Universidade Federal do Paraná

Phylogenetics
An Introduction

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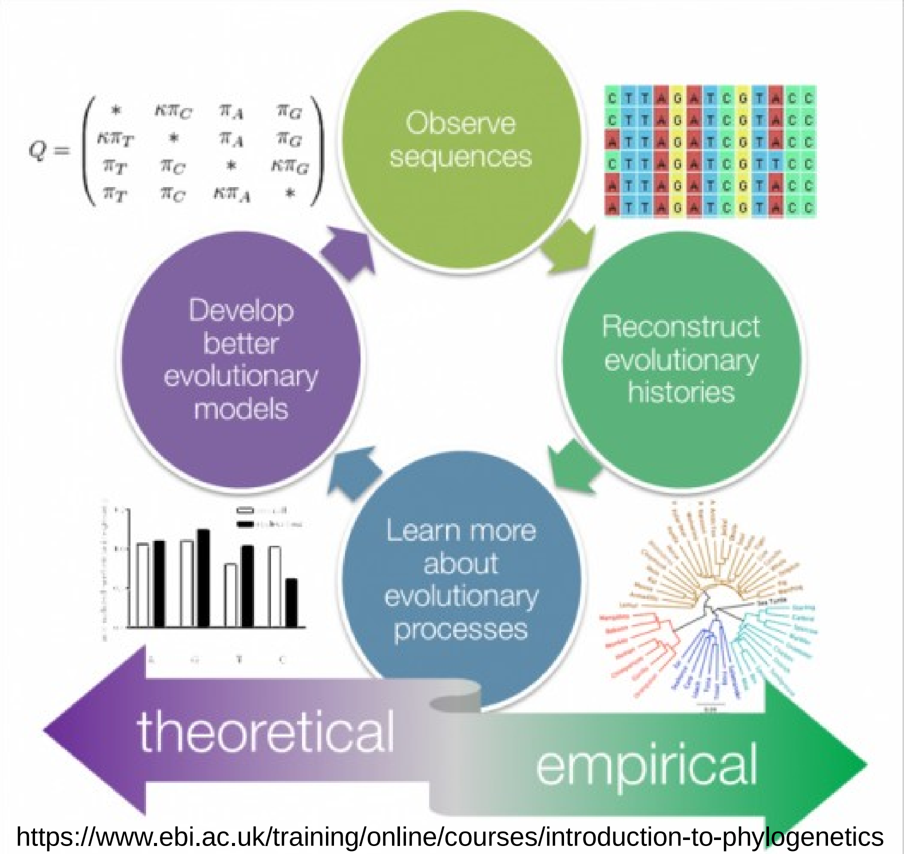
What is phylogenetics?

- Study of evolutionary relationships among biological entities
  - species
  - individuals
  - genes

The major elements of phylogenetics



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Why use molecular data?

- Today, almost all evolutionary relationships are inferred from molecular sequence data
- This is because:
  - DNA is the inherited material
  - We can now easily, quickly, inexpensively and reliably sequence genetic material
  - Sequences are highly specific and are often information rich

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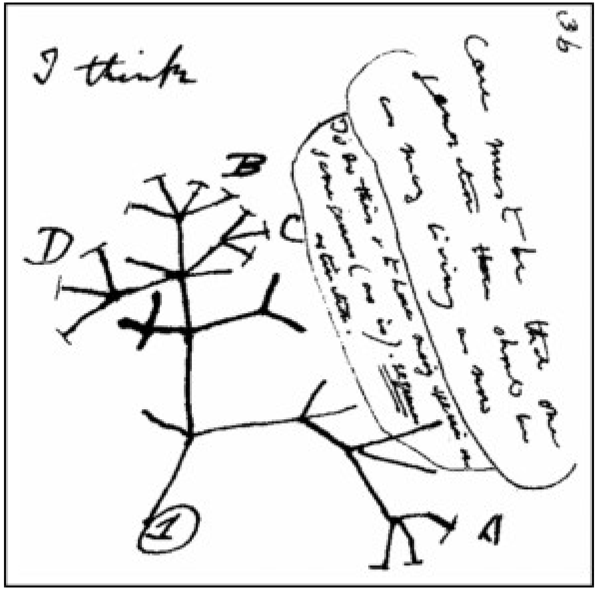
Tree thinking

