ape: analysis of phylogenetics and evolution

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Program

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	II	Data Structures in ape
	III	Data I/O with ape
	IV	Data Manipulation
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	VI	DNA distances
	VII	Tree estimation
	VIII	Molecular Dating
	IX	Overview on Comparative Methods
Day 3		Work on Personal Data

I Introduction to R

Starting with random data (and reminding a few statistical basics):

```
> rnorm(10)
 [1]  0.7945464  1.2347852 -0.6727558 -0.1248683 -1.0731775
 [6]  0.8186927  1.2732294 -0.3175405 -1.0760822  0.5352055
> a <- rnorm(10)
> b <- rnorm(10)
> a
 [1]  1.4626831  0.7438223  0.7839932 -0.5920665 -0.7460782
 [6]  2.3744954 -0.4458595 -0.2753595 -1.0668780 -1.0060478
> mean(a)
[1] 0.1232704
> mean(b)
[1] -0.4187831
```

Is this difference statistically significant?

```
> t.test(a, b)

        Welch Two Sample t-test

data:  a and b
t = 1.2553, df = 15.023, p-value = 0.2285
alternative hypothesis: true difference in means is not equal t
95 percent confidence interval:
 -0.3781917  1.4622988
sample estimates:
 mean of x  mean of y
 0.1232704 -0.4187831
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sample estimates:
 mean of x  mean of y
 0.1232704 -0.4187831
```

What if the test were one-tailed?

```
> t.test(a, b, alternative = "greater")

        Welch Two Sample t-test
```

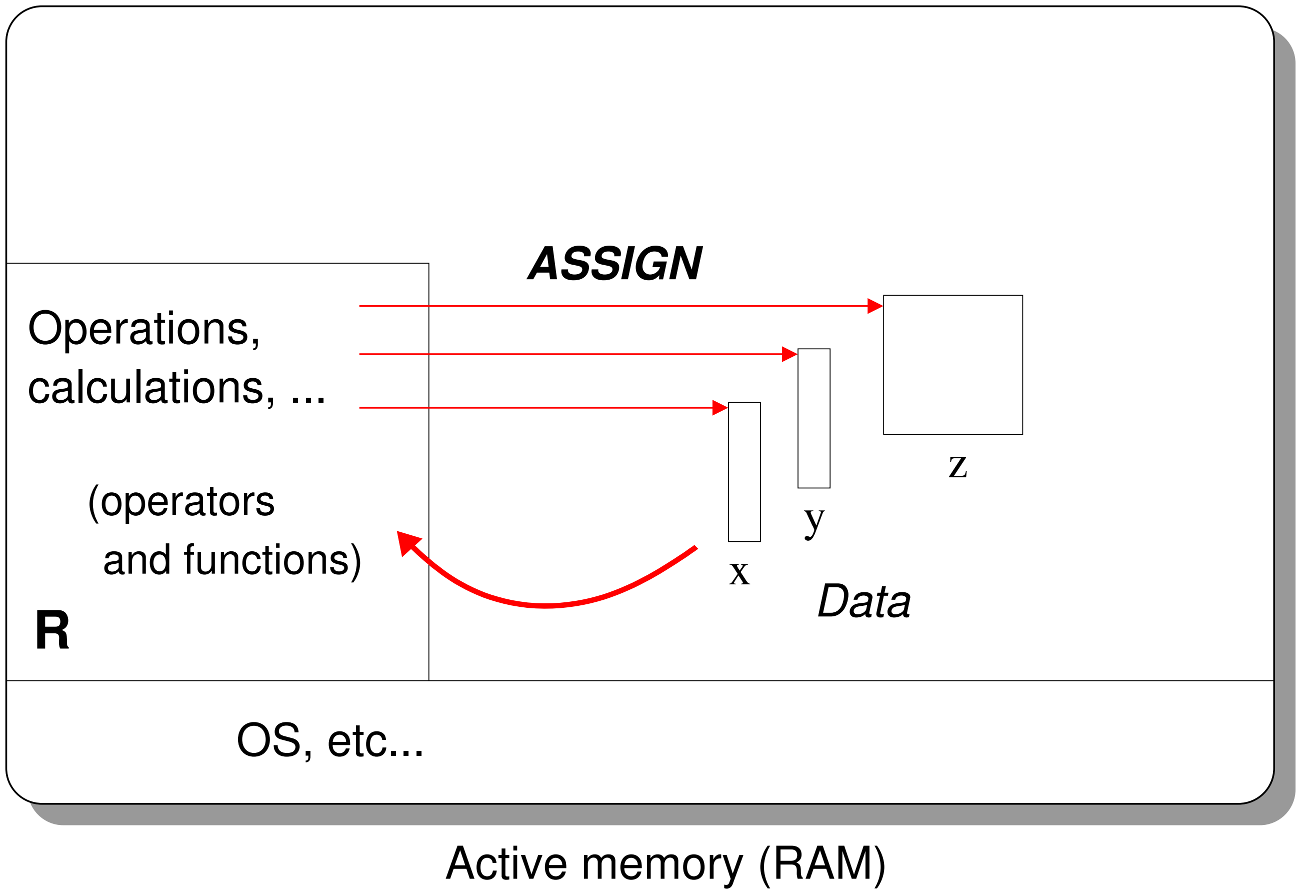
```
data:  a and b
t = 1.2553, df = 15.023, p-value = 0.1143
alternative hypothesis: true difference in means is greater tha
95 percent confidence interval:
 -0.2148436        Inf
sample estimates:
 mean of x  mean of y 
 0.1232704 -0.4187831
```

```
data:  a and b
t = 1.2553, df = 15.023, p-value = 0.1143
alternative hypothesis: true difference in means is greater tha
95 percent confidence interval:
 -0.2148436        Inf
sample estimates:
 mean of x  mean of y
 0.1232704 -0.4187831
```

