Phylogenetic Trees

Phylogeny

- PHYLOGENY (coined 1866 Haeckel)
  1. the line of descent or evolutionary development of any plant or animal species
  2. the origin and evolution of a division, group or race of animals or plants

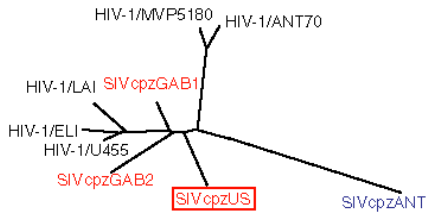
Goals

- Understand evolutionary history
  - Origin of Europeans
- Assist in epidemiology
  - of infectious diseases
  - of genetic defects
- Aid in prediction of function of novel genes
- Biodiversity studies
- Understanding microbial ecologies

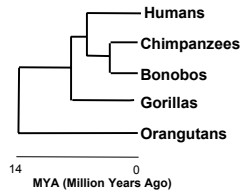
Mitochondria and Phylogeny

- **Mitochondrial DNA (mtDNA)**: Extra-nuclear DNA, transmitted through maternal lineage.
  - Allows tracing of a single genetic line
- 16.5 Kb circular DNA contains genes: coding for 13 proteins, 22 tRNA genes, 2 rRNA genes.
- mtDNA has a pointwise mutation substitution rate 10 times faster than nuclear DNA: provides a way to infer relationships between closely related individuals

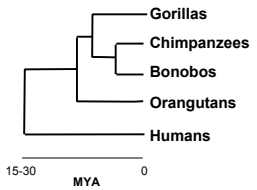
HIV-1 Origins



Which species are the closest living relatives of modern humans?



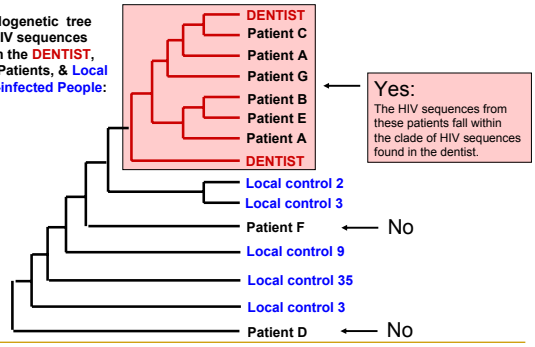
Mitochondrial DNA, most nuclear DNA-encoded genes, and DNA/DNA hybridization all show that bonobos and chimpanzees are related more closely to humans than either are to gorillas.



The pre-molecular view was that the great apes (chimpanzees, gorillas and orangutans) formed a clade separate from humans, and that humans diverged from the apes at least 15-30 MYA.

Did the *Florida Dentist* infect his patients with HIV?

Phylogenetic tree of HIV sequences from the DENTIST, his Patients, & Local HIV-infected People:



Yes: The HIV sequences from these patients fall within the clade of HIV sequences found in the dentist.

No

No

From Ou et al. (1992) and Page & Holmes (1998)

Gene Tree *vs.* Species Tree

- The evolutionary history of genes reflects that of species that carry them, except if :
  - horizontal transfer = gene transfer between species (*e.g.* bacteria, mitochondria)
  - Gene duplication : orthology/ paralogy

Orthology / Paralogy

speciation

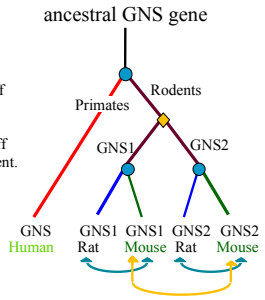
duplication

Homology: two genes are homologous iff they have a common ancestor.

Orthology: two genes are orthologous iff they diverged following a speciation event.

Paralogy: two genes are paralogous iff they diverged following a duplication event.