- One the first sketches of a phylogenetic tree was made by Charles Darwin



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Aspects of phylogenies

- Topology – inferred from phylogeny
  - Branches – their length can be inferred from phylogeny
  - Nodes
    - Tips
    - Internal nodes
    - Root
- Confidence

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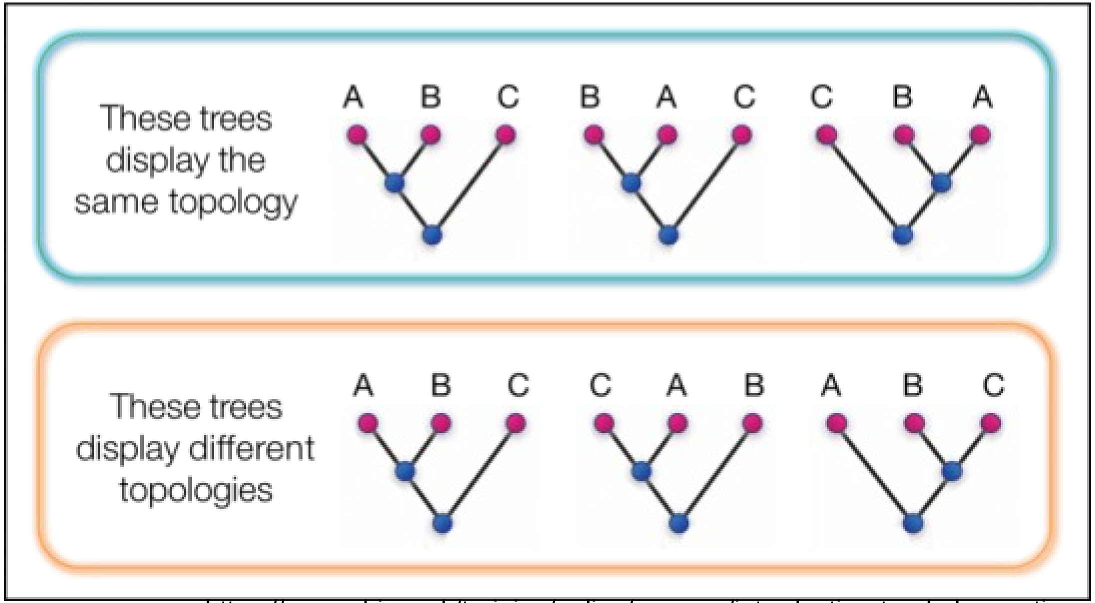
Topology

- Is the branching structure of the tree
- It is of particular biological significance because
  - indicates patterns of relatedness among taxa
  - trees with the same topology have the same biological interpretation



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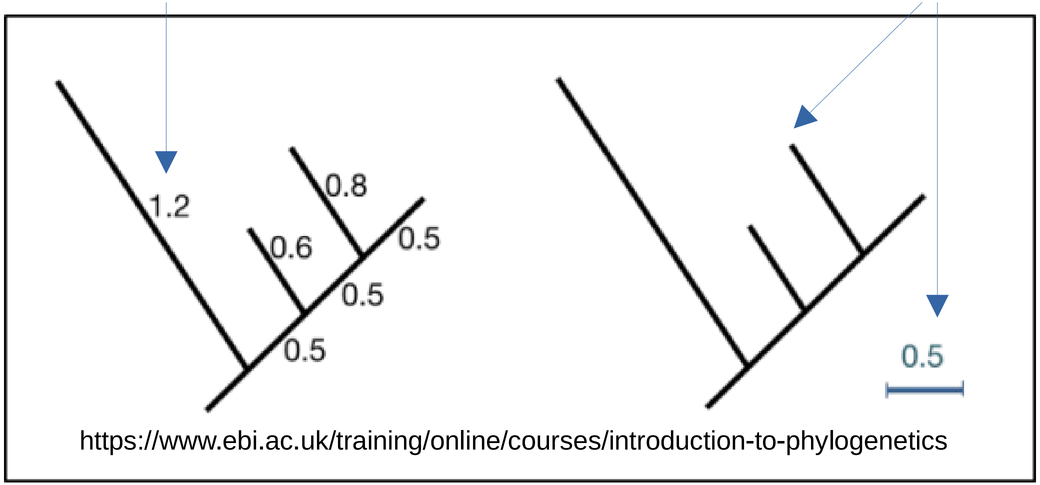
Branches

