Bristol Bioinformatics Workshop 2018

Tom Williams, Gary Barker, Tom Batstone

5th-9th November, 2018

Course aims

- Introduction to **a general approach** to doing bioinformatics
- **Core concepts** and signposts to the literature
- **Practical experience** to get your hands dirty

Course structure

- **Day 1 (today)**
  Introduction
  Comparative genomics (theory)
  Comparative genomics (GUI/web practical)

- **Day 2:**
  Introduction to UNIX
  Comparative genomics (practical)
  Phylogenetics (theory)
- **Day 3:**
  Phylogenetics (practical)

Today's schedule

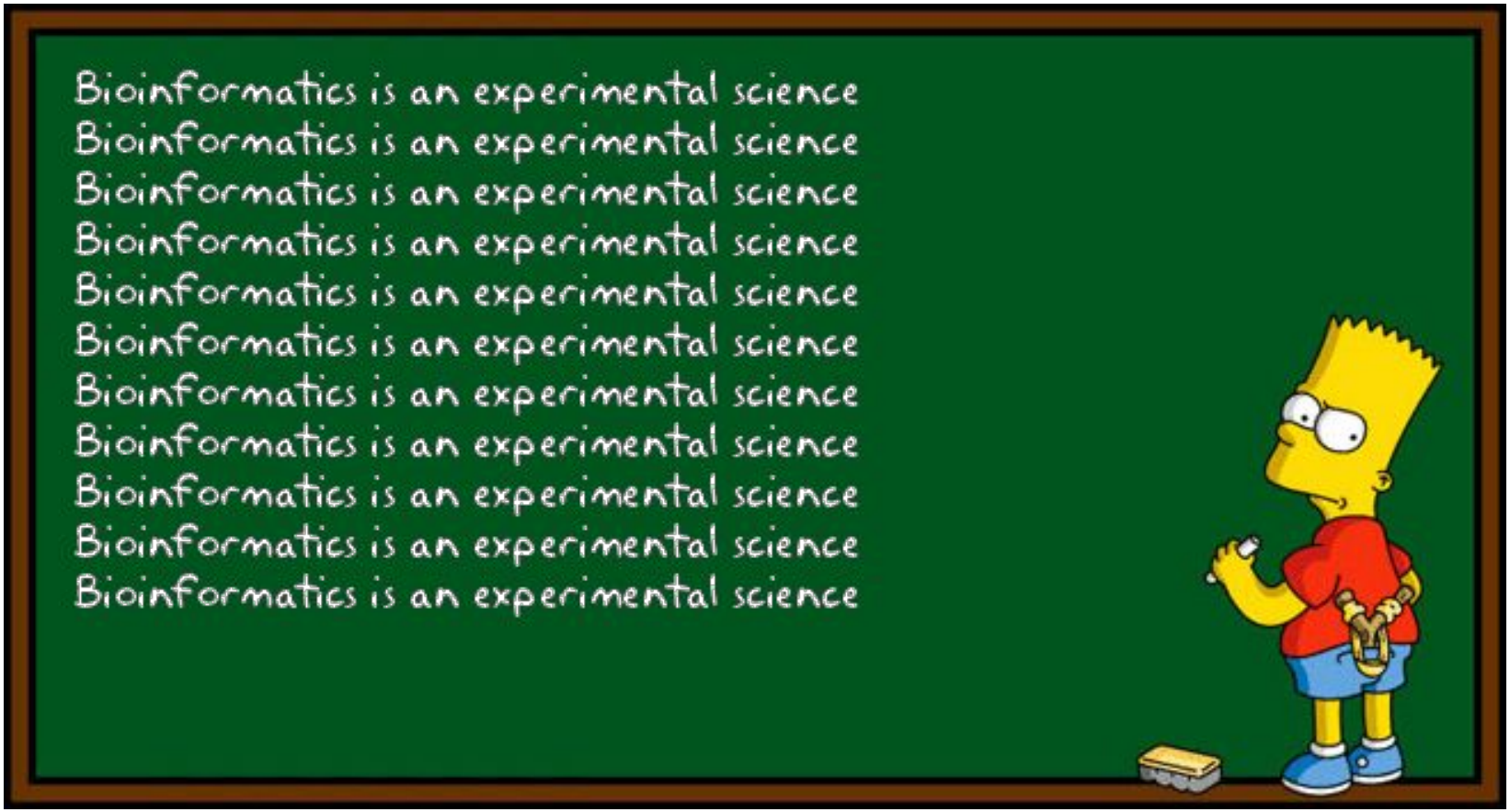
Time	Material	Lecturer
10.00-10.30	Course introduction and overview	Tom W.
10.30-13.00	DNA sequencing, genome assembly and comparative genomics	Gary B.
14.00-17.00	Introduction to phylogenetics	Tom W.

Protip

Participation is key to getting the most out of this kind of course.

Please ask (and answer) questions!

The basic idea



Doing science: a workflow

Collect, collect, collect, collect, collect, collect, collect, analyze, Nature

We use computers to explore data or test hypotheses

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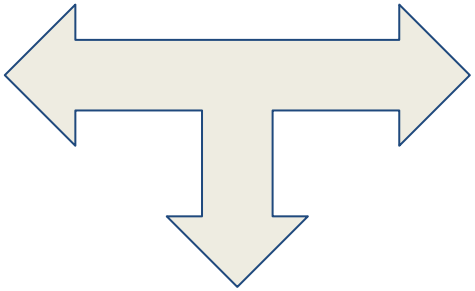
Doing science in an integrated way



“An **hour** of #bioinformatics **can save** a week in the **lab**. ALSO An **hour in the lab can save** a week of #bioinformatics” - Torsten Seeman

Two kinds of questions frequently encountered when doing bioinformatics

Scientific questions:

- Is my hypothesis testable, and with what data?
- What assumptions can I make when analyzing the data?
- Which of the available analysis methods is most appropriate?

Is what I'm doing sensible?

Technical questions:

- What software/methods are available for problem X?
- How do I install this software on my computer?
- How do I fix this error message?
- How do I make it give me the answer?

Why won't it $%$£"!# work?

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Why won't it $%$£"!# work?

Bioinformatics: in sum

- Computers provide powerful tools for analyzing data
- They aren’t black boxes, into which you feed data and out of which the result comes
- They can speed up (not replace) our thinking, and make many types of analysis possible
- Be as critical as you would be in any other area of science

Introduction to molecular phylogenetics

(a) Core concepts

Tom Williams

Schedule for the phylogenetics material

Today: Core concepts in phylogenetics.
Core concepts: games and activities.

Friday morning: A workflow for phylogenetics.
Some theory.
The molecular clock.

Friday afternoon: **Phylogenetics practical**.
Principles of phylogenomics.
Games and activities.

Core concepts in molecular phylogenetics

- Some justification for doing phylogenetics
- Homology and analogy
- Interpreting phylogenetic trees
- Naming groups on trees
- The root: a very special node on the tree

Some quizzes.

What is phylogenetics?

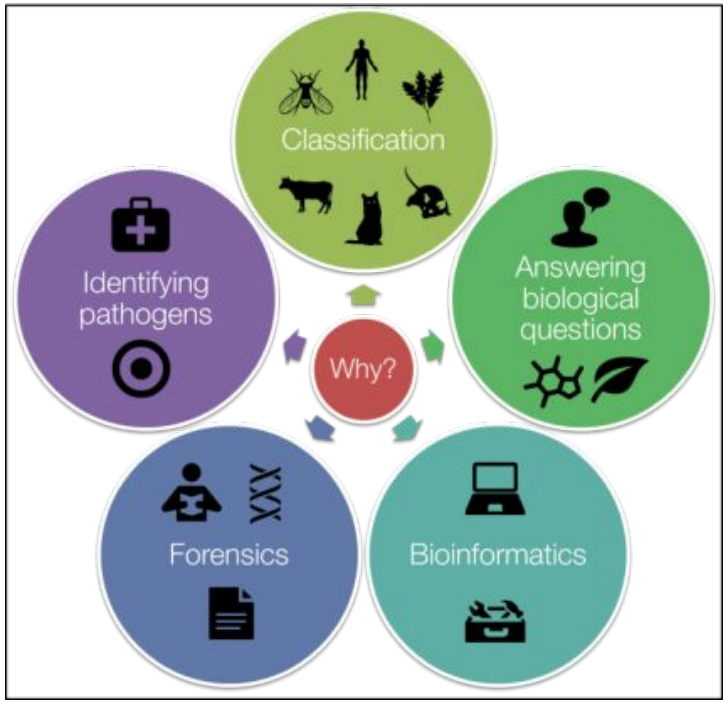
The study of the evolutionary relationships among evolving entities (species, genomes, genes, individuals).

"The time will come, though I shall not live to see it, when we shall have fairly true genealogical trees of each great kingdom of nature"

- Darwin (1857)

"One of the grand biological ideas is to be able to work out the complete detailed quantitative phylogenetic tree --- the history of the origins of all living species, back to the very beginning."

- Dayhoff and Eck (1972)



(Various practical applications)

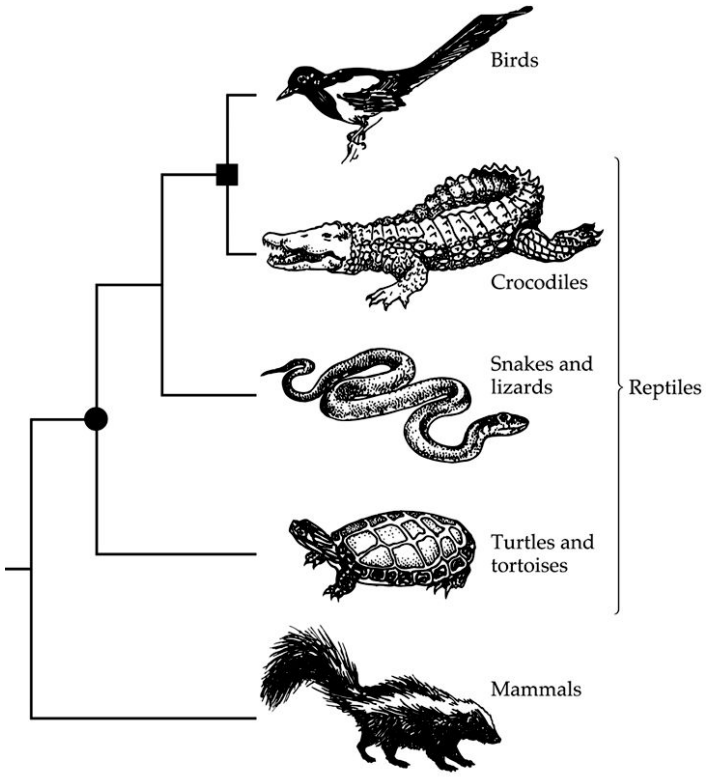
What is a phylogeny (evolutionary tree)?

A branching diagram representing the genealogical relationships among species

Can be inferred using:

- Genetic (molecular) data
- Morphological data
- Any kind of character/trait data

Normally, we want to infer a tree (or trees) that make sense of the character data



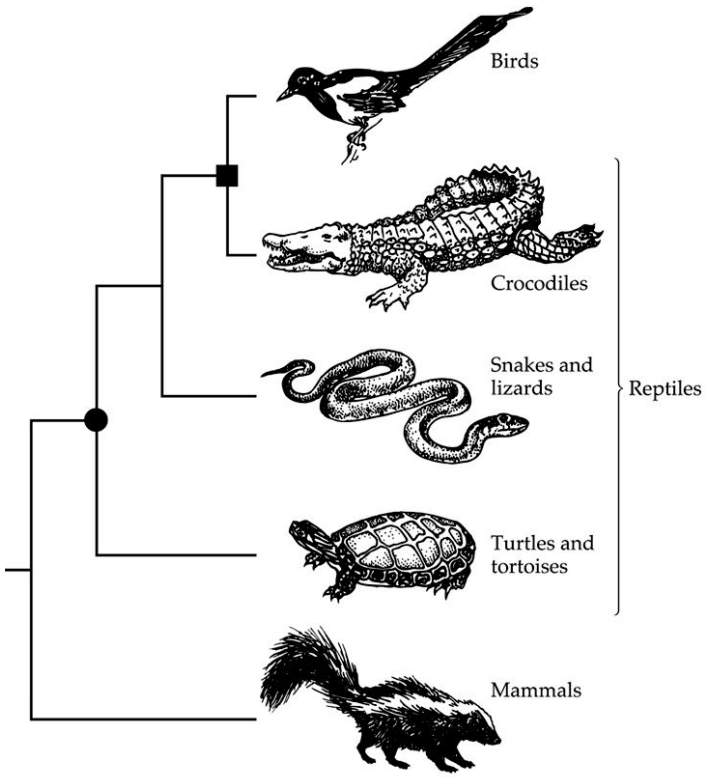
1. Homologous vs. analogous characters

- Homology is similarity due to common ancestry

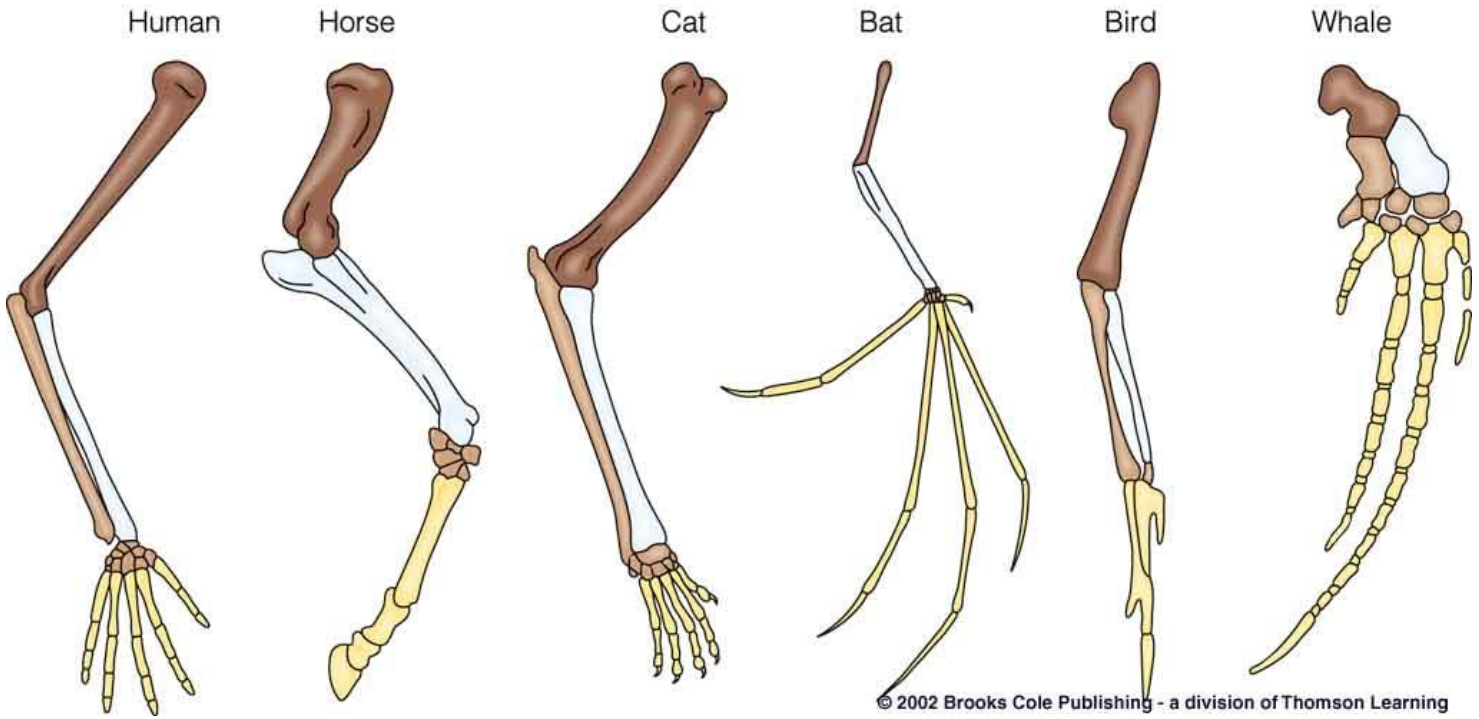
  "the same organ under every variety of form and function" (Owen)

- Analogy is similarity due to chance or convergent evolution.

