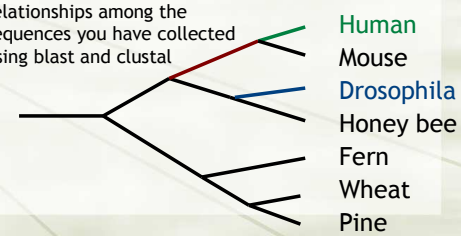
# Phylogenetics

BIOL3004 electives

## Evolution

- The aim is to reconstruct the evolutionary history and relationships among the sequences you have collected using blast and clustal




## Sequences evolve over time

- Changes include point mutations and insertions and deletions.
- Changes are subject to natural selection - the differences you observe are the combination several processes
  - Speciation creates branches - the sequences are now in two different species, and can evolve separately
  - Genes can be duplicated within the genome, and the two copies become distinct - eg specialised functions.
  - Genes can be lost from a lineage
  - Genes are sometimes transferred between lineages

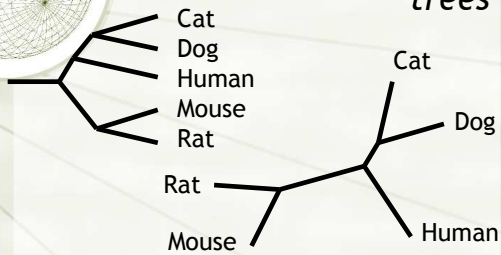
## Sequence evolution terminology

- Sequence **similarity** is a measure of identical or conserved amino acids
- Sequence **homology** indicates descent from a shared common ancestor
- Sequences are **orthologous** if they not only descend from a common ancestor, but have the same function, unaltered during that time
- Sequences are **paralogous** if they descend from a common ancestor, but have altered function or are the result of gene duplication.
- (rare) A **xenologue** is transferred from another species.

## Constructing a phylogenetic tree from a MSA

- There are three main classes of methods:
  - distance matrix methods (ClustalX, Phylip)
  - parsimony (Phylip)
  - maximum likelihood - too slow and complex for an introductory project
- Additionally:
  - testing reliability of tree (bootstrapping)
  - drawing and presenting trees (TreeView)

## Rooted and unrooted (radial) trees




The rooted tree includes information about time and origin

## Distance matrix methods

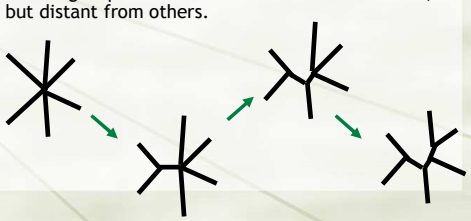
- The sequences in the MSA are compared to each other pairwise and it is determined how different they are to each other
- The pairwise distance matrix contains the results of all the comparisons.

| | | | | | |
|---|---|---|---|---|---|
| Human | 0 | 8 | 8 | 8 | 8 |
| Mouse | 8 | 0 | 3 | 9 | 9 |
| Rat | 8 | 3 | 0 | 8 | 8 |
| Dog | 8 | 9 | 8 | 0 | 2 |
| Cat | 8 | 9 | 8 | 2 | 0 |

```
Human   GGTTATCCTACATGTATA
Mouse   ACTTGTCCAACGCGGACA
Rat     ACTCGTCCAACGTGCACA
Dog     AGCTGCCTTACGTACATA
Cat     AGCTGTCTTACGTACGTA
```

## Distance matrix methods

- The evolutionary tree is then reconstructed based on the pairwise distances.
- ClustalX uses the "neighbor-joining" algorithm, choosing sequences that are similar to each other, but distant from others.



## Constructing a distance tree in ClustalX

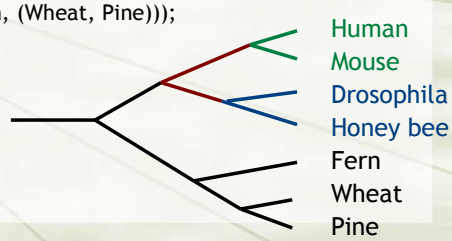
- Make sure the sequences in the window are aligned (either just recently aligned, or by importing a saved alignment).
- Go to the "Tree" menu.
- Turn on the "Correct for multiple substitutions" option (your sequences are unlikely to be similar enough not to need this)
- Run "Draw Tree"
- Output is filename.ph, a tree in New Hampshire (Newick) Format
- You can also try the other options and compare the trees you get.