Orthology → functional equivalence



Building Phylogenies: Phenotype Information has problems

- Can be difficult to observe
  - Bacteria
- Difficult to compare diverse species
  - Plants, bacteria, animals

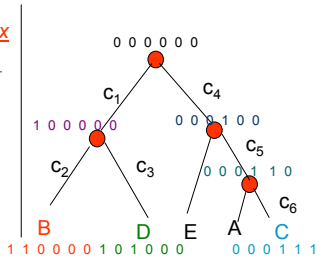
Data for Building Phylogenies

- Characteristics
  - Traits (continuous or discrete)
  - Biomolecular features
  - *character state matrix*
- Numerical distance estimates
  - *distance matrix*

Example of Character-based Phylogeny

A character state matrix

Taxon	c_1	c_2	c_3	c_4	c_5	c_6
A	0	0	0	1	1	0
B	1	1	0	0	0	0
C	0	0	0	1	1	1
D	1	0	1	0	0	0
E	0	0	0	1	0	0



Different Kinds of Trees

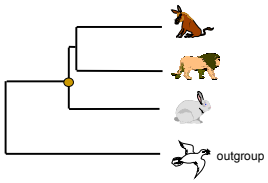
- Order of evolution
  - Rooted: indicates direction of evolution
  - Unrooted: only reflects the distance
- Rate of evolution
  - Edge lengths: distance (scaled trees)
    - Molecular clock: constant rate of evolution
  - Unscaled trees

Rooted and Unrooted Trees

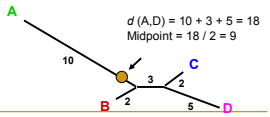
- Most phylogenetic methods produce unrooted trees. This is because they detect differences between sequences, but have no means to orient residue changes relatively to time.
- Two means to root an unrooted tree :
  - The outgroup method : include in the analysis a group of sequences known *a priori* to be external to the group under study; the root is by necessity on the branch joining the outgroup to other sequences.
  - Make the molecular clock hypothesis : all lineages are supposed to have evolved with the same speed since divergence from their common ancestor. Root the tree at the midway point between the two most distant taxa in the tree, as determined by branch lengths. The root is at the equidistant point from all tree leaves.

Rooting unrooted trees

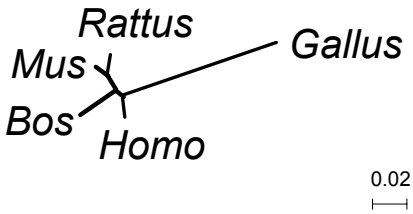
By outgroup:



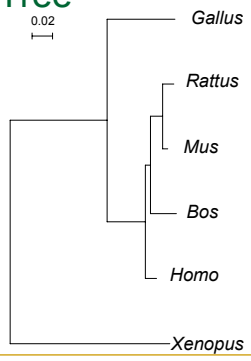
By midpoint or distance:

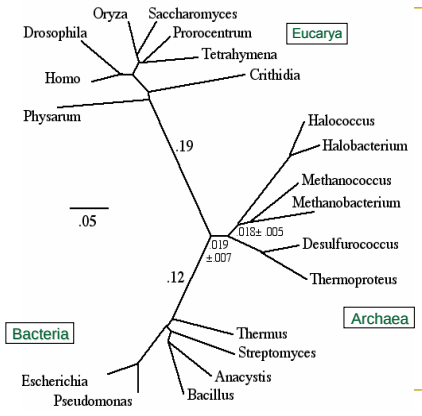


Unrooted Tree



Rooted Tree





Universal phylogeny

deduced from comparison of SSU and LSU rRNA sequences (2508 homologous sites) using Kimura's 2-parameter distance and the NJ method.

The absence of root in this tree is expressed using a circular design.

Tree building Methods

- Character-based methods
  - Maximum parsimony
  - Maximum likelihood
- Distance-based methods
  - UPGMA
  - NJ

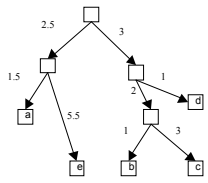
Distance Matrix Methods

- Given a pairwise distace matrix D
- Produce a tree such that the *path distance* between leaves i and j (sum of edge weights in the path between i and j) equals d_{ij}
- Optimize the error between d and D
  - Least square error metric: LSQ
  - LSQ(d,D) = $\Sigma \Sigma (d_{ij} - D_{ij})^2$
  - NP-complete
- Heuristics (usually based on agglomerative (group by group) clustering)
  - UPGMA
  - NJ
  - Both assume additive distances
    - implies that distance is a metric
      - symmetry
      - triangle inequality
      - d(x,y) = 0 iff x = y
      - d(x,y) >= 0