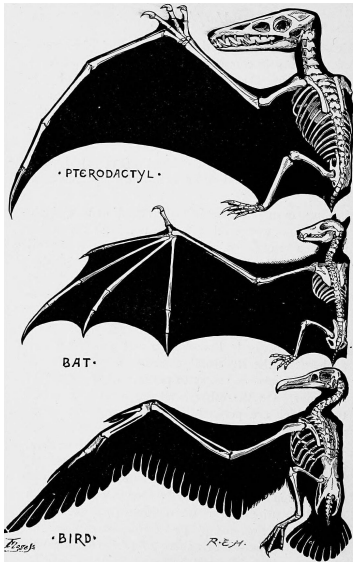
  Superficial or misleading similarities.



Vertebrate limbs are homologous



Are these wings homologous?



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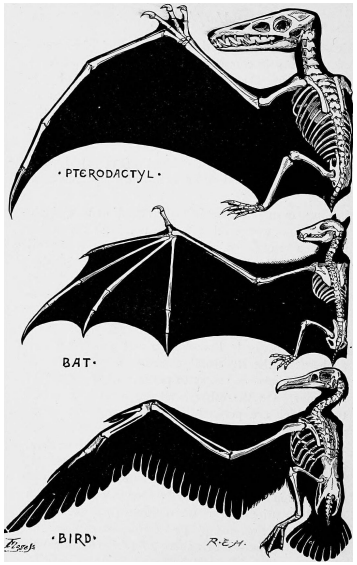
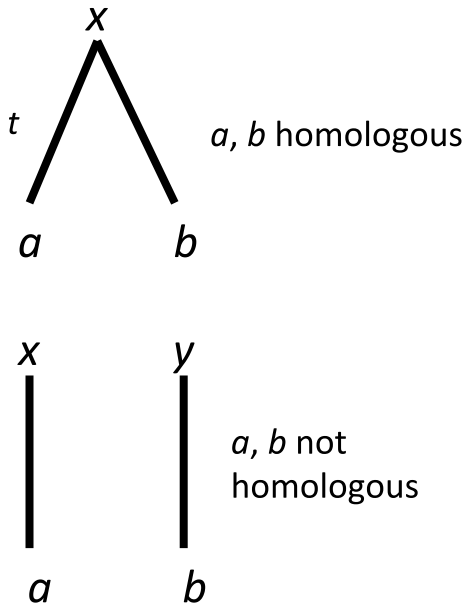


No: they do not descend from a common ancestral wing in the common ancestor of these animals.
They evolved independently for the same reasons (flight).

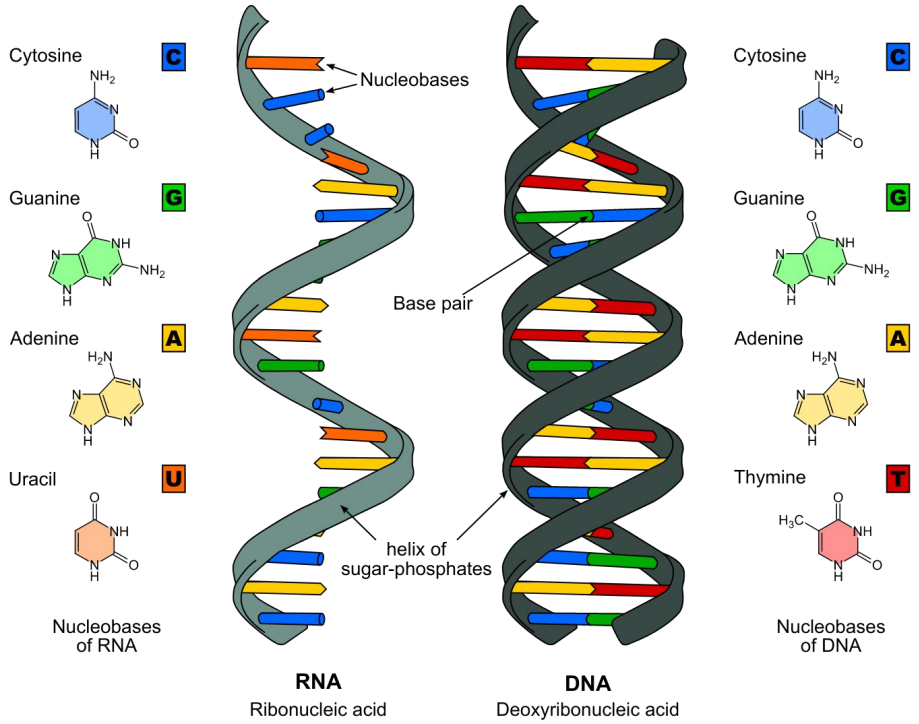
Homology

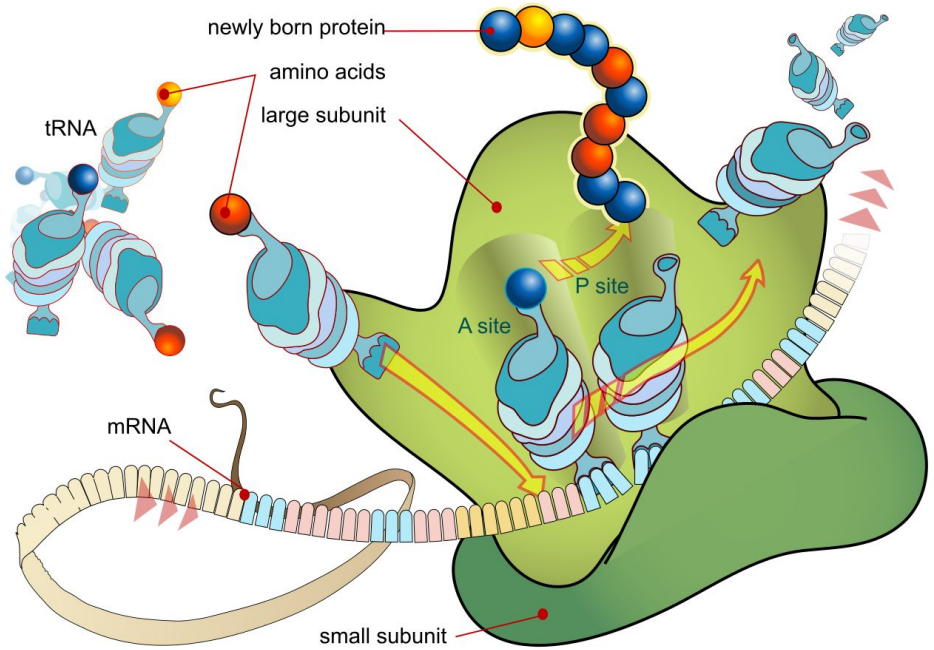
- Homologous characters can tell us about evolutionary relationships
- Homologous gene sequences: descend from a common ancestor
- A statement of homology is an evolutionary hypothesis.

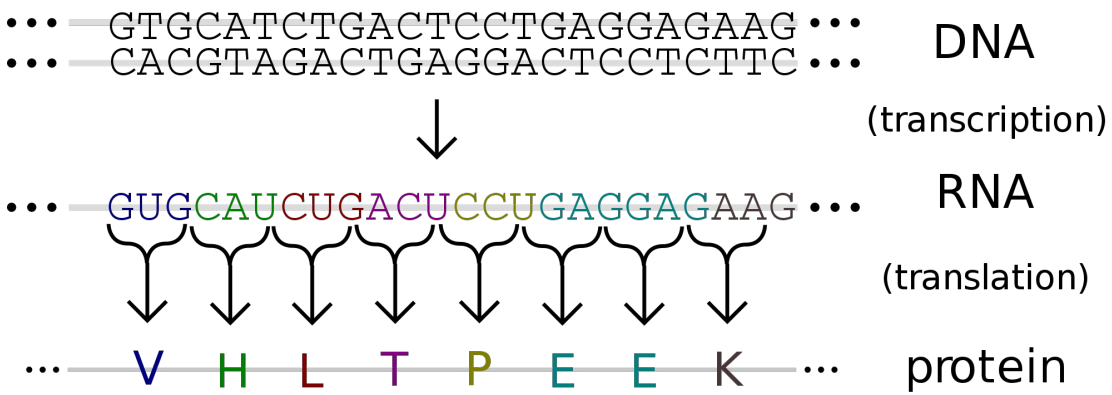
- Homology is **either-or**: sequences either share a common ancestor or they don't.
- But, homology is often inferred from sequence similarity (e.g. BLAST searching).



Molecular sequences provide digital information, many homologous characters





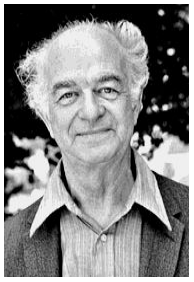


Wikimedia

DNA sequences can be used to make phylogenetic trees

Common ancestor

Gene A Sequence



GAACTCGAC

GATCTCGAC

GATCTGGGC

GCTCTGGGC

Common ancestor of vertebrates

GCTCTGCGTA

We use alignment to construct hypotheses of homology for sequence data

ATTGGCG

AGGAG

Sequences!

ATTGGCG

A--GGAG

Alignment!

AGGCG

+TT

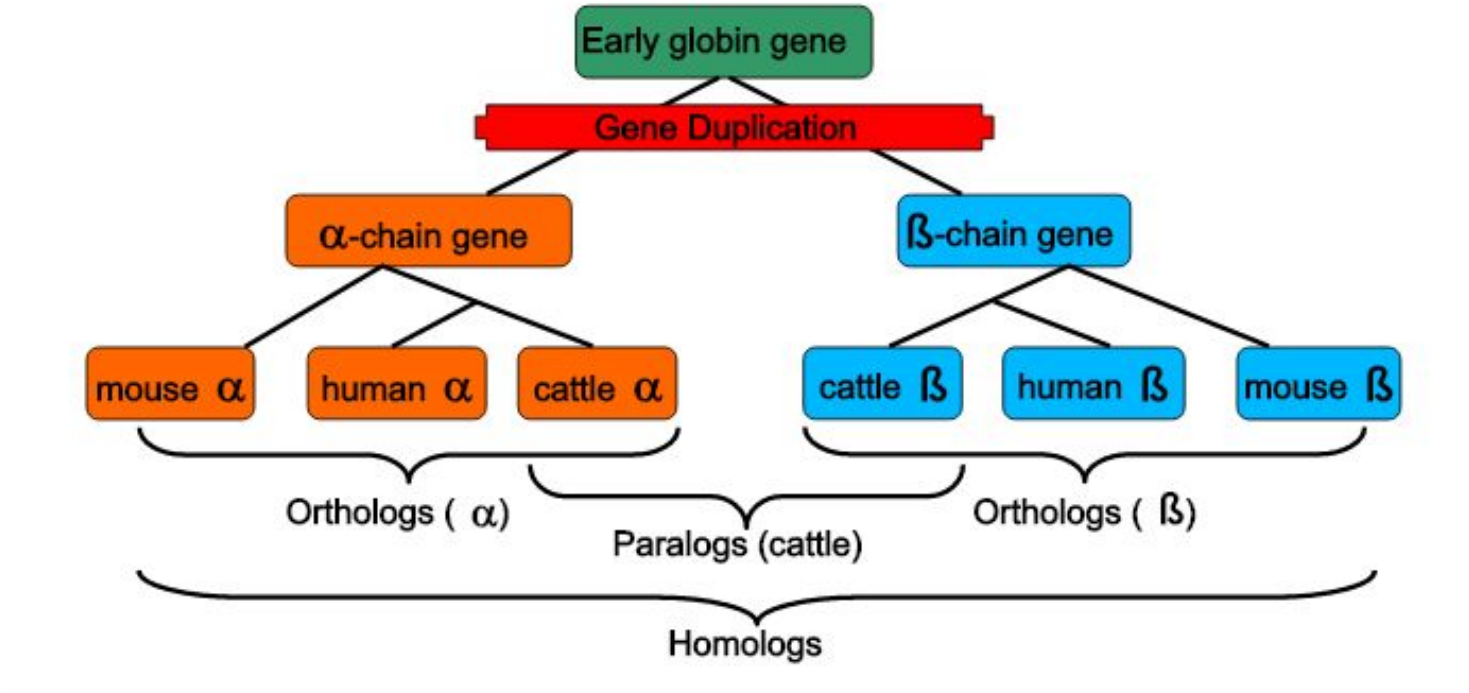
C=>A

ATTGGCG

A--GGAG

Homology!

Types of homology: orthology and paralogy



Orthologues: homologues related through a speciation event.