- Show the path of transmission of genetic information
- **Branch lengths** indicate genetic change
  - the longer the branch, the more genetic change (or divergence) has occurred
  - estimates the average number of nucleotide or protein substitutions per site



Branch lengths shown

Branch lengths scaled

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How do we estimate genetic change?

- A naïve method
  - align pairs of sequences
  - count up the number of differences
  - divide by the sequence length
- Assumes that...
  - Every substitution was observed
    - Does not account multiple substitutions
  - Every substitution is equally likely
- Evolutionary models correct for these unrealistic situations

Human **ATGTTGACTC**
Mouse **ATGCTGACTC**

1 difference
10 sites
1/10 = 0.1 substitutions per site (*p* distance)

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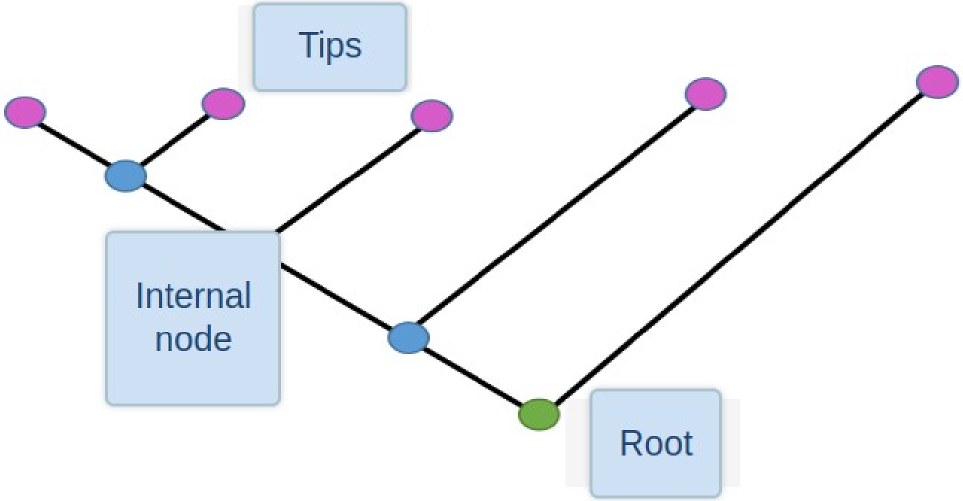
Nodes

- For the particular group of organisms being studied
  - Tips = currently sequences/organisms
  - Internal node = ancestral sequences/organisms
  - Root = common ancestral sequence/organism



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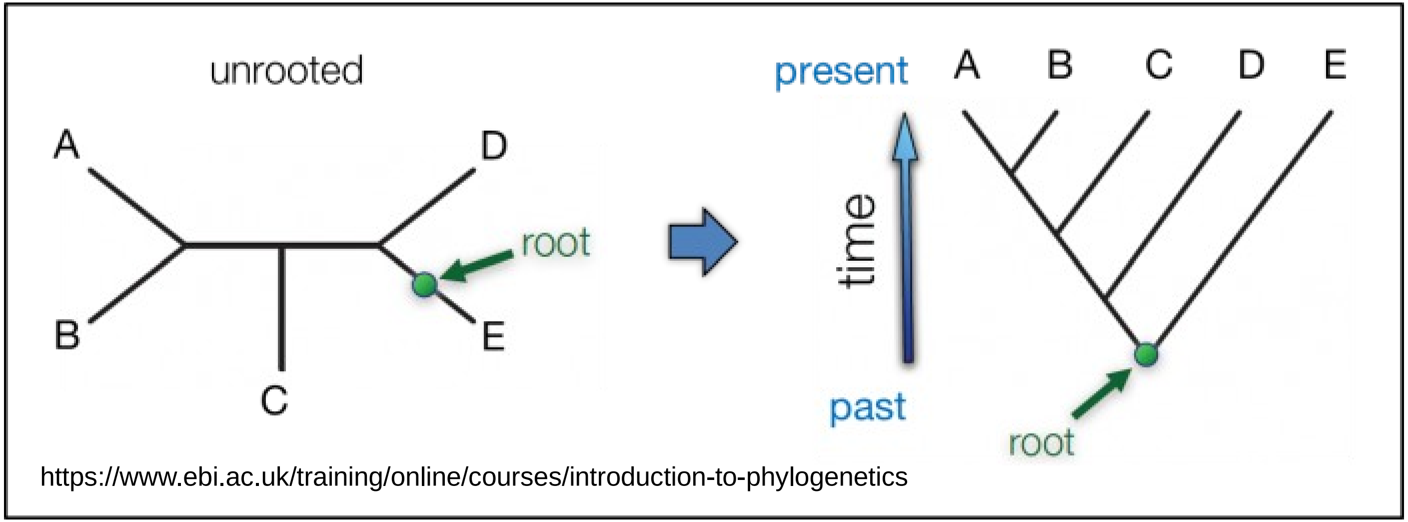
Internal nodes and root are hypothetical (inferred by phylogenetic methods)

