Project 2: Augmenting functional information about human genes using probabilistic phylogenetic modeling

George G. Vega Yon
vegayon@usc.edu

Department of Preventive Medicine

University of Southern California

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Agenda

On Genes and Trees

Model

Peeling algorithm

The `aphylo` R package

The `amcmc` R package

Bayesian Estimation of the parameters

Concluding Remarks

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Overview

- A **GO annotation** is an association between a gene and a GO (Gene Ontology) term describing its function, e.g: A gene can be annotated with the GO term `GO:0016049`, which denotes *cellular growth*.
- **Phylogenetic Tree** represents "inferred evolutionary relationships among various biological species or other entities" (wiki), in this context, our entities are genes.
- **PANTHER Classification System** (PantherDB), part of the Gene Ontology Consortium, consists on a database of $\sim$ 15,000 phylogenetic trees (gene families), and can be linked to the GO terms.

Overview (cont.)

- Manual Curation of GO terms is good but infeasible: Out of all the genes present in PantherDB, only ~9% has been annotated (17 years of work)
- Today, we present a model that uses both: (1) existing gene functional annotations, and (2) phylogenetic trees to infer annotations on un-annotated genes in a *probabilistic way* (so it is not a 0/1 prediction).
- This predicted functional information will serve as prior covariates in Projects 1 and 3.

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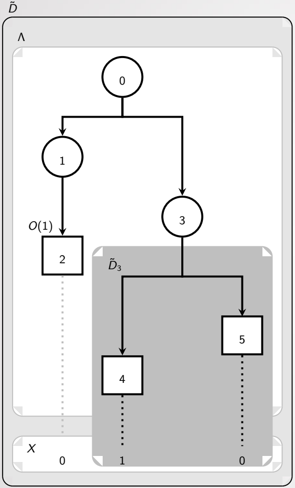
Some definitions



Symbol	Description
$\tilde{D}$	Observed Annotated Tree
Λ	Partially ordered phylogenetic tree (PO tree)
$O(n)$	Offspring of node n
$\tilde{D}_n$	n-induced Annotated Sub-tree
X	Experimental annotation

Where

$$x_{lp} = \begin{cases} 1 & \text{if the function } p \text{ is believed to be present} \\ 0 & \text{if the function } p \text{ is believed to be absent} \\ 9 & \text{if we don't have information for this node} \end{cases}$$

Formal definitions

A probabilistic model of function propagation

1. For any given node, we can write down the probability of observing a *functional state* as a function of some model parameters and its offspring.
2. This version of our model has five parameters (probabilities):

   2.1 Root node had a function: π,
   2.2 Gain of function: μ_0,
   2.3 Loss of function: μ_1.
   2.4 Misclassification of:

   - A missing function as present, ψ_0, and
   - A present function as missing, ψ_1

   All five parameters are assumed to be equal across functions, this is, $\pi, \mu_0, \mu_1, \psi_0$, and ψ_1 are assumed to be independent of the functions that are analyzed.
3. In this presentation, we will focus on the case that $P = 1$.

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Peeling phylogenies

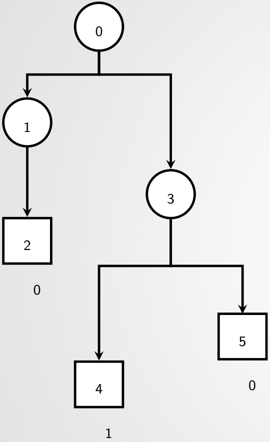
Given an Experimentally Annotated (PO) Phylogenetic Tree, the likelihood computation on a single function is as follows.

