Opinionated Lessons in Statistics

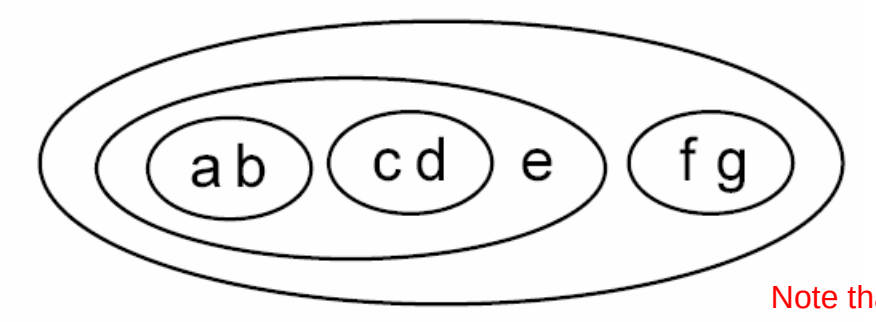
by Bill Press

#51 Hierarchical Classification

Classification by Phylogenetic Trees

This is more general than just biology. Useful any time that you want do discover or visualize "closeness" relationships.

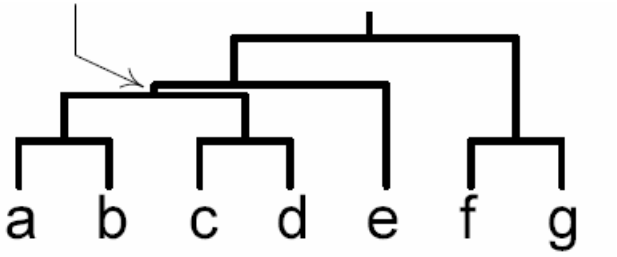
Can be qualitative (groupings, no distances):



(((ab)(cd)e)(fg))

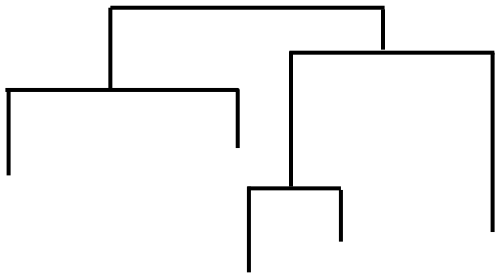
Note that binary tree is general case if allow zero distances

Or quantitative (a tree model for distances):



Note that the tree model is a much more restricted representation than a general distance matrix.

"additive tree"



N nodes
$2N-2$ branch lengths (prove by induction)

distance matrix

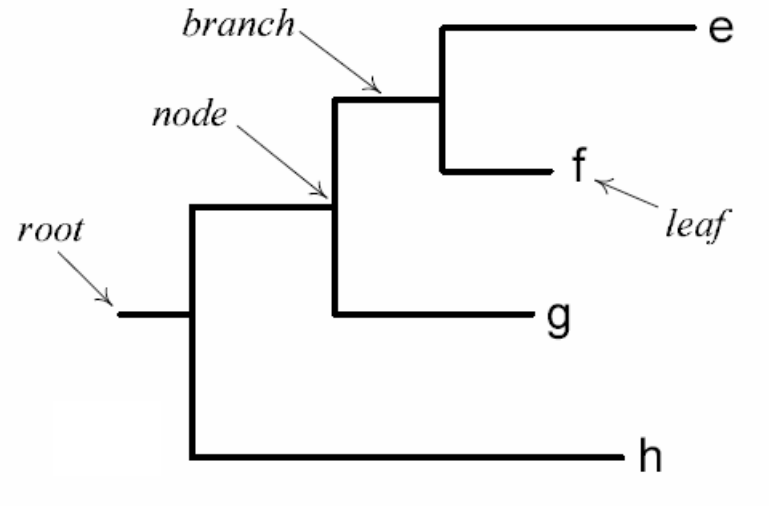
N nodes
$N(N-1)/2$ distances

$$\begin{pmatrix} 0 & d_{01} & d_{02} & d_{03} & \cdots \\ d_{01} & 0 & d_{12} & d_{13} & \cdots \\ d_{02} & d_{12} & 0 & d_{23} & \cdots \\ \cdots & & & & \end{pmatrix}$$

$$\begin{aligned} d_{ij} &\geq 0 && \text{(positivity)} \\ d_{ii} &= 0 && \text{(zero self-distance)} \\ d_{ij} &= d_{ji} && \text{(symmetry)} \\ d_{ik} &\leq d_{ij} + d_{jk} && \text{(triangle inequality)} \end{aligned}$$

Facts (some amazing) about additive trees

- Can get distance matrix from tree
  - not amazing
- Can test whether a distance matrix came from an additive tree
  - the four point test
  - slightly amazing, but $O(N^4)$
- There is an efficient $O(N^2)$ algorithm for actually constructing the tree
  - neighbor joining (NJ)
  - also reduces the four point test to $O(N^2)$
  - amazing!
- Applied to distance matrices that are not additive trees, NJ produces a good approximation to the closest additive tree
  - which is often itself a remarkably good approximation to the full distance matrix
  - really amazing since the exact problem is known to be NP
  - not completely understood!



