Phylogenetics in R
package phangorn

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Introduction

Phylogenetics with phangorn (and ape)
- Importing data and trees
- Distance methods
- Maximum Parsimony
- Maximum Likelihood

One tree can’t rule them all
- Comparing tree
- Partition Models
- Hadamard Conjugation and Splits

Simulating trees and sequences

Summary
This talk is mainly about the two packages *ape* and *phangorn*. There are many other phylogenetic packages on CRAN (some are for very specific tasks) e.g.:

- *phylobase* (nice plot functions), *apTreeshape*, *geiger*, *ouch*, *ade4*

An overview over many packages is given at:
http://www.cran.r-project.org/web/views/Phylogenetics.html

For handling biological data:

- *seqinr*
- *ShortRead* (bioconductor)
- many bioconductor packages for meta-data, annotations etc.
Overview of R-packages for phylogenetics

phylogeny reconstruction:

- ape (NJ, fastme)
- phangorn (ML, MP, Network methods, Hadamard)
- Hierarchical clustering `hclust` in package `stats`  
  `upgma` is just a wrapper around `hclust`
Phylogenetics in R

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Outline

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Summary
Loading data

read.dna in *ape* reads in nucleotide data (phylip and fasta), read.aa amino acids and read.nexus.data nexus files. The files are either of class DNAbin or a list

```r
> data <- read.dna("data.phy")
> data <- read.dna("data.fas", format = "fasta")
> data <- read.nexus.data("data.nex")
> data <- as.phyDat(data)
> data <- read.phyDat(data, format = "phylip",
+     type = "DNA")
```

`read.phyDat` is a wrapper around the other function and transforms object into class `phyDat`. Nexus files come in lot of different dialects. Splitstree has a quite good nexus parser, so importing into and exporting from Splitstree often helps to make them readable to other software.
ape also offers to functions to read in trees:
▶ `read.tree` for reading trees in Newick format
▶ `read.nexus` for reading trees in Nexus format

There are also some functions to convert between different tree formats in R, e.g. `hclust`. 
Distance methods

There are many different distance based methods available nj, fastme.bal and fastme.ols in ape and upgma, wpgma in phangorn (based on code from hclust)

```r
> library(phangorn)
> library(multicore)
> data(Laurasiatherian)
> dm = dist.dna(as.DNAbin(Laurasiatherian),
+    model = "JC69")
> treeUPGMA = upgma(dm)
> treeNJ = nj(dm)
> treeFME = fastme.bal(dm)
```
Plotting trees

We can plot these trees

```r
> par(mfrow = c(2, 2), mar = c(2, 2, 4, + 2))
> plot(treeUPGMA)
> title("UPGMA")
> plot(treeUPGMA, type = "fan")
> title("UPGMA (fan)"")
> plot(treeNJ, type = "unrooted", main = "NJ")
> title("NJ")
> plot(treeFME, type = "unrooted", main = "fastME")
> title("fastME")
```
Plotting trees

Plotting trees in R has some advantages, make set up favorite set up once and reuse it. `plot.phylo` offers a big variety of styles.

```r
> args(plot.phylo)

function (x, type = "phylogram", use.edge.length = TRUE, show.tip.label = TRUE, show.node.label = FALSE, edge.color = "black", edge.width = 1, edge.lty = 1, font = 3, cex = par("cex"), adj = NULL, srt = 0, no.margin = FALSE, root.edge = FALSE, label.offset = 0, underscore = FALSE, x.lim = NULL, y.lim = NULL, direction = "rightwards", lab4ut = "horizontal", tip.color = "black", ...)

NULL

`phylobase` offers also nice plotting with annotations, but less variety yet.
Maximum Parsimony

parsimony returns the parsimony score.

