# Tutorial using BEAST v2.7.7

contraband tutorial

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Total-evidence dating and trait-evolution evolutionary inference using phylogenetic multivariate Brownian motion models

# 1 Background

**Bird's-eye view**. This tutorial shows how to use the **contraband** package in **BEAST2** to model continuous trait evolution along a phylogeny with Brownian motion. Unlike methods that assume a "known", fixed tree, **contraband** lets you estimate the tempo and mode of trait evolution simultaneously with both species relationships and divergence times.

#### 1.1 What is contraband for

In this tutorial, we will walk you through running a simple analysis with the contraband (continuous traits brownian models) BEAST2 package. As the name suggests, contraband implements Brownian motion (BM) models for the evolution of continuous traits on a phylogeny.

To understand how these models can be useful to evolutionary biologists, let's put our X-ray goggles on and look at the core of the **contraband** package: the probability density function (pdf) of the multivariate Brownian motion model – the same pdf used for a multivariate normal distribution:

$$f(\mathbf{M}|\mathbf{V}, \mathbf{y_0}) = \frac{1}{(2\pi)^{nk/2} |\mathbf{V}|^{1/2}} \exp\left(-\frac{1}{2}(\operatorname{vec}(\mathbf{M}) - \mathbf{y_0})^{\mathrm{T}} \mathbf{V}^{-1}(\operatorname{vec}(\mathbf{M}) - \mathbf{y_0})\right),$$
(1)

This equation simply gives us the probability of observing our data  $\mathbf{M}$  – that is, one or more continuous traits – given two key parameters: (i) the expected value vector (or mean vector),  $\mathbf{y}_0$ , and (ii) the variance-covariance matrix,  $\mathbf{V}$ . If you have tried a few of the other Taming the BEAST tutorials, these two parameters are the quantities whose posterior probability distributions we want to approximate via Markov Chain Monte Carlo (MCMC).

In phylogenetics, V is typically decomposed as  $V = \Sigma \otimes T$ , where  $\Sigma$  describes the variance and covariance structure of the traits, and T represents phylogenetic relatedness. In essence, T captures the phylogeny itself – the shared evolutionary history among species.

In many software tools, especially those implemented in R and using frequentist methods, the phylogeny (T) is not estimated but instead fixed to a tree point estimate from the literature. The downside of this approach is that the continuous trait data can only inform our estimates of trait evolution parameters,  $y_0$  and  $\Sigma$  – not the phylogeny itself.

While it is possible to take this approach in BEAST 2 as well, its hierarchical Bayesian framework allows us to go further: we can co-estimate T (i.e., the species tree or phylogeny) together with the parameters of trait evolution. This means we can infer trait-evolution parameters **alongside** the species divergence times and phylogenetic relationships captured in T. In other words, **contraband** is a tool not only for studying how continuous traits evolve, but also for estimating the topology and divergence times of phylogenies.

The estimation of divergence times using multiple types of data – for example, molecular sequences combined with discrete and/or continuous morphological traits – is known as *total-evidence dating* (TED; Ronquist et al. 2012). Among other things, contraband is a TED method. It is designed to help evolutionary biologists leverage continuous traits to reconstruct species evolutionary histories, including both divergence times and the tempo and mode of phenotypic evolution.

#### 1.2 A quick peek under the hood

Later in this tutorial, you will be placing prior distributions on a series of parameters, as well as making modeling decisions related to things like the correlation between characters, for example, or the intraspecific variance in trait values. Setting up such an analysis can quickly become overwhelming, so in this section we will introduce a few implementation and statistical details to help you understand what comes next.

While it is possible to directly compute the value of equation (1) via matrix algebra, this is computationally expensive. Instead, contraband saves us time by using an alternative mathematical formulation (Mitov et al. 2020) and a dynamic programming algorithm. The details do not matter for this tutorial, but it is important to re-write equation (1) as:

$$f(\mathbf{M}|\mathbf{V}, \mathbf{y_0}) = f(\mathbf{M}|\Phi, \mathbf{y_0}, \mathbf{r}, \boldsymbol{\rho}, c_m, \mathbf{b}_m, \boldsymbol{\theta})$$
(2)

As mentioned above, **M** contains our continuous-character data, it is a matrix whose dimensions are the number of species  $\times$  the number of characters. On the right-hand side of this equation, you should further recognize some of the terms that have direct counterparts in models used for molecular evolution, e.g., those involved in the morphological clock model. These are the morphological global clock rate  $(c_m)$  and relative branch rates  $(\mathbf{b}_m)$ . Other parameters, however, are unique to multivariate Brownian models, like the character values from all characters  $(\mathbf{y}_0)$  at the root of the tree  $(\Phi)$ , a vector containing all relative character-specific evolutionary rates  $(\mathbf{r})$ , and the between-character correlation matrix  $(\boldsymbol{\rho})$ . All of these parameters can in principle be estimated with MCMC, but the accuracy of and uncertainty about our estimates will be a function of our data set size, which include the number of traits and of species (more details can be found in Zhang et al. 2024), as well as analysis running times.

Among the most challenging parameters to estimate is  $\rho$ . For example, attempting to estimate  $\rho$  with MCMC means we have a potentially very large number of parameters that will be very hard to identify unless one has a very large phylogeny (which in turn would make the analysis prohibitively slow). One thing we can do is obtain intraspecific character data from one of the species in the phylogeny, and then estimate character correlations from that. In short, one can obtain an estimate of  $\rho$ ,  $\hat{\rho}$ , from characters scored in many individuals of a single species, and then assume this estimate to be true and constant across the phylogeny – i.e., there is no MCMC sampling of character correlation parameters.