Four point test:
For all distinct *i,j,k,l* there is at least one tie among the three sums of the form $d_{ij}+d_{kl}$

above: [eg]+[fh] = [eh]+[fg]
can you see why this always works?

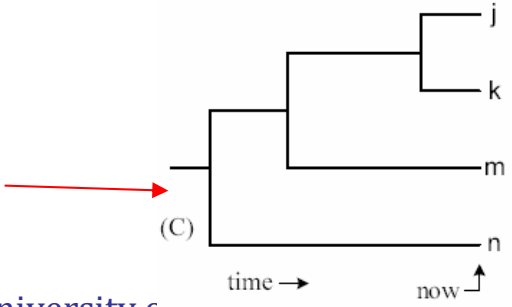
NJ is one of several "agglomerative methods"

- Initialize N active clusters with one leaf node in each
- Then repeat exactly N-2 times:
  - find the two active clusters that are closest by some prescribed[1] distance measure
  - create a new active cluster that combines the two
  - connect the new active cluster (parent) to the two (children)
    - specify branch lengths by some prescription[2]
  - delete the two children from the active list
  - compute by some prescription[1] distances from the new cluster to the other clusters

Different agglomerative methods differ only in the two[1,2] prescriptions.

NJ is an agglomerative method that is exact for additive trees.

WPGMA is an agglomerative method that is exact for ultrametric trees.



I downloaded a (literally) random piece of the human genome that has alignments to 21 other vertebrates

```
hg18.chr1                        1550026 128 + 247249719 CCCTCTTGCAGTGTCACACTGAGTCCC…
panTro2.chr1                     1550081 128 + 229974691 CCCTCTTGCAGTGTCACACTGAGTCCC…
rheMac2.chr1                     4687633 128 + 228252215 CCCTCTTGCAGTGTCACGCTGAGTCCC…
tupBel1.scaffold_142366.1-9639      6335 128 -      9639 CTCTCTTGCAGTGTCACGCTGAGTCCC…
eriEur1.scaffold_329849             4248 128 -     16173 CTTCCTTGTAGGGTGACGCTGAGCCCC…
bosTau3.chr16                   24854618 126 -  72834534 --TTCCCGTAGGGTCATGCTGAGTCCC…
equCab1.chrUn                  152279254 126 - 405909753 --CTTTTGCAGGGTAATGCTGAGTCCC…
felCat3.scaffold_207897             9337 126 -     11103 --CTCTTGCAGGGTCACGCTGAGTCCC…
canFam2.chr5                    59689323 126 +  91976430 --TTCTTGCAGGGTCACGCTGAGTCCC…
mm8.chr4                          526108 126 - 155029701 --CTCTTACAGGGTTACGCTGAGCCCC…
rn4.chr5                          600229 126 - 173096209 --CTCTTACAGGGTTACGCTGAGTCCC…
echTel1.scaffold_295407             3308 128 +      9723 CTCTCCTGCAGCATCACCCTGAGCCCC…
monDom4.chr2                   103757048 128 + 541556283 CTCTCTTGCAGAGTCACCGTGAGTGCC…
ornAna1.Contig30834                 338 126 -      8660 --CCCTTGCAGAGTCTCCGTGAGTCCC…
anoCar1.scaffold_2684             12615 123 -     15861 CCTCCTTGCAGAGTTACTGTGAGTCCG…
galGal3.chr21                   4917466 120 -   6959642 ---TTTTTCAGAGTCCTAGTGAGTCCT…
xenTro2.scaffold_414             492398 124 +   1070475 TTTTTGTGCAGAGTCATACTGAGCACT…
fr2.chrUn                      185937056 125 - 400509343 TGTCGTCACAGAGTGAGTTTGGCGCCG…
tetNig1.chrUn_random            28277029 125 + 171761319 TCTCATCACAGAGTGAGTTTGGCGCCG…
danRer4.chrNA_random             3175416 123 + 208014280 CTTCTTCTCAGGGTGAGTTTGACGCCG…
gasAcu1.chrXII                   5652141 126 +  18401067 TCCCATCACAGGGTGTGCTTGGCACCG…
oryLat1.chr7                    29242227 128 -  29492121 TGTCATTGCAGAGTGAGCCTGACACCG…
```

http://genome.ucsc.edu

Here's the secret decoder ring for the genome names
the number is the assembly number (like version number)

human	Homo sapiens	hg18
chimpanzee	Pan troglodytes	panTro2
rhesus	Macaca mulatta	rheMac2
bushbaby	Otolemur garnetti	otoGar1
tree shrew	Tupaia belangeri	tupBel1
mouse	Mus musculus	mm8
rat	Rattus norvegicus	rn4
guinea pig	Cavia porcellus	cavPor2
rabbit	Oryctolagus cuniculus	oryCun1
shrew	Sorex araneus	sorAra1
hedgehog	Erinaceus europaeus	eriEur1
dog	Canis familiaris	canFam2
cat	Felis catus	felCat3
horse	Equus caballus	equCab1
cow	Bos taurus	bosTau3
armadillo	Dasypus novemcinctus	dasNov1
elephant	Loxodonta africana	loxAfr1
tenrec	Echinops telfairi	echTel1
opossum	Monodelphis domestica	monDom4
platypus	Ornithorhychus anatinus	ornAna1
lizard	Anolis carolinensis	anoCar1
chicken	Gallus gallus	galGal3
frog	Xenopus tropicalis	xenTro2
fugu	Takifugu rubripes	fr2
tetraodon	Tetraodon nigroviridis	tetNig1
stickleback	Gasterosteus aculeatus	gasAcu1
medaka	Oryzias latipes	oryLat1
zebrafish	Danio rerio	danRer4

