

# Package ‘RRphylo’

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**Type** Package

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**Description** Functions for phylogenetic analysis (Castiglione et al, 2018 <[doi:10.1111/2041-210X.12954](https://doi.org/10.1111/2041-210X.12954)>). The functions perform the estimation of phenotypic evolutionary rates, identification of phenotypic evolutionary rate shifts, quantification of direction and size of evolutionary change in multivariate traits, the computation of ontogenetic shape vectors and test for morphological convergence.

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## Description

**RRphylo** provides tools for phylogenetic comparative analysis. The main functions allow estimation of phenotypic evolutionary rates, identification of shifts in rate of evolution, quantification of direction and size of evolutionary change of multivariate traits, and computation of species ontogenetic vectors. Additionally, there are functions for simulating phenotypic data, manipulating phylogenetic trees, and retrieving information from phylogenies. Finally, there are functions to plot and test rate shifts at particular nodes.

The complete list of functions can be displayed with `library(help = RRphylo)`

## Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

## References

Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Serio, C., Di Febbraro, M., & Raia, P. (2018). A new method for testing evolutionary rate variation and shifts in phenotypic evolution. *Methods in Ecology and Evolution*, 9: 974-983. doi:10.1111/2041-210X.12954

Piras, P., Silvestro, D., Carotenuto, F., Castiglione, S., Kotsakis, A., Maiorino, L., Melchionna, M., Mondanaro, A., Sansalone, G., Serio, C., Vero, V.A., & Raia, P. (2018). Evolution of the saber-tooth mandible: A deadly ecomorphological specialization. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 496: 166-174.

## Description

This function computes and compares ontogenetic vectors among species in a tree.

## Usage

```
angle.matrix(RR,node,Y=NULL,select.axes=c("no","yes"),
             type=c("phenotypes","rates"),cova=NULL,clus=0.5)
```

## Arguments

RR	an object produced by <a href="#">RRphylo</a> .
node	the number identifying the most recent common ancestor to all the species the user wants ontogenetic vectors be computed.
Y	multivariate trait values at tips.
select.axes	if "yes", Y variables are individually regressed against developmental stages and only significant variables are retained to compute ontogenetic vectors. All variables are retained otherwise.
type	specifies weather to perform the analysis on phenotypic ("phenotypes") or rate ("rates") vectors.
cova	the covariate to be indicated if its effect on rate values must be accounted for. Contrary to <a href="#">RRphylo</a> , cova needs to be as long as the number of tips in the tree. As the covariate only affects rates computation, there is no covariate to provide when type = "phenotypes".
clus	the proportion of clusters to be used in parallel computing. To run the single-threaded version of <code>angle.matrix</code> set <code>clus = 0</code> .

## Details

The `angle.matrix` function takes as objects a phylogenetic tree (retrieved directly from an [RRphylo](#) object), including the different ontogenetic stages of each species as polytomies. Names at tips must be written as species ID and stage number separated by the underscore. The [RRphylo](#) object `angle.matrix` is fed with is just used to extract the dichotomized version of the phylogeny. This is necessary because node numbers change randomly at dichotomizing non-binary trees. However, when performing `angle.matrix` with the covariate the [RRphylo](#) object must be produced without accounting for the covariate. Furthermore, as the covariate only affects the rates computation, it makes no sense to use it when computing vectors for phenotypic variables. Once angles and vectors are computed, `angle.matrix` performs two tests by means of standard major axis (SMA) regression. For each species pair, the "biogenetic test" verifies whether the angle between species grows during development, meaning that the two species becomes less similar to each other during growth. The "paedomorphosis test" tells whether there is heterochronic shape change in the data. Under paedomorphosis, the adult stages of one (paedomorphic) species will resemble the juvenile stages of the other (peramorphic) species. The test regresses the angles formed by the shapes at different ontogenetic stages of a species to the shape at the youngest stage of the other in the pair, against age. Then, it tests whether the two regression lines (one per species) have different slopes, and whether they have different signs. If the regression lines point to different directions, it means that one of the two species in the pair resembles, with age, the juveniles of the other, indicating paedomorphosis. Ontogenetic vectors of individual species are further computed, in reference to the MRCA of the pair, and to the first stage of each species (i.e. intraspecifically). Importantly, the size of the ontogenetic vectors of rates tell whether the two species differ in terms of developmental rate, which is crucial to understand which process is behind paedomorphosis, where it applies. While performing the analysis, the function prints messages on-screen informing about tests results. If `select.axes = "yes"`, informs the user about which phenotypic variables are used. Secondly, it specifies whether ontogenetic vectors to MRCA, and intraspecific ontogenetic vectors significantly differ in angle or size between species pairs. Then, for each species pair, it indicates if the biogenetic law and paedomorphosis apply.

**Value**

A list containing 4 objects:

1. **\$regression.matrix** a 'list' including 'angles between species' and 'angles between species to MRCA' matrices for all possible combinations of species pairs from the two sides descending from the MRCA. For each matrix, corresponding biogenetic and paedomorphosis tests are reported.
2. **\$angles.2.MRCA.and.vector.size** a 'data.frame' including angles between the resultant vector of species and the MRCA and the size of the resultant vector computed from species to MRCA, per stage per species.
3. **\$ontogenetic.vectors2MRCA** a 'data.frame' including angle, size, and corresponding x and y components, of ontogenetic vectors computed between each species and the MRCA. For both angle and size, the p-value for the difference between species pairs is reported.
4. **\$ontogenetic.vectors.to.1st.stage** a 'list' containing:
  - \$matrices: for all possible combinations of species pairs from the two sides descending from the MRCA, the upper triangle of the matrix contains the angles between different ontogenetic stages for the first species. The same applies to the lower triangle, but for the second species.
  - \$vectors: for all possible combinations of species pairs from the two sides descending from the MRCA, angles and sizes of ontogenetic vectors computed to the first stage of each species. For both, the p-value for the difference between the species pair is reported.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
## Not run:
data("DataApes")
DataApes$PCstage->PCstage
DataApes$Tstage->Tstage
DataApes$CentroidSize->CS

cc<- 2/parallel::detectCores()
RRphylo(tree=Tstage,y=PCstage,clus=cc)->RR
# Case 1. without accounting for the effect of a covariate

# Case 1.1 selecting shape variables that show significant relationship with age
# on phenotypic vectors
angle.matrix(RR,node=72,Y=PCstage,select.axes="yes",type="phenotypes",clus=cc)
# on rates vectors
angle.matrix(RR,node=72,Y=PCstage,select.axes="yes",type="rates",clus=cc)

# Case 1.2 using all shape variables
# on phenotypic vectors
angle.matrix(RR,node=72,Y=PCstage,select.axes="no",type="phenotypes",clus=cc)
# on rates vectors
```

```

angle.matrix(RR,node=72,Y=PCstage,select.axes="no",type="rates",clus=cc)

# Case 2. accounting for the effect of a covariate (on rates vectors only)

# Case 2.1 selecting shape variables that show significant relationship with age
angle.matrix(RR,node=72,Y=PCstage,select.axes="yes",type="rates", cova=CS,clus=cc)

# Case 2.2 using all shape variables
angle.matrix(RR,node=72,Y=PCstage,select.axes="no",type="rates",cova=CS,clus=cc)

## End(Not run)

```

---

conv.map

*Mapping morphological convergence on 3D surfaces*


---

## Description

Given vectors of RW (or PC) scores, the function selects the RW(PC) axes which best account for convergence and maps convergent areas on the corresponding 3D surfaces.

## Usage

```

conv.map(dataset,pcs,mshape,conv=NULL, exclude=NULL,out.rem=TRUE,
show.consensus=FALSE, plot=TRUE,col="blue",names = TRUE)

```

## Arguments

dataset	data frame (or matrix) with the RW (or PC) scores of the group or species to be compared.
pcs	RW (or PC) vectors (eigenvectors of the covariance matrix) of all the samples.
mshape	the Consensus configuration.
conv	a named character vector indicating convergent species as (indicated as "conv" in dataset) and not convergent species (indicated as "noconv").
exclude	integer: the index number of the RW (or PC) to be excluded from the comparison.
out.rem	logical: if TRUE triangles with outlying area difference are removed.
show.consensus	logical: if TRUE, the Consensus configuration is included in the comparison.
plot	logical: if TRUE, the pairwise comparisons are be plotted. For more than 5 pairwise comparisons, the plot is not shown.
col	character: the colour for the plot.
names	logical: if TRUE, the names of the groups or species are displayed in the 3d plot.

## Details

conv.map automatically builds a 3D mesh on the mean shape calculated from the Relative Warp Analysis (RWA) or Principal Component Analysis (PCA) (Schlager 2017) by applying the function `vcgBallPivoting` (**Rvcg**). conv.map further gives the opportunity to exclude some RW (or PC) axes from the analysis because, for example, in most cases the first axes are mainly related to high-order morphological differences driven by phylogeny and size variations. conv.map finds and plots the strength of convergence on 3D surfaces. An output of conv.map (if the dataset contains a number equal or lower than 5 items) is an interactive plot mapping the convergence on the 3D models. In the upper triangle of the 3D multiple layouts the rows representing the reference models and the columns the target models. On the contrary, on the lower triangle the rows correspond to the target models and the columns the reference models. In the calculation of the differences of areas we supply the possibility to find and remove outliers from the vectors of areas calculated on the reference and target surfaces. We suggest considering this possibility if the mesh may contain degenerate facets.

## Value

The function returns a list including:

- **\$angle.compare** data frame including the real angles between the given shape vectors, the angles conv computed between vectors of the selected RWs (or PCs), the angles between vectors of the non-selected RWs (or PCs), the difference conv, and its p values.
- **\$selected.pcs** RWs (or PCs) axes selected for convergence.
- **\$average.dist** symmetric matrix of pairwise distances between 3D surfaces.
- **\$surface1** list of coloured surfaces, if two meshes are given, it represents convergence between mesh A and B charted on mesh A.
- **\$surface2** list of coloured surfaces, if two meshes are given, it represents convergence between mesh A and B charted on mesh B.
- **\$scale** the value used to set the colour gradient, computed as the maximum of all differences between each surface and the mean shape.

## Author(s)

Marina Melchionna, Antonio Profico, Silvia Castiglione, Carmela Serio, Gabriele Sansalone, Pasquale Raia

## References

Schlager, S. (2017). *Morpho and Rvcg—Shape Analysis in R: R-Packages for geometric morphometrics, shape analysis and surface manipulations*. In: Statistical shape and deformation analysis. Academic Press.

## See Also

[search.conv vignette](#) ; [relWarps](#) ; [procSym](#)

**Examples**

```

## Not run:
data(DataSimians)
DataSimians$pca->pca

## Case 1. Convergent species only
dato<-pca$PCscores[c(1,4),]

CM<-conv.map(dataset = dato,
             pcs = pca$PCs,
             mshape = pca$mshape,
             show.consensus = TRUE)

## Case 2. Convergent and non-convergent species
dato<-pca$PCscores[c(1,4,7),]
conv<-c("conv", "conv", "noconv")
names(conv)<-rownames(dato)

CM<-conv.map(dataset = dato,
             pcs = pca$PCs,
             mshape = pca$mshape,
             conv = conv,
             show.consensus = TRUE,
             col = "orange")

## End(Not run)

```

---

cutPhylo

*Cut the phylogeny at a given age or node*


---

**Description**

The function cuts all the branches of the phylogeny which are younger than a specific age or node (i.e. the age of the node).

**Usage**

```
cutPhylo(tree, age=NULL, node=NULL, keep.lineage=TRUE)
```

**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
age	the age (in terms of time distance from the recent) at which the tree must be cut
node	the node whose age must be used as cutting limit.
keep.lineage	logical specifying whether lineages with no descendant tip must be retained (see example below). Default is TRUE.



**Details**

When an entire lineage is cut (i.e. one or more nodes along a path) and `keep.lineages = TRUE`, the leaves left are labeled as "l" followed by a number.

**Value**

The function returns the cut phylogeny and plots it into the graphic device. The time axis keeps the root age of the original tree.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**See Also**

[cutPhylo vignette](#)

**Examples**

```
## Not run:
library(ape)

set.seed(22)
rtree(100)->tree
3->age

cutPhylo(tree,age=age)->t1
cutPhylo(tree,age=age,keep.lineage=FALSE)->t1
cutPhylo(tree,node=151)->t2
cutPhylo(tree,node=151,keep.lineage=FALSE)->t2

## End(Not run)
```

---

DataApes

*Example dataset*

---

**Description**

Geometric morphometrics shape data regarding Apes' facial skeleton and Apes phylogentic trees (*Profico et al. 2017*).

**Usage**

```
data(DataApes)
```

**Format**

A list containing:

**\$PCstage** A data frame containing 38 shape variables for Apes' facial skull at different ontogenetic stages.

**\$PCadult** A data frame containing 3 shape variables for Apes' facial skull.

**\$Tstage** Phylogenetic tree of Apes including the different ontogenetic stages of each species as polytomies.

**\$Tadult** Phylogenetic tree of Apes.

**\$CentroidSize** numeric vector of Centroid Size values of 'PCstage'.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**References**

Profico, A., Piras, P., Buzi, C., Di Vincenzo, F., Lattarini, F., Melchionna, M., Veneziano, A., Raia, P. & Manzi, G. (2017). The evolution of cranial base and face in Cercopithecoidea and Hominoidea: Modularity and morphological integration. *American journal of primatology*,79: e22721.

---

DataCetaceans

*Example dataset*

---

**Description**

Cetaceans' body and brain mass, and phylogenetic tree (Serio et al. 2019).

**Usage**

`data(DataCetaceans)`

**Format**

A list containing:

**treecet** Cetaceans phylogenetic tree.

**masscet** numeric vector of cetaceans body masses (ln g).

**brainmasscet** numeric vector of cetaceans brain masses (ln g).

**aceMyst** body mass (ln g) for *Mystacodon selenensis*, used as node prior at the ancestor of the Mysticeti.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

## References

Serio, C., Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Di Febbraro, M., & Raia, P. (2019). Macroevolution of Toothed Whales Exceptional Relative Brain Size. *Evolutionary Biology*, 46: 332-342. doi:10.1007/s11692-019-09485-7

---

DataFelids

*Example dataset*

---

## Description

Geometric morphometrics shape data regarding felids' mandible and phylogentic tree (Piras *et al.*, 2018).

## Usage