Depending on the dimensions of  $\hat{\rho}$ , however, it can become unwieldly: it may become nearly singular, its determinant approaching 0, and its inverse blowing up. (Down the line, we obviously cannot compute  $f(\mathbf{M}|\mathbf{V}, \mathbf{y}_0)$ !) Here, we can borrow a technique often referred to as "regularization": we can "shrink"  $\hat{\rho}$  towards the identity matrix  $\mathbf{I}$  – which represents the correlation matrix of a data set where characters are uncorrelated – and obtain what we call a ridge estimator of  $\rho$ ,  $\rho^*$ . Doing so effectively shrinks the off-diagonals of the correlation matrix, making it better conditioned; the extent to which we "shrink"  $\hat{\rho}$  towards the identity matrix is captured in a tuning ("shrinkage") parameter,  $\delta$ . (As you will see later in this tutorial, we will have to specify  $\delta$  to run one of our inference analyses.) The more uncertain we are about character correlations, because we have way too many characters for way too few species, say, the

larger  $\delta$  should be. At any rate, we will not have to worry too much about the details of how to obtain  $\delta$ . There are methods for doing just that in the literature, and we will use them.

Overall, here is a list of the continuous-trait model parameters that we want to estimate, and for which we will need to place prior distributions on:

- 1) Character-specific evolutionary rates, r,
- 2) Character correlations,  $\rho$ ,
- 3) Ancestral state values,  $y_0$ ,
- 4) Morphological clock parameters, relative branch rates  $\boldsymbol{b}_m$  and global evolutionary rate  $c_m$

In what follows, we will guide you through the explicit steps – including installation of dependencies and post-processing tools – that will (i) set up the analysis for inferring the above parameters, and (ii) help you process and visualize the results.

# 2 Programs used in this exercise

# **BEAST2** - Bayesian Evolutionary Analysis Sampling Trees2

BEAST2 (http://www.beast2.org) is a free software package for Bayesian evolutionary analysis of molecular sequences using MCMC and strictly oriented toward inference using rooted, time-measured phylogenetic trees. This tutorial is written for BEAST v2.7.7 (Bouckaert et al. 2019).

# BEAUti2 - Bayesian Evolutionary Analysis Utility

BEAUti2 is the successor of BEAUti, a graphical user interface tool that makes it easy to generate BEAST2 XML configuration files (these files are necessary to specify and run MCMC analyses). It is provided as a part of the BEAST2 package so you do not need to install it separately. Both BEAST2 and BEAUti2 are written in Java, meaning that these programs can not only be integrated at their codebase level, but that they are also cross-platform: the exact same code runs on all platforms. Although the screenshots used in this tutorial have been taken on a Mac OS X computer, both BEAST2 and BEAUti2 will have the same layout and functionality under other operating systems like Windows and Linux.

#### TreeAnnotator

TreeAnnotator is a program we will use to produce a summary tree from a posterior sample of trees obtained via MCMC. We will also use this program to summarize and visualize the posterior estimates of other tree-related parameters (e.g., node heights). TreeAnnotator is also provided as a part of the BEAST2 package so you do not need to install it separately.

#### Tracer

Tracer (http://tree.bio.ed.ac.uk/software/tracer) is used to summarize the posterior estimates of the various parameters sampled via MCMC. This program can be used for visual inspection of MCMC chains and to assess their convergence. Tracer makes it easy to calculate parameter median estimates, their 95% highest posterior density (95%-HPD) intervals, their effective sample sizes (ESS), and their correlation with other parameters.

# FigTree

The last program we will use is FigTree (http://tree.bio.ed.ac.uk/software/figtree). FigTree was designed so that users can easily visualize trees and draw publication-quality figures. FigTree interprets the annotations created by TreeAnnotator and associated to summary tree nodes; this allows the researcher to easily visualize and compare node-based statistics (e.g., posterior probabilities). We will use FigTree v1.4.4.

# 3 Setting up

# 3.1 Installing dependencies

Total-evidence dating of phylogenies is a complex task that requires a series of models, a few of which are implemented in their own BEAST2 packages. The main package for this tutorial is called **contraband** and it implements Brownian motion models for the evolution of multiple characters on phylogenetic trees.

In order to install contraband, we have to download it using the BEAST2 package manager. Open BEAUti2, go to *File* >> *Manage Packages*, and click on the contraband link and then clicking *Instal-*l/Upgrade (Figure 1).

