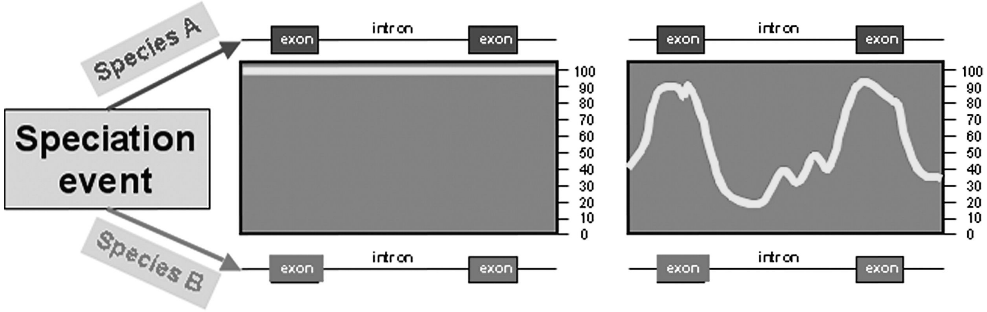
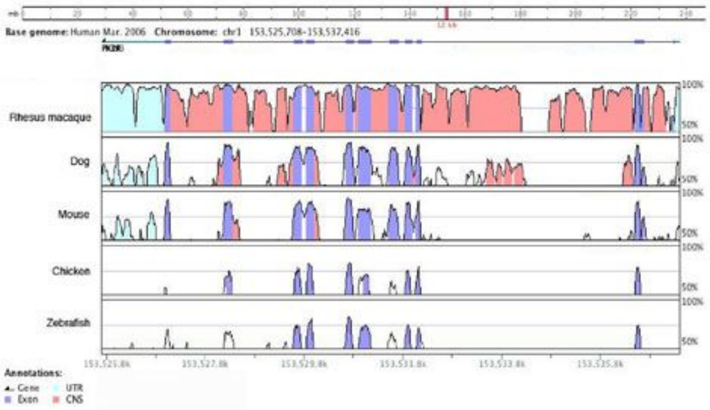
Week 4: Comparative genomics

- Whole genome alignment
  - MUMmer & Suffix trees
- Gene/species trees
  - Phylogenetic trees
  - Gene orthology & functional analysis