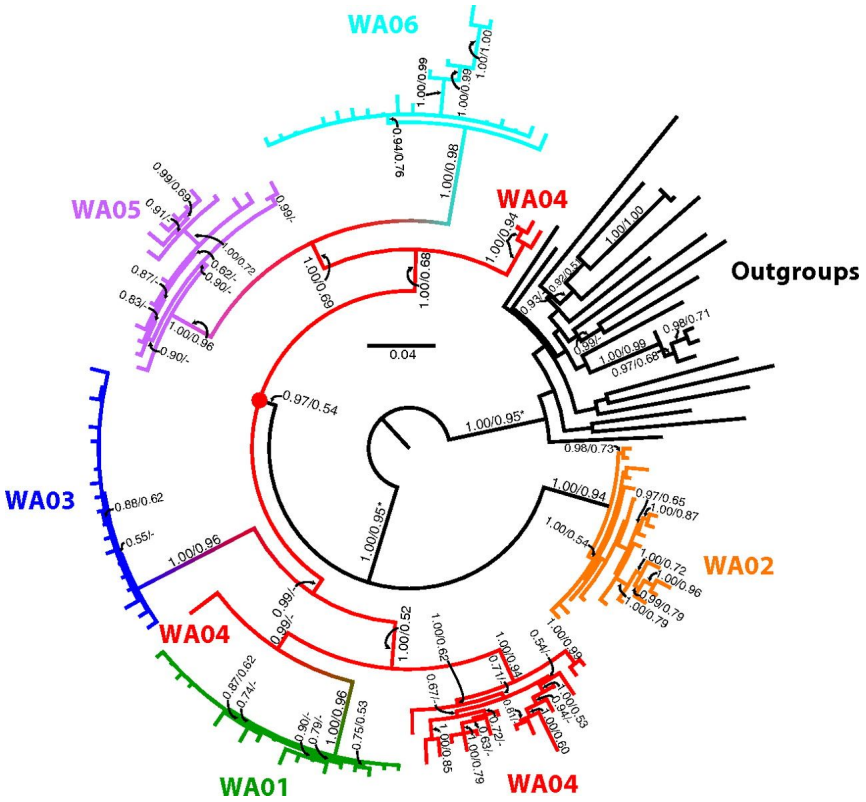
Paralogues: homologues related through a duplication event.

(Don't mix them in your analyses!)

2. Interpreting phylogenetic trees

What kind of information can we learn from phylogenies?

- Information about relationships (who is mostly closely related to whom?)



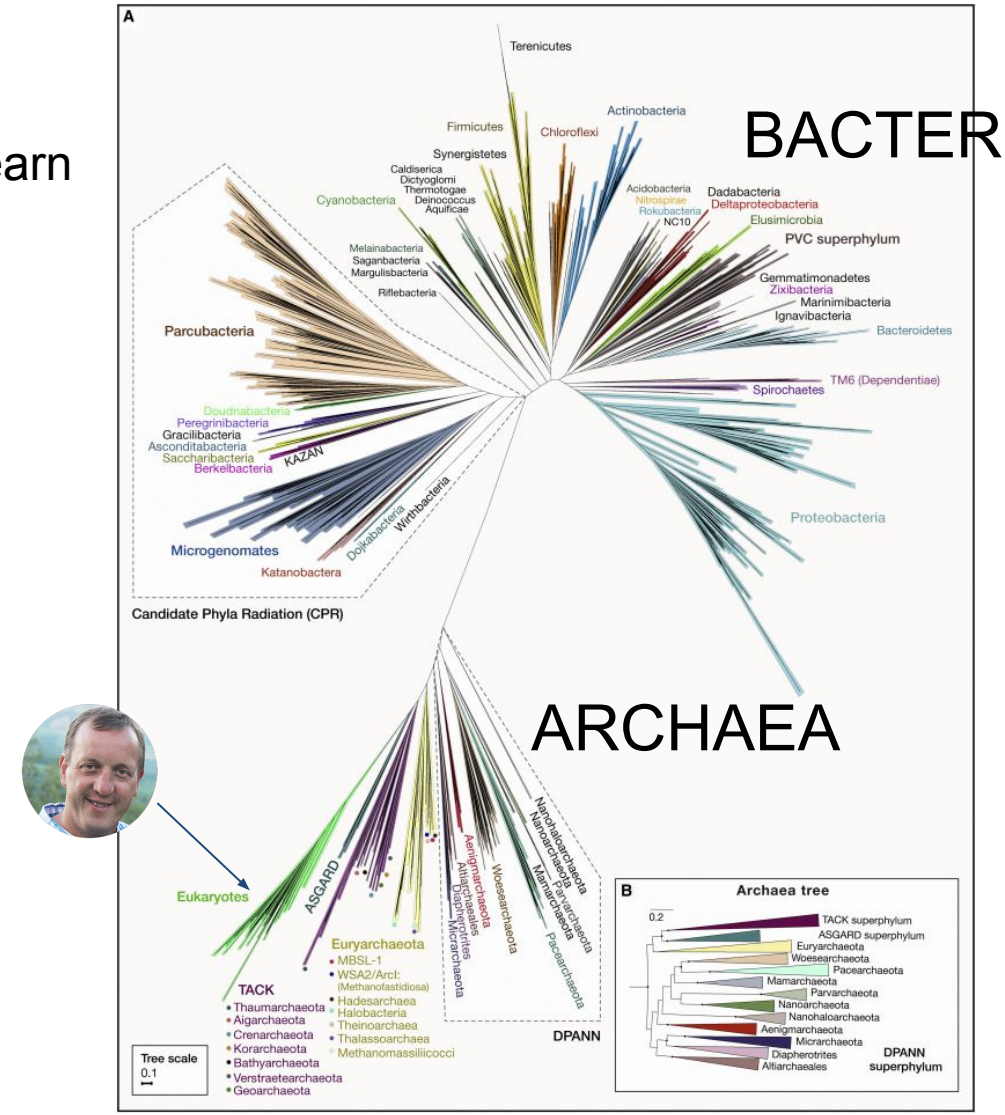
E.g. **epidemiology**
(HIV-1 env gene)

Scaduto et al. (2010)
PNAS

Interpreting phylogenetic trees

What kind of information can we learn from phylogenies?

- Information about genetic (or other) diversity

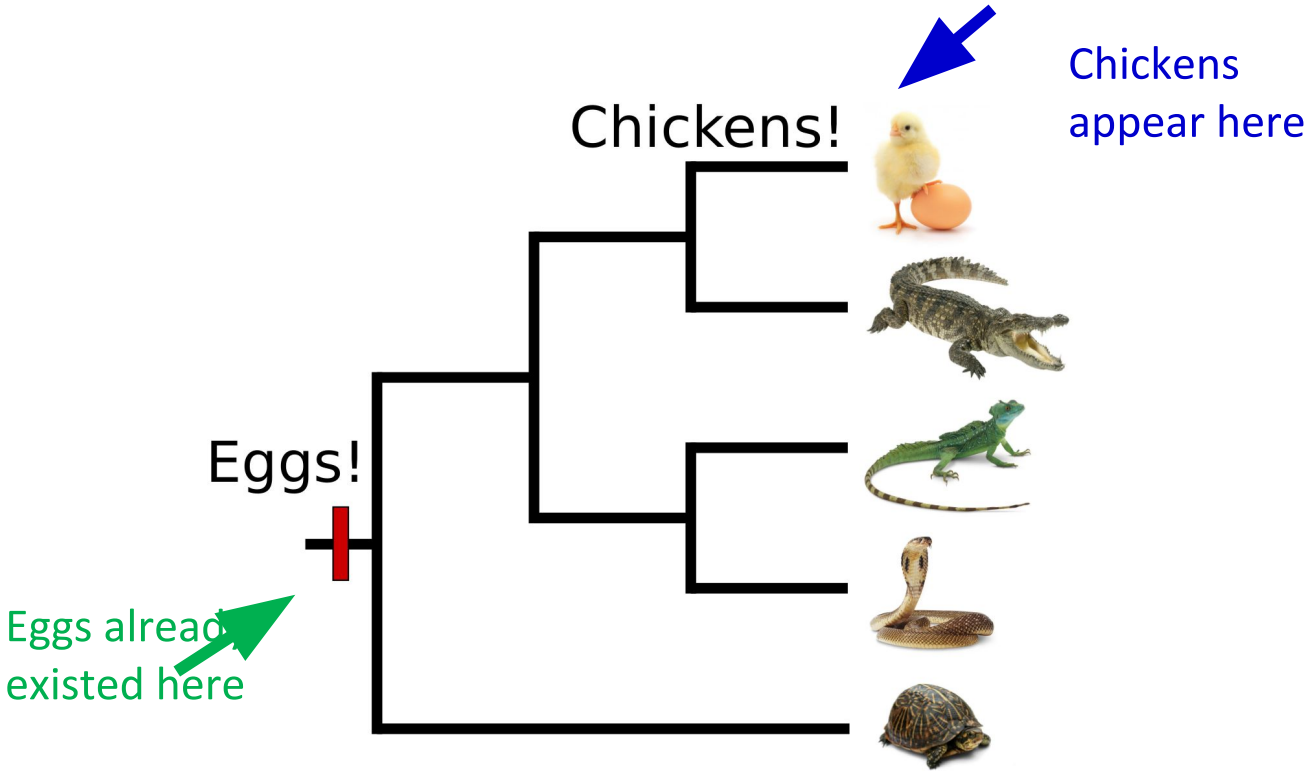


Castelle and Banfield (2018)

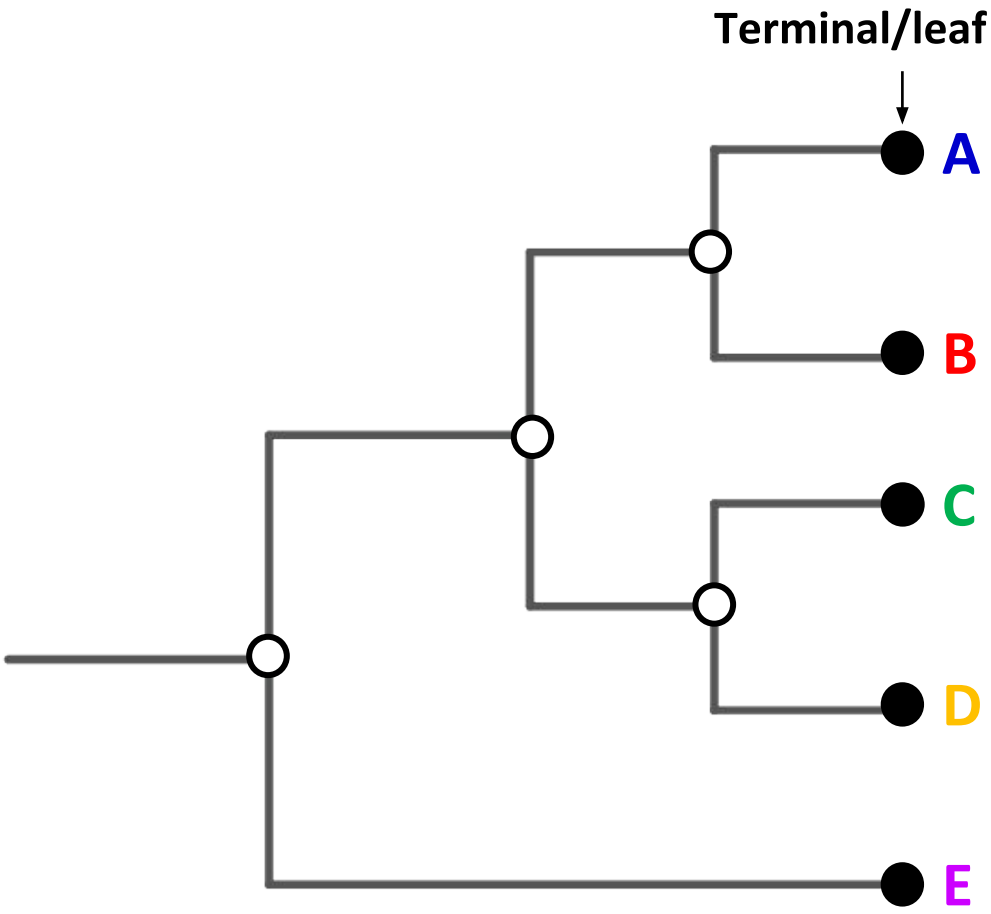
Interpreting phylogenetic trees

What kind of information can we learn from phylogenies?

- The history of trait evolution



Anatomy of a phylogenetic tree



OTUs
(individual genes, populations, species etc.)

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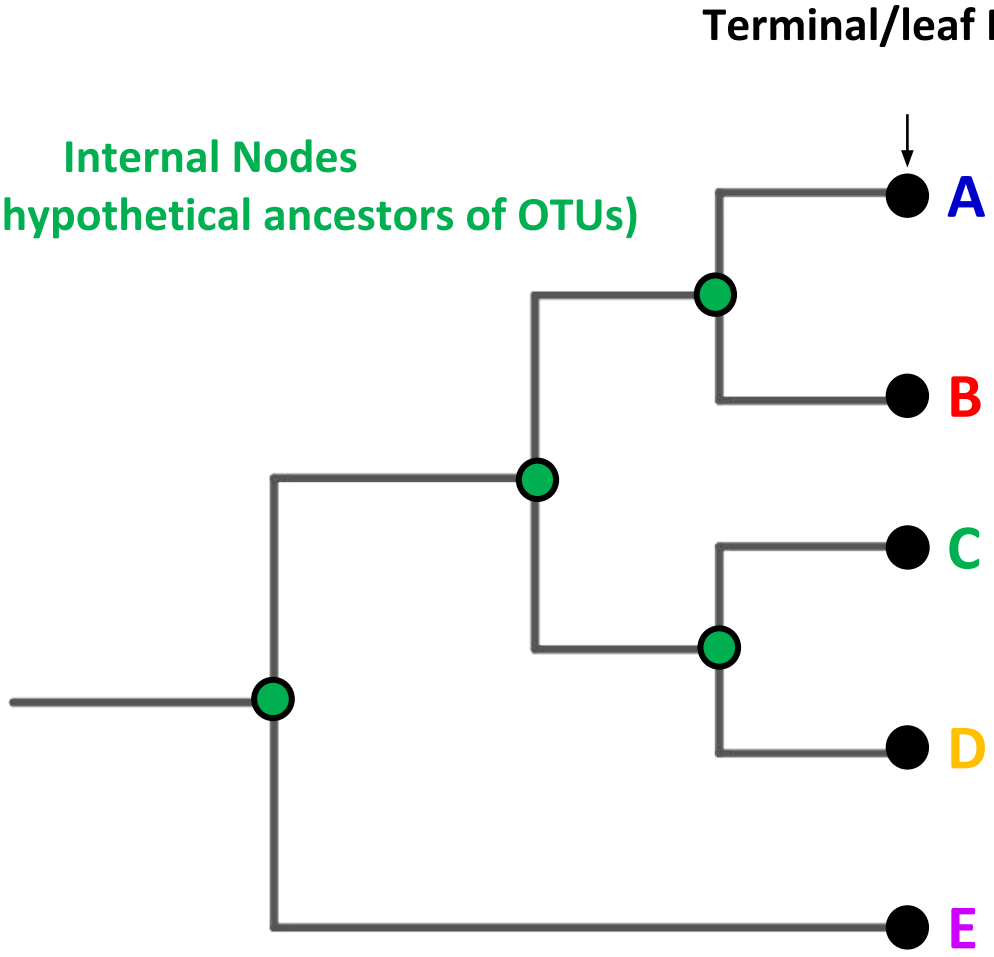