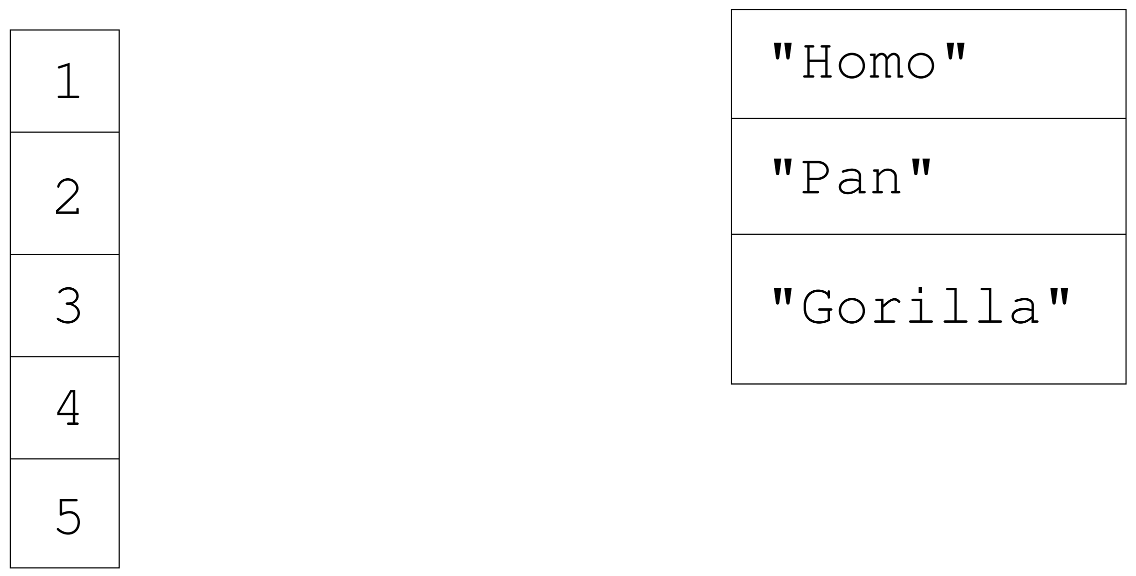
Was this second test necessary?

```
> curve(dt(x, df = 15.023), from = -3, to = 3)
> abline(v = 1.2553, col = "blue")
```

How R works



Vectors are the fundamental data structures in R.



length (= 5)

mode (= "numeric")

length (= 3)

mode (= "character")

Attributes

length: number of elements ***mode***: numeric, character, logical (complex, raw)

Vectors are combined to build more complex data structures:

- ***matrix***: “square” vector
- ***factor***: “qualitative” variable
- ***data frame***: vectors and/or factors all of same length
- ***list***: any object(s)

Generally, these structures are not built by the user, but returned by a function.

Reading/Writing Files from the Disk

`read.table` reads tabular data in text files and returns a data frame (many options);

`scan` reads any kind of data in text files;

`write.table` writes a data frame in tabular form;

`write` writes simply a vector;

... and many specialized packages on CRAN.

The indexing system of vectors: [ ]

- Numerical: positive (extract, modify and/or ‘elongate’) OR negative (extract only).

- Logical: the vector of logical indices is possibly recycled (without warning) to extract, modify and/or elongate.

- With ***names*** (= vector of mode character); to extract or modify.

