PastView user's Manual 1.1

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Goals of PastView and terminology

Strains sampling / databank

sequences
molecular level

character states
« phenotypic » level
(geographic origin, resistance to a treatment, life history traits, …)

phylogenetic tree

primary annotations

PastView analysis:

**Ancestral
sequence**

1

**Ancestral
annotation**

**Rooted
Tree**

2

Transitions

1 Compute (or import) and display
ancestral annotations

2 Analyse transitions of ancestral annotations

identify evolutionary
scenarios such as the
spread of a disease, the
dynamic of a drug
resistance, or shifts of
ecological habitats.

Installing and running PastView

1 **www.pastview.org** and scroll to the download and install section
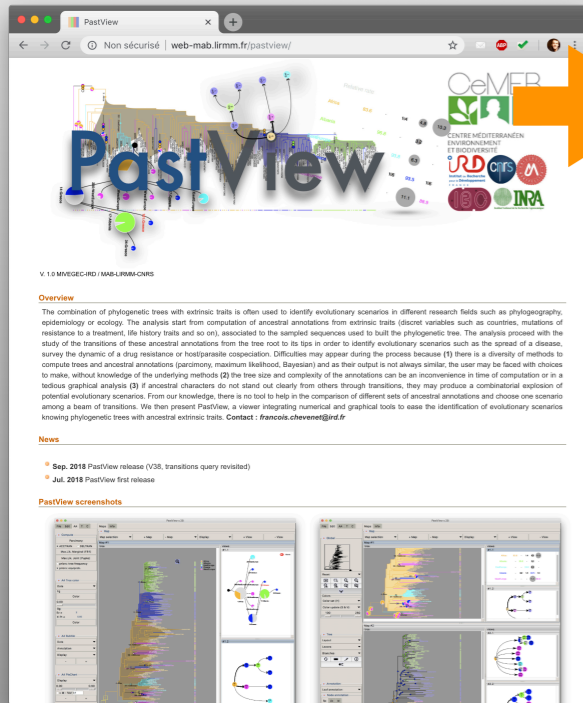
2 download and install tcl/tk

How to load & run PastView ?

1) Download & Install Tcl/Tk: OSX Linux Windows
2) Download PastView package (source code, multi-platforms)
3) under Linux or OSX: from a terminal, goto to the downloaded PastView folder, then command "wish", then from the wish prompt, command "source pastview.tcl". Under Windows: double click the pastview.tcl file.

3 download and extract pastview.zip

```
Last login: Mon Nov 19 14:29:50 on ttys000
[MacBook-Pro-de-Francois:~ chevenet$ cd Desktop
[MacBook-Pro-de-Francois:Desktop chevenet$ cd pastview
[MacBook-Pro-de-Francois:pastview chevenet$ wish
% source pastview.tcl
PastView v.39
% []
```

4 OS Console, command
« wish », cd pastview folder
command « source
pastview.tcl »
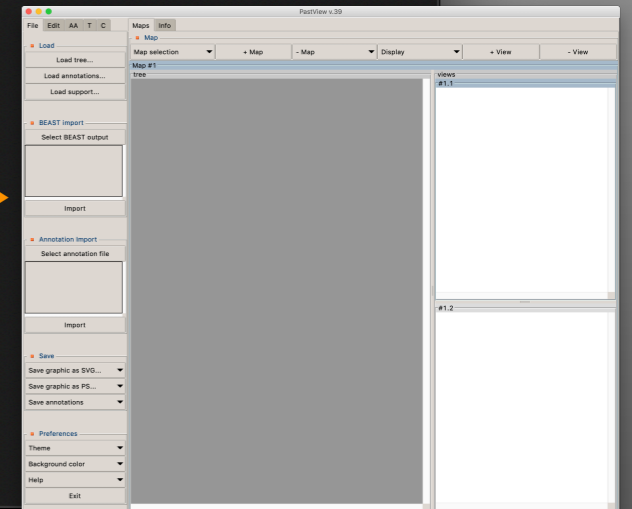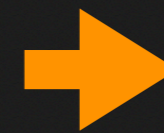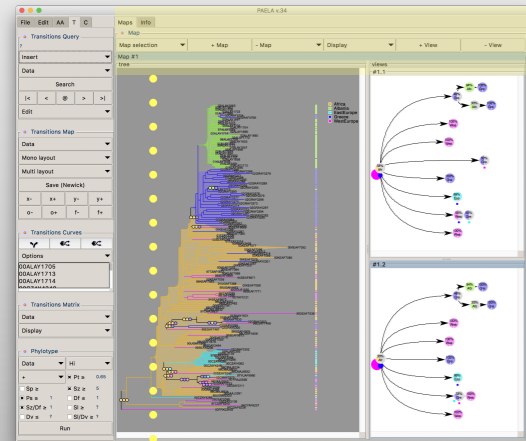
Overview of the PastView interface
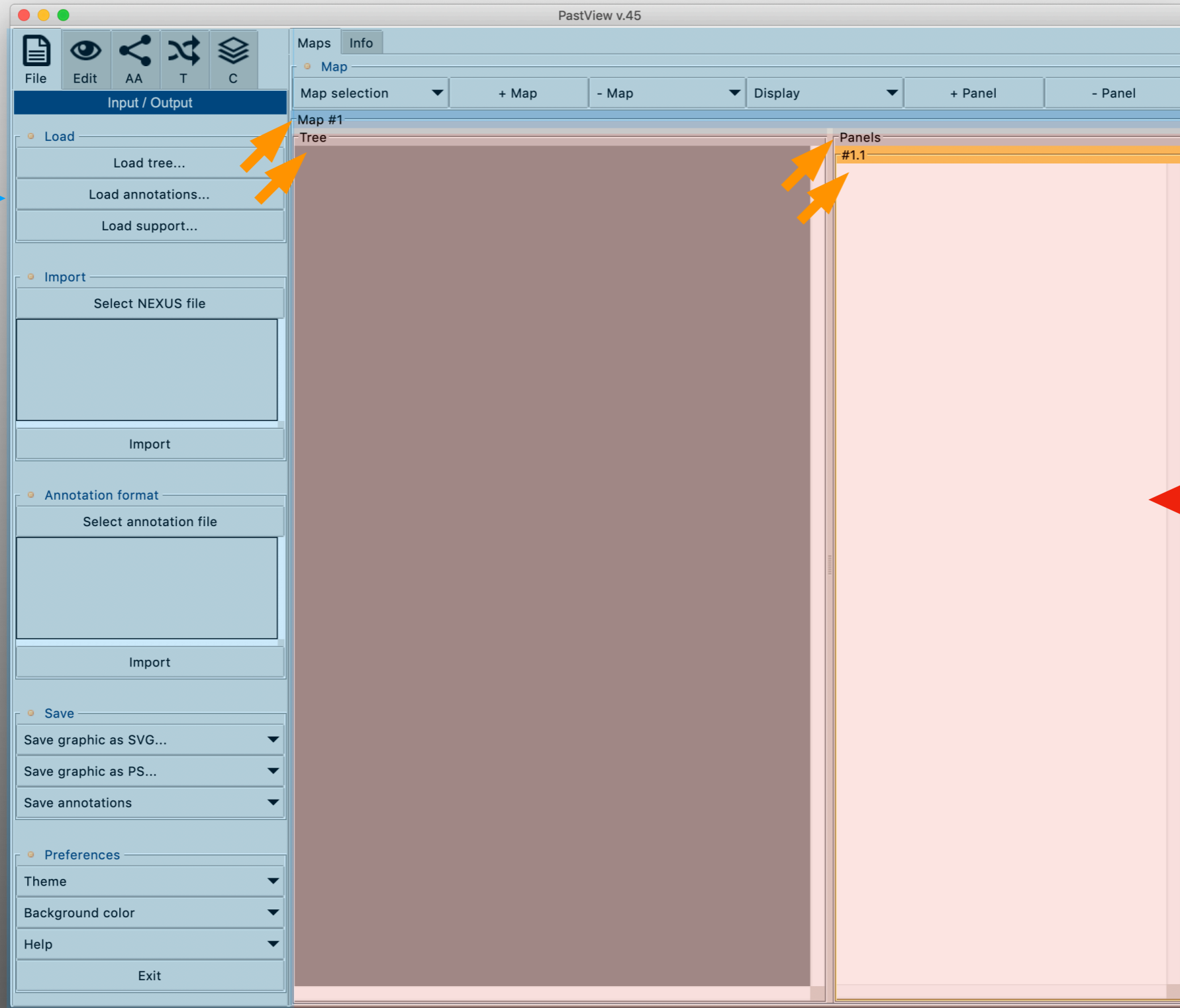
Controls

Data visualization, a « map » is sub-divided into one tree view and one or more secondary view(s)
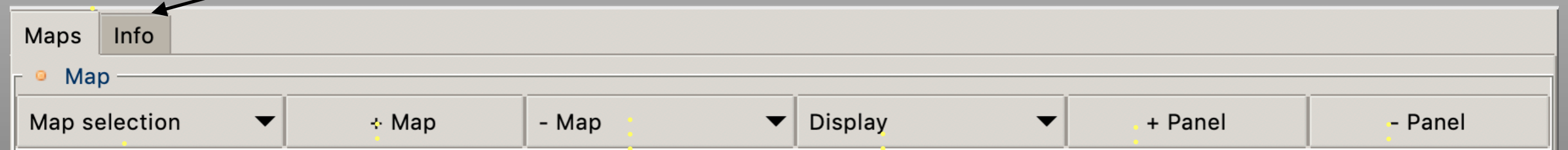
To set a view as a current view (for instance as a target for a new output, clic on its id)

PastView toolboxes

Canvas management (drawing spaces)

The info tab output textual results

Maps	Info

🔸 Map

Map selection ▼	⊹ Map	– Map ▼	Display ▼	+ Panel	– Panel

Select a map
(this can be done by
clicking on the name
of the map)

Delete the current map

Add a new view to
the current map

Display/Hide map(s)

Delete the current
view of the current
map

Add a new map

5

Overview of the PastView interface
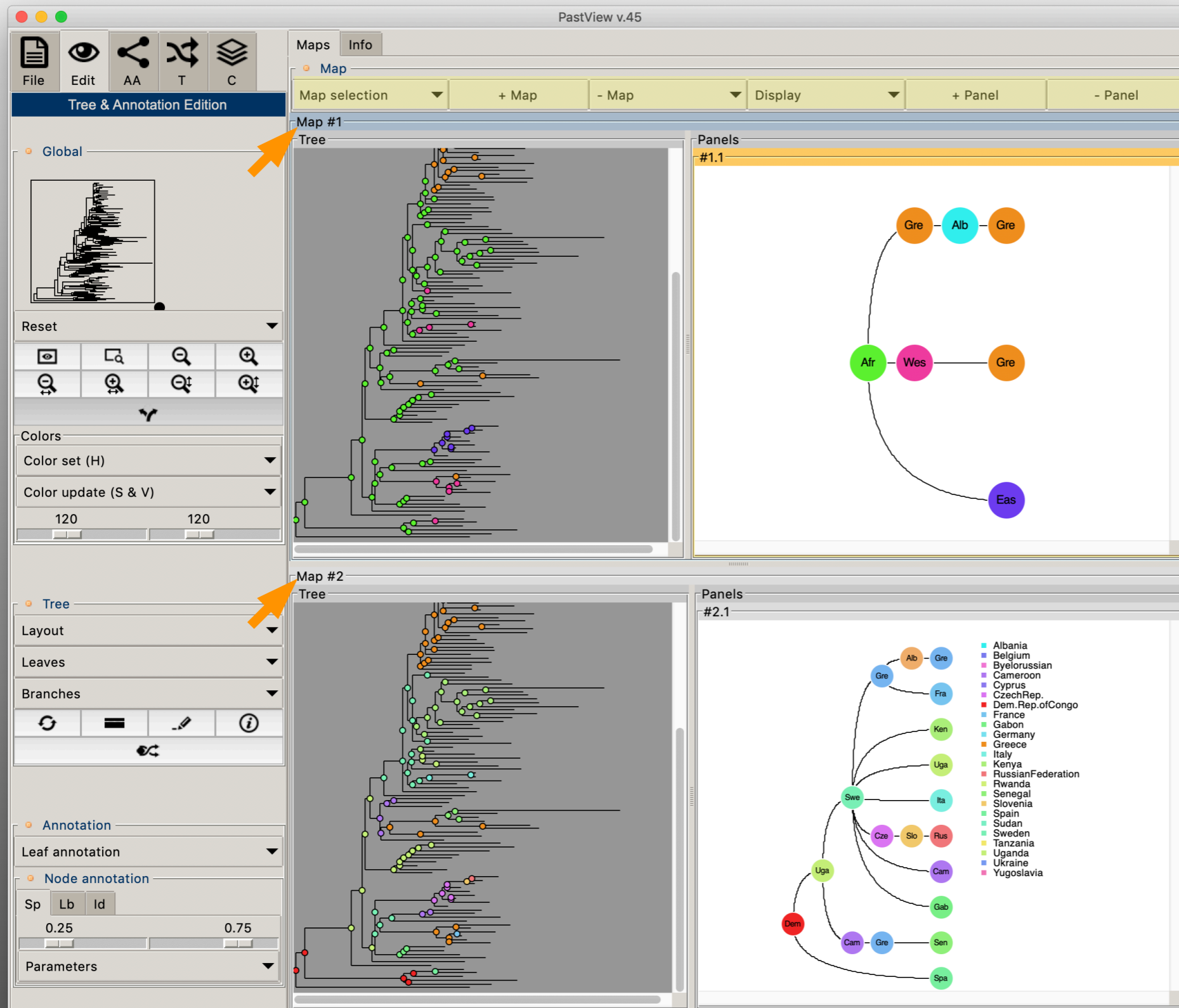

PastView manages multiple maps

A map is dedicated to a maximum of three sets of ancestral annotations : a set from ML marginal likelihood or import (eg NEXUS import, each node having multiple annotations with probabilities), a set from ML joint likelihood and a set from parsimony

To add a map, clic the button « + Map », to delete a map use the « - Map » menu. To re-order the map display, use the « Display » button.

To set a map as the current map, either use the « Map selection » menu or clic on the Map name (Map #1, Map #2 …) The current map is highlight in blue

PastView toolboxes 5 families of controls:
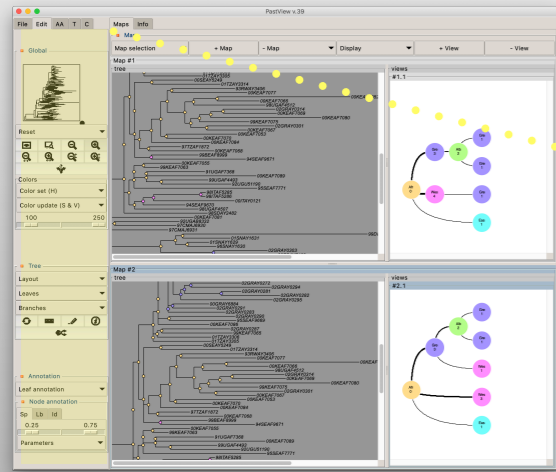
« File » « Edit » « AA » « T » « C »

« File » — Input / Output

Load
- Load tree...
- Load annotations...
- Load support...

Import
- Select NEXUS file
- Import

Annotation format
- Select annotation file
- Import

Save
- Save graphic as SVG...
- Save graphic as PS...
- Save annotations

Preferences
- Theme
- Background color
- Help
- Exit

« Edit » — Tree & Annotation Edition

Global
- Reset

Colors
- Color set (H)
- Color update (S & V)
- 120   120

Tree
- Layout
- Leaves
- Branches

Annotation
- Leaf annotation

Node annotation
Sp | Lb | Id
0.25   0.75
- Parameters

« AA » — Compute & Display Ancestral Annotations

Compute
- Parsimony
- ACCTRAN   DELTRAN
- F81-like
- Joint scenario
- priors: tree frequency
- priors: equiprob.