(the line ends)

```
AAGGGGCATCTTCCAGGGAGCGAAGGTGGTGCGAGGCCCCGACTGGGAGTGGGGCTCACAGGATGGTGAGTGGAG---
AAGGGGCATCTTCCAGGGAGCGAAGGTGGTGCGAGGCCCCGACTGGGAGTGGGGCTCACAGGATGGTGAGTGGAG---
AAGGGGCATCTTCCAGGGCGCGAAGGTGGTGCGAGGCCCCGACTGGGAGTGGGGCTCACAGGATGGTGAGTGGAG---
AAAGGGCATCTTCCAGGGGGCAAAGGTGGTGCGAGGCCCCGACTGGGAGTGGGGCTCACAGGACGGTGAGTGGGG---
ACGGGGCATCTTCCAGGGTGCGAAGGTGGTGCGGGGTCCTGACTGGGAGTGGGGCTCCCAGGATGGTGAGTGGGG---
AAGGGGCATCTTCCAGGGGGCGAAGGTGGTTCGGGGCCCCGACTGGGAGTGGGGCTCACAGGATGGTGAGTGGGG---
GAGGGGCATCTTCCAGGGGGCAAAGGTGGTACGGGGCCCCGACTGGGAGTGGGGCTCACAGGACGGTGAGTGGGG---
GAGGGGCATCTTCCAGGGGGCGAAGGTGGTACGGGGTCCTGACTGGGAGTGGGGCTCGCAGGACGGTGAGTGGGG---
GAGGGGCATCTTCCAGGGGGCGAAGGTGGTGCGGGGCCCTGACTGGGAGTGGGGTTCGCAGGATGGTGAGTGAGG---
GAGGGGCATCTTTCAAGGAGCTAAGGTGGTACGAGGCCCTGACTGGGAATGGGGCTCACAAGATGGTGAGTGGTG---
GAGGGGCATCTTTCAAGGAGCAAAAGTGGTACGAGGCCCTGACTGGGAATGGGGCTCACAAGATGGTGAGTGGTG---
GAGGGGCATCTTCCAAGGGGCAAAGGTGGTGCGAGGCCCCGACTGGGAATGGGGCTCTCAAGACGGTGAGTGAGG---
GAGGGGCATCTTCCAAGGCGCCAAGGTCCTCCGGGGCCCAGACTGGGAATGGGGCAATCAGGATGGTGAGTGGAG---
GAGAGGGATCTTCCAGGGTGCCAAGGTGCTCCGGGGCCCAGACTGGGAGTGGGGCAATCAGGACGGTAAGTGGGG---
GAAGGGAACATTTCAGGGCGCAAAAGTGGTCCGGGGCCCCGACTGGGAATGGGGCAACCAAGACGGTAAG--------
AAAAGGGACTTTCCAGGGGGCTAAAGTAGTCCGTGGCCCAGACTGGGAATGGGGTAACCAGGATGGTAAG--------
AAAAGGAATCTTCCAAGGTGCAAAAGTGGTGCGTGGTCCTGACTGGGAATGGGGAAACCAAGATGGTATGT-------
CAAAGGGATTTTCCAGGGCGTTAAAGTTGTTCGAGGACCTGACTGGGACTGGGGTAACCAAGACGGTGAGT---G---
AAAGGGGATTTTCCAGGGCGTTAAAGTCGTTCGAGGACCTGACTGGGACTGGGGTAACCAAGACGGTGAGT---G---
GAAGGGAATCTTTCAGGGAGTAAAGGTGGTGCGGGGACCCGACTGGGACTGGGGGAACCAGGACGGTGAG--------
CAAAGGAATCTTCCAGGGGGTCAAAGTGGTCCGTGGACCCGATTGGGATTGGGGCAACCAAGACGGTGAGT---GG--
GAAAGGAATCTTCCAGGGCGTGAAGGTGGTTCGGGGACCCGACTGGGACTGGGGGAACCAGGACGGTGAGT---GGCG
```

file posted on course website as hammingdata.txt

(Fractional) Hamming distance is the fraction of positions that are different

```
Doub hamming(char *a, char *b) {
     Int i,neq=0,na=strlen(a), nb=strlen(b);
     if (na != nb) throw("hamming: strings unequal length");
     for (i=0;i<na;i++) if (a[i] != b[i]) neq++;
     return Doub(neq)/na;
}
```