Name	Installed	Latest	Dependencies	Link		
odtree	0.0.2	0.0.2	BEAST.base, BEAST.app	8940	Birth-death sequential sampling	^
BEAST_CLASSIC		1.6.4	BEAST.app, BEAST.base	819	BEAST classes ported from BEAST 1 in	wrappers
BEASTLabs	2.0.3	2.0.3	BEAST.base, BEAST.app	819	BEAST utilities, such as Script, multim	onophyletic constraints
BEASTvntr		0.2.0	BEAST.app, BEAST.base	89	Variable Number of Tandem Repeat d	ata, such as microsattelites
BICEPS	1.1.2	1.1.2	BEAST.base, BEAST.app	89	Bayesian Integrated Coalescent Epoch	PlotS + Yule Skyline
oModelTest	1.3.3	1.3.3	BEAST.app, BEAST.base	8960	Bayesian model test for nucleotide sub	ist models, gamma rate he
BREAK_AWAY		1.2.0	BEASTLabs, GEO_SPHERE, BEA	8950	break-away model of phylogegraphy	
BREATH		0.0.1	BEAST.app, BEAST.base	819	Bayesian Reconstruction and Evolution	nary Analysis of Transmiss
CA		2.1.0	BEAST.base, BEAST.app	88	Bayesian estimation of clade ages bas	ed on probabilities of fossi
CCD	1.0.3	1.0.3	BEAST.app, BEAST.base	89	Sensible tree set summary/point estim	ates.
ClaDS		2.1.2	BEAST.base, BEAST.app	88	Implementation of the ClaDS birth-dea	th tree prior with sampled
CoalRe		1.0.4	BEAST.app, BEAST.base	8950	Inference of Recombination networks	
CodonSubstModels		2.0.0	BEAST.base, BEAST.app	849	Codon substitution models for DNA	
contacTrees		1.2.0	BEASTLabs, BICEPS, BEAST.bas	83	Phylogenetic model with horizontal tra	nsfer for linguistics
				89		us trait evolution
CorrelatedTrait		0.0.5	BEAST.app, BEAST_CLASSIC, B	89	Analysis for a fixed tree or fixed tree se	t
CoupledMCMC		1.2.2	BEAST.base, BEAST.app	8840	Adaptive coupled MCMC (adaptive pa	rallel tempering or MC3)
CubeVB		1.0.2	BEASTLabs, BEAST.app, BEAST	849	Variational Bayes on a cube	
DENIM		1.1.1	BEAST.app, BEAST.base	85	Divergence Estimation Notwithstandin	g ILS and Migration
EpiInf		8.0.0	SA, BEAST.base, BEAST.app, feast	89	BD/SIR/SIS epidemic trajectory inferen	ce.
EpochClockModel		0.0.2	BEAST.base, BEAST.app	88	Add clock models that have different r	nean rates at different epo
Experimenter		0.2.0	BEAST.base, BEASTLabs, BEAST	8840	Experimenter	
FastRelaxedClockLogNormal	1.2.0	1.2.0	BEAST.app, BEAST.base	849	Relaxed clock that works well with larg	e data 🔍
						>
✓ Latest In	stall/Upgrade		Uninstall Package re	positorie	es ?	Close

Figure 1: Downloading the contraband package.

This tutorial will also make use of a few other packages; these are sampled-ancestors and morph-models. The first implements speciation and fossilization models (for evaluating the probability of phylogenies themselves), and the second implements models for the evolution of discrete morphological characters. The SA and MM packages have two dedicated tutorials on the Taming the BEAST website (Divergence Time Estimation tutorial and Total Evidence tutorial) and will not be discussed further in the present exercise. The newly installed packages will become available in BEAUti2 once you close and restart the program.

# 3.2 The data

The data sets used in this tutorial include three types of data – molecular, discrete morphology and continuous morphology – scored for up to 27 Carnivore species (11 of which are extinct and 16 of which are extant).

### **3.2.1** Continuous characters

Our TED analysis will leverage a published geometric-morphometric data set consisting of 29 threedimensional (3D) cranium landmarks (Álvarez–Carretero et al. 2019), each dimension of which will be treated as a separate continuous character (i.e., we will have a total of 87 continuous characters). This data can be found in file carnivora\_continuous\_27.nex attached to this tutorial.

The same cranium landmarks have also been scored in 21 *Vulpes vulpes* (one of the focal carnivore species) individuals. This intraspecific data will be used in the analyses to bypass the estimation of character correlations ( $\rho$ ), and can be found in the attached file vulpes\_continuous\_data.txt.

#### **3.2.2** Discrete characters

12 species of interest have discrete morphological characters that describe their basicranial, dental and postcranial anatomical features (carnivora\_discrete\_27.nex) (Barrett et al. 2021). There are 183 features in total and the number of character states ranges from 0 to 3.

#### **3.2.3** Molecular sequences

The molecular sequences of 12 mitochondrial genes for 14 species of interest were collected from the NCBI database, concatenated and aligned using MAFFT (carnivora\_dna\_27.fasta).

# 4 Practical Part I: Bayesian phylogenetic inference using multiple continuous morphological characters (and more!)

The following two sections will walk you through the required steps for setting up a couple of different analyses. The first shall carry out Bayesian inference of divergence times using continuous morphology only; the main player here is the Brownian model implemented in the **contraband** package. In the second analysis we will set up an integrative model with components for each type of data (described above) – this will be a full-blown total-evidence dating analysis with all the data we have. Like in other tutorials, we will use BEAUti2 as a tool for specifying BEAST2 .XML files for running our analyses.

# 4.1 Setting up the analysis in BEAUti

# 4.1.1 Loading the Carnivoran Continuous data

The continuous characters can be found in the data folder named carnivora\_continuous\_27.nex. It can be either dragged and dropped into BEAUti "Partitions" panel or added using BEAUti's menu system via *File* >> *Load Continuous Data*. Once the characters are loaded successfully into BEAUTi, the panel will show the number of species and number of characters in the columns "Taxa" and "Sites" correspondingly (Figure 2).

						💓 BE	AUti 2: S	tandard						
Partitions	Continuou	s Model Ti	p Dates	Site Mode	I Clo	ick Model	Priors	MCMC						
Link Site Mo	dels Unlin	k Site Mode	ls Link C	lock Models	Unlin	k Clock Model	s Link	Trees Unli	nk Trees					
Na	ne		File	Таха	Sites	Data Type		Site Model		Clock Mo	del	Tree		Am
arnivora_cor	tinuous_27	carnivora_	continuous_	_27 27	87	continuous	carnive	ora_continuol	-	camivora_contin	uoi 👻	carnivora_continue	n 👻	
	Split													
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_					_		_	_	_		_		_	_

Figure 2: Loading the continuous data.

