

# The PhyloSim package

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## Contents

<b>1</b>	<b>Getting help</b>	<b>2</b>
<b>2</b>	<b>Basic examples</b>	<b>2</b>
2.1	Simulating substitutions under the JC69 model . . . . .	2
2.1.1	Compact example . . . . .	2
2.1.2	“Unrolled” example . . . . .	3
2.2	Simulating substitutions under the HKY model . . . . .	5
2.3	Simulating among-sites rate variation . . . . .	7
2.3.1	Simulating under the discrete gamma (GTR+d $\Gamma$ ) model . . . . .	7
2.3.2	Simulating under the invariants and discrete gamma (GTR+I+d $\Gamma$ ) model . . . . .	9
2.4	Simulating indels . . . . .	10
2.4.1	Insertion and deletions having the same length distribution . . . . .	10
2.4.2	Insertion and deletions having different length distributions . . . . .	11
2.4.3	Simulating indels under selective constraints . . . . .	12
2.5	Simulating partitions . . . . .	14
2.6	Simulating heterotachy . . . . .	15
2.7	Simulating many replicates . . . . .	18
2.8	Simulating many replicates in parallel . . . . .	19
<b>3</b>	<b>Advanced examples</b>	<b>21</b>
3.1	Simulating “domains” and heterogeneous evolution . . . . .	21
3.2	Evolving codon sequences . . . . .	27
3.3	Implementing a new process . . . . .	32
3.4	Evolving a genomic region containing a “gene” . . . . .	34
<b>4</b>	<b>Details of the fast field deletion model</b>	<b>41</b>

# 1 Getting help

- PhyloSim is extensively documented; the documentation of the `PhyloSim` class (as invoked by `help("PhyloSim")`) is probably a good entry point for beginners and also contains some more basic examples.
- Serious users might consider reading all class documentations which provide more focused examples.
- Additional example scripts can be found at <http://github.com/sbotond/phylosim/tree/master/examples>
- The `ll()` method list the methods and virtual field implemented in the immediate class of an object which is useful as “proto-documentation”.

# 2 Basic examples

The following examples illustrate how to set up simple simulations.

First load the package:

```
> library(phylosim)
> set.seed(833)
```

Create the tree files used in the basic examples:

```
> cat("((t1:0.3,t2:0.3):0.2,t3:0.1);",file="3taxa.nwk")
> cat("((t1:0.3,t2:0.3):0.2,(t3:0.1,t4:0.1):0.4);",file="4taxa.nwk")
```

## 2.1 Simulating substitutions under the JC69 model

We will simulate the evolution of nucleotide sequences of length 50 along a tree with 3 tips under the JC69 model. The following two examples are essentially equivalent.

### 2.1.1 Compact example

```
> Simulate(PhyloSim(
+   root.seq=sampleStates( NucleotideSequence(len=50,proc=list(list(JC69())) ),
+   phylo=read.tree("3taxa.nwk")
+ ))$alignment
```

Simulating edge 1 of 4

Simulating edge 2 of 4

Simulating edge 3 of 4

Simulating edge 4 of 4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Root node 4	"T"	"C"	"T"	"T"	"T"	"G"	"A"	"A"	"G"	"A"	"G"	"T"	"G"	"T"	"C"	"G"	"T"	"T"	"C"
t3	"T"	"C"	"T"	"C"	"T"	"G"	"A"	"A"	"G"	"A"	"G"	"A"	"G"	"T"	"C"	"G"	"T"	"T"	"C"
Node 5	"T"	"G"	"T"	"T"	"T"	"G"	"A"	"A"	"G"	"A"	"G"	"T"	"G"	"T"	"A"	"G"	"T"	"T"	"C"
t2	"T"	"G"	"T"	"T"	"T"	"G"	"C"	"T"	"G"	"A"	"C"	"T"	"A"	"T"	"T"	"G"	"T"	"T"	"C"
t1	"T"	"T"	"T"	"T"	"T"	"G"	"A"	"A"	"G"	"A"	"G"	"A"	"G"	"T"	"A"	"G"	"T"	"T"	"C"
Root node 4	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
t3	"C"	"A"	"G"	"G"	"C"	"C"	"T"	"T"	"T"	"T"	"G"	"T"	"G"	"A"	"C"	"T"	"C"	"A"	"T"
	"C"	"A"	"G"	"G"	"C"	"C"	"T"	"T"	"T"	"T"	"G"	"T"	"G"	"A"	"C"	"T"	"C"	"A"	"T"

Node 5	"C"	"A"	"A"	"G"	"A"	"A"	"T"	"T"	"T"	"T"	"G"	"T"	"G"	"A"	"C"	"G"	"T"	"C"	"T"
t2	"C"	"A"	"A"	"G"	"A"	"A"	"T"	"T"	"T"	"C"	"C"	"T"	"A"	"A"	"C"	"G"	"T"	"G"	"G"
t1	"C"	"A"	"A"	"G"	"A"	"A"	"A"	"T"	"T"	"T"	"G"	"A"	"G"	"A"	"C"	"C"	"G"	"C"	"T"
	39	40	41	42	43	44	45	46	47	48	49	50							
Root node 4	"A"	"C"	"T"	"G"	"C"	"A"	"G"	"T"	"A"	"T"	"A"	"A"							
t3	"A"	"C"	"T"	"G"	"C"	"A"	"G"	"T"	"A"	"T"	"A"	"A"							
Node 5	"A"	"C"	"T"	"G"	"C"	"A"	"G"	"T"	"A"	"T"	"C"	"A"							
t2	"C"	"C"	"T"	"G"	"C"	"A"	"A"	"T"	"A"	"T"	"C"	"G"							
t1	"A"	"A"	"C"	"G"	"G"	"A"	"G"	"T"	"A"	"T"	"C"	"A"							

### 2.1.2 “Unrolled” example

Construct the root sequence object:

```
> root.seq<-NucleotideSequence(length=50)
```

Print out the root sequence:

```
> print(root.seq)
```

```
[1] "????????????????????????????????????????????????????????"
```

Note, that all states are still undefined.

Construct a JC69 substitution process object:

```
> p<-JC69()
```

Attach the substitution process to root sequence:

```
> attachProcess(root.seq,p)
```

Sample the site states from the equilibrium distribution of the JC69 substitution process:

```
> sampleStates(root.seq)
```

Print out the root sequence, now with the sampled states:

```
> print(root.seq)
```

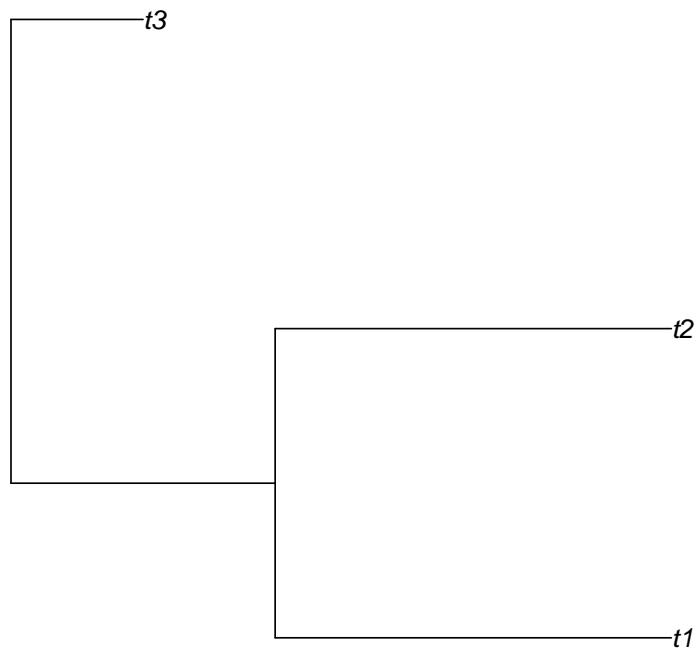
```
[1] "CCGTAGTAGGCACGTCGCCGTCGTCTAGTGACCCGACGGGATTAGTGATT"
```

Read in tree from file using APE:

```
> tree<-read.tree("3taxa.nwk")
```

Plot the tree:

```
> plot(tree)
```



Construct a PhyloSim object:

```
> sim<-PhyloSim()
```

Set the phylo object:

```
> sim$phylo<-tree
```

Set the root sequence:

```
> sim$rootSeq<-root.seq
```

Run the simulation:

```
> Simulate(sim)
```

```
Simulating edge 1 of 4  
Simulating edge 2 of 4  
Simulating edge 3 of 4  
Simulating edge 4 of 4
```

Display the resulting alignment matrix:

```
> sim$alignment
```

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Root	node 4	"C"	"C"	"G"	"T"	"A"	"G"	"T"	"A"	"G"	"G"	"C"	"A"	"C"	"G"	"T"	"C"	"G"	"C"	"C"
t3		"C"	"C"	"G"	"T"	"A"	"G"	"T"	"A"	"G"	"G"	"C"	"C"	"A"	"G"	"T"	"C"	"G"	"C"	"C"
Node 5		"C"	"C"	"G"	"T"	"A"	"G"	"T"	"A"	"G"	"G"	"C"	"G"	"C"	"G"	"T"	"C"	"G"	"A"	"C"
t2		"T"	"C"	"G"	"T"	"A"	"G"	"T"	"A"	"A"	"C"	"T"	"G"	"C"	"G"	"T"	"C"	"G"	"A"	"C"
t1		"C"	"T"	"T"	"T"	"T"	"G"	"T"	"A"	"G"	"G"	"G"	"T"	"C"	"C"	"T"	"C"	"G"	"A"	"C"
		20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Root	node 4	"G"	"T"	"C"	"G"	"T"	"C"	"T"	"A"	"G"	"T"	"G"	"A"	"C"	"C"	"C"	"G"	"A"	"C"	"G"
t3		"G"	"T"	"G"	"G"	"T"	"C"	"T"	"A"	"G"	"T"	"C"	"A"	"C"	"C"	"C"	"G"	"A"	"C"	"G"
Node 5		"G"	"A"	"C"	"G"	"T"	"C"	"T"	"G"	"C"	"T"	"A"	"A"	"C"	"C"	"C"	"G"	"A"	"C"	"G"
t2		"C"	"A"	"C"	"G"	"T"	"C"	"T"	"G"	"C"	"C"	"A"	"G"	"G"	"C"	"C"	"G"	"A"	"C"	"G"
t1		"T"	"A"	"C"	"G"	"T"	"A"	"T"	"G"	"C"	"T"	"G"	"A"	"G"	"T"	"C"	"G"	"A"	"C"	"G"
		39	40	41	42	43	44	45	46	47	48	49	50							
Root	node 4	"G"	"G"	"A"	"T"	"T"	"A"	"G"	"T"	"G"	"A"	"T"	"T"							
t3		"G"	"G"	"A"	"T"	"T"	"A"	"G"	"T"	"G"	"C"	"T"	"T"							
Node 5		"G"	"C"	"A"	"T"	"T"	"T"	"G"	"T"	"G"	"A"	"T"	"T"							
t2		"G"	"C"	"A"	"G"	"T"	"C"	"A"	"T"	"G"	"C"	"T"	"T"							
t1		"T"	"C"	"A"	"G"	"A"	"T"	"G"	"T"	"G"	"A"	"T"	"T"							