Distance Measures

- DNA sequences
  - Percent Identities
- Protein sequences
  - PAM matrix

Example Tree and Additive Matrix



	a	b	c	d	e
A	0	10	12	8	7
B		0	4	4	14
C			0	6	16
D				0	12
E					0

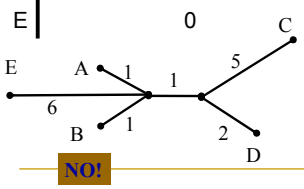
There exists a tree with additive distances

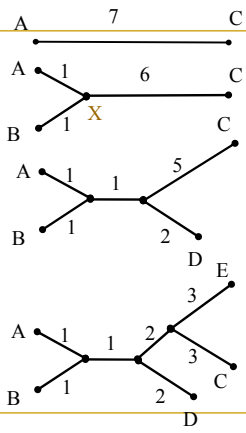
Additive Trees from Additive Matrices

- Verify that the distance matrix is additive
- Choose a pair of objects, which results in the first path in the tree.
- Choose a third object and establish the linear equations to let the object branch off the path.
- Choose a pair of leaves in the tree constructed so far and compute the point at which a newly chosen object is inserted.
  1. The new path branches off an existing node in the tree: Do the insertion step once more in the branching path.
  2. The new path branches off an edge in the tree: This insertion is finished.

Example

	A	B	C	D	E
A	0	2	7	4	7
B		0	7	4	7
C			0	7	6
D				0	7
E					0





Approximating Additive Matrices

In practice, the distance matrix between molecular sequences will not be additive.

An additive tree T whose distance matrix approximates the given one is used.

The methods for exact tree reconstruction provide an inventory for heuristics for tree construction based on approximating additive metrics.

Heuristics give exact results when operating on additive metrics.

UPGMA

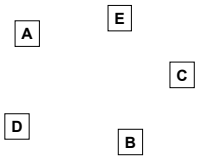
- Unweighted Pair-Group Method with Arithmetic Mean
  - Sokal and Michener 1958
- Agglomerative clustering
- Ultrametric tree
  - distances from root to all leaves are equal
- Cluster distances defined as

$$d_{AB} = \frac{1}{|A||B|} \sum_{a \in A} \sum_{b \in B} d_{ab}$$

UPGMA Step 1

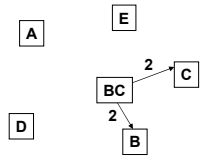
combine B and C

Choose two clusters with minimum distance and combine them



	A	B	C	D	E
A	0	10	12	8	7
B		0	4	4	14
C			0	6	16
D				0	12
E					0

Updating distance matrices



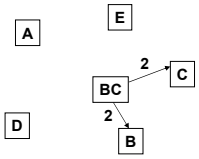
	A	BC	D	E
A	0	11	8	7
BC		0	5	15
D			0	12
E				0

Distance of new cluster to nodes in the cluster is half of original distance

Distance of new cluster to other clusters is weighted mean of individual distances