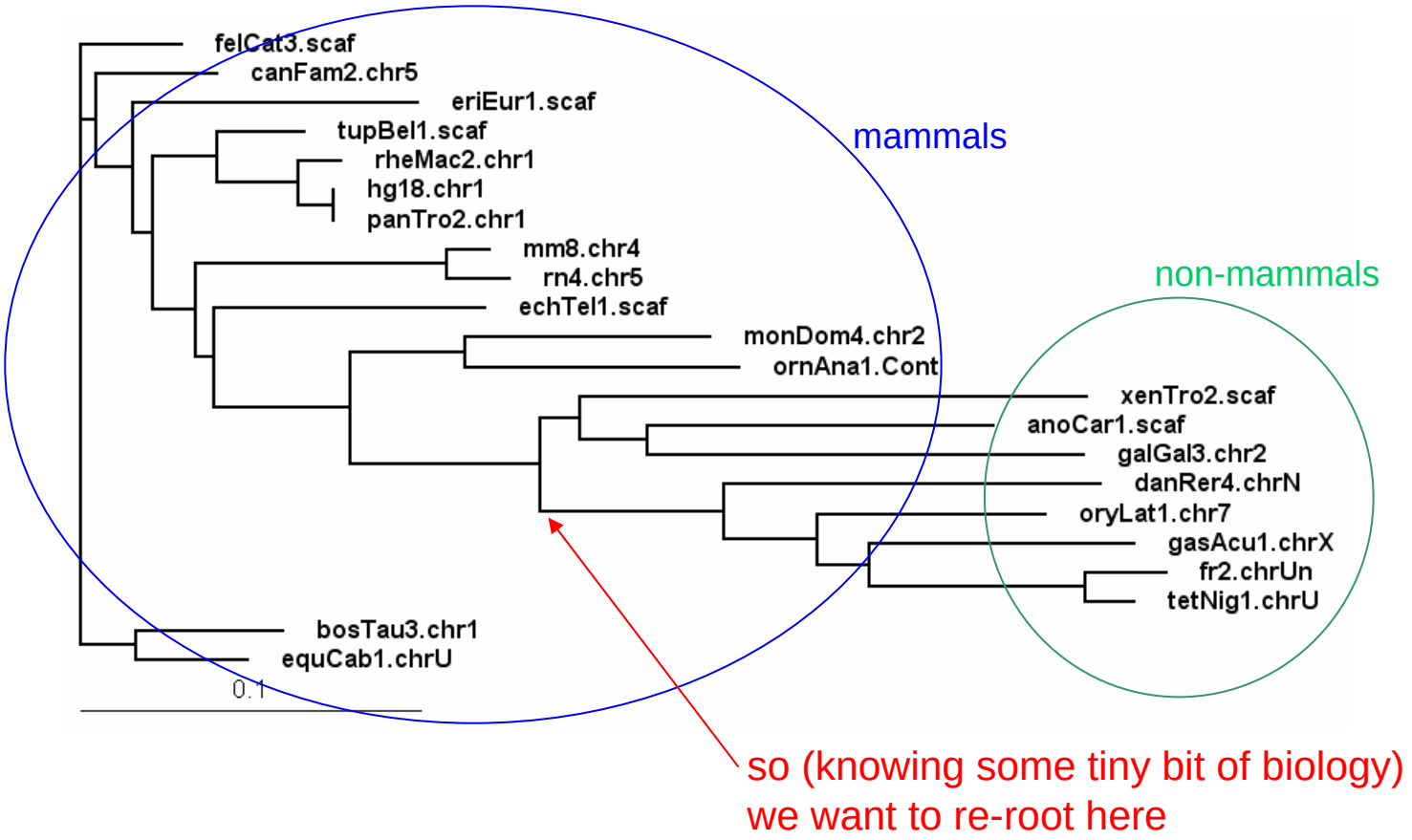
in percent (rounded)

0	hg18.chr1	0	0	2	6	13	12	13	11	11	15	15	15	21	24	32	34	34	37	35	34	34	32
1	panTro2.chr1	0	0	2	6	13	12	13	11	11	15	15	15	21	24	32	34	34	37	35	34	34	32
2	rheMac2.chr1	2	2	0	6	13	12	13	11	11	15	16	15	21	24	32	35	35	37	35	36	35	32
3	tupBel1.scaf	6	6	6	0	14	11	9	10	11	16	16	11	22	23	30	34	33	35	34	32	34	31
4	eriEur1.scaf	13	13	13	14	0	15	18	13	12	19	21	20	27	25	35	40	31	37	36	32	37	32
5	bosTau3.chr1	12	12	12	11	15	0	8	10	9	18	19	18	26	25	34	34	34	37	36	36	37	32
6	equCab1.chrU	13	13	13	9	18	8	0	7	10	15	15	15	24	22	31	33	34	37	37	33	34	31
7	felCat3.scaf	11	11	11	10	13	10	7	0	7	14	15	15	22	19	33	35	34	37	36	34	34	31
8	canFam2.chr5	11	11	11	11	12	9	10	7	0	15	16	16	24	23	32	31	33	37	36	34	37	31
9	mm8.chr4	15	15	15	16	19	18	15	14	15	0	3	17	23	27	31	34	34	35	34	37	35	35
10	rn4.chr5	15	15	16	16	21	19	15	15	16	3	0	18	22	27	29	33	34	36	35	36	35	37
11	echTel1.scaf	15	15	15	11	20	18	15	15	16	17	18	0	21	24	30	38	33	37	36	34	34	32
12	monDom4.chr2	21	21	21	22	27	26	24	22	24	23	22	21	0	15	27	30	35	36	34	34	34	31
13	ornAna1.Cont	24	24	24	23	25	25	22	19	23	27	27	24	15	0	27	28	36	35	36	34	31	30
14	anoCar1.scaf	32	32	32	30	35	34	31	33	32	31	29	30	27	27	0	23	28	30	28	25	31	27
15	galGal3.chr2	34	34	35	34	40	34	33	35	31	34	33	38	30	28	23	0	28	31	31	32	34	35
16	xenTro2.scaf	34	34	35	33	31	34	34	34	33	34	34	33	35	36	28	28	0	34	34	33	35	33
17	fr2.chrUn	37	37	37	35	37	37	37	37	37	35	36	37	36	35	30	31	34	0	4	22	16	16
18	tetNig1.chrU	35	35	35	34	36	36	37	36	36	34	35	36	34	36	28	31	34	4	0	21	16	17
19	danRer4.chrN	34	34	36	32	32	36	33	34	34	37	36	34	34	34	25	32	33	22	21	0	24	21
20	gasAcu1.chrX	34	34	35	34	37	37	34	34	37	35	35	34	34	31	31	34	35	16	16	24	0	16
21	oryLat1.chr7	32	32	32	31	32	32	31	31	31	35	37	32	31	30	27	35	33	16	17	21	16	0