AA Tree color
- Data
- Fg
- Color
- 0.00
- Bg
- Sz ≥   2
- Pt ≥   0.65
- Color

AA Bubble
- Data
- Annotation
- Display
- -   +

AA PieChart
- Display
- 0.00   0.90
- > M - %M   0.1
- -   +

Filters
- Filter

« T » — Transitions of Ancestral Annotations

Transitions Query
?
- Insert
- Data
- Search
- |< < > >|
- Edit

Transitions Map
- Data
- Full   options...
- Compressed   options...
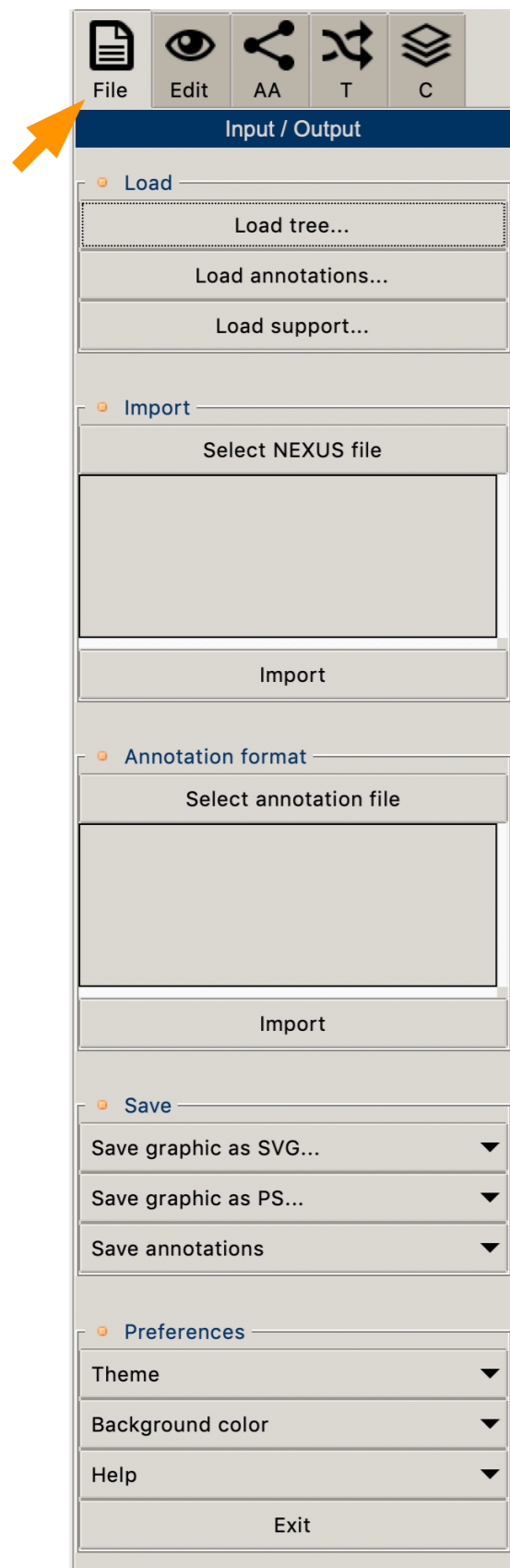- Save (Newick)

Transitions Matrix
- Data
- Display

« C » — Comparative Analysis

Intra MAP Inconsistencies
- Display On/Off
- -   +

Inter MAP Inconsistencies
- AA distribution from
- Bubble
- Piecharts
- -   +

Input/Output

Tree & Annotations Edition

Compute & display Ancestral Annotations (AA)

Transitions of Ancestral Annotation

Comparative analysis

7

« File » toolbox overview

The « File » toolbox : Input/Output

Loading tree and annotations (primary annotations with or without ancestral annotations)

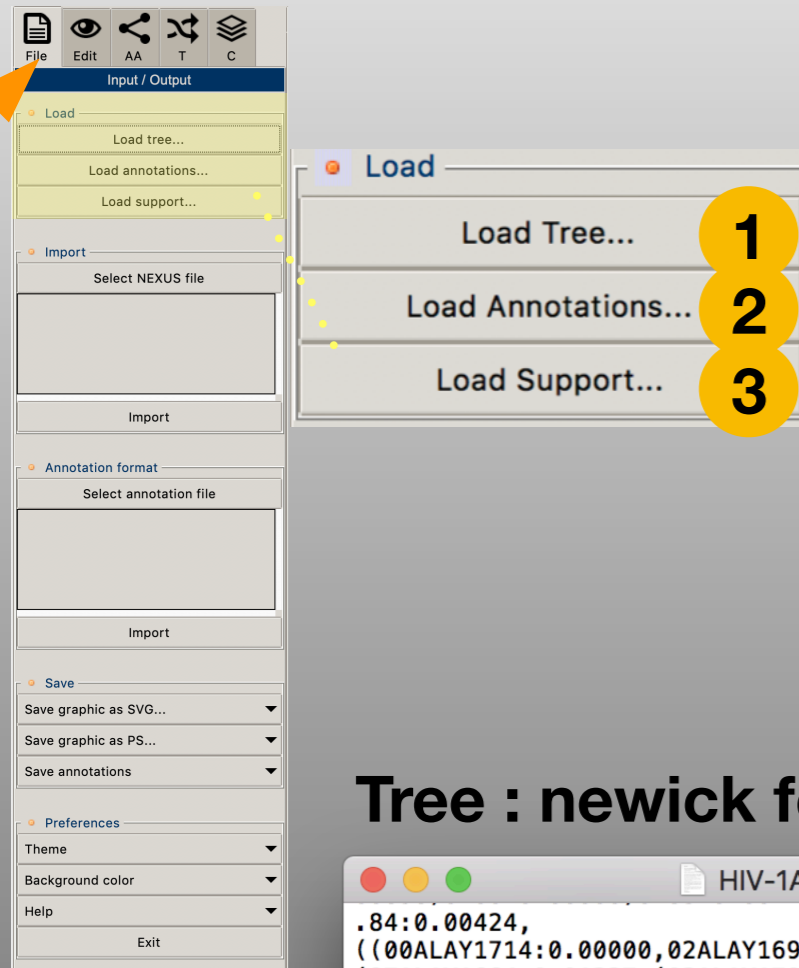**Importation from NEXUS format (tree, primary and ancestral annotations)**

Translation of a strainsXvariables annotations matrix to a strainsXmodalities matrix

Saving graphics and annotations

Miscellaneous controls (default values for the graphical user's interface)

« File » toolbox, Load   Case #1

1 Clic the « Load tree… » button and select a tree following the Newick format. The tree is displayed in the current map (tree canvas)

2 Clic the « Load annotations… » button and select an annotation file following the PastView format (see below). In this example the annotation file contains primary annotations only.

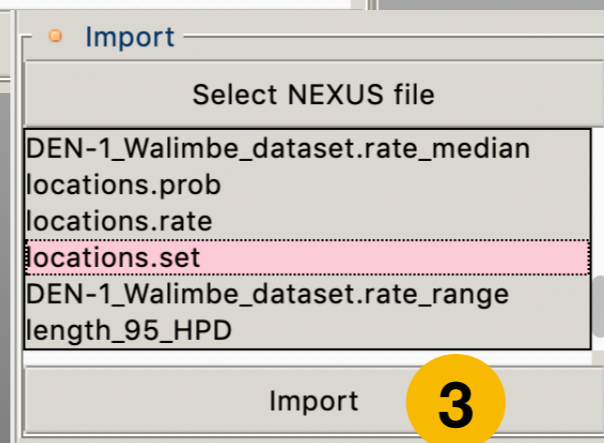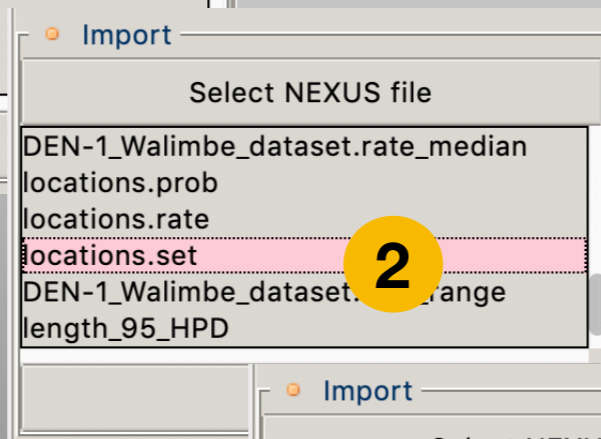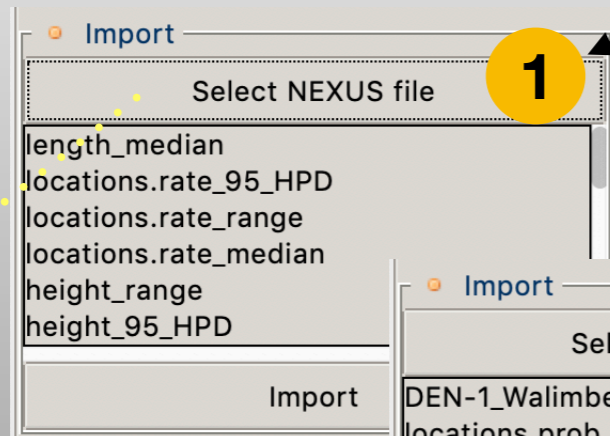**3** Clic the « Load support… » button if support are available. The format follows the annotation format (see example in case #2)
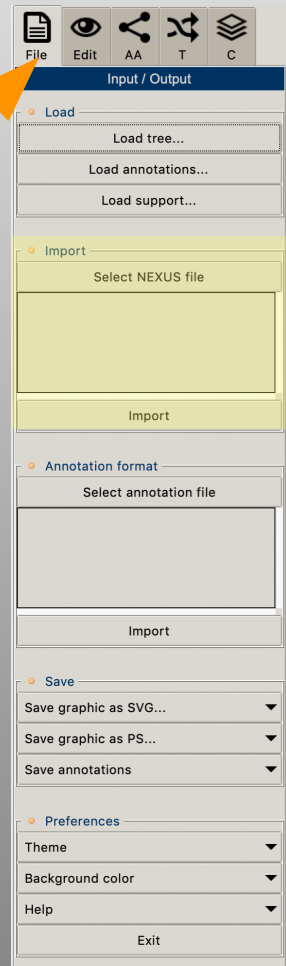
Tree : newick format

```
.84:0.00424,
((00ALAY1714:0.00000,02ALAY1696:0.03541)0.81:0.00425,
(97ALAY1664:0.01327,(03ALAY1704:0.03110,
((00ALAY1705:0.00858,01ALAY1652:0.00000)0.89:0.00875,
((02ALAY1711:0.00000,02ALAY1697:0.00853)0.00:0.00000,
(00GRAF5768:0.03063,00GRAF5754:0.00871)0.75:0.00480)0.89:0.
00868)0.00:0.00000)0.62:0.00442)0.79:0.00420)0.00:0.00000)0
.00:0.00000,
((01ALAY1689:0.00413,03ALAY1703:0.01753)0.88:0.00867,
(03ALAY1706:0.00860,
(03ALAY1662:0.00429,03ALAY1663:0.00877)0.75:0.00426)0.80:0.
00432)0.00:0.00000)0.00:0.00000)0.96:0.01745)0.00:0.00000)0
.
81:0.00435)0.88:0.00873)0.00:0.00000)0.74:0.00429)0.89:0.01
264)0.77:0.00447)0.00:0.00000)0.60:0.01845)0.78:0.01376)0.6
7:0.00449)0.60:0.00303)0.69:0.01023)0.88:0.04473)1.0:0.0088
5);
```

Annotations : CSV , primary annotations

```
ID,Africa,Albania,EastEurope,Greece,WestEurope
00ALAY1705,0,1,0,0,0
00ALAY1705,0,1,0,0,0
00ALAY1705,0,1,0,0,0
00ALAY1713,0,1,0,0,0
00ALAY1713,0,1,0,0,0
00ALAY1714,0,1,0,0,0
00ALAY1714,0,1,0,0,0
```
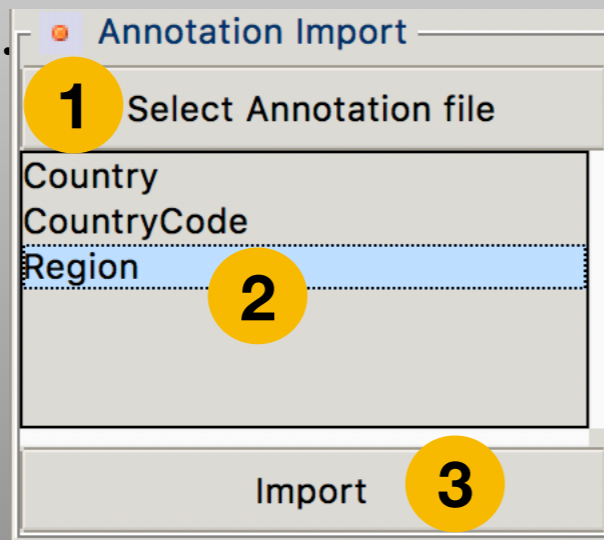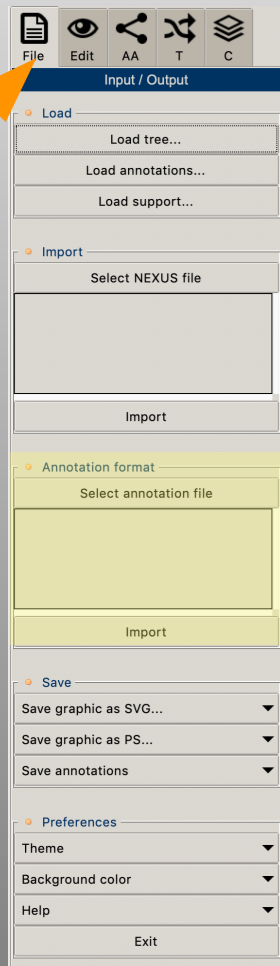
« File » toolbox, Load   Case #2

1 Clic the « Load tree… » button and select a tree following the Newick format. The tree is displayed in the current map (tree canvas)

2 Clic the « Load annotations… » button and select an annotation file with ancestral annotations, following the PastView format (see below). In this example ancestral annotations are saved by using unique IDs to put in regards node of the tree with annotations. These IDs must be used in the newick string in place of the support values (before the « : » character)

3 Clic the « Load support… » button if support are available. The format follows the annotation format (with IDs)

Tree : newick format with IDs

Annotations : CSV , primary annotations and ancestral annotations (with IDs)

« File » toolbox, NEXUS import

1 Clic the « Select NEXUS file » button and select a NEXUS output, wait for the parsing of the data

2 Select the variable of annotation to extract

3 Clic the « Import » button. The tree and the annotations will be automatically loaded but 3 files will also be created in the same file's path than the NEXUS file: the tree, the annotations and the support values.

« File » toolbox, annotations format translation

Select the Strains x Variables file (1) then
select one of its variable to extract, in this example
the « Region » variable (2), and
Clic the Import button (3)

Strains x Variables matrix

Strains x Modalities matrix

« File » toolbox, saving graphics
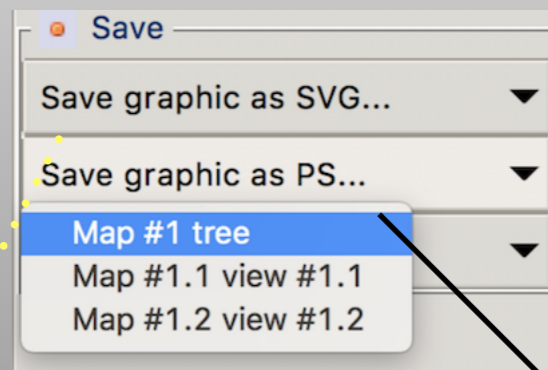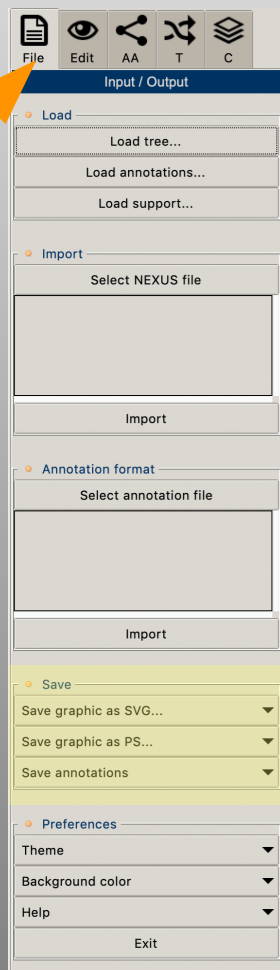
Select the map and the canvas to be saved

Graphics can be saved to SVG or PostScript formats

Annotations are saved to CSV format
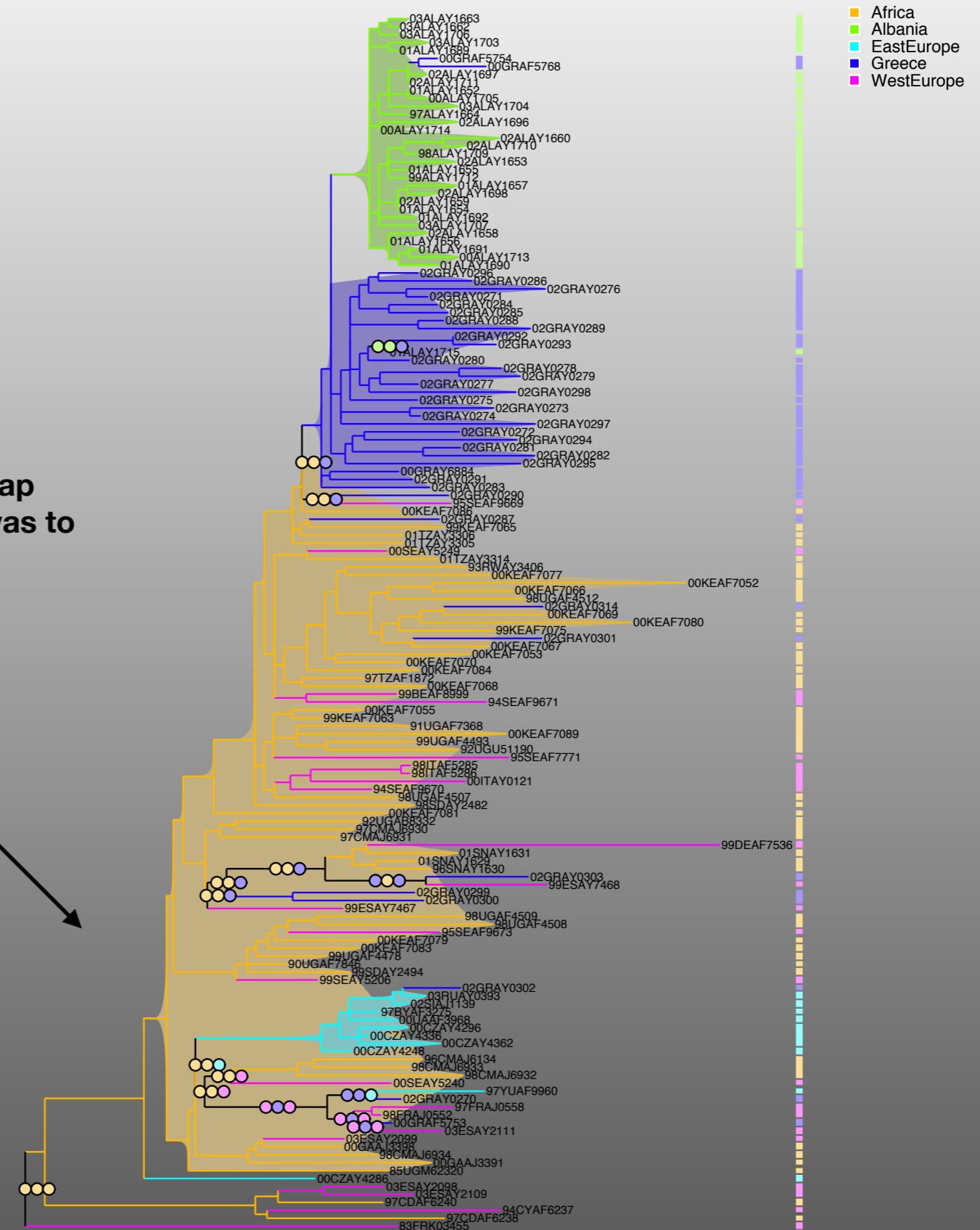
« File » toolbox, preferences
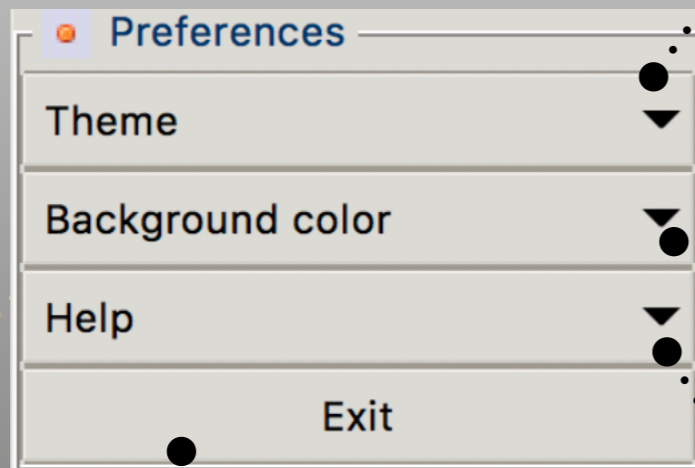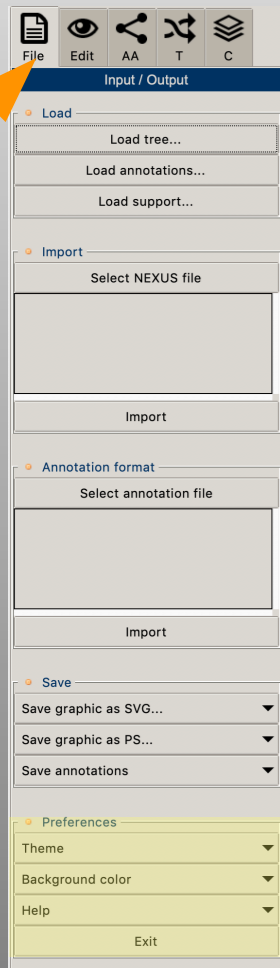
Different themes for the PastView
graphical user interface