Extraction and subsetting of matrices, data frames, and lists

- `[, ]` (the 3 systems) for matrices and data frames but:
  - elongation impossible,
  - `drop = TRUE` by default.

  There are no names but `colnames` and/or `rownames`. (Still names for lists.)

- Extraction from a data frame or a list: `$` (with names) `[[` (numerical or with names).

- Subsetting from a data frame or a list: `[` (the 3 systems).

  `subset` is a fenction to do the same kind of operation to select rows and/or columns of a matrix or a data frame.

Manipulating Character String

```
> paste("site", 1:3)
[1] "site 1" "site 2" "site 3"
> paste("site", 1:3, sep = "")
[1] "site1" "site2" "site3"
> paste(letters[24:26], 1:3, sep = " < ")
[1] "x < 1" "y < 2" "z < 3"
> paste(1:5, "x", letters[1:2], sep = "+")
[1] "1+x+a" "2+x+b" "3+x+a" "4+x+b" "5+x+a"
> paste(LETTERS, collapse = "")
[1] "ABCDEFGHIJKLMNOPQRSTUVWXYZ"
> paste(1:5, collapse = "-")
[1] "1-2-3-4-5"
> paste("x", 1:5, sep = "*", collapse = "; ")
[1] "x*1; x*2; x*3; x*4; x*5"
```

Character substitution:

```
> sub("a", "?", c("aaa", "aba"))
[1] "?aa" "?ba"
> gsub("a", "?", c("aaa", "aba"))
[1] "???" "?b?"
```

Character substitution:

```
> sub("a", "?", c("aaa", "aba"))
[1] "?aa" "?ba"
> gsub("a", "?", c("aaa", "aba"))
[1] "???" "?b?"
```

Pattern searching:

```
> grep("A", LETTERS)
[1] 1
> grep("A", LETTERS, value = TRUE)
[1] "A"
> grep("a", LETTERS, value = TRUE)
character(0)
> grep("a", LETTERS, value = TRUE, ignore.case = TRUE)
[1] "A"
```

```
> x <- 1:3
> names(x) <- c("Pan paniscus", "Pan troglodytes",
+               "Homo sapiens")
> x["^Pan "]
<NA>
  NA
> sel <- grep("^Pan ", names(x))
> x[sel]
   Pan paniscus Pan troglodytes
              1               2
```

Managing R scripts

A good editor allows to:

1. highlight the syntax,
2. show matching parentheses, brackets, and braces,
3. add and edit comments,
4. eventually send lines or blocks of commands directly into R.

Two main advantages in using scripts: repeating analyses, and adding comments.

To repeat analyses from a script:

1. copy/paste towards the console;
2. send the commands to R (if possible);
3. `source("script_Aedes_morpho_Dakar.R")`
4. `R CMD BATCH script_Aedes_morpho_Dakar.R`

With `R CMD BATCH`, the commands and the results are in the same file (‘script_Aedes_morpho_Dakar.Rout’).

With `source` or `R CMD BATCH`, the comments must start with `#`.

Alternatively:

```
if (FALSE) {
..........
... block of commands not executed
........
}
```

Add commands in a script

1. copy/paste from the console;
2. `savehistory("R_script_today.R")` saves the commands of the current session on the disk. If no file name is specified, '.Rhistory' will be used.

⚠ It is strongly recommended to add spaces around operators:

```
x > -1              x>-1
x < -1              x<-1   # :(
```

Add commands in a script

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```
x > -1                x>-1
x < -1                x<-1   # :(
```

Add messages in a script

```
message("Starting calculations...")
cat("n =", n, "\n")
```

`stop("......")`: stops the execution and prints `"......"`

R Programming

First program in R: assessing type I error rate of the t-test

```
p <- numeric(1000)
for (i in 1:1000) {
    x <- rnorm(20)
    y <- rnorm(20)
    p[i] <- t.test(x, y)$p.value
}
hist(p)
sum(p < 0.05)
```

Q: How would you assess type II error rate?

II Data Structures in ape

Three things to know about ape…

- close to R’s logic $\Rightarrow$ integration with standard statistical methods
- concerns a continuum of users from “low” to “high” levels
- “evolving”: new methods, bug fixing, web site

… and many others `http://ape.mpl.ird.fr/`

ape’s friends

- ade4, apTreeshape, seqinr
  close connections (data conversions, very few overlap in methods)

- RMesquite
  soon to be released (based on R–Java communication)

- geiger, laser, phangorn, phylobase, . . .
  use ape and ade4 data structures

Trees

```
> library(ape)
> tr <- rtree(20)
> tr

Phylogenetic tree with 20 tips and 19 internal nodes.