1. Create an matrix Pr of size $2 \times |N|$,
2. For node $n \in \{|N|, |N|-1, \dots, 1, 0\}$ (the peeling sequence) do:
   - 2.1 For $z_n \in \{0, 1\}$ do:
     - 2.1.1 Set $\text{Pr}_{n,z_n} = \begin{cases} \Pr\left(Z_n = z_n \mid X_n = X_n\right) & \text{If n is a leaf} \\ \Pr\left(Z_n = z_n \,\middle|\, \tilde{D}_n\right) & \text{otherwise} \end{cases}$
     - 2.1.2 Next z_n
   - 2.2 Next n
3. At this point the matrix Pr should be completely filled, so following (3), we can compute

$$
\text{L}\left(\psi, \mu, \pi \middle| \tilde{D}\right) = \sum_{z_0 \in \{0,1\}} \Pr\left(Z_0 = z_0 \mid \pi\right)\text{Pr}_{0,z_0}
$$

Let's see an example! details

Peeling algorithm

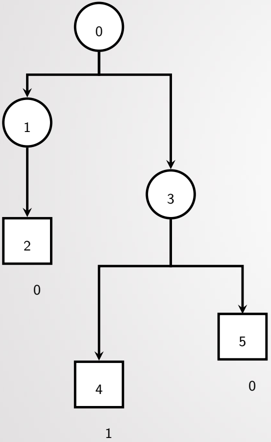


- Let's calculate the likelihood of observing this tree with the following parameters:

$$
\begin{aligned}
\psi_0 &= 0.1\\
\psi_1 &= 0.05\\
\mu_0 &= 0.04\\
\mu_1 &= 0.01\\
\pi &= 0.5
\end{aligned}
$$

Peeling algorithm (cont. 1)

$\psi_0 = 0.1 \qquad \psi_1 = 0.05 \qquad \mu_0 = 0.04 \qquad \mu_1 = 0.01 \qquad \pi = 0.5$



	State 0	State 1
0		
1		
2	0.9000	0.0500
3		
4	0.1000	0.9500
5	0.9000	0.0500

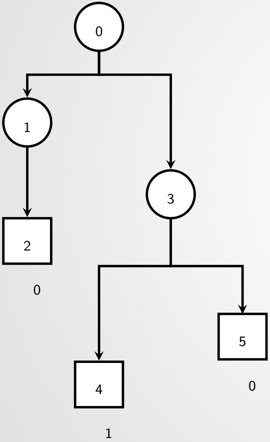
$$\Pr(Z_2 = 0 \mid X_2 = 0) = 1 - \psi_0 \qquad = 0.9$$
$$\Pr(Z_2 = 1 \mid X_2 = 0) = \psi_1 \qquad = 0.05$$

$$\Pr(Z_4 = 0 \mid X_4 = 1) = \psi_0 \qquad = 0.1$$
$$\Pr(Z_4 = 1 \mid X_4 = 1) = 1 - \psi_1 \qquad = 0.95$$

$$\Pr(Z_5 = 0 \mid X_5 = 0) = 1 - \psi_0 \qquad = 0.9$$
$$\Pr(Z_5 = 1 \mid X_5 = 0) = \psi_1 \qquad = 0.05$$

Peeling algorithm (cont. 2)

$\psi_0 = 0.1 \qquad \psi_1 = 0.05 \qquad \mu_0 = 0.04 \qquad \mu_1 = 0.01 \qquad \pi = 0.5$



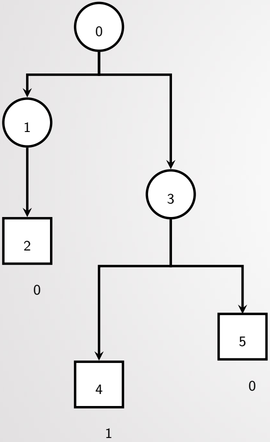
	State 0	State 1
0		
1	0.8660	0.0585
2	0.9000	0.0500
3		
4	0.1000	0.9500
5	0.9000	0.0500

$$\begin{aligned}\Pr\left(Z_1 = 0 \;\middle|\; \tilde{D}_1\right) &= \Pr(Z_2 = 0 \mid X_2 = 0)(1 - \mu_0) + \\ &\quad \Pr(Z_2 = 1 \mid X_2 = 0)\mu_0 \\ &= 0.9000 \times 0.96 + 0.0500 \times 0.04 = 0.866\end{aligned}$$

$$\begin{aligned}\Pr\left(Z_1 = 1 \;\middle|\; \tilde{D}_1\right) &= \Pr(Z_2 = 0 \mid X_2 = 0)\mu_1 + \\ &\quad \Pr(Z_2 = 1 \mid X_2 = 0)(1 - \mu_1) \\ &= 0.9000 \times 0.01 + 0.0500 \times 0.99 = 0.0585\end{aligned}$$