## New Hampshire (Newick) notation

- Evolutionary trees are easiest to work with and understand as diagrams.
- However, not all evolutionary programs can draw trees and diagrams can't be transferred between programs.
- New Hampshire format is the format used for text description of trees and to allow easy movement between programs.
- It is useful to be familiar with this format and be able to translate between it and tree diagrams.

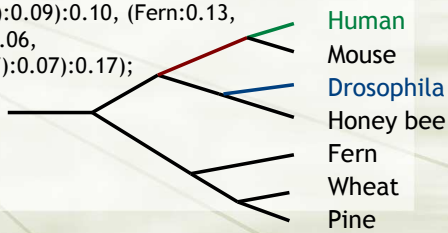
## New Hampshire (Newick) format for trees

((Human, Mouse), (Drosophila, Honey bee)), (Fern, (Wheat, Pine)));



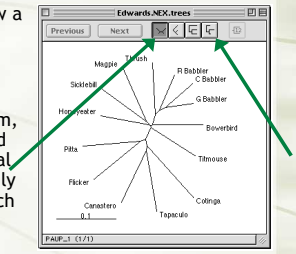

## New Hampshire (Newick) format with branch lengths

(((Human:0.05, Mouse:0.06) :0.14, (Drosophila:0.11, Honey bee:0.11):0.09):0.10, (Fern:0.13, (Wheat:0.06, Pine:0.07):0.07):0.17);



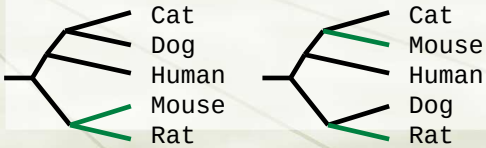
## TreeView

- Use TreeView to draw a tree in the New Hampshire format
- TreeView draws four types of trees: radial (unrooted), cladogram, square cladogram and phylogram. Use radial and phylogram, as only these two show branch lengths correctly.




## Parsimony analysis

- The principle of parsimony is to find the tree which has the smallest number of changes required to get the multiple sequence alignment.
- Eg a position where cat, dog and human have Glycine and mouse and rat have Threonine




## Parsimony in Phylip

- You need an output file from clustal for phylip input (.phy)
- The phylip program is called **protpars** (**dnapars**)
- The program calculates the parsimony score for a given tree, then tries other trees and sees if the score is improved.
- Parsimony (particularly for many sequences) is typically slower than distance methods.

```
Protein parsimony algorithm, version 3.6

Setting for this run:
  U                 Search for best tree?  Yes
  J   Randomize input order of sequences?  No. Use input order
  O                        Outgroup root?  No, use as outgroup species  1
  T              Use Threshold parsimony?  No, use ordinary parsimony
```

## Phylip in general

- Phylip doesn't have a fancy user-interface. In windows, you will get a plain blue window with some text options, firstly, to enter the name of the input data file (eg mydata.phy)
- When you run a phylip program, you will see a menu list of options, and you type in the letter on the left to change that option.
- When there are multiple options for a letter, keep entering that letter to cycle through the options.
- Type "y" once you have changed the required options to run the phylip program.

## Phylip menu

```
Protein parsimony algorithm, version 3.6

Setting for this run:
  U                 Search for best tree?  Yes
  J   Randomize input order of sequences?  No. Use input order
  O                        Outgroup root?  No, use as outgroup species 1
  T              Use Threshold parsimony?  No, use ordinary parsimony
  C                Use which genetic code?  Universal
  W                        Sites weighted?  No
  M           Analyze multiple data sets?  No
  I          Input sequences interleaved?  Yes
  0  Terminal type (IBM PC, ANSI, none)?  ANSI
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                 Print out tree         Yes
  4        Print out steps in each site    No
  5  Print sequences at all nodes of tree  No
  6       Write out trees onto tree file?  Yes

Are these settings correct? (type Y or the letter for one to change)
```

## Phylip output

- Phylip always outputs a file called **outfile** and sometimes an **outtree** from each program. This will overwrite and replace any previous files with the same name.
- After running any phylip program, immediately rename the outfile (and outtree) files to something unique, and useful; eg mydata.dst, mydata.nei, mydatatree.prs, mydatatree.nei
- These files are all text files, and you can read them using Wordpad or any other text editor.
- The **outtree** files are Newick format you can open in Treeview.