```
data(DataFelids)
```

## Format

A list containing:

**\$PCscoresfel** A data frame containing 83 shape variables for felids' mandible.

**\$treefel** Phylogenetic tree of felids.

## Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

## References

Piras, P., Silvestro, D., Carotenuto, F., Castiglione, S., Kotsakis, A., Maiorino, L., Melchionna, M., Mondanaro, A., Sansalone, G., Serio, C., Vero, V. A., & Raia, P. (2018). Evolution of the saber-tooth mandible: A deadly ecomorphological specialization. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 496, 166-174.

DataOrnithodirans      *Example dataset*

---

**Description**

Ornithodirans' body mass, phylogenetic tree and locomotory type (*Castiglione et al 2018*).

**Usage**

```
data(DataOrnithodirans)
```

**Format**

A list containing:

**treedino** Ornithodirans phylogenetic tree.

**massdino** numeric vector of ornithodirans body masses.

**statedino** vector of ornithodirans locomotory type.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**References**

Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Serio, C., Di Febbraro, M., & Raia, P.(2018). A new method for testing evolutionary rate variation and shifts in phenotypic evolution. *Methods in Ecology and Evolution*, 9: 974-983.doi:10.1111/2041-210X.12954

---

DataSimians      *Example dataset*

---

**Description**

The output of Procrustes superimposition as performed by the function `procSym` on 9 simians faces and the phylogenetic tree for such species.

**Usage**

```
data(DataSimians)
```

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

---

DataUng

*Example dataset*

---

### Description

Geometric morphometrics shape data regarding mandible and phylogentic tree of 'Ungulatomorpha' (Raia et al., 2010).

### Usage

```
data(DataUng)
```

### Format

A list containing:

**\$PCscoresung** A data frame containing 205 shape variables for mandible of 'Ungulatomorpha'.

**\$treeung** Phylogenetic tree of 'Ungulatomorpha'.

**\$stateung** vector of 'Ungulatomorpha' feeding type.

### Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### References

Raia, P., Carotenuto, F., Meloro, C., Piras, P., & Pushkina, D. (2010). The shape of contention: adaptation, history, and contingency in ungulate mandibles. *Evolution*, 64: 1489-1503.

---

distNodes

*Finding distance between nodes and tips*

---

### Description

The function computes the distance between pairs of nodes, pairs of tips, or between nodes and tips. The distance is meant as both patristic distance and the number of nodes intervening between the pair.

### Usage

```
distNodes(tree,node=NULL,cclus=0.5)
```

**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
node	either a single node/tip or a pair of nodes/tips.
clus	the proportion of clusters to be used in parallel computing. To run the single-threaded version of <code>distNodes</code> set <code>clus = 0</code> .

**Value**

If `node` is specified, the function returns a data frame with distances between the focal node/tip and the other nodes/tips on the tree (or for the selected pair only). Otherwise, the function returns a matrix containing the number of nodes intervening between each pair of nodes and tips.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data("DataApes")
DataApes$Tstage->Tstage

cc<- 2/parallel::detectCores()
distNodes(tree=Tstage,clus=cc)
distNodes(tree=Tstage,node=64,clus=cc)
distNodes(tree=Tstage,node="Tro_2",clus=cc)
distNodes(tree=Tstage,node=c(64,48),clus=cc)
distNodes(tree=Tstage,node=c(64,"Tro_2"),clus=cc)
```

---

evo.dir

*Phylogenetic vector analysis of phenotypic change*

---

**Description**

This function quantifies direction, size and rate of evolutionary change of multivariate traits along node-to-tip paths and between species.

**Usage**

```
evo.dir(RR,angle.dimension=c("rates","phenotypes"),
  y.type=c("original","RR"),y=NULL,pair.type=c("node","tips"),pair=NULL,
  node=NULL,random=c("yes","no"),nrep=100)
```

## Arguments

RR	an object produced by <code>RRphylo</code> .
angle.dimension	specifies whether vectors of "rates" or "phenotypes" are used.
y.type	must be indicated when <code>angle.dimension = "phenotypes"</code> . If "original", it uses the phenotypes as provided by the user, if "RR" it uses <code>RR\$predicted.phenotypes</code> .
y	specifies the phenotypes to be provided if <code>y.type = "original"</code> .
pair.type	either "node" or "tips". Angles are computed between each possible couple of species descending from a specified node ("node"), or between a given couple of species ("tips").
pair	species pair to be specified if <code>pair.type = "tips"</code> . It needs to be written as in the example below.
node	node number to be specified if <code>pair.type = "node"</code> . Notice the node number must refer to the dichotomic version of the original tree, as produced by <code>RRphylo</code> .
random	whether to perform randomization test ("yes"/"no").
nrep	number of replications must be indicated if <code>random = "yes"</code> . It is set at 100 by default.

## Details

The way `evo.dir` computes vectors depends on whether phenotypes or rates are used as variables. `RRphylo` rates along a path are aligned along a chain of ancestor/descendant relationships. As such, each rate vector origin coincides to the tip of its ancestor, and the resultant of the path is given by vector addition. In contrast, phenotypic vectors are computed with reference to a common origin (i.e. the consensus shape in a geometric morphometrics). In this latter case, vector subtraction (rather than addition) will define the resultant of the evolutionary direction. It is important to realize that resultants could be at any angle even if the species (the terminal vectors) have similar phenotypes, because path resultants, rather than individual phenotypes, are being contrasted. However, the function also provides the angle between individual phenotypes as `'angle.between.species'`. To perform randomization test (`random = "yes"`), the evolutionary directions of the two species are collapsed together. Then, for each variable, the median is found, and random paths of the same size as the original paths are produced sampling at random from the 47.5th to the 52.5th percentile around the medians. This way, a random distribution of angles is obtained under the hypothesis that the two directions are actually parallel. The `'angle.direction'` represents the angle formed by the species phenotype and a vector of 1s (as long as the number of variables representing the phenotype). This way, each species phenotype is contrasted to the same vector. The `'angle.direction'` values could be inspected to test whether individual species phenotypes evolve towards similar directions.

## Value

Under all specs, `evo.dir` returns a 'list' object. The length of the list is one if `pair.type = "tips"`. If `pair.type = "node"`, the list is as long as the number of all possible species pairs descending from the node. Each element of the list contains:

**angle.path.A** angle of the resultant vector of species A to MRCA

**vector.size.species.A** size of the resultant vector of species A to MRCA  
**angle.path.B** angle of the resultant vector of species B to MRCA  
**vector.size.species.B** size of the resultant vector of species B to MRCA  
**angle.between.species.to.mrca** angle between the species paths resultant vectors to the MRCA  
**angle.between.species** angle between species vectors (as they are, without computing the path)  
**MRCA** the node identifying the most recent common ancestor of A and B  
**angle.direction.A** angle of the vector of species A (as it is, without computing the path) to a fixed reference vector (the same for all species)  
**vec.size.direction.A** size of the vector of species A  
**angle.direction.B** angle of the vector of species B (as it is, without computing the path) to a fixed reference vector (the same for all species)  
**vec.size.direction.B** size of the vector of species B  
 If random = "yes", results also include p-values for the angles.

### Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### Examples

```
## Not run:
data("DataApes")
DataApes$PCstage->PCstage
DataApes$Tstage->Tstage
cc<- 2/parallel::detectCores()

RRphylo(tree=Tstage,y=PCstage, clus=cc)->RR

# Case 1. Without performing randomization test

# Case 1.1 Computing angles between rate vectors
# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="rates",pair.type="node",node=72 ,
  random="no")
# for a given couple of species
evo.dir(RR,angle.dimension="rates",pair.type="tips",
  pair= c("Sap_1","Tro_2"),random="no")

# Case 1.2 computing angles between phenotypic vectors provided by the user
# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="phenotypes",y.type="original",
  y=PCstage,pair.type="node",node=72,random="no")
# for a given couple of species
evo.dir(RR,angle.dimension="phenotypes",y.type="original",
  y=PCstage,pair.type="tips",pair=c("Sap_1","Tro_2"),random="no")

# Case 1.3 computing angles between phenotypic vectors produced by "RRphylo"
```



```

# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="phenotypes",y.type="RR",
pair.type="node",node=72,random="no")
# for a given couple of species
evo.dir(RR,angle.dimension="phenotypes",y.type="RR",
pair.type="tips",pair=c("Sap_1","Tro_2"),random="no")

# Case 2. Performing randomization test

# Case 2.1 Computing angles between rate vectors
# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="rates",pair.type="node",node=72 ,
random="yes",nrep=10)

# for a given couple of species
evo.dir(RR,angle.dimension="rates",pair.type="tips",
pair= c("Sap_1","Tro_2"),random="yes",nrep=10)

# Case 2.2 computing angles between phenotypic vectors provided by the user
# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="phenotypes",y.type="original",
y=PCstage,pair.type="node",node=72,random="yes",nrep=10)

# for a given couple of species
evo.dir(RR,angle.dimension="phenotypes",y.type="original",
y=PCstage,pair.type="tips",pair=c("Sap_1","Tro_2"),random="yes",nrep=10)

# Case 2.3 computing angles between phenotypic vectors produced by "RRphylo"
# for each possible couple of species descending from node 72
evo.dir(RR,angle.dimension="phenotypes",y.type="RR",
pair.type="node",node=72,random="yes",nrep=10)

# for a given couple of species
evo.dir(RR,angle.dimension="phenotypes",y.type="RR",
pair.type="tips",pair=c("Sap_1","Tro_2"),random="yes",nrep=10)

## End(Not run)

```

---

fix.poly

*Resolving polytomies to non-zero length branches*


---

## Description

The function either collapses clades under a polytomy or resolves polytomous clades to non-zero length branches, dichotomous clades.

## Usage

```
fix.poly(tree,type=c("collapse","resolve"),node=NULL,tol=1e-10,random=TRUE)
```

**Arguments**

tree	a phylogenetic tree.
type	either 'collapse' to create polytomies to one or more specific nodes or 'resolve' to resolve (fix) all the polytomies within the tree or to one or more specific nodes.
node	the node in the tree where a polytomy should be created or fixed, either. If type='resolve' and node=NULL all the polytomies present in the tree are resolved.
tol	the tolerance to consider a branch length significantly greater than zero, set at 1e-10 by default. If type='resolve', all the branch lengths smaller than tol are treated as polytomies.
random	a logical value specifying whether to resolve the polytomies randomly (the default) or in the order they appear in the tree (if random = FALSE).

**Details**

Under type='resolve' polytomous clades are resolved adding non-zero length branches to each new node. The evolutionary time attached to the new nodes is partitioned equally below the dichotomized clade.

**Value**

A phylogenetic tree with randomly fixed (i.e. type='resolve') polytomies or created polytomies (i.e. type='collapse').

**Author(s)**

Silvia Castiglione, Pasquale Raia, Carmela Serio

**References**

Castiglione, S., Serio, C., Piccolo, M., Mondanaro, A., Melchionna, M., Di Febbraro, M., Sansalone, G., Wroe, S., & Raia, P. (2020). The influence of domestication, insularity and sociality on the tempo and mode of brain size evolution in mammals. *Biological Journal of the Linnean Society*, in press. doi:10.1093/biolinnean/blaa186

**See Also**

[fix.poly vignette](#);

**Examples**

```
## Not run:
require(ape)

data("DataCetaceans")
DataCetaceans$treecet->treecet

# Resolve all the polytomies within Cetaceans phylogeny
```

```

fix.poly(treecet,type="resolve")->treecet.fixed
par(mfrow=c(1,2))
plot(treecet,no.margin=TRUE,show.tip.label=FALSE)
plot(treecet.fixed,no.margin=TRUE,show.tip.label=FALSE)

# Resolve the polytomies pertaining the genus Kentriodon
fix.poly(treecet,type="resolve",node=221)->treecet.fixed2
par(mfrow=c(1,2))
plot(treecet,no.margin=TRUE,show.tip.label=FALSE)
plot(treecet.fixed2,no.margin=TRUE,show.tip.label=FALSE)

# Collapse Delphinidae into a polytomous clade
fix.poly(treecet,type="collapse",node=179)->treecet.collapsed
par(mfrow=c(1,2))
plot(treecet,no.margin=TRUE,show.tip.label=FALSE)
plot(treecet.collapsed,no.margin=TRUE,show.tip.label=FALSE)

## End(Not run)

```

---

getMommy

*Upward tip or node to root path*


---

### Description

This function is a wrapper around **phytools** `getDescendants` (Revell 2012). It returns the node path from a given node or species to the root of the phylogeny.

### Usage

```
getMommy(tree,N,curr=NULL)
```

### Arguments

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
N	the number of node or tip to perform the function on. Notice the function only works with number, not tip labels.
curr	has not to be provided by the user.

### Details

The object 'curr' is created inside the function in order to produce an array of nodes on the path.

### Value

The function produces a vector of node numbers as integers, collated from a node or a tip towards the tree root.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**References**

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3: 217-223.doi:10.1111/j.2041-210X.2011.00169.x

**Examples**

```
data("DataApes")
DataApes$Tstage->Tstage

getMommy(tree=Tstage,N=12)
```

---

getSis

*Get sister clade*

---

**Description**

The function identifies and returns the sister clade of a given node/tip.

**Usage**

```
getSis(tree,n,printZoom=TRUE)
```

**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
n	number of focal node or name of focal tip.
printZoom	if TRUE the function plots the tree section of interest.

**Value**

The sister node number or sister tip name. In case of polytomies, the function returns a vector.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data(DataOrnithodirans)
DataOrnithodirans$treedino->treedino
getSis(tree=treedino,n=677,printZoom=FALSE)
getSis(tree=treedino,n="Shenzhoupterus_chaoyangensis",printZoom=FALSE)
```

---

makeFossil	<i>Make fossil species on a phylogeny</i>
------------	---

---

**Description**

This function takes an object of class 'phylo' and randomly changes the lengths of the leaves.

**Usage**

```
makeFossil(tree,p=0.5,ex=0.5)
```

**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
p	the proportion of tips involved. By default it is half of the number of tips.
ex	the multiplying parameter to change the leaf lengths. It is set at 0.5 by default.

**Value**

The function produces a phylogeny having the same backbone of the original one.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data("DataApes")
DataApes$Tstage->Tstage

makeFossil(tree=Tstage)
```

---

makeL	<i>Matrix of branch lengths along root-to-tip paths</i>
-------	---

---

**Description**

This function produces a  $n * m$  matrix, where  $n$ =number of tips and  $m$ =number of branches (i.e.  $n + \text{number of nodes}$ ). Each row represents the branch lengths aligned along a root-to-tip path.

