A quick review

- Significance of similarity scores (P-values)
  - Empirical null score distribution
  - Extreme value distribution
- Multiple-testing correction (Bonferroni) and E-values
FROM THE ABSTRACT:
P values and accompanying methods ... are creating challenges in biomedical science ... However, many of the claims that these reports highlight are likely false. Recognizing the major importance of the statistical significance conundrum, the American Statistical Association (ASA) published a statement on P values in 2016. The status quo is widely believed to be problematic, but how exactly to fix the problem is far more contentious.
Human: CGAAT-CGA-TTCA
Chimp: C-A-TACGAGT-CA
Gorilla: --A-TGCGT-TGCA
Orangutan: --A-TGCGT-GGCA
Phylogenetic Trees

Genome 373
Genomic Informatics
Elhanan Borenstein
I think

There is a line of descent from A to B. The first gradation, B & D rather greater distinction. Then genus would be formed.
Defining what a “tree” means

- **branch points**
- **root**
- **ancestral sequence**
- **branches**

... (horizontal) branch lengths sequence is proportional to divergence

**Note:** Many drawing practices exist

**Note:** A tree has topology and distances
Are these topologically different trees?
Are these topologically different trees?

Topologically, these are the SAME tree. In general, two trees are the same if they can be inter-converted by branch rotations.
Branch lengths and evolutionary divergence time

```
  C04F2.1
   /   \
  /     \
DC2.6   F08E10.3
      /     \
     /       \
  F08E10.6  C46E10.10

  /   \    /   \
  /     \  /     \n5      10.5 10 18

C31B8.3
```

Time (?)

Rooted and unrooted trees

**Rooted tree** (all real trees are rooted):

- **branch points**
- **root**
- **ancestral sequence**
- **branches**

leaves or tips (eg sequences)

```
  DC2.6
   C04F2.1
     F08E10.3
     F08E10.6
     C46E10.10
       E03D2.3
       C17E7.2
         C31B8.3
```

Time (?)

... (horizontal) branch lengths sequence is proportional to divergence

**Unrooted tree** (used when the root isn’t known):

```
  A
   D
     B
   C
     E
```

time radiates out from somewhere (probably near the center)
Why is inferring phylogeny a hard problem?

(assume, for example, we are trying to infer the phylogenetic tree for 20 primate species)
The number of tree topologies grows extremely fast

3 leaves
3 branches
1 internal node
1 topology
(3 insertions)
The number of tree topologies grows extremely fast

- 3 leaves, 3 branches, 1 internal node, 1 topology (3 insertions)
- 4 leaves, 5 branches, 2 internal nodes, 3 topologies (x3) (5 insertions)
- 5 leaves, 7 branches, 3 internal nodes, 15 topologies (x5) (7 insertions)
The number of tree topologies grows extremely fast

In general, an unrooted tree with $N$ leaves has:

- $2N - 3$ total branches
- $N$ leaf branches
- $N - 3$ internal branches
- $N - 2$ internal nodes
- $3*5*7*...*(2N-5) \sim O(N!)$ topologies
There are many rooted trees for each unrooted tree

For each unrooted tree, there are $2N - 3$ times as many rooted trees, where $N$ is the number of leaves ($\# \text{ branches} = 2N - 3$).

The number of tree topologies grows extremely fast
There are many rooted trees for each unrooted tree

For each unrooted tree, there are 2N - 3 times as many rooted trees, where N is the number of leaves (# branches = 2N - 3).

The number of tree topologies grows extremely fast

20 leaves - 564,480,989,588,730,591,336,960,000,000,000 topologies
How can you infer a tree?

- Many methods available, we will talk about:
  - Distance trees
  - Parsimony trees

- Others include:
  - Maximum-likelihood trees
  - Bayesian trees
Distance matrix methods

- Methods based on a set of pairwise distances typically from a multiple alignment.

<table>
<thead>
<tr>
<th></th>
<th>human</th>
<th>chimp</th>
<th>gorilla</th>
<th>orang</th>
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<td>4/6</td>
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Human - chimp has 2 changes out of 6 sites
Human - orang has 4 changes of out 6 sites etc.

- Many different metrics can be used!!
Approach:
Try to build the tree whose **distances** best match the real distances
Trees and distances

Time (?)

Distance Matrix:

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Best Match?

• "Best match" based on **least squares** of real pairwise distances compared to the tree distances:

Let $D_m$ be the measured distances.

Let $D_t$ be the tree distances.

Find the tree that minimizes:

$$\sum_{i=1}^{N} (D_t - D_m)^2$$
Why not enumerate and score all trees?
The UPGMA algorithm
(Unweighted Pair Group Method with Arithmetic Mean)

1) generate a table of pairwise sequence distances and assign each sequence to a list of N tree nodes.

2) look through current list of nodes (initially these are all leaf nodes) for the pair with the smallest distance.

3) merge the closest pair, remove the pair of nodes from the list and add the merged node to the list.

4) repeat until only one node left in list - it is the root.
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\[ D_{n1,n2} = \frac{1}{N} \sum_i \sum_j d_{ij} \]

where \( i \) is each leaf of \( n1 \) (node1), \( j \) is each leaf of \( n2 \) (node2), and \( N \) is the number of distances summed

(in words, this is just the arithmetic average of the distances between all the leaves in one node and all the leaves in the other node)
The UPGMA algorithm

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<tr>
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UPGMA
(Unweighted Pair Group Method with Arithmetic Mean)
The Molecular Clock

- UPGMA assumes a constant rate of the molecular clock across the entire tree!
  - The sum of times down a path to any leaf is the same

- This assumption may not be correct ... and will lead to incorrect tree reconstruction.
Neighbor-Joining (NJ) Algorithm

- Essentially similar to UPGMA, but correction for distance to other leaves is made.

- Specifically, for sets of leaves $i$ and $j$, we denote the set of all other leaves as $L$, and the size of that set as $|L|$, and we compute the corrected distance $D_{ij}$ as:

$$D_{ij} = d_{ij} - (r_i + r_j)$$

where

$$r_i = \frac{1}{|L|} \sum_{k \in L} d_{ik}$$

(the mean distance from $i$ to all 'other' leaves)
But wait, there’s one more problem

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Raw distance correction

• As two DNA sequences diverge, it is easy to see that their maximum raw distance is ~0.75 (assuming equal nt frequencies, ¼ of residues will be identical even if unrelated sequences).

• We would like to use the "true" distance, rather than raw distance.

• This graph shows evolutionary distance related to raw distance:
Jukes-Cantor model

Jukes-Cantor model:

\[ D = -\frac{3}{4} \ln \left(1 - \frac{4}{3} D_{\text{raw}} \right) \]

- \( D_{\text{raw}} \) is the raw distance (what we directly measure)
- \( D \) is the corrected distance (what we want)
Distance trees - summary

• Convert each pairwise raw distance to a corrected distance.

• Build tree as before (UPGMA algorithm).

• Notice that these methods don't need to consider all tree topologies - they are very fast, even for large trees.