Construct the tree and write it out in a standard format

```
MatDoub dist(nseq,nseq);
for (i=0;i<nseq;i++) for (j=0;j<nseq;j++) dist[i][j] = hamming(&seq[i][0],&seq[j][0]);
Phylo_nj mytree(dist);
newick(mytree,species,"d:\\MyCompStatNotes\\mytree.phy");
```

Now view it in (e.g.) TreeView NJ trees are intrinsically unrooted!

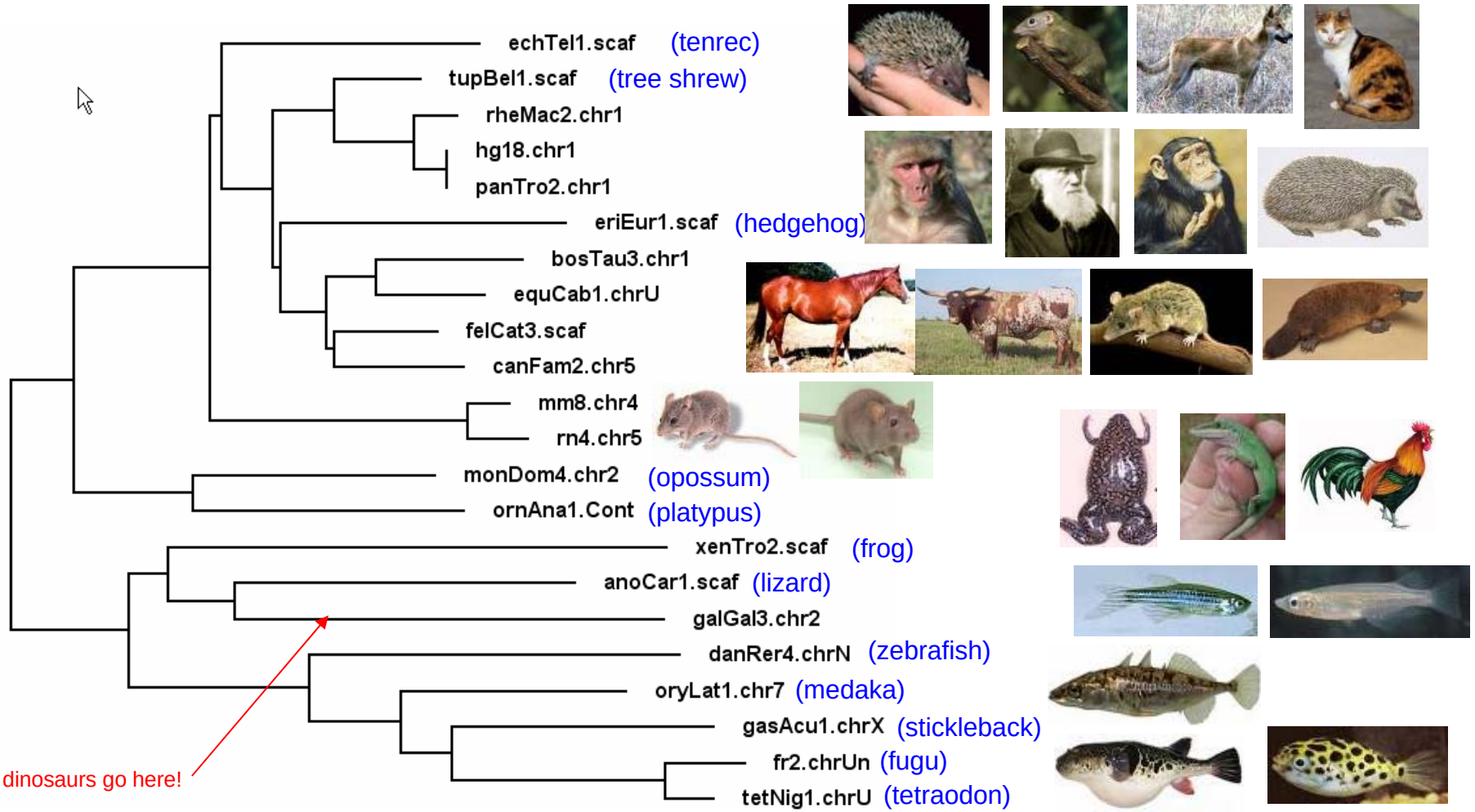