Peeling algorithm (cont. 3)

$\psi_0 = 0.1 \qquad \psi_1 = 0.05 \qquad \mu_0 = 0.04 \qquad \mu_1 = 0.01 \qquad \pi = 0.5$



	State 0	State 1
0	0.0947	0.0037
1	0.8660	0.0585
2	0.9000	0.0500
3	0.1160	0.0551
4	0.1000	0.9500
5	0.9000	0.0500

$$\Pr\left(Z_3 = 0 \mid \tilde{D}_3\right) = \prod_{m \in \{4,5\}} \sum_{z_m \in \{0,1\}} \Pr\left(Z_m = z_m \mid \tilde{D}_m\right) \Pr\left(Z_m = z_m \mid Z_3 = 0\right)$$

$$= \left(0.1(1-\mu_0) + 0.95 \times \mu_0\right) \times \left(0.9(1-\mu_0) + 0.05 \times \mu_0\right)$$

$$= (0.1(1-0.04) + 0.95 \times 0.04) \times (0.9(1-0.04) + 0.05 \times 0.04)$$

$$= 0.116$$

Finally, the likelihood of this tree is:

$$\mathrm{L}\left(\psi, \mu, \Pi \mid \tilde{D}\right) = (1-\pi)\Pr\left(Z_0 = 0 \mid \tilde{D}_0\right) + \pi \Pr\left(Z_0 = 1 \mid \tilde{D}_0\right)$$

$$= (1-0.5) \times 0.0947 + 0.5 \times 0.0037 = 0.0492$$

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`aphylo` in a nutshell

- Provides a representation of *annotated* partially ordered trees.
- Interacts with the ape package (most used Phylogenetics R package with ~25K downloads/month)
- Implements the loglikelihood calculation of our model (with C++ under-the-hood).

Examples: Simulating Trees

```r
set.seed(80)
tree <- sim_tree(5)
tree
```

```
##
## A PARTIALLY ORDERED PHYLOGENETIC TREE
##
##   # Internal nodes: 4
##   # Leaf nodes    : 5
##
##   Leaf nodes labels:
##     4, 5, 6, 7, 8.
##
##   Internal nodes labels:
##     0, 1, 2, 3.
```

```r
atree <- sim_annotated_tree(
  tree = tree, P = 2,
  psi  = c(.05, .05),
  mu   = c(.2, .1),
  Pi   = .01
  )

atree
```

```
##
## A PARTIALLY ORDERED PHYLOGENETIC TREE
##
##   # Internal nodes: 4
##   # Leaf nodes    : 5
##
##   Leaf nodes labels:
##     4, 5, 6, 7, 8.
##
##   Internal nodes labels:
##     0, 1, 2, 3.
##
## ANNOTATIONS:
##      fun0000 fun0001
```

Examples: Visualizing annotated data

```
plot(atree)
```

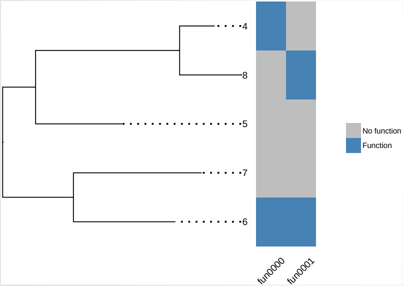

Figure 1: Visualization of annotations and tree structure.

```
plot_LogLike(atree)
```

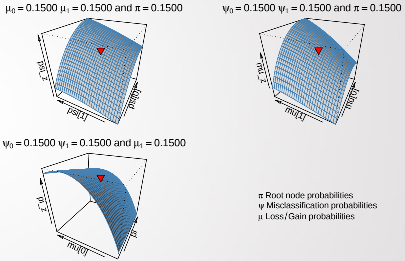

Figure 2: LogLikelihood surface of the simulated data

Example: Tree pruning

- The peeling algorithm requires visiting all nodes in a tree.
- The fact is, we don't need to go through branches with no annotations, as these are uninformative. So we can prune them, e.g.:

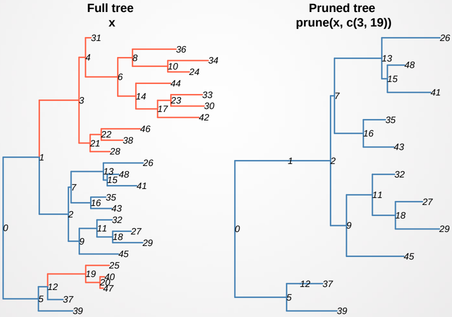

Figure 3: Pruning trees. In the original none of the leaf nodes under 3 and 9 have annotations. After pruning those branches, we go from having 49 nodes, to have 21

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Yet another MCMC package

You may be wondering why, well:

1. Allows running multiple chains simultaneously (parallel)
2. Overall faster than other Metrop MCMC algorithms (from our experience)
3. Planning to include other types of kernels (the Handbook of MCMC)
4. Implements reflective boundaries random-walk kernel

Example: MCMC

```r
# Loading the packages
library(amcmc)
library(coda)

# Defining the ll function (data was already defined)
ll <- function(x, D) {
  x <- log(dnorm(D, x[1], x[2]))
  sum(x)
}

ans <- MCMC(
  # Ll function and the starting parameters
  ll, c(mu=1, sigma=1),
  # How many steps, thinning, and burn-in
  nbatch = 1e5, thin=10, burnin = 1e4,
  # Kernel parameters
  scale = .1, ub = 10, lb = c(-10, 0),
  # How many parallel chains
  nchains = 4,
  # Further arguments passed to ll
  D=D
  )
```

Example: MCMC (cont. 1)

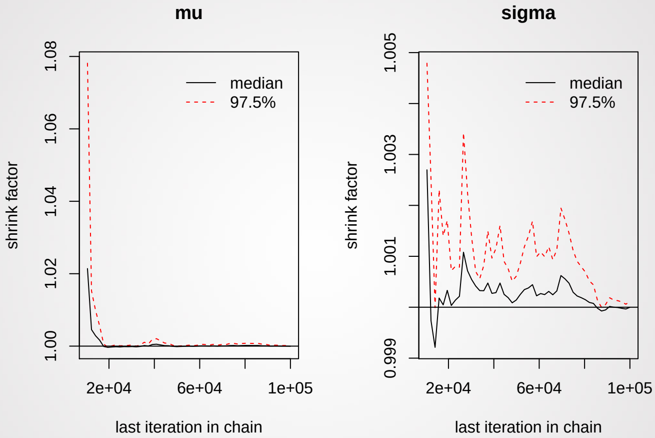

Figure 4: Gelman diagnostic for convergence. The closer to 1, the better the convergence. Rule of thumb: A chain has a reasonable convergence if it has a Potential Scale Reduction Factor (PSRF) below 1.15.

Example: MCMC (cont. 2)

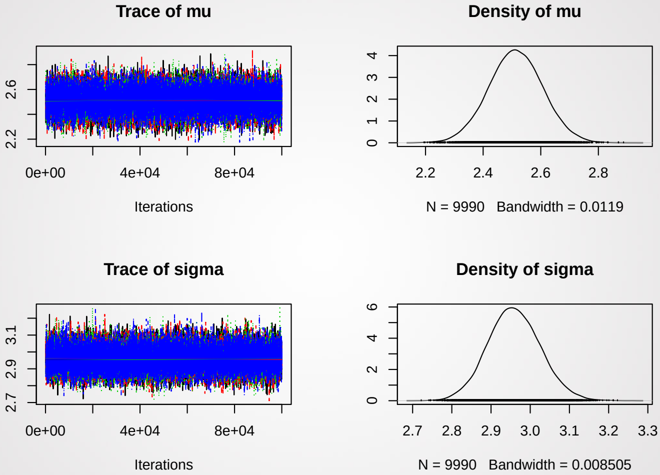

Figure 5: Posterior distribution

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Putting all together

Let's start by reading some data

```
# Reading the data
path <- system.file("tree.tree", package="aphylo")
dat <- read_panther(path)

# The tree
dat$tree
```

```
##
## Phylogenetic tree with 145 tips and 107 internal nodes.
##
## Tip labels:
##  AN5:MONBE|Gene=28576|UniProtKB=A9V8K6, AN7:SCHPO|PomBase=SPAC25B8.12c|UniProtKB=Q9UTA6
## Node labels:
##  AN0, AN1, AN2, AN3, AN4, AN6, ...
##
## Rooted; includes branch lengths.
```