Tree canvas backround color
View canvas background color

By default, the canvas background color
for the tree is grey, this can be changed
by using the « background color » menu

By default, « the tooltips » popup
window is activated

Exit PastView

« Edit » toolbox overview
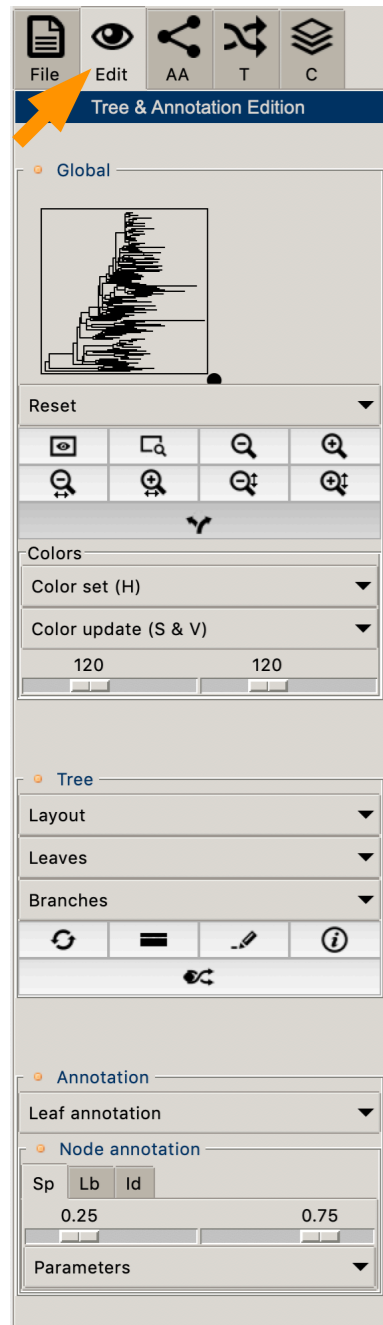
Edition of tree AND annotations (reset, zooms, move, colors…)

Tree Edition: tree layouts, leaves graphical feature's (font, color). The controls of a menu operates at a global level. The « iconic » button controls operate on the fly to the nodes of the tree

Edition of annotation: display primary annotations in regards of the tips of the tree, display support, branches lengths (taking into account threshold values)

« Edit » toolbox   Global
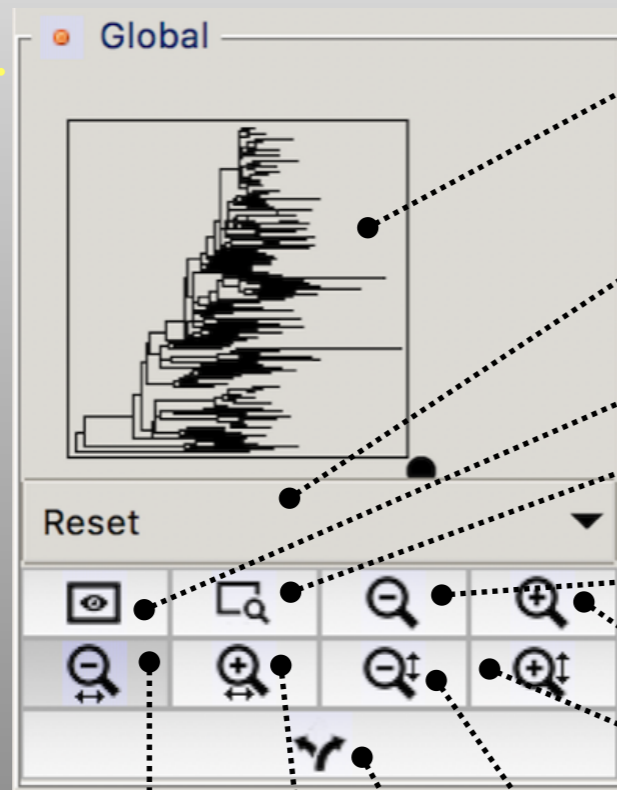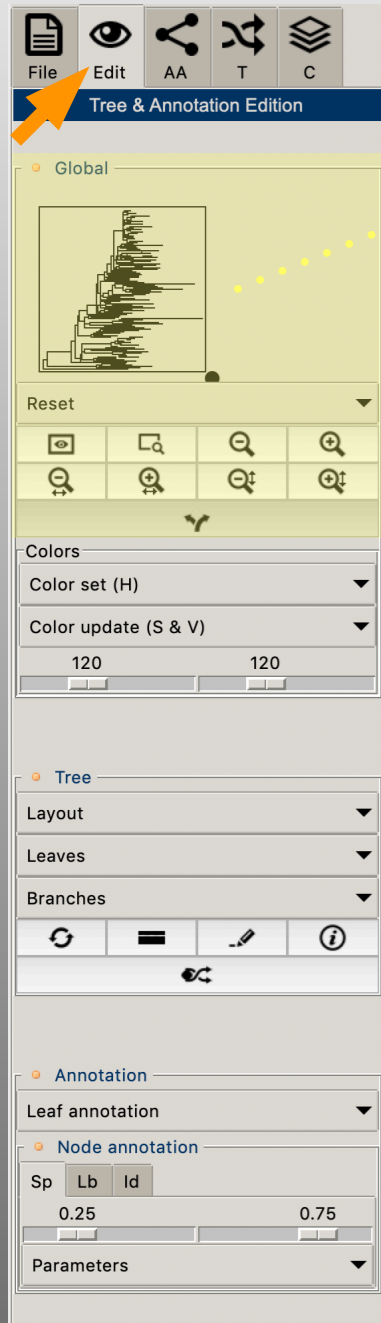
Global view of the tree
with moveable and
resizable brush (zoom)

Reset menu

Reset all

Tree foreground color
Tree background color
Line width

Leave foreground color
Leave annotation

Map tree
Map views

Fit to window

Zoom In Frame

Zoom Out X&Y

Zoom In X&Y

Zoom In Y

Activate a tool then apply it on canvas (maps or views

Zoom Out X

Move

Zoom In X

Zoom Out Y

« Edit » toolbox Global

Africa
Albania
EastEurope
Greece
WestEurope

Add Legend to Map
Delete Legend from Map
Add Legend to Board
Delete Legend from Board

Select an annotation to change « on the fly » the Hue value of the color associated to the annotation

Colors

Color set (H)

Color update (S & V)

86   195

saturation   value

Tree BG
Tree FG
Piechart
Bubble
✓ Tips
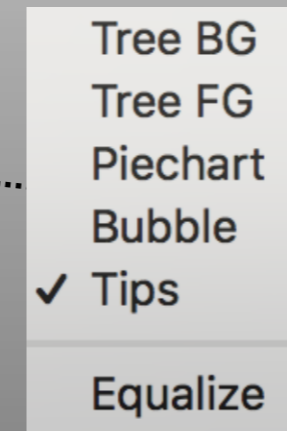
Equalize

Saturation and Value changes are apply to all annotations but in a different way according to graphical items : tree background, tree foreground, piecharts, bubble, tips labels
Use the « Equalize » command to set the same S and V values to these different items

File Edit AA T C
Tree & Annotation Edition

Global

Reset

Colors
Color set (H)
Color update (S & V)
120   120

Tree
Layout
Leaves
Branches

Annotation
Leaf annotation
Node annotation
Sp Lb Id
0.25   0.75
Parameters

« Edit » toolbox   Tree

Laderize up
Laderize down

Rotation

On/Off
Font
Color

Size -
Size +

Size 1
Size 2
Size 3

Tree

Layout

Leaves

Branches
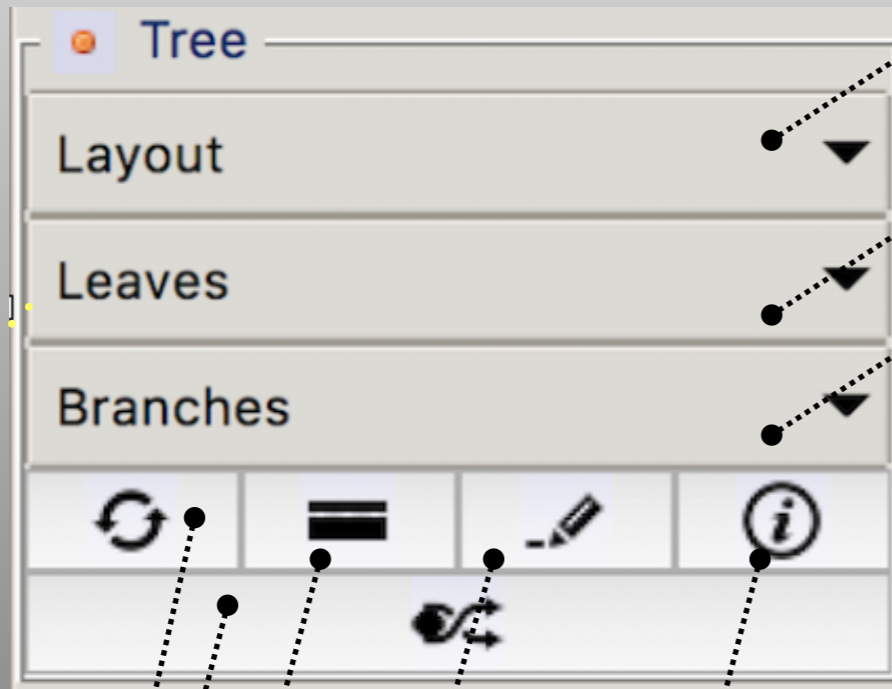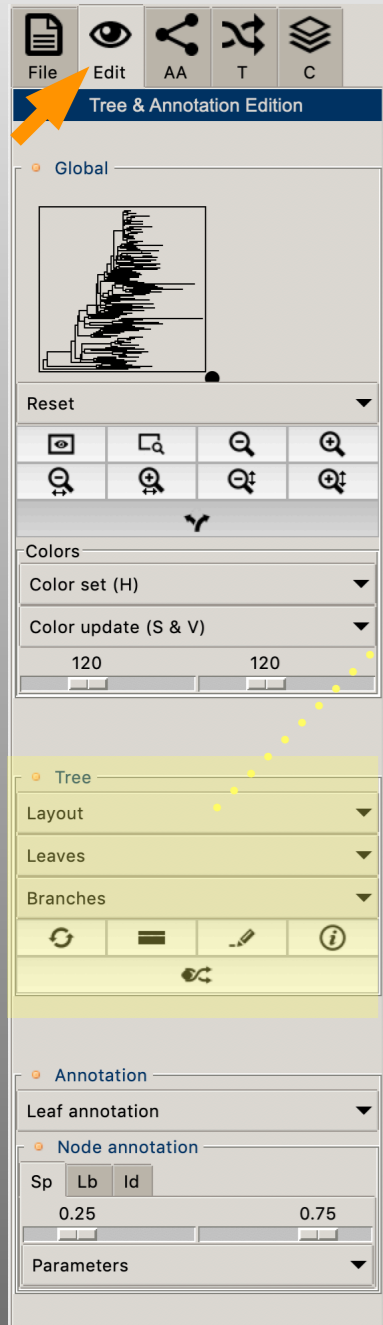
Swap
Root
Line size
D1 = + Shift-D1 = -
Foreground color
Info
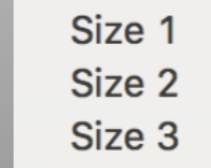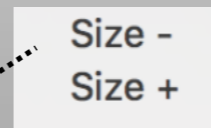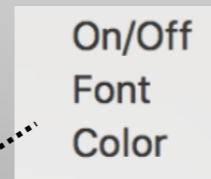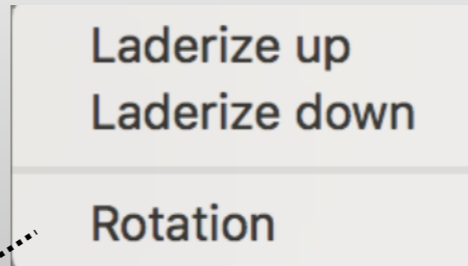
Activate a tool then apply it on node(s) (maps or views

« Edit » toolbox Annotations
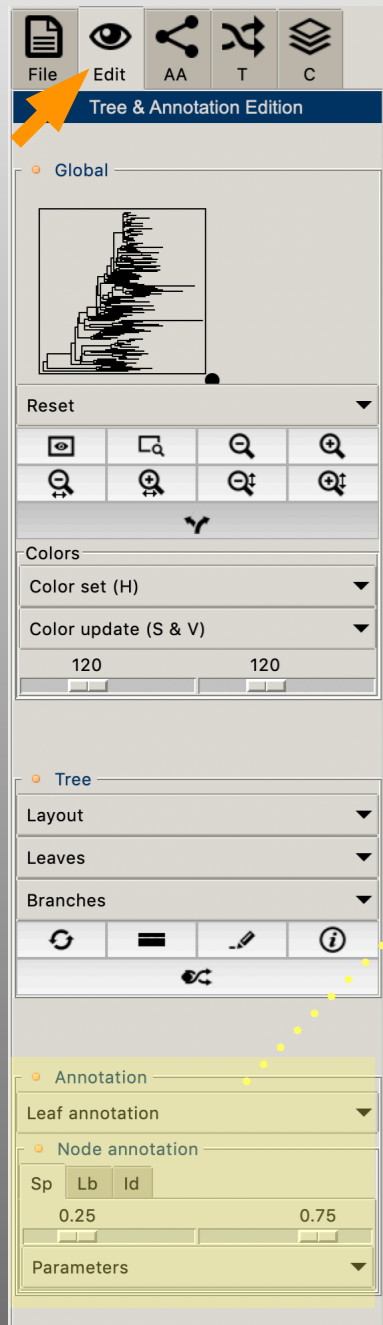
Color labels
Color brackets with text
Color brackets without text

Annotation

Leaf annotation

Node annotation

Sp Lb Id

0.25   0.75

Parameters
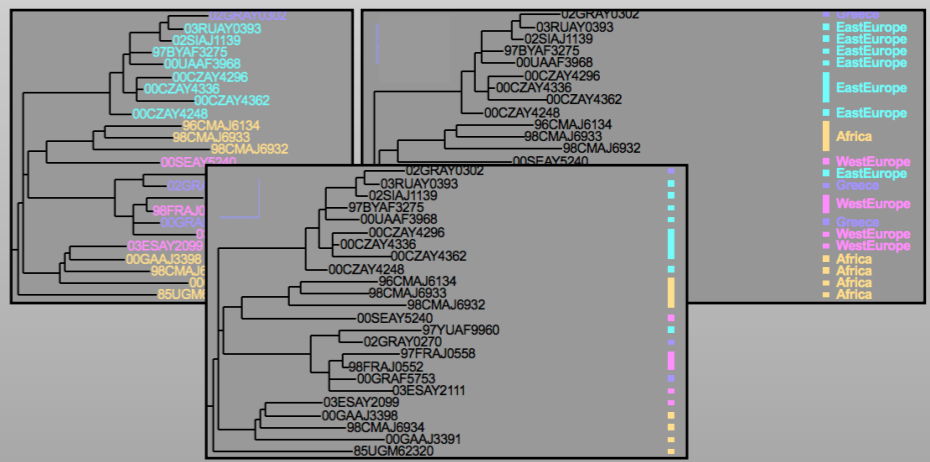
Display Support values,
branch lengths, node ID