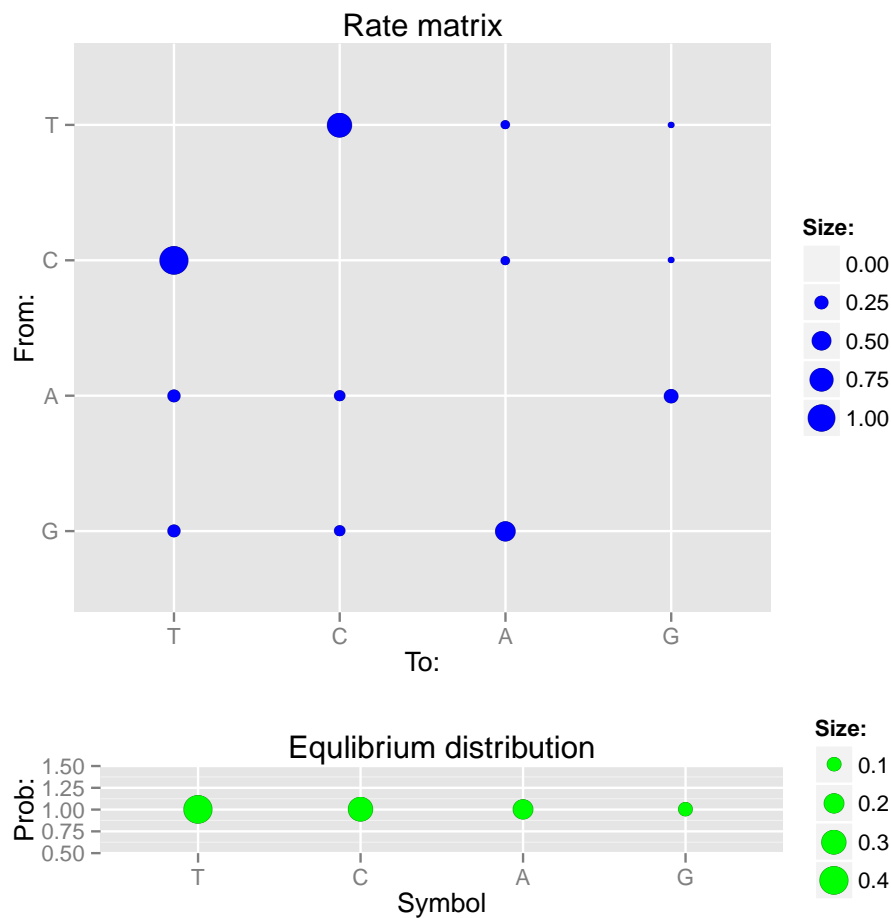
## 2.2 Simulating substitutions under the HKY model

Construct an HKY substitution process object:

```
> p<-HKY(rate.params=list( "Alpha"=10,"Beta"=2),
+         base.freqs=c(4,3,2,1)/10
+         )
```

Get a plot of the instantaneous substitution matrix and equilibrium distribution of the p process (bubble plot):

```
> plot(p,scale=0.5)
```



Construct the root sequence, attach the substitution process to root sequence via the constructor and sample states:

```
> root.len50.seq<-NucleotideSequence(length=50,processes=list(list(p)))
> sampleStates(root.len50.seq)
```

Print out root sequence:

```
> print(root.len50.seq)
```

```
[1] "TTGTATTTAGTCTGTCAATTGTAATACCTCTTCCCACAGTGACTTTACTT"
```

Construct a PhyloSim object, set the phylo object and the root sequence:

```
> sim<-PhyloSim(
+   root.seq=root.len50.seq,
+   phylo=read.tree("3taxa.nwk")
+ );
```

Run simulation:

```
> Simulate(sim)
```

```

Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4

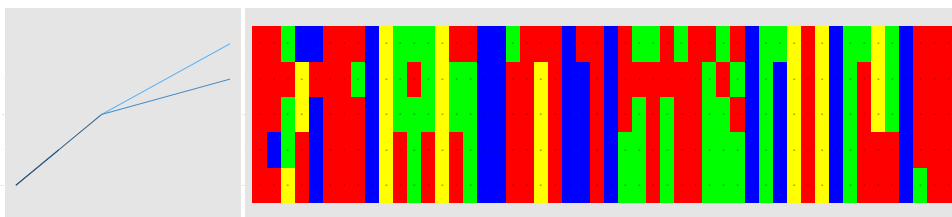
```

Save the resulting alignment (fasta format):

```
> saveAlignment(sim,file="HKY_sim.fas")
```

Plot the alignment alongside the tree (including sequences at ancestral nodes):

```
> plot(sim,num.pages=1)
```



## 2.3 Simulating among-sites rate variation

### 2.3.1 Simulating under the discrete gamma (GTR+dΓ) model

Construct a GTR subsection process object:

```

> p<-GTR(
+       rate.params=list(
+           "a"=1, "b"=2, "c"=3,
+           "d"=1, "e"=2, "f"=3
+       ),
+       base.freqs=c(2,2,1,1)/6
+ )

```

Summary of object p:

```
> summary(p)
```

```

Name: Anonymous
Id: GTR:Anonymous:90011480
Alphabet:
  Type: Nucleotide
  Symbols: T C A G
Rate parameters: a = 1, b = 2, c = 3, d = 1, e = 2, f = 3
Unscaled rate matrix:

```

	T	C	A	G
T	-1.1666667	0.3333333	0.3333333	0.5000000
C	0.3333333	-0.8333333	0.1666667	0.3333333
A	0.6666667	0.3333333	-1.5000000	0.5000000
G	1.0000000	0.6666667	0.5000000	-2.1666667

Equilibrium distribution:

	T	C	A	G
Prob:	0.3333333	0.3333333	0.1666667	0.1666667

Site specific parameters (1):

Id: rate.multiplier  
Name: Rate multiplier  
Type: numeric  
Default value: 1

Construct the root sequence, attach substitution process:

```
> root.seq<-NucleotideSequence(length=50,processes=list( list(p) ))
```

Sample rate multipliers from a discrete gamma distribution with 4 categories and shape parameter 0.5:

```
> plusGamma(root.seq,p,0.5)
```

Get the sampled rate multipliers:

```
> getRateMultipliers(root.seq,p);
```

```
[1] 0.03338775 0.82026848 2.89442785 0.82026848 0.82026848 0.03338775 0.03338775  
[8] 0.03338775 0.03338775 0.82026848 0.82026848 0.25191592 0.03338775 0.82026848  
[15] 2.89442785 0.82026848 0.03338775 2.89442785 0.25191592 0.82026848 0.03338775  
[22] 0.82026848 2.89442785 2.89442785 0.03338775 0.82026848 0.25191592 0.25191592  
[29] 0.82026848 0.03338775 2.89442785 0.03338775 0.25191592 0.03338775 0.03338775  
[36] 0.03338775 0.03338775 0.25191592 2.89442785 0.25191592 0.03338775 0.82026848  
[43] 0.82026848 2.89442785 0.03338775 0.03338775 0.03338775 0.25191592 0.82026848  
[50] 0.82026848
```

Construct the PhyloSim object, sample states and set the phylo object:

```
> sim<-PhyloSim(  
+   root.seq=sampleStates(root.seq),  
+   phylo=read.tree("3taxa.nwk")  
+ )
```

Run the simulation:

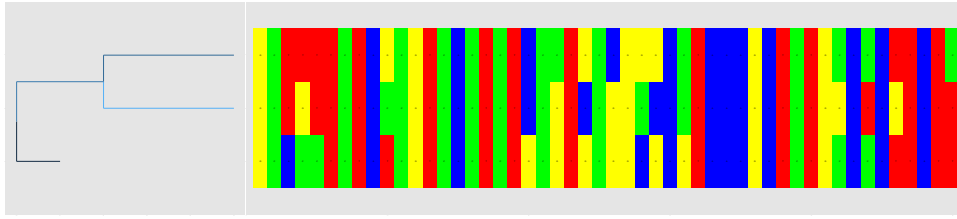
```
> Simulate(sim)
```

```
Simulating edge 1 of 4  
Simulating edge 2 of 4  
Simulating edge 3 of 4  
Simulating edge 4 of 4
```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```
> plot(sim,num.pages=1,plot.ancestors=FALSE)
```





Save the resulting alignment, omitting sequences at internal nodes:

```
> saveAlignment(sim,file="Gamma_sim.fas",skip.internal=TRUE)
```

### 2.3.2 Simulating under the invariants and discrete gamma (GTR+I+d $\Gamma$ ) model

We will reuse the root sequence object and GTR object from the previous example. The process is already attached, but we need to clear the states to have a new root sequence.

Clear the states of the root sequence object and sample a set of new states:

```
> clearStates(root.seq)
> sampleStates(root.seq)
```

Sample rate multipliers from a +I+d $\Gamma$  model:

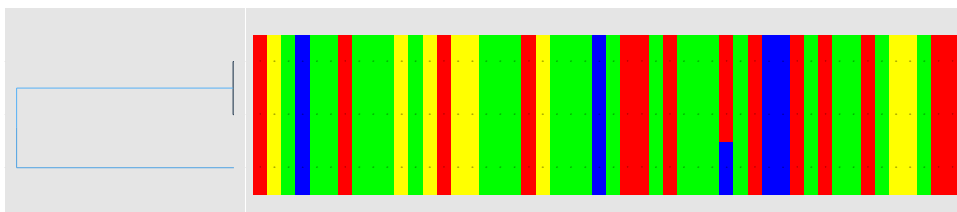
```
> plusInvGamma(root.seq,p,pinv=0.8,shape=0.5)
```

Deal with the rest of the simulation (note that for variety we use a random coalescent tree in this example):

```
> sim<-Simulate(
+   PhyloSim(
+     root.seq=root.seq,
+     phylo=rcoal(3)
+   )
+ )
```

```
Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4
```

```
> plot(sim,num.pages=1,plot.ancestors=FALSE)
```



Save the resulting alignment, skip sequences from internal nodes:

```
> saveAlignment(sim,file="InvGamma_sim.fas",skip.internal=TRUE)
```

## 2.4 Simulating indels

### 2.4.1 Insertion and deletions having the same length distribution

Set up the substitution process and the root sequence:

```
> p<-JC69()
> root.seq<-NucleotideSequence(len=50,processes=list(list(p)))
> sampleStates(root.seq)
```

Construct a deletion process proposing deletions with rate 0.25 according to a discrete length distribution:

```
> d<-DiscreteDeletor(
+           rate=0.25,
+           sizes=c(1,2),
+           probs=c(1/2,1/2)
+ )
```

Construct an insertion process proposing insertions with rate 0.25 according to a discrete length distribution:

```
> i<-DiscreteInsertor(
+           rate=0.25,
+           sizes=c(1,2),
+           probs=c(1/2,1/2)
+ )
```

Set the template sequence for the insertion process:

```
> i$templateSeq<-NucleotideSequence(length=2,processes=list( list(p) ))
```

The states of the template sequence are undefined. The actual states will be sampled from the equilibrium distribution of the attached substitution process before performing the insertion.

Attaching the indel processes:

```
> attachProcess(root.seq,d)
> attachProcess(root.seq,i)
```

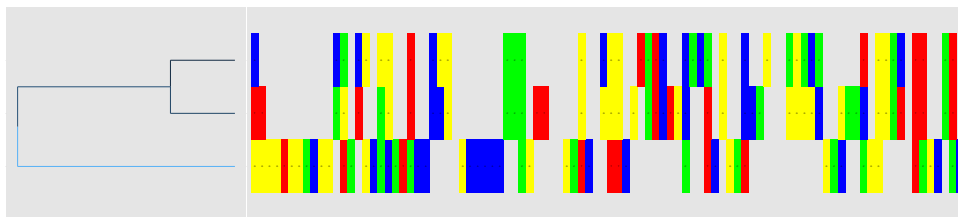
Construct the PhyloSim object, set the phylo object (random coalescent tree for three taxa) and run the simulation:

```
> sim<-Simulate(PhyloSim(
+   root.seq=root.seq,
+   phylo=rcoal(3)
+ ))
```

```
Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4
```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```
> plot(sim,num.pages=1,plot.ancestors=FALSE)
```



Save the resulting alignment, skip sequences from internal nodes:

```
> saveAlignment(sim,file="indel1_sim.fas",skip.internal=TRUE)
```

## 2.4.2 Insertion and deletions having different length distributions

The following code reuses the objects constructed in the previous example to simulate deletions and insertions with different length distributions.