#### 4.1.2 Setting the fossil ages (Tip Dates)

Since the data set contains fossil species, we will need to open the "Tip Dates" panel and then select the "Use tip dates" checkbox to specify the fossil ages. This can be done in multiple ways. In our case, we can obtain the date information from the species names. We can tell BEAUti to use these by clicking the *Auto-configure* button. The fossil ages appear following the second underscore "\_\_" in the species name. To extract these times, select "use everything", then select "after last" from the drop-down box to the right, and input "\_\_" (without the quotes) in the text box immediately to the right, as shown in the figure below Figure 3. Clicking "OK" should now populate the table with the fossil ages extracted from the species names.

In the populated table, the two columns **Date** and **Height** should now have values between 0.0 and 35.55 in million years (Figure 4).

#### 4.1.3 Setting the Brownian motion Model

As introduced above, the parameters under the Brownian motion model include trait evolutionary rate (Sigmasq), trait correlations (Correlation) and ancestral states at the root (Root Values). Here we assume that all characters share one evolutionary rate. Therefore, we put a tick in the box in front of the "One Rate Only".

As elaborated in the background section above, there are a few key Brownian motion model parameters that concern us. Namely:

1. the character values at the root of the tree,  $y_0$ ,

<ul> <li>use everything</li> </ul>	after last	<b>-</b>
split on character	_	and take group(s): 1 🔹
use regular expression		
read from file		Browse
Add fixed value	1900	
Unless less than	20	
then add	2000	

Figure 3: Guessing sampling times.

Y		Ŷ	Ϋ́Υ.		
Partitions	Continuous Model Tip Dates 💿 Si	te Model Clock Model	Priors MCMC		
✓ Use tip dat	es				
Dates specified	t:      numerically as year	Before the present	✓ Auto-configure	Clear	
	as dates with format dd/M/yy	уу 👻 ?			
	Taxon		Date (raw valu	ue) Age/Height	
Aelurodon_ferc	x_13.13		13.13	13.13	-
Canis_dirus_0.	0285		0.0285	0.0285	
Epicyon_hayde	ni_11.95		11.95	11.95	
Hesperocyon_g	gregarius_35.55		35.55	35.55	
Paraenhydrocy	on_josephi_25.615		25.615	25.615	
Tomarctus_hip	pophaga_14.785		14.785	14.785	
Enhydrocyon_p	pahinsintewakpa_28.55		28.55	28.55	
Cuon_alpinus_	0.0		0.0	0.0	
Speothos_vena	aticus_0.0		0.0	0.0	
Canis_lupus_0	.0		0.0	0.0	
Cerdocyon_the	ous_0.0		0.0	0.0	
Otocyon_mega	alotis_0.0		0.0	0.0	
Vulpes_vulpes_	_0.0		0.0	0.0	
Ursus_america	nus_0.0		0.0	0.0	
Ailurus_fulgens	s_0.0		0.0	0.0	
Nandinia_binot	ata_0.0		0.0	0.0	
Paradoxurus_h	ermaphroditus_0.0		0.0	0.0	
Hyaenictitheriu	m_wongii_6.65		6.65	6.65	
Smilodon_fatal	is_0.0285		0.0285	0.0285	
Canis_latrans_	0.0		0.0	0.0	
Smilodon_popu	ulator_0.39		0.39	0.39	
Puma_concolo	r_0.0		0.0	0.0	
Dantham las (	2.0		A A	0.0	~

Figure 4: Fossil ages.

- 2. the character correlations,  $\rho$ , and
- 3. the character-specific evolutionary rates,  $\boldsymbol{r}$ .

We want to estimate all these parameters (so check all the "estimate" boxes), but for simplicity, we will assume that all characters are evolving at the same rate. Therefore, we want to check the "One Rate Only" box. Parameter values at the start of the MCMC chain can be specified in the text boxes next to their corresponding parameters names; these should not matter for analysis convergence, and we will just use arbitrary, round values (Fig. 5).

Include Root	I Include Root ode Math Sigmasq 1.0 ● ♥ estimate Correlation 0.1 ● ♥ estimate ♥ One Rate Only Upper Matrix	Include Root Sigmasq 1.0 ● ✓ estimate Correlation 0.1 ● ✓ estimate ✓ One Rate Only Upper Matrix	Include Root de Math Sigmasq 1.0 • I estimate Correlation 0.1 • I estimate I one Rate Only Upper Matrix	•••				100 E	BEAUti 2: S	tandard		
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Correlation 0.1 • V estimate	Correlation O.1 OR Rate Only Upper Matrix	Correlation One Rate Only Upper Matrix	Correlation 0.1 • V estimate Upper Matrix	Include Ro	oot							
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One Rate Only Upper Matrix	One Rate Only Upper Matrix	One Rate Only Upper Matrix	One Rate Only Upper Matrix			Correlation	ı.	0.1		💿 🗸 estimate		
Upper Matrix	Upper Matrix	Upper Matrix	Upper Matrix			One Ra	ate Only					
Root Values 0.0	Root Values 0.0 estimate	Root Values 0.0 ( estimate	Root Values			_						
						Root Value	s	0.0		💿 🗸 estimate		

Figure 5: BM model parameter specifications.

#### 4.1.4 Setting the Clock model

The morphological clock model we will use in this analysis is the well known uncorrelated log-normal clock Drummond et al. 2006, under which each branch of the species tree is assumed to have its own relative evolutionary rate, with rates being identically and independently distributed.