Two thresholds (inf. and sup.) to select support values

Support On/Off

Color
Tree foreground color
✓ Tree annotate

how to display the support values

« AA » toolbox overview AA = Ancestral Annotations
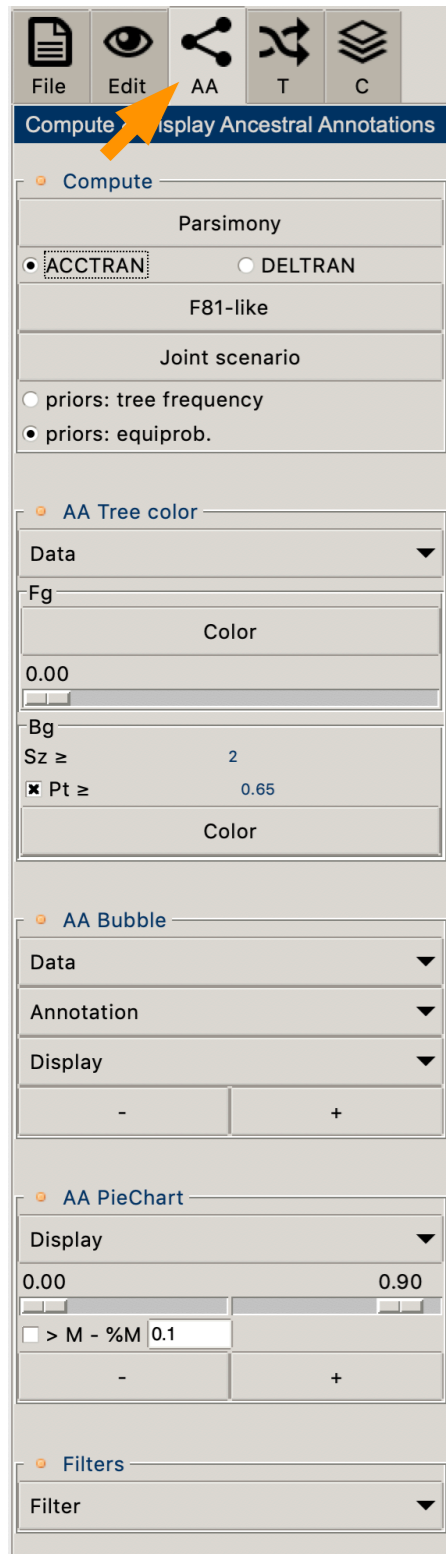
Computation of ancestral annotations knowing a tree and primary annotations, by parsimony and maximum likelihood (marginal and jointed methods)

Displaying ancestral annotations

Highlight nodes with several filters

AA toolbox: compute ancestral annotations

Parsimony
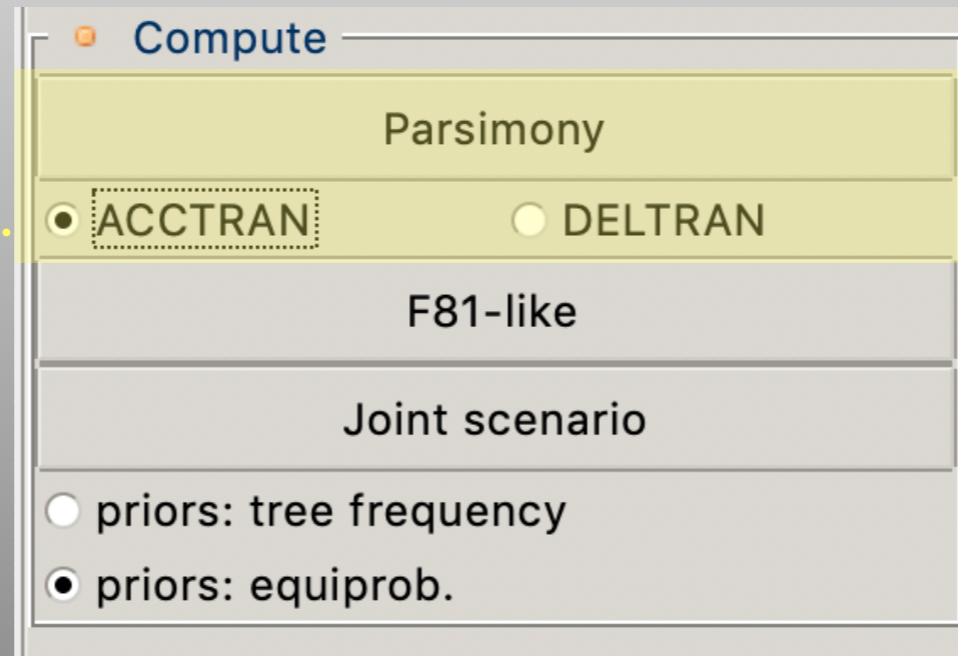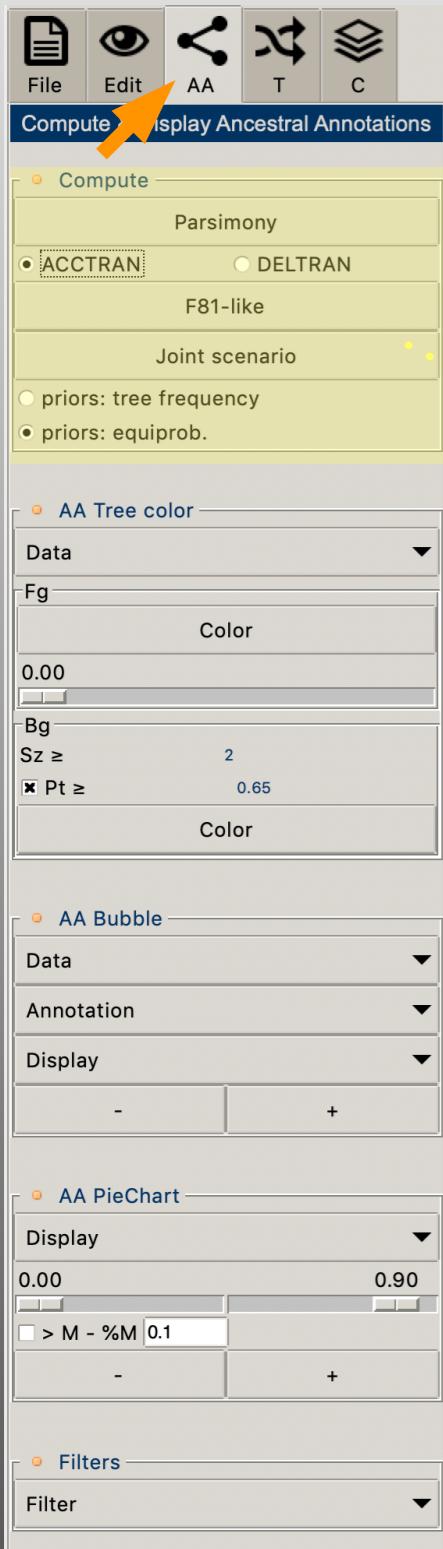
Click the « Parsimony » button, the AA will computed for the current tree (map)

2

Select the option

1

Computing AA by parsimony is fast but without any model of evolution but ambiguities may appear (several AA for a node)

AA toolbox: compute ancestral annotations

Maximun likelihood, F-81 like, marginal
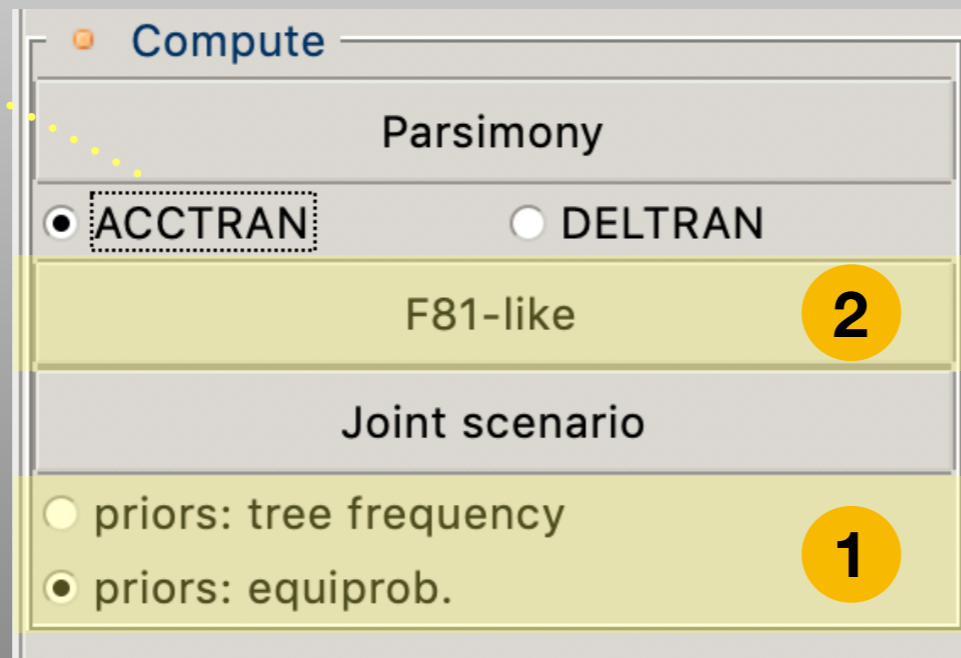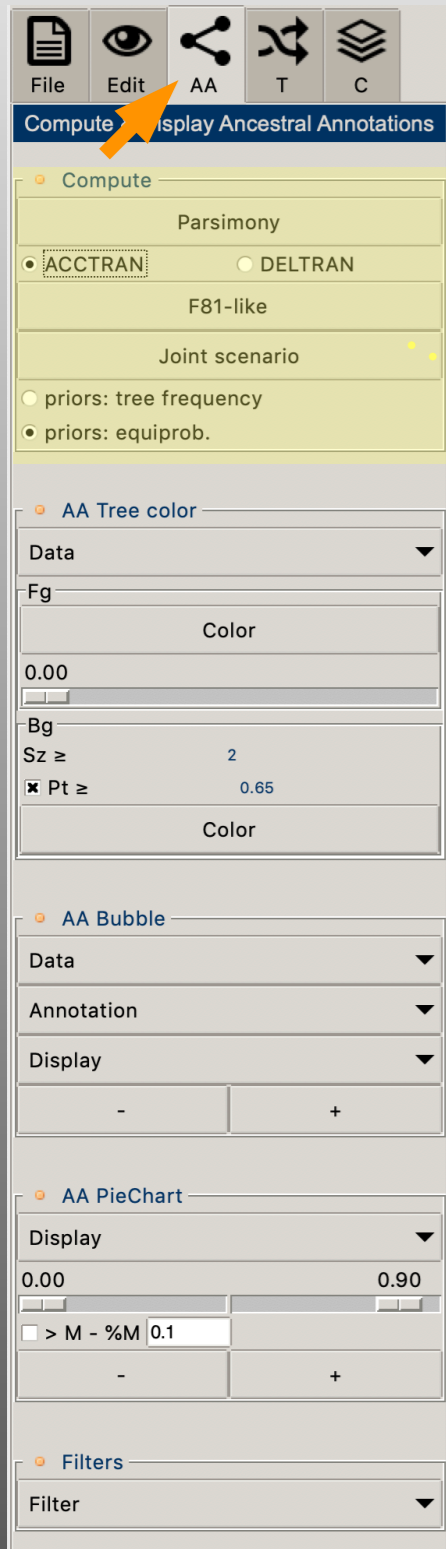
1 Select the priors option

2 Click the « Max.Lik. Marginal (F81) » button, the ancestral annotations will be computed for the current tree (map)

AA toolbox: compute ancestral annotations

Maximun likelihood, joint
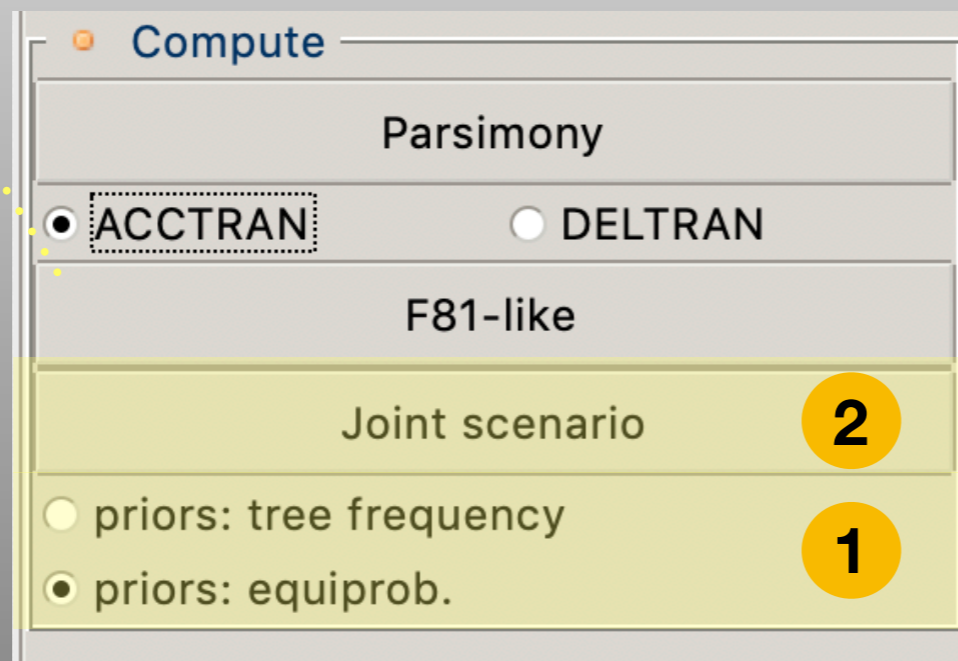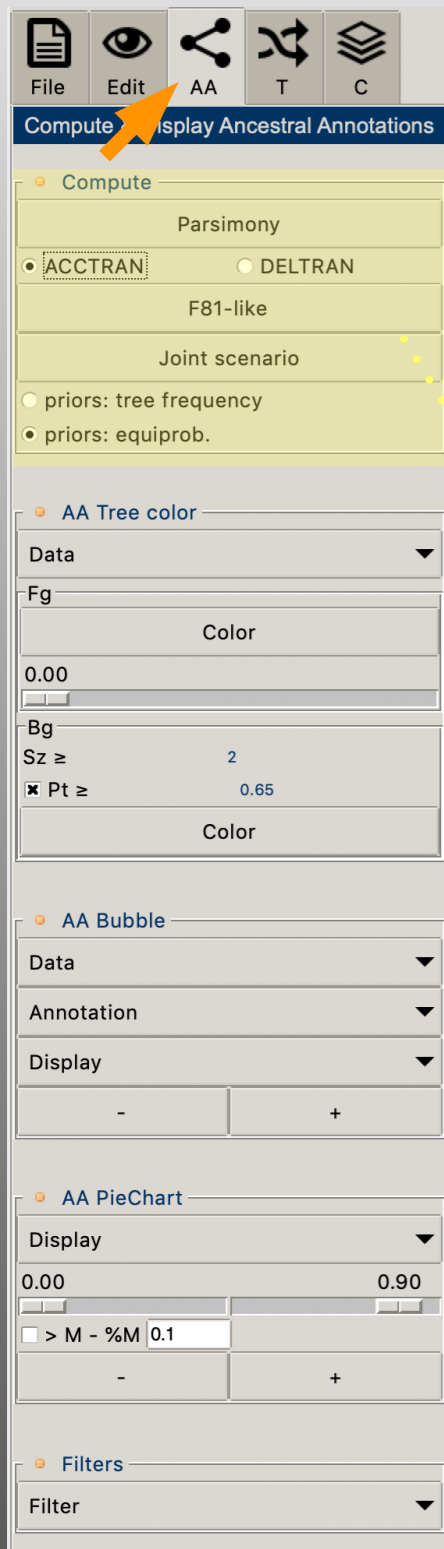
1 Select the priors option