Root

- Is the **most recent common ancestor** (**MRCA**) of all of the *taxa* in the tree
    - the oldest part of the tree
    - tells us the direction of evolution
- Most of phylogenetic methods do not estimate the position of the root
    - in part because this increases the number of possible trees



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Rooting a tree

- There are two main approaches that we can use to root a tree
  - **Outgroup rooting**
  - **Midpoint rooting**

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Outgroup rooting

- Include one or more sequences that we know are more distantly related to our sequences of interest than they are to one another
- The root estimate is then simply the point at which our outgroup(s) join the rest of our tree of interest
- The best possible outgroups are those most closely related to our sequences of interest
    - If outgroups are too distantly related then they can be unreliable as they may be difficult to align or become saturated with substitutions

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Midpoint rooting

- Assume that all sequences evolve at the same rate
  - this assumption does not hold for many biological datasets!
- The root is positioned at the midpoint between the two longest branches

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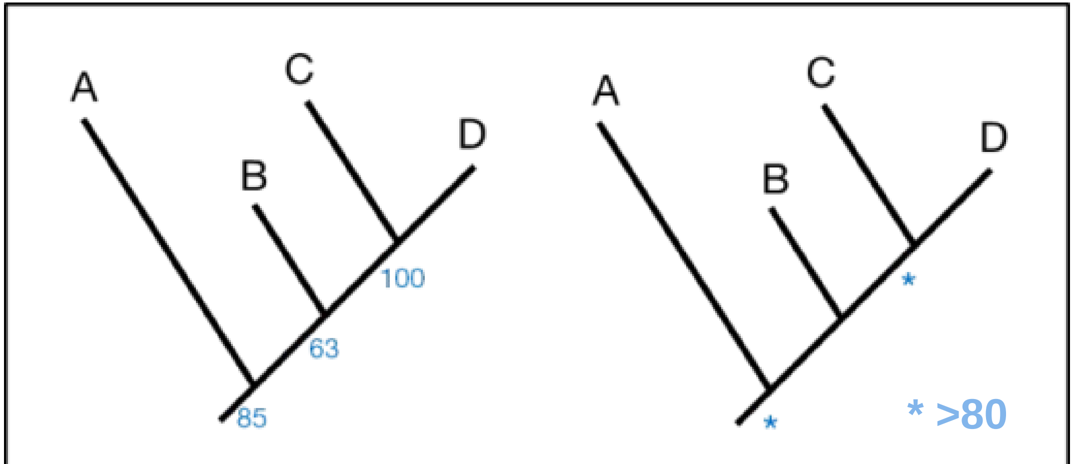
Approaches for estimating confidence