TreeView can also display unrooted trees without specifying a root

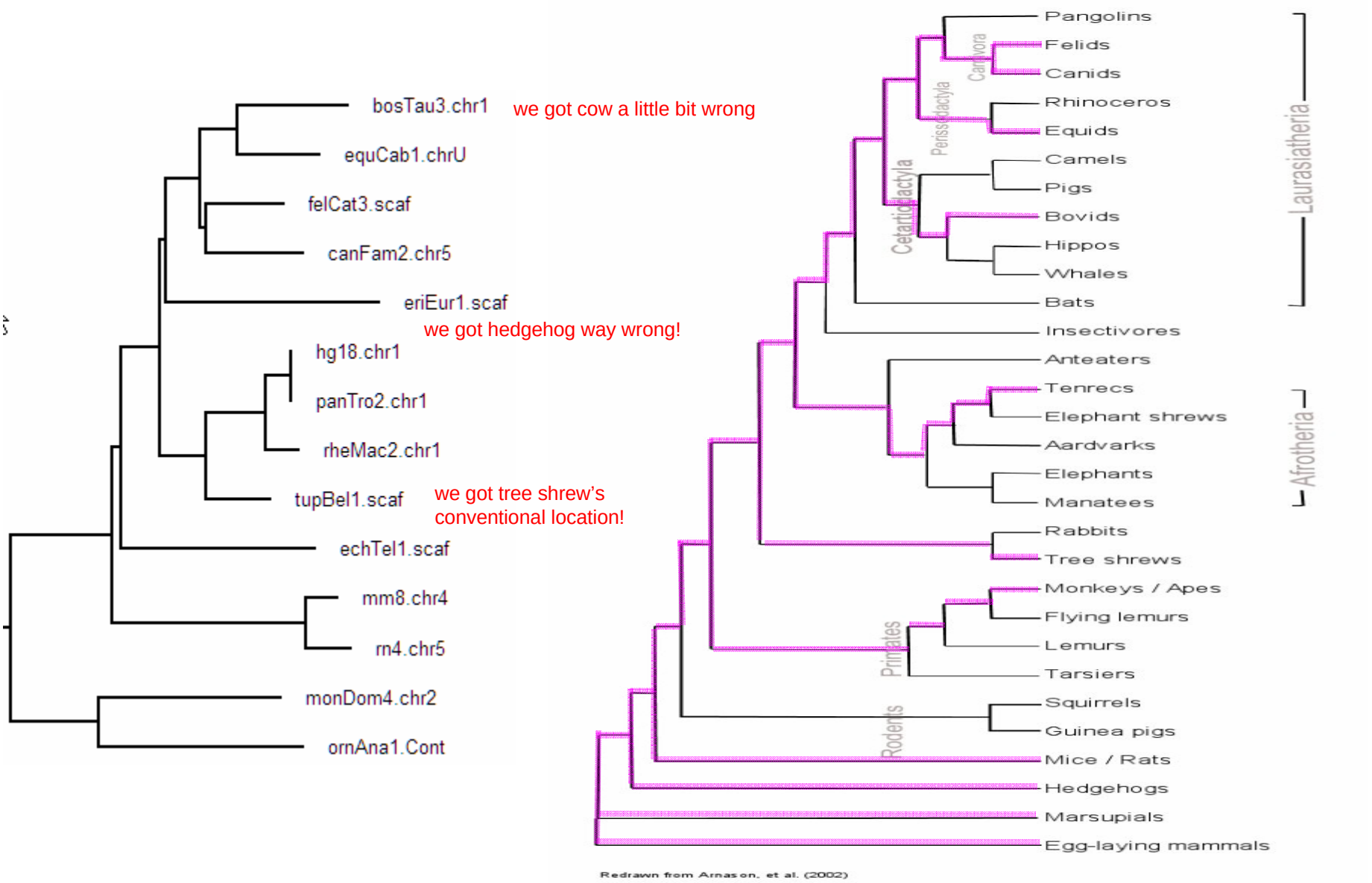
NR3's re-rooting method is kludgy (serious bio software much user friendlier, but I ran out of time writing that section of NR3!)

```
Phylo_nj mypretree(dist);
Phylo_nj mytree(dist,mypretree.comancestor(16,18));
newick(mytree,species,"d:\\MyCompStatNotes\\mytree.phy");
```