2 Click the « Max.Lik. Joint (Pupko) » button, the ancestral annotations will be computed for the current tree (map)

AA toolbox: displaying ancestral annotations

Foreground color the tree
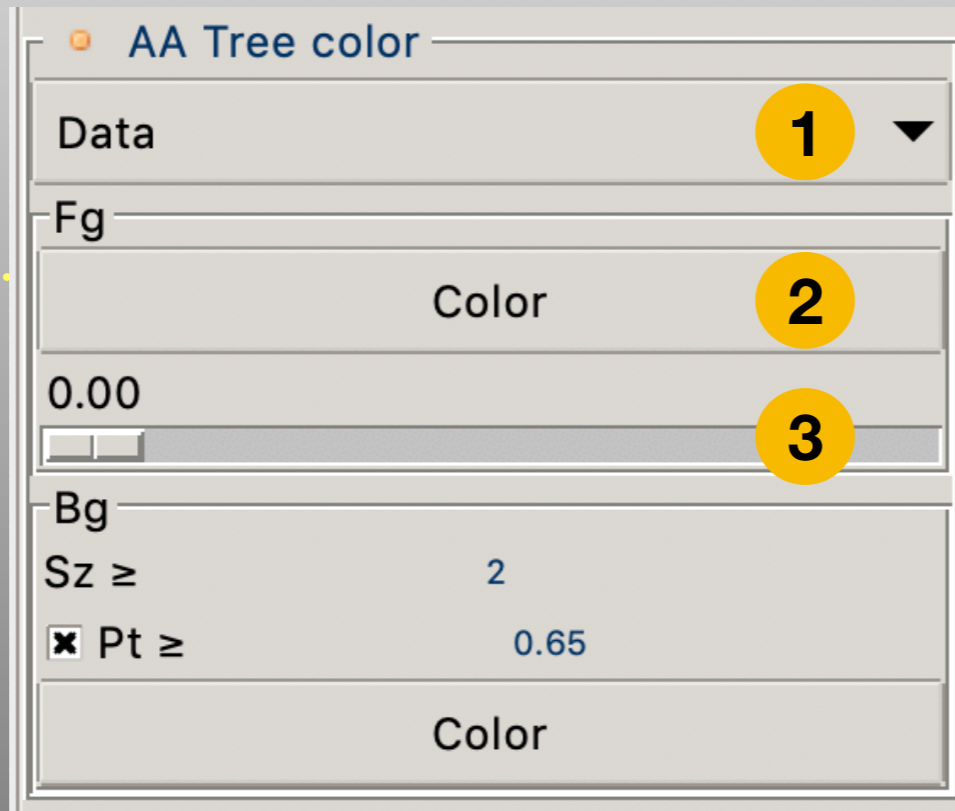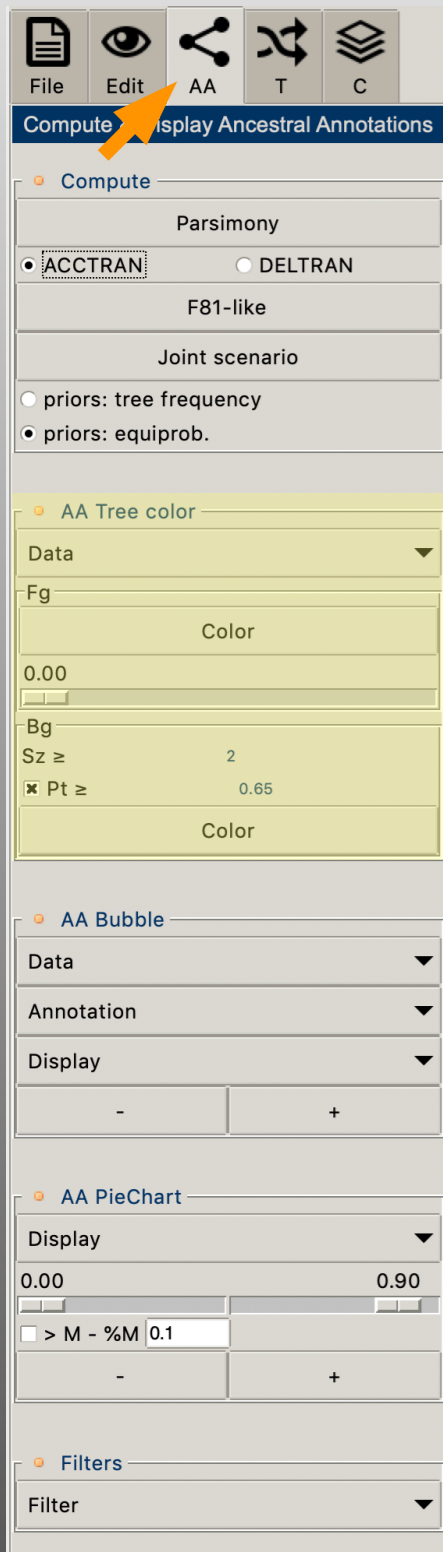
1) select the family of ancestral annotation to use for highlighting the tree : parsimony, majority of marginal maximum likelihood (or Bayesian), joint maximum likelihood
2) Clic the « Color » button to color the tree
3) Adjust the threshold (only for ancestral annotation with likelihood values), the ancestral annotations with a likelihood value under this threshold value will be black color coded.
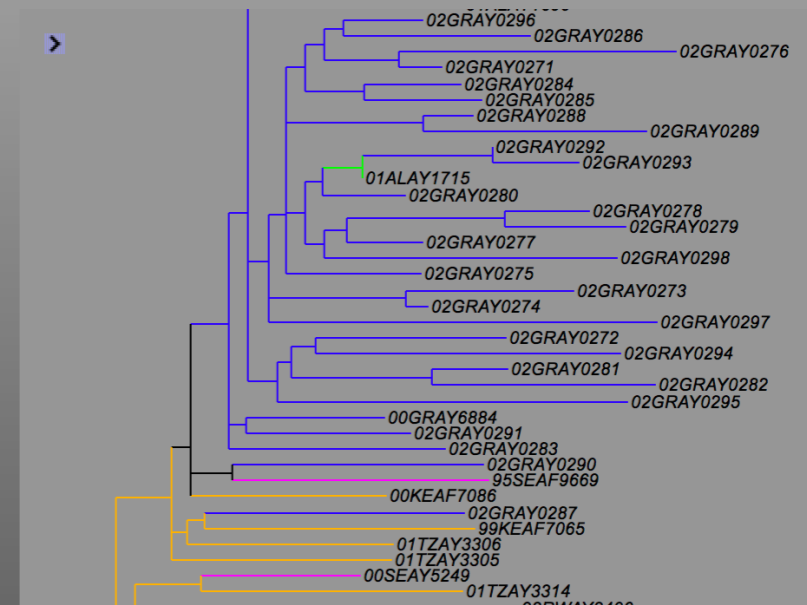
Note: to reset the tree color, see the « Reset » menu , tab « Edit »

AA toolbox: displaying ancestral annotations

Background color the tree
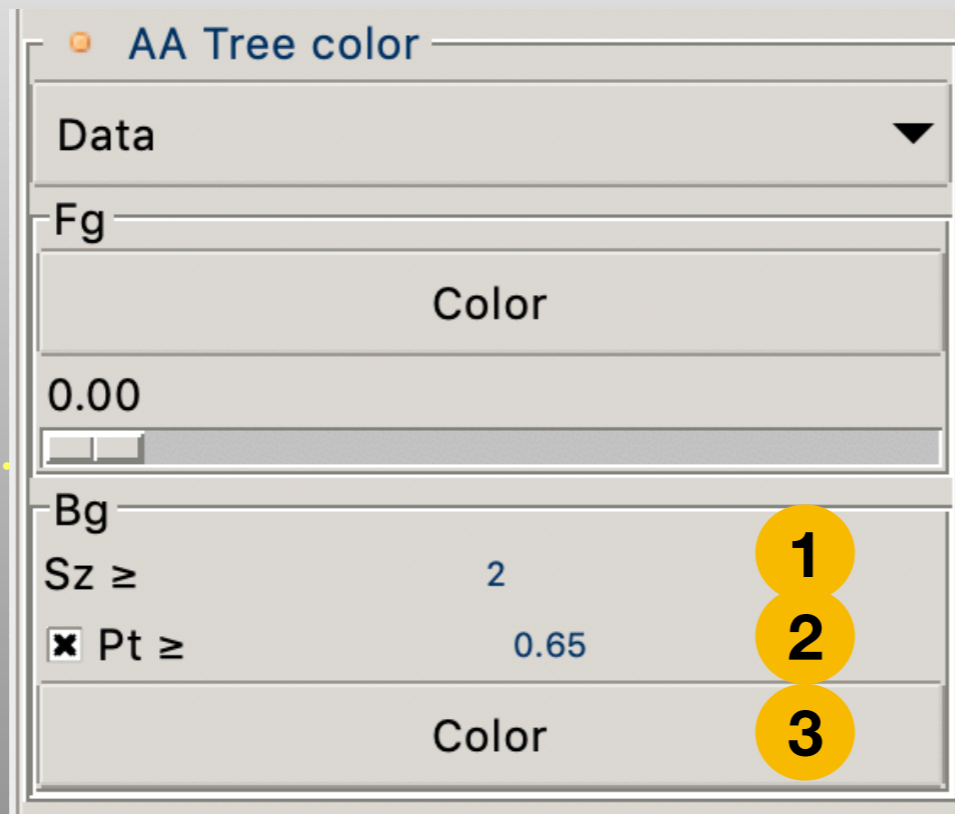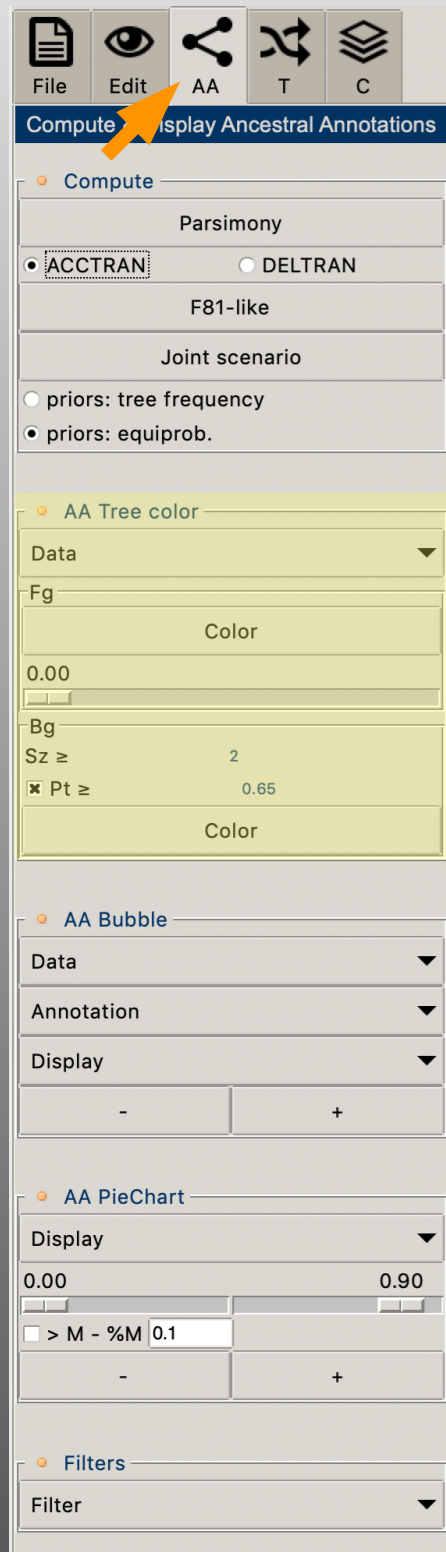
1) set a threshold value for the Sz criteria (see below)
2) set a threshold value for the Pt criteria. Pt is a probably threshold below which the node will be considered to be not annotated by an annotation

Sz = 3
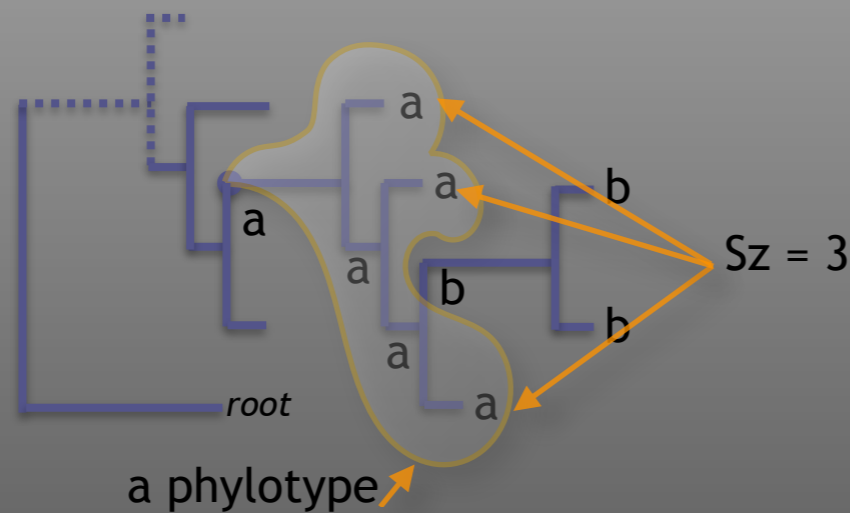
a phylotype

For more details related to phylotype, see
Searching for Virus Phylotypes
Chevenet F, Jung M, Peeters M, de Oliveira T, Gascuel O.
Bioinformatics (2013) Volume 29, Issue 5Pp. 561-570.
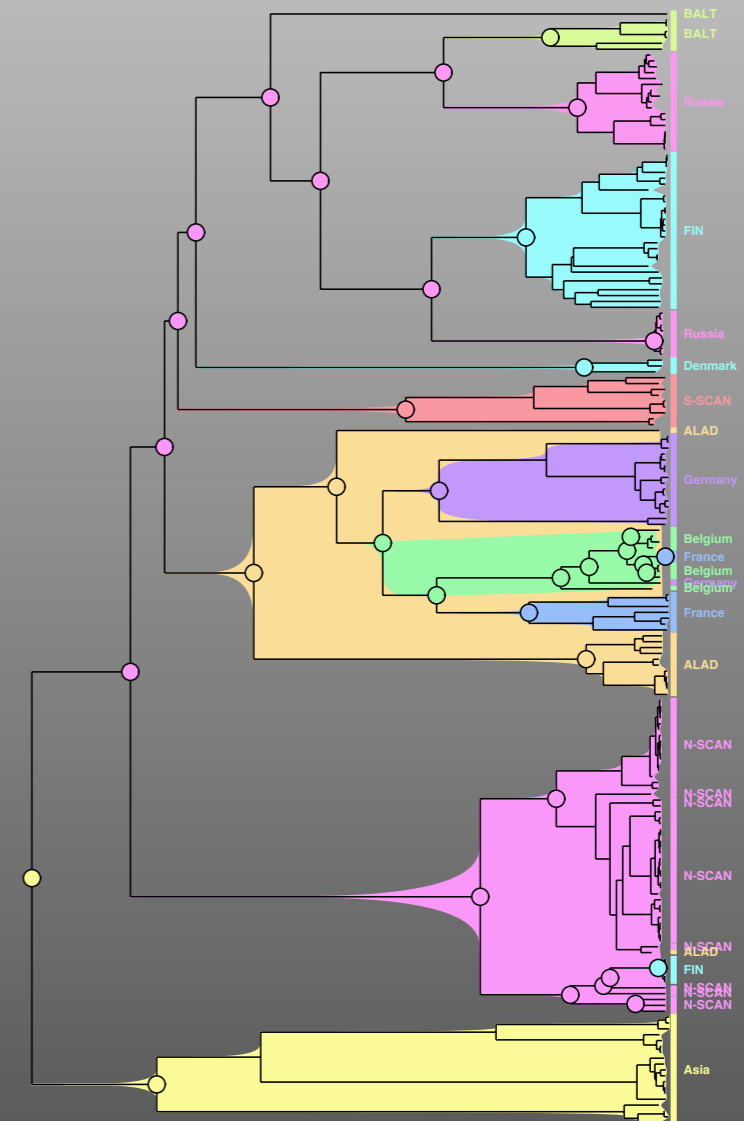
AA toolbox: displaying ancestral annotations

Add « bubbles » according to ancestral annotations
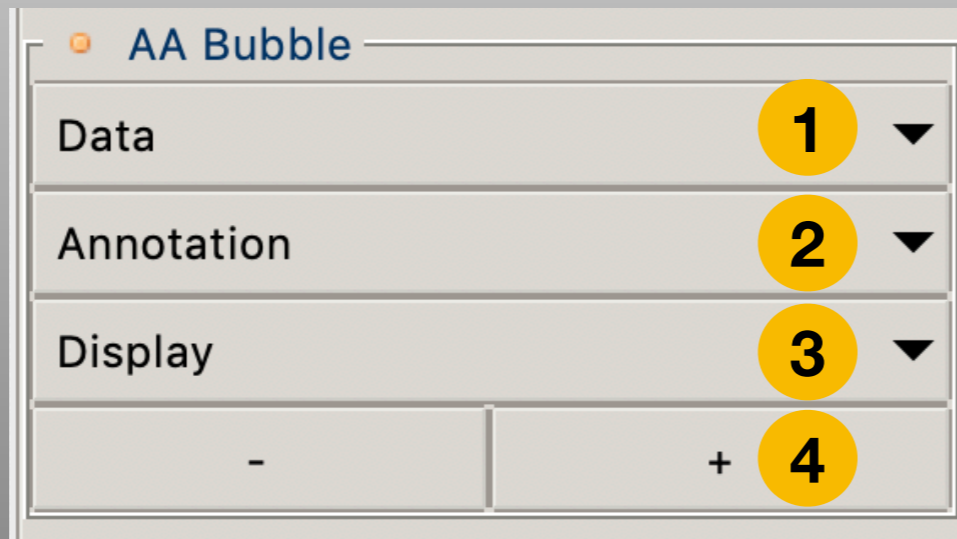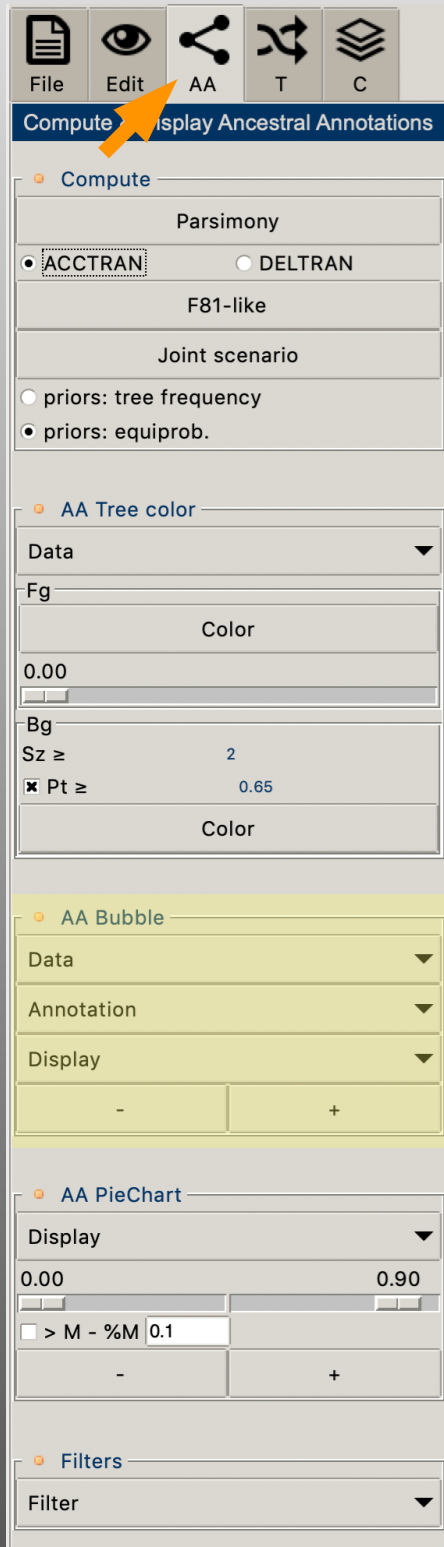
1) select the family of ancestral annotation (eg parsimony, maximum likelihood, etc.)
2) select the annotation(s) to consider
3) the « Display » menu enables **a)** to display (ON) or not (OFF) the « bubbles », b**)** to display a simplified view. With the simplified view, if a cluster of strains share the same annotations, only one bubble is displayed at its root
4) Adjust the size of the bubbles with the « - » or « + » buttons
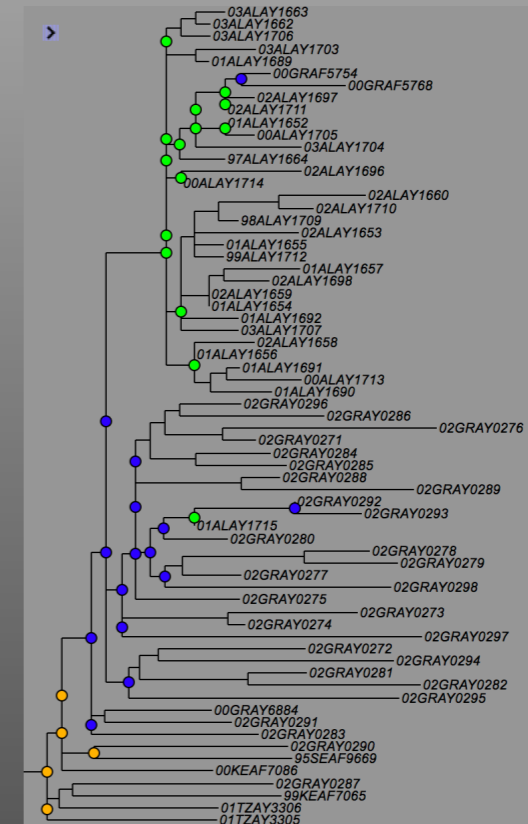
AA toolbox: displaying ancestral annotations