- Several approaches to estimate confidence in tree topology...
  - Bootstraps
  - Likelihood
  - Bayesian
- Confidence estimates refer to the internal branches

typical representations of
confidence on part of a phylogeny



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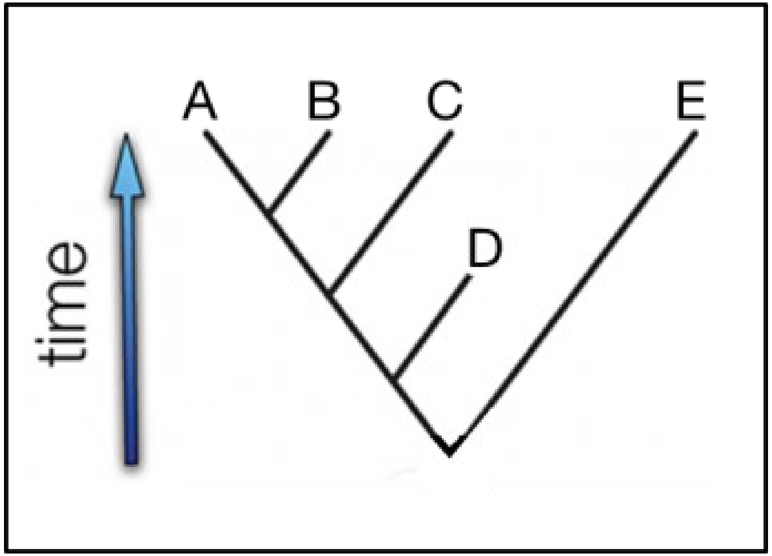
Relating distance, rate and time

- Evolutionary rate and time are confounded
  - The genetic change is calculated by a combination of the rate of substitution and the time that has elapsed
  - **Genetic change (substitutions/site) =** **Evolutionary rate (substitutions/site/year) x Divergence time (years)**



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without any other prior information about the tree, we do not know whether branch **D** represents an extinct or frozen lineage that was sampled a long time ago; a lineage with a relatively slow evolutionary rate; or some combination of both

Evolutionary rate and time are confounded

- Genetic change/distance – **substitutions/site**
  - Calculated by a combination of…
    - Rate substitution (**substitutions/site/year**)
    - Time that has elapsed (**years**)
    - **GENETIC CHANGE =**
      EVOLUTIONARY RATE X DIVERGENCE TIME

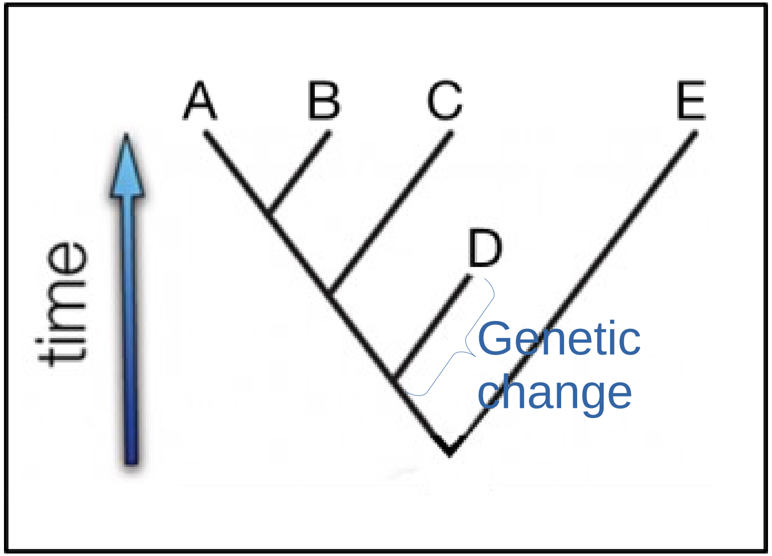
Genetic change → the branch lengths estimated in a phylogenetic tree

Relating distance, rate and time

Genetic change	**=**	**Evolutionary rate**	**X**	**Divergence time**
(substitutions/site)		(substitutions/site/year)		(years)

Not possible to know whether branch **D** represents

→ an extinct lineage
→ a lineage with a relatively slow evolutionary rate
→ or some combination of both