```r
> trees = structure(list(treeFME, treeNJ, 
+    treeUPGMA), class = "multiPhylo")
> parsimony(trees, Laurasiatherian)

[1]  9751  9776 10015
```

These functions are vectorized and can also take multiPhylo objects.

phangorn contains the possibility to search for the better parsimony trees:

```r
> trB <- optim.parsimony(treeFME, Laurasiatherian)
> parsimony(trB, Laurasiatherian)

[1]  9731
```

Searching is so far slow (in comparison to Paup*) and only NNI moves are implemented. To find a lower bound of the pscore - use Min-Max Squeeze (Holland et al. 2005).
We can compute the likelihood given the data:

```r
> fit <- pml(treeNJ, Laurasiatherian)
> fit <- update(fit, k = 4, inv = 0.2)
```

The function `optim.pml` is used to optimize the different parameter.

```r
> fit2 <- optim.pml(fit, optNni = TRUE, optGamma = TRUE, optInv = TRUE, model = "GTR")
```
Maximum Likelihood

The function `pml` returns an object of class `pml`. The design differs from most phylogeny packages, but closer to R functions like `lm` or `glm`. There exist several generic functions for further analysis of these objects:

```r
> methods(class = "pml")
```

[1] `anova.pml`*  `logLik.pml`*  `plot.pml`*
[4] `print.pml`*  `update.pml`*  `vcov.pml`*

Non-visible functions are asterisked and other generic functions like AIC work on these objects.
Before running the analysis we should have checked which model to use:

```r
> mT = modelTest(treeFME, Laurasiatherian, +
+               c("JC", "GTR"))
> mT
```

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>logLik</th>
<th>AIC</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>JC</td>
<td>-58142.27</td>
<td>116466.54</td>
<td>117018.40</td>
</tr>
<tr>
<td>2</td>
<td>JC+I</td>
<td>-55277.59</td>
<td>110739.18</td>
<td>111297.09</td>
</tr>
<tr>
<td>3</td>
<td>JC+G</td>
<td>-53136.57</td>
<td>106461.14</td>
<td>107031.18</td>
</tr>
<tr>
<td>4</td>
<td>JC+G+I</td>
<td>-53775.30</td>
<td>107740.61</td>
<td>108316.72</td>
</tr>
<tr>
<td>5</td>
<td>GTR</td>
<td>-54907.98</td>
<td>110013.96</td>
<td>110614.33</td>
</tr>
<tr>
<td>6</td>
<td>GTR+I</td>
<td>-51957.25</td>
<td>104114.51</td>
<td>104720.94</td>
</tr>
<tr>
<td>7</td>
<td>GTR+G</td>
<td>-49039.82</td>
<td>98283.65</td>
<td>98902.21</td>
</tr>
<tr>
<td>8</td>
<td>GTR+G+I</td>
<td>-48671.55</td>
<td>97549.09</td>
<td>98173.72</td>
</tr>
</tbody>
</table>
It is also possible to produce bootstrap samples. The function `bootstrap.pml` makes use of the *multicore* package (under Linux and without GUI interface).

```r
> bs <- bootstrap.pml(fit2, bs = 100, optNni = TRUE)
> plotBS(fit2$tree, bs)
```
Tree with bootstrap values
Cheat Sheet

Putting things together here is a script for a standard ML analysis:

```r
library(multicore)
library(phangorn)
dat = read.phyDat("myfile")
dm = dist.ml(dat)
tree = fastme.bal(dm)
(mT = modelTest(tree, dat))
fit = pml(tree, dat, k = 4, inv = 0.2)
fit = optim.pml(fit, optNni = TRUE, optGamma = TRUE,
               optInv = TRUE, model = "GTR")
bs = bootstrap.pml(fit, bs = 100, optNni = TRUE)
plotBS(fit$tree, bs)
```
One tree can’t rule them all
Comparing trees

treedist returns several tree distance measures, RF.dist is a fast and more memory efficient implementation of the Robinson-Foulds distance for big trees (10,000 taxa)

```r
> tree1 = unroot(rtree(100))
> tree2 = unroot(rtree(100))
> treedist(tree1, tree2)

  symmetric.difference         194.000000
  branch.score.difference      9.308153
  path.difference              442.497458
  quadratic.path.difference    224.744427

> RF.dist(tree1, tree2)
[1] 194
```

An alternative is dist.topo in ape.
Partition Models

Partition Models are frequently used to adjust for differences in codon positions. In the next example we allow different rates for different genes.