Tip labels:
        t12, t18, t2, t13, t9, t8, ...

Rooted; includes branch lengths.
> plot(tr)
```

Trees

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Tip labels:
        t12, t18, t2, t13, t9, t8, ...

Rooted; includes branch lengths.
> plot(tr)


> is.rooted(tr)
[1] TRUE
> is.binary.tree(tr)
[1] TRUE
```

```
> is.ultrametric(tr)
[1] FALSE
> tu <- rcoal(20)
> is.ultrametric(tu)
[1] TRUE
```

```
> is.ultrametric(tr)
[1] FALSE
> tu <- rcoal(20)
> is.ultrametric(tu)
[1] TRUE
```

Lists of trees:

```
> TR <- rmtree(10, 20)
> TR
10 phylogenetic trees

> plot(TR)
```

DNA Sequences

DNA sequences in ape are stored in a special format (in the RAM; not on the disk).

```
> x <- c("a", "c", "g", "t")
> x <- as.DNAbin(x)
> x
1 DNA sequence in binary format.
> base.freq(x)
   a    c    g    t
0.25 0.25 0.25 0.25
```

What is not (yet) in ape:

- Amino acid sequences: see the package seqinr
- Allelic data (under development): see the package adegenet
- SNPs, microarray data, genotype–phenotype files, etc: see Bioconductor
- Networks (reticulated trees)
- Trees with singleton nodes (of degree 2)

The current tree data structure can be generalized for the last two.

III Input/Output with ape

`read.tree` reads tree(s) in Newick format

`read.nexus` same in Nexus format

`read.dna` reads DNA sequences in Phylip, FASTA, or Clustal formats (see the option `format`)

`read.GenBank` reads DNA sequences from GenBank giving accession number(s)

`write.tree` writes tree(s) in Newick format

`write.nexus` same in Nexus format

`write.dna` reads DNA sequences in Phylip or FASTA formats (see the option `format`)

IV Data Manipulation with ape

```
> TR[1:5]
5 phylogenetic trees

> TR[[1]]

Phylogenetic tree with 20 tips and 19 internal nodes.

Tip labels:
        t2, t16, t17, t1, t19, t10, ...

Rooted; includes branch lengths.
```

Special functions for trees: `root`, `unroot`, `drop.tip`.

```
> data(woodmouse)
> summary(woodmouse)
15 DNA sequences in binary format stored in a matrix.

All sequences of same length: 965

Labels: No305 No304 No306 No0906S No0908S No0909S ...

Base composition:
    a     c     g     t
0.307 0.261 0.126 0.306
> dim(woodmouse)
[1]  15 965
> dim(woodmouse[, 1:300])
[1]  15 300
> dim(woodmouse[, c(TRUE, TRUE, FALSE)])
[1]  15 644
> dim(woodmouse[, !c(TRUE, TRUE, FALSE)])
[1]  15 321
```

Non-aligned sequences (i.e., from a FASTA file or from GenBank) are stored in a list.

Aligned sequences (i.e., from a Phylip or a Clustal file) are stored in a matrix.

`as.matrix` converts a list of sequences into a matrix if all sequences are of the same length.

Exercices IV

1. Get the sequences from GenBank with accession numbers DQ082330-DQ082374. Examine this data set and see if you can compute distances straight from it.
2. Align these sequences with Clustal (X or W). Read back the sequences into R.
3. Write eventually these commands into a script file, and repeat them for AF006387–AF006459 with only the odd numbers.
4. Put both data sets into a single matrix, possibly dropping some observations.

V Tree Plotting

`plot` is a ***generic*** function.

```
> tree.carni <- read.nexus("carnprune3-1.nex")
> plot(tree.carni)
```

Many options: see `?plot.phylo`

Tree labelling and annotation: `add.scale.bar`, `axisPhylo`, `nodelabels`, `tiplabels`, `edgelabels` with many options.

Exploring big trees:

```
> zoom(tree.carni, 1:10)
> g1 <- grep("Felis", tree.carni$tip.label)
> g2 <- grep("Panthera", tree.carni$tip.label)
> zoom(tree.carni, list(g1, g2))
```

VI DNA Distances

```
> args(dist.dna)
function (x, model = "K80", variance = FALSE, gamma = FALSE,
    pairwise.deletion = FALSE, base.freq = NULL,
    as.matrix = FALSE)
```

See also the function `dist` to compute “geometric” distances. In both cases only the lower triangle of the distance matrix is returned (except if the distance is asymmetric), and is stored in a vector.