you specify the root by a common ancestor of nodes, after viewing a trial tree



Compare to a more careful phylogeny based on Mitochondrial sequence

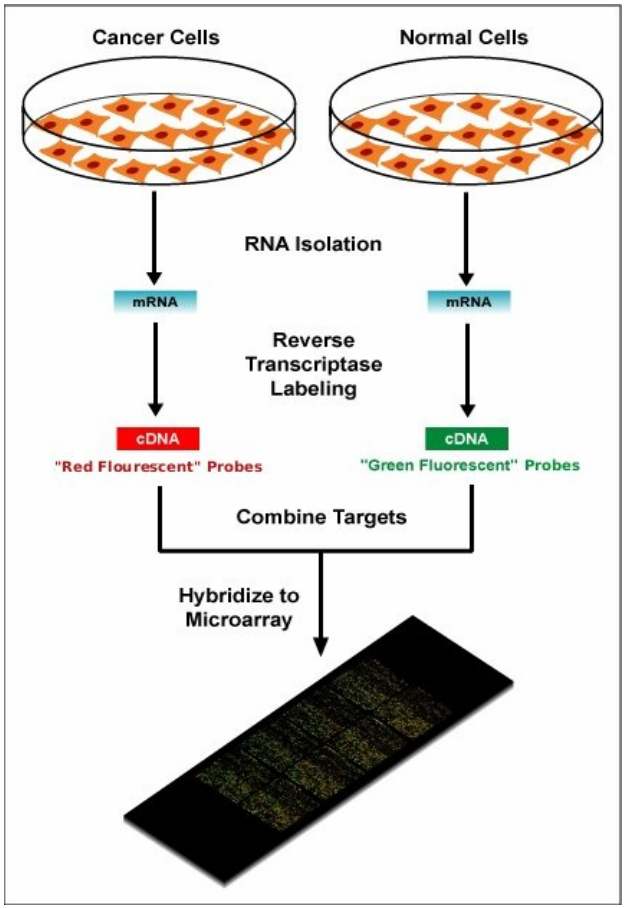


Hierarchical clustering methods are also commonly used on gene expression data

One color or single-channel systems:

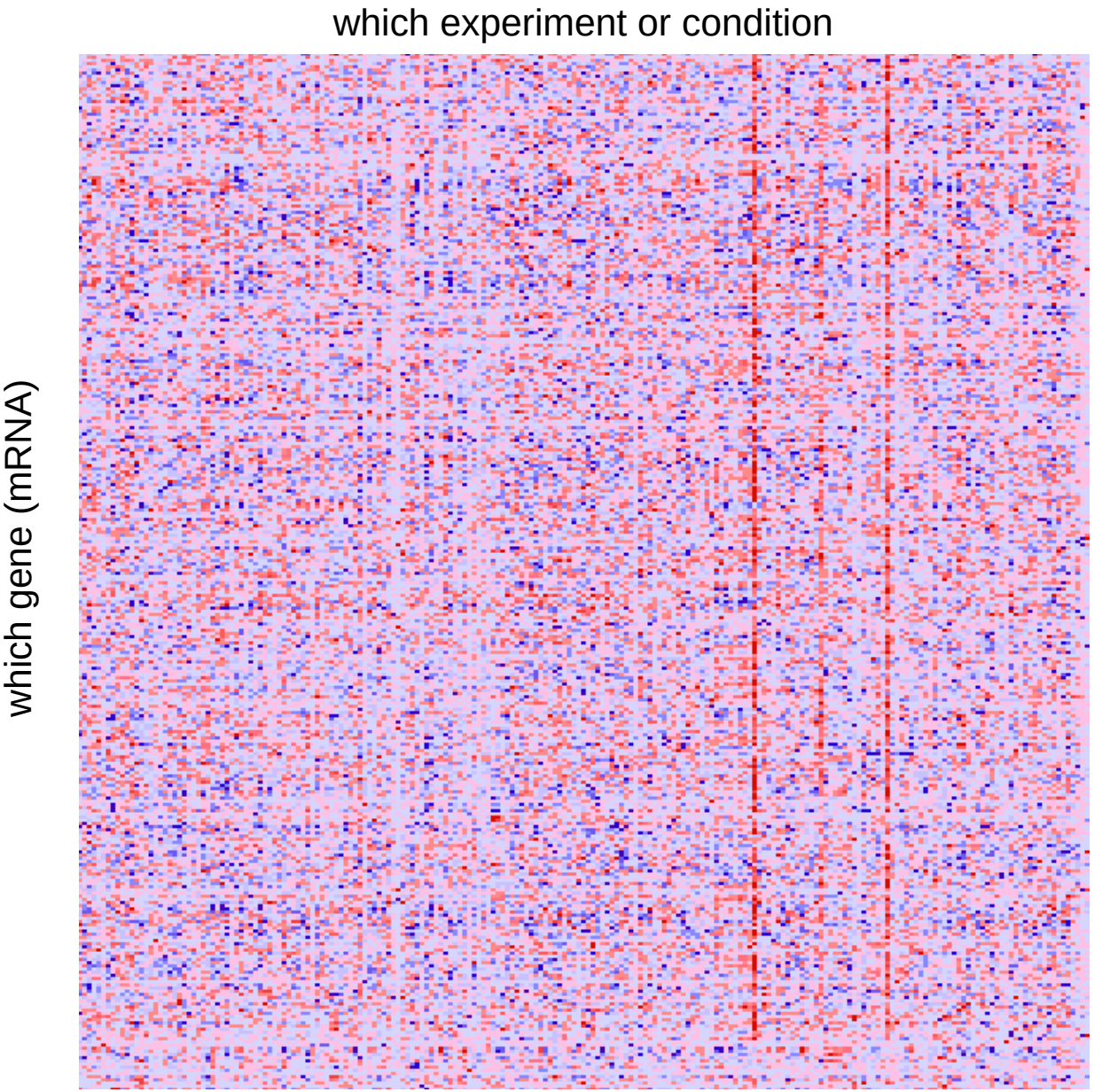
"Absolute" measurements of mRNA concentrations can be compared across multiple experiments. But, there is an imperfectly known binding specificity for each spot.