# 4.1.5 Specifying the priors

In the "Priors" panel, we select "Fossilized Birth Death Model" (Heath et al. 2014; Gavryushkina et al. 2014) as the tree prior and leave the rest of the parameters at their default prior distributions.

# 4.1.6 Specifying the MCMC chain length (MCMC)

Here we can set the length of the MCMC chain and after how many iterations the parameter and trees a logged. For this dataset, 2 million iterations should be sufficient. In order to have enough samples but not create too large files, we can set the logEvery to 2000, so we have 1001 samples overall. Next, we have to save the \*.xml file under *File* >> *Save as*.

Patitions Continuous Model     Tip Dates Site Model     Optimised Relaxed Clock     Mean clock rate     10			🔤 BEAUti 2:	2: Standard	
	Partitions Continuous Model	Tip Dates Site Model	Clock Model 💿 Priors	s MCMC	
Mean clock rate	Optimised Relaxed Clock	-			
	Mean clock rate	1.0	/ estimate		

Figure 6: Setting the initial clock rate.

Partition	Is Continuous Model	Tip Dates	Site Model	Clock Mod	lel Priors	МСМС	
) T	Free.t:carnivora_continuous	_27	Fossilized Birth Deat	h Model	•		
•	ORCRates.c:carnivora_conti	nuous	Log Normal[1.0,0.2]	•	initial = [0.5] [1	.0E-100,∞]	Optimised lognormal relaxed clock rates of partition c:carnivora_contin
•	ORCsigma.c:carnivora_cont	inuous	Gamma[5.0,0.05]	•	initial = [0.2	[0.0,∞]	Optimised lognormal relaxed clock stdev of partition c:carnivora_contin
•	DRCucldMean.c:camivora_c	contin	Gamma[0.1,100.0]	•	initial = [1.0	] [-∞,∞]	Optimised lognormal relaxed clock mean of partition c:carnivora_conti
•	covValues.t:carnivora_contir	nuous	Beta[2.0,2.0]	•	initial = [0.1]	[-1.0,1.0]	Prior on trait correlations for partition s:carnivora_continuous_27
•	diversificationRateFBD.t:can	nivora	Uniform[0.0,Infinity]	•	initial = [1.0	][0.0,∞]	prior on diversificationRateFBD.t:carnivora_continuous_27
•	originFBD.t:carnivora_contin	uous	Uniform[0.0,Infinity]	•	initial = [100.	0] [0.0,∞]	prior on originFBD.t:carnivora_continuous_27
• r	ootValues.t:carnivora_conti	nuous	Normal[0.0,0.5]	•	initial = [0.0	] [-00,00]	Prior on trait root values for partition s:carnivora_continuous_27
▶ s	samplingProportionFBD.t:ca	mivora	Uniform[0.0,1.0]	•	initial = [0.5]	[0.0,1.0]	prior on samplingProportionFBD.t:carnivora_continuous_27
▶ t	urnoverFBD.t:carnivora_cor	itinuo	Uniform[0.0,1.0]	•	initial = [0.5]	[0.0,1.0]	prior on turnoverFBD.t:carnivora_continuous_27
•	varValues.t:carnivora_contin	uous	Log Normal[0.2,0.5]	•	initial = [1.0	] [-∞,∞]	Prior on trait evolutionary rate for partition s:carnivora_continuous_27
						+ Add Prior	

Figure 7: Setting the tree model and priors on parameters.

Partitions MCMC Chain Length Store Every	Continuous Model		Site Model	Clock Model	Priors	MCMC ③	
Chain Length							
Store Every		1000000	D	۲			
		-1	۲				
Pre Burnin		0					
	tion Attempts	10					
		10					
▶ tracel	ug						
▶ scree	nlog						
▶ treelo	g.t:carnivora_continuc	ous_27					
•							
Sample F	rom Prior 💿						

Figure 8: saving the \*.xml file.

# 4.2 Running the Analysis using BEAST2

Run the \*.xml file using BEAST2. The analysis should take about 6 to 7 minutes.

#### 4.3 Analysing the results

For this section either use the output files from your own analysis or use finished runs from the *precooked*runs folder.

Follow the steps below:

#### 4.3.1 Analysing the log file using Tracer

First, we can open the \*.log file in tracer to check if the MCMC has converged. The ESS value should be above 200 for almost all parameters and especially for the posterior estimates (Figure 9). This is clearly not the case here and this analysis should be run for much longer to reach convergence.

Next, examine the posterior estimates for the following parameters:

- The estimated evolutionary rate (Figure 10),
- The estimated character correlations (Figure 11),
- The estimated ancestral states at the root (Figure 12),
- The inferred morphological clock model (Figure 13).

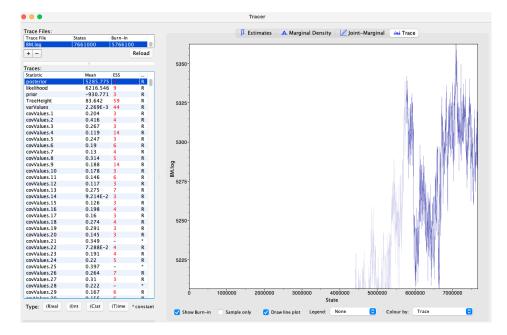


Figure 9: Checking if the chain converged.