Construct a new insertion process proposing insertions of Cs with rate 0.5 according to a discrete length distribution:

```
> i2<-DiscreteInsertor(
+     rate=0.25,
+     sizes=c(1,2,3,4),
+     probs=c(1,2,3,4)/10,
+     template.seq=NucleotideSequence(string="C")
+ )
```

Clear the states of the root sequence object:

```
> clearStates(root.seq)
```

Define a new set of processes (p - substitution, d - deletion, i2 - the new insertion process) for the root sequence using the `processes` virtual field:

```
> root.seq$processes<-list(list(i2, d, p))
```

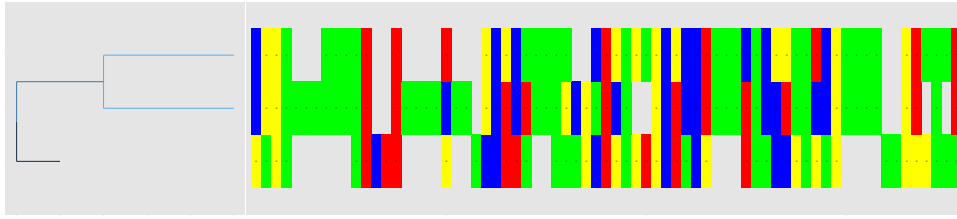
Construct the `PhyloSim` object, sample states, set the phylo object and run the simulation:

```
> sim<-Simulate(PhyloSim(
+     root.seq=sampleStates(root.seq),
+     phylo=read.tree("3taxa.nwk")
+ ))
```

```
Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4
```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```
> plot(sim,num.pages=1,plot.ancestors=FALSE)
```



Save the resulting alignment, skip sequences from internal nodes:

```
> saveAlignment(sim,file="indel2_sim.fas",skip.internal=TRUE)
```

### 2.4.3 Simulating indels under selective constraints

Set up the substitution process and the root sequence:

```
> p<-JC69()
> root.seq<-NucleotideSequence(len=100,processes=list(list(p)))
> sampleStates(root.seq)
```

Construct a deletion process proposing deletions with rate 1 according to a discrete length distribution:

```
> d<-DiscreteDeletor(
+           rate=1,
+           sizes=c(1,2,3),
+           probs=c(3/6,2/6,1/6)
+ )
```

Construct an insertion process proposing insertions with rate 1 according to a discrete length distribution:

```
> i<-DiscreteInsertor(
+           rate=1,
+           sizes=c(1,2,3),
+           probs=c(3/6,2/6,1/6)
+ )
```

Set the template sequence for the insertion process:

```
> i$templateSeq<-NucleotideSequence(length=2,processes=list( list(p) ))
```

Attaching the indel processes:

```
> attachProcess(root.seq,d)
> attachProcess(root.seq,i)
```

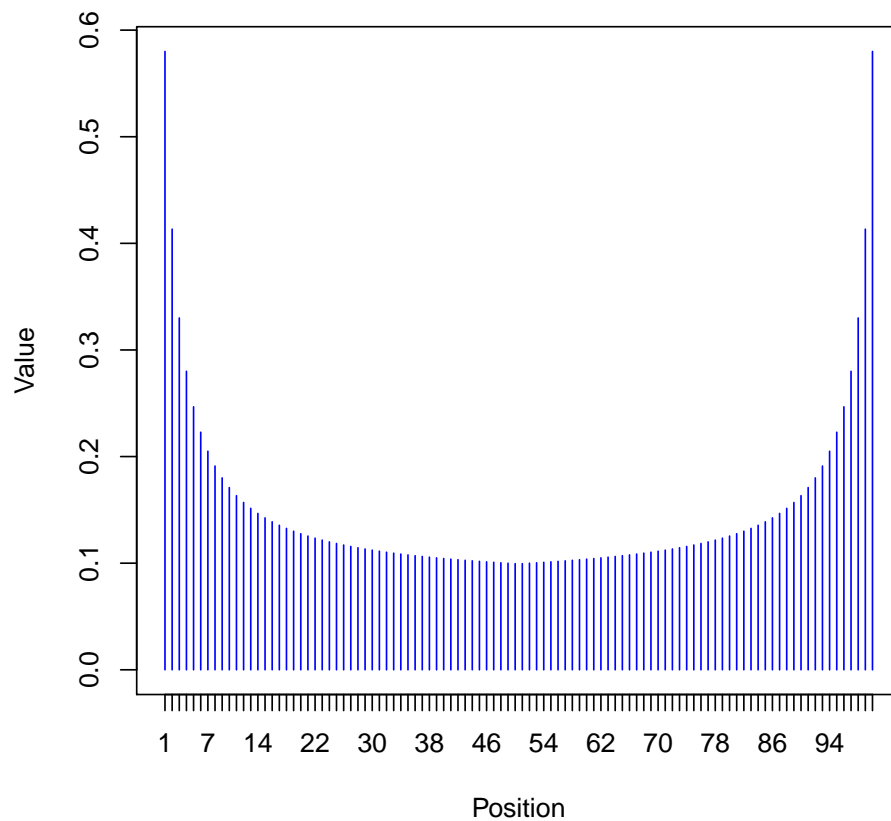
Set deletion tolerance values:

```
> setDeletionTolerance(root.seq,d,0.08 + c(1/2:51,1/51:2))
```

Plot deletion tolerance values:

```
> plotParametersAtSites(root.seq,d,"deletion.tolerance")
```

## parameter deletion.tolerance for process DiscreteDeletor:Anonymo



Set insertion tolerance values:

```
> setInsertionTolerance(root.seq,i,0.08 + c(1/2:51,1/51:2))
```

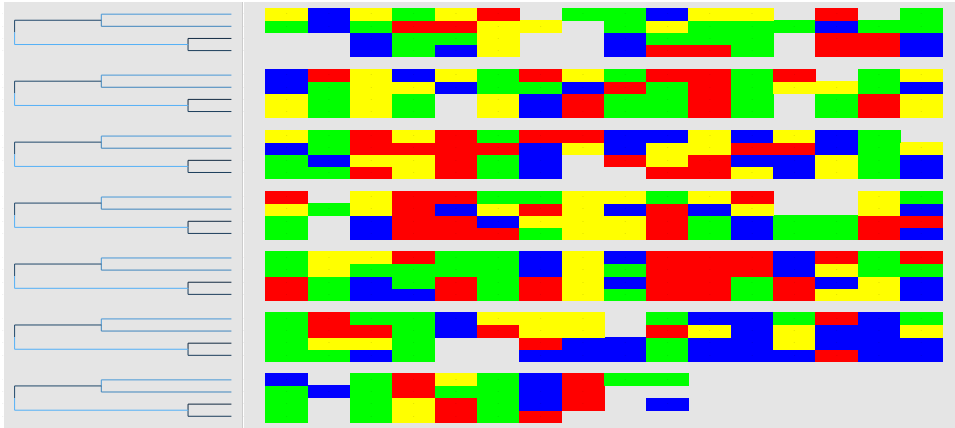
Construct the PhyloSim object, read phylo object from file:

```
> sim<-Simulate(PhyloSim(  
+     root.seq=root.seq,  
+     phylo=read.tree("4taxa.nwk")  
+ ))
```

```
Simulating edge 1 of 6  
Simulating edge 2 of 6  
Simulating edge 3 of 6  
Simulating edge 4 of 6  
Simulating edge 5 of 6  
Simulating edge 6 of 6
```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```
> plot(sim,plot.ancestors=FALSE)
```



Save the resulting alignment, skip sequences from internal nodes:

```
> saveAlignment(sim, file="indel3_sim.fas", skip.internal=TRUE)
```

## 2.5 Simulating partitions

The following example demonstrates how to use the processes and site- and process-specific parameters to simulate “partitions” with different properties.

We will simulate four partitions:

- Partition 1: sites in range 1:25 evolving by JC+d $\Gamma$  with a shape parameter  $\alpha = 1$
- Partition 2: sites in range 26:50 evolving by JC+d $\Gamma$  with a shape parameter  $\alpha = 0.5$
- Partition 3: sites in range 51:75 evolving by HKY+d $\Gamma$  with a shape parameter  $\alpha = 1$
- Partition 4: sites in range 76:100 evolving by HKY+d $\Gamma$  with a shape parameter  $\alpha = 0.5$

First construct two substitution process objects:

```
> jc69<-JC69()
> hky<-HKY(rate.params=list( "Alpha"=5,"Beta"=2),
+               base.freqs=c(4,3,2,1)/10
+               )
```

Construct a root sequence object of length 100:

```
> root.seq<-NucleotideSequence(length=100)
```

Attach process jc69 to range 1:50:

```
> attachProcess(root.seq, jc69, 1:50)
```

Attach process hky to range 51:100:

```
> attachProcess(root.seq, hky, 51:100)
```

Sample rate multipliers in the four partitions:

```

> plusGamma(root.seq,jc69,1,1:25)
> plusGamma(root.seq,jc69,0.5,26:50)
> plusGamma(root.seq,hky,1,51:75)
> plusGamma(root.seq,hky,0.5,76:100)

```

Construct the PhyloSim object, sample states, set root sequence, set the phylo object (random coalescent tree for three taxa) and run the simulation:

```

> sim<-Simulate(PhyloSim(
+   root.seq=sampleStates(root.seq),
+   phylo=rcoal(3)
+ ))

```

```

Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4

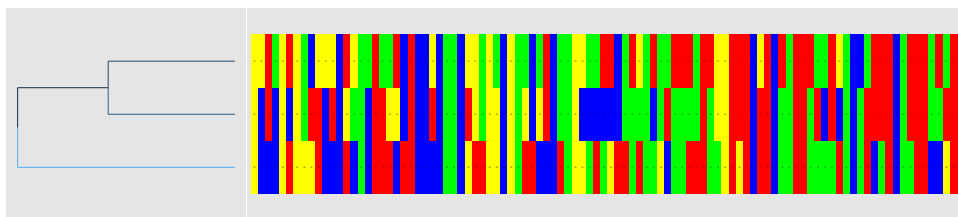
```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```

> plot(sim,num.pages=1,plot.ancestors=FALSE)

```



## 2.6 Simulating heterotachy

Set up the substitution process:

```

> p<-F84(base.freqs=c(1,2,3,4));
> p$kappa<-1;

```

Set up the root sequence:

```

> root.seq<-NucleotideSequence(length=50,processes=list(list(p)));

```

Sample the rate multipliers from a +dΓ model with shape parameter 1.0:

```

> plusGamma(root.seq,p,1)

```

Read a tree from file:

```

> tree<-read.tree("4taxa.nwk")

```

Construct the PhyloSim object, sample states, set root sequence, and set the phylo object:

```
> sim<-PhyloSim(
+   root.seq=sampleStates(root.seq),
+   phylo=tree
+ )
```

A “node hook” is a function which accepts a **Sequence** object through the named argument “seq” and returns a **Sequence** object. After simulating the branch leading to the node, the resulting **Sequence** object is passed to the node hook and the returned object is used to simulate the downstream branches.

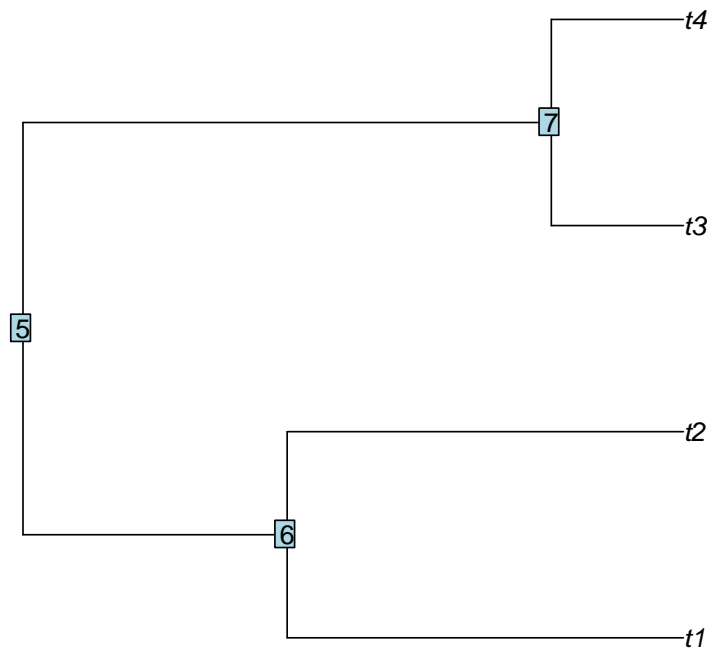
Create a node hook function which will create heterotachy by resampling the site rates from the +dΓ model with shape parameter 1.0:

```
> node.hook <- function(seq) {
+
+   if(!isAttached(seq$sites[[1]],p)){
+       return(seq);
+   }
+
+   cat("Resampling rate multipliers!\n");
+   plusGamma(seq,p,1)
+   return(seq)
+ }
```

Plot the PhyloSim object:

```
> plot(sim)
```





Notice that node 6 is the ancestor of taxa **t1** and **t2**.

Attach the hook to node 6:

```
> attachHookToNode(sim, node = 6, fun = node.hook)
```

Run the simulation:

```
> Simulate(sim)
```

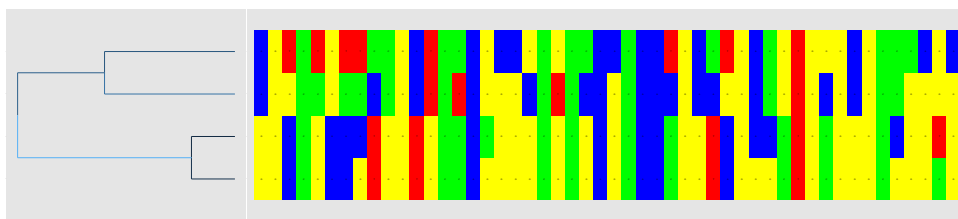
```

Simulating edge 1 of 6
Resampling rate multipliers!
Simulating edge 2 of 6
Simulating edge 3 of 6
Simulating edge 4 of 6
Simulating edge 5 of 6
Simulating edge 6 of 6

```

Plot the alignment alongside the tree, skip sequences at ancestral nodes:

```
> plot(sim,num.pages=1,plot.ancestors=FALSE)
```



## 2.7 Simulating many replicates

Constructing `Sequence` objects with a large number of sites is expensive, so it is a good idea to do that outside the cycle when simulating many replicates with the same root sequence length.

Reusing the root sequence object is easy, but do not forget to do the modifications needed to get independent simulations (e.g. clearing the states of the root sequence, resampling the rate multipliers).

The following code illustrates how to simulate many replicates under the JC69+d $\Gamma$  model.

Construct the root sequence object and attach the substitution process:

```
> p<-JC69();
> root.seq<-NucleotideSequence(length=10)
> attachProcess(root.seq,p)
```

Read the required phylogeny from file (this will remain fixed in the simulated replicates):

```
> tree<-read.tree("3taxa.nwk");
```

Simulate three replicates. Note that the states are cleared and resampled; the rate multipliers are resampled as well. The resulting alignments are stored in files `aln_1.fas`, `aln_2.fas`, `aln_3.fas`.

```
> for(i in 1:3){
+   cat(paste("\n\nSimulating replication ",i,"\n\n",sep=""))
+
+   clearStates(root.seq)
+   plusGamma(root.seq,p,0.25)
+   sampleStates(root.seq)
+
+   sim<-Simulate(PhyloSim(
+     root.seq=root.seq,
+     phylo=tree
+   ))
+
+   saveAlignment(sim,file=paste("aln_",i,".fas",sep=""))
+ }
```

Simulating replication 1

```
Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4
```

Simulating replication 2

```

Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4

```

```

Simulating replication 3

```

```

Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4

```

## 2.8 Simulating many replicates in parallel

The the speed of the above method for simulating replicates can be improved on a multicore machine by running many replicates in parallel by using the `mclapply` method from the `multicore` package (currently not available on Windows operating systems).

Under default settings, the `mclapply` method launches one replication per core and this approach needs enough memory to run all of them in parallel.

The following code illustrates how to simulate many replicates in parallel under the JC69+dΓ model.

Construct the root sequence object and attach the substitution process:

```

> p<-JC69();
> root.seq<-NucleotideSequence(length=50)
> attachProcess(root.seq,p)

```

Read the required phylogeny from file (this will remain fixed in the simulated replicates):

```

> tree<-read.tree("3taxa.nwk");

```

Function to simulate a single replication:

```

> sim.replicate<-function(i){
+   name<-paste("replication_",i,sep="")
+   clearStates(root.seq)
+   plusGamma(root.seq,p,0.25)
+   sampleStates(root.seq)
+
+   sim<-Simulate(PhyloSim(
+     name=name,
+     root.seq=root.seq,
+     phylo=tree,
+   ),
+     quiet=TRUE
+   )
+
+   saveAlignment(sim,file=paste("aln_",i,".fas",sep=""))
+   return(sim)
+   # return(TRUE)
+ }

```

Note that the states are cleared and resampled; the rate multipliers are resampled as well. The resulting alignments are stored in files `aln_1.fas`, `aln_2.fas`, `aln_3.fas`.

Memory can be saved by throwing away the objects generated by the replication by returning `TRUE` (or any other fixed value) from the `sim.replicate` function.

Load the `multicore` package if available:

```
> have.mcore <- is.element("multicore", installed.packages()[,1])
> if(have.mcore){
+   library(multicore)
+ }
```

Run replicates in parallel, print the resulting `PhyloSimobjects`:

```
> if(have.mcore){
+   nr.replicates<-3
+   res.objects<-mclapply(1:nr.replicates, sim.replicate)
+   print(res.objects)
+ }
```

```
[[1]]
[1] "PhyloSim:replication_1:92691720"
```

```
[[2]]
[1] "PhyloSim:replication_2:74469992"
```

```
[[3]]
[1] "PhyloSim:replication_3:101996080"
```

Print one of the resulting alignments:

```
> if(have.mcore){
+   print(res.objects[[1]]$alignment)
+ }
```

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Root node 4	"C"	"G"	"T"	"G"	"G"	"G"	"A"	"T"	"A"	"A"	"A"	"G"	"A"	"A"	"C"	"A"	"A"	"C"	"G"
t3	"C"	"G"	"T"	"G"	"G"	"G"	"A"	"T"	"A"	"A"	"C"	"G"	"A"	"A"	"G"	"A"	"A"	"C"	"G"
Node 5	"C"	"G"	"T"	"G"	"G"	"G"	"A"	"T"	"A"	"A"	"G"	"G"	"A"	"A"	"T"	"A"	"C"	"C"	"G"
t2	"C"	"G"	"T"	"G"	"G"	"T"	"A"	"T"	"A"	"A"	"T"	"A"	"A"	"A"	"A"	"A"	"C"	"T"	"G"
t1	"C"	"G"	"T"	"G"	"G"	"G"	"A"	"T"	"A"	"A"	"T"	"A"	"A"	"A"	"T"	"A"	"A"	"C"	"G"
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Root node 4	"A"	"T"	"C"	"C"	"G"	"C"	"G"	"T"	"A"	"A"	"A"	"T"	"A"	"G"	"T"	"C"	"C"	"C"	"G"
t3	"A"	"T"	"C"	"C"	"G"	"C"	"G"	"A"	"A"	"A"	"A"	"T"	"G"	"G"	"T"	"C"	"C"	"G"	"G"
Node 5	"A"	"T"	"C"	"C"	"G"	"C"	"G"	"T"	"C"	"A"	"A"	"T"	"C"	"G"	"T"	"A"	"T"	"C"	"G"
t2	"A"	"T"	"C"	"C"	"G"	"G"	"G"	"T"	"C"	"A"	"A"	"T"	"T"	"G"	"T"	"G"	"C"	"C"	"G"
t1	"G"	"T"	"C"	"C"	"G"	"T"	"A"	"T"	"C"	"A"	"A"	"T"	"C"	"G"	"T"	"C"	"C"	"C"	"G"
	39	40	41	42	43	44	45	46	47	48	49	50							
Root node 4	"G"	"T"	"G"	"C"	"A"	"T"	"C"	"T"	"G"	"A"	"C"	"A"							
t3	"G"	"T"	"G"	"C"	"A"	"T"	"C"	"T"	"G"	"A"	"C"	"C"							
Node 5	"G"	"T"	"G"	"C"	"A"	"T"	"C"	"T"	"G"	"A"	"C"	"A"							
t2	"G"	"G"	"C"	"T"	"A"	"T"	"C"	"T"	"G"	"A"	"C"	"T"							
t1	"G"	"T"	"G"	"C"	"A"	"T"	"C"	"T"	"G"	"A"	"C"	"T"							

```
>
```

### 3 Advanced examples

Creating the tree file used in the advanced examples:

```
> cat("(((t2:0.1231,t4:0.1231):0.2131,(t3:0.0284,t5:0.0284):0.3078):0.1698,t1:0.5060);",file="smalld
```

#### 3.1 Simulating “domains” and heterogeneous evolution

The following code illustrates how to set up a more complicated simulation of amino acid sequences involving “domains” and heterogeneous evolution.

Use the `ll()` method to list the methods and virtual fields implemented in the `Site` class:

```
> ll(Site())
```

```
Class: Site
Inherits from: PSRoot Object
Fields (0):
Virtual fields (7):
  alphabet
  ancestral
  events
  processes
  sequence
  state
  totalRate
Methods implemented in Site (8):
  as.character
  attachProcess
  checkConsistency
  detachProcess
  flagTotalRate
  is
  isAttached
  summary
```

Enable the “fast & careless mode”:

```
> PSIM_FAST <- TRUE
```

Construct substitution process objects:

```
> wag <- WAG()
> jtt <- JTT()
> lg <- LG()
> pam <- PAM()
```

Summary of the object `wag`:

```
> summary(wag)
```

```
Name: Anonymous
Id: WAG:Anonymous:86541640
```

Alphabet:

Type: Amino acid

Symbols: A R N D C Q E G H I L K M F P S T W Y V

PAML data file:: PAMLdat/wag.dat

Unscaled rate matrix: not shown

Equilibrium distribution:

	A	R	N	D	C	Q	E	G
Prob:	0.08662791	0.043972	0.0390894	0.05704511	0.0193078	0.0367281	0.05805891	0.08325181
	H	I	L	K	M	F	P	S
Prob:	0.0244313	0.048466	0.08620901	0.06202861	0.0195027	0.0384319	0.0457631	0.06951791
	T	W	Y	V				
Prob:	0.06101271	0.0143859	0.0352742	0.07089561				

Site specific parameters (1):

Id: rate.multiplier

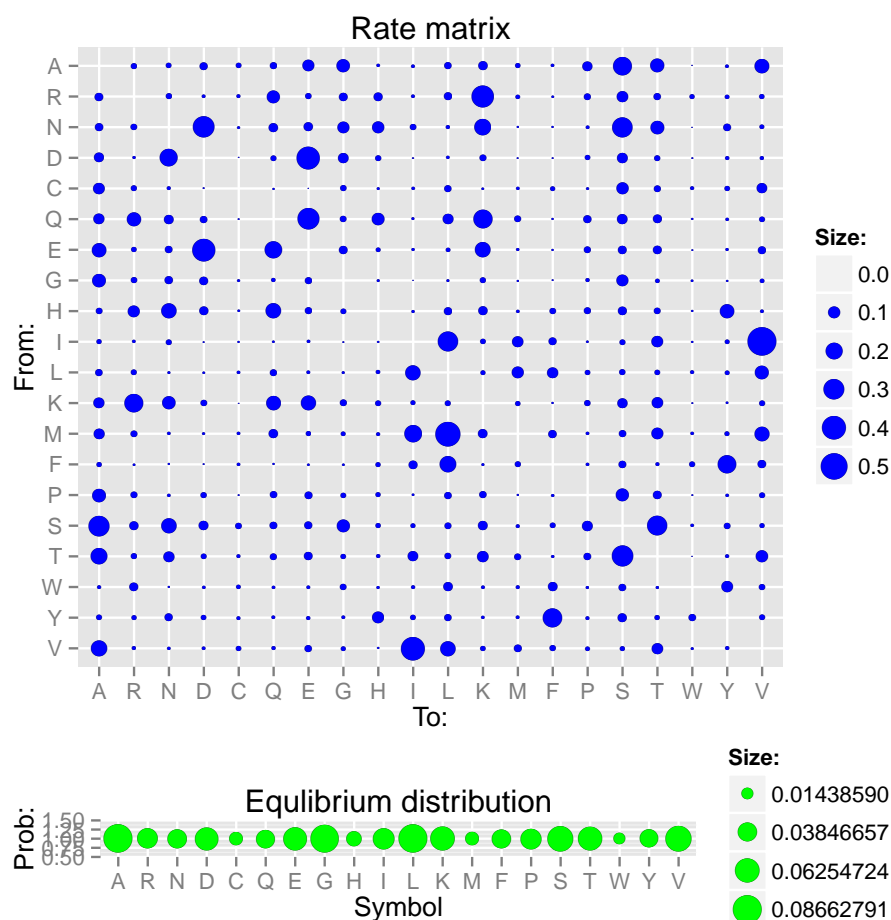
Name: Rate multiplier

Type: numeric

Default value: 1

Get a plot of the instantaneous substitution matrix and equilibrium distribution of the `wag` process (bubble plot):