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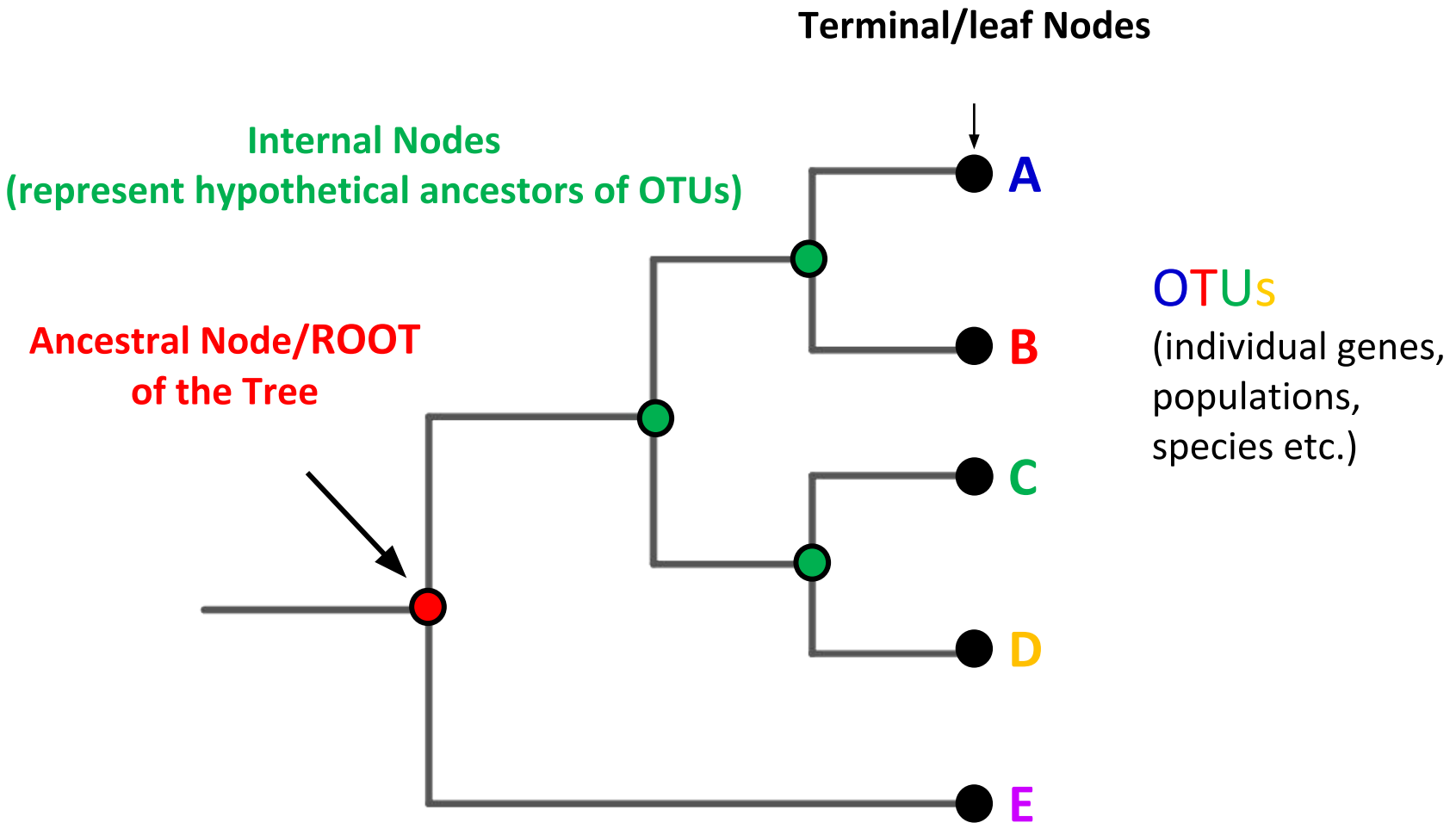

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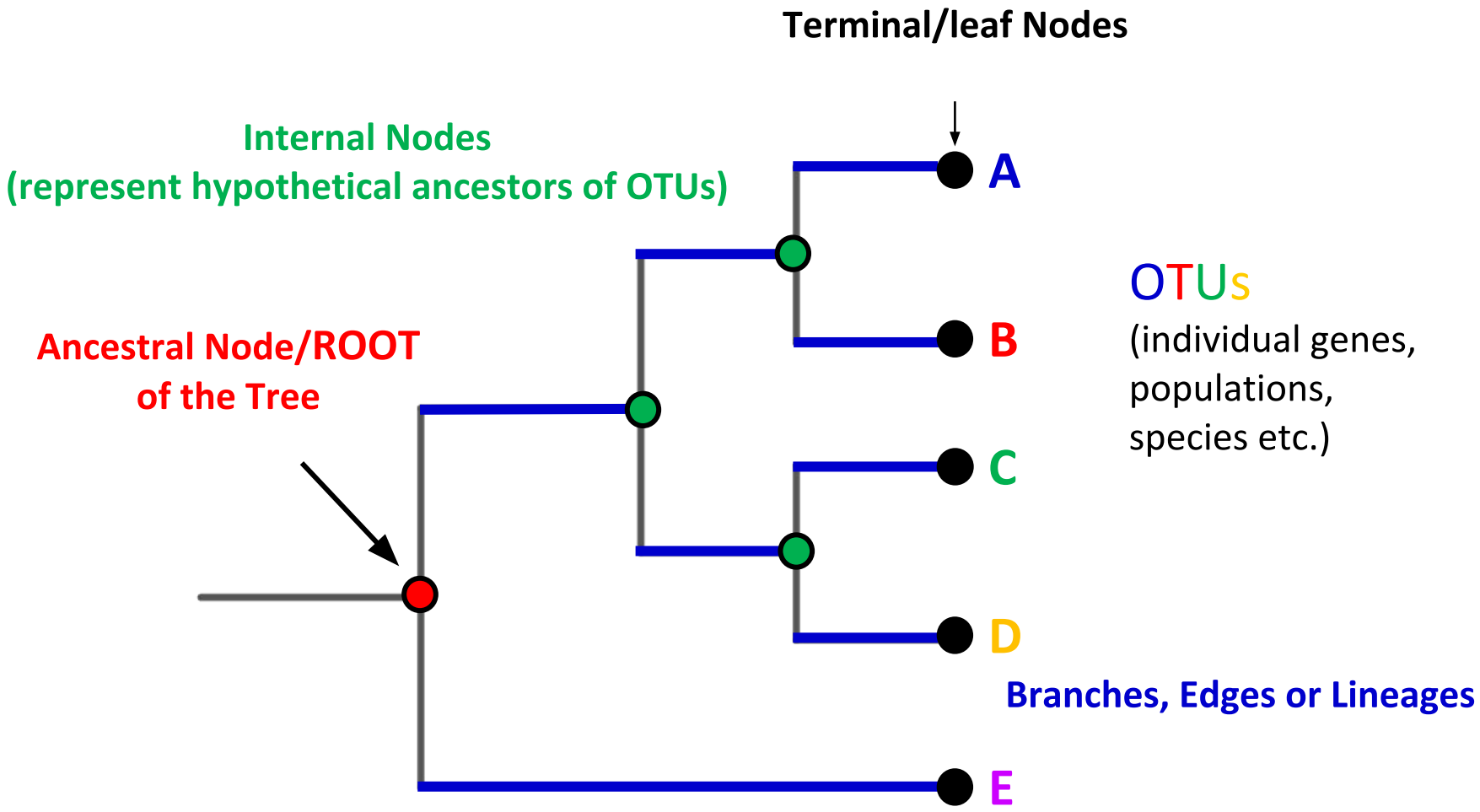
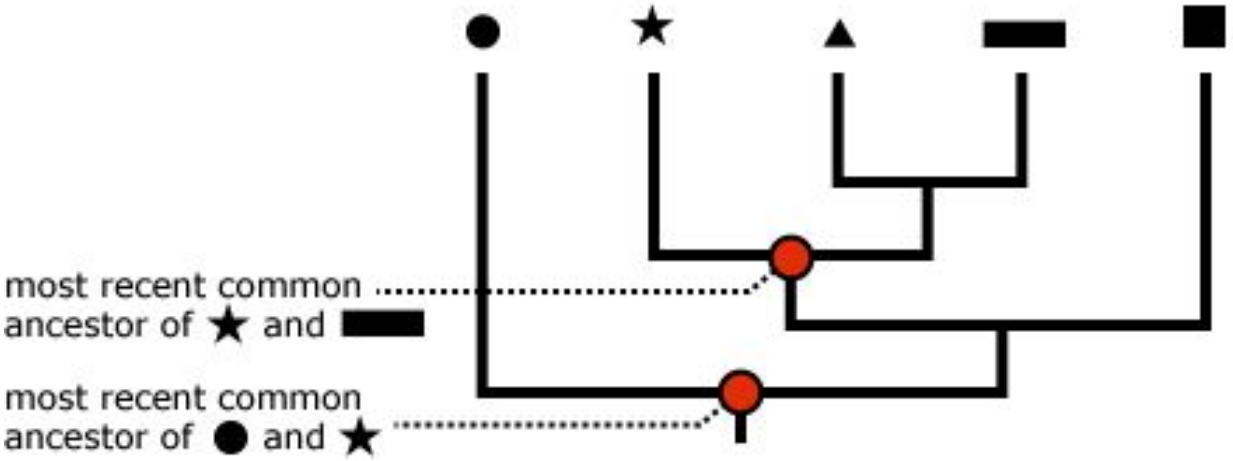
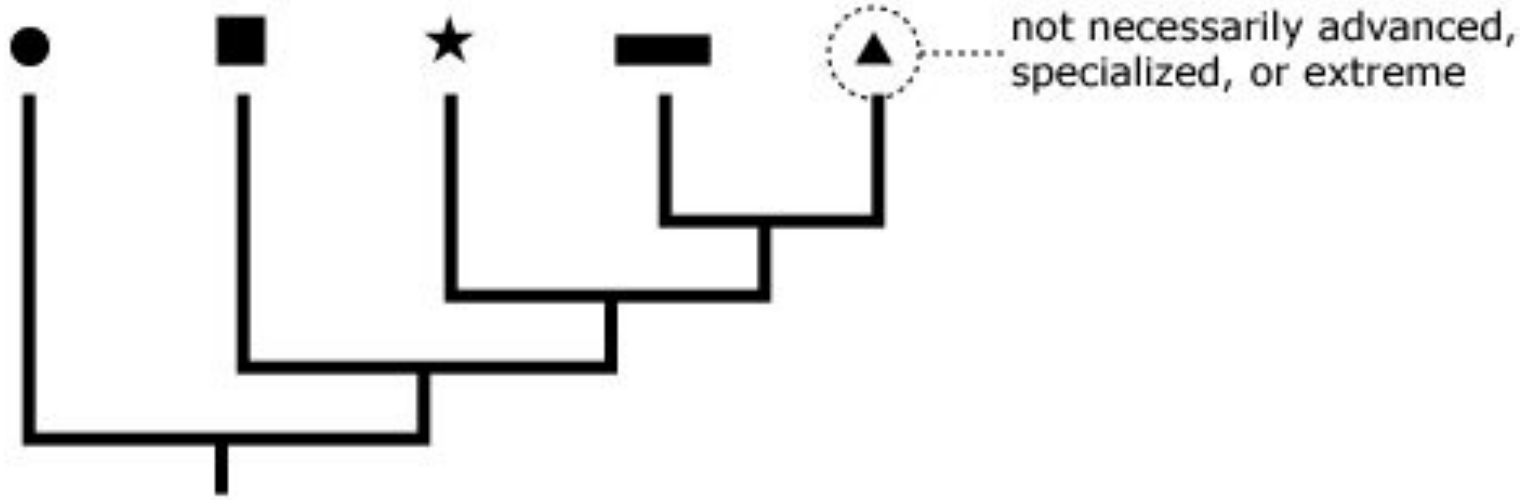


Reading trees: patterns of relatedness



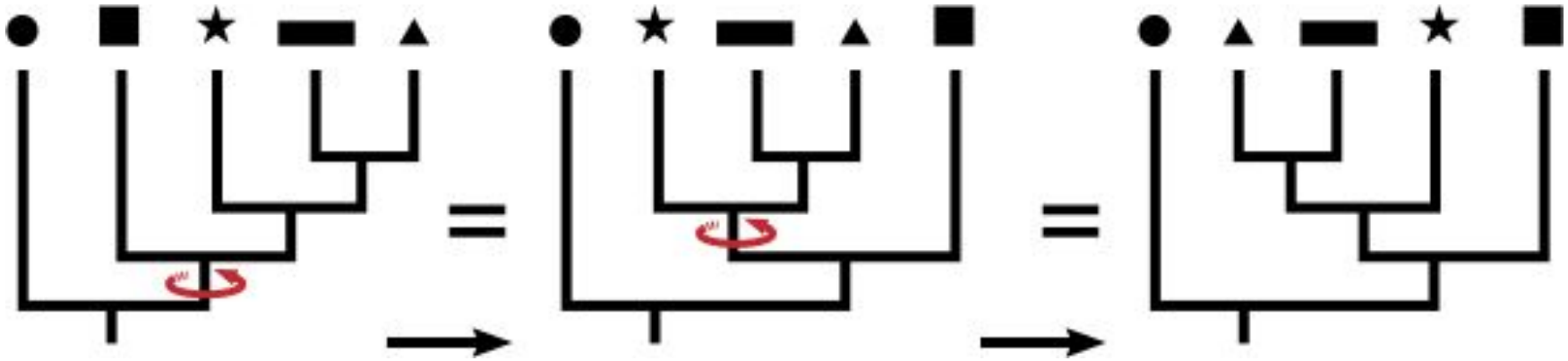
The more recently two species share a common ancestor, the more closely related they are.