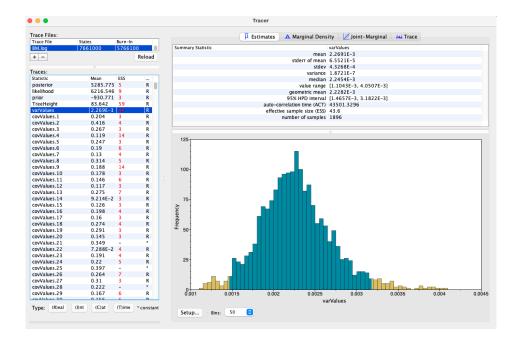


Figure 10: Evolutionary rate shared by 87 characters.

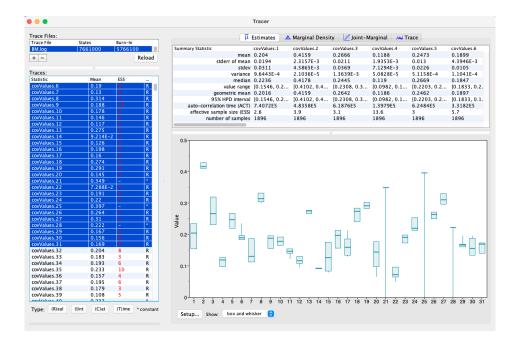


Figure 11: Character correlations among 87 characters.

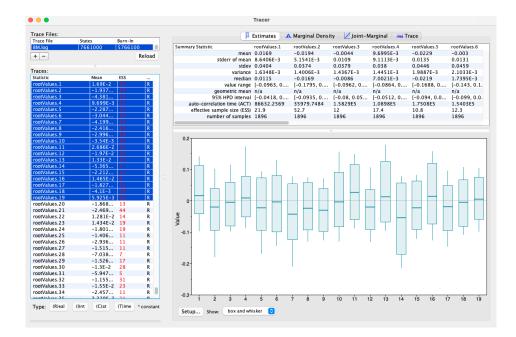


Figure 12: 87 trait values at the root of the tree.

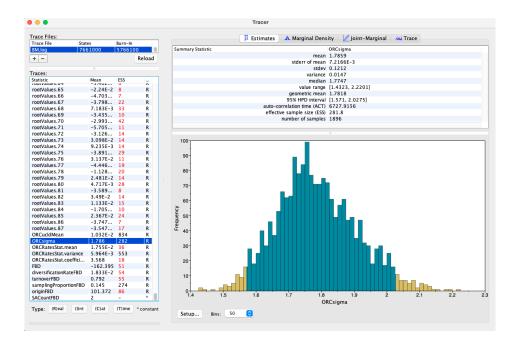


Figure 13: Comparing the inferred migration rates.

#### 4.3.2 Constructing the summary tree using TreeAnnotator

Open **TreeAnnotator** and then set the options including **Burnin percentage**, **Target tree type**, **Node heights**, **Input Tree File** and the **Output File**. Use the logged trees in the file carnivora\_continuous\_27\_tree\_bm $\leftrightarrow$ .trees as **Input Tree File**. Name output file carnivora\_continuous\_27\_bm\_mcc.tree.

Use the logged trees in the file carnivora\_continuous\_27\_tree\_bm.trees as Input Tree File. Name output file carnivora\_continuous\_27\_bm\_mcc.tree.

After clicking **Run** the program should summarize the trees.

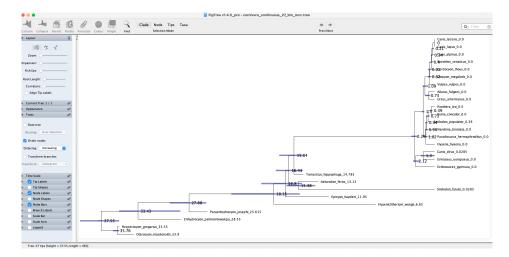


Figure 14: Summarised MCC tree of logged trees under the Brownian motion model.

	r	Y	Ŷ	Ĩ		Y							
Partitions		ific Tip Dates	Site Mo		Clock M		10 II						
Link Site Mo			Link Clock		Unlink	Clock Models	Link Trees Unlin	k Tree					
	ime	File		Таха	Sites	Data Type	Site Model		Clock Model		Tree		Aml
carnivora_co	ntinuous_27	carnivora_conti	nuous_27	27	87	continuous	carnivora_continuol	*	carnivora_continuo	*	carnivora_continuol	*	
carnivora_dn	a_27_1,2	carnivora_dna_	27	27	7320	nucleotide	carnivora_dna_27_'	-	carnivora_dna_27_'	•	camivora_continuou	•	
carnivora_dn	a_27_3	carnivora_dna_	27	27	3660	nucleotide	carnivora_dna_27_:	-	carnivora_dna_27_:	•	camivora_continuo	-	
carnivora_dis	crete_272	camivora_discr	ete_27	27	156	standard	carnivora_discrete_	•	carnivora_discrete_	•	camivora_continuo	•	
arnivora_dis	crete_273	carnivora_discr	ete_27	27	31	standard	carnivora_discrete_	-	carnivora_discrete_	-	camivora_continuou	-	
carnivora_dis	crete_274	carnivora_discr	ete_27	27	5	standard	carnivora_discrete_	-	carnivora_discrete_	•	camivora_continuo	•	

Figure 15: Loading continuous characters, molecular sequences and discrete characters.

