359805/H3N8/...
- A/canine/VT/2782/2013/2013-10-10/KM359810/H3N8/...
- A/canine/Vermont/278213/2013/2013-10-10/H3N8/...
- A/canine/Wyoming/86033/2007/2007-02-14/HQ917...
- A/canine/Wyoming/86955/2007/2007-02-27/JX253...
- A/canine/Zhejiang/S34/2015/2015-01-06/MH01858...
- A/dog/Jacksonville/C3/2005/2005-10-10/H3N8/...
- A/duck/Miami/F3/2005/2005-10-10/H3N8/...

434 taxa excluded

Select: taxon set...

Included Taxa

- A/Equus\_caballus/Ulaanbaatar/1201/2013/2013-07-...
- A/Equus\_caballus/Ulaanbaatar/1202/2013/2013-07-...
- A/Equus\_caballus/Ulaanbaatar/1203/2013/2013-07-...
- A/Equus\_caballus/Ulaanbaatar/2/2011/2011-07-05/...
- A/Equus\_caballus/Ulaanbaatar/3/2011/2011-07-05/...
- A/Equus\_caballus/Ulaanbaatar/4/2011/2011-07-05/...
- A/Equus\_caballus/Ulaanbaatar/5/2011/2011-07-05/...
- A/Equus\_caballus/Ulaanbaatar/6/2011/2011-07-05/...
- A/Equus\_ferus\_caballus/USA/176576/2019/2019-09-...
- A/Equus\_ferus\_caballus/USA/218776/2018/2018-12-...
- A/Equus\_ferus\_caballus/USA/48519/2019/2019-03-2...
- A/Equus\_ferus\_caballus/USA/51053/2019/2019-03-3...
- A/Equus\_ferus\_caballus/USA/53954/2019/2019-04-0...
- A/Equus\_ferus\_caballus/USA/72778/2019/2019-04-2...
- A/Equus\_ferus\_caballus/USA/82889/2019/2019-05-0...
- A/donkey/Nigeria/VRD19\_ZM20\_T10\_19R5459-5/201...
- A/donkey/Shandong/1/2017/2017-03-27/MG132047...
- A/donkey/Xinjiang/5/2007/2007-12-12/EU794575/H3N8...
- A/equine/Ahmedabad/1/2009/2009-04-26/GU3961...
- A/equine/Ain/1/2014/2014-10-14/KY241318/H3N8/France/...
- A/equine/Alaska/29759/1991/1991-10-10/H3N8/...
- A/equine/Algiers/1/1972/1972-10-10/H3N8/Alge...
- A/equine/Algiers/1972/1972-10-10/H3N8/Alge...
- A/equine/Almaty/26/2007/2007-09-03/GU53266/H3...
- A/equine/Ankara/1/2013/2013-07-17/MF067527/H3...
- A/equine/Argentina/1/93/1993/139913/H3N8/Argent...
- A/equine/Argentina/E1134-7/2001/2001-07-31/KX8...
- A/equine/Argentina/E131/1994/1994-05-24/KX8153...
- A/equine/Argentina/E226/1995/1995-06-02/KX8153...
- A/equine/Argentina/E2345-1/2012/2012-07-09/KX8...
- A/equine/Argentina/E2345-5/2012/2012-07-09/KX8...

0 taxa included

Select: taxon set...

Data: 434 taxa, 3 partitions

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2. Now navigate to the “clocks” panel. For the default clock rate, select “fixed local clock” instead of strict clock.
3. Now, navigate to the “priors” panel. You should now see a new set of priors for the clock rates. We should change the default for the “canine\_taxa” and “equine\_taxa” clock rates, which are specified as “default.canine\_taxa.rate” and “default.equine\_taxa.rate”. Because our a priori assumption of the clock rates is that they should not deviate too far beyond the overall influenza clock rate, we can use the same prior we used for the strict clock in Analysis 1, a Lognormal with a mean of 0.01, with mean in real space.

The screenshot shows the BEAUti interface with the 'Priors' tab selected. A table lists various parameters and their assigned priors. The 'default.canine\_taxa.rate' parameter is highlighted, showing a LogNormal prior with a mean of 0.01 and a standard deviation of 1.0. A dialog box titled 'Prior for Parameter default.canine\_taxa.rate' is open, allowing the user to select a Lognormal prior distribution. The dialog box includes fields for the initial value (1.0), mean/stdev in real space (checked), mean (0.01), stdev (1.0), and offset (0.0). A plot of the prior distribution is shown at the bottom of the dialog box. The plot shows a sharp peak at 0.01, indicating a high probability of the clock rate being close to this value. The x-axis is labeled 'Quantiles' and ranges from 0 to 0.1. The y-axis ranges from 0 to 80. The plot also displays the following quantiles: 2.5%: 2.61E-7, 5%: 6.792E-7, Median: 1E-4, 95%: 0.015, 97.5%: 0.038. The dialog box has 'Cancel' and 'OK' buttons.

Parameter	Prior	Bound	Description
tmrca(canine_taxa)	* Using Tree Prior	n/a	tmrca statistic for taxon set canine_taxa on tree defa
tmrca(equine_taxa)	* Using Tree Prior	n/a	tmrca statistic for taxon set equine_taxa on tree defa
kappa	* LogNormal [1, 1.25], initial=2	[0, ∞]	HKY transition-transversion parameter
frequencies	* Dirichlet [1, 1]	[0, ∞]	base frequencies
alpha	* Exponential [0.5], initial=0.5	[0, ∞]	gamma shape parameter
default.clock.rate	LogNormal [0.01, 1], initial=1	[0, ∞]	substitution rate
default.canine_taxa.rate	LogNormal [0.01, 1], initial=1	[0, ∞]	substitution rate
default.equine_taxa.rate	LogNormal [0.01, 1], initial=1	[0, ∞]	substitution rate
region.clock.rate	Approx. Reference Prior, initi...	[0, ∞]	substitution rate
host.clock.rate	Approx. Reference Prior, initi...	[0, ∞]	substitution rate
treeModel.rootHeight	* Using Tree Prior in [59.1835...	[59.18356...	root height of the tree
skyride.precision	* Gamma [0.001, 1000], initial...	[0, ∞]	GMRF Bayesian skyride precision
region.nonZeroRates	Poisson [9]	n/a	the number of non-zero rates for BSSVS
region.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	discrete state frequencies
region.rates	* Gamma [1, 1], initial=1	[0, ∞]	discrete trait instantaneous transition rates
host.nonZeroRates	* Poisson [1]	n/a	the number of non-zero rates for BSSVS
host.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	discrete state frequencies
host.rates	* Gamma [1, 1], initial=1	[0, ∞]	discrete trait instantaneous transition rates
region.root.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	discrete state root frequencies
host.root.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	discrete state root frequencies

Link parameters together   Link parameters into a hierarchical model   Unlink parameters

\* Marked parameters currently have a default prior distribution. You should check that these are appropriate.

Data: 434 taxa, 3 partitions

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4. Save the xml as before, and launch!