Reading trees: patterns of relatedness



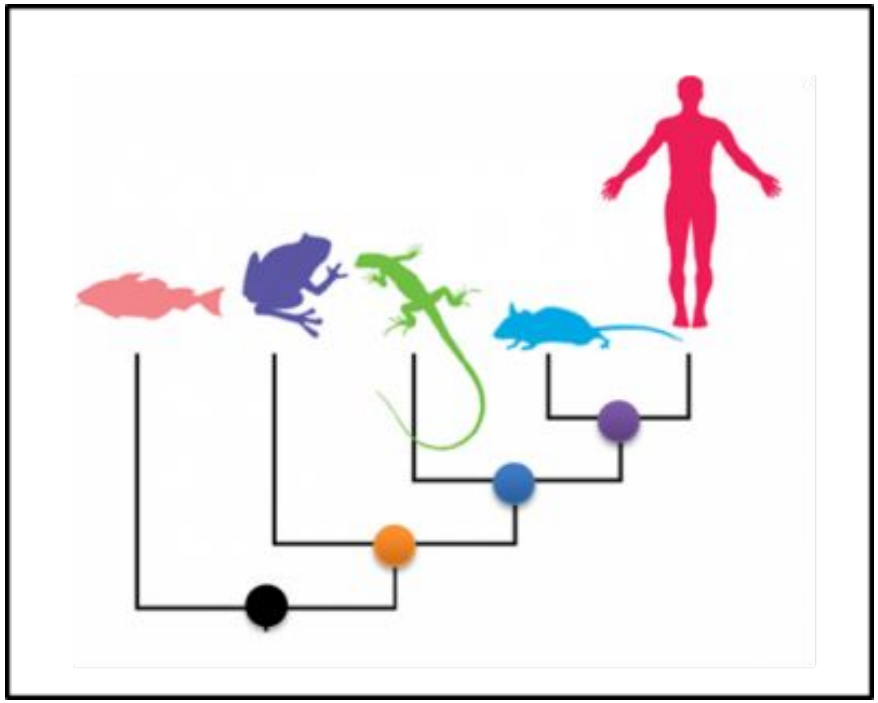
Trees depict evolutionary relationships, not evolutionary progress (!)

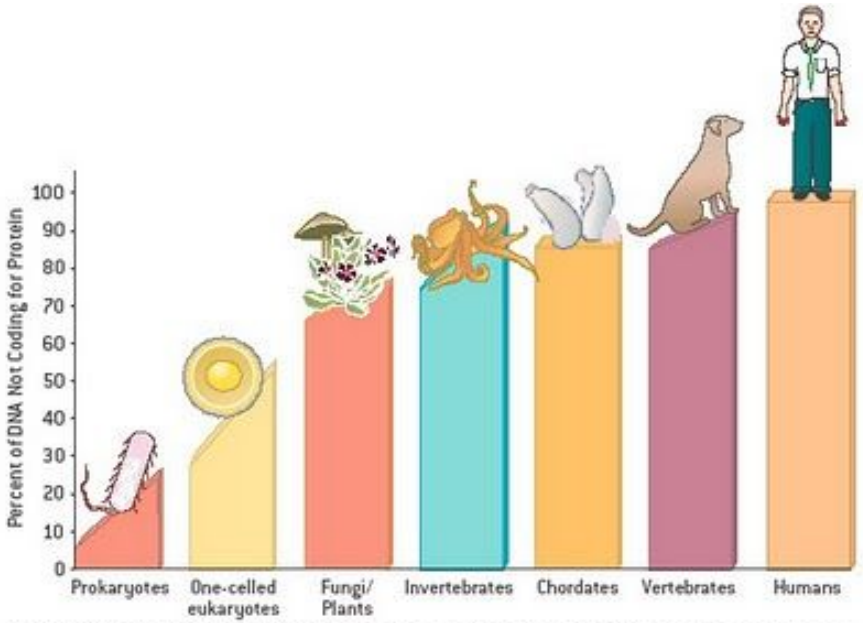
Reading trees: patterns of relatedness



Rotation around internal nodes produces equivalent trees.

Beware of “Dog’s Ass” plots

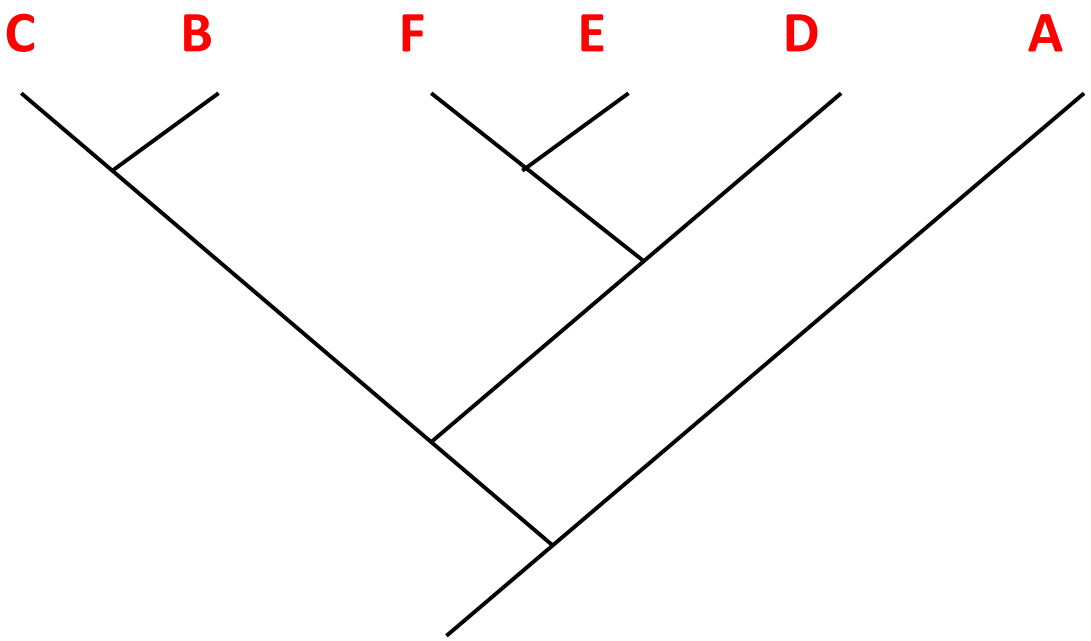




NONPROTEIN-CODING SEQUENCES make up only a small fraction of the DNA of prokaryotes. Among eukaryotes, as their complexity increases, generally so, too, does the proportion of their DNA that does not code for protein. The noncoding sequences have been considered junk, but perhaps it actually helps to explain organisms' complexity.

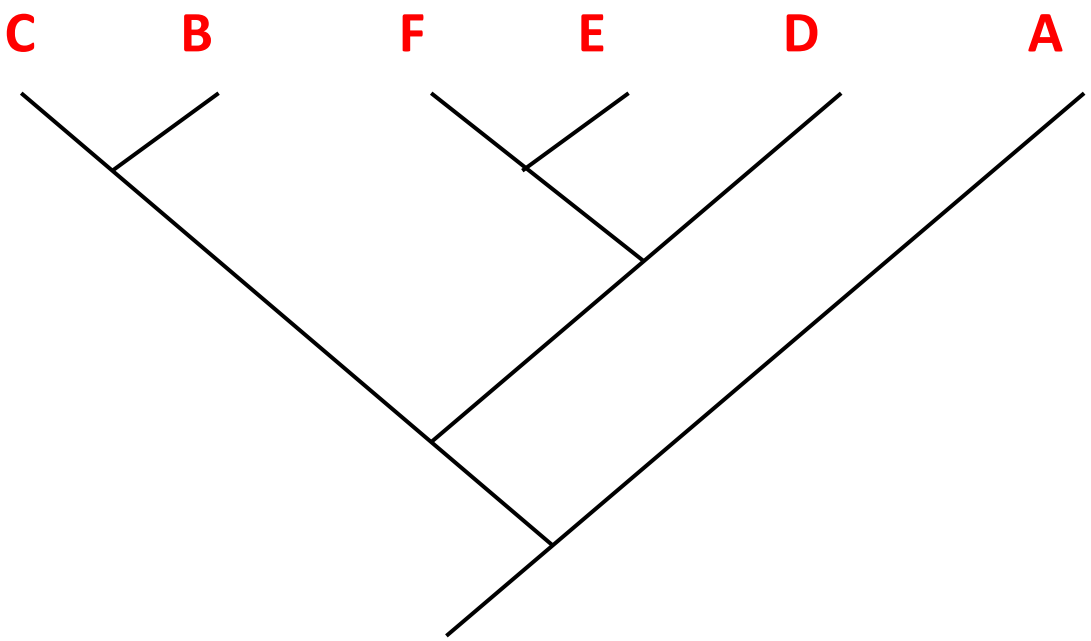
http://www.genomicron.evolverzone.com/2007/09/dogs-ass-plots-daps/

Quiz: reading trees



Is “E” more closely related to “D” or to “F”?
Is “E” more closely related to “B” or to “A”?
Is “E” more closely related to “B” or to “C”?

Quiz: reading trees



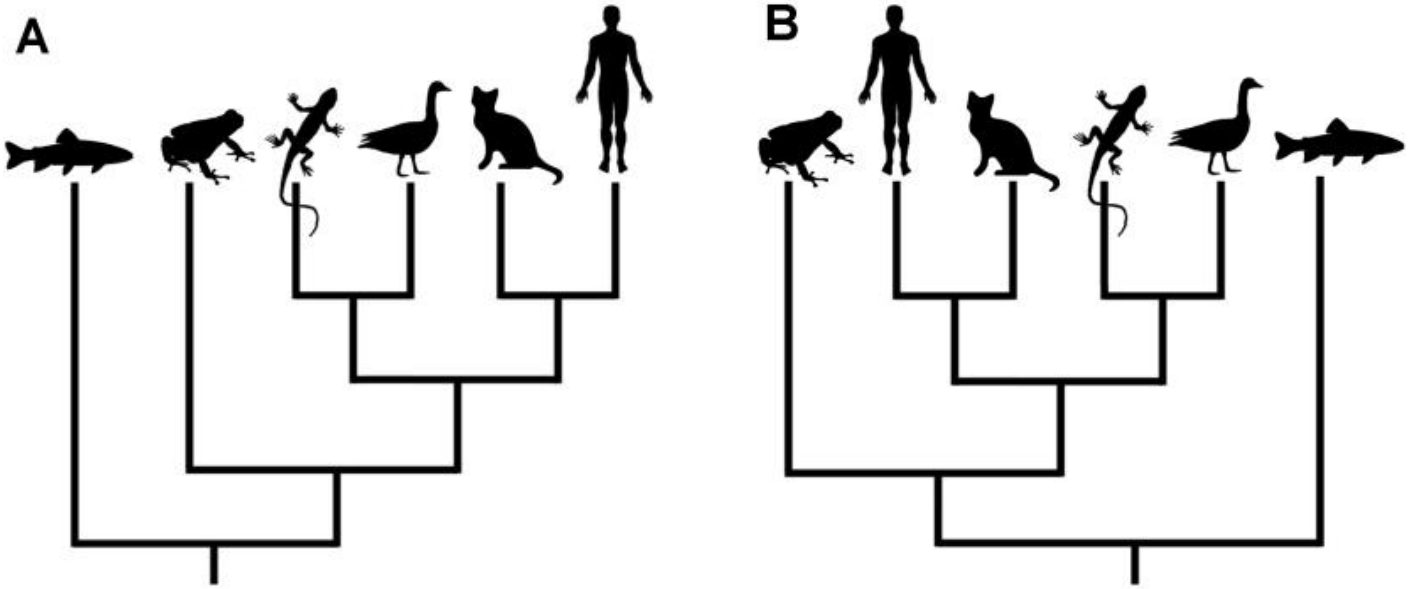
“E” more closely related to “F”

“E” more closely related to “B”

“E” more closely related to neither (equally to “B” & “C”)

Quiz: reading trees

Find the differences between the two trees!



Quiz: reading trees

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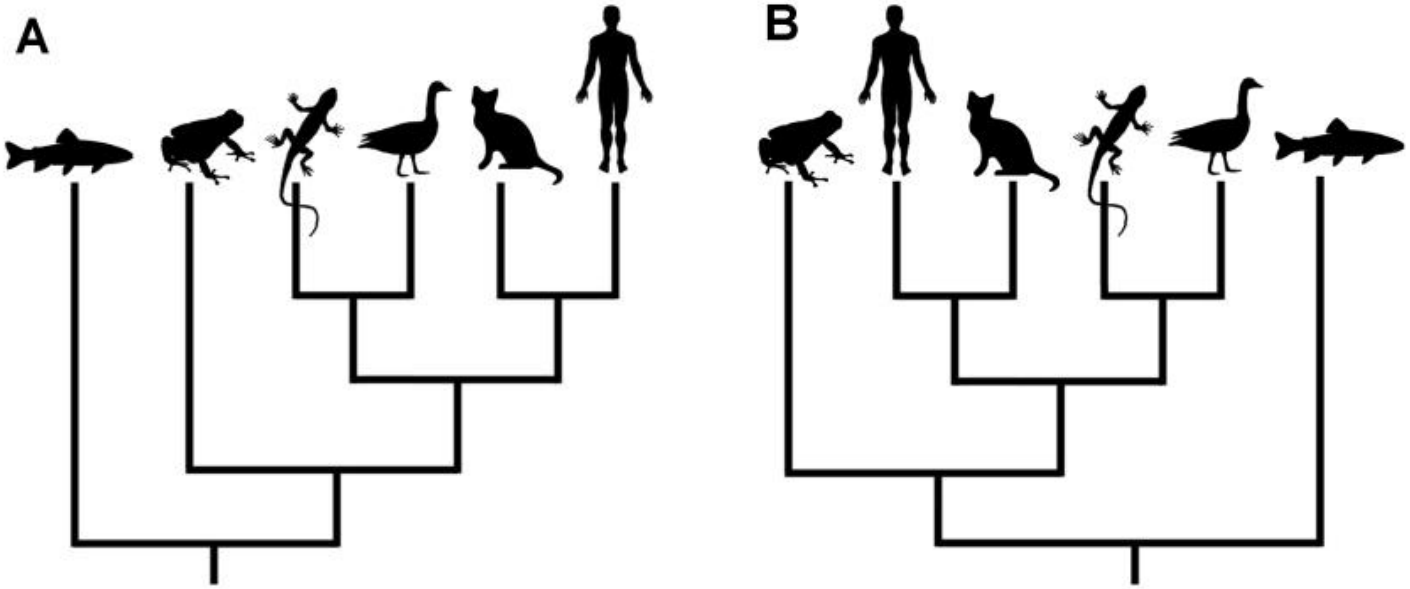
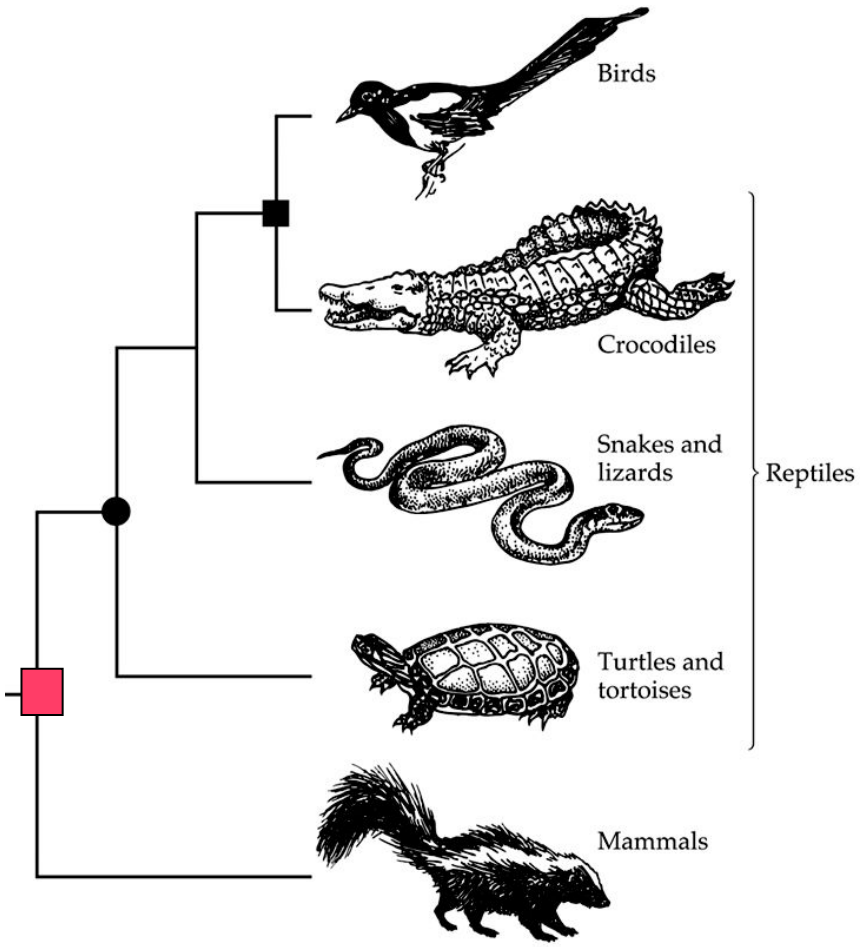