**Usage**

```
makeL(tree)
```

**Arguments**

tree                    a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.

**Value**

The function returns a  $n * m$  matrix of branch lengths for all root-to-tip paths in the tree (one per species).

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data("DataApes")
DataApes$Tstage->Tstage

makeL(tree=Tstage)
```

---

makeL1

*Matrix of branch lengths along a root-to-node path*

---

**Description**

This function produces a  $n * n$  matrix, where n=number of internal branches. Each row represents the branch lengths aligned along a root-to-node path.

**Usage**

```
makeL1(tree)
```

**Arguments**

tree                    a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.

**Value**

The function returns a  $n * n$  matrix of branch lengths for all root-to-node paths (one per each node of the tree).

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data("DataApes")
DataApes$Tstage->Tstage

makeL1(tree=Tstage)
```

---

node.paths

*Tracing nodes along paths*

---

**Description**

Given a vector of nodes, the function collates nodes along individual lineages from the youngest (i.e. furthest from the tree root) to the oldest.

**Usage**

```
node.paths(tree, vec)
```

**Arguments**

tree            a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.  
vec             a vector of node numbers

**Value**

A list of node paths, each starting from the youngest node (i.e. furthest from the tree root) and ending to the oldest along the path.

**Author(s)**

Silvia Castiglione, Pasquale Raia

**Examples**

```
require(ape)

rtree(100)->tree
sample(seq(Ntip(tree)+1,Ntip(tree)+Nnode(tree)),20)->nods
plot(tree,show.tip.label=FALSE)
nodelabels(node=nods,frame="n",col="red")
node.paths(tree=tree, vec=nods)
```

overfitRR

*Testing RRphylo methods overfit***Description**

Testing the robustness of `search.trend` (Castiglione et al. 2019a), `search.shift` (Castiglione et al. 2018), `search.conv` (Castiglione et al. 2019b), and `PGLS_fossil` results to sampling effects and phylogenetic uncertainty.

**Usage**

```
overfitRR(RR,y,s=0.25,swap.args=NULL,trend.args=NULL,shift.args=NULL,conv.args=NULL,
pgls.args=NULL,aces=NULL,x1=NULL,aces.x1=NULL,cov=NULL,rootV=NULL,nsim=100,clus=.5)
```

**Arguments**

RR	an object produced by <code>RRphylo</code> .
y	a named vector of phenotypes.
s	the percentage of tips to be cut off. It is set at 25% by default.
swap.args	a list of arguments to be passed to the function <code>swapONE</code> , including <code>list(si=NULL,si2=NULL,node=NULL)</code> . If <code>swap.arg</code> is unspecified, the function automatically sets both <code>si</code> and <code>si2</code> to 0.1.
trend.args	a list of arguments specific to the function <code>search.trend</code> , including <code>list(node=NULL,x1.residuals=FA)</code> . If a trend for the whole tree is to be tested, type <code>trend.args = list()</code> . No trend is tested if left unspecified.
shift.args	a list of arguments specific to the function <code>search.shift</code> , including <code>list(node=NULL,state=NULL)</code> . Arguments <code>node</code> and <code>state</code> can be specified at the same time.
conv.args	a list of arguments specific to the function <code>search.conv</code> , including <code>list(node=NULL,state=NULL,declust=FALSE)</code> . Arguments <code>node</code> and <code>state</code> can be specified at the same time.
pgls.args	a list of arguments specific to the function <code>PGLS_fossil</code> , including <code>list(modform,data,tree=FALSE,RR=TRUE)</code> . If <code>tree=TRUE</code> , <code>PGLS_fossil</code> is performed by using the <code>RRphylo</code> output <code>tree</code> as <code>tree</code> argument. If <code>RR=TRUE</code> , <code>PGLS_fossil</code> is performed by using the <code>RRphylo</code> output as <code>RR</code> argument. Arguments <code>tree</code> and <code>RR</code> can be <code>TRUE</code> at the same time.
aces	if used to produce the <code>RR</code> object, the vector of those ancestral character values at nodes known in advance must be specified. Names correspond to the nodes in the tree.
x1	the additional predictor to be specified if the <code>RR</code> object has been created using an additional predictor (i.e. multiple version of <code>RRphylo</code> ). 'x1' vector must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running <code>RRphylo</code> on the predictor as well, and taking the vector of ancestral states and tip values to form the <code>x1</code> .



<code>aces.x1</code>	a named vector of ancestral character values at nodes for <code>x1</code> . It must be indicated if the RR object has been created using both <code>aces</code> and <code>x1</code> . Names correspond to the nodes in the tree.
<code>cov</code>	if used to produce the RR object, the covariate must be specified. As in <code>RRphylo</code> , the covariate vector must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running <code>RRphylo</code> on the covariate as well, and taking the vector of ancestral states and tip values to form the covariate.
<code>rootV</code>	if used to produce the RR object, the phenotypic value at the tree root must be specified.
<code>nsim</code>	number of simulations to be performed. It is set at 100 by default.
<code>clus</code>	the proportion of clusters to be used in parallel computing. To run the single-threaded version of <code>overfitRR</code> set <code>clus = 0</code> .

## Details

Methods using a large number of parameters risk being overfit. This usually translates in poor fitting with data and trees other than the those originally used. With `RRphylo` methods this risk is usually very low. However, the user can assess how robust the results got by applying `search.shift`, `search.trend`, `search.conv` or `PGLS_fossil` are by running `overfitRR`. With the latter, the original tree and data are subsampled by specifying a `s` parameter, that is the proportion of tips to be removed from the tree. In some cases, though, removing as many tips as imposed by `s` would delete too many tips right in clades and/or states under testing. In these cases, the function maintains no less than 5 species at least in each clade/state under testing (or all species if there is less), reducing the sampling parameter `s` if necessary. Internally, `overfitRR` further shuffles the tree by using the function `swapONE`. Thereby, both the potential for overfit and phylogenetic uncertainty are accounted for straight away.

## Value

The function returns a 'RRphyloList' object containing:

**\$mean.sampling** the mean proportion of species actually removed from the tree over the iterations.

**\$tree.list** a 'multiPhylo' list including the trees generated within `overfitRR`

**\$RR.list** a 'RRphyloList' including the results of each `RRphylo` performed within `overfitRR`

**\$rootCI** the 95% confidence interval around the root value.

**\$ace.regressions** a 'RRphyloList' including the results of linear regression between ancestral state estimates before and after the subsampling.

**\$conv.results** a list including results for `search.conv` performed under clade and state conditions. If a node pair is specified within `conv.args`, the `$clade` object contains the percentage of simulations producing significant p-values for convergence between the clades. If a state vector is supplied within `conv.args`, the object `$state` contains the percentage of simulations producing significant p-values for convergence within (single state) or between states (multiple states).

**\$shift.results** a list including results for `search.shift` performed under clade and sparse conditions. If one or more nodes are specified within `shift.args`, the `$clade` object contains for each node the percentage of simulations producing significant p-value separated by shift sign, and the same figures by considering all the specified nodes as evolving under a single rate (`all.clades`). If a

state vector is supplied within `shift.args`, the object `$sparse` contains the percentage of simulations producing significant p-value separated by shift sign (`$p.states`).

**\$trend.results** a list including the percentage of simulations showing significant p-values for phenotypes versus age and absolute rates versus age regressions for the entire tree separated by slope sign (`$tree`). If one or more nodes are specified within `trend.args`, the list also includes the same results at nodes (`$node`) and the results for comparison between nodes (`$comparison`).

**\$pgls.results** two 'RRphyloList' objects including results of PGLS\_fossil performed by using the phylogeny as it is (`$tree`) or rescaled according to the RRphylo rates (`$RR`).

### Author(s)

Silvia Castiglione, Carmela Serio, Pasquale Raia

### References

Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Serio, C., Di Febbraro, M., & Raia, P. (2018). A new method for testing evolutionary rate variation and shifts in phenotypic evolution. *Methods in Ecology and Evolution*, 9: 974-983. doi:10.1111/2041-210X.12954

Castiglione, S., Serio, C., Mondanaro, A., Di Febbraro, M., Profico, A., Girardi, G., & Raia, P. (2019a) Simultaneous detection of macroevolutionary patterns in phenotypic means and rate of change with and within phylogenetic trees including extinct species. *PLoS ONE*, 14: e0210101. <https://doi.org/10.1371/journal.pone.0210101>

Castiglione, S., Serio, C., Tamagnini, D., Melchionna, M., Mondanaro, A., Di Febbraro, M., Profico, A., Piras, P., Barattolo, F., & Raia, P. (2019b). A new, fast method to search for morphological convergence with shape data. *PLoS ONE*, 14, e0226949. <https://doi.org/10.1371/journal.pone.0226949>

### See Also

[overfitRR vignette](#) ; [search.trend vignette](#) ; [search.shift vignette](#) ; [search.conv vignette](#) ;

### Examples

```
## Not run:
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino
DataOrnithodirans$massdino->massdino
DataOrnithodirans$statedino->statedino
cc<- 2/parallel::detectCores()

# Extract Pterosaurs tree and data
library(ape)
extract.clade(treedino,746)->treeptero
massdino[match(treeptero$tip.label,names(massdino))]->massptero
massptero[match(treeptero$tip.label,names(massptero))]->massptero

RRphylo(tree=treedino,y=massdino)->dinoRates
RRphylo(tree=treeptero,y=log(massptero))->RRptero

# Case 1 search.shift under both "clade" and "sparse" condition
```

```

search.shift(RR=dinoRates, status.type= "clade",
             filename=paste(tempdir(),"SSnode",sep="/"))->SSnode
search.shift(RR=dinoRates, status.type= "sparse", state=statedino,
             filename=paste(tempdir(),"SSstate",sep="/"))->SSstate

overfitRR(RR=dinoRates,y=massdino,swap.args =list(si=0.2,si2=0.2),
          shift.args = list(node=rownames(SSnode$single.clades),state=statedino),
          nsim=10,clus=cc)->orr.ss

# Case 2 search.trend on the entire tree
search.trend(RR=RRptero, y=log(massptero),nsim=100,clus=cc,cov=NULL,node=NULL,
             filename=paste(tempdir(),"STtree",sep="/"),ConfInt=FALSE)->STtree

overfitRR(RR=RRptero,y=log(massptero),swap.args =list(si=0.2,si2=0.2),
          trend.args = list(),nsim=10,clus=cc)->orr.st1

# Case 3 search.trend at specified nodescov=NULL,
search.trend(RR=RRptero, y=log(massptero),node=143,clus=cc,
             filename=paste(tempdir(),"STtree",sep="/"),ConfInt=FALSE)->STnode

overfitRR(RR=RRptero,y=log(massptero),
          trend.args = list(node=143),nsim=10,clus=cc)->orr.st2

# Case 4 overfitRR on multiple RRphylo
data("DataCetaceans")
DataCetaceans$treecet->treecet
DataCetaceans$masscet->masscet
DataCetaceans$brainmasscet->brainmasscet
DataCetaceans$aceMyst->aceMyst

ape::drop.tip(treecet,treecet$tip.label[-match(names(brainmasscet),
                                               treecet$tip.label)])->treecet.multi
masscet[match(treecet.multi$tip.label,names(masscet))]->masscet.multi

RRphylo(tree=treecet.multi,y=masscet.multi)->RRmass.multi
RRmass.multi$aces[,1]->acemass.multi
c(acemass.multi,masscet.multi)->x1.mass

RRphylo(tree=treecet.multi,y=brainmasscet,x1=x1.mass)->RRmulti
search.trend(RR=RRmulti, y=brainmasscet,x1=x1.mass,clus=cc,
             filename=paste(tempdir(),"STtree",sep="/"))->STcet
overfitRR(RR=RRmulti,y=brainmasscet,trend.args = list(),
          x1=x1.mass,nsim=10,clus=cc)->orr.st3

search.trend(RR=RRmulti, y=brainmasscet,x1=x1.mass,x1.residuals=TRUE,
             clus=cc,filename=paste(tempdir(),"STcet.resi",sep="/"))->STcet.resi
overfitRR(RR=RRmulti,y=brainmasscet,trend.args = list(x1.residuals=TRUE),
          x1=x1.mass,nsim=10,clus=cc)->orr.st4

# Case 5 searching convergence between clades and within a single state
data("DataFelids")
DataFelids$PCscoresfel->PCscoresfel
DataFelids$treefel->treefel

```

```

DataFelids$statefel->statefel

RRphylo(tree=treefel,y=PCscoresfel,clus=cc)->RRfel
search.conv(RR=RRfel, y=PCscoresfel, min.dim=5, min.dist="node9",
            filename = paste(tempdir(),"SC.clade",sep="/"),clus=cc)->SC.clade
as.numeric(c(rownames(SC.clade[[1]])[1],as.numeric(as.character(SC.clade[[1]])[1,1])))->conv.nodes

overfitRR(RR=RRfel, y=PCscoresfel,conv.args =
list(node=conv.nodes,state=statefel,declust=TRUE),nsim=10,clus=cc)->orr.sc

# Case 6 overfitRR on PGLS_fossil
library(phytools)
rtree(100)->tree
fastBM(tree)->resp
fastBM(tree,nsim=3)->resp.multi
fastBM(tree)->pred1
fastBM(tree)->pred2

PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp,x2=pred1,x1=pred2),tree=tree)->pgls_noRR

RRphylo::RRphylo(tree,resp)->RR
PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp,x2=pred1,x1=pred2),tree=tree,RR=RR)->pgls_RR

overfitRR(RR=RR,y=resp,
          pgl.args=list(modform=y1~x1+x2,data=list(y1=resp,x2=pred1,x1=pred2),
                    tree=TRUE,RR=TRUE),nsim=10,clus=cc)->orr.pgl1

PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp.multi,x2=pred1,x1=pred2),tree=tree)->pgls2_noRR
cc<- 2/parallel::detectCores()
RRphylo::RRphylo(tree,resp.multi,clus=cc)->RR
PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp.multi,x2=pred1,x1=pred2),tree=tree,RR=RR)->pgls2_RR

overfitRR(RR=RR,y=resp.multi,
          pgl.args=list(modform=y1~x1+x2,data=list(y1=resp.multi,x2=pred1,x1=pred2),
                    tree=TRUE,RR=TRUE),nsim=10,clus=cc)->orr.pgl2

## End(Not run)

```

---

PGLS\_fossil

*Phylogenetic Generalized Least Square with fossil phylogenies*


---

## Description

The function performs pgl for non-ultrametric trees using either Pagel's lambda transform, Brownian Motion or [RRphylo](#) rates to change the correlation structure.