# 5 Practical Part II: Parameter and State inference using combined data with the Brownian motion model combined with a shrinkage method

# 5.1 Setting up the analysis in BEAUti

#### 5.1.1 Loading the Carnivoran data sets

We first load the continuous data and parse the fossil ages as in sections 4.1.1 and 4.1.2. Then, in the "Partitions" panel, we also load the Carnivoran molecular sequences via File >> Import Alignment. Finally, we add the discrete characters by File >> Add Morphological Data. As is shown in Figure 15, the two rows with "nucleotide" as Data Type indicate that the molecular sequences are partitioned based on the codon positions, i.e., first and second codon positions for the first partition and the third codon positions for the second partition. Likewise, the last three rows with "standard" as Data Type indicate that the discrete data are partitioned based on the number of states the characters have. In this data set, the discrete characters are described by the states the number of which ranges from 2 to 4.

#### 5.1.2 Setting the Shrinkage Model

In the "Shrinkage Model" panel, we will need to fill in three components of the model. First, the shrinkage parameter is given by a constant value in the box to the right of "Delta". Second, the continuous characters from 21 *Vulpes vulpes* individuals are given in the block of "Population Traits". To be more specific, the trait data should be written in one-line data separated by spaces. In addition, the number of traits is given by "Minordimension" and should be consistent with the dimension of the continuous data in the "Partitions" panel. Third, the added individual trait values are not only used for estimating correlations,

but also normalizing the continuous data of the 19 carnivoran species. Therefore, we put a  $\checkmark$  in the box in front of "Include Pop Var" (Figure 16).

beta 0.636349274: ✓ Include Pop Var Delta 0.0 Computation Traits 0.0 Lower 0.0 Upper 0.0 Value 0.120118943693226 0.0 Dimension 1827 Minordimension 87 Estimate	•••					BEAUt	2: Standa
Include Pop Var  Value Include Include Pop Var  Value Include Include Pop Var  Include Pop	Partitions	Instraspecific	Tip Dates	Site Model	Clock Model	Priors	MCMC
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10       Image: Constraints         Lower       Image: Constraints         Upper       Image: Constraints         Value       0.120118943693226 0.0         Dimension       1827         Minordimension       1827         Estimate       Image: Constraints	V Include Pe	op Var					
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Dimension 1827 Minordimension 87 Estimate							
Minordimension 87			Value	0.1201189436	693226 0.0		
Estimate			Dimen	sion	1	827	
			Minor	dimension	8	7	
Sigmasq 1.0 estimate			Es	timate			
	Sigmasq		10		estimate		
			1.0				

Figure 16: Setting the shrinkage model.

#### 5.1.3 Setting the Substitution Model

In the "Site Model" panel, we assume an HKY+Gamma model for nucleotide substitutions by specifying 4 categories under "Gamma Category Count" Figure 17. In addition, we assume Mk models (Lewis 2001) for the discrete characters, as is shown in Figure 18.

#### 5.1.4 Setting the Clock model

Similar to section 4.1.4, we assume a relaxed clock model for each data partition. The specifications are shown in Figure 19.

#### 5.1.5 Specifying the priors

First, we select "Fossilized Birth Death Model" from the drop-down menu and set it as the tree prior. Then we again retain the default priors for the rest of the parameters (Figure 20).

# 5.1.6 Specifying the MCMC chain length (MCMC)

Here we can set the length of the MCMC chain and after how many iterations the parameter and trees a logged. For this dataset, 2 million iterations should be sufficient. In order to have enough samples but not create too large files, we can set the logEvery to 2000, so we have 1001 samples overall. Next, we have to save the \*.xm1 file under *File* >> *Save as*.

• • •					🔤 BEAUti :	i 2: Standard
Partitions	Instraspecific	Tip Dates	Site Model 💿	Clock Model	Priors	MCMC
Partition		Gam	nma Site Model			▼
carnivora_co		Subst	itution Rate			1 • estimate
carnivora_dn		00030	autorrate		1.0	Counde
carnivora_dn carnivora_dis		Gamn	na Category Coun	t	4	0
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carnivora_dis		Shape	e		1.0	⑦ ● ✓ estimate
our in or o_uro	0.000_27.4	Propo	ortion Invariant		0.0	Image:
		Subst	t Model		HKY 👻	3
					Kappa	2.0 (3) (estimate
					Frequencies	Estimated 🔹 💿 🐵
		V Fix	mean substitutior	n rate 😑		

Figure 17: Setting site models for molecular sequences and discrete characters.

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17_1,2 17_3 te_272 te_273	Gamr Propo	na Category Coun ortion Invariant		0	() () ()	
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te_272 te_273	Propo	ortion Invariant	nt (	•	3	٥
te_273	Propo	ortion Invariant		•		٢
	Subst	: Model		Lewis MK	¥	
			-			
	Fix	mean substitution	on rate 🦰			
		Fb	✓ Fix mean substitutie	🖉 Fix mean substitution rate ●	V Fix mean substitution rate	V Fix mean substitution rate

Figure 18: Setting site models for discrete characters.

• • •					BEAUti 2: Standard				
Partitions	Instraspecific	Tip Dates	Site Model	Clock Model @	Priors	MCMC			
Partition			Optimised Relaxed C	lock 👻					
carnivora_continuous_27		м	Mean clock rate		.0	<ul> <li>Image: Image of the second second</li></ul>			
carnivora_dna_27_1,2 carnivora_dna_27_3					.0				
carnivora_dis									

Figure 19: Setting the clock models for continuous data, molecular data and discrete data.