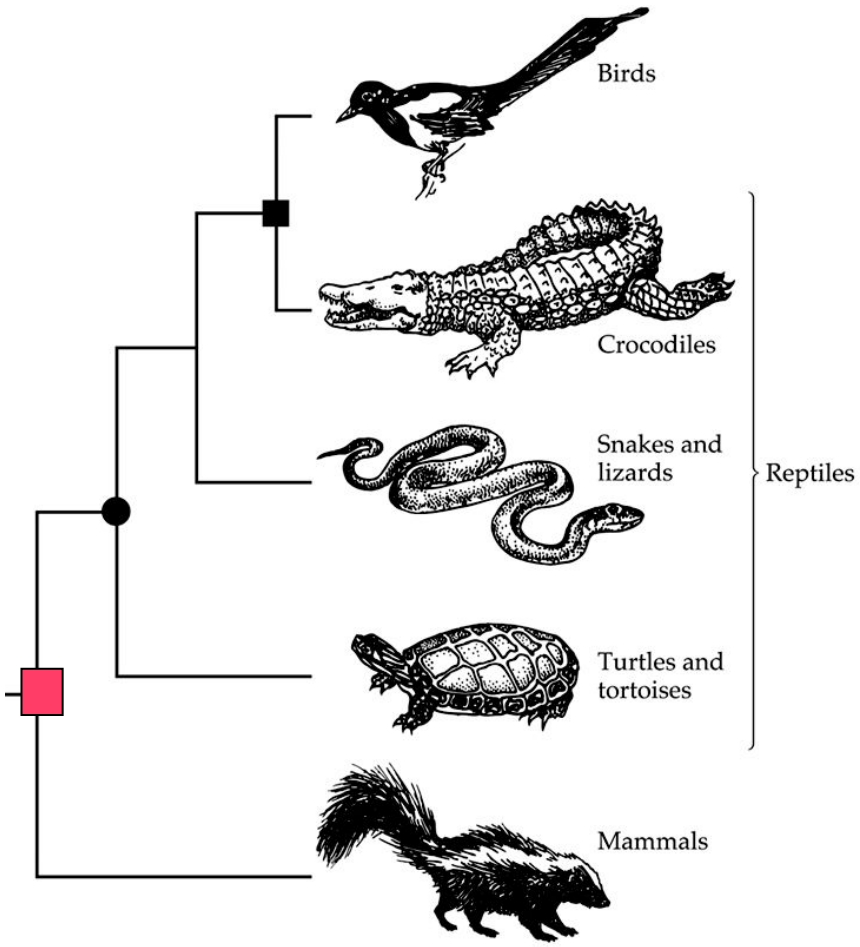
They are the same.

3. Naming groups on trees



Monophyletic group: a node and all its descendants (e.g. “amniotes”)

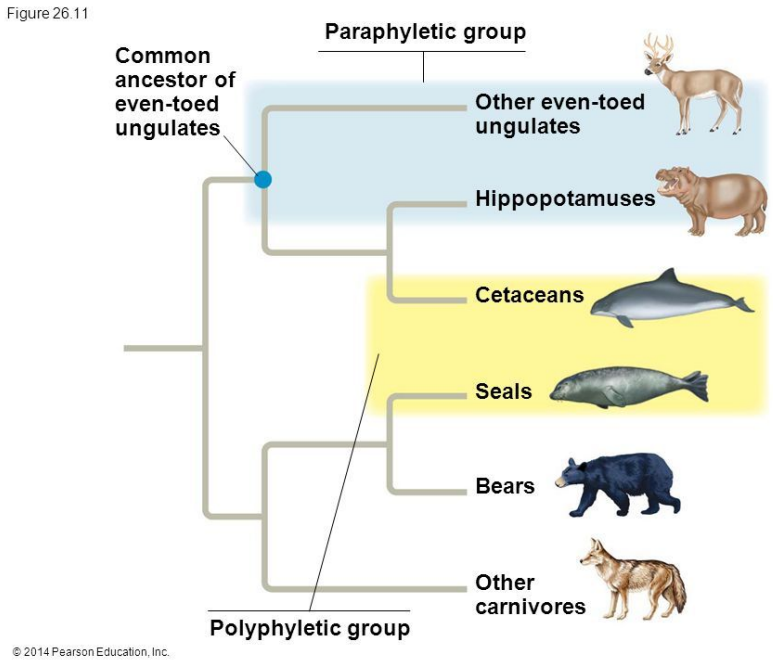
Naming groups on trees



Monophyletic group: a node and all its descendants (e.g. “amniotes”)

Paraphyletic group: a node and some of its descendants (e.g. “reptiles”, “prokaryotes”)

Naming groups on trees

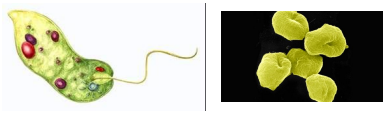


Monophyletic group: a node and all its descendants (e.g. “amniotes”)

Paraphyletic group: a node and some of its descendants (e.g. “reptiles”, “prokaryotes”)

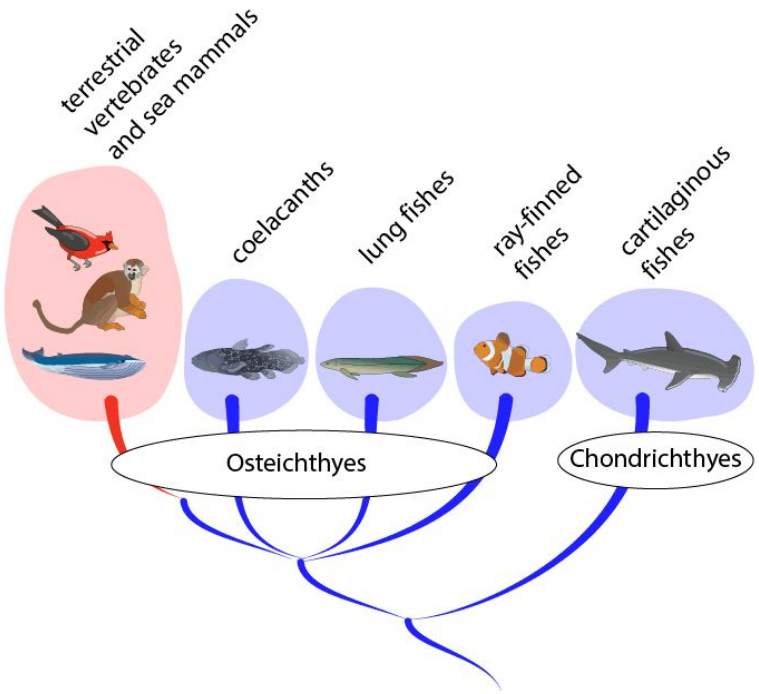
Polyphyletic group: some taxa, but not their most recent common ancestor (e.g “marine mammals”)

(Don’t feel bad if your favourite group is paraphyletic! Join the club!)



Quiz: naming groups

What kind of group are fishes?



(a) Monophyletic
(b) Paraphyletic
(c) Polyphyletic

Quiz: naming groups

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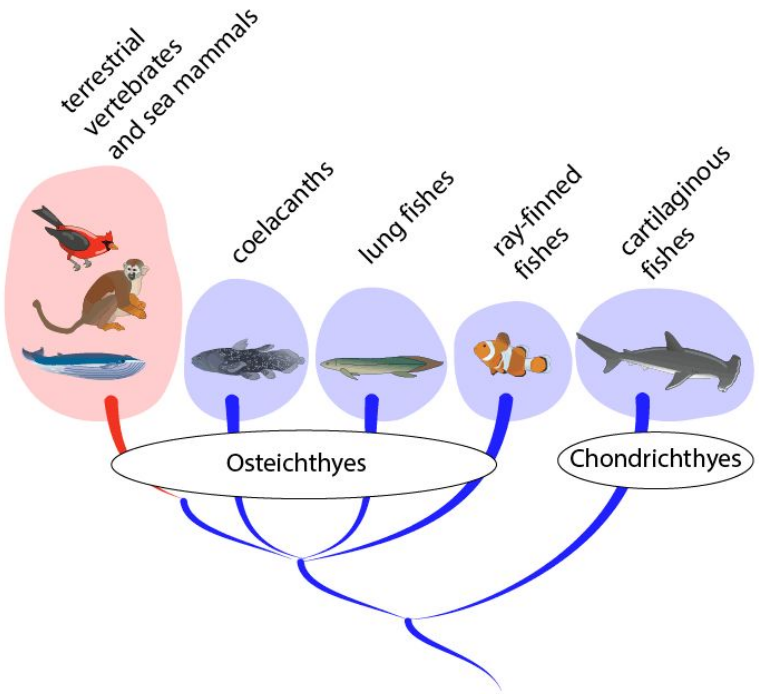
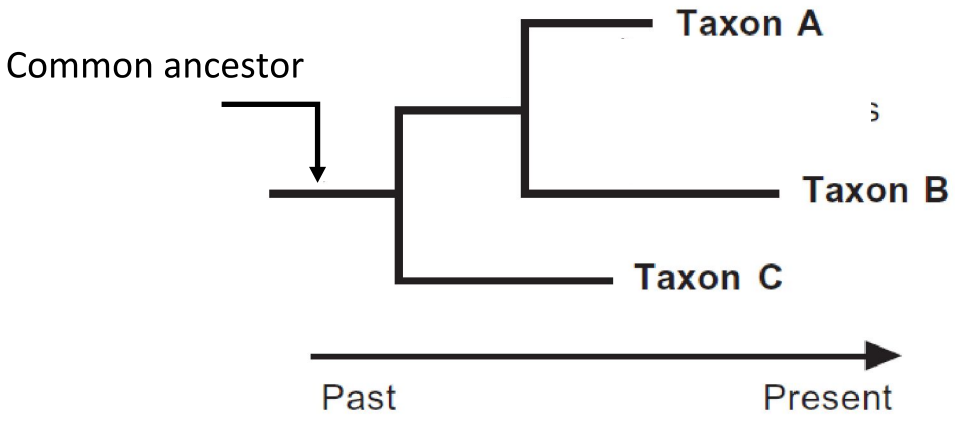


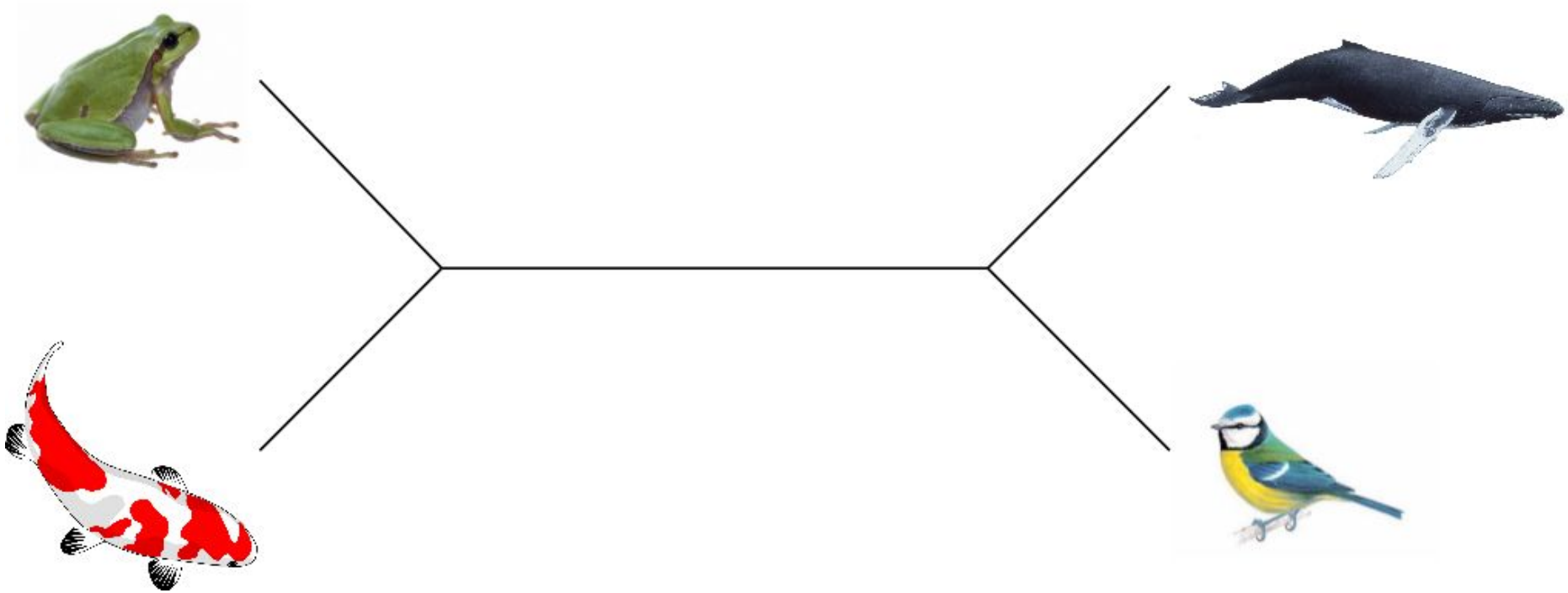
(a) Monophyletic
(b) Paraphyletic
(c) Polyphyletic