```r
> data(yeast)
> dm.y <- dist.logDet(yeast)
> tree.y <- NJ(dm.y)
> fits <- pml(tree.y, yeast)
> fits <- optim.pml(fits)
> weight = xtabs(~index + genes, attr(yeast, +   "index"))

> fit.part <- pmlPart(edge ~ rate, fits, +   weight = weight[, 1:10])
```
### Partition Models

We can compare the different partitions with the Shimodaira-Hasegawa test.

```r
> set.seed(123)
> sh.p <- SH.test(fit.part)
> sh.p[1:9, ]
```

<table>
<thead>
<tr>
<th>Partition</th>
<th>Trees</th>
<th>ln L</th>
<th>Diff ln L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1,]</td>
<td>1</td>
<td>-10142.89</td>
<td>2.155272</td>
<td>0.8793</td>
</tr>
<tr>
<td>[2,]</td>
<td>1</td>
<td>-10188.93</td>
<td>48.190589</td>
<td>0.0374</td>
</tr>
<tr>
<td>[3,]</td>
<td>1</td>
<td>-10173.94</td>
<td>33.202811</td>
<td>0.0906</td>
</tr>
<tr>
<td>[4,]</td>
<td>1</td>
<td>-10191.95</td>
<td>51.217693</td>
<td>0.0312</td>
</tr>
<tr>
<td>[5,]</td>
<td>1</td>
<td>-10170.99</td>
<td>30.255435</td>
<td>0.1171</td>
</tr>
<tr>
<td>[6,]</td>
<td>1</td>
<td>-10192.16</td>
<td>51.427521</td>
<td>0.0367</td>
</tr>
<tr>
<td>[7,]</td>
<td>1</td>
<td>-10157.74</td>
<td>16.998760</td>
<td>0.2305</td>
</tr>
<tr>
<td>[8,]</td>
<td>1</td>
<td>-10198.69</td>
<td>57.956076</td>
<td>0.0201</td>
</tr>
<tr>
<td>[9,]</td>
<td>1</td>
<td>-10179.33</td>
<td>38.588812</td>
<td>0.0651</td>
</tr>
</tbody>
</table>

We observe that the first gene does not differ significantly from the other.
Clustering genes

If number of partitions is too high to justify a different rate for each gene, the pmlCluster clusters groups genes together which are similar.

```r
> set.seed(111)
> fit.cluster <- pmlCluster(edge ~ rate, + fits, weight = weight, p = 4)
```
### Clustering genes

```r
> set.seed(321)
> sh.c <- SH.test(fit.cluster)
> sh.c
```

<table>
<thead>
<tr>
<th>Partition</th>
<th>Trees</th>
<th>ln L</th>
<th>Diff ln L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1,]</td>
<td>1</td>
<td>-193624.9</td>
<td>1923.0837</td>
<td>0e+00</td>
</tr>
<tr>
<td>[2,]</td>
<td>1</td>
<td>-192675.5</td>
<td>973.6675</td>
<td>0e+00</td>
</tr>
<tr>
<td>[3,]</td>
<td>1</td>
<td>-192205.5</td>
<td>503.6238</td>
<td>0e+00</td>
</tr>
<tr>
<td>[4,]</td>
<td>2</td>
<td>-165790.1</td>
<td>1505.8416</td>
<td>0e+00</td>
</tr>
<tr>
<td>[5,]</td>
<td>2</td>
<td>-168746.2</td>
<td>4461.8649</td>
<td>0e+00</td>
</tr>
<tr>
<td>[6,]</td>
<td>2</td>
<td>-164647.5</td>
<td>363.2420</td>
<td>0e+00</td>
</tr>
<tr>
<td>[7,]</td>
<td>3</td>
<td>-168918.4</td>
<td>908.3175</td>
<td>0e+00</td>
</tr>
<tr>
<td>[8,]</td>
<td>3</td>
<td>-173249.8</td>
<td>5239.7492</td>
<td>0e+00</td>
</tr>
<tr>
<td>[9,]</td>
<td>3</td>
<td>-170676.9</td>
<td>2666.7813</td>
<td>0e+00</td>
</tr>
<tr>
<td>[10,]</td>
<td>4</td>
<td>-208419.1</td>
<td>512.5676</td>
<td>0e+00</td>
</tr>
<tr>
<td>[11,]</td>
<td>4</td>
<td>-208380.8</td>
<td>474.2584</td>
<td>6e-04</td>
</tr>
<tr>
<td>[12,]</td>
<td>4</td>
<td>-210897.2</td>
<td>2990.6184</td>
<td>0e+00</td>
</tr>
</tbody>
</table>

Now we cannot reject the hypothesis that all clusters differ.
Partition Models

- The partition models is quite general. One can easily specify which parameters get optimized for each partition and which for all together.
- For given trees (gene/bootstrap/MCMC etc.) estimated from sequence data one can estimate rates for changing their ecological niches (alpine, coastal environment etc.). In this case the trees not even need to have the same taxon set.
- If to many partitions exist pmlCluster can group them in clusters with similar trees/parameters.
Hadamard conjugation

Hadamard conjugation is a analytical tool to analyze relations between observed sequence patterns and edge weights.