Putting all together (cont.)

```r
# Extra annotations
head(dat$internal_nodes_annotations)
```

```
##     branch_length type           ancestor duplication
## AN0            NA    S               LUCA       FALSE
## AN1         0.057    S Archaea-Eukaryota       FALSE
## AN2         0.244    S          Eukaryota       FALSE
## AN3         0.436    S           Unikonts       FALSE
## AN4         0.417    S       Opisthokonts       FALSE
## AN6         0.684    D               <NA>        TRUE
```

Putting all together: MCMC of the model

In this example, using data from PANTHERDB, we will simulate a single function and use the aphylo_mcmc function for obtaining parameter estimates

```r
tree <- dat$tree

# Simulating a function
set.seed(123)
atree <- sim_annotated_tree(
  tree= as_po_tree(tree),
  Pi = .05, mu = c(.1, .05), psi = c(.01, .02)
)

# Estimation
ans <- aphylo_mcmc(
  params  = rep(.05, 5),
  dat     = atree,
  # Passing a Beta prior
  priors  = function(p) dbeta(p, 2, 20),
  # Parameters for the MCMC
  control = list(nchain=4, nbatch=1e4, thin=20, burnin=1e3)
  )
```

Putting all together: MCMC of the model (cont. 1)

```
ans
```

```
##
## ESTIMATION OF ANNOTATED PHYLOGENETIC TREE
## ll:  -57.0072,
## Method used: mcmc (10000 iterations)
## Leafs
##  # of Functions 1
##  # of 0:    99 (68%)
##  # of 1:    46 (32%)
##
##          Estimate  Std. Error
##  psi[0]    0.0527      0.0289
##  psi[1]    0.0502      0.0312
##  mu[0]     0.0777      0.0226
##  mu[1]     0.0397      0.0266
##  Pi        0.0907      0.0620
```

How good is our prediction

```r
# Looking at the posterior probabilities
head(predict(ans, what="leafs"))
```

```
##                                                                  fun0000
## AN87:STAA8|EnsemblGenome=SAOUHSC_01375|UniProtKB=Q2FYR0          0.06121
## AN88:DEIRA|EnsemblGenome=DR_2147|UniProtKB=Q9RSH7                0.06033
## AN219:LEPIN|EnsemblGenome=LB_007|UniProtKB=Q8EY50                0.06032
## AN223:CHLTR|EnsemblGenome=CT_103|UniProtKB=O84105                0.06032
## AN29:CHLRE|EnsemblGenome=CHLREDRAFT_196269|UniProtKB=A8HYJ4 0.10564
## AN65:PYRAE|EnsemblGenome=PAE3495|UniProtKB=Q8ZT04                0.10611
```

```r
# And to the prediction score
prediction_score(ans)
```

```
## PREDICTION SCORE: ANNOTATED PHYLOGENETIC TREE
## Observed : 0.06 (146.89)
## Random   : 0.25 (591.44)
## Best     : 0.00 (0.00)
## Worse    : 1.00 (2365.77)
## ---------------------------------------------------------------------------
## Values between 0 and 1, 0 being best. Absolute scores in parenthesis.
```

How good is our prediction (cont. 1)

```
plot(prediction_score(ans), main="")
```

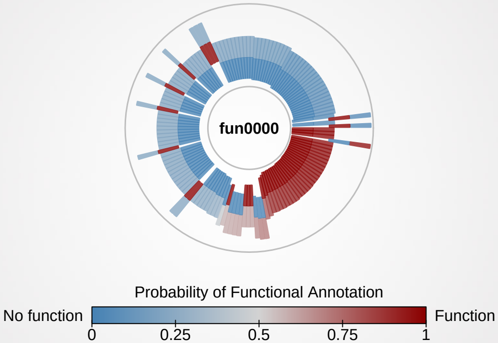


Figure 6: Predicted versus Observed values. Each slice of the pie represents a gene, the outer half of a slice is the predicted value, while the inner half is the observed value. Good predictions will coincide in color and show the slice closer to the center of the plot.