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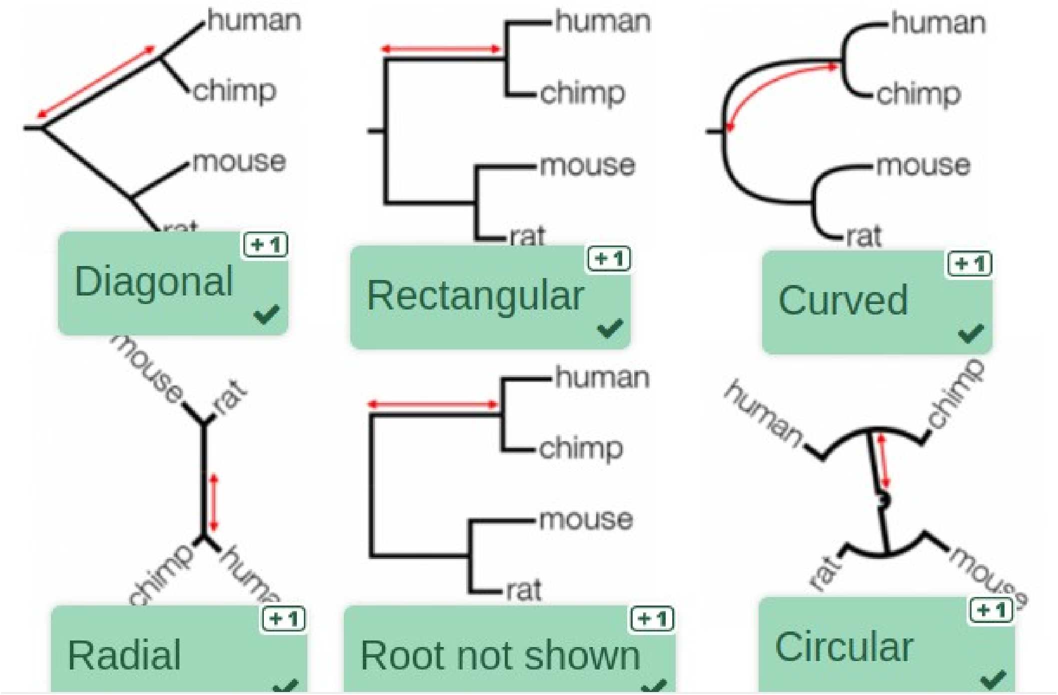
Alternative representation of phylogenies

- The same topology can be drawn in lots of different ways
- Red lines indicate the same branch in each representation
- Trees can be rotated on the page and still depict the same tree
- These trees are not drawn to the same scale.



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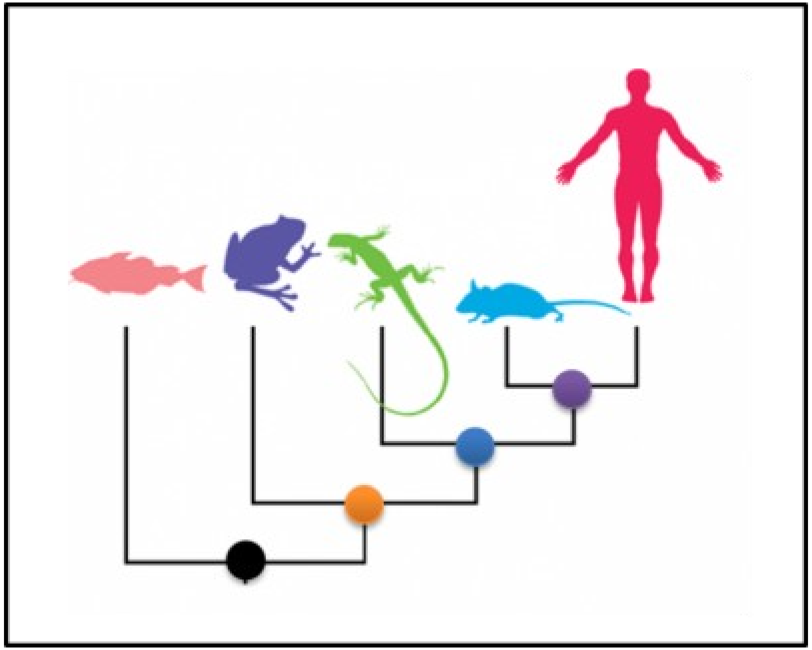
Interpreting patterns of relatedness