Add « piecharts » according to ancestral annotations

1 « Display » menu: display (ON) or not (OFF) the piecharts

2 display a simplified view by using two thresholds. The first threshold (left) set a value for probabilities of annotations below which the annotation will be aggregated to the « black » portion of the piechart. Like this, piecharts will be restricted to higher probabilities. The second threshold (right) set a value above which, piecharts will not be displayed. These thresholds are dynamic (madifying their values will automatically updates the tree display)

3 filter, help to display piecharts for nodes with contentious annotations

4 Adjust the size of the piecharts with the « - » or « + » buttons

M-%M : if two or more annotations are near the majority then the piechart is displayed. Here « near » mean a probability higher than the probability of the majority minus a percentage of it. The percentage is a user setting

AA toolbox: filters

Indentify nodes with particular characteristics regarding their ancestral annotations

File Edit AA T C

Compute / Display Ancestral Annotations

Compute

Parsimony

○ ACCTRAN   ○ DELTRAN

F81-like

Joint scenario

○ priors: tree frequency
● priors: equiprob.

AA Tree color

Data

Fg

Color

0.00

Bg
Sz ≥   2
☒ Pt ≥   0.65

Color

AA Bubble

Data

Annotation

Display

−   +

AA PieChart

Display

0.00   0.90

☐ > M − %M 0.1

−   +

Filters

Filter

Filters

Filter

> M − ∂ **2**

Majo ≠ Pupk.

A(n) ≠ A(father) (majo.)

A(n) ≠ A(father) (pupko)

Symbol **1**

Reset

1 Set the symbol used to highlight nodes

2 Select a filter

Filter symbol

Type

● Rectangle
○ Oval
○ Text
○ Arrow

Size/Position & Color

10

10

Color

In this example, ancestral annotations have been computed (maximum likelihood F81, marginal). The « > M−∂/2 » filter is applied, it highlights nodes (green rectangles) having one or more ancestral annotation(s) having a probability near the majority one. First, the standard variation (∂) of all the probabilities of the ancestral annotations presented at a node is computed. Then if a node has at least one ancestral annotation with a probability superior to the probability of the majority one minus half the standard variation, then the node is highlight

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« T » toolbox, transitions

highlight tree transition paths knowing a sequence of annotations

transition map = a summarized view of all the transitions in the tree

crossing matrices of annotation with computation of several indices

« T » toolbox, transition query

How to highlight tree transition paths knowing a sequence of annotations

Transitions Query

Africa Greece Albania

Insert ②

Data ①

Search ③

|< < @ > >|

Edit

Query string, enter manually key words here and/or select annotation from the insert menu. The query string may includes generic characters such as *

Clic the « Search » button to highlight paths corresponding to the evolutionary scenario depicted in the query string. Exemple of a query (HIV-1A majority on ML marginal): Africa then Greece then Albania

✓ Majority
Pupko
Parcimony

Select the data to be used

Clear
*
Africa
Albania
EastEurope
Greece
WestEurope

The select menu enables the selection of annotations to add to the query string

Africa Greece Albania
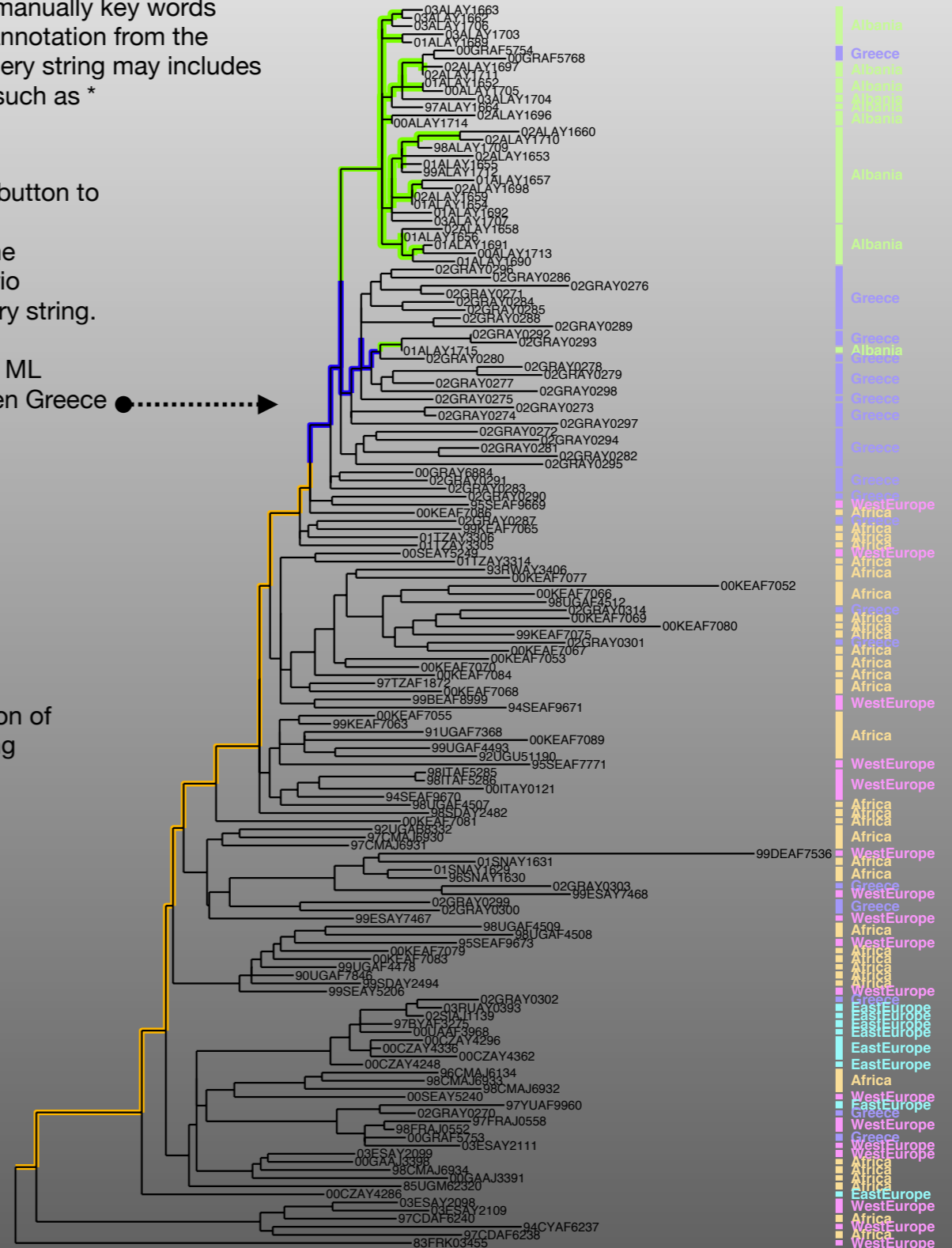
Reset history
Reset highlight
Load transitions queries...
Save history...

Alternate

Query history, saving/loading queries

Navigate the query history by using these buttons

« T » toolbox, transition map

transition map = a synthetic view of all the transitions in the tree

Phylogenetic tree with AA

Transition map

Select the data to be used

Radial
✓ Slanted
Rectangular

Generation
✓ Branch lengths

Bubble
✓ Piecharts

✓ Tips % (annotation)
✓ Annotation (3 char.)
✓ Arrow (on/off)

Radial
✓ Slanted
Rectangular

✓ Number of transitions(s) - Edge size
✓ Number of transitions(s) - Text
✓ Annotation (3 char.)
✓ Arrow (on/off)

Clic these buttons to adjust the map layout:
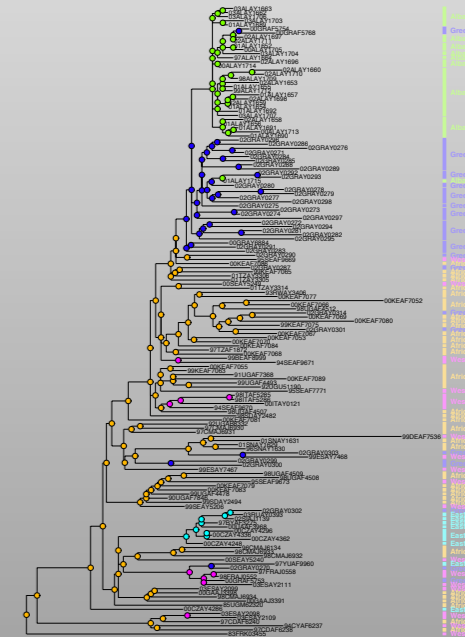
X-, X+ abscissa scale factor

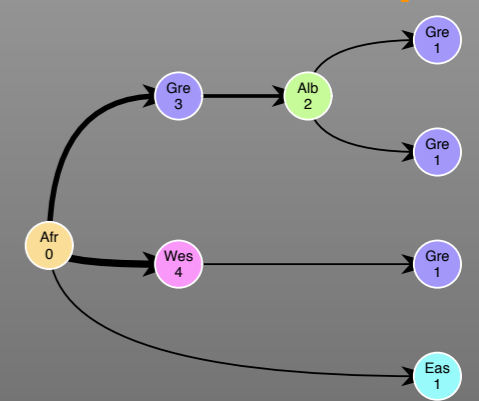Y-, Y+ ordinate scale factor

O-, O+ bubble scale factor

F-, F+ font size

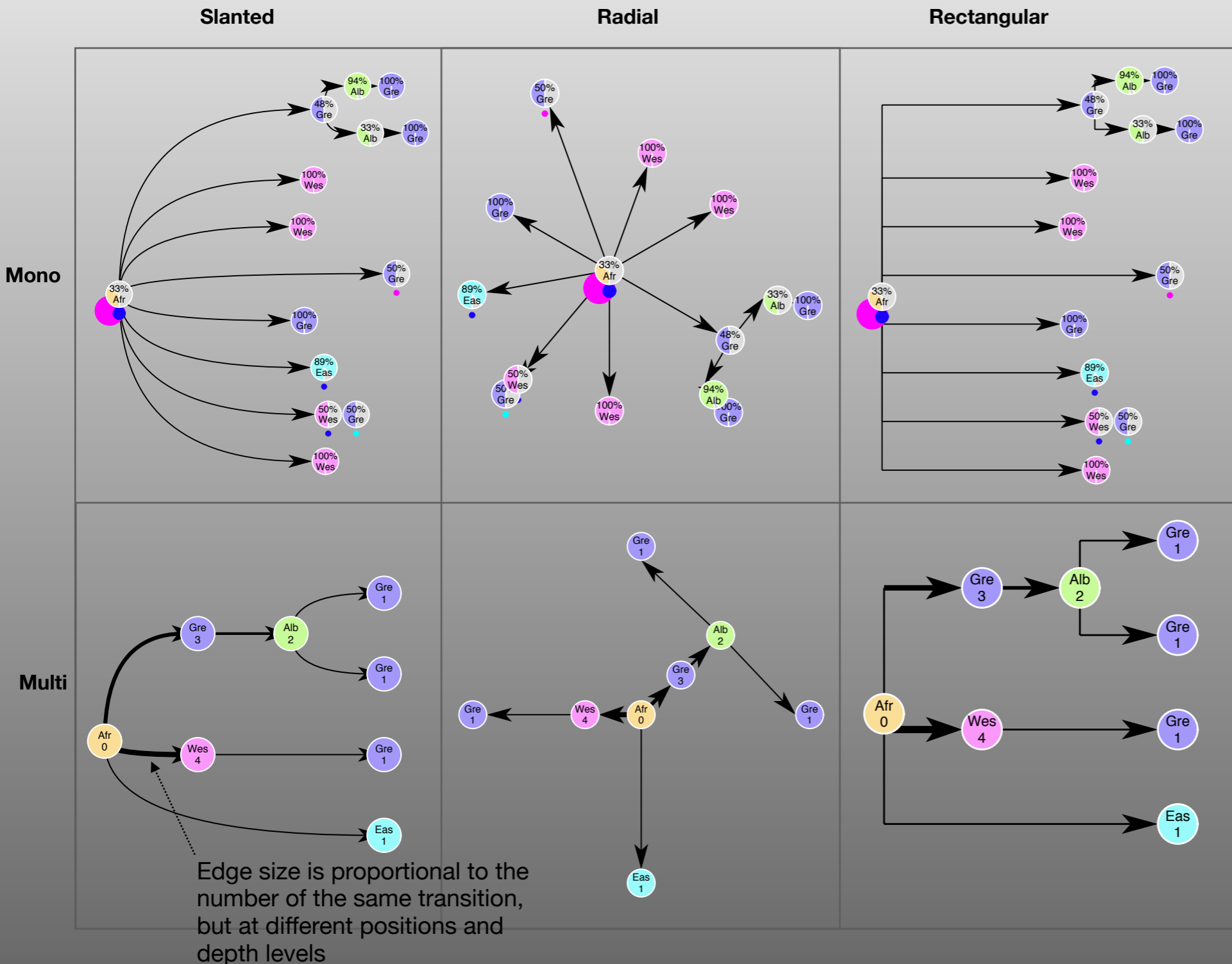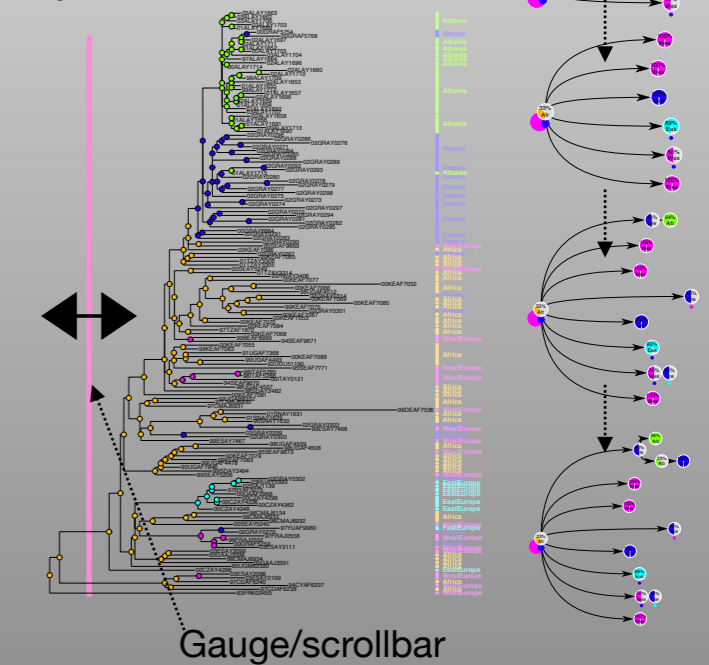A transition map can be saved under the standard Newick format

« T » toolbox, transition map

Transition map layouts

Mono layout count every transitions in the tree, edges of the map can be proportional to the branch lengths of the tree. Piecharts with a percentage display the proportion of subsequents strains annotated with the same ancestral annotation. Mono map can be computed « on the fly » knowing a depth level selected by the user (drag a gauge)

Slanted / Radial / Rectangular — Mono / Multi layouts

Edge size is proportional to the number of the same transition, but at different positions and depth levels

Dynamic mode

Gauge/scrollbar

Multi layout are more synthetic views. Node can display the number of the same transition (but at a different position and/or a different depth level in the tree)