4. The root: a very special node on the tree

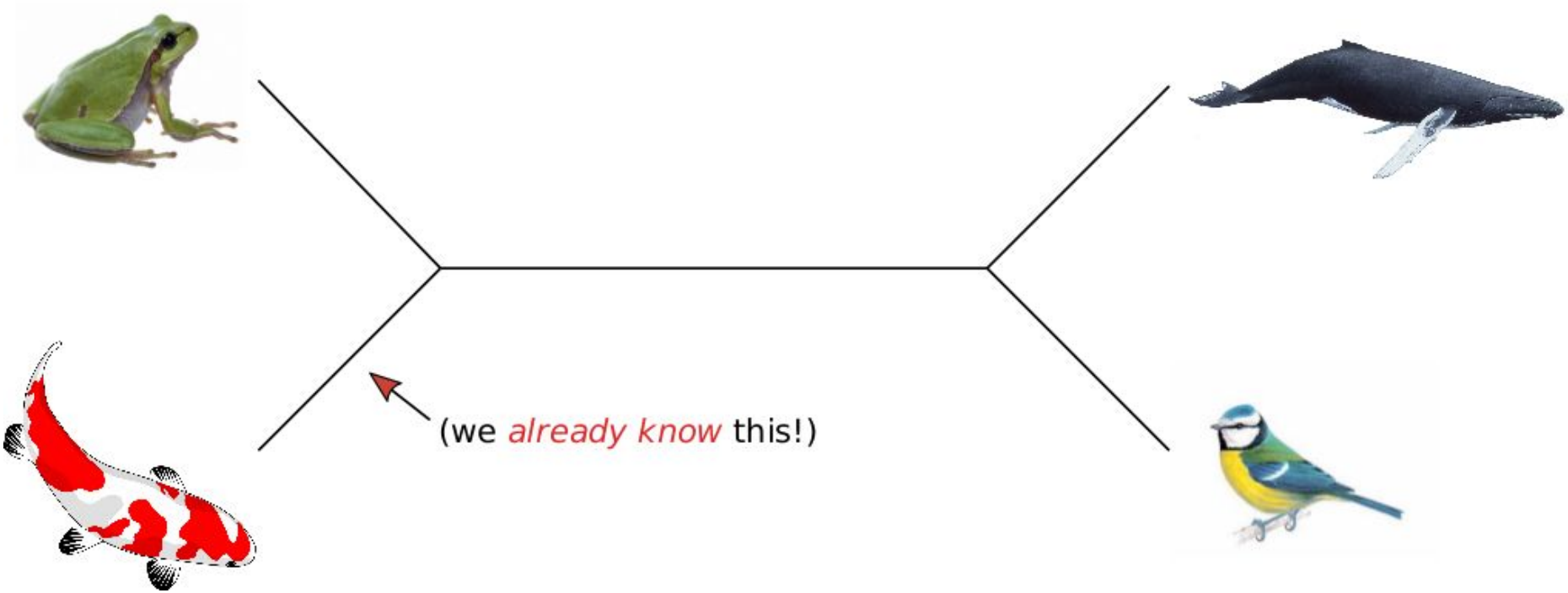


- The root is the oldest point on the tree (most recent common ancestor of all taxa). It orients the tree in time.
- All inferences about ancestor-descendant relationships **and relatedness** depend on the root position.
- Most phylogenetic methods do not estimate the root!

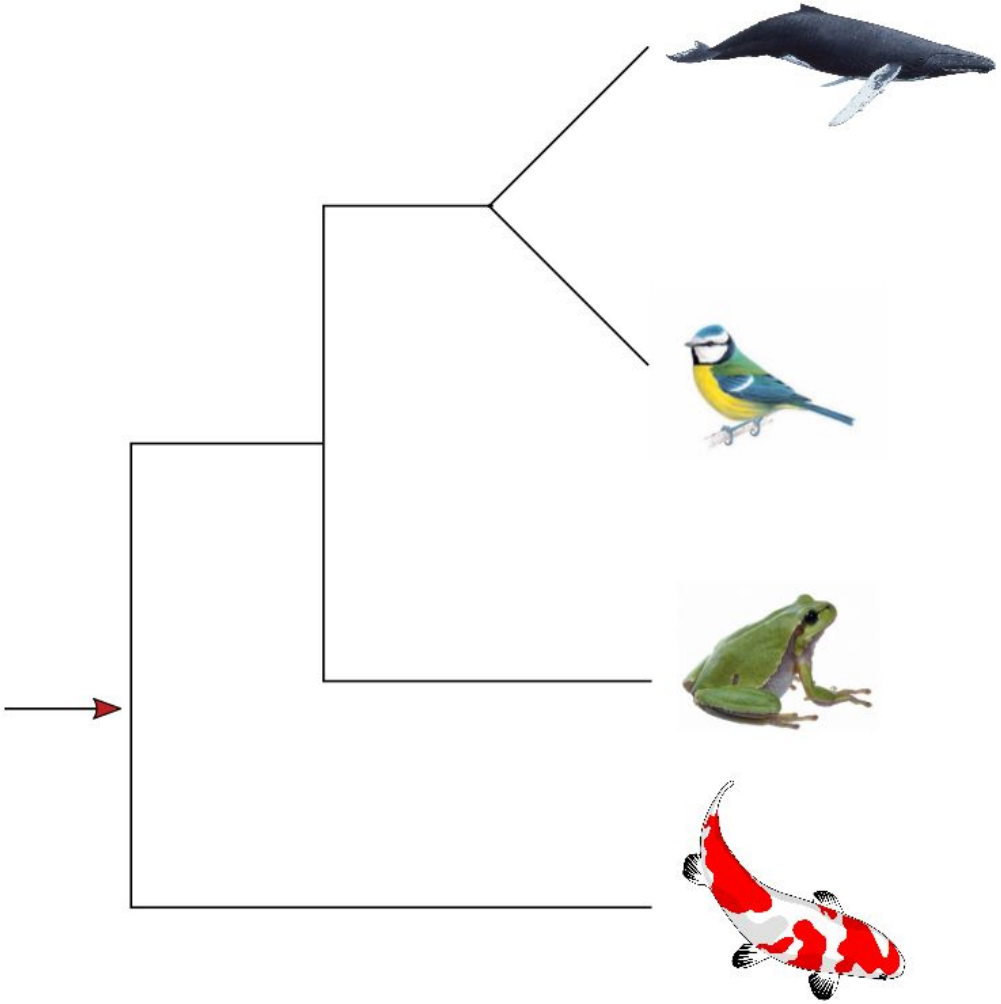
Rooting an unrooted tree using *a priori* knowledge (outgroup)



Rooting an unrooted tree using *a priori* knowledge (outgroup)



Rooting an unrooted tree using *a priori* knowledge (outgroup)



- Most packages will arbitrarily root the tree on some branch, for no particular reason
- You need to re-root on the outgroup branch

This doesn't apply if you use a method that can infer the root:

- Molecular clock
- Gene tree-species tree reconciliation
- Non-reversible/ non-stationary phylogenetic model
- Some pop. gen. settings (if you know the ancestral/derived allele)

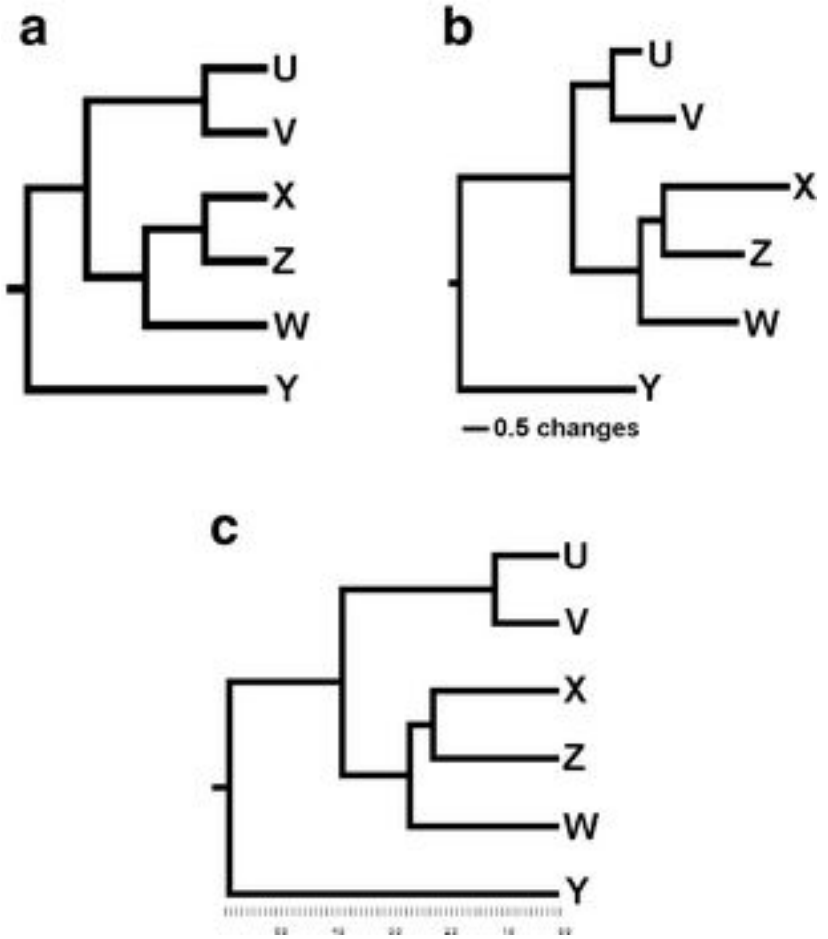
Interpreting branch lengths

Depending on the method of analysis, branch lengths can have different interpretations.

(a) **Cladogram**: just the topology matters (not so useful).

(b) **Phylogram**: branch lengths correspond to genetic distance (changes/alignment site).

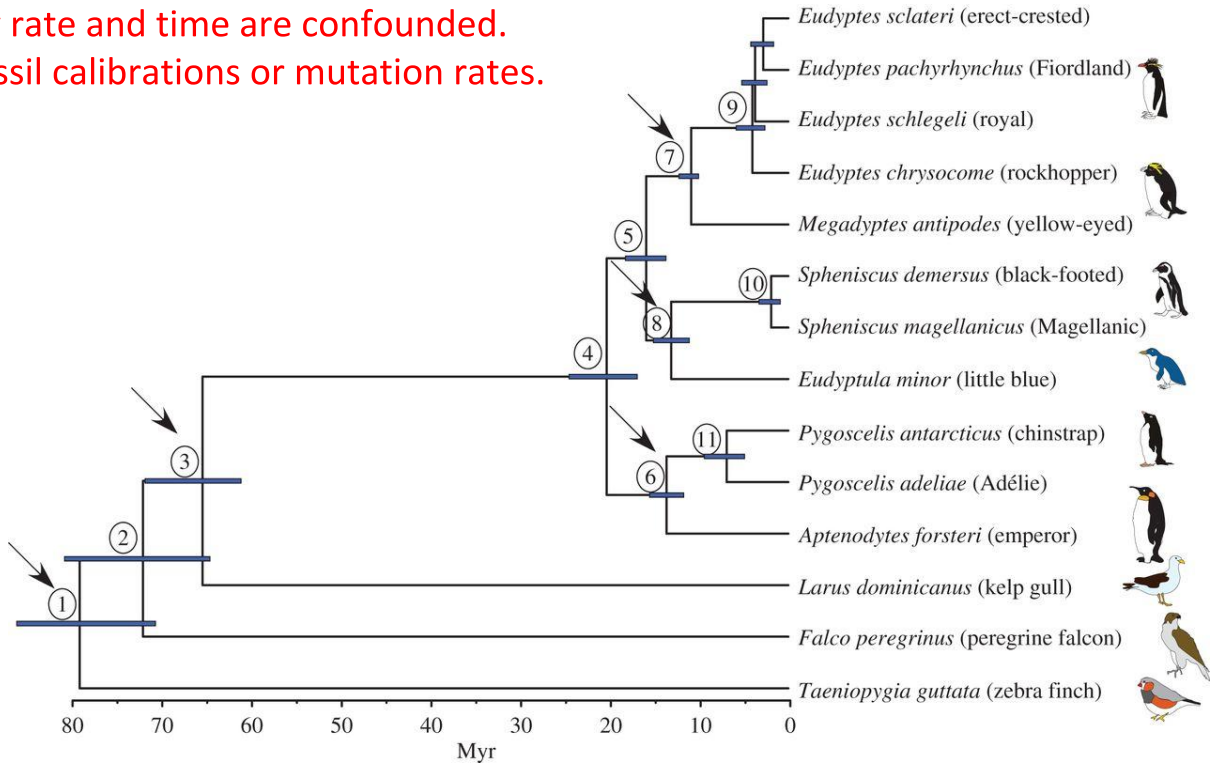
(c) **Chronogram**: branch lengths correspond to relative or absolute times (number of years)



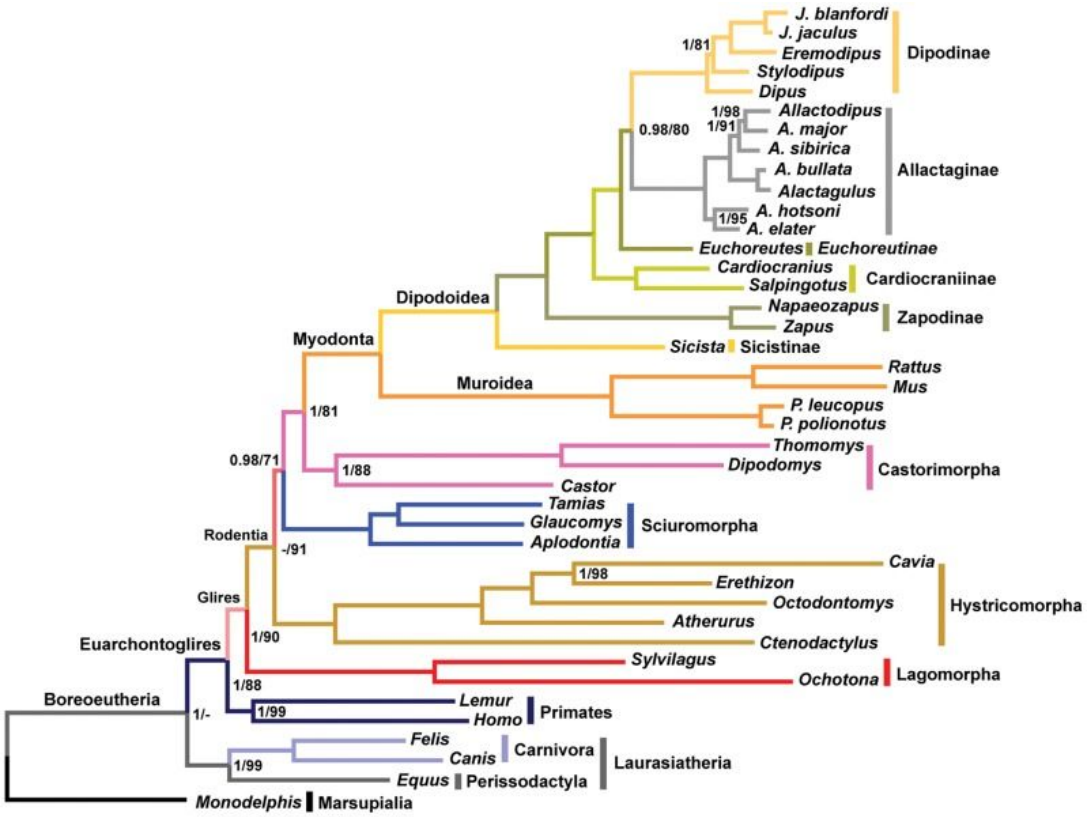
Chronograms: branch lengths in time (estimated with a molecular clock)

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

Evolutionary rate and time are confounded.
Need e.g. fossil calibrations or mutation rates.