- The key to interpreting patterns of relatedness in an evolutionary context is to trace back to the point in the tree where taxa share their most recent common ancestor (MRCA)



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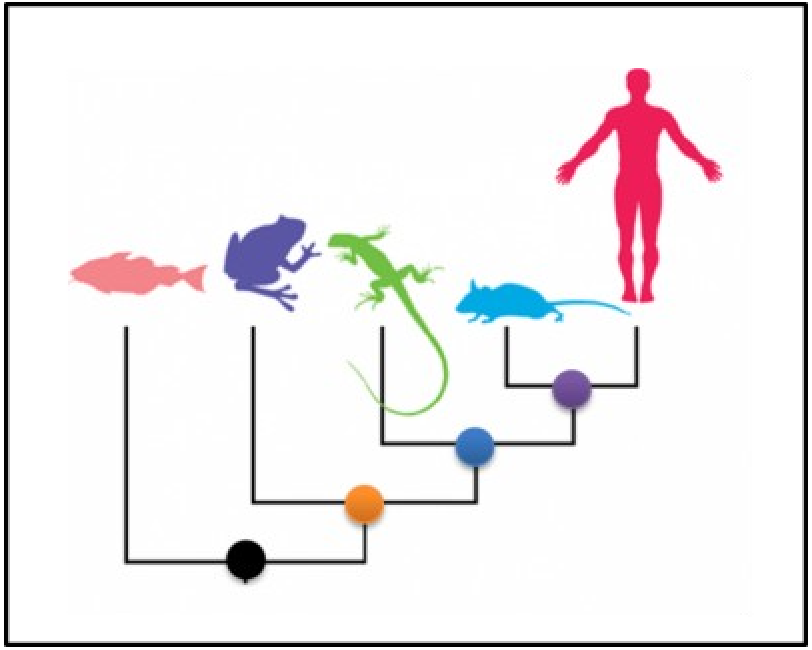
Interpreting patterns of relatedness

- From figure tthe following statements can be made
  - Humans are closer to mice than to lizards
    - Because humans and mice share a common ancestor more recently (purple spot)
  - Frogs are closer to lizards then to fish
    - Because frogs and lizards share a common ancestor more recently (orange spot)
  - Fish are equally related to mice and frogs
    - Mice and frogs share the same common ancestor (black spot) with fish
    - So neither species is more closely related to fish
- Remember
  - These interpretations rely on our tree being rooted because the root is needed to define the direction of evolution
  - If we rotate the branches to change the topology of the tree then the tree still has the same biological meaning and evolutionary relationships



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Further reading

- Phylogenetics – an Introduction
  - Online Tutorial – EMBL-EBI Training
  - https://www.ebi.ac.uk/training/online/courses/introduction-to-phylogenetics
- How to read a phylogenetic tree
  - https://artic.network/how-to-read-a-tree.html
- Understanding Evolution
  - UC Museum of Paleontology – University of California, Berkeley
  - https://evolution.berkeley.edu/teach-evolution
  - Misconceptions about evolution (https://evolution.berkeley.edu/teach-evolution/misconceptions-about-evolution)

Further reading

- Baum DA, Smith SD, Donovan SS. Evolution. The tree-thinking challenge. Science (New York, N.Y.). 2005 Nov;310(5750):979-980. DOI: 10.1126/science.1117727. PMID: 16284166.
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- Chapter 1. Molecular Evolution and phylogeny: what, when, why and how? Kolekar, P; Kale, M; Kulkarni-Kale, U. In: Lopes, HS; Cruz, LM (ed.) Computational biology and applied bioinformatics. InTech, 2011
  - https://www.intechopen.com/books/249
- Chapter 5. Protein Function Prediction. Cruz LM et al. Kaufmann, M.; Klinger, C.; Savelsbergh, A. (eds.) Functional Genomics - Methods and Protocols. Third Edition - Methods in Molecular Biology 1654 - Springer protocols. Humana Press.
- Barry G. Hall, Building Phylogenetic Trees from Molecular Data with MEGA, Molecular Biology and Evolution, Volume 30, Issue 5, May 2013, Pages 1229–1235
  - https://doi.org/10.1093/molbev/mst012