Pointing a node from a transition map highlight the corresponding subparts of the tree

« T » toolbox, matrices of transitions

Matrices of transitions are matrices crossing annotations and giving several indices between couple of annotations
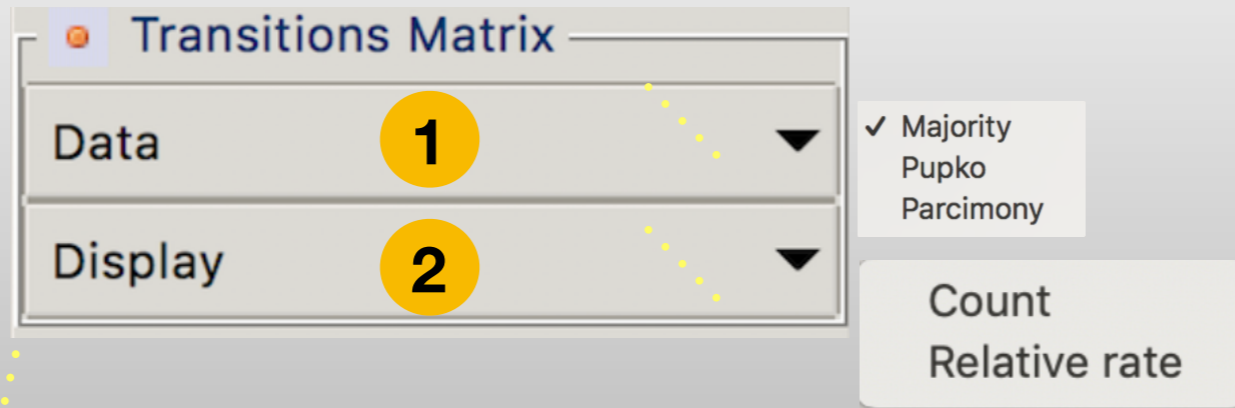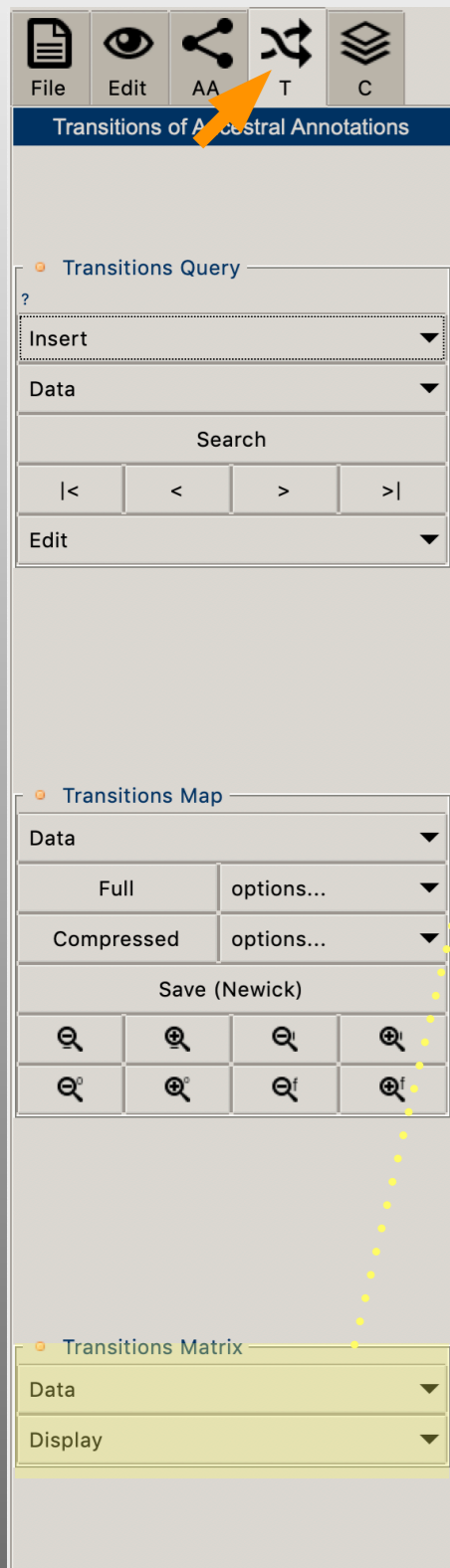
1 Select the family of data to be used to compute the matrices of transitions

2 Select the transition matrix to compute, it will be displayed in the current view

- The matrix « Count » counts the number of transitions from the annotation « a » to the annotation « b ».

- The matrix « Relative rate » count-based estimation of the relative transition rate, where raw counts are normalized and divided by state priors :

$$\partial ab \;=\; \frac{N\,a{\to}b}{N\,a.\; *\; \Pi b}$$
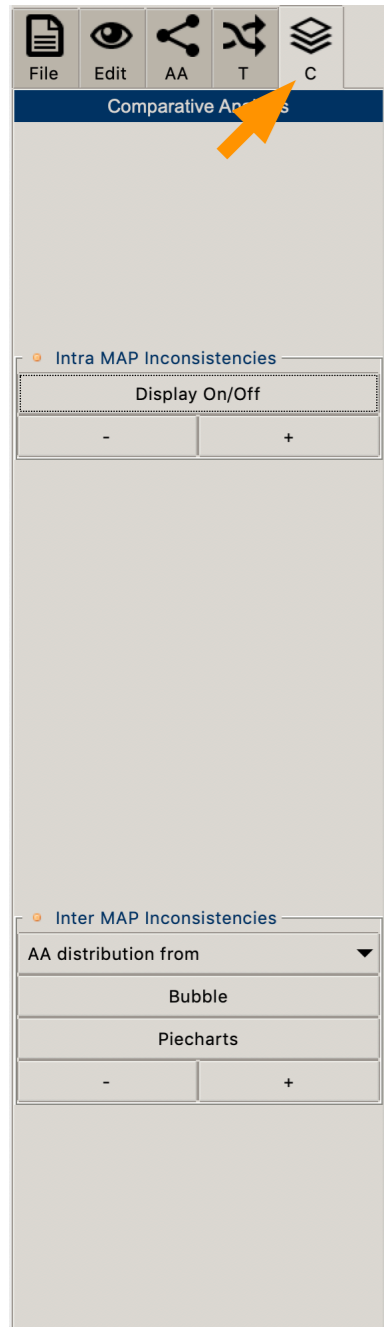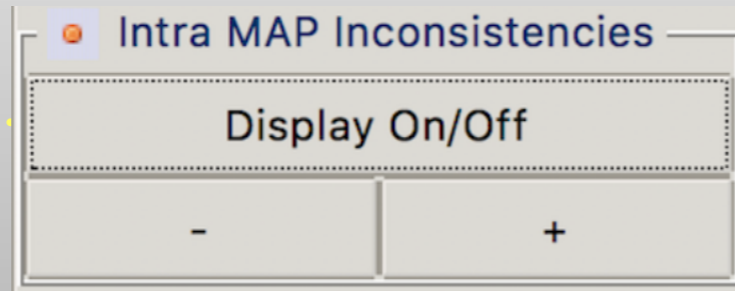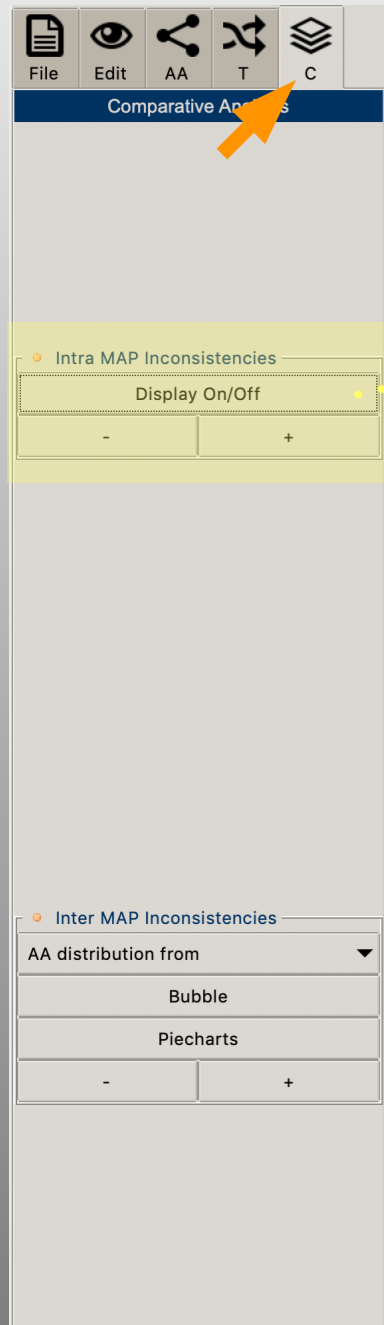
« C » toolbox

Graphical comparison of ancestral annotations from a map

**Graphical comparison of ancestral annotations from several maps**

« C » toolbox

Intra Map comparison

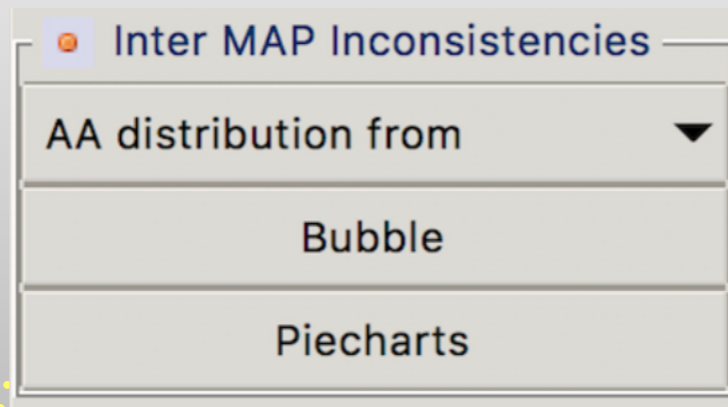### Intra MAP Inconsistencies

Display On/Off

Adjust the size of the bubbles with the « - » or « + » buttons

In this example, the primary annotations have been used to compute (PastView) three sets of ancestral annotations: ML marginal majority, ML joint and Parsimony (DELTRANS). If the ancestral annotations are the same between the three data sets for a node, the tree is foreground color-coded according to the color associated to the annotation. If not, colored bubbles are drawn showing the inconsistencies between the methods (the white/grey color indicates ambiguities for the parsimony method)
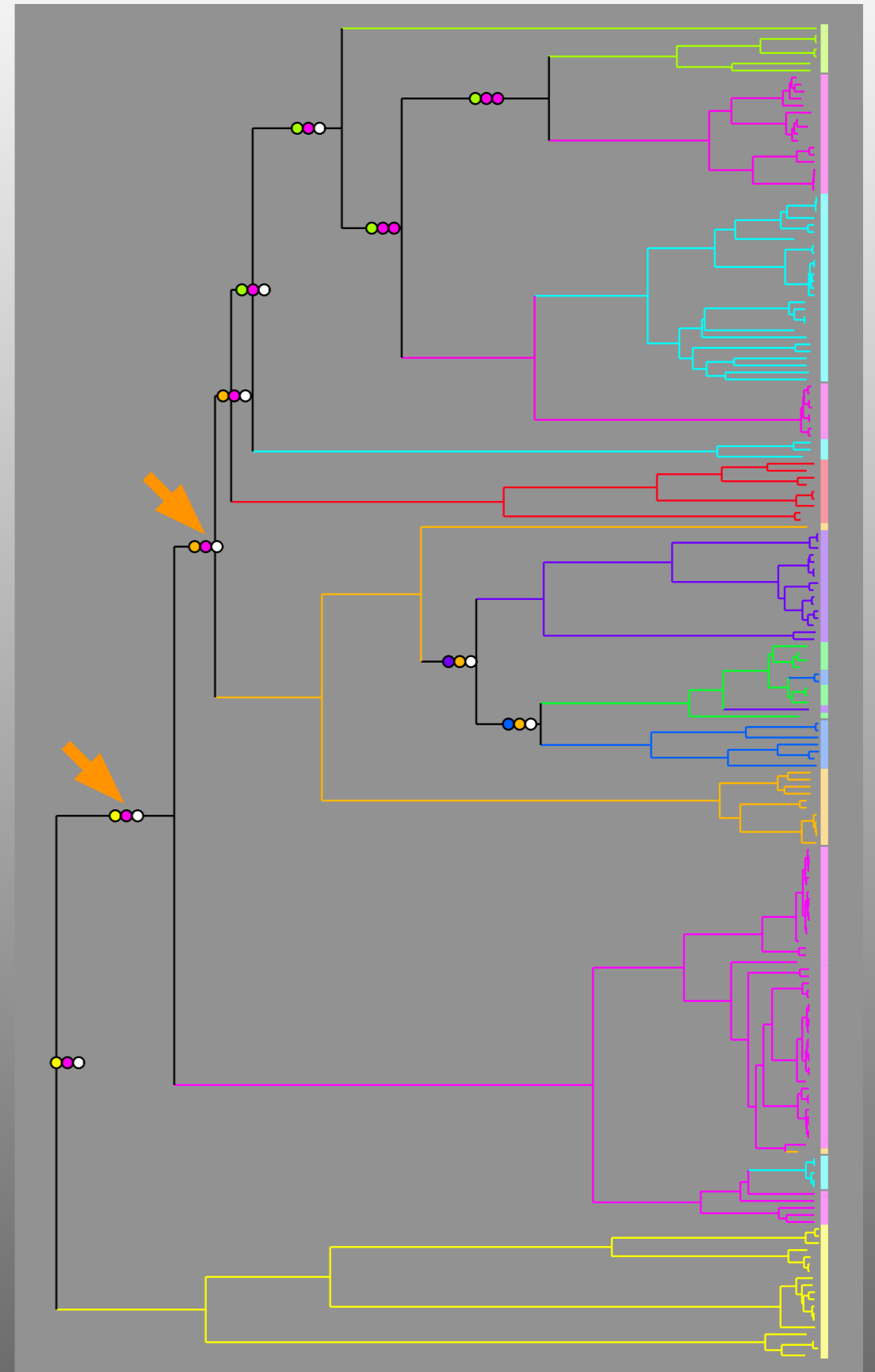
« C » toolbox
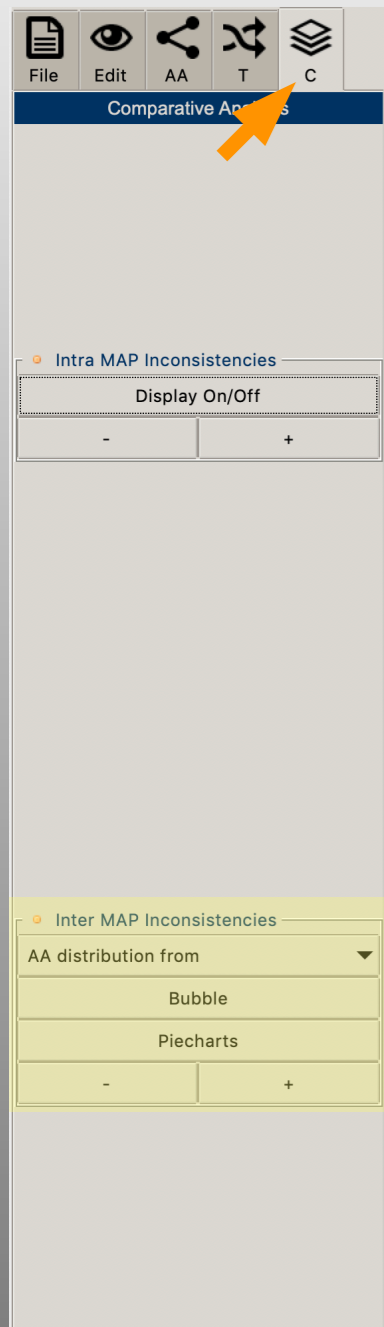