```r
> data(yeast)
> dat = as.character(yeast)
> dat[dat == "a" | dat == "g"] = "r"
> dat[dat == "c" | dat == "t"] = "y"
> dat = phyDat(dat, type = "USER", levels = c("r", + "y"))
> sp = h2st(dat)
> write.nexus.splits(sp, file = "splits_for_SP_SpectroNet.nex")
> lento(sp)
```

Conflicting splits can be represented by an lento plot or split graphs (via SpectroNet or SplitsTree). Problem: works only for up to 24 taxa.
Lentoplot

The lento plot offers a nice possibility to illustrate these conflicting signals:
Simulating trees and sequences
User defined data formats

The data format `phyDat` is very general and it is easy to construct user defined data formats. For example following would generate data where gaps "-" are coded as a fifth state.

```r
> dat = phyDat(dat, "USER", levels = c("a", "c", "g", "t", "-"))
```

This data can used with all the parsimony or maximum likelihood methods.
Simulating trees

ape contains some functions to simulate trees:

> tree5 = unroot(rtree(5))
> treeNNI = nni(tree5)

nni in phangorn generates all trees which are one Nearest Neighbor Interchange away.
Distribution of parsimony scores

Original Tree

- t5
- t1
- t3
- t4
- t2

- t5
- t1
- t3
- t4
- t2

- t5
- t1
- t3
- t4
- t2

- t5
- t1
- t3
- t4
- t2

- t5
- t1
- t3
- t4
- t2
Simulating trees

With the function `allTrees` in `phangorn` constructs all possible trees (up to 10 taxa), what can be interesting for simulation studies.

```r
> trees = allTrees(7, tip = names(yeast)[-8])
> length(trees)
[1] 945
> pscores = parsimony(trees, yeast)
> plot(hist(pscores))
```
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Distribution of parsimony scores

Histogram of pscores
Simulating sequences

```r
> tree3 = read.tree(text = "((a:.3, b:.3):.4, c:.7);")
> dat3 = simSeq(tree3, l = 9)
> as.character(dat3)

  a  "g"  "g"  "a"  "g"  "a"  "a"  "t"  "a"  "g"
  b  "c"  "a"  "a"  "g"  "g"  "a"  "t"  "a"  "a"
  c  "c"  "t"  "t"  "c"  "g"  "g"  "t"  "a"  "g"

simSeq can be used to produce parametric bootstrap samples:
> dat0 = simSeq(fit$tree, Q = fit$Q, bf = fit$bf)
```
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+ large number of functions available
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+ large number of functions available
+ very general framework and easy to extend
+ fast to prototype new models
  - for big trees when speed is essential: RAXML, Garli
  - no Bayesian analysis (yet)
In case of help, suggestions, bugs, help with special models (mixtures / partitions), new feature requests etc. feel free to contact me:
klaus.schliep@snv.jussieu.fr
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