Uncertainty in phylogenetic trees: support values



- Bootstraps (ML; 0-100) and posterior probabilities (Bayesian; 0-1)
- Different interpretations; higher is better
- Don’t draw strong conclusions if the support values are low
- Apply to splits (branches) on the tree, not nodes
- Sometimes useful to summarise on same tree diagram
- Always think about supports, and include them in your published phylogenies!

Bioinformatics Workshop Day 3: Schedule

Time	Event	Lecturer
10-12.30	Phylogenetics workflow & theory	Tom W.
1.30-3.30	Phylogenetics practical	Tom W.
3.45-4.30	Phylogenomics: theory and some games	Tom W.

Introduction to molecular phylogenetics

(b) A basic workflow

Tom Williams

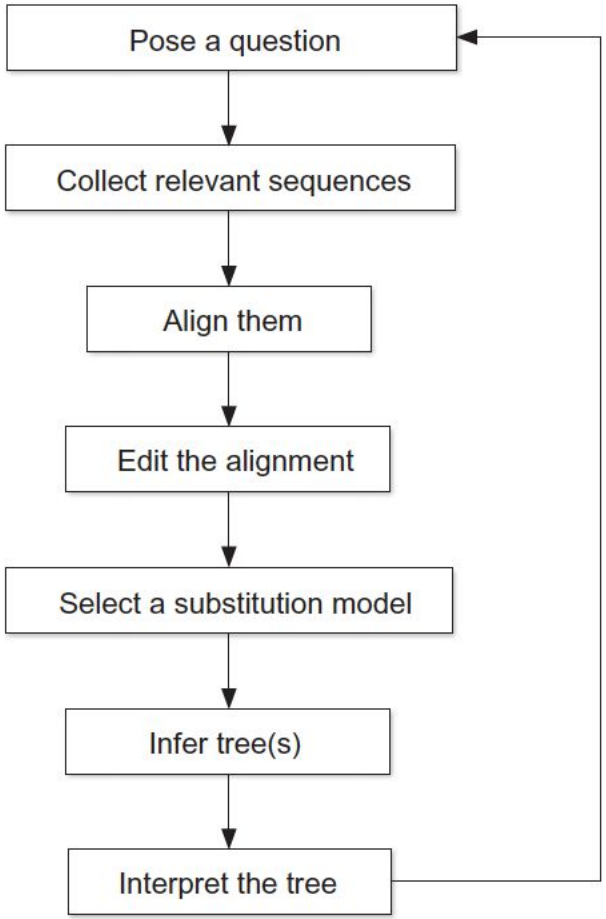
A basic workflow for molecular phylogenetics

- Typical analysis steps
- Some discussion of the methodology at each step

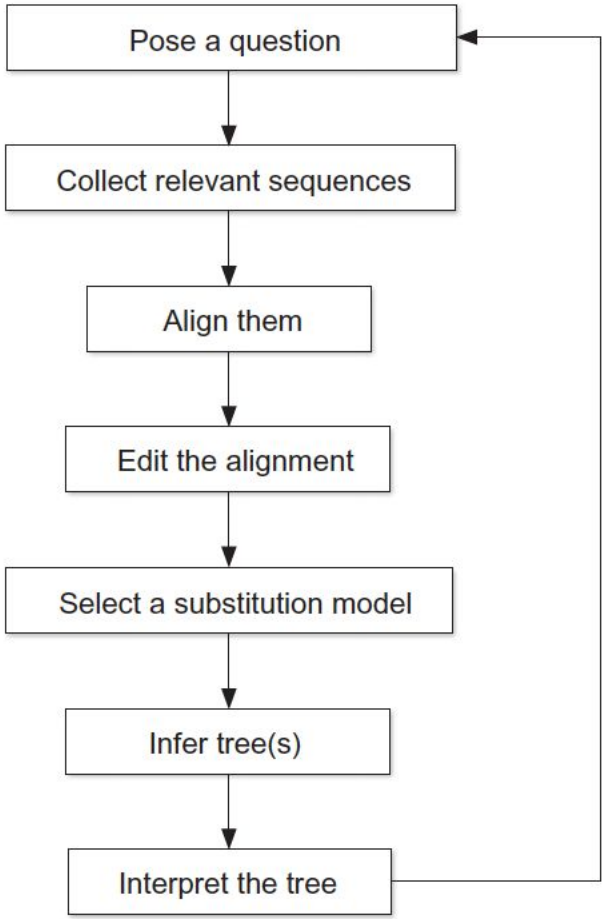
A basic workflow for molecular phylogenetics

- Typical analysis steps
- Some discussion of the methodology at each step
- Core ideas/elements
- Somewhat more technical material

Molecular phylogenetics: a possible flowchart

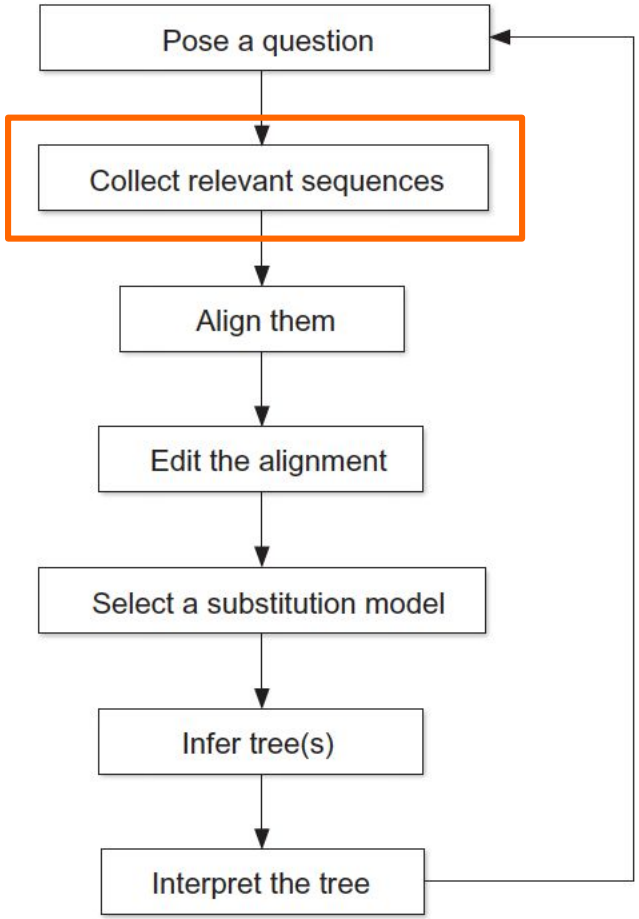


Molecular phylogenetics: a possible flowchart



A critical approach to analysis is key: don't fall into the black box!

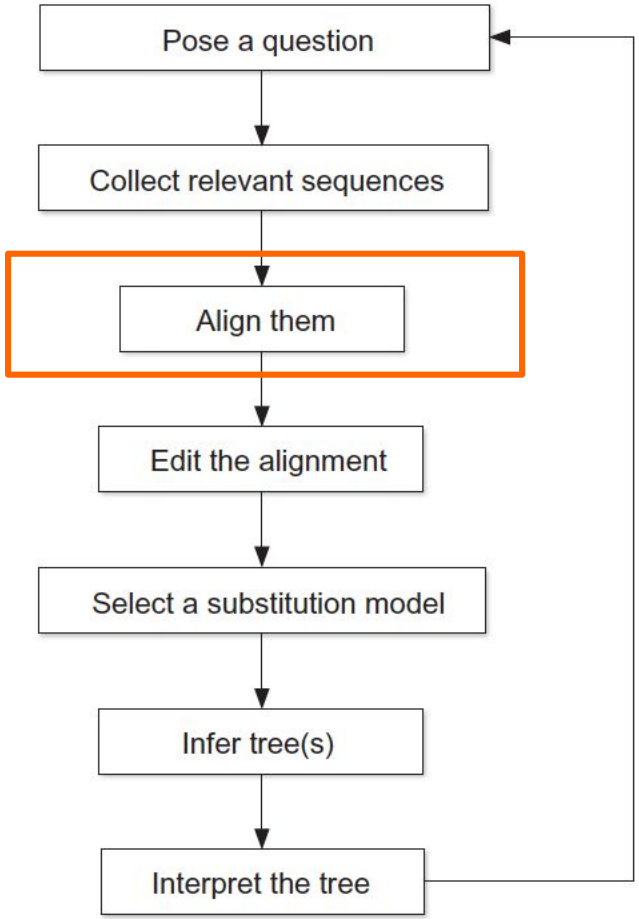
Molecular phylogenetics: a possible flowchart



Did I miss any important ones?

A critical approach to analysis is key: don't fall into the black box!

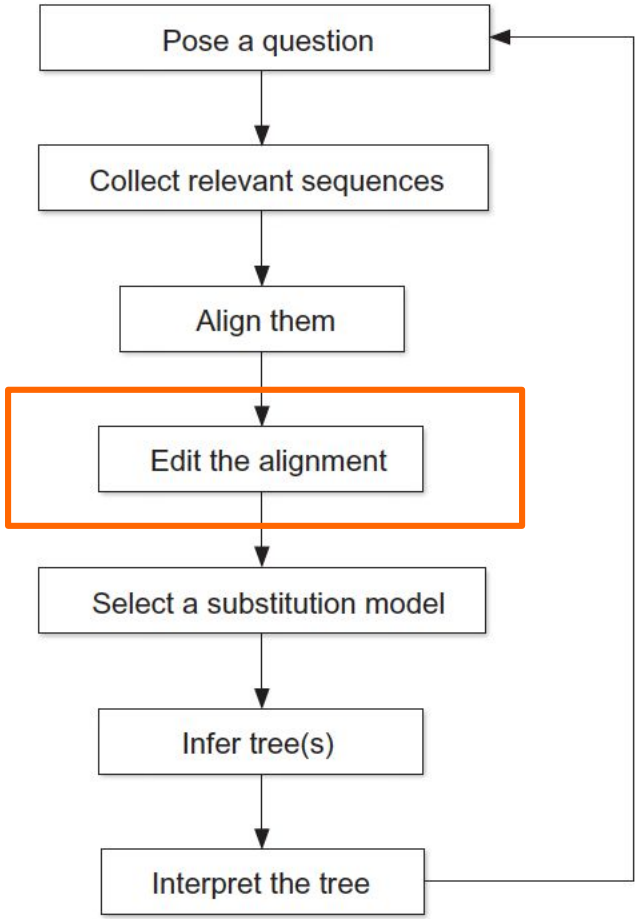
Molecular phylogenetics: a possible flowchart



How certain is my alignment?
Would other alignments give a different tree?

A critical approach to analysis is key: don't fall into the black box!

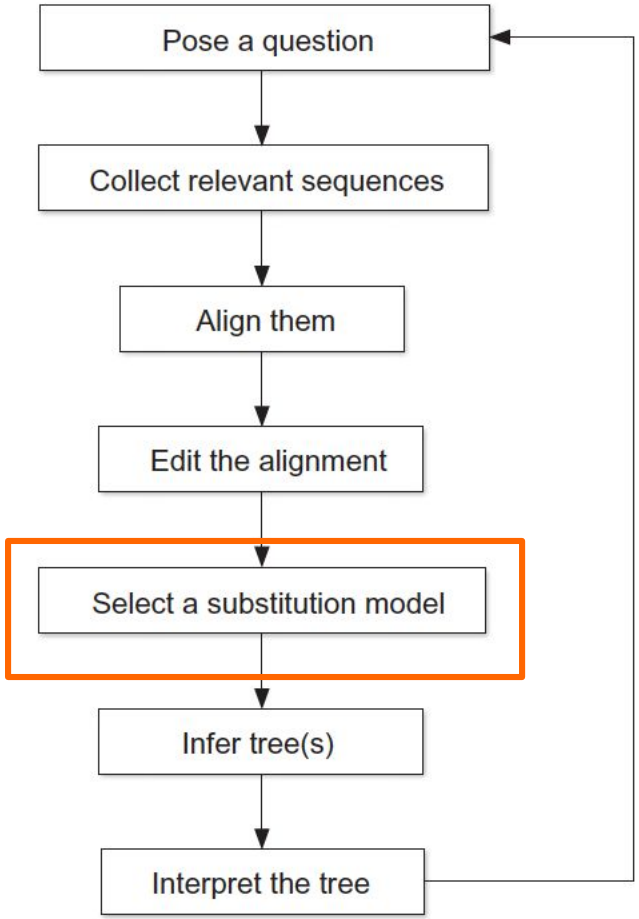
Molecular phylogenetics: a possible flowchart



Which bits, if any, should I remove?

A critical approach to analysis is key: don't fall into the black box!