## Usage

```
PGLS_fossil(modform,data,tree,RR=NULL)
```

**Arguments**

modform	the formula for the regression model.
data	a list of named vectors including response and predictor variables as named in modform.
tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
RR	the result of RRphylo performed on the response variable. If NULL the function fits Pagel's lambda in the regression for univariate data or uses the tree variance covariance matrix in the multivariate case. If RR is specified, tree branches are rescaled to the absolute branch-wise rate values calculated by RRphylo to transform the variance-covariance matrix.

**Details**

With univariate data, the user may want to use either Pagel's lambda or RRphylo rates to transform the correlation structure. In the former case, the lambda transform is fitted to the data (Revell, 2010). In the latter case, branch lengths are multiplied by absolute rates as computed by RRphylo to accommodate rate variation across the tree. In the multivariate case, the variance-covariance structure is either left unaltered by keeping RR = NULL (Adams and Collyer, 2015) or changed according to the norm-2 vector of rates computed for each phenotype by specifying the RR object.

**Value**

Fitted pgl parameters and significance.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**References**

- Revell, L.J. (2010). Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution*, 1, 319-329. <https://doi.org/10.1111/j.2041-210X.2010.00044.x>
- Adams, D.C., & Collyer, M. L. (2017). Multivariate phylogenetic comparative methods: evaluations, comparisons, and recommendations. *Systematic Biology*, 67, 14-31. <https://doi.org/10.1093/sysbio/syx055>

**See Also**

[RRphylo vignette](#)

**Examples**

```
## Not run:
library(ape)
library(phytools)

rtree(100)->tree
fastBM(tree)->resp
fastBM(tree,nsim=3)->resp.multi
```

```

fastBM(tree)->pred1
fastBM(tree)->pred2

PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp,x2=pred1,x1=pred2),tree=tree)->pgls_noRR

RRphylo::RRphylo(tree,resp)->RR
PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp,x2=pred1,x1=pred2),tree=tree,RR=RR)->pgls_RR

# To derive log-likelihood and AIC for PGLS_fossil outputs performed on univariate data
# without including the RR object, the function AIC can be applied
AIC(pgl_s_noRR)

# For univariate with RR and multivariate models, the function "model.comparison"
# from the package RRPP must be used.
RRPP::model.comparison(pgl_s_RR,type = "logLik")

PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp.multi,x2=pred1,x1=pred2),tree=tree)->pgls2_noRR
cc<- 2/parallel::detectCores()
RRphylo::RRphylo(tree,resp.multi,clus=cc)->RR
PGLS_fossil(modform=y1~x1+x2,data=list(y1=resp.multi,x2=pred1,x1=pred2),tree=tree,RR=RR)->pgls2_RR

RRPP::model.comparison(pgl_s2_noRR,pgls2_RR,type = "logLik")

## End(Not run)

```

---

phyloclust

*Test for phylogenetic clustering*


---

## Description

The function tests the presence of phylogenetic clustering for species within a focal state.

## Usage

```
phyloclust(tree, state, focal, nsim=100)
```

## Arguments

tree	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous.
state	the named vector of tip states.
focal	the focal state to be tested for phylogenetic clustering.
nsim	number of simulations to perform the phylogenetic clustering test.

**Details**

To test for phylogenetic clustering, the function computes the mean cophenetic (i.e. evolutionary time) distance between all the species under the focal state. Such value is compared to a random distribution of time distances obtained by sampling `nsim` times as many random tips as those under the focal state. In the presence of significant phylogenetic clustering, tips under the focal state are randomly removed until the `p` becomes  $>0.05$  or only 3 tips are left.

**Value**

The function returns a list including the p-value (`$p`) for the test of phylogenetic clustering and a `$declusterized` object containing the declusterized versions of the original tree and state vector (i.e. tips are removed as to make  $p>0.05$ ) and the vector of removed species.

**Author(s)**

Silvia Castiglione, Pasquale Raia

**Examples**

```
data("DataFelids")
DataFelids$treefel->treefel
DataFelids$statefel->statefel

phyloclust(tree=treefel,state=statefel,focal="saber")
```

---

plotRates

*Plot RRphylo rates at a specified node*

---

**Description**

The function `plotRates` plots the [RRphylo](#) rates computed for a given clade as compared to the rates computed for the rest of the tree.

**Usage**

```
plotRates(RR,node,export.tiff =FALSE,foldername=NULL,filename=NULL)
```

**Arguments**

<code>RR</code>	an object produced by <a href="#">RRphylo</a> .
<code>node</code>	the node subtending the clade of interest.
<code>export.tiff</code>	if TRUE the function save a "rate_bars.tiff" file inside the working directory. It is TRUE by default.
<code>foldername</code>	has been deprecated; please see the argument <code>filename</code> instead.
<code>filename</code>	a character indicating the name of the tiff file and the path where it is to be saved. If no path is indicated the file is stored in the working directory.

**Value**

The function produces two barplots. On the left side, the rates (in absolute values) computed for the focal clade (blue) are plotted against the rates of the rest of the tree (green). On the right side, the absolute rates of individual branches of the focal clade are collated in increasing rate value (blue bars), and contrasted to the average rate computed over the rest of the tree branches (the vertical red line). It also returns the rate values for both nodes and species descending from the focal node.

**See Also**

[RRphylo vignette](#)

**Examples**

```
data("DataApes")
DataApes$PCstage->PCstage
DataApes$Tstage->Tstage
cc<- 2/parallel::detectCores()

RRphylo(tree=Tstage,y=PCstage,clus=cc)->RR

plotRates(RR,node=72,export.tiff = FALSE)
```

---

random.evolvability.test

*Randomization test for phylogenetic structuring in evolvability*

---

**Description**

The function is a wrapper around the function [MeanMatrixStatistics](#) from the package **evolgg** (Melo *et al.* 2015). It estimates ancestral character at internal nodes either according to Brownian Motion or by means of [RRphylo](#) (see the argument `node.estimation`), then performs [MeanMatrixStatistics](#) to calculate: Mean Squared Correlation, ICV, Autonomy, ConditionalEvolvability, Constraints, Evolvability, Flexibility, Pc1Percent, and Respondability. To assess the importance of phylogenetic structuring (signal) on Respondability Evolvability, and Flexibility, the function performs a randomization test by randomly shuffling the species on tree and replicating the analyses `nsim` times. A p-value is computed by contrasting the real metrics to the ones derived by randomization.

**Usage**

```
random.evolvability.test(tree,data,node.estimation=c("RR","BM"),
  aces=NULL,iterations=1000,nsim=100,clus=0.5)
```



**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous.
data	a matrix or data.frame of phenotypic data having species as rownames
node.estimation	specify the method to compute ancestral character at nodes. It can be one of "RR", to compute ancestral states by mean of RRphylo, or "BM", to use <b>phytools</b> ' function fastAnc ( <i>Paradis &amp; Schliep 2019</i> ) to estimate ancestral characters at nodes according to Brownian Motion.
aces	a named matrix of known ancestral character values at nodes. Names correspond to the nodes in the tree.
iterations	the iterations argument to be indicated in MeanMatrixStatistics
nsim	the number of simulations to be performed for the randomization test, by default nrep is set at 100.
clus	the proportion of clusters to be used in parallel computing. To run the single-threaded version of NOME set clus = 0.

**Value**

The function returns a list object including (`$means`) the mean values for all statistics as produced by MeanMatrixStatistics and (`$means`) the significance levels for Responsibility, Evolvability, and Flexibility.

**Author(s)**

Silvia Castiglione, Gabriele Sansalone, Pasquale Raia

**References**

- Melo, D., Garcia, G., Hubbe, A., Assis, A. P., & Marroig, G. (2015). EvolQG-An R package for evolutionary quantitative genetics. *F1000Research*, 4.
- Revell, L. J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217-223.

**See Also**

[RRphylo vignette](#)

**Examples**

```
## Not run:
library(ape)
library(phytools)

rtree(30)->tree
fastBM(tree,nsim=4)->y

random.evolvability.test(tree=tree,data=y,node.estimation="RR")->rEvTest
```

```
## End(Not run)
```

---

```
rate.map
```

*Mapping rate and direction of phenotypic change on 3D surfaces.*

---

## Description

Given vectors of RW (or PC) scores, the function selects the RW(PC) axes linked to highest (and lowest) evolutionary rate values and reconstructs the morphology weighted on them. In this way, `rate.map` shows where and how the phenotype changed the most between any pair of taxa.

## Usage

```
rate.map(x, RR, PCscores, pcs, mshape, out.rem = TRUE,
        shape.diff=FALSE, show.names=TRUE)
```

## Arguments

<code>x</code>	the species/nodes to be compared; it can be a single species, or a vector containing two species or a species and any of its parental nodes.
<code>RR</code>	an object generated by using the <a href="#">RRphylo</a> function
<code>PCscores</code>	PC scores.
<code>pcs</code>	RW (or PC) vectors (eigenvectors of the covariance matrix) of all the samples.
<code>mshape</code>	the Consensus configuration.
<code>out.rem</code>	logical: if TRUE mesh triangles with outlying area difference are removed.
<code>shape.diff</code>	logical: if TRUE, the mesh area differences are displayed in an additional 3d plot.
<code>show.names</code>	logical: if TRUE, the names of the species are displayed in the 3d plot.

## Details

After selecting the PC axes, `rate.map` automatically builds a 3D mesh on the mean shape calculated from the Relative Warp Analysis (RWA) or Principal Component Analysis (PCA) (*Schlager 2017*) by applying the function [vcgBallPivoting](#) (**Rvcg**). Then, it compares the area differences between corresponding triangles of the 3D surfaces reconstructed for the species and surface of the mrca. Finally, `rate.map` returns a 3D plot showing such comparisons displayed on shape of the mrca used as the reference. The colour gradient goes from blue to red, where blue areas represent expansion of the mesh, while the red areas represent contractions of the mesh triangles. In the calculation of the differences of areas we supply the possibility to find and remove outliers from the vectors of areas calculated on the reference and target surfaces. We suggest considering this possibility if the mesh may contain degenerate facets. Additionally, `rate.map` allows to investigate the pure morphological comparison of shapes by excluding the evolutionary rate component by setting the argument `show.diff = TRUE`. In this case, a second 3D plot will be displayed highlighting area differences in terms of expansion (green) and contraction (yellow).

**Value**

The function returns a list including:

- **\$selected** a list of PCs axes selected for higher evolutionary rates for each species.
- **\$surfaces** a list of reconstructed coloured surfaces of the given species and of the most recent common ancestor.

**Author(s)**

Marina Melchionna, Antonio Profico, Silvia Castiglione, Gabriele Sansalone, Pasquale Raia

**References**

Schlager, S. (2017). *Morpho and Rvcg-Shape Analysis in R: R-Packages for geometric morphometrics, shape analysis and surface manipulations*. In: Statistical shape and deformation analysis. Academic Press.

**See Also**

[RRphylo vignette](#) ; [relWarps](#) ; [procSym](#)

**Examples**

```
## Not run:
data(DataSimians)
DataSimians$pca->pca
DataSimians$tree->tree
dato<-pca$PCscores

RRphylo(tree,dato)->RR

Rmap<-rate.map(x=c("Pan_troglodytes","Gorilla_gorilla"),RR=RR, PCscores=dato,
               pcs=pca$PCs, mshape=pca$mshape, shape.diff = TRUE)

## End(Not run)
```

---

```
retrieve.angles
```

```
Extracting a user-specified subset of the evo.dir results
```

---

**Description**

This function takes the result list produced by [evo.dir](#) as the input, and extracts a specific subset of it. The user may specify whether to extract the set of angles between species resultant vectors and the MRCA, the size of resultant vectors, or the set of angles between species.

**Usage**

```
retrieve.angles(angles.res,wishlist=c("anglesMRCA","angleDir","angles.between.species"),
  random=c("yes","no"),focus=c("node","species","both","none"),
  node=NULL,species=NULL,write2csv=c("no","yes"),csvfile=NULL)
```

**Arguments**

angles.res	the object resulting from <code>evo.dir</code> function.
wishlist	specifies whether to extract angles and sizes ("anglesMRCA") of resultant vectors between individual species and the MRCA, angles and sizes ("angleDir") of vectors between individual species and a fixed reference vector (the same for all species), or angles between species resultant vectors ("angles.between.species").
random	it needs to be "yes" if 'angles.res' object contains randomization results.
focus	it can be "node", "species", "both", or "none", whether the user wants the results for a focal node, or for a given species, for both, or just wants to visualize everything.
node	must be indicated if focus = "node" or "both". As for <code>evo.dir</code> , the node number must refer to the dichotomic version of the original tree, as produced by <code>RRphylo</code> .
species	must be indicated if focus = "species" or "both".
write2csv	has been deprecated; please see the argument <code>csvfile</code> instead.
csvfile	if results should be saved to a .csv file, a character indicating the name of the csv file and the path where it is to be saved. If no path is indicated the file is stored in the working directory. If left unspecified, no file will be saved.

**Details**

`retrieve.angles` allows to focalize the extraction to a particular node, species, or both. Otherwise it returns the whole dataset.

**Value**

`retrieve.angles` outputs an object of class 'data.frame'.