Two-color systems (e.g., Agilent):

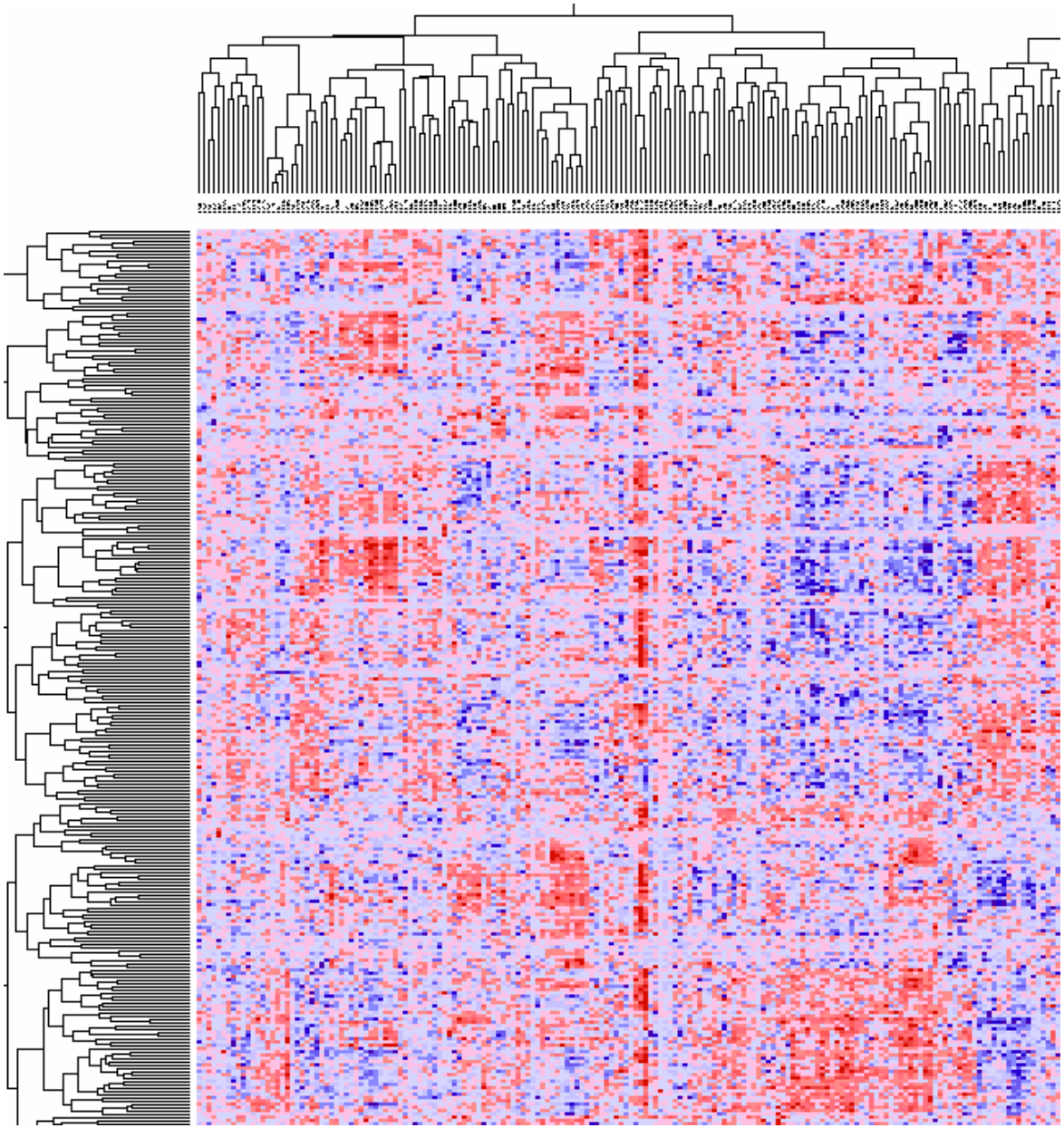


Hybridization is independent of the fluorophor, so red-green ratio directly measures ratio of mRNA concentrations between the two samples. (Overall intensity depends on binding specificity and is considered irrelevant.)

Gene expression data repeated over many conditions look like this:



Hierarchical clustering is often used to identify related genes and/or related experimental conditions



- the assumption is that related genes have similar expression profiles
- permutations of rows and columns are done independently and don't interfere with each other
- distance matrix is usually Pearson correlation coefficient
- "pairwise complete linkage", not NJ, is commonly used
- there are public (free) servers for doing the analysis and returning nice pictures (and detailed data files), for example:

http://genepattern.broad.mit.edu

GenePattern
A platform for integrative genomics