A simulation study

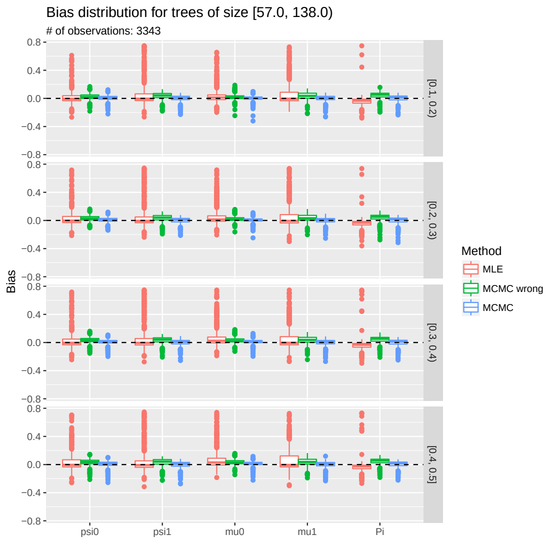
Setup

- Simulation study using ∼13,000 families from PantherDB
- Using a Beta 1/20 prior, we simulated annotations:
  - Draw a set of the parameters $\{\psi_0, \psi_1, \mu_0, \mu_1, \pi\}$,
  - Simulated annotations using our model's Data Generating Process,
  - Randomly removed $p \in [.1, .5]$ proportion of annotations.
- With that data, we did parameter estimation and computed prediction scores using
  - MLE
  - MCMC with the right prior (Beta 1/20), and
  - MCMC with the wrong prior (Beta 1/10, twice the mean as the right prior).

Convergence

A simulation study

Bias



A simulation study

Prediction scores

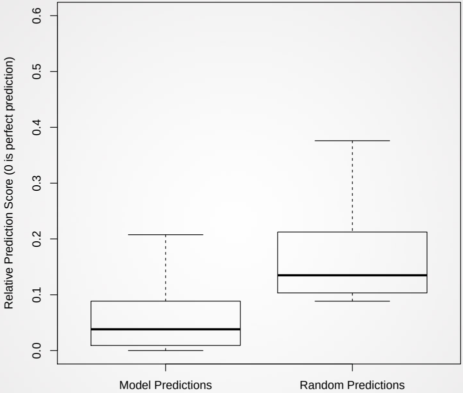


Figure 7: Distribution of prediction scores. The random prediction scores were computed analytically with parameter $p = 0.3$ (as resulting from the DGP).

Concluding Remarks

- A parsimonious model of gene functions: easy to apply on a large scale (we already ran some simulations using all 14,000 trees from the Panther DB).
- Already implemented, we are currently in the stage of writing the paper and setting up the simulation study.
- For the next steps, we are evaluating whether to include or how to include:
    - Type of node: speciation, duplication, horizontal transfer.
    - Branch lengths
    - Correlation structure between functions
    - Using Taxon Constraints to improve predictions
    - Hierarchical model: Use fully annotated trees by curators as prior information.

Thank you!

Project 2: Augmenting functional information about human genes using probabilistic phylogenetic modeling