If `wishlist = "anglesMRCA"`, the data frame includes:

- **MRCA** the most recent common ancestor the angle is computed to
- **species** species ID
- **angle** the angle between the resultant vector of species and the MRCA
- **vector.size** the size of the resultant vector computed from species to MRCA

If `wishlist = "angleDir"`, the data frame includes:

- **MRCA** the most recent common ancestor the vector is computed to
- **species** species ID
- **angle.direction** the angle between the vector of species and a fixed reference

- **vector.size** the size of the vector of species

If `wishlist = "angles.between.species"`, the data frame includes:

- **MRCA** the most recent common ancestor the vector is computed from
- **species** pair IDs of the species pair the "angle between species" is computed for
- **angleBTWspecies2MRCA** angle between species resultant vectors to MRCA
- **anglesBTWspecies** angle between species resultant vectors

### Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### Examples

```
## Not run:
  data("DataApes")
  DataApes$PCstage->PCstage
  DataApes$Tstage->Tstage

  cc<- 2/parallel::detectCores()
  RRphylo(tree=Tstage,y=PCstage,clus=cc)->RR

# Case 1. "evo.dir" without performing randomization
  evo.dir(RR,angle.dimension="rates",pair.type="node",
  node= 57,random="no")->evo.p

# Case 1.1 angles and sizes of resultant vectors between individual species and the MRCA:
# for a focal node
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="no",focus="node",
  node=68)
# for a focal species
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="no",focus="species",
  species="Sap")
# for both focal node and species
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="no",focus="both",
  node=68,species="Sap")
# without any specific requirement
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="no",focus="none")

# Case 1.2 angles and sizes of vectors between individual species
#and a fixed reference vector:
# for a focal node
  retrieve.angles(evo.p,wishlist="angleDir",random="no",focus="node",
  node=68)
# for a focal species
  retrieve.angles(evo.p,wishlist="angleDir",random="no",focus="species",
  species="Sap")
# for both focal node and species
  retrieve.angles(evo.p,wishlist="angleDir",random="no",focus="both",
  node=68,species="Sap")
```

```

# without any specific requirement
  retrieve.angles(evo.p,wishlist="angleDir",random="no",focus="none")

# Case 1.3 angles between species resultant vectors:
# for a focal node
  retrieve.angles(evo.p,wishlist="angles.between.species",random="no",
  focus="node", node=68)
# for a focal species
  retrieve.angles(evo.p,wishlist="angles.between.species",random="no",
  focus="species", species="Sap")
# for both focal node and species
  retrieve.angles(evo.p,wishlist="angles.between.species",random="no",
  focus="both",node=68,species="Sap")
# without any specific requirement
  retrieve.angles(evo.p,wishlist="angles.between.species",random="no",
  focus="none")

# Case 2. "evo.dir" with performing randomization
  evo.dir(RR,angle.dimension="rates",pair.type="node",node=57,
  random="yes",nrep=10)->evo.p

# Case 2.1 angles and sizes of resultant vectors between individual species and the MRCA:
# for a focal node
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="yes",focus="node",
  node=68)
# for a focal species
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="yes", focus="species",
  species="Sap")
# for both focal node and species
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="yes",focus="both",
  node=68,species="Sap")
# without any specific requirement
  retrieve.angles(evo.p,wishlist="anglesMRCA",random="yes",focus="none")

# Case 2.2 angles and sizes of vectors between individual species and a fixed reference vector:
# for a focal node
  retrieve.angles(evo.p,wishlist="angleDir",random="yes",focus="node",
  node=68)
# for a focal species
  retrieve.angles(evo.p,wishlist="angleDir",random="yes",focus="species",
  species="Sap")
# for both focal node and species
  retrieve.angles(evo.p,wishlist="angleDir",random="yes",focus="both",
  node=68, species="Sap")
# without any specific requirement
  retrieve.angles(evo.p,wishlist="angleDir",random="yes",focus="none")

# Case 2.3 retrieve angles between species resultant vectors:
# for a focal node
  retrieve.angles(evo.p,wishlist="angles.between.species",random="yes",
  focus="node", node=68)
# for a focal species

```

```

    retrieve.angles(evo.p,wishlist="angles.between.species",random="yes",
    focus="species", species="Sap")
# for both focal node and species
    retrieve.angles(evo.p,wishlist="angles.between.species",random="yes",
    focus="both",node=68,species="Sap")
# without any specific requirement
    retrieve.angles(evo.p,wishlist="angles.between.species",random="yes",
    focus="none")

## End(Not run)

```

---

RRphylo

*Evolutionary rates computation along phylogenies*


---

## Description

The function `RRphylo` (Castiglione *et al.* 2018) performs the phylogenetic ridge regression. It takes a tree and a vector of tip data (phenotypes) as entries, calculates the regularization factor, produces the matrices of tip to root (`makeL`), and node to root distances (`makeL1`), the vector of ancestral state estimates, the vector of predicted phenotypes, and the rates vector for all the branches of the tree. For multivariate data, rates are given as both one vector per variable, and as a multivariate vector obtained by computing the Euclidean Norm of individual rate vectors.

## Usage

```
RRphylo(tree,y,cov=NULL,rootV=NULL,aces=NULL,x1=NULL,aces.x1=NULL,c1us=0.5)
```

## Arguments

<code>tree</code>	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous.
<code>y</code>	either a single vector variable or a multivariate dataset of class 'matrix'. In any case, <code>y</code> must be named. In case of categorical variable, this should be supplied to the function as a numeric vector.
<code>cov</code>	the covariate to be indicated if its effect on the rates must be accounted for. In this case residuals of the covariate versus the rates are used as rates. 'cov' must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running <code>RRphylo</code> on the covariate as well, and taking the vector of ancestral states and tip values to form the covariate, as in the example below. See <a href="#">RRphylo vignette - covariate</a> for details.
<code>rootV</code>	phenotypic value (values if multivariate) at the tree root. If <code>rootV=NULL</code> the function 'learns' about the root value from the 10% tips being closest in time to the tree root, weighted by their temporal distance from the root itself (close tips phenotypes weigh more than more distant tips).
<code>aces</code>	a named vector (or matrix if <code>y</code> is multivariate) of ancestral character values at nodes. Names correspond to the nodes in the tree. See <a href="#">RRphylo vignette - aces</a> for details.

<code>x1</code>	the additional predictor(s) to be indicated to perform the multiple version of RRphylo. 'x1' vector/matrix must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running RRphylo on the predictors (separately for each predictor) as well, and taking the vector of ancestral states and tip values to form the x1. See <a href="#">RRphylo vignette - predictor</a> for details.
<code>aces.x1</code>	a named vector/matrix of ancestral character values at nodes for x1. It must be indicated if both aces and x1 are specified. Names/rownames correspond to the nodes in the tree.
<code>clus</code>	the proportion of clusters to be used in parallel computing (only if y is multivariate). To run the single-threaded version of RRphylo set <code>clus = 0</code> .

### Value

**tree** the tree used by RRphylo. The fully dichotomous version of the tree argument. For trees with polytomies, the tree is resolved by using `multi2di` function in the package **ape**. If the latter is a dichotomous tree, the two trees will be identical.

**tip.path** a  $n * m$  matrix, where  $n$ =number of tips and  $m$ =number of branches (i.e.  $2*n-1$ ). Each row represents the branch lengths along a root-to-tip path.

**node.path** a  $n * n$  matrix, where  $n$ =number of internal branches. Each row represents the branch lengths along a root-to-node path.

**rates** single rate values computed for each branch of the tree. If y is a single vector variable, rates are equal to `multiple.rates`. If y is a multivariate dataset, rates are computed as the square root of the sum of squares of each row of `multiple.rates`.

**aces** the phenotypes reconstructed at nodes.

**predicted.phenotypes** the vector of estimated tip values. It is a matrix in the case of multivariate data.

**multiple.rates** a  $n * m$  matrix, where  $n$ = number of branches (i.e.  $n*2-1$ ) and  $m$  = number of variables. For each branch, the column entries represent the evolutionary rate.

**lambda** the regularization factor fitted within RRphylo by the inner function `optL`. With multivariate data, several `optL` runs are performed. Hence, the function provides a single lambda for each individual variable.

**ace.values** if aces are specified, the function returns a dataframe containing the corresponding node number on the RRphylo tree for each node, along with estimated values.

**x1.rate** if x1 is specified, the function returns the partial regression coefficient for x1.

### Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### References

Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Serio, C., Di Febbraro, M., & Raia, P.(2018). A new method for testing evolutionary rate variation and shifts in phenotypic evolution. *Methods in Ecology and Evolution*, 9: 974-983.doi:10.1111/2041-210X.12954



Serio, C., Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Di Febbraro, M., & Raia, P. (2019). Macroevolution of toothed whales exceptional relative brain size. *Evolutionary Biology*, 46: 332-342. doi:10.1007/s11692-019-09485-7

Melchionna, M., Mondanaro, A., Serio, C., Castiglione, S., Di Febbraro, M., Rook, L., Diniz-Filho, J.A.F., Manzi, G., Profico, A., Sansalone, G., & Raia, P. (2020). Macroevolutionary trends of brain mass in Primates. *Biological Journal of the Linnean Society*, 129: 14-25. doi:10.1093/biolinnean/blz161

Castiglione, S., Serio, C., Mondanaro, A., Melchionna, M., Carotenuto, F., Di Febbraro, M., Profico, A., Tamagnini, D., & Raia, P. (2020). Ancestral State Estimation with Phylogenetic Ridge Regression. *Evolutionary Biology*, 47 (3), 220-232. doi:10.1007/s11692-020-09505-x

Castiglione, S., Serio, C., Piccolo, M., Mondanaro, A., Melchionna, M., Di Febbraro, M., Sansalone, G., Wroe, S., & Raia, P. (2020). The influence of domestication, insularity and sociality on the tempo and mode of brain size evolution in mammals. *Biological Journal of the Linnean Society*, in press. doi:10.1093/biolinnean/blaa186

## Examples

```
## Not run:
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino
DataOrnithodirans$massdino->massdino

# Case 1. "RRphylo" without accounting for the effect of a covariate
RRphylo(tree=treedino,y=massdino)->RRcova

# Case 2. "RRphylo" accounting for the effect of a covariate
# "RRphylo" on the covariate in order to retrieve ancestral state values
c(RRcova$aces,massdino)->cov.values
c(rownames(RRcova$aces),names(massdino))->names(cov.values)

RRphylo(tree=treedino,y=massdino,cov=cov.values)->RR

# Case 3. "RRphylo" specifying the ancestral states
data("DataCetaceans")
DataCetaceans$treecet->treecet
DataCetaceans$masscet->masscet
DataCetaceans$brainmasscet->brainmasscet
DataCetaceans$aceMyst->aceMyst

RRphylo(tree=treecet,y=masscet,aces=aceMyst)->RR

# Case 4. Multiple "RRphylo"
library(ape)
drop.tip(treecet,treecet$tip.label[-match(names(brainmasscet),treecet$tip.label)])->treecet.multi
masscet[match(treecet.multi$tip.label,names(masscet))]->masscet.multi

RRphylo(tree=treecet.multi, y=masscet.multi)->RRmass.multi
RRmass.multi$aces[,1]->acemass.multi
c(acemass.multi,masscet.multi)->x1.mass

RRphylo(tree=treecet.multi,y=brainmasscet,x1=x1.mass)->RR
```

```

# Case 5. Categorical and multiple "RRphylo" with 2 additional predictors
library(phytools)
library(geiger)

set.seed(1458)
rtree(50)->tree
fastBM(tree)->y
jitter(y)*10->y1
rep(1,length(y))->y2
y2[sample(1:50,20)]<-2
names(y2)<-names(y)

c(RRphylo(tree,y1)$aces[,1],y1)->x1

RRphylo(tree,y2)->RRcat ### this is the RRphylo on the categorical variable
c(RRcat$aces[,1],y2)->x2

cbind(c(jitter(mean(y1[tips(tree,83)])),1),
      c(jitter(mean(y1[tips(tree,53)])),2))->acex
c(jitter(mean(y[tips(tree,83)])),jitter(mean(y[tips(tree,53)])))->acesy
names(acesy)<-rownames(acex)<-c(83,53)

RRphylo(tree,y,aces=acesy,x1=cbind(x1,x2),aces.x1 = acex)

## End(Not run)

```

---

scaleTree

*Phylogenetic tree calibration*

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## Description

The function is a wrapper around the functions "scalePhylo", "assign.ages", and "assign.brlen" written by Gene Hunt (<http://paleobiology.si.edu/staff/individuals/hunt.cfm>). It rescales tree branch lengths according to given calibration dates.

## Usage

```
scaleTree(tree, tip.ages, node.ages=NULL, min.branch=0.1)
```

## Arguments

tree	a phylogenetic tree. The tree needs not to be ultrametric and fully dichotomous.
tip.ages	a named vector including the ages (i.e. distance from the youngest tip within the tree) of the tips to be changed. If unspecified, the function assumes all the tips are correctly placed with respect to the root.
node.ages	a named vector including the ages (i.e. distance from the youngest tip within the tree) of the nodes to be changed. If no calibration date for nodes is supplied, the function shifts node position only where needed to fit tip ages.
min.branch	the minimum branch length that will be imposed for shifted nodes.

**Value**

Rescaled phylogentic tree.

**Author(s)**

Silvia Castiglione, Pasquale Raia, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**See Also**

[scaleTree vignette](#)

**Examples**

```
library(ape)
library(phytools)
library(geiger)

data("DataFelids")
DataFelids$treefel->tree

max(nodeHeights(tree))->H

#### Example 1 ####
rep(0,4)->tipAges
names(tipAges)<-tips(tree,146)
scaleTree(tree,tipAges)->treeS1

edge.col<-rep("black",nrow(tree$edge))
edge.col[which(treeS1$edge[,2]%in%getDescendants(treeS1,146))]<-"red"

layout(2:1)
plot(tree,edge.color = edge.col,show.tip.label=FALSE)
plot(treeS1,edge.color = edge.col,show.tip.label=FALSE)

#### Example 2 ####
nodeAges<-c(23.5,15.6)
names(nodeAges)<-c(85,139)
scaleTree(tree,node.ages=nodeAges)->treeS2

edge.col<-rep("black",nrow(tree$edge))
edge.col[which(treeS1$edge[,2]%in%c(getDescendants(treeS1,85),
                                   getDescendants(treeS1,139)))]<-"red"

layout(2:1)
plot(tree,edge.color = edge.col,show.tip.label=FALSE)
nodelabels(bg="w",frame="n",node=c(85,139),col="green")
plot(treeS2,edge.color = edge.col,show.tip.label=FALSE)
nodelabels(bg="w",frame="n",node=c(85,139),col="green")

#### Example 3 ####
```

```

16->nodeAges
names(nodeAges)<-"145"
tipAges<-19
names(tipAges)<-tree$tip.label[1]
scaleTree(tree,tip.ages = tipAges,node.ages=nodeAges)->treeS3

edge.col<-rep("black",nrow(tree$edge))
edge.col[which(trees3$edge[,2]%in%c(1,getMommy(tree,1),
                                getDescendants(trees3,145)))]<-"red"

layout(2:1)
plot(tree,edge.color = edge.col,show.tip.label=FALSE)
nodelabels(bg="w",frame="n",node=145,col="green")
plot(treeS3,edge.color = edge.col,show.tip.label=FALSE)
nodelabels(bg="w",frame="n",node=145,col="green")

```

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search.conv

*Searching for morphological convergence among species and clades*


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## Description

The function scans a phylogenetic tree looking for morphological convergence between entire clades or species evolving under specific states.