## Distance methods in Phylip

- You can also construct a neighbor-joining tree using phylip programs.
- The same (.phy) input file is used, but the program to run is **protdist** (**dnadist**). The **outfile** contains the distance matrix (intermediate step).
- This outfile becomes the infile to **neighbor**, which calculates the neighbour-joining tree.
- Phylip allows a number of options, such as the distance method used.

## Testing the quality of a tree

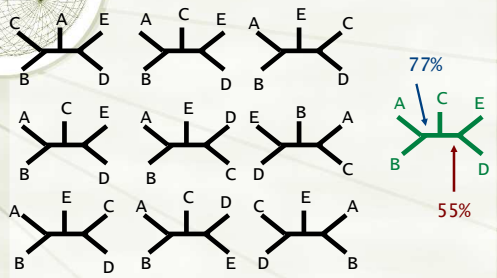
- You can now compare the distance matrix tree to the parsimony tree. They may not be the same, but are the differences important?
- Is there enough evidence in the MSA to support one branching pattern over another?
- You can "bootstrap" your distance tree to see how reliable different parts of the tree are


http://www.mouthmag.com/issues/58/number58.htm

## Bootstrapping

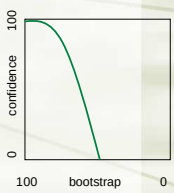
- From the original multiple sequence alignment, "pseudoreplicate" multiple sequence alignments are created by randomly selecting columns.
- Statistically, these pseudoreplicates are similar, but not identical, to the original
- For each pseudoreplicate, the tree is calculated.
- For each branch in the original tree, we count how many times the pseudoreplicate trees have the same branch.
- Note that we are repeating the complete analysis multiple times - this can be slow!

## Bootstrapped tree




## What do the bootstrap values mean?

- Bootstrap values for phylogenetic trees do not follow typical statistical behaviour
- Bootstrap value 95% : actually close to 100% confidence in that branch
- Bootstrap value 75% : often close to 95% confidence
- Bootstrap value 60% : much lower confidence
- Less than 50% bootstrap: no confidence in that branch over an alternative




## Bootstrapping in ClustalX

- First, calculate your tree as before ("Draw tree") producing filename.ph
- Then, select the "Bootstrap tree" option in the Tree menu. Try 100 bootstraps first. If that goes quickly, rerun with 1000 bootstraps.
- The output is called filename.phb, and is also a Newick tree, but it includes the bootstrap values.
- Be careful when bootstrapping. Make sure you are bootstrapping with the same options you drew the tree with, and that the tree is in filename.ph.
- Bootstrapping in Phylip: full details at http://foo.maths.uq.edu.au/twiki/bin/view/Know/PhylipBootstrapping

## *Treeview with bootstrap values*

- In ClustalW, Tree menu, Output tree format options, change "Phylip bootstrap position" from BRANCH to NODE.
- Bootstrap tree (filename.phb)
- Import filename.phb into Treeview, displaying as phylogram
- Select display option "Show internal edge labels"
- Ignore the "TRICHOTOMY" at the base of the tree, or edit filename.phb first, deleting it.

## *Examining the alignment for unusual regions*

- Expect there to be "families" or clusters of sequences with similar patterns from close species. These are often visible in the clustal alignment.
- However, some sequences may appear part of one family in one region of the alignment, and part of another in another part.
- To test the difference between regions, prepare smaller alignments containing the separate regions, and construct the distance tree with bootstrap values for each in ClustalX

## *References*

- Phylogenetics is a huge field, with a large number of techniques and software
- Methods are covered in more depth in MATH2210 and BIOL3014
- Commonly-used software: Phylip PAUP* MEGA
- Index to phylogenetic software at http://evolution.genetics.washington.edu/phylip/software.html
- Books: Felsenstein; Li & Graur; Nei & Kumar