```
> plot(wag, scale = 0.8)
```



Construct a continuous deleter process:

```
> cont.del <- ContinuousDeletor(rate = 0.6, max.length = 10,
+   dist = expression(rnorm(1, mean = 5, sd = 3)))
```

Construct the template sequence for the `cont.ins.lg` insertion process:

```
> templ.seq.wag <- AminoAcidSequence(length = 10)
```

Clone the template sequence for the `cont.ins.wag` process:

```
> templ.seq.lg <- clone(templ.seq.wag)
```

Construct continuous insertor process object `cont.ins.wag`:

```
> cont.ins.wag <- ContinuousInserter(rate = 0.6, max.length = 10,
+   dist = expression(rnorm(1, mean = 5, sd = 3)))
```

Construct continuous insertor process object `cont.ins.lg`:

```
> cont.ins.lg <- ContinuousInserter(rate = 0.6, max.length = 10,
+   dist = expression(rnorm(1, mean = 5, sd = 3)))
```

Set up the template sequences for the insertion processes:

```
> processes.site.wag<-list(wag, cont.ins.wag, cont.del)
> processes.site.lg<-list(lg, cont.ins.lg, cont.del)
> templ.seq.wag$processes <- list(processes.site.wag)
> templ.seq.lg$processes <- list(processes.site.lg)
```

Now the `cont.ins.lg` process samples the states from the equilibrium distribution of the LG model and `cont.ins.wag` samples the states from the WAG model.

Disabling write protection for the insertion processes:

```
> cont.ins.wag$writeProtected <- FALSE
> cont.ins.lg$writeProtected <- FALSE
```

Set the template sequence for the insertion processes:

```
> cont.ins.wag$templateSeq <- templ.seq.wag
> cont.ins.lg$templateSeq <- templ.seq.lg
```

Setting up the insert hook for the insertion processes:

Insert hook functions are called just before inserting the sequence generated by the insertion process. This function allows for arbitrary modifications to be made to the inserted sequence object. In this case the insert hook functions sample the site-process specific rate multipliers of the substitution processes from an invariants plus discrete gamma (+I+dΓ) model:

```
> cont.ins.wag$insertHook <- function(seq, target.seq, event.pos,
+   insert.pos) {
+   plusInvGamma(seq, process = wag, pinv = 0.1, shape = 1)
+   return(seq)
+ }
```

```
> cont.ins.lg$insertHook <- function(seq, target.seq, event.pos,
+   insert.pos) {
+   plusInvGamma(seq, process = lg, pinv = 0.1, shape = 1)
+   return(seq)
+ }
```

Now the processes are in place, so it is time to set up the root sequence.

```
> aa.seq <- AminoAcidSequence(length = 60)
```

Now we will create a pattern of processes. The “left linker”, “core” and “right linker” regions evolve by different sets of processes. The core region has no indel processes attached, so its length will remain constant:

```
> process.pattern <- c(rep(list(list(wag, cont.del, cont.ins.wag)),
+   times = 20), rep(list(list(jtt)), times = 20), rep(list(list(lg,
+   cont.del, cont.ins.lg)), times = 20))
```

Apply the process pattern to the root sequence:

```
> aa.seq$processes <- process.pattern
```

Set up site specific rates by iterating over sites and sampling rates from a substitution process specific distribution:

```
> for (i in 1:aa.seq$length) {
+   if (isAttached(aa.seq$sites[[i]], jtt)) {
+     setRateMultipliers(aa.seq, jtt, qnorm(runif(1,min=0.5,max=1),mean=0.001,sd=0.01), index = i)
+   }
+   else if (isAttached(aa.seq$sites[[i]], wag)) {
+     plusInvGamma(aa.seq, process = wag, pinv = 0.1, shape = 1,
+       index = i)
+   }
+   else if (isAttached(aa.seq$sites[[i]], lg)) {
+     plusInvGamma(aa.seq, process = lg, pinv = 0.1, shape = 1,
+       index = i)
+   }
+ }
```

Sample the states of the root sequence from the attached substitution processes:

```
> sampleStates(aa.seq)
> print(aa.seq)
```

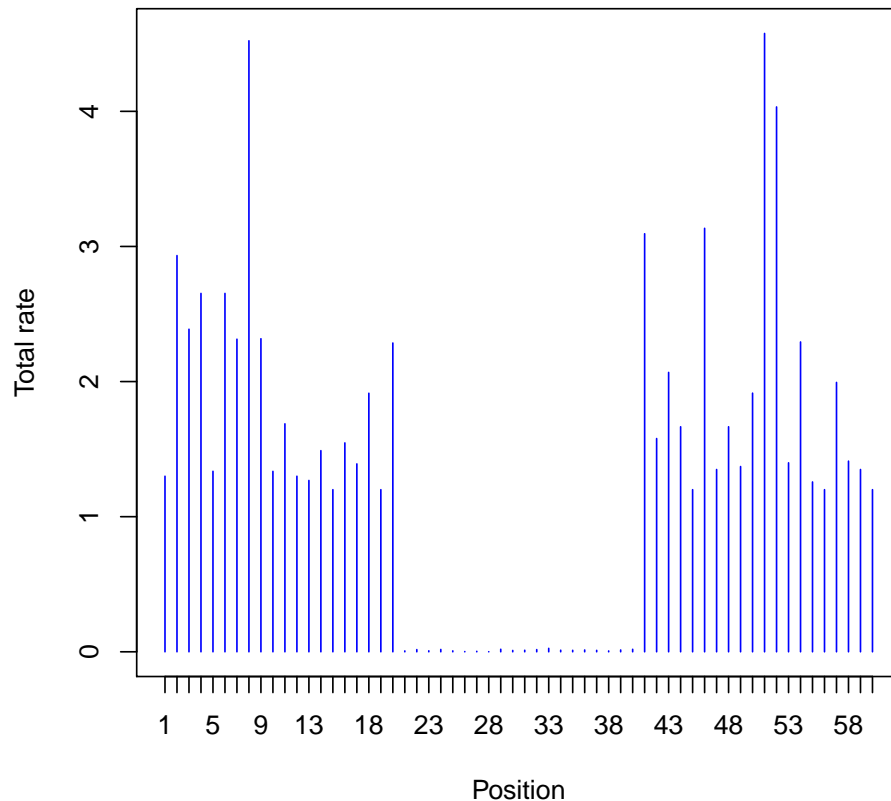
```
[1] "YLGHNHNSADCYGPQLSFYVLQIVEEHGQESDTHYHTAAQLLCKMFAKQYINPHPELMAT"
```

Plot the total rates of the sites:

```
> plot(aa.seq)
```



## otal rate plot for sequence AminoAcidSequence:Anonymous:757



Read in a tree using the `ape` package:

```
> tree <- read.tree(file = "smalldemotree.nwk")
```

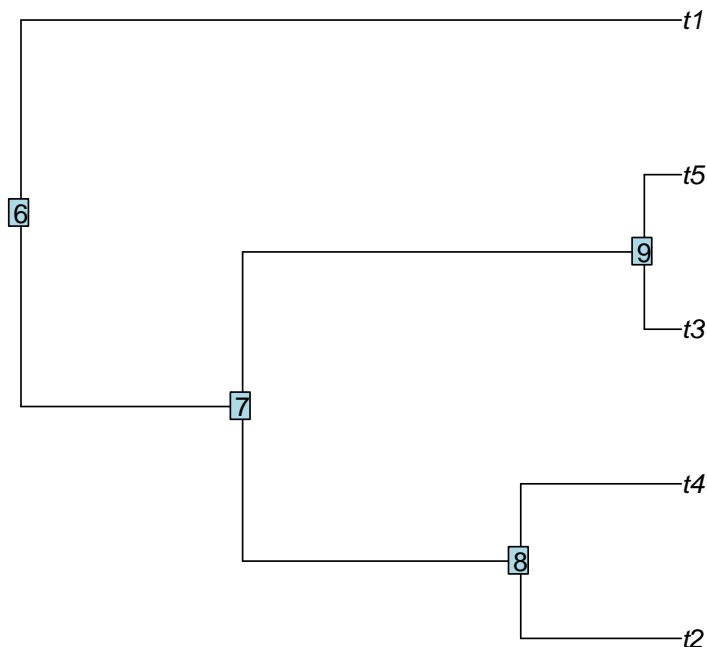
Construct the simulation object and get an object summary:

```
> sim <- PhyloSim(phylo = tree, root.seq = aa.seq)
> summary(sim)
```

```
Name: Anonymous
Id: PhyloSim:Anonymous:80099912
Root Sequence big rate: 79.0086948512488
Tree length: 1.4997
Phylo object details:
  Phylogenetic tree with 5 tips and 4 internal nodes.
  Tip labels:
  [1] "t2" "t4" "t3" "t5" "t1"
  Rooted; includes branch lengths.
Alignment: undefined
Log file: PhyloSim_Anonymous_80099912.log
Log level: -1
```

Plot the simulation object (tree with node labels):

```
> plot(sim)
```



A “node hook” is a function which accepts a **Sequence** object through the named argument “seq” and returns a **Sequence** object. After simulating the branch leading to the node, the resulting **Sequence** object is passed to the node hook and the returned object is used to simulate the downstream branches.

Create a node hook function:

```
> node.hook <- function(seq) {
+   for (site in seq$sites) {
+     if (isAttached(site, jtt)) {
+       attachProcess(site, pam)
+     }
+   }
+   return(seq)
+ }
```

Attach the hook to node 8:

```
> attachHookToNode(sim, node = 8, fun = node.hook)
```

This `node.hook` function will attach the `pam` substitution process to all sites which have the `jtt` process attached (the “core” region). The affected sites will evolve with a doubled rate by a combination of substitution processes (`jtt` and `pam`) in the clade defined by the node - (`t4`, `t2`).

Run the simulation:

```
> Simulate(sim)
```

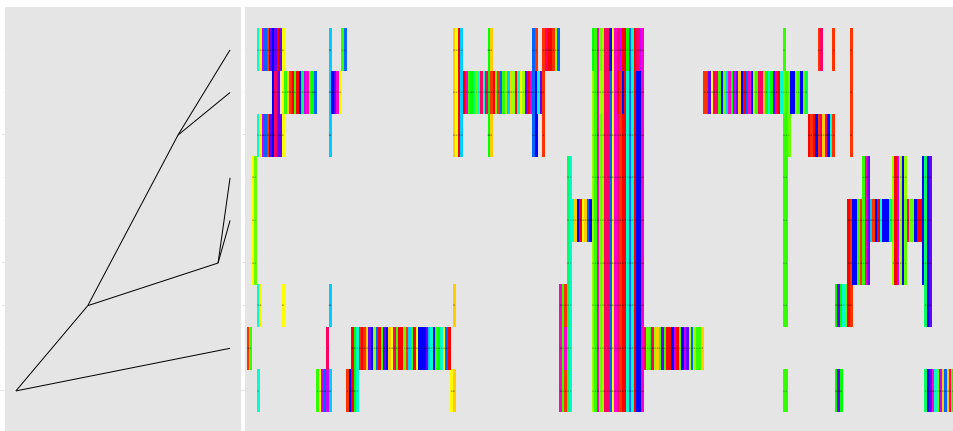
```
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!! WARNING: fast & careless mode is on, most of the error checking is omitted! !!
!! Please note that this also disables the saving of branch statistics.      !!
!! You can go back to normal mode by deleting the PSIM_FAST object.         !!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
Simulating edge 1 of 8
Simulating edge 2 of 8
Simulating edge 3 of 8
Simulating edge 4 of 8
Simulating edge 5 of 8
Simulating edge 6 of 8
Simulating edge 7 of 8
Simulating edge 8 of 8
```

Save the resulting alignment, omitting the internal nodes:

```
> saveAlignment(sim, file = "example_V3.1_aln.fas", skip.internal = TRUE)
```

Plot the resulting alignment alongside the tree (including sequences at internal nodes):

```
> plot(sim,num.pages=1)
```



Disable fast mode:

```
> rm(PSIM_FAST)
```

## 3.2 Evolving codon sequences

Enable “fast & careless” mode:

```
> PSIM_FAST <- TRUE
```

Construct a GY94 codon substitution model:

```
> p <- GY94()
```

Set the transition/transversion rate ratio:

```
> p$kappa = 2
```

Sample codon frequencies from a normal distribution:

```
> codon.freqs <- abs(rnorm(61, mean = 10, sd = 3))
> codon.freqs <- codon.freqs/sum(codon.freqs)
> p$equDist <- codon.freqs
```

Get object summary for p:

```
> summary(p)
```

Name: Anonymous

Id: GY94:Anonymous:105859360

Kappa: 2

Genetic code table id: 1

Alphabet:

  Type: Standard

  Symbols: AAA AAC AAG AAT ACA ACC ACG ACT AGA AGC AGG AGT ATA ATC ATG ATT CAA CAC CAG CAT CCA CCC C

Unscaled rate matrix: not shown

Equilibrium distribution:

	AAA	AAC	AAG	AAT	ACA	ACC	ACG
Prob:	0.01660395	0.01908665	0.01436825	0.01354432	0.02117309	0.01629473	0.01151528
	ACT	AGA	AGC	AGG	AGT	ATA	ATC
Prob:	0.02199895	0.0182126	0.02426235	0.01237077	0.02093671	0.01766806	0.02163061
	ATG	ATT	CAA	CAC	CAG	CAT	CCA
Prob:	0.02015422	0.01785793	0.01547921	0.02069444	0.01700808	0.002783545	0.02362818
	CCC	CCG	CCT	CGA	CGC	CGG	CGT
Prob:	0.02288464	0.009998127	0.008604262	0.01876636	0.01226162	0.01143051	0.01396217
	CTA	CTC	CTG	CTT	GAA	GAC	GAG
Prob:	0.01468434	0.01302523	0.01431409	0.02228846	0.0191214	0.0138515	0.01020915
	GAT	GCA	GCC	GCG	GCT	GGA	GGC
Prob:	0.0211715	0.01680518	0.02113407	0.02076435	0.01502911	0.01146576	0.01705024
	GGG	GGT	GTA	GTC	GTG	GTT	TAC
Prob:	0.0153531	0.01623546	0.0196913	0.02042429	0.01738206	0.01215839	0.01455958
	TAT	TCA	TCC	TCG	TCT	TGC	TGG
Prob:	0.02219488	0.009279575	0.01831496	0.00805714	0.01138013	0.02478062	0.01459087
	TGT	TTA	TTC	TTG	TTT		
Prob:	0.01452467	0.01877213	0.01460328	0.01330227	0.01830131		

Site specific parameters (2):

  Id: rate.multiplier

  Name: Rate multiplier

  Type: numeric

  Default value: 1

  Id: omega

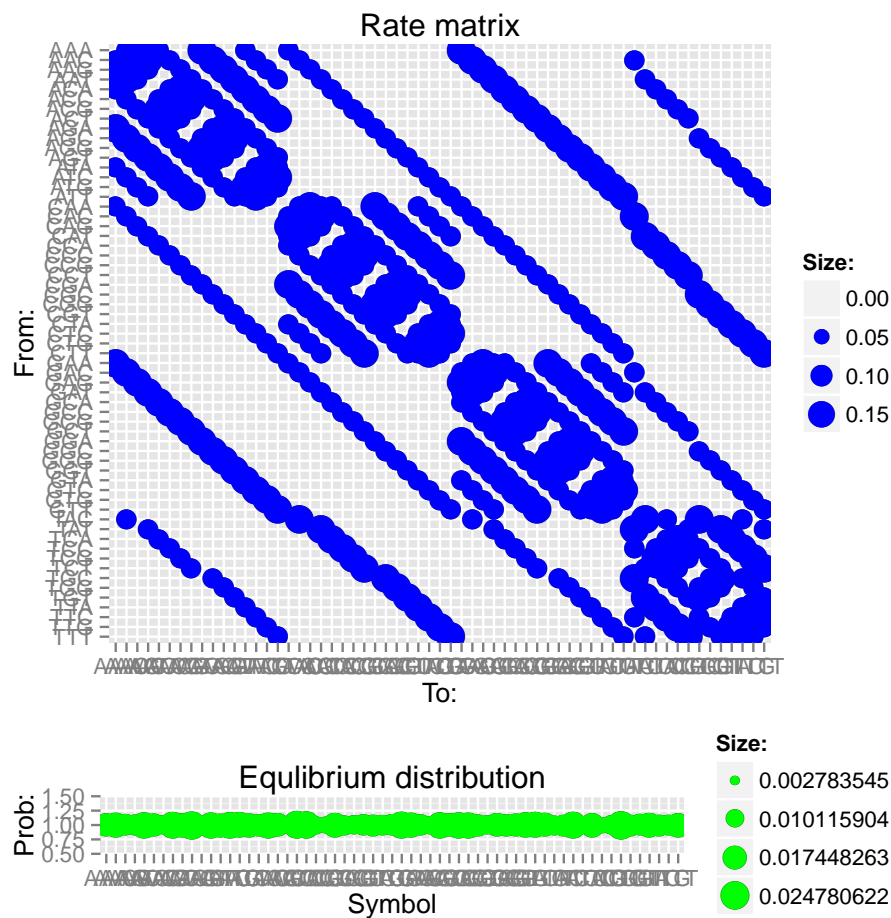
  Name: Omega

  Type: numeric

  Default value: 1

Get a bubble plot of p:

```
> plot(p,scale=0.5)
```



Construct a discrete deleter process:

```
> d<-DiscreteDeletor(
+   rate=1,
+   sizes=1:4,
+   probs=c(4,3,2,1)/10
+ );
```

Construct a discrete insertor process inserting neutrally evolving sites:

```
> i<-DiscreteInsertor(
+   rate=1.5,
+   sizes=1:4,
+   probs=c(4,3,2,1)/10,
+   template.seq=CodonSequence(length=4,processes=list(list(p)))
+ );
```

Construct root sequence and attach process p:

```
> s<-CodonSequence(length=30,processes=list(list(p)))
```

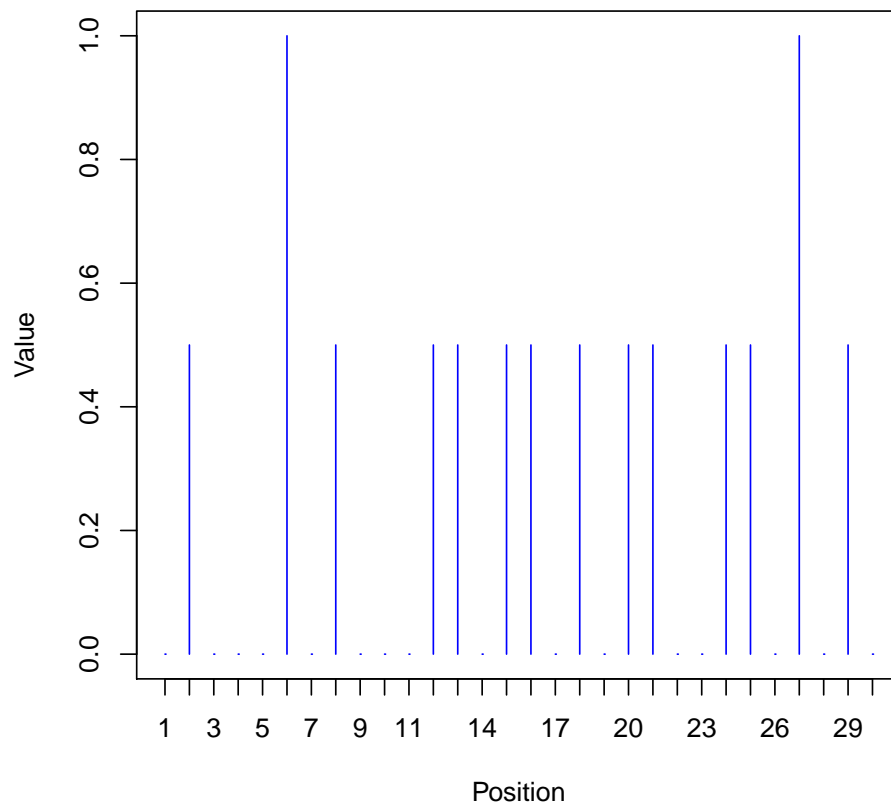
Sample omegas from a discrete model:

```
> omegaVarM3(s,p,omegas=c(0,0.5,1),probs=c(2/4,1/4,1/4))
```

Plot the omega values across sites:

```
> plotParametersAtSites(s,p,"omega");
```

### Plot of parameter omega for process GY94:Anonymous:1058593



Sample states:

```
> sampleStates(s)
```

Construct the simulation object:

```
> sim <- PhyloSim(root.seq = s, phylo = read.tree("smalldemotree.nwk"))
```

Create a node hook function and attach to node 8:

```
> node.hook <- function(seq) {  
+   setOmegas(seq, p, 1)  
+   attachProcess(seq, d)  
+   attachProcess(seq, i)  
+   return(seq)  
+ }
```

```
+ }
> attachHookToNode(sim, node = 8, fun = node.hook)
```

The `node.hook` function sets all omegas to 1 and attaches the insertion process `i` and deletion process `d`. Hence the sequences will evolve neutrally with indels in the clade defined by node 8 - (`t4`, `t2`).

Disable fast mode just before simulation in order to preserve branch statistics:

```
> rm(PSIM_FAST)
```

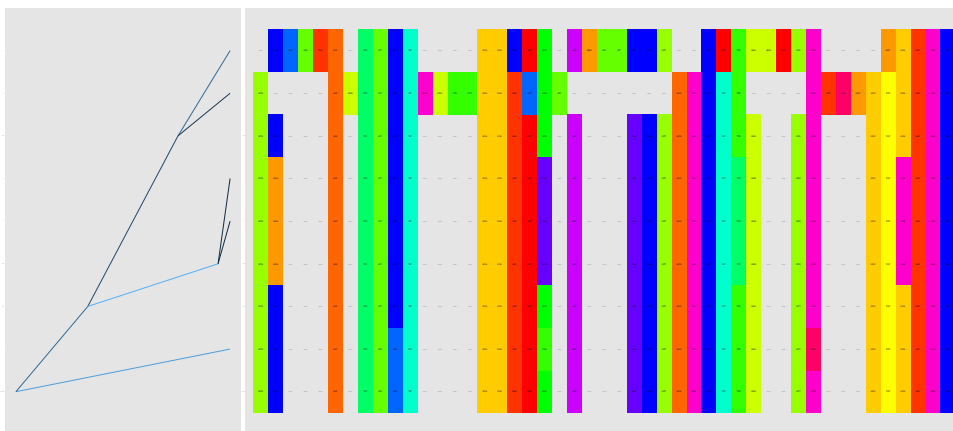
Run the simulation:

```
> Simulate(sim)
```

```
Simulating edge 1 of 8
Simulating edge 2 of 8
Simulating edge 3 of 8
Simulating edge 4 of 8
Simulating edge 5 of 8
Simulating edge 6 of 8
Simulating edge 7 of 8
Simulating edge 8 of 8
```

Plot the resulting alignment alongside the tree:

```
> plot(sim,num.pages=1)
```

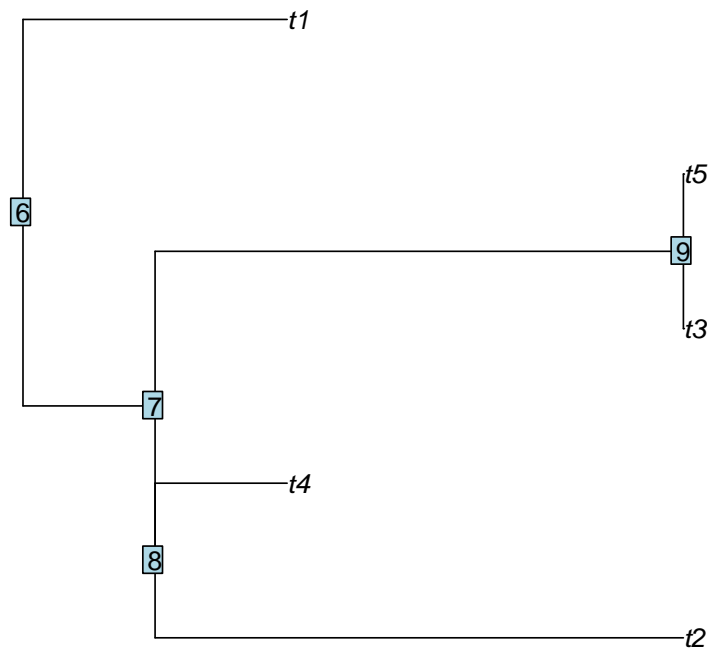


Export the nonsynonymous substitution counts as a phylo object:

```
> nsyn.subst<-exportStatTree(sim,"nr.nsyn.subst")
```

Plot the exported phylo object:

```
> plot(nsyn.subst)
> nodelabels()
```



Save the resulting alignment:

```
> saveAlignment(sim, file = "example_V3.2_aln.fas", )
```

### 3.3 Implementing a new process

The following code demonstrates how to implement a process which performs inverted duplications. In this example we simply replace the function object stored in the `generateBy` virtual field of a `GeneralInsertor` object. Alternatively, we could have defined a new class and set the insert generating function in the constructor method.

Enable fast & careless mode:

```
> PSIM_FAST<-TRUE;
```

Construct a `DiscreteInsertor` process:

```
> ivd<-DiscreteInsertor(rate=0.06,sizes=c(4,6),probs=c(2/3,1/3));
```

Set template sequence just to make the process object happy:

```
> ivd$templateSeq<-NucleotideSequence(length=1);
```



Replace the function object stored in the `generateBy` virtual field. See the documentation of the `GeneralInserter` class:

```
> ivd$generateBy<-function(process=NA,length=NA,target.seq=NA,event.pos=NA,insert.pos=NA){
+   # get the target sequence length
+   target.length<-target.seq$length;
+   # construct a vector with the positions to copy:
+   positions<-(insert.pos+1):(insert.pos + length)
+   # discard illegal positions:
+   positions<-positions[ positions > 0 & positions <= target.length];
+   # copy subsequence
+   insert<-copySubSequence(target.seq,positions,process);
+   # reverse complement sequence,
+   # take care, the class of this objects is "Sequence":
+   revComp.NucleotideSequence(insert);
+   # do not allow nested insertions:
+   setRateMultipliers(insert,ivd,0);
+   # return insert
+   return(insert);
+ }
```

Now we have a process which performs inverted duplications.

Construct a JC69 process object:

```
> p<-JC69();
```

Construct root sequence object:

```
> s<-NucleotideSequence(length=50)
```

Attach processes via virtual field:

```
> s$processes<-list(list(p,ivd))
```

Sample states from the equilibrium distribution of the attached processes:

```
> sampleStates(s)
```

Detach the substitution process:

```
> detachProcess(s,p)
```

Create among-sites rate variation for the inverted duplication process by sampling rate multipliers from an I+G model:

```
> plusInvGamma(s,ivd,pinv=0.7,shape=0.5)
```

Construct simulation object:

```
> sim<-PhyloSim(root.seq=s, phylo=read.tree("smalldemotree.nwk"));
```

Run simulation:

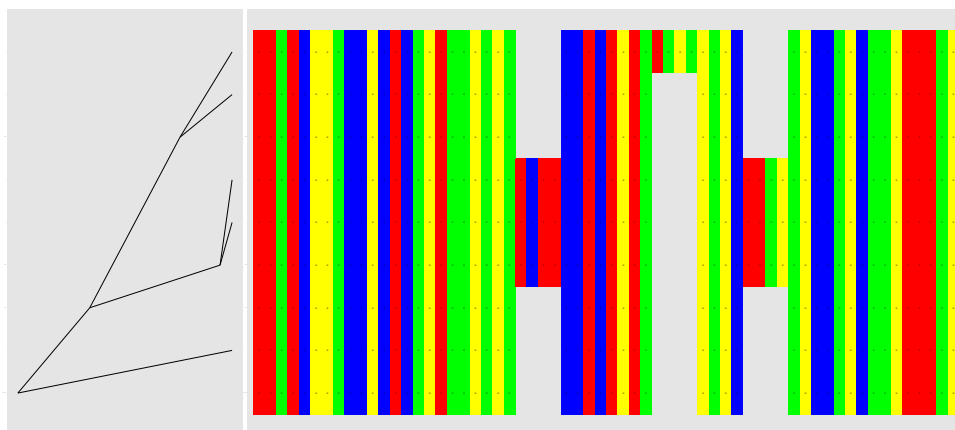
```
> Simulate(sim)
```

```
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!! WARNING: fast & careless mode is on, most of the error checking is omitted! !!
!! Please note that this also disables the saving of branch statistics.      !!
!! You can go back to normal mode by deleting the PSIM_FAST object.          !!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
```

```
Simulating edge 1 of 8
Simulating edge 2 of 8
Simulating edge 3 of 8
Simulating edge 4 of 8
Simulating edge 5 of 8
Simulating edge 6 of 8
Simulating edge 7 of 8
Simulating edge 8 of 8
```

Plot tree and alignment:

```
> plot(sim,num.pages=1)
```



Save alignment:

```
> saveAlignment(sim,file="example_V3.3.fas");
```

Disable fast & careless mode:

```
> rm(PSIM_FAST);
```

### 3.4 Evolving a genomic region containing a “gene”

The following code demonstrates how to simulate a genomic region containing a gene with introns and noncoding regions.

We will simulate the following features:

- NC1 (length: 1000 nucleotide sites) - noncoding region 1, evolving under a GTR substitution model with deletions and insertions (of Cs only).
- E1 (length: 400 codon sites) - exon 1, evolving under a nucleotide-scaled GY94 codon model with small indels.

- I1-5 (length: 200 nucleotide sites) - introns 1-5, evolving under a F84 substitution model with indels
- E2-5 (length: 200 codon sites) - exons 2-5, evolving under a nucleotide-scaled GY94 codon model.
- E6 (length: 400 codon sites) - exon 6, evolving under a nucleotide-scaled GY94 codon model with small indels.
- NC2 (length: 1400 nucleotide sites) - noncoding region 2, evolving under a K80 substitution model.
- Invariable start codon and splice sites.
- A special substitution process for stop codons.

The total length of the Sequence object is 5000. We will also simulate heterogeneity of nucleotide substitution rates, indel rates and omega ratios.

Enable fast & careless mode:

```
> PSIM_FAST<-TRUE;
```

Create a list holding partition information and set partition properties:

```
> part<-list();
```

Partition NC1:

```
> part$nc1<-list(
+     "type"="noncoding",
+     "len"=1000,
+     "subst"=NA,
+     "ins"=NA,
+     "del"=NA,
+     "gamma.shape"=0.5
+ );
> part$nc1$subst<-GTR(
+     rate.params=list(
+         "a"=1, "b"=2, "c"=3,
+         "d"=1, "e"=2, "f"=3
+     ),
+     base.freqs=c(1.5,1,1.5,1)/5
+ )
> part$nc1$ins<-DiscreteInsertor(
+     rate=0.025,sizes=1:6,
+     probs=6:1/21,
+     template.seq=NucleotideSequence(string="CCCCC")
+ );
> part$nc1$del<-DiscreteDeletor(
+     rate=0.025,
+     sizes=1:6,
+     probs=6:1/21
+ );
```

Partition E1:

```
> part$e1<-list(
+     "type"="first.exon",
```

```

+         "len"=400,
+         "subst"=NA,
+         "ins"=NA,
+         "del"=NA,
+         "omegas"=c(0,1,1.1),
+         "omega.probs"=c(4,2,1)/5
+     );

```

Construct a “nucleotide-scaled” codon model which can be used in mixed sequences:

```

> part$e1$subst<-GY94(kappa=2,scale.nuc=TRUE);
> part$e1$ins<-DiscreteInsertor(
+     rate=0.025,
+     sizes=1:2,
+     probs=c(2/3,1/3),
+     template.seq=CodonSequence(length=2,processes=list(list(part$e1$subst)))
+ );
> part$e1$del<-DiscreteDeletor(
+     rate=0.025,
+     sizes=1:2,
+     probs=c(2/3,1/3)
+ );
> f84<-F84();
> f84$kappa<-1.8;
> gy94.e2_5<-clone(part$e1$subst);
> gy94.e2_5$kappa<-1.5

```

Partitions E2-5 and I1-4:

```

> del.introns<-DiscreteDeletor(
+     rate=0.025,
+     sizes=1:8,
+     probs=8:1/36
+ );
> ins.introns<-DiscreteInsertor(
+     rate=0.025,
+     sizes=1:8,
+     probs=8:1/36,
+     template.seq=NucleotideSequence(length=8,processes=list(list(f84)))
+ );
> for(i in 1:4){
+     part[[paste("i",i,sep="")]]<-list(
+         "type"="intron",
+         "len"=200,
+         "subst"=f84,
+         "ins"=ins.introns,
+         "del"=del.introns,
+         "gamma.shape"=abs(rnorm(1,mean=1,sd=0.2))
+     )
+     part[[paste("e",i+1,sep="")]]<-list(
+         "type"="exon",
+         "len"=200,
+         "subst"=gy94.e2_5,
+         "ins"=NA,
+         "del"=NA,

```

```

+           "omegas"=c(0,0.5),
+           "omega.probs"=c(3/4,1/4)
+       )
+
+ }

```

Partition I5:

```

> part$i5<-list(
+     "type"="intron",
+     "len"=200,
+     "subst"=f84,
+     "ins"=ins.introns,
+     "del"=del.introns,
+     "gamma.shape"=abs(rnorm(1,mean=1,sd=0.1))
+ )
> rm(f84,gy94.e2_5,del.introns,ins.introns);

```

Partition E6:

```

> part$e6<-list(
+     "type"="last.exon",
+     "len"=400,
+     "subst"=NA,
+     "ins"=NA,
+     "del"=NA,
+     "omegas"=c(0,1,1.1),
+     "omega.probs"=c(4,2,1)/5
+ );
> tmp<-clone(part$e1$subst);
> tmp$kappa<-0.5;
> part$e6$subst<-tmp;
> part$e6$del<-DiscreteDeletor(
+     rate=0.025,
+     sizes=1:4,
+     probs=c(4,3,2,1)/10
+ );
> part$e6$ins<-DiscreteInsertor(
+     rate=0.025,
+     sizes=1:2,
+     probs=c(1/3,2/3),
+     template.seq=CodonSequence(length=2,processes=list(list(part$e6$subst)))
+ );

```

Partition NC2:

```

> part$nc2<-list(
+     "type"="noncoding",
+     "len"=1400,
+     "subst"=K80(rate.params=list(Alpha = 2, Beta = 1)),
+     "ins"=NA,
+     "del"=NA,
+     "gamma.shape"=0.5
+ );

```

Construct root sequence object:

```
> s<-Sequence(length=5000);
```

Construct Alphabet objects:

```
> nuc<-NucleotideAlphabet();  
> cod<-CodonAlphabet();
```

Construct an Alphabet object containing the stop codons:

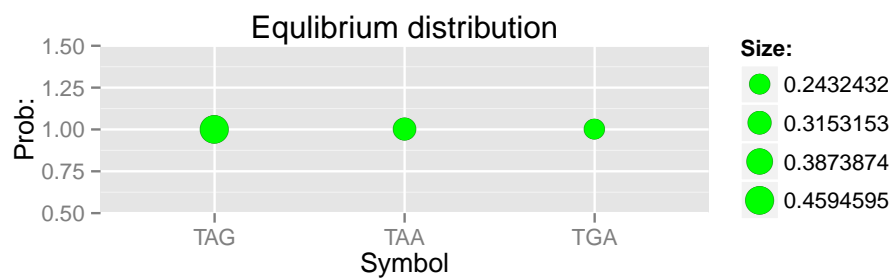
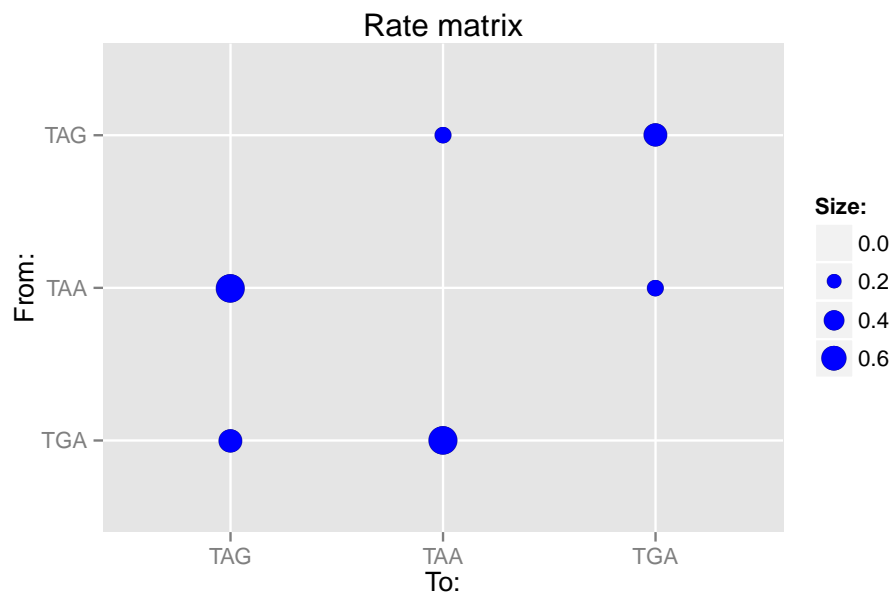
```
> stop.alphabet<-Alphabet(symbols=c("TAG","TAA","TGA"));
```

Construct a substitution process acting on stop codons only:

```
> stop.subst<-GeneralSubstitution(  
+       alphabet=stop.alphabet,  
+       rate.list=list(  
+           "TAG->TAA "=1,  
+           "TAG->TGA "=2,  
+           "TAA->TAG "=3,  
+           "TAA->TGA "=1,  
+           "TGA->TAG "=2,  
+           "TGA->TAA "=3  
+       )  
+ );
```

Get a bubble plot of stop.subst:

```
> plot(stop.subst,scale=0.5);
```



Iterate over partitions, set up processes and rate multipliers, fix start codon and splice sites, attach the `stop.subst` process to stop codon:

```
> pos<-0;
> for(i in part){
+   beg<-pos+1;
+   end<-pos+i$len;
+   range<-beg:end;
+
+
+   if( (i$type=="noncoding") | (i$type=="intron") ){
+     setAlphabets(s,list(nuc),range);
+   }
+   else {
+     setAlphabets(s,list(cod),range);
+   }
+
+   if(!is.na(i$del)){
+     attachProcess(s,i$del,range);
+   }
+   if(!is.na(i$ins)){
+     attachProcess(s,i$ins,range);
+   }
+
+   if( (i$type=="noncoding") | (i$type=="intron")){
```

```

+         attachProcess(s,i$subst,range);
+
+         plusInvGamma(this=s,process=i$subst,pinv=0.6,shape=i$gamma.shape,range);
+         if(i$type=="intron"){
+             # fix splicing sites
+             setStates(s,c("G","T","A","G"),c(beg,beg+1,end-1,end));
+             setRateMultipliers(s,i$subst,0,c(beg,beg+1,end-1,end));
+             setInsertionTolerance(s,i$ins,0,c(beg,beg+1,end-1,end));
+             setDeletionTolerance(s,i$del,0,c(beg,beg+1,end-1,end));
+         }
+     }
+     else if(i$type=="exon"){
+         attachProcess(s,i$subst,range);
+
+         omegaVarM3.CodonSequence(s,i$subst,i$omegas,i$omega.probs,range);
+     }
+     if(i$type=="first.exon"){
+         attachProcess(s,i$subst,range);
+         # Fix start codon:
+         setStates(s,"ATG",beg);
+         setRateMultipliers(s,i$subst,0,beg)
+         setInsertionTolerance(s,i$ins,0,beg);
+         setDeletionTolerance(s,i$del,0,beg);
+     }
+     if(i$type=="last.exon"){
+         attachProcess(s,i$subst,range);
+         # Detach GY94 from last site:
+         detachProcess(s,i$subst,end);
+         # Replace alphabet:
+         setAlphabets(s,list(stop.alphabet),end);
+         # Attach stop codon process:
+         attachProcess(s,stop.subst,end);
+         # Sample stop codon:
+         sampleStates(s,end);
+         # Protect against indels:
+         setInsertionTolerance(s,i$ins,0,end);
+         setDeletionTolerance(s,i$del,0,end);
+     }
+
+     pos<-end;
+ }

```

Sample remaining site states:

```
> sampleStates(s);
```

Generate a random coalescent tree with 3 leaves and construct simulation object:

```
> sim<-PhyloSim(root.seq=s, phylo=rcoal(3));
```

Scale tree length to 0.15:

```
> scaleTree(sim,0.15/sim$treeLength);
```

Run simulation:

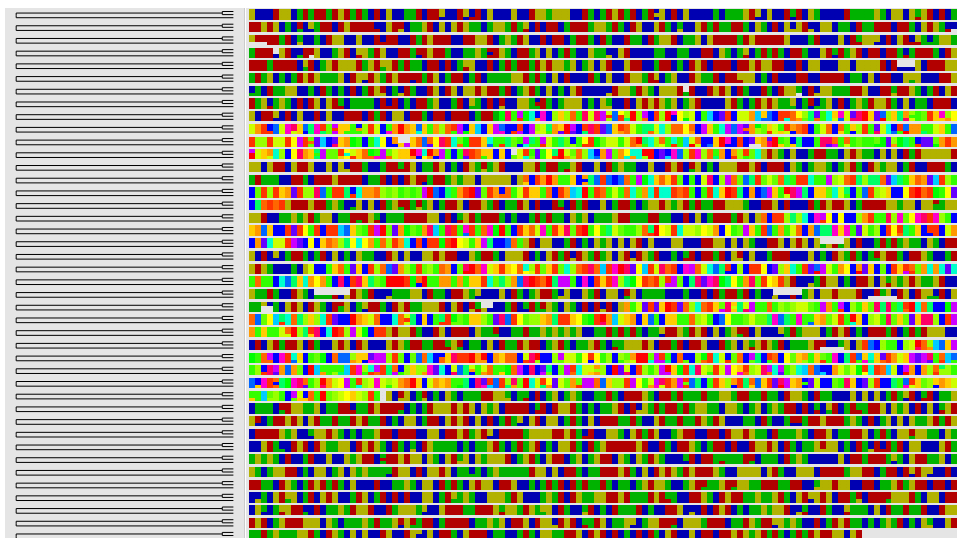


```
> Simulate(sim)
```

```
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!! WARNING: fast & careless mode is on, most of the error checking is omitted! !!
!!   Please note that this also disables the saving of branch statistics.      !!
!!   You can go back to normal mode by deleting the PSIM_FAST object.         !!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
Simulating edge 1 of 4
Simulating edge 2 of 4
Simulating edge 3 of 4
Simulating edge 4 of 4
```

Plot tree and alignment, omitting ancestral sequences:

```
> plot(sim,num.pages="auto",plot.ancestors=FALSE)
```



Save alignment:

```
> saveAlignment(sim,file="example_V3.4.fas");
```

Disable fast & careless mode:

```
> rm(PSIM_FAST)
```

## 4 Details of the fast field deletion model

A natural way to incorporate deletions into the Gillespie framework is to assign an individual rate to every possible deletion event. Modelling in this manner is extremely general but requires a lot of specification: not only individual sites' tolerance to deletion but also of how they interact with neighbouring sites. Instead we propose a more restricted "field model" of deletion that generalises previous work to allow the rate at which deletions occur to vary across the sequence but only requires one parameter per site – its deletion tolerance – to be specified. Under this model, deletions are proposed in same manner as other events, specifying a rate of occurrence and a distribution of lengths and then assuming that the location and orientation of the deletion is chosen uniformly, but proposed deletions may then be rejected based on sites they propose to remove.

Firstly consider only single-site deletions and let each site,  $i$ , in the sequence have an associated deletion tolerance parameter,  $d_i \in [0, 1]$ , representing the probability that it is actually deleted given that a deletion is proposed. A proposal / acceptance step is equivalent to just proposing at a slower rate, so this is just a special case of the most general model but one that can be implemented efficiently as a single Gillespie event (a deletion occurred somewhere) rather than a large number of slower events (treating every possible deletion separately). Sites where  $d_i = 1$  are deleted at the background rate, sites with  $d_i < 1$  are deleted more slowly, and sites with  $d_i = 0$  are never deleted. For proposed deletions that span multiple sites,  $\mathcal{I}$ , each site is considered independently and the proposed deletion is accepted if and only if every site accepts it: the total probability of acceptance is therefore  $\prod_{i \in \mathcal{I}} d_i$ .

It is natural to think of the background rate of deletion as a neutral rate but this is not necessary and can lead to the Gillespie algorithm becoming inefficient: for example, an extremely deletion intolerant sequence will reject almost all deletions proposed and so waste many steps. Instead we can rescale the process (“fast field deletion model”) so that deletions are proposed at a rate equal to what would occur if the entire sequence had a deletion tolerance equal to its most tolerant site (deletion tolerance  $d$ ) and then accept a deletion spanning sites  $\mathcal{I}$  with probability  $d_i/d$ . Table 1 gives the rate scaling factor and distribution of deletion lengths after scaling for a variety of distributions that could be used to model deletion length. In most cases, the rescaled distribution of deletion lengths is a member of the same family as the “neutral” process.

Distribution	Density	Scale factor	Rescaled distribution
Geometric, $\text{Geom}(\lambda)$	$\lambda^{k-1}(1 - \lambda)$	$d \frac{1-\lambda}{1-d\lambda}$	$\text{Geom}(d\lambda)$
Poisson + 1, $\text{Po}_{+1}(\lambda)$	$\frac{e^{-\lambda} \lambda^{k-1}}{\Gamma(k)}$	$d e^{-\lambda(1-d)}$	$\text{Po}_{+1}(d\lambda)$
Conway-Maxwell Poisson + 1, $\text{CMP}_{+1}(\lambda, \nu)$	$\frac{\lambda^{k-1}}{\Gamma(k)^\nu \mathcal{Z}(\lambda, \nu)}$	$d \frac{\mathcal{Z}(\lambda, \nu)}{\mathcal{Z}(d\lambda, \nu)}$	$\text{CMP}_{+1}(d\lambda, \nu)$
Negative Binomial + 1, $\text{NB}_{+1}(\lambda, r)$	$\frac{\Gamma(r+k-1)}{\Gamma(k)\Gamma(r)} (1 - \lambda)^r \lambda^{k-1}$	$d \frac{(1-\lambda)^r}{(1-d\lambda)^r}$	$\text{NB}_{+1}(d\lambda, r)$

Table 1: The rate scaling factor and distribution of deletion lengths after scaling for a variety of distributions that could be used to model deletion length.