Comparative genomics

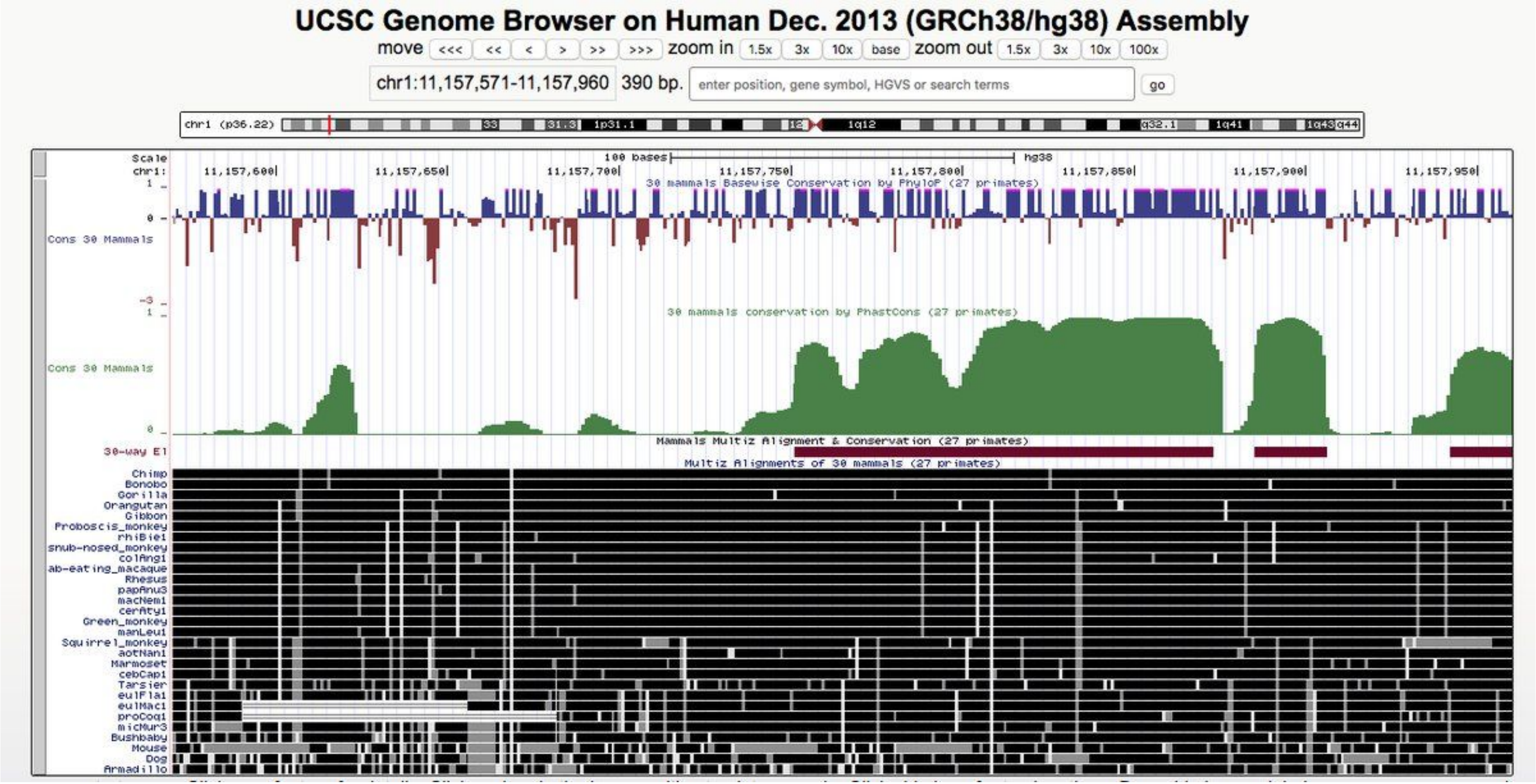




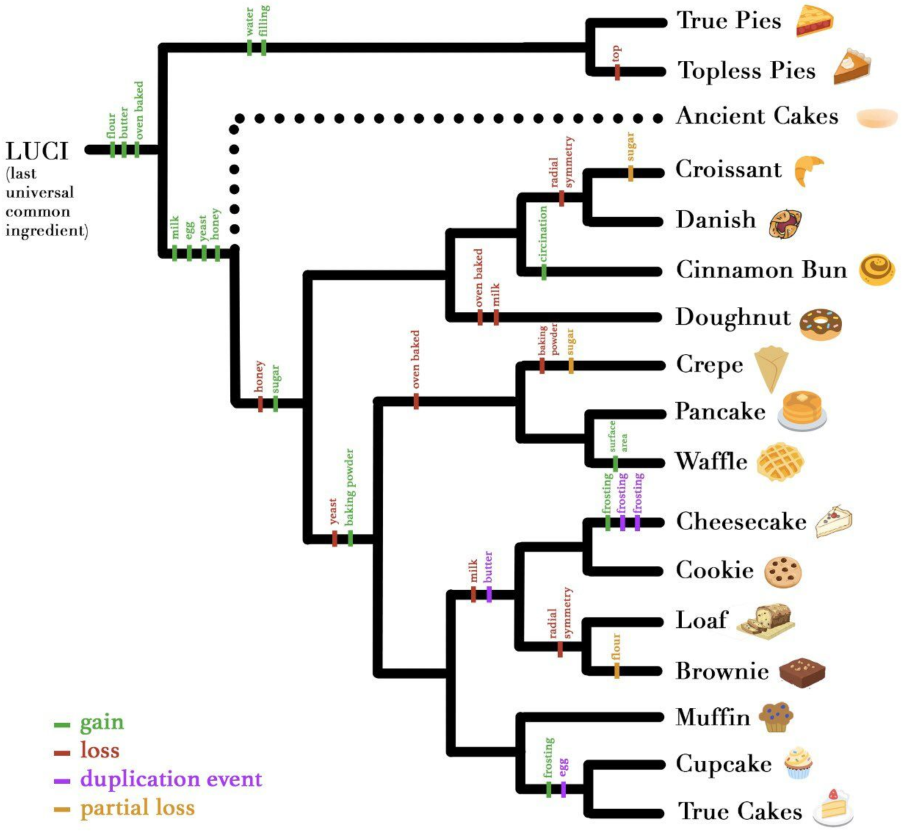
Miller et al., (2004) Ann. Rev. Genomics & Hum. Gen.
Nature Scitable

Comparative genomics

Multiple alignments and measurements of evolutionary conservation for 30 species (27 primates)

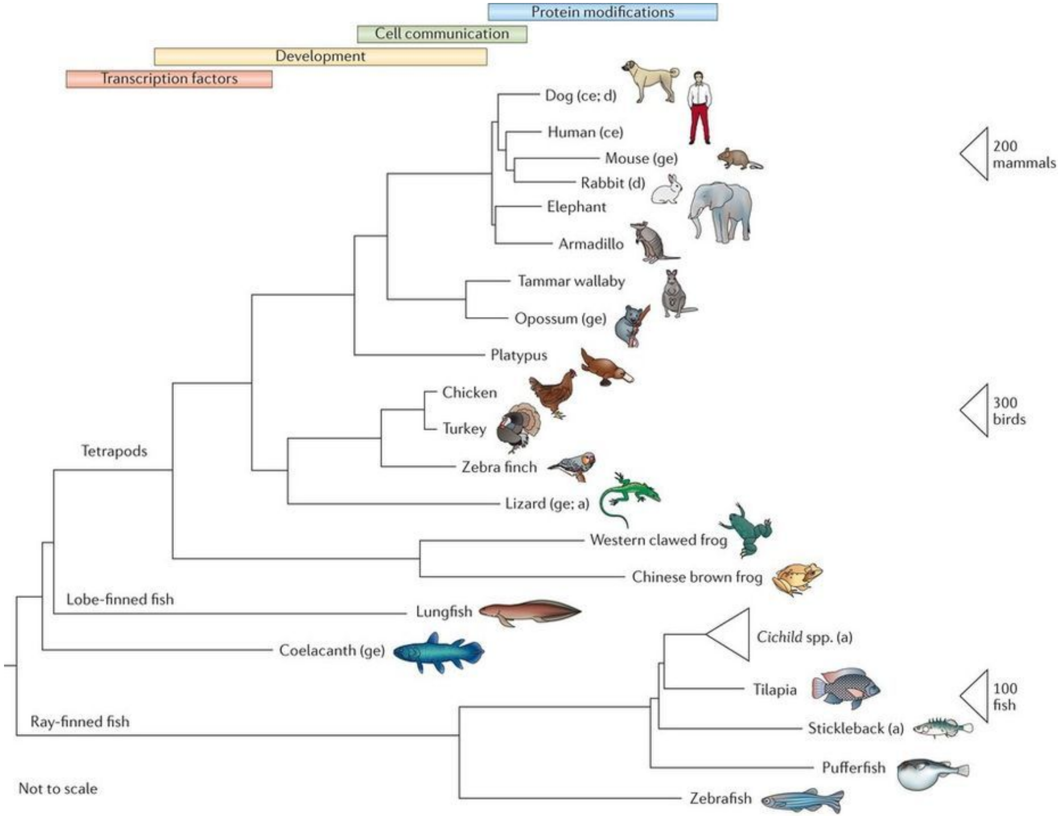


“Evolutionary” relationships between baked goods



On the Origin of Baked Goods
by Means of Natural Consumption

Evolutionary relationships between species



a = adaptation

d = domestication

ge = genome evolution

ce = convergent evolution

Meadows & Lindblad-Toh (2017) Nat. Rev. Gen.