George G. Vega Yon
vegayon@usc.edu

Department of Preventive Medicine

University of Southern California

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Formal definitions

go back

1. Phylogenetic tree: In our case, we talk about partially ordered phylogenetic tree, in particular, $\Lambda \equiv (N, E)$ is a tuple of nodes N, and edges
$$E \equiv \{(n, m) \in N \times N : n \mapsto m, n < m\}$$
2. Offspring of n: $O(n) \equiv \{m \in N : (n, m) \in E, n \in N\}$
3. Parent node of m: $r(m) \equiv \{n \in N : (n, m) \in E, m \in N\}$
4. Leaf nodes: $L(\Lambda) \equiv \{m \in N : O(m) = \{\emptyset\}\}$
5. Annotations: Given P functions, $Z \equiv \{z_n \in \{0, 1\}^P : n \in L(\Lambda)\}$
6. Annotated Phylogenetic Tree $D \equiv (\Lambda, Z)$
7. Observed Annotated Annotations $X = \{x_l\}_{l \in L(\Lambda)}$,
8. Experimentally Annotated Phylogenetic Tree $\tilde{D} \equiv (\Lambda, X)$

Leaf node probabilities

go back

- The probability of the leaf nodes having annotations z_l conditional on the observed annotation is

$$\Pr\left(Z_l = z_l \mid X_l = x_l\right) = \begin{cases} \psi & \text{if } x_l \neq z_l \\ 1 - \psi & \text{otherwise} \end{cases} \tag{1}$$

Where ψ can be either ψ_0 (mislabelling a zero), or ψ_1 (mislabelling a one).

Internal node probabilities

go back

- In the case of the internal nodes, the probability of a given state is defined in terms of the gain/loss probabilities

$$\Pr\left(Z_n = z_{lp} \;\middle|\; Z_{r(n)} = z_{r(n)}\right) = \begin{cases} \mu & \text{if } z_n \neq z_{r(n)} \\ 1-\mu & \text{otherwise} \end{cases}$$

  Where μ can be either μ_0 (gain), or μ_1 (loss).

- Assuming independence accross offspring, we can write

$$\Pr\left(Z_n = z_n \;\middle|\; \tilde{D}_n\right) = \prod_{m\in O(n)} \sum_{z_m\in\{0,1\}} \Pr\left(Z_m = z_m \;\middle|\; \tilde{D}_m\right) \Pr\left(Z_m = z_m \mid Z_n = z_n\right) \tag{2}$$

  Notice that if m is a leaf node, then $\Pr\left(Z_m = z_m \;\middle|\; \tilde{D}_m\right) = \Pr\left(Z_m = z_m \mid X_m = x_m\right)$.

Likelihood of the tree

go back

- Once the computation reaches the root node, $n = 0$, equations (1) and (2):

$$\Pr\left(Z_l = z_l \mid \tilde{D}_l\right) = \Pr\left(Z_l = z_{lp} \,\middle|\, X_l = x_l\right) \tag{1}$$

$$\Pr\left(Z_n = z_n \mid \tilde{D}_n\right) = \prod_{m \in O(n)} \sum_{z_m \in \{0,1\}} \Pr\left(Z_m = z_m \mid \tilde{D}_m\right) \Pr\left(Z_m = z_m \mid Z_n = z_n\right) \tag{2}$$

Allow us writing the likelihood of the entire tree

$$\mathrm{L}\left(\psi, \mu, \pi \middle| \tilde{D}\right) = \sum_{z_0 \in \{0,1\}} \Pr\left(Z_0 = z_0 \mid \pi\right) \Pr\left(Z_0 = z_0 \,\middle|\, \tilde{D}_0\right) \tag{3}$$

Where $\Pr\left(Z_0 = z_0 \mid \pi\right) = \pi^{z_0} \left(1 - \pi\right)^{1 - z_0}$

A simulation study

Convergence

go back

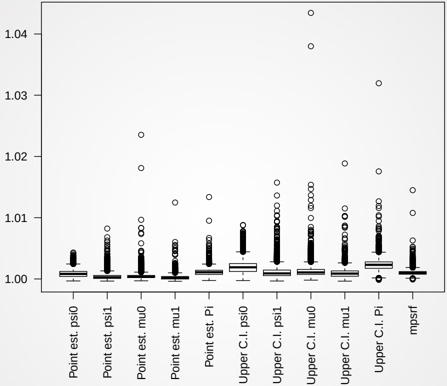

Figure 8: Gelman diagnostic for convergence. The closer to 1, the better the convergence. Rule of thumb: A chain has a reasonable convergence if it has a Potential Scale Reduction Factor (PSRF) below 1.15.