Adjust the size of the bubbles with the « - » or « + » buttons

In this example, the primary annotations have been used to compute ML marginal and Bayesian ancestral annotations (import from a NEXUS output). For each node, If the ancestral annotations are the same (the majority ones) between the two methods, the tree is foreground color-coded according to the color associated to the annotation. If not, piecharts are drawn showing the inconsistencies.
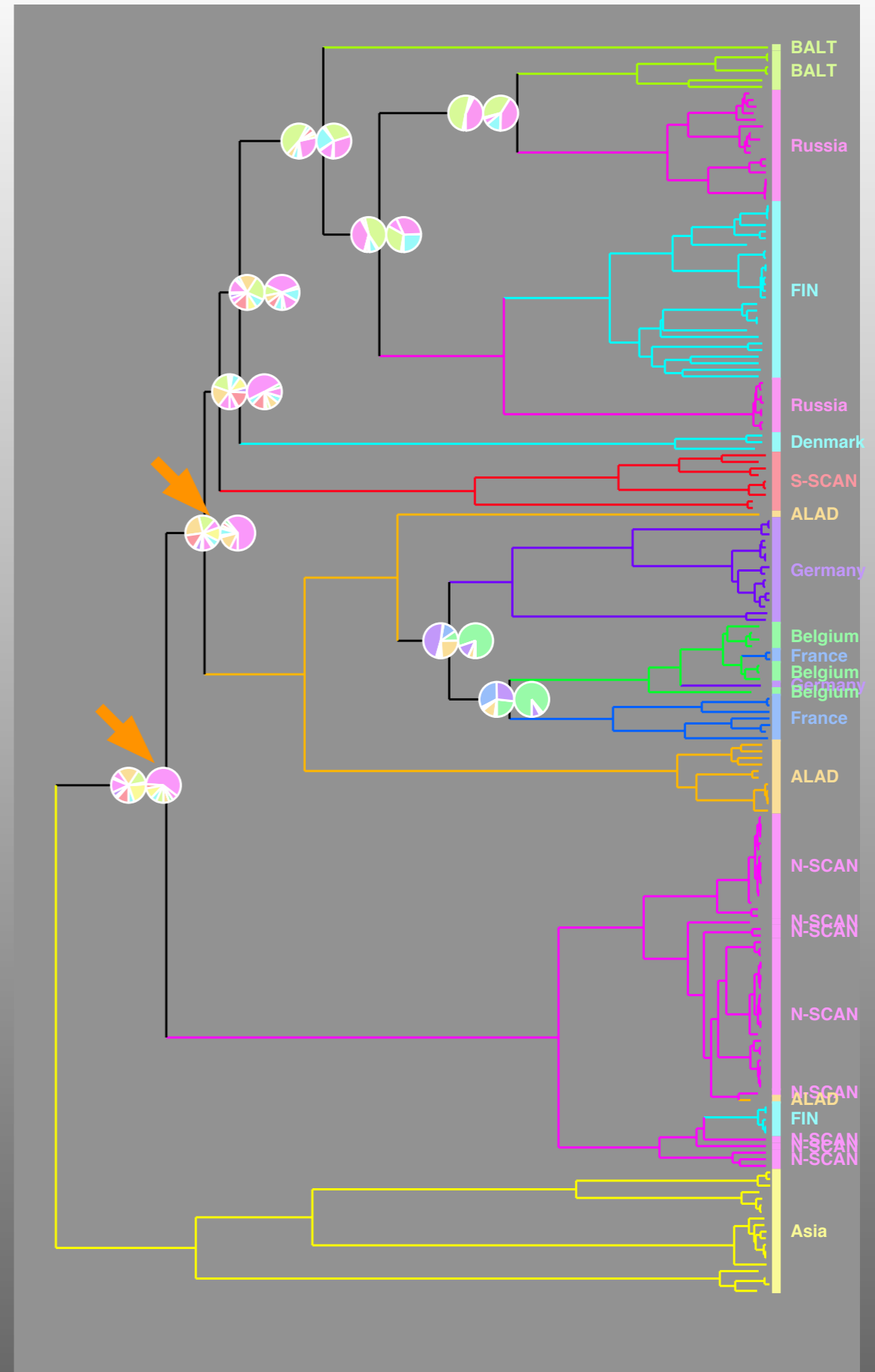
Appendix

Computing the marginal posterior probabilities of every state for each of the tree nodes (F-81-like) and the joint ancestral scenario with the maximal posterior probability

Computing the marginal posteriors probabilities (F81-like)

Probability of changes along a branch of length t is expressed as:

$PC(i{\rightarrow}j/l)=(1-e^{-\mu l})\pi_{j \text{ if } j \neq i}$

$= e^{-\mu l} + (1-e^{-\mu l})\pi_{i \text{ otherwise}}$

With πi equilibrium frequency (priors)

user supplied, roughly estimated from the state frequencies observed at the tree tips or equal probability

with $\mu=1/(1-\sum_{i}\pi_{i}^{2})$ (normalization factor)

Computing the marginal posteriors probabilities is done in four steps:

1 tree rescaling. maximize the tree likelihood to find the scaling factor with iterative calls to a Pruning (bottom-up) with a gold number or Brent search. The tree is then rescaling with the found scaling factor

2 bottom-up computation of 'down' conditional likelihoods (post-order recursion using the pruning algorithm

3 top-down computation of 'up' conditional likelihoods (pre-order recursion)

4 computation of the states marginal posterior probabilities knowing the 'down' and 'up' conditional likelihoods

Computing the marginal posteriors probabilities (F81-like)

1 tree rescaling. maximize the tree likelihood to find the scaling factor with iterative calls to a Pruning (bottom-up) with a gold number or Brent search. The tree is then rescaling with the found scaling factor. G, D = Left and Right descendant respectively

$$L(T)=\sum_i \pi_i \; Ldown(T/i)$$

$$Ldown(T/i) = \left[\; \sum_j PC(i \to j/l_g)Ldown(G/j) \; \right] * \left[\; \sum_j PC(i \to j/l_d)Ldown(D/j) \; \right]$$

$Ldown(F/i) = 1$ if the tip F is annotated with i, 0 otherwise

2 bottom-up computation of 'down' conditional likelihoods (post-order recursion using the pruning algorithm

3 top-down computation of 'up' conditional likelihoods (pre-order recursion)

The 'up' likelihood of G is calculated from the 'down' likelihood of D and the 'up' likelihood of their common father, and vice versa.

$$Lup(G/i) = \left[\; \sum_j PC(i \to j/l_g)Lup(T/j) \left[\; \sum_k PC(j \to k/l_d)Ldown(D/k) \; \right] \; \right]$$

$$Lup(D/i) = \left[\; \sum_j PC(i \to j/l_d)Lup(T/j) \left[\; \sum_k PC(j \to k/l_g)Ldown(G/k) \; \right] \; \right]$$

$Lup(T/j) = 1$ si T is the whole tree

4 computation of the states marginal posterior probabilities knowing the 'down' and 'up' conditional likelihoods

$$Post(N,i)=\pi_i \; Ldown(N/i) \; Lup(N/i) \; / \; L(T)$$

$$L(T)=\sum_i \pi_i \; Ldown(N/i) \; Lup(N/i)$$

$$L(T)=\sum_{i}\pi_i\, Ldown(T/i)$$

$$Ldown(T/i) = \left[\ \sum_{j}PC(i{\to}j/l_g)Ldown(G/j)\ \right] * \left[\ \sum_{j}PC(i{\to}j/l_d)Ldown(D/j)\ \right]$$

$Ldown(F/i)$ = 1 if the tip F is annotated with i, 0 otherwise

L = πA * LnA + πB * LnB

States A and B
G left descendant
D right descendant

```
Ln A
Ln B
```
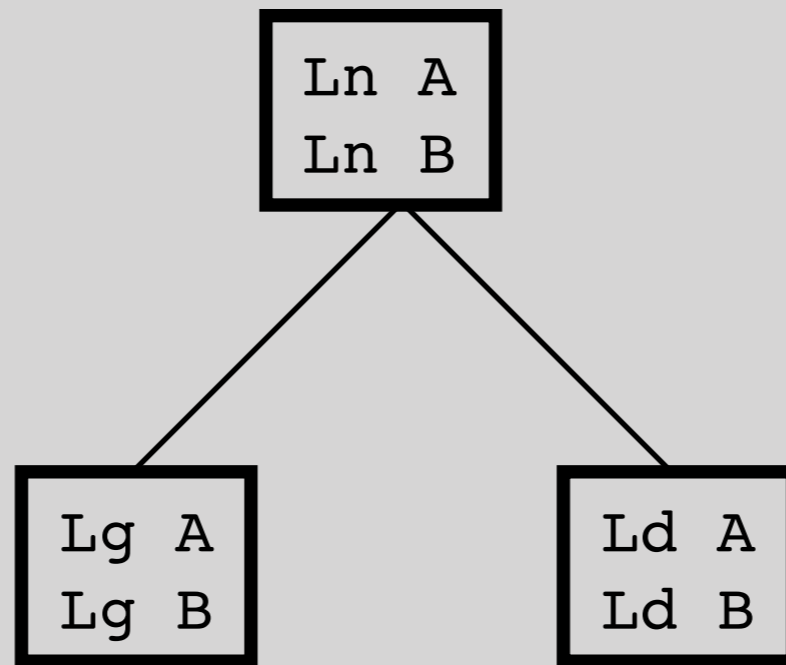
Conditional Likelihood
Knowing A, Left
descendant

```
Lg A          Ld A
Lg B          Ld B
```

« post order »

LnA = (LgA*PC(A->A)+LgB*PC(A->B))*(LdA*PC(A->A)+LdB*PC(A->B)

LnB = (LgA*PC(B->A)+LgB*PC(B->B))*(LdA*PC(B->A)+LdB*PC(B->B)

Computing the joint scenario, example (Yang, 1995) (Pupko, 2000)

Tree scaling, then : Up + Down

Up
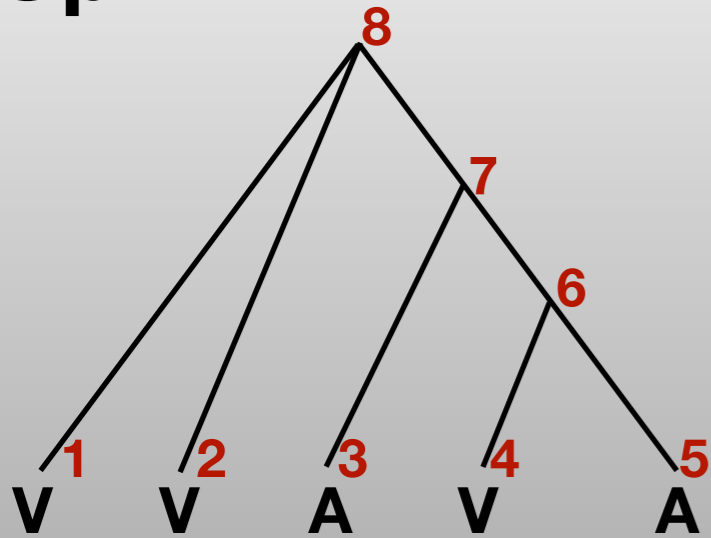
Priors

$\pi_A = 0.4$ (2/5)

$\pi_V = 0.6$ (3/5)

Probabilities of change

PC	To A	V
From		
A	0.7	0.3
V	0.45	0.55

1
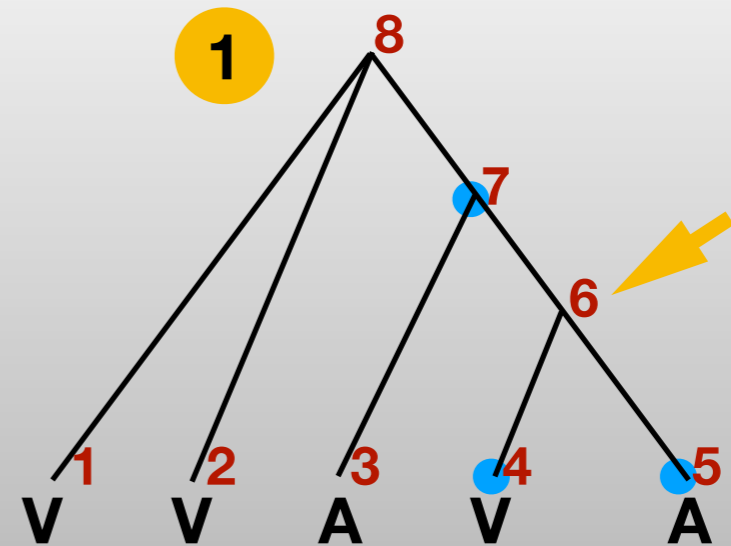
If 7 is A
  If 6 is A L6= 0.7x0.3x0.7= 0.147 ➡ A
  If 6 is V L6= 0.3x0.55x0.45= 0.07425
If 7 is V
  If 6 is A L6= 0.45x0.3x0.7= 0.0945
  If 6 is V L6= 0.55x0.55x0.45= 0.1361 ➡ V

2
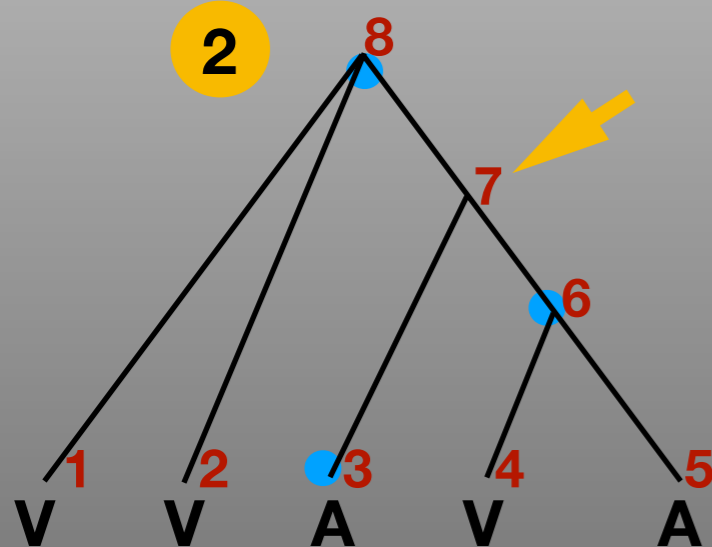
If 8 is A
  If 7 is A L7= 0.7x0.7x0.147= 0.072 ➡ A
  If 7 is V L7= 0.3x0.7x0.1361= 0.02856
If 8 is V
  If 7 is A L7= 0.45x0.7x0.147= 0.0463 ➡ A
  If 7 is V L7= 0.55x0.45x0.1361= 0.03366

3
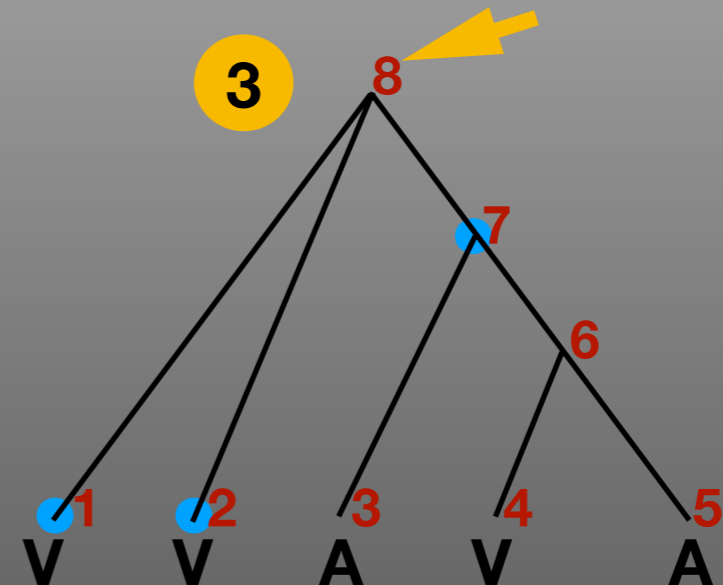
If 8 is A L8 = 0.3x0.3x0.072x0.4=0.0026
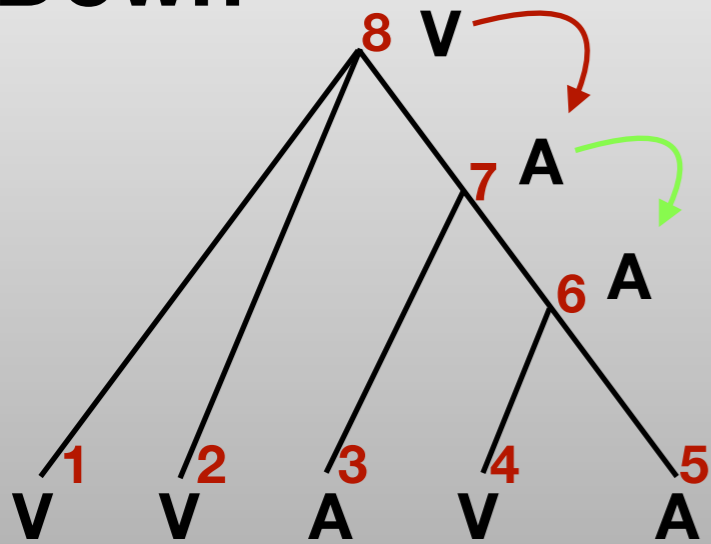If 8 is V L8 = 0.55x0.55x0.0463x0.6=0.0084 ➡ V

Computing the joint scenario, example   (Yang, 1995) (Pupko, 2000)

Tree scaling, then : Up + Down

Down

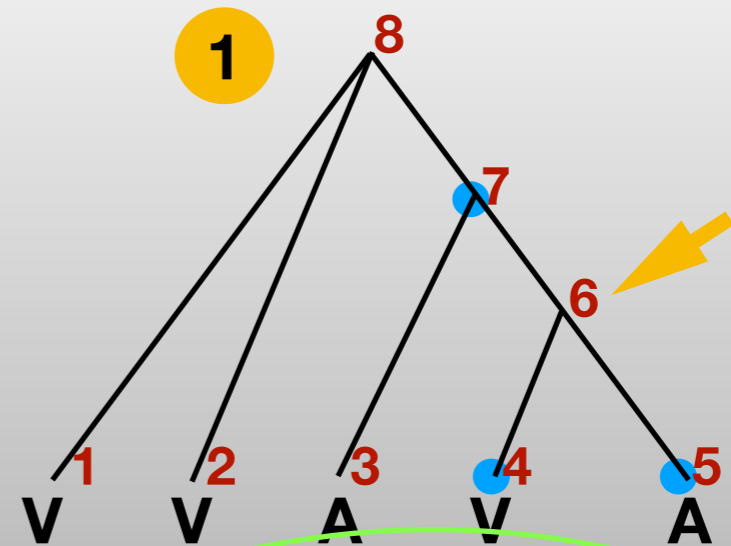| $\pi_A =$	0.4 (2/5)
$\pi_V =$	0.6 (3/5)

PC De	Vers A	V
A	0.7	0.3
V	0.45	0.55

1

If 7 is A
  If 6 is A L6= 0.7x0.3x0.7= 0.147
  If 6 is V L6= 0.3x0.55x0.45= 0.07425

If 7 is V

  If 6 is A L6= 0.45x0.3x0.7= 0.0945
  If 6 is V L6= 0.55x0.55x0.45= 0.1361

2

If 8 is A
  If 7 is A L7= 0.7x0.7x0.147= 0.072
  If 7 is V L7= 0.3x0.7x0.1361= 0.02856

If 8 is V

  If 7 is A L7= 0.45x0.7x0.147= 0.0463
  If 7 is V L7= 0.55x0.45x0.1361= 0.03366

3

If 8 is A L8 = 0.3x0.3x0.072x0.4=0.0026
If 8 is V L8 = 0.55x0.55x0.0463x0.6=0.0084

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