## Usage

```

search.conv(RR=NULL,tree=NULL,y,nodes=NULL,state=NULL,aceV=NULL,
            min.dim=NULL,max.dim=NULL,min.dist=NULL,declust=FALSE,nsim=1000,rsim=1000,
            clus=.5,foldername=NULL,filename)

```

## Arguments

RR	an object produced by <a href="#">RRphylo</a> . This is not indicated if convergence among states is tested.
tree	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous. This is not indicated if convergence among clades is tested.
y	a multivariate phenotype. The object y should be either a matrix or dataframe with species names as rownames.
nodes	node pair to be tested. If unspecified, the function automatically searches for convergence among clades. Notice the node number must refer to the dichotomic version of the original tree, as produced by <a href="#">RRphylo</a> .
state	the named vector of tip states. The function tests for convergence within a single state or among different states (this latter case is especially meant to test for iterative evolution as for example the appearance of repeated morphotypes into different clades). In both cases, the state for non-focal species (i.e. not belonging to any convergent group) must be indicated as "nostate".

aceV	phenotypic values at internal nodes. The object aceV should be either a matrix or dataframe with nodes (referred to the dichotomic version of the original tree, as produced by RRphylo) as rownames. If aceV are not indicated, ancestral phenotypes are estimated via RRphylo.
min.dim	the minimum size of the clades to be compared. When nodes is indicated, it is the minimum size of the smallest clades in nodes, otherwise it is set at one tenth of the tree size.
max.dim	the maximum size of the clades to be compared. When nodes is indicated, it is min.dim*2 if the largest clade in nodes is smaller than this value, otherwise it corresponds to the size of the largest clade. Without nodes it is set at one third of the tree size.
min.dist	the minimum distance between the clades to be compared. When nodes is indicated, it is the distance between the pair. Under the automatic mode, the user can choose whether time distance or node distance (i.e. the number of nodes intervening between the pair) should be used. If time distance has to be considered, min.dist should be a character argument containing the word "time" and then the actual time distance to be used. The same is true for node distance, but the word "node" must precede the node distance to be used. For example, if the user want to test only clades more distant than 10 time units, the argument should be "time10". If clades separated by more than 8 nodes has to be tested, the argument min.dist should be "node8". If left unspecified, it automatically searches for convergence between clades separated by a number of nodes bigger than one tenth of the tree size.
declust	if species under a given state (or a pair of states) to be tested for convergence are phylogenetically closer than expected by chance, trait similarity might depend on proximity rather than true convergence. In this case, by setting declust = TRUE, tips under the focal state (or states) are removed randomly until clustering disappears. A minimum of 3 species per state is enforced to remain anyway.
nsim	number of simulations to perform sampling within the theta random distribution. It is set at 1000 by default.
rsim	number of simulations to be performed to produce the random distribution of theta values. It is set at 1000 by default.
clus	the proportion of clusters to be used in parallel computing. To run the single-threaded version of search.conv set clus = 0.
foldername	has been deprecated; please see the argument filename instead.
filename	a character indicating the name of the pdf file and the path where it is to be saved. If no path is indicated the file is stored in the working directory

## Details

Regardless the case (either 'state' or 'clade'), the function stores a plot into the folder specified by filename. If convergence among clades is tested, the clade pair plotted corresponds to those clades with the smallest \$average distance from group centroid. The figure shows the Euclidean distances computed between the MRCAs of the clades and the mean Euclidean distance computed between all the tips belonging to the converging clades, as compared to the distribution of these same figures across the rest of the tree. Furthermore, the function stores the PC1/PC2 plot obtained

by PCA of the species phenotypes. Convergent clades are indicated by colored convex hulls. Large colored dots represent the mean phenotypes per clade (i.e. their group centroids). Eventually, a modified traitgram plot is produced, highlighting the branches of the clades found to converge. In both PCA and traitgram, asterisks represent the ancestral phenotypes of the individual clades. If convergence among states is tested, the function produces a PC plot with colored convex hulls enclosing species belonging to different states. Furthermore, it generates circular plots of the mean angle between states (blue lines) and the range of random angles (gray shaded area). The p-value for the convergence test is printed within the circular plots.

## Value

If convergence between clades is tested, the function returns a list including:

- **\$node pairs**: a dataframe containing for each pair of nodes:
  - ang.bydist.tip: the mean theta angle between clades divided by the time distance.
  - ang.conv: the mean theta angle between clades plus the angle between aces, divided by the time distance.
  - ang.ace: the angle between aces.
  - ang.tip: the mean theta angle between clades.
  - nod.dist: the distance intervening between clades in terms of number of nodes.
  - time.dist: the time distance intervening between the clades.
  - p.ang.bydist: the p-value computed for ang.bydist.tip.
  - p.ang.conv: the p-value computed for ang.conv.
  - clade.size: the size of clades.
- **\$node pairs comparison**: pairwise comparison between significantly convergent pairs (all pairs if no instance of significance was found) performed on the distance from group centroids (the mean phenotype per clade).
- **\$average distance from group centroids**: smaller average distances mean less variable phenotypes within the pair.

If convergence between (or within a single state) states is tested, the function returns a dataframe including for each pair of states (or single state):

- ang.state: the mean theta angle between species belonging to different states (or within a single state).
- ang.state.time: the mean of theta angle between species belonging to different states (or within a single state) divided by time distance.
- p.ang.state: the p-value computed for ang.state.
- p.ang.state.time: the p-value computed for ang.state.time.

## Author(s)

Silvia Castiglione, Carmela Serio, Pasquale Raia, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto, Paolo Piras, Davide Tamagnini

## References

Castiglione, S., Serio, C., Tamagnini, D., Melchionna, M., Mondanaro, A., Di Febbraro, M., Profico, A., Piras, P., Barattolo, F., & Raia, P. (2019). A new, fast method to search for morphological convergence with shape data. *PLoS ONE*, 14, e0226949. <https://doi.org/10.1371/journal.pone.0226949>

## See Also

[search.conv vignette](#)

## Examples

```
## Not run:
data("DataFelids")
DataFelids$PCscoresfel->PCscoresfel
DataFelids$treefel->treefel
DataFelids$statefel->statefel
cc<- 2/parallel::detectCores()

RRphylo(treefel,PCscoresfel,clus=cc)->RRfel

## Case 1. searching convergence between clades
# by setting min.dist as node distance
search.conv(RR=RRfel, y=PCscoresfel, min.dim=5, min.dist="node9",
            filename = paste(tempdir(), "SCclade_nd", sep="/"),clus=cc)
# by setting min.dist as time distance
search.conv(RR=RRfel, y=PCscoresfel, min.dim=5, min.dist="time38",
            filename = paste(tempdir(), "SCclade_td", sep="/"),clus=cc)

## Case 2. searching convergence within a single state
search.conv(tree=treefel, y=PCscoresfel, state=statefel, declust=TRUE,
            filename = paste(tempdir(), "SCstate", sep="/"),clus=cc)

## End(Not run)
```

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search.shift

*Locating shifts in phenotypic evolutionary rates*

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## Description

The function `search.shift` (Castiglione et al. 2018) tests whether individual clades or isolated tips dispersed through the phylogeny evolve at different `RRphylo` rates as compared to the rest of the tree. Instances of rate shifts may be automatically found.

## Usage

```
search.shift(RR, status.type = c("clade", "sparse"), node = NULL, state
            = NULL, cov = NULL, nrep = 1000, f = NULL, foldername=NULL, filename)
```

## Arguments

RR	an object fitted by the function <a href="#">RRphylo</a> .
status.type	whether the "clade" or "sparse" condition must be tested.
node	under the "clade" condition, the node (clade) to be tested for the rate shift. When multiple nodes are tested, they need to be written as in the example below. If node is left unspecified, the function performs under the 'auto-recognize' feature, meaning it will automatically test individual clades for deviation of their rates from the background rate of the rest of the tree (see details).
state	the state of the tips specified under the "sparse" condition.
cov	the covariate to be indicated if its effect on rate values must be accounted for. Contrary to <a href="#">RRphylo</a> , cov needs to be as long as the number of tips of the tree.
nrep	the number of simulations to be performed for the rate shift test, by default nrep is set at 1000.
f	the size of the smallest clade to be tested. By default, nodes subtending to one tenth of the tree tips are tested.
foldername	has been deprecated; please see the argument filename instead.
filename	a character indicating the name of the pdf file and the path where it is to be saved. If no path is indicated the file is stored in the working directory

## Details

The function `search.shift` takes the object produced by [RRphylo](#). Two different conditions of rate change can be investigated. Under the "clade" condition the vector of node or nodes subjected to the shift must be provided. Alternatively, under the "sparse" case the (named) vector of states (indicating which tips are or are not evolving under the rate shift according to the tested hypothesis) must be indicated. In the "clade" case, the function may perform an 'auto-recognize' feature. Under such specification, the function automatically tests individual clades (from clades as large as one half of the tree down to a specified clade size) for deviation of their rates from the background rate of the rest of the tree, which is identical to the "clade" case. An inclusive clade with significantly high rates is likely to include descending clades with similarly significantly high rates. Hence, with 'auto-recognize' the `search.shift` function is written as to scan clades individually and to select only the node subtending to the highest difference in mean absolute rates as compared to the rest of the tree. A plot of the tree highlighting nodes subtending to significantly different rates is automatically produced. Caution must be put into interpreting the 'auto-recognize' results. For instance, a clade with small rates and another with large rates could be individuated even under BM. This does not mean these clades are actual instances for rate shifts. Clades must be tested on their own without the 'auto-recognize' feature, which serves as guidance to the investigator, when no strong a priori hypothesis to be tested is advanced. The 'auto-recognize' feature is not meant to provide a test for a specific hypothesis. It serves as an optional guidance to understand whether and which clades show significantly large or small rates as compared to the rest of the tree. Individual clades are tested at once, meaning that significant instances of rate variation elsewhere on the tree are ignored. Conversely, running the "clade" condition without including the 'auto-recognize' feature, multiple clades presumed to evolve under the same shift are tested together, meaning that their rates are collectively contrasted to the rest of the tree, albeit they can additionally be compared to each other upon request. Under both the "clade" and "sparse" conditions, multiple clades could be specified at once, and optionally tested individually (for deviation of rates) against the rates of



the rest of the tree and against each other. The histogram of random differences of mean rates distribution along with the position of the real difference of means is automatically generated by `search.shift`. Regardless of which condition is specified, the function output produces the real difference of means, and their significance value.

## Value

Under all circumstances, `search.shift` provides a vector of `$rates`. If 'cov' values are provided, rates are regressed against the covariate and the residuals of such regression form the vector `$rates`. Otherwise, `$rates` are the same rates as with the RR argument.

Under "clade" case without specifying nodes (i.e. 'auto-recognize') a list including:

**\$all.clades** for each detected node, the data-frame includes the average rate difference (computed as the mean rate over all branches subtended by the node minus the average rate for the rest of the tree) and the probability that it do represent a real shift. Probabilities are contrasted to simulations shuffling the rates across the tree branches for a number of replicates specified by the argument `nrep`. Note that the p-values refer to the number of times the real average rates are larger (or smaller) than the rates averaged over the rest of the tree, divided by the number of simulations. Hence, large rates are significantly larger than the rest of the tree (at  $\alpha = 0.05$ ), when the probability is  $> 0.975$ ; and small rates are significantly small for  $p < 0.025$ .

**\$single.clades** the same as with 'all.clades' but restricted to the largest/smallest rate values along a single clade (i.e. nested clades with smaller rate shifts are excluded). Large rates are significantly larger than the rest of the tree (at  $\alpha = 0.05$ ), when the probability is  $> 0.975$ ; and small rates are significantly small for  $p < 0.025$ .

Under "clade" condition by specifying the node argument:

**\$all.clades.together** if more than one node is tested, this specifies the average rate difference and the significance of the rate shift, by considering all the specified nodes as evolving under a single rate. As with the 'auto-recognize' feature, large rates are significantly larger than the rest of the tree (at  $\alpha = 0.05$ ), when the probability is  $> 0.975$ ; and small rates are significantly small for  $p < 0.025$ .

**\$single.clades** this gives the significance for individual clades, tested separately. As previously, large rates are significantly larger than the rest of the tree (at  $\alpha = 0.05$ ), when the probability is  $> 0.975$ ; and small rates are significantly small for  $p < 0.025$ .

Under the "sparse" condition:

**\$state.results** for each state, the data-frame includes the average rate difference (computed as the mean rate over all leaves evolving under a given state, minus the average rate for each other state or the rest of the tree) and the probability that the shift is real. Large rates are significantly larger (at  $\alpha = 0.05$ ), when the probability is  $> 0.975$ ; and small rates are significantly small for  $p < 0.025$ . States are compared pairwise.

## Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

## References

Castiglione, S., Tesone, G., Piccolo, M., Melchionna, M., Mondanaro, A., Serio, C., Di Febbraro, M., & Raia, P.(2018). A new method for testing evolutionary rate variation and shifts in phenotypic evolution. *Methods in Ecology and Evolution*, 9: 974-983.doi:10.1111/2041-210X.12954

## See Also

[search.shift vignette](#)

## Examples

```
## Not run:
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino
DataOrnithodirans$massdino->massdino
DataOrnithodirans$statedino->statedino

RRphylo(tree=treedino,y=massdino)->dinoRates

# Case 1. Without accounting for the effect of a covariate

# Case 1.1 "clade" condition
# with auto-recognize
search.shift(RR=dinoRates,status.type="clade",
             filename=paste(tempdir(), "SSauto", sep="/"))
# testing two hypothetical clades
search.shift(RR=dinoRates,status.type="clade",node=c(696,746),
             filename=paste(tempdir(), "SSclade", sep="/"))

# Case 1.2 "sparse" condition
# testing the sparse condition.
search.shift(RR=dinoRates,status.type="sparse",state=statedino,
             filename=paste(tempdir(), "SSsparse", sep="/"))

# Case 2. Accounting for the effect of a covariate

# Case 2.1 "clade" condition
search.shift(RR=dinoRates,status.type="clade",cov=massdino,
             filename=paste(tempdir(), "SSclade_cov", sep="/"))

# Case 2.2 "sparse" condition
search.shift(RR=dinoRates,status.type="sparse",state=statedino,cov=massdino,
             filename=paste(tempdir(), "SSstate_cov", sep="/"))

## End(Not run)
```

---

search.trend	<i>Searching for evolutionary trends in phenotypes and rates</i>
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### Description

This function searches for evolutionary trends in the phenotypic mean and the evolutionary rates for the entire tree and individual clades.

### Usage

```
search.trend(RR,y,x1=NULL,x1.residuals = FALSE,
             node=NULL,cov=NULL,nsim=100,clus=0.5,ConfInt=FALSE,foldername=NULL,filename)
```

### Arguments

RR	an object produced by <a href="#">RRphylo</a> .
y	the named vector (or matrix if multivariate) of phenotypes.
x1	the additional predictor to be specified if the RR object has been created using an additional predictor (i.e. multiple version of <a href="#">RRphylo</a> ). 'x1' vector must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running <a href="#">RRphylo</a> on the predictor as well, and taking the vector of ancestral states and tip values to form the x1. Note: only one predictor at once can be specified.
x1.residuals	logical specifying whether the residuals of regression between y and x1 should be inspected for a phenotypic trend (see details and examples below). Default is FALSE.
node	the node number of individual clades to be specifically tested and contrasted to each other. It is NULL by default. Notice the node number must refer to the dichotomic version of the original tree, as produced by <a href="#">RRphylo</a> .
cov	the covariate values to be specified if the RR object has been created using a covariate for rates calculation. As for <a href="#">RRphylo</a> , 'cov' must be as long as the number of nodes plus the number of tips of the tree, which can be obtained by running <a href="#">RRphylo</a> on the covariate as well, and taking the vector of ancestral states and tip values to form the covariate (see the example below).
nsim	number of simulations to be performed. It is set at 100 by default.
clus	the proportion of clusters to be used in parallel computing. To run the single-threaded version of <code>search.trend</code> set <code>clus = 0</code> .
ConfInt	if TRUE, the function returns 95% confidence intervals around phenotypes and rates produced according to the Brownian motion model of evolution. It is FALSE by default.
foldername	has been deprecated; please see the argument <code>filename</code> instead.
filename	a character indicating the name of the pdf file and the path where it is to be saved. If no path is indicated the file is stored in the working directory

## Details

The function simultaneously returns the regression of phenotypes and phenotypic evolutionary rates against age tested against Brownian motion simulations to assess significance. To this aim rates are rescaled in the 0-1 range and then logged. The function stores the rates (both rescaled and unscaled absolute values) versus age regression and the phenotype versus age regression plots as .pdf files. In the plots, the 95% confidence intervals of phenotypes and rates simulated under the Brownian motion for each node are plotted as shaded areas. Regression lines are printed for all regressions. To assess significance, slopes are compared to a family of simulated slopes (BMslopes, where the number of simulations is equal to `nsim`), generated under the Brownian motion, using the `fastBM` function in the package **phytools**. Individual nodes are compared to the rest of the tree in different ways depending on whether phenotypes or rates (always unscaled in this case) versus age regressions are tested. With the former, the regression slopes for individual clades and the slope difference between clades is contrasted to slopes obtained through Brownian motion simulations. For the latter, regression models are tested and contrasted to each other referring to estimated marginal means, by using the `emmeans` function in the package **emmeans**.

The **multiple regression version of RRphylo** allows to incorporate the effect of an additional predictor in the computation of evolutionary rates without altering the ancestral character estimation. Thus, when a multiple `RRphylo` output is fed to `search.trend`, the predictor effect is accounted for on the absolute evolutionary rates, but not on the phenotype. However, in some situations the user might want to factor out the predictor effect on phenotypes as well. Under the latter circumstance, by setting the argument `x1.residuals = TRUE`, the residuals of the response to predictor regression are used as to represent the phenotype.

## Value

The function returns a list object containing:

**\$trends.data** a 'RRphyloList' object including:

1. `$phenotypeVStime`: a data frame of phenotypic values (or `y` versus `x1` regression residuals if `x1.residuals=TRUE`) and their distance from the tree root for each node (i.e. ancestral states) and tip of the tree.
2. `$absrateVStime`: a data frame of `RRphylo` rates and the distance from the tree root (age). If `y` is multivariate, it also includes the multiple rates for each `y` vector. If node is specified, each branch is classified as belonging to an indicated clade.
3. `$rescaledrateVStime`: a data frame of rescaled `RRphylo` rates and the distance from the tree root (age). If `y` is multivariate, it also includes the multiple rates for each `y` vector. If node is specified, each branch is classified as belonging to an indicated clade. NAs correspond either to very small values or to outliers which are excluded from the analysis.

**\$phenotypic.regression** results of phenotype (`y` versus `x1` regression residuals) versus age regression. It reports a p-value for the regression slope between the variables (`p.real`), a p-value computed contrasting the real slope to Brownian motion simulations (`p.random`), and a parameter indicating the deviation of the phenotypic mean from the root value in terms of the number of standard deviations of the trait distribution (`dev`). `dev` is 0 under Brownian Motion. Only `p.random` should be inspected to assess significance.

**\$rate.regression** results of the rates (rescaled absolute values) versus age regression. It reports a p-value for the regression between the variables (`p.real`), a p-value computed contrasting the real

slope to Brownian motion simulations (p.random), and a parameter indicating the ratio between the range of phenotypic values and the range of such values halfway along the tree height, divided to the same figure under Brownian motion (spread). spread is 1 under Brownian Motion. Only p.random should be inspected to assess significance.

**\$ConfInts** a 'RRphyloList' object including the 95% confidence intervals around phenotypes and rates (both rescaled and unscaled absolute rates) produced according to the Brownian motion model of evolution.

If specified, individual nodes are tested as the whole tree, the results are summarized in the objects:

**\$node.phenotypic.regression** results of phenotype (or y versus x1 regression residuals) versus age regression through node. It reports the slope for the regression between the variables at node (slope), a p-value computed contrasting the real slope to Brownian motion simulations (p.random), the difference between estimated marginal means predictions for the group and for the rest of the tree (emm.difference), and a p-value for the emm.difference (p.emm).

**\$node.rate.regression** results of the rates (absolute values) versus age regression through node. It reports the difference between estimated marginal means predictions for the group and for the rest of the tree (emm.difference), a p-value for the emm.difference (p.emm), the difference between regression slopes for the group and for the rest of the tree (slope.difference), and a p-value for the slope.difference (p.slope).

If more than one node is specified, the object **\$group.comparison** reports the same results as \$node.phenotypic.regression and \$node.rate.regression obtained by comparing individual clades to each other.

### Author(s)

Silvia Castiglione, Carmela Serio, Pasquale Raia, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### References

Castiglione, S., Serio, C., Mondanaro, A., Di Febbraro, M., Profico, A., Girardi, G., & Raia, P. (2019) Simultaneous detection of macroevolutionary patterns in phenotypic means and rate of change with and within phylogenetic trees including extinct species. *PLoS ONE*, 14: e0210101. <https://doi.org/10.1371/journal.pone.0210101>

### See Also

[search.trend vignette](#)

### Examples

```
## Not run:
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino
DataOrnithodirans$massdino->massdino
cc<- 2/parallel::detectCores()

# Extract Pterosaurs tree and data
library(ape)
```

```

extract.clade(treedino,746)->treeptero
massdino[match(treeptero$tip.label,names(massdino))]->massptero
massptero[match(treeptero$tip.label,names(massptero))]->massptero

# Case 1. "RRphylo" whitout accounting for the effect of a covariate
RRphylo(tree=treeptero,y=log(massptero))->RRptero

# Case 1.1. "search.trend" whitout indicating nodes to be tested for trends
search.trend(RR=RRptero, y=log(massptero), nsim=100, clus=cc,
             filename=paste(tempdir(), "ST", sep="/"),cov=NULL,ConfInt=FALSE,node=NULL)

# Case 1.2. "search.trend" indicating nodes to be specifically tested for trends
search.trend(RR=RRptero, y=log(massptero), nsim=100, node=143, clus=cc,
             filename=paste(tempdir(), "STnode", sep="/"),cov=NULL,ConfInt=FALSE)

# Case 2. "RRphylo" accounting for the effect of a covariate
# "RRphylo" on the covariate in order to retrieve ancestral state values
RRphylo(tree=treeptero,y=log(massptero))->RRptero
c(RRptero$aces,log(massptero))->cov.values
names(cov.values)<-c(rownames(RRptero$aces),names(massptero))
RRphylo(tree=treeptero,y=log(massptero),cov=cov.values)->RRpteroCov

# Case 2.1. "search.trend" whitout indicating nodes to be tested for trends
search.trend(RR=RRpteroCov, y=log(massptero), nsim=100, clus=cc,
             filename=paste(tempdir(), "ST_cov", sep="/"),ConfInt=FALSE,cov=cov.values)

# Case 2.2. "search.trend" indicating nodes to be specifically tested for trends
search.trend(RR=RRpteroCov, y=log(massptero), nsim=100, node=143, clus=cc,
             filename=paste(tempdir(), "STnode_cov", sep="/"),ConfInt=FALSE,cov=cov.values)

# Case 3. "search.trend" on multiple "RRphylo"
data("DataCetaceans")
DataCetaceans$treecet->treecet
DataCetaceans$masscet->masscet
DataCetaceans$brainmasscet->brainmasscet
DataCetaceans$aceMyst->aceMyst

drop.tip(treecet,treecet$tip.label[!match(names(brainmasscet),treecet$tip.label)])->treecet.multi
masscet[match(treecet.multi$tip.label,names(masscet))]->masscet.multi

RRphylo(tree=treecet.multi,y=masscet.multi)->RRmass.multi
RRmass.multi$aces[,1]->acemass.multi
c(acemass.multi,masscet.multi)->x1.mass

RRphylo(tree=treecet.multi,y=brainmasscet,x1=x1.mass)->RRmulti

# incorporating the effect of body size at inspecting trends in absolute evolutionary rates
search.trend(RR=RRmulti, y=brainmasscet,x1=x1.mass,clus=cc,
             filename=paste(tempdir(), "STmulti_rate", sep="/"))

# incorporating the effect of body size at inspecting trends in both absolute evolutionary

```

```
# rates and phenotypic values (by using brain versus body mass regression residuals)
search.trend(RR=RRmulti, y=brainmasscet,x1=x1.mass,x1.residuals=TRUE,clus=cc,
             filename=paste(tempdir(), "STmulti", sep="/"))

## End(Not run)
```

setBM

*Producing simulated phenotypes with trends***Description**

The function setBM is wrapper around **phytools** fastBM function, which generates BM simulated phenotypes with or without a trend.

**Usage**

```
setBM(tree, nY = 1, s2 = 1, a = 0, type = c("", "brown", "trend",
      "drift"), trend.type = c("linear", "stepwise"), tr = 10, t.shift = 0.5,
      es=2, ds=1)
```

**Arguments**

tree	a phylogenetic tree.
nY	the number of phenotypes to simulate.
s2	value of the Brownian rate to use in the simulations.
a	the phenotype at the tree root.
type	the type of phenotype to simulate. With the option "brown" the phenotype will have no trend in the phenotypic mean or in the rate of evolution. A variation in the phenotypic mean over time (a phenotypic trend) is obtained by selecting the option "drift". A trend in the rate of evolution produces an increased variance in the residuals over time. This is obtained by specifying the option "trend".
trend.type	two kinds of heteroscedastic residuals are generated under the "trend" type. The option "linear" produces an exponential linear increase (or decrease) in heteroscedasticity, whereas the "stepwise" option produces an increase (or decrease) after a specified point in time.
tr	the intensity of the trend with the "stepwise" option is controlled by the tr argument. The scalar tr is the multiplier of the branches extending after the shift point as indicated by t.shift.
t.shift	the relative time distance from the tree root where the stepwise change in the rate of evolution is indicated to apply.
es	when trend.type="linear", es is a scalar representing the exponent at which the evolutionary time (i.e. distance from the root) scales to change to phenotypic variance. With es = 1 the phenotypic rate will be trendless, with es < 1 the variance of the phenotypes will decrease exponentially towards the present and the other way around with es > 1.

`ds` a scalar indicating the change in phenotypic mean in the unit time, in `type="drift"` case. With `ds = 0` the phenotype will be trendless, with `ds < 0` the phenotypic mean will decrease exponentially towards the present and the other way around with `ds > 0`.

### Details

Note that `setBM` differs from `fastBM` in that the produced phenotypes are checked for the existence of a temporal trend in the phenotype. The user may specify whether she wants trendless data (option `"brown"`), phenotypes trending in time (option `"drift"`), or phenotypes whose variance increases/decreases exponentially over time, consistently with the existence of a trend in the rate of evolution (option `"trend"`). In the latter case, the user may indicate the intensity of the trend (by applying different values of `es`), and whether it should occur after a given proportion of the tree height (hence a given point back in time, specified by the argument `t.shift`). Trees in `setBM` are treated as non ultrametric. If an ultrametric tree is fed to the function, `setBM` alters slightly the leaf lengths multiplying randomly half of the leaves by  $1 * 10e-3$ , in order to make it non-ultrametric.

### Value

Either an object of class `'array'` containing a single phenotype or an object of class `'matrix'` of  $n$  phenotypes as columns, where  $n$  is indicated as `nY = n`.

### Author(s)

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

### Examples

```
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino

setBM(tree=treedino, nY= 1, type="brown")
setBM(tree=treedino, nY= 1, type="drift", ds=2)
setBM(tree=treedino, nY= 1, type="trend", trend.type="linear", es=2)
```

---

sizedsubtree

*Find a node subtending to a clade of desired size*

---

### Description

The function `sizedsubtree` scans a phylogenetic tree to randomly find nodes subtending to a subtree of desired minimum size, up to one half of the tree size (number of tips).