Approaches for constructing phylogenetic trees

Distance-based methods

- UPGMA & Neighbor-Joining
- Calculate pairwise distances & then build tree

Character-based methods

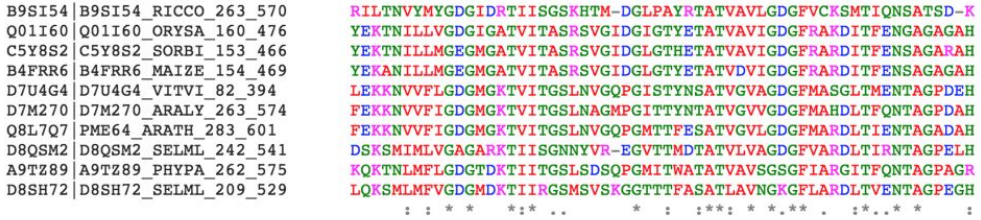
- Maximum parsimony & Maximum likelihood
- Directly build tree by coupling tree proposal & scoring

Distance-based methods for constructing phylogenetic trees

Distance-based methods

- UPGMA & Neighbor-Joining
- Calculate pairwise distances & then build tree

Multiple sequence alignment



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B9SI54|B9SI54_RICCO_263_570   RILTNVYMYGDGIDRTIISGSKHTM-DGLPAYRTATVAVLGDGFVCKSMTIQNSATSD-K
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C5Y8S2|C5Y8S2_SORBI_153_466   YEKTNILLMGEGMGATVITASRSVGIDGLGTHETATVAVIGDGFRARDITFENSAGARAH
B4FRR6|B4FRR6_MAIZE_154_469   YEKANILLMGEGMGATVITASRSVGIDGLGTYETATVDVIGDGFRARDITFENSAGAGAH
D7U4G4|D7U4G4_VITVI_82_394    LEKKNVVFLGDGMGKTVITGSLNVGQPGISTYNSATVGVAGDGFMASGLTMENTAGPDEH
D7M270|D7M270_ARALY_263_574   FEKKNVVFIGDGMGKTVITGSLNAGMPGITTYNTATVGVVGDGFMAHDLTFQNTAGPDAH
Q8L7Q7|PME64_ARATH_283_601    FEKKNVVFIGDGMGKTVITGSLNVGQPGMTTFESATVGVLGDGFMARDLTIENTAGADAH
D8QSM2|D8QSM2_SELML_242_541   DSKSMIMLVGAGARKTIISGNNYVR-EGVTTMDTATVLVAGDGFVARDLTIRNTAGPELH
A9TZ89|A9TZ89_PHYPA_262_575   KQKTNLMFLGDGTDKTIITGSLSDSQPGMITWATATVAVSGSGFIARGITFQNTAGPAGR
D8SH72|D8SH72_SELML_209_529   LQKSMLMFVGDGMDKTIIRGSMSVSKGGTTTFASATLAVNGKGFLARDLTVENTAGPEGH
                                   : : * *   *:* ..       *  :  :**: * *.** .  :*..* *    :
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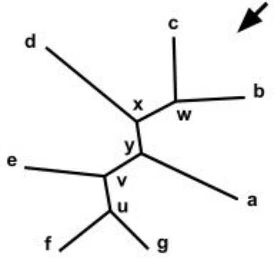
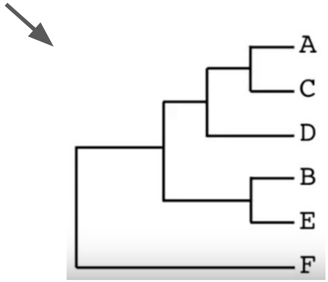
	A	B	C	D	E	F
A		7	2	5	6	9
B			6	6	1	8
C				5	7	8
D					5	7
E						8
F						





Evolutionary relationships between genes in different species

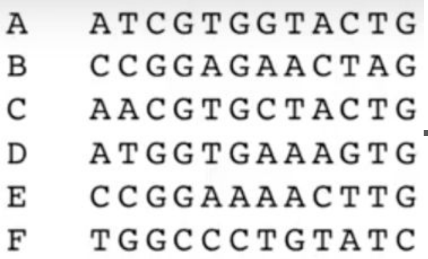
UPGMA (Unweighted Pair Group Method with Arithmetic Mean)

- Rooted tree
- Assumes constant-rate

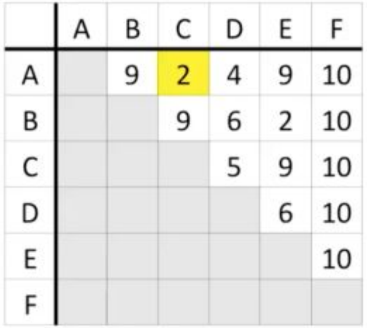
Distance b/w any two clusters A and B, each of size = the mean distance between elements of each cluster

$$\frac{1}{|\mathcal{A}| \cdot |\mathcal{B}|} \sum_{x\in\mathcal{A}} \sum_{y\in\mathcal{B}} d(x,y)$$