What is the influence of missing data and gaps?

```
> d <- dist.dna(woodmouse)
> dp <- dist.dna(woodmouse, pairwise.deletion = TRUE)
> summary(d)
    Min.  1st Qu.   Median     Mean  3rd Qu.     Max.
0.002201 0.009979 0.013350 0.013120 0.016740 0.022410
> summary(dp)
    Min.  1st Qu.   Median     Mean  3rd Qu.     Max.
0.002086 0.010520 0.013690 0.013350 0.016920 0.022280
> cor(d, dp)
[1] 0.9879206
> plot(d, dp)
> abline(a = 0, b = 1, lty = 3)
```

What is the influence of multiple substitutions?

```
> djc <- dist.dna(woodmouse, "JC69")
> dr <- dist.dna(woodmouse, "raw")
> plot(djc, dr)
> abline(a = 0, b = 1, lty = 3)
```

What is the influence of multiple substitutions?

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> djc <- dist.dna(woodmouse, "JC69")
> dr <- dist.dna(woodmouse, "raw")
> plot(djc, dr)
> abline(a = 0, b = 1, lty = 3)
```

Exercices VI

1. Analyse the three data sets prepared previously with evolutionary distances. Do any sort of analyses and explorations you may find useful.

VII Tree Estimation

Distance Methods

Three methods available in ape: `nj`, `bionj`, `fastme.bal` (+ `fastme.ols`)

```
> tr.k80 <- nj(d)
> plot(tr.k80)
```

Does a different substitution model result in a different topology?

```
> tr.jc69 <- nj(djc)
> dist.topo(tr.k80, tr.jc69)
[1] 2
> plot(tc <- consensus(tr.k80, tr.jc69))
```

Bootstrapping the NJ tree:

```
> args(boot.phylo)
function (phy, x, FUN, B = 100, block = 1, trees = FALSE)
> f <- function(x) nj(dist.dna(x))
> bp <- boot.phylo(tr.k80, woodmouse, f)
> bp
 [1] 100  25  48  49  54  47  74  65  86  89  88 100  48
```

```
> par(mfcol = c(1, 2))
> plot(tr.k80)
> nodelabels(bp)
> plot(tc)
```

What about the FastME method?

```
> tr.me.k80 <- fastme.bal(d)
> dist.topo(tr.k80, tr.me.k80)
[1] 6
> dist.topo(root(tr.k80, "No305"), tr.me.k80)
[1] 0
```

The topologies are the same.

Maximum Likelihood Methods

ML phylogeny estimation in R is in its early days. ape has an interface with PHYML:

```
> args(phymltest)
function (seqfile, format = "interleaved", itree = NULL,
    exclude = NULL, execname, path2exec = NULL)
```

The trees estimated by PHYML can then be read into R with `read.tree`.

Bootstrapping is better done from PHYML; the tree then read with `read.tree` has the bootstrap values in the element `node.label`.

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Bootstrapping is better done from PHYML; the tree then read with `read.tree` has the bootstrap values in the element `node.label`.

The function `mlphylo` is in a beta-stage, and will provide soon various facilities (data partitioning, LRTs, . . .).

Note the package phangorn which has an implementation similar to PHYML (NNI-rearrangements).

Other Methods

The package phangorn has parsimony methods for DNA sequences.

Bayesian methods may be implemented using existing tools in R (e.g., likelihood calculations, generating random trees).

FastME is currently based on NNI-rearrangements. A version based on SPR- and TBR-rearrangements has been written and is under testing.

Other Methods

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Exercices VII

1. Continue the analysis of the three data sets. Apply the same guidelines than in the previous sections.

VIII Molecular Dating

Two main methods are available in ape:

1. The penalized likelihood (PL) method:

```
> args(chronopl)
function (phy, lambda, age.min = 1, age.max = NULL,
     node = "root", S = 1, tol = 1e-08, CV = FALSE,
     eval.max = 500, iter.max = 500, ...)
```

2. The mean path lengths method:

```
> args(chronoMPL)
function (phy, se = TRUE, test = TRUE)
```

(`NPRS` is obsolete and will be soon removed.)

Exercices VIII

1. Continue the analysis of the three data sets. Apply the same guidelines than in the previous section.

IX Overview on Comparative Methods

```
> source("tree_macro.R")
```

You may have to resize the graphic window!

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> source("tree_macro.R")
```

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Exercices IX

1. Do a phylogenetic comparative analysis of Carnivora body mass and litter size with the files ‘carni_data.txt’ and ‘carnprune3-1.nex’. You will use the function `pic` as a starting point.

Many thanks to Céline, Selma & Christian for the organisation of this workshop, and the University of Graz for hosting it.