### Usage

```
sizedsubtree(tree, Size=NULL, time.limit=10)
```



**Arguments**

<code>tree</code>	a phylogenetic tree.
<code>Size</code>	the desired size of the tree subtending to the extracted node. By default, the minimum tree size is set at one tenth of the tree size (i.e. number of tips).
<code>time.limit</code>	specifies a limit to the searching time, a warning message is thrown if the limit is reached.

**Details**

The argument `time.limit` sets the searching time. The algorithm stops if that limit is reached, avoiding recursive search when no solution is in fact possible.

**Value**

A node subtending to a subtree of desired minimum size.

**Author(s)**

Pasquale Raia, Silvia Castiglione, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**Examples**

```
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino

sizedsubtree(tree=treedino,Size=40)
```

---

StableTraitsR

*Run StableTraits from within R*

---

**Description**

This function runs `StableTraits` and `StableTraitsSum` (*Elliot and Mooers 2014*) from within the R environment and returns its output into the workspace.

**Usage**

```
StableTraitsR(tree,y,path,output=NULL,aces=NULL,argST=NULL,argSTS=NULL)
```

**Arguments**

<code>tree</code>	a phylogenetic tree. The tree needs not to be either ultrametric or fully dichotomous.
<code>y</code>	a named vector of phenotypic trait.

path	the folder path where the StableTraits output will be stored. Notice that the input tree and data (modified automatically if the original tree is not fully dichotomous or if aces are specified) will be stored in this folder as well.
output	name of the output to be returned, if unspecified it will be named "output".
aces	a named vector of ancestral character values at nodes specified in advance. Names correspond to the nodes in the tree.
argST	a list of further arguments passed to StableTraits. If the argument has no value (for example "brownian") it must be specified as TRUE.
argSTS	list of further arguments passed to StableTraitsSum. If the argument has no value (for example "brownian") it must be specified as TRUE.

### Details

The StableTraits software is available at <https://mickelliot.com/>, along with instructions for compilation. Once it is installed, the user must set as R working directory the folder where the StableTraits software are installed. Further information about the arguments and outputs of StableTraits and StableTraitsSum can be found at <https://mickelliot.com/>. StableTraitsR automatically recognizes which Operating System is running on the computer (it has been tested successfully on MacOS and Windows machines).

### Value

The function returns a 'list' containing the output of StableTraits and StableTraitsSum.

**\$progress** a table reporting the DIC and PRSF diagnostics.

**\$rates\_tree** a copy of the original tree with branch lengths set to the evolutionary rate imputed by the stable reconstruction. Specifically, each branch length is equal to the absolute difference in the stable reconstruction occurring on that branch divided by the square root of the input branch length.

**\$rates** the original branch lengths, evolutionary rates, node height and (optionally) scaled branch lengths.

**\$aces** the median estimates of ancestral states and stable parameters along with the 95% credible interval.

**\$brownian\_tree** if "brownian" is TRUE in argSTS, a copy of the original tree with branch lengths set such that the Brownian motion reconstruction of the character on this tree is approximately the same as the stable ancestral reconstruction.

**\$ace.prior.values** if aces is specified, the function returns a dataframe containing the corresponding node number on the RRphylo tree for each node, the original (preset) and the estimated values, and the 95% credible interval.

### Author(s)

Silvia Castiglione, Carmela Serio, Pasquale Raia

### References

Elliot, M. G., & Mooers, A. Ø. (2014). Inferring ancestral states without assuming neutrality or gradualism using a stable model of continuous character evolution. *BMC evolutionary biology*, 14: 226. doi.org/10.1186/s12862-014-0226-8

**Examples**

```
## Not run:
library(ape)
library(phytools)

# Set as working directory the folder where StableTraits software are stored
# setwd("~/StableTraits")

dir.create("Analyses")
rtree(100)->tree
fastBM(tree)->y
c(1,2,3)->acev
sample(Ntip(tree)+seq(1:Nnode(tree)),3)->names(acev)
StableTraitsR(tree,y,path="Analyses/",output="my_output",aces=acev,
argST=list(iterations=500000,chains=4),argSTS=list(brownian=TRUE))->ST

## End(Not run)
```

---

swap.phylo	<i>Test the effect of phylogenetic uncertainty on rate shifts found at a particular node</i>
------------	--

---

**Description**

The function uses a number of alternative phylogenies with altered (as compared to the reference tree) topology and branch lengths tests whether the tips descending from the specified node (node) have statistically different rates from the rest of the tree. A phenotypic vector *y* must be supplied. Eventually, the effect of a covariate could be included.

**Usage**

```
swap.phylo(tree, si=0.5, si2=0.5, node, y, rts, nrep=100, cov=NULL, clus=0.5)
```

**Arguments**

<i>tree</i>	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous.
<i>si</i>	the proportion of tips whose topologic arrangement will be swapped.
<i>si2</i>	the proportion of nodes whose age will be changed.
<i>node</i>	the focal node to be tested.
<i>y</i>	the phenotype under testing.
<i>rts</i>	the rates found by <a href="#">RRphylo</a> on the original tree.
<i>nrep</i>	the number of simulated trees to be produced.
<i>cov</i>	the covariate to be indicated if its effect on rate values must be accounted for. Contrary to <a href="#">RRphylo</a> , <i>cov</i> needs to be as long as the number of tips of the tree.
<i>clus</i>	the proportion of clusters to be used in parallel computing (only if <i>y</i> is multi-variate). To run the single-threaded version of <code>swap.phylo</code> set <i>clus</i> = 0.

## Details

swap.phylo changes the tree topology and branch lengths to a level specified by the user. Up to half of the tips, and half of the branch lengths can be changed randomly. The function provides a 'swapped' tree, yet, importantly, once a shift in the rate of evolution has been found by [RRphylo](#), this function can be used to test whether the shift depends on the tree topology and branch lengths. It runs RRphylo on swapped trees (default is 100) and then calculates the absolute rate difference between all the branches of the shifted node and the rest of the tree. A t-test is eventually performed to assess significance.

## Value

The function returns a 'list' object containing:

**\$p.swap** the probability that the rates at node are different from rates at the rest of the tree.

**\$rates** the distribution of rates per branch as calculated by RRphylo on 'swapped' phylogenies.

## Examples

```
## Not run:
  data("DataApes")
  DataApes$PCstage->PCstage
  DataApes$Tstage->Tstage
  DataApes$CentroidSize->CS
  cc<- 2/parallel::detectCores()

# Case 1. swap.phylo without accounting for the effect of a covariate
RRphylo(tree=Tstage,y=PCstage,clus=cc)->RR
RR$rates->rr
swap.phylo(Tstage,node=61,y=PCstage,rts=rr,clus=cc)

# Case 2. swap.phylo accounting for the effect of a covariate
RRphylo(tree=Tstage,y=CS,clus=cc)->RRcova
c(RRcova$aces,CS)->cov.values
c(rownames(RRcova$aces),names(CS))->names(cov.values)
RRphylo(tree=Tstage,y=PCstage,cov=cov.values,clus=cc)->RR
RR$rates->rr
swap.phylo(Tstage,node=61,y=PCstage,rts=rr,cov=CS,clus=cc)

## End(Not run)
```

---

swapONE

*Create alternative phylogenies from a given tree*

---

## Description

The function produces an alternative phylogeny with altered topology and branch length, and computes the Kuhner-Felsenstein (Kuhner & Felsenstein 1994) distance between original and 'swapped' tree.

**Usage**

```
swapONE(tree,node=NULL,si=0.5,si2=0.5,plot.swap=FALSE)
```

**Arguments**

tree	a phylogenetic tree. The tree needs not to be ultrametric or fully dichotomous.
node	if specified, the clades subtended by such node(s) are imposed to be monophyletic. In this case, the function can still swap tips <i>within</i> the clade.
si	the proportion of tips whose topologic arrangement will be swapped.
si2	the proportion of nodes whose age will be changed.
plot.swap	if TRUE, the function plots the swapped tree. Swapped positions appear in red. Nodes with altered ages appear in green.

**Details**

swapONE changes the tree topology and branch lengths. Up to half of the tips, and half of the branch lengths can be changed randomly. Each randomly selected node is allowed to move up to 2 nodes apart from its original position.

**Value**

The function returns a list containing the 'swapped' version of the original tree, and the Kuhner-Felsenstein distance between the trees.

**Author(s)**

Silvia Castiglione, Pasquale Raia, Carmela Serio, Alessandro Mondanaro, Marina Melchionna, Mirko Di Febbraro, Antonio Profico, Francesco Carotenuto

**References**

Kuhner, M. K. & Felsenstein, J. (1994). A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates, *Molecular Biology and Evolution*, 11: 459-468.

**Examples**

```
## Not run:
data("DataOrnithodirans")
DataOrnithodirans$treedino->treedino

## Case 1. change the topology and the branch lengths for the entire tree
swapONE(tree=treedino,si=0.5,si2=0.5,plot.swap=FALSE)

## Case 2. change the topology and the branch lengths of the
## tree by keeping the monophyly of a specific clade
swapONE(tree=treedino,node=422,si=0.5,si2=0.5,plot.swap=FALSE)

## End(Not run)
```

tree.merger

*Fast addition of tips and clades on an existing tree***Description**

The function attaches new tips and/or clades derived from a source phylogeny to a pre-existing backbone tree.

**Usage**

```
tree.merger(backbone,data,source.tree=NULL,age.offset=NULL,tip.ages =
  NULL, node.ages = NULL,min.branch=NULL,plot=TRUE,filename=NULL)
```

**Arguments**

backbone	the backbone tree to attach tips/clades on.
data	a dataset including as columns: <ol style="list-style-type: none"> <li>1. bind = the tips/clades to be attached;</li> <li>2. reference = the reference tip or clade where 'bind' must be attached;</li> <li>3. poly = logical specifying if 'bind' and 'reference' should form a polytomous clade.</li> </ol> <p>See details for further explanations.</p>
source.tree	the tree where 'bind' clades are to be extracted from. If no clade has to be attached, it can be left unspecified.
age.offset	if the most recent age (i.e. the maximum distance from the tree root) differs between the source and the backbone trees, the "age.offset" is the difference between them in this exact order (source minus backbone). It is positive when the backbone tree attains younger age than the source tree, and vice-versa.
tip.ages	as in <a href="#">scaleTree</a> , a named vector including the ages (i.e. the time distance from the youngest tip within the tree) of the tips. If unspecified, the function assumes all the tips on the backbone tree are correctly placed and places all the new tips at the maximum distance from the tree root (i.e. the present if the tips are extant).
node.ages	as in <a href="#">scaleTree</a> , a named vector including the ages (i.e. the time distance from the youngest tip within the tree) of the nodes. The nodes must be defined by collating the names of the two phylogenetically furthest tips it subtends to, separated by a "-" (see examples). If no calibration date for nodes is supplied, the function may shift the node position back in time as to place new tips/clades and to fit tip ages.
min.branch	as in <a href="#">scaleTree</a> , the minimum branch length that will be imposed for shifted nodes.
plot	if TRUE, the function produces an interactive plotting device to check the placing of each bind.
filename	if plot=TRUE and provided a filename (with or without the path), the function stores a pdf file showing the plot of the entire phylogeny.

## Details

The function attaches tips and/or clades from the source tree to the backbone tree according to the data object. Within the latter, a clade, either to be bound or to be the reference, must be indicated by collating the names of the two phylogenetically furthest tips belonging to it, separated by a "-". Duplicated 'bind' produce error. Tips/clades set to be attached to the same 'reference' are considered to represent a polytomy. Tips set as 'bind' which are already on the backbone tree are removed from the latter and placed according to the 'reference'. See examples and [vignette](#) for clarifications.

## Value

Merged phylogenetic tree.

## Author(s)

Silvia Castiglione, Carmela Serio, Pasquale Raia

## References

aaa

## See Also

[tree.merger vignette](#); [scaleTree vignette](#);

## Examples

```
## Not run:
require(ape)
require(geiger)
DataCetaceans$treecet->tree
data.frame(bind=c("Balaena_mysticetus-Caperea_marginata",
  "Aetiocetus_weltoni",
  "Saghacetus_osiris",
  "Zygorhiza_kochii",
  "Ambulocetus_natans",
  "Kentriodon_pernix",
  "Kentriodon_schneideri",
  "Kentriodon_obscurus",
  "Tursiops_truncatus-Delphinus_delphis",
  "Kogia_sima",
  "Grampus_griseus"),
reference=c("Fucaia_buelli-Aetiocetus_weltoni",
  "Aetiocetus_cotylalveus",
  "Fucaia_buelli-Tursiops_truncatus",
  "Saghacetus_osiris-Fucaia_buelli",
  "Dalanistes_ahmedi-Fucaia_buelli",
  "Kentriodon_schneideri",
  "Phocoena_phocoena-Delphinus_delphis",
  "Kentriodon_schneideri",
  "Stenella_attenuata-Stenella_longirostris"),
```

```

        "Kogia_breviceps",
        "Globicephala_melas-Pseudorca_crassidens"),
poly=c(FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE,
        FALSE))->dato

c(Aetiocetus_weltoni=28.0,
  Saghacetus_osiris=33.9,
  Zygorhiza_kochii=34.0,
  Ambulocetus_natans=40.4,
  Kentriodon_pernix=15.9,
  Kentriodon_schneideri=11.61,
  Kentriodon_obscurus=13.65)->tip.ages
c("Ambulocetus_natans-Fucaia_buelli"=52.6,
  "Balaena_mysticetus-Caperea_marginata"=21.5)->node.ages

# remove some tips from the original tree and create a source tree
drop.tip(tree,c(names(tip.ages),
                tips(tree,131)[-which(tips(tree,131)%in%
                                     c("Caperea_marginata", "Eubalaena_australis"))],
                tips(tree,195)[-which(tips(tree,195)=="Tursiops_aduncus")]))->backtree
drop.tip(tree,which(!tree$tip.label%in%c(names(tip.ages),
                                         tips(tree,131),
                                         tips(tree,195))))->sourcetree

plot(backtree,cex=.6)
plot(sourcetree,cex=.6)

tree.merger(backbone=backtree,data=dato,source.tree=sourcetree,
            tip.ages=tip.ages,node.ages = node.ages, plot=TRUE)->treeM

## End(Not run)

```



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