					BEAUti 2:	Standard	
Partitions Instraspecific Tip Dates			Site Model C	lock Model	Priors 💿	MCMC	
Tree.t:carnivora_continuous_27			Fossilized Birth Death Model				
ORCRates.c:carnivora_continuous			Log Normal[1.0,0.2]		initial = [0.5] [1.0E-100,∞]		Optimised lognormal relaxed clock rates of partition c:carnivora_con
•	ORCRates.c:carnivora_discrete_27		Log Normal[1.0,0.2]		initial = [0.5] [1.0E-100,∞]		Optimised lognormal relaxed clock rates of partition c:carnivora_disc
•	ORCRates.c:carnivora_dn	a_27_1,2 l	Log Normal[1.0,0.	2] 🔻	initial = [0.5]	[1.0E-100,∞]	Optimised lognormal relaxed clock rates of partition c:carnivora_dna
•	ORCRates.c:carnivora_dn	a_27_3 l	Log Normal[1.0,0.	2] 🔻	initial = [0.5]	[1.0E-100,∞]	Optimised lognormal relaxed clock rates of partition c:camivora_dna
•	ORCsigma.c:camivora_continuous		Gamma[5.0,0.05] •		initial = $[0.2] [0.0, \infty]$		Optimised lognormal relaxed clock stdev of partition c:carnivora_cor
•	ORCsigma.c:carnivora_discrete_27		Gamma[5.0,0.05] -		initial = [0.2] [0.0,∞]		Optimised lognormal relaxed clock stdev of partition c:carnivora_dis
•	ORCsigma.c:carnivora_dn	a_27_1,2	Gamma[5.0,0.05]	•	initial = [0.	.2] [0.0,∞]	Optimised lognormal relaxed clock stdev of partition c:carnivora_dna
•	ORCsigma.c:carnivora_dn	a_27_3	Gamma[5.0,0.05]	•	initial = [0.	.2] [0.0,∞]	Optimised lognormal relaxed clock stdev of partition c:camivora_dna
•	ORCucldMean.c:carnivora	_contin	Gamma[0.1,100.0	] •	initial = [1.	.0] [-∞,∞]	Optimised lognormal relaxed clock mean of partition c:carnivora_con
•	ORCucldMean.c:carnivora	_discret	Gamma[0.1,100.0	] •	initial = [1.	.0] [-∞,∞]	Optimised lognormal relaxed clock mean of partition c:carnivora_dis
•	ORCucldMean.c:carnivora	_dna_2	Gamma[0.1,100.0	] •	initial = [1.	.0][-∞,∞]	Optimised lognormal relaxed clock mean of partition c:carnivora_dn
•	ORCucldMean.c:carnivora	_dna_2	Gamma[0.1,100.0	] •	initial = [1.	.0] [-∞,∞]	Optimised lognormal relaxed clock mean of partition c:carnivora_dn
▶ .	diversificationRateFBD.t:c	amivora	Uniform[0.0.Infinit	vi 👻	initial = [1.	.01[0.0.∞]	prior on diversificationRateFBD.t:carnivora_continuous_27

Figure 20: Setting up tree model and the prior distributions.

# 5.2 Running the Analysis using BEAST2

Run the \*.xm1 using BEAST2 or use finished runs from the *precooked-runs* folder. The analysis should take about 6 to 7 minutes.

# 5.3 Analysing the results

For this section either use the output files from your own analysis or use finished runs from the *precooked-runs* folder.

• Examine the posterior estimates for the inferred clock models for continuous data, discrete data and molecular sequences in Tracer (Figure 21 and Figure 22)

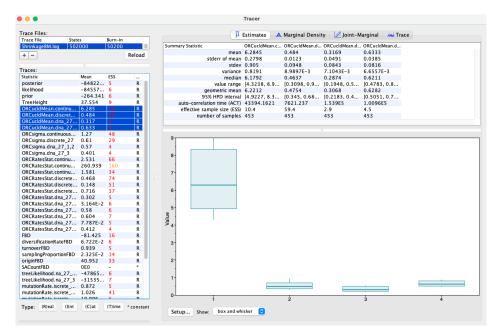


Figure 21: Estimated clock rates.

• Construct the summary tree using TreeAnnotator (Figure 23)

# 6 Errors that can occur (Work in progress)

One of the errors message that can occur regularly is the following: Infinity likelihood

Negative branch length

Unequal likelihoods

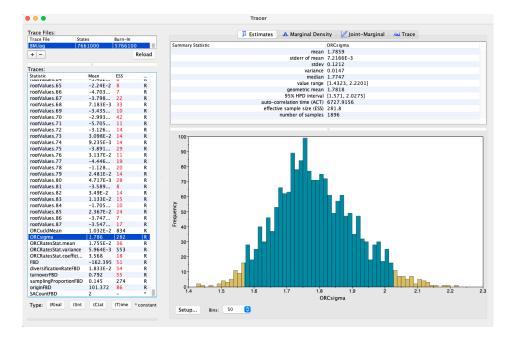


Figure 22: Estimated standard deviations.

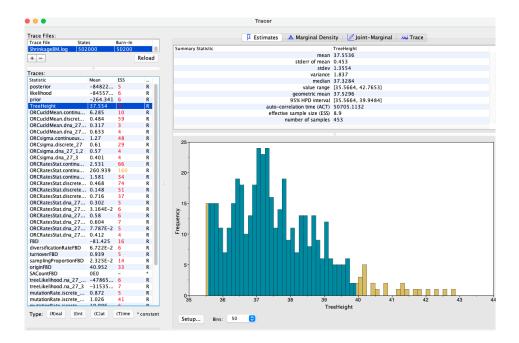


Figure 23: Summarised MCC trees estimated from the combined data sets.

**(c)** This tutorial was written by Rong Zhang and Fábio K. Mendes for Taming the BEAST and is licensed under a Creative Commons Attribution 4.0 International License.

Version dated: July 15, 2025

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