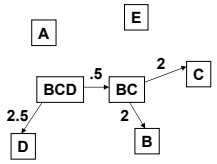
UPGMA step 2

combine BC and D



	A	BC	D	E
A	0	11	8	7
BC		0	5	15
D			0	12
E				0

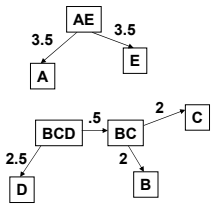
Updating distance matrices



	A	BCD	E
A	0	10	7
BCD		0	14
E			0

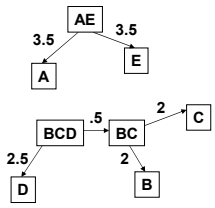
UPGMA step 3

combine A and E



	A	BCD	E
A	0	10	7
BCD		0	14
E			0

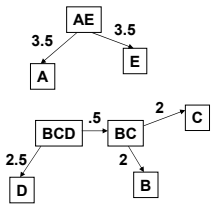
Updating distance matrices



	AE	BCD
AE	0	12
BCD		0

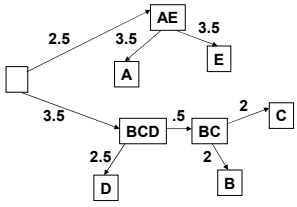
UPGMA step 4

combine AE and BCD



	AE	BCD
AE	0	12
BCD		0

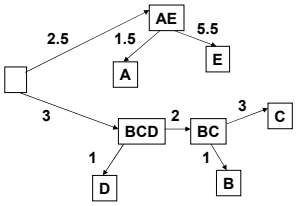
UPGMA Result



produced tree

	A	B	C	D	E
A	0	10	12	8	7
B		0	4	4	14
C			0	6	16
D				0	12
E					0

Actual tree



actual tree

	A	B	C	D	E
A	0	10	12	8	7
B		0	4	4	14
C			0	6	16
D				0	12
E					0

Limitations of UPGMA

- Ultrametric tree
  - Path distance from the root to each leaf is the same
- Ultrametric distance
  - Usual metric conditions
  - d(x,y) <= max[d(x,z), d(y,z)]
    - 2 largest distances in any group of 3 are equal
    - meaning in a tree setting?
- UPGMA works correctly for ultrametric distances

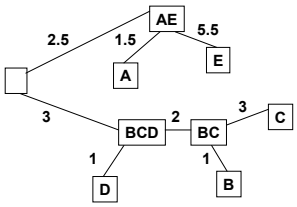
Neighbor Joining (NJ)

- Saitou and Nei, 1987
  - Join clusters that are close to each other and also far from the rest
- Produces unrooted tree
- NJ is a fast method, even for hundreds of sequences.
- The NJ tree is an approximation of the minimum evolution tree (that whose total branch length is minimum).
- In that sense, the NJ method is very similar to parsimony methods because branch lengths represent substitutions.
- NJ always finds the correct tree if distances are additive (tree-like).
- NJ performs well when substitution rates vary among lineages. Thus NJ should find the correct tree if distances are well estimated.

Algorithm

- Define $u_i = \sum_{k \neq i} D_{ik} / (n-2)$
  - measure of average distance from other nodes
- Iterate until 2 nodes are left
  - choose pair (i,j) with smallest $D_{ij} - u_i - u_j$
    - close to each other and far from others
  - merge to a new node (ij) and update distance matrix
    - $D_{k,(ij)} = (D_{ik} + D_{jk} - D_{ij})/2$ -- consider the tree paths
    - $D_{i,(ij)} = (D_{ij} + u_i - u_j)/2$ -- similarly
    - $D_{j,(ij)} = D_{ij} - D_{i,(ij)}$ -- similarly
  - delete nodes i and j
- For the final group (i,j), use D_{ij} as the edge weight.

Neighbor-Joining Result



actual tree

	A	B	C	D	E
A	0	10	12	8	7
B		0	4	4	14
C			0	6	16
D				0	12
E					0

WWW Resources

- PHYLIP : an extensive package of programs for all platforms
  `http://evolution.genetics.washington.edu/phylip.html`
- CLUSTALX : beyond alignment, it also performs NJ
- PAUP* : a very performing commercial package
  `http://paup.csit.fsu.edu/index.html`
- PHYLO_WIN : a graphical interface, for unix only
  `http://pbil.univ-lyon1.fr/software/phylowin.html`
- MrBayes : Bayesian phylogenetic analysis
  `http://morphbank.ebc.uu.se/mrbayes/`
- PHYML : fast maximum likelihood tree building
  `http://www.lirmm.fr/~guindon/phyml.html`
- WWW-interface at Institut Pasteur, Paris
  `http://bioweb.pasteur.fr/seqanal/phylogeny`
- Tree drawing
  NJPLOT (for all platforms)
  `http://pbil.univ-lyon1.fr/software/njplot.html`