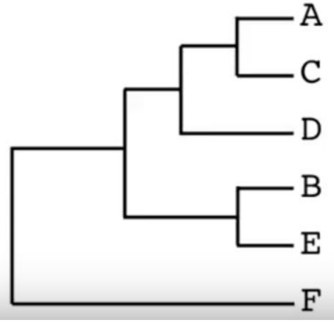
$$d_{(\mathcal{A}\cup\mathcal{B}),X} = \frac{|\mathcal{A}| \cdot d_{\mathcal{A},X} + |\mathcal{B}| \cdot d_{\mathcal{B},X}}{|\mathcal{A}| + |\mathcal{B}|}$$



A	ATCGTGGTACTG
B	CCGGAGAACTAG
C	AACGTGCTACTG
D	ATGGTGAAAGTG
E	CCGGAAAACTTG
F	TGGCCCTGTATC



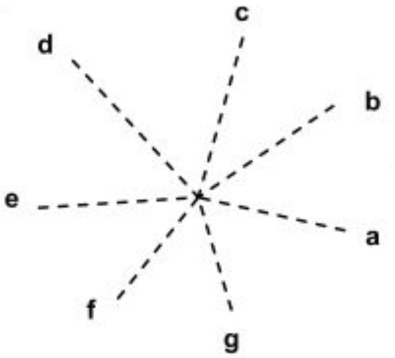
	A	B	C	D	E	F
A		9	2	4	9	10
B			9	6	2	10
C				5	9	10
D					6	10
E						10
F						



Evolutionary relationships between genes in different species

Neighbor-Joining

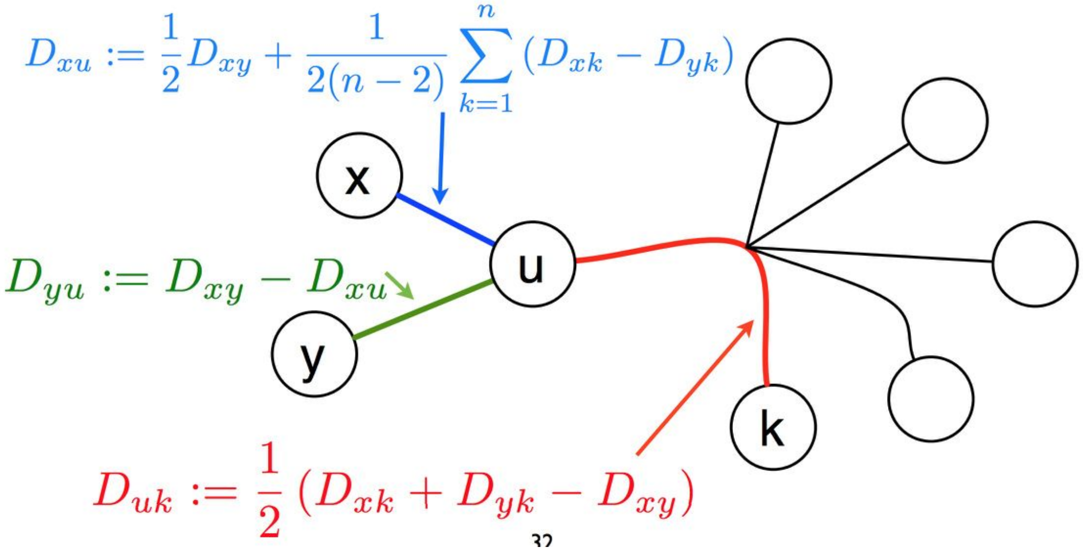
- Unrooted tree
- Does not assumes constant-rate



Choose x, y to merge that minimize:

$$Q(x,y) := (n-2)D_{xy} - \left(\sum_{k=1}^{n} D_{xk} + \sum_{k=1}^{n} D_{yk}\right)$$

Update lengths:



$$D_{xu} := \frac{1}{2}D_{xy} + \frac{1}{2(n-2)}\sum_{k=1}^{n}(D_{xk} - D_{yk})$$

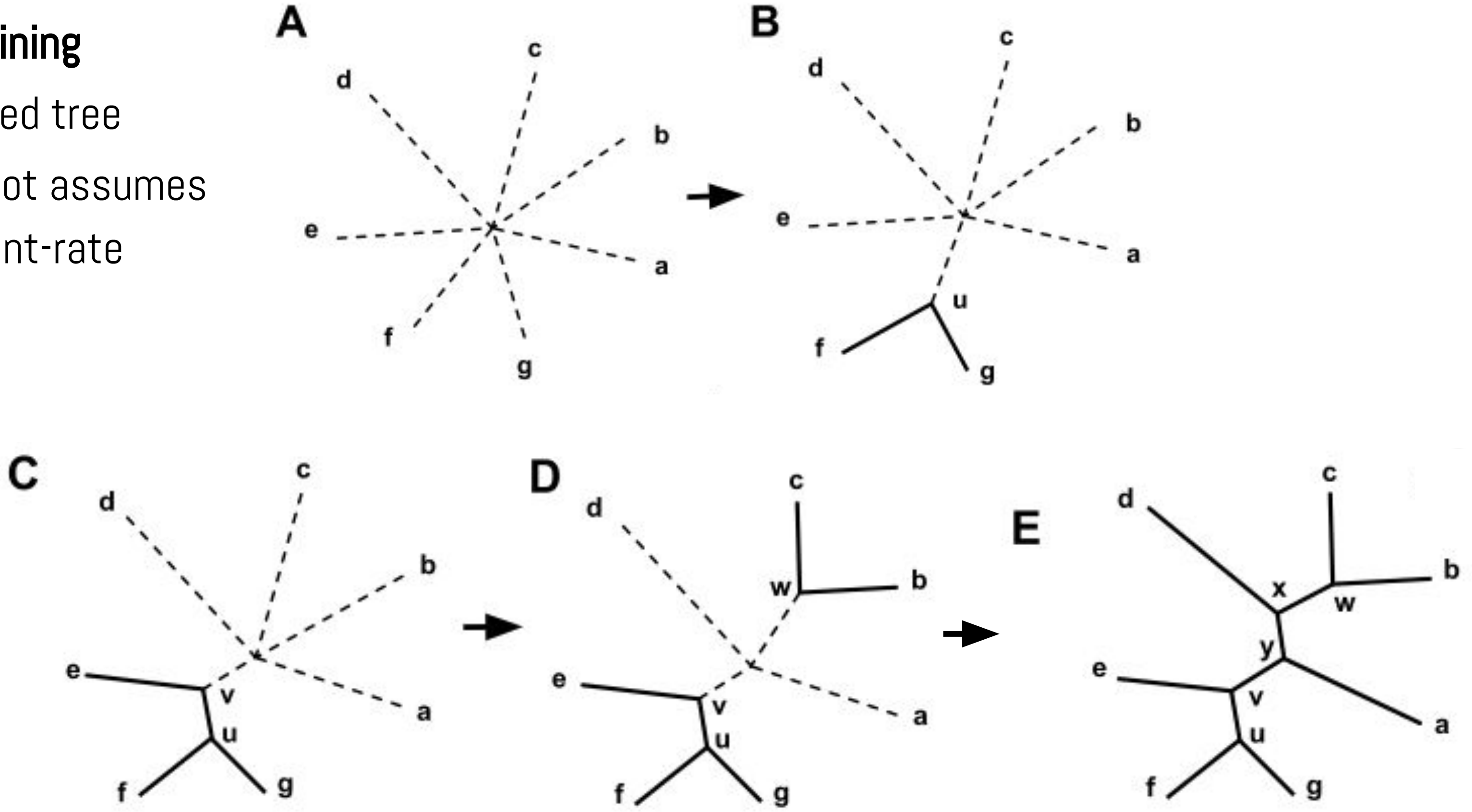
$$D_{yu} := D_{xy} - D_{xu}$$

$$D_{uk} := \frac{1}{2}(D_{xk} + D_{yk} - D_{xy})$$

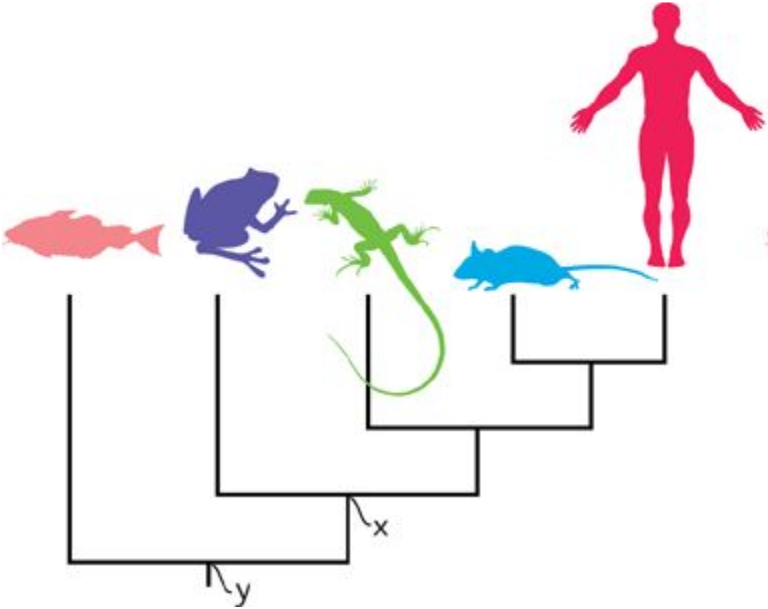
Evolutionary relationships between genes in different species

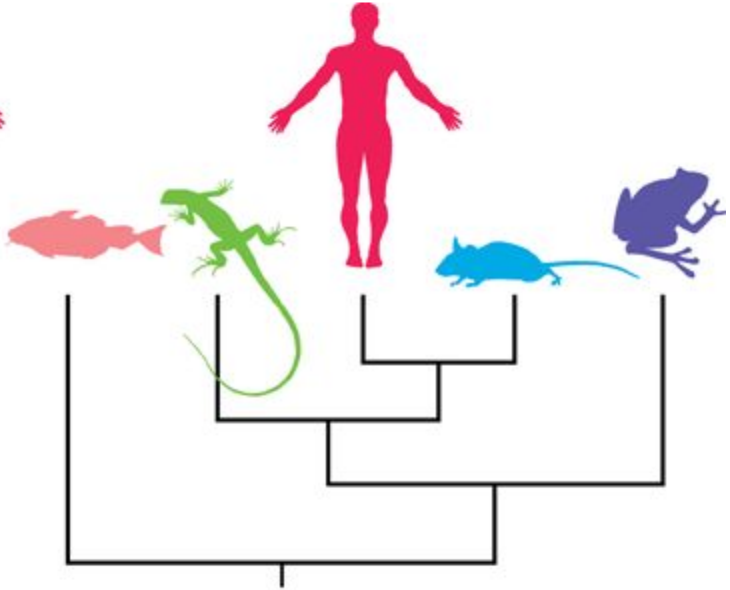
Neighbor-Joining

- Unrooted tree
- Does not assumes constant-rate



Interpreting a tree



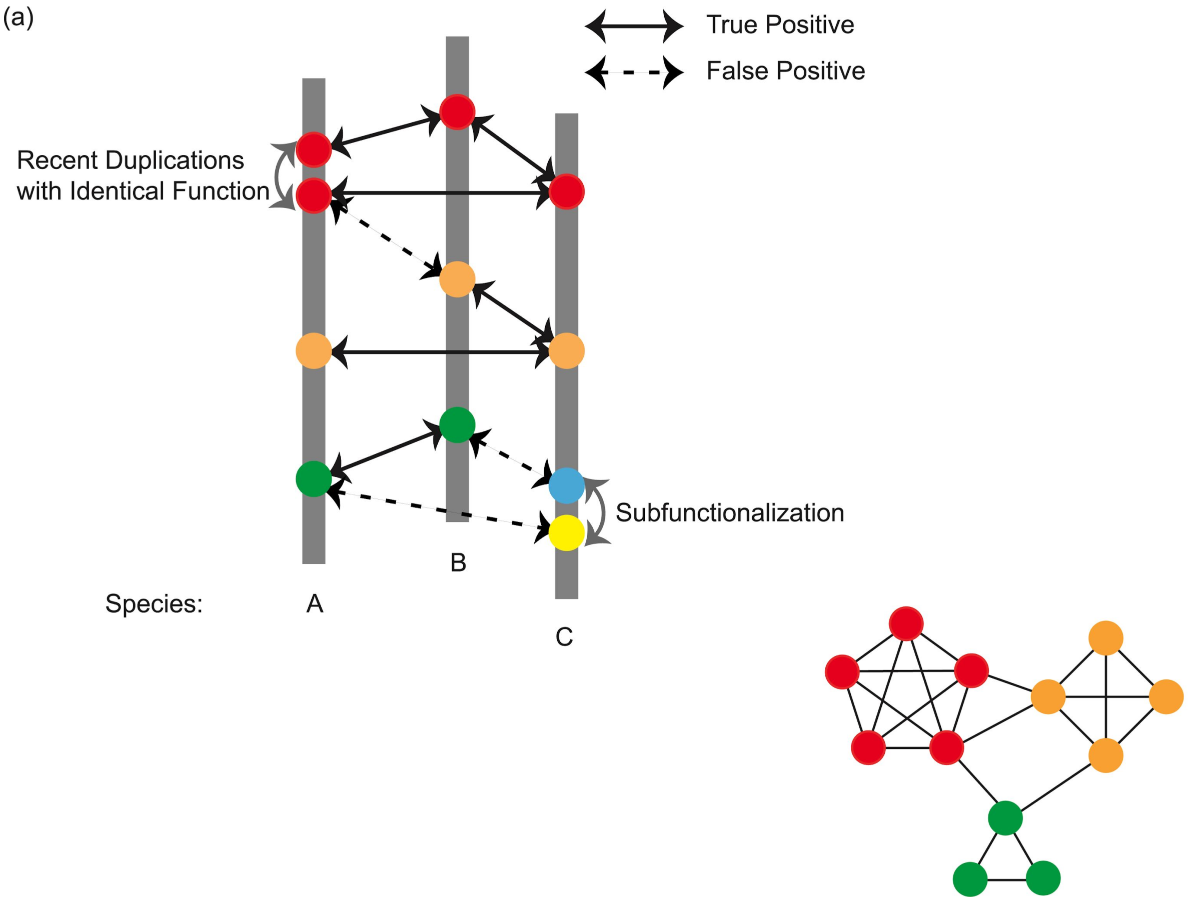


https://science.sciencemag.org/content/310/5750/979

Evolutionary relationships between genes in different species

Evolutionary relationships:

- Orthologs
- Paralogs
  - Subfunctionalization
  - Neofunctionalization



Complicated evolutionary processes:

- gene speciation
- gene duplication
- gene fusion and fission
- horizontal gene transfer
- whole gene deletion

Upcoming project deadline: Project topic due on Jan 31

- Discuss with me *and* any other PI; Read recent papers.
- Briefly describe a project idea:
    - Title
    - Project advisor (if someone outside class)
    - 250-word abstract addressing the following 4 Qs:
        - What is the problem?
        - How is it addressed currently & what are the limitations?
        - What is your approach to addressing it & why is likely to be successful?
        - If successful, why does it matter (what is the impact)?
- Submit a PDF.

Approaches for constructing phylogenetic trees

Distance-based methods

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- Calculate pairwise distances & then build tree

Character-based methods

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Distance-based methods for constructing phylogenetic trees

High computational efficiency (esp. NJ).

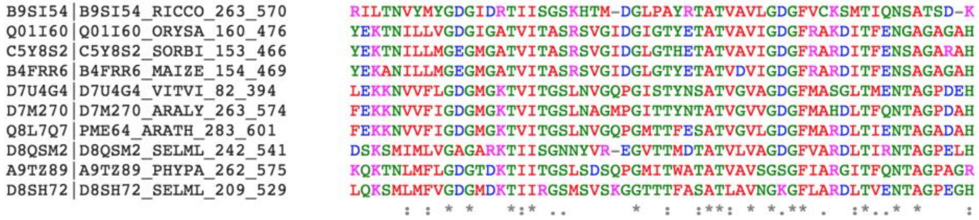
- Useful for analysing large data sets with low levels of sequence divergence.

Can perform poorly for very divergent sequences.

- Large distances involve large sampling errors, and most distance methods (such as NJ) do not account for the high variances of large distance estimates.

Need a realistic substitution model to calculate the pairwise distances. Also sensitive to gaps in the sequence alignment.

Multiple sequence alignment



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B9SI54|B9SI54_RICCO_263_570   RILTNVYMYGDGIDRTIISGSKHTM-DGLPAYRTATVAVLGDGFVCKSMTIQNSATSD-K
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                                   : : * *   *:* ..      *  :  :**: * *.** .  :*..* *    :
```

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C				5	7	8
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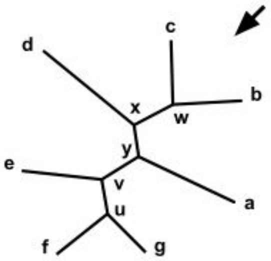
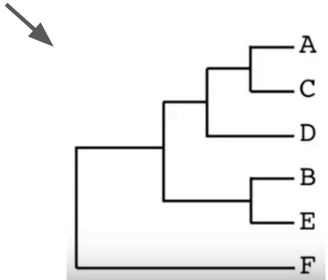
E						8
F						





Rooted vs. Unrooted trees

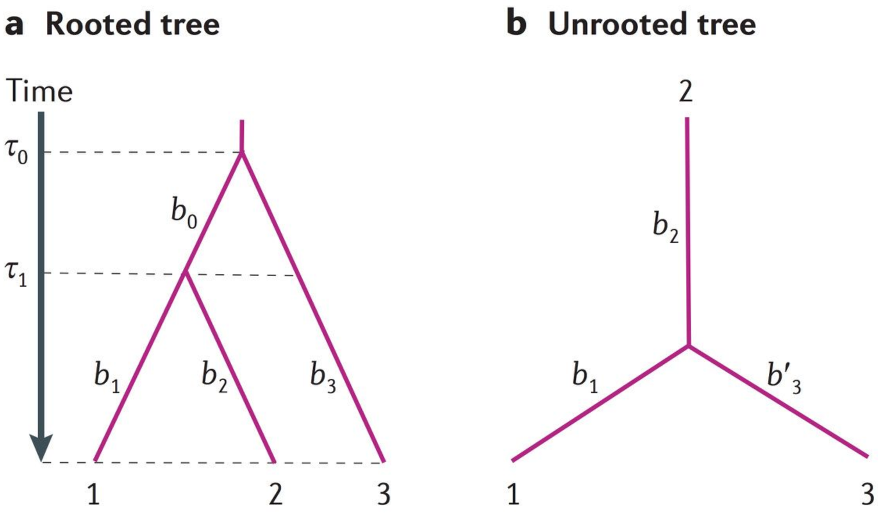
Substitution rate is constant over time or among lineages → the molecular clock holds.

The tree will then have a root (inferring rooted tree is called molecular clock rooting).

- The tree will be ultrametric: distances from the tips of the tree to the root are all equal (b0 + b1 = b0 + b2 = b3).

A rooted tree for *s* species:

- Can then be represented by the ages of the *s* – 1 ancestral nodes
- Involves *s* – 1 branch-length parameters.



For distantly related species, the clock hypothesis should not be assumed.

Yang & Rannala (2012) Nat. Rev. Genet.

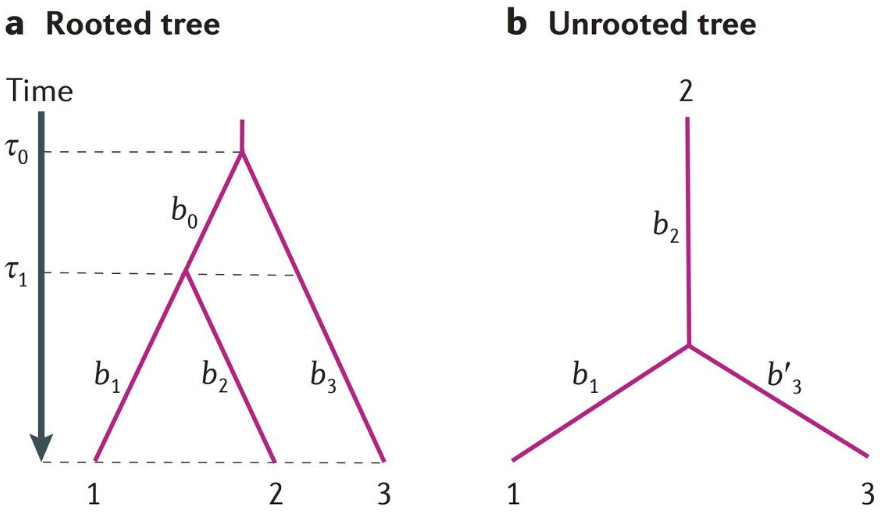
Rooted vs. Unrooted trees

If every branch on the tree is allowed to have an independent evolutionary rate → unrooted trees.

An unrooted tree for *s* species has 2*s* – 3 branch length parameters.

Rooting a tree using outgroup rooting:

- Include outgroup species (a species/genes known to be more distantly related than the species/genes of interest).
- Root is located along the branch that leads to the outgroup so that the tree for the ingroup species is rooted.



Yang & Rannala (2012) Nat. Rev. Genet.

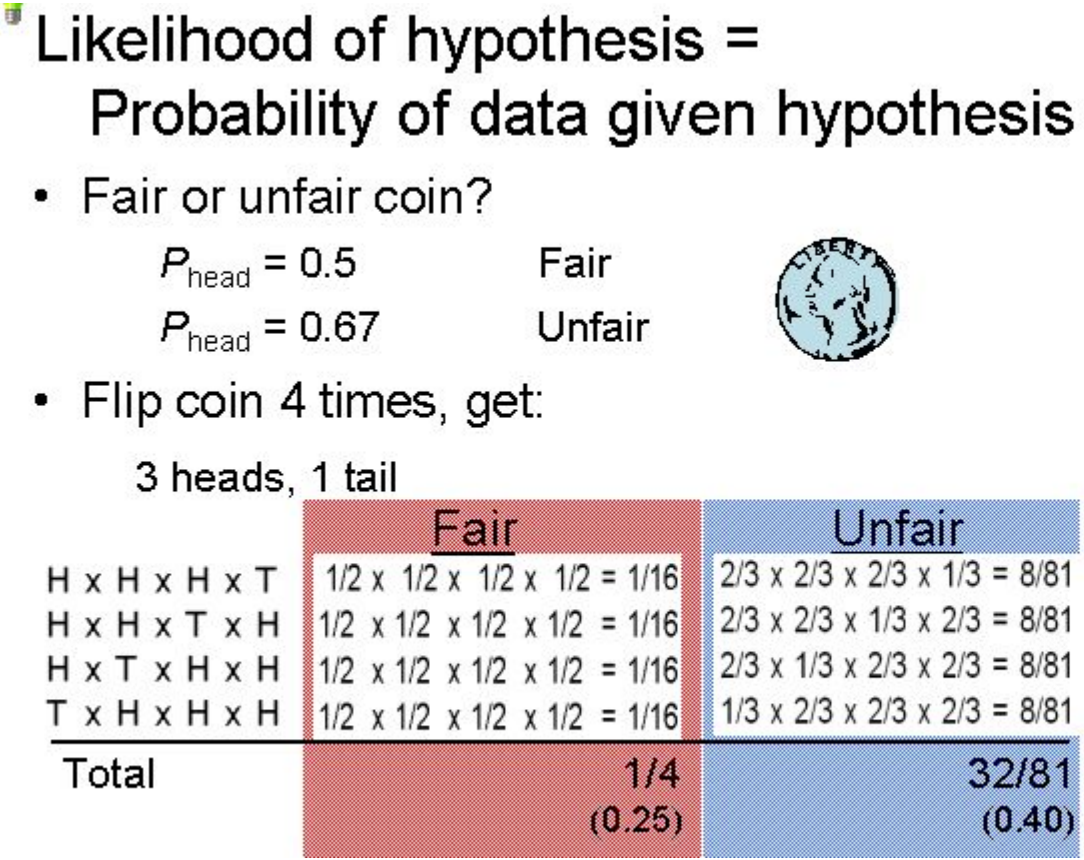
Maximum likelihood

A general statistical method for estimating unknown parameters of a probabilistic model by maximizing a function, so that under the assumed model, the observed data is most probable.

- Given data, assume it comes from a model (E.g. normal distribution).
- Likelihood ~ the probability of observing the data given the model: **P(Data | Model)**.
- Examine this likelihood function to see where it is greatest (meaning, different values of the parameters of the model: e.g. μ & σ).
- The values of the parameters at that point is the **maximum likelihood estimate** of the parameters (found numerically by some iterative optimization procedure).

MLEs have desirable asymptotic properties: Unbiased, Consistent (approach true values), & Efficient (have the smallest variance among unbiased estimates).

Maximum likelihood



Likelihood of hypothesis =
Probability of data given hypothesis

- Fair or unfair coin?

$P_{\text{head}} = 0.5$ Fair

$P_{\text{head}} = 0.67$ Unfair

- Flip coin 4 times, get:

3 heads, 1 tail

	Fair	Unfair
H x H x H x T	1/2 x 1/2 x 1/2 x 1/2 = 1/16	2/3 x 2/3 x 2/3 x 1/3 = 8/81
H x H x T x H	1/2 x 1/2 x 1/2 x 1/2 = 1/16	2/3 x 2/3 x 1/3 x 2/3 = 8/81
H x T x H x H	1/2 x 1/2 x 1/2 x 1/2 = 1/16	2/3 x 1/3 x 2/3 x 2/3 = 8/81
T x H x H x H	1/2 x 1/2 x 1/2 x 1/2 = 1/16	1/3 x 2/3 x 2/3 x 2/3 = 8/81
Total	1/4 (0.25)	32/81 (0.40)

https://www.ncbi.nlm.nih.gov/Class/NAWBIS/Modules/Phylogenetics/phylo15.html

Maximum likelihood for tree estimation

Model: The tree; Parameters: The tree's branch lengths.

Use a specific substitution model:

- Assume independent evolution of sites in the sequence → likelihood = product of the probabilities for different sites.
- Probability at any particular site = average over the unobserved character states at the ancestral nodes.

ML for tree inference is equivalent to comparing many statistical models, each with the same number of parameters.

Two optimization steps:

1. Optimization of branch lengths to calculate the tree score for each candidate tree.
2. A search in the tree space for the maximum likelihood tree.

Yang & Rannala (2012) Nat. Rev. Genet.

Maximum likelihood for tree estimation

ML is used exclusively these days for inferring deep phylogenies using conserved proteins.

- All model assumptions are explicit, so that they can be evaluated and improved.
- Availability of a rich repertoire of sophisticated evolutionary models.
  - Including models that accommodate variable amino acid substitution rates among sites or different amino acid frequencies among sites.
- Great for understanding the process of sequence evolution.
  - The likelihood ratio test can be used to:
    - Examine the fit of evolutionary models
    - Test interesting biological hypotheses (e.g. molecular clock) and selection affecting protein evolution.

Yang & Rannala (2012) Nat. Rev. Genet.

Maximum likelihood for tree estimation

There are some drawbacks!

- The attractive asymptotic properties of MLEs apply to parameter estimation when the true tree is given but not to the maximum likelihood tree.
- The likelihood calculation, particularly tree search under the likelihood criterion, is computationally demanding.
- The method has potentially poor statistical properties if the model is mis-specified.

Yang & Rannala (2012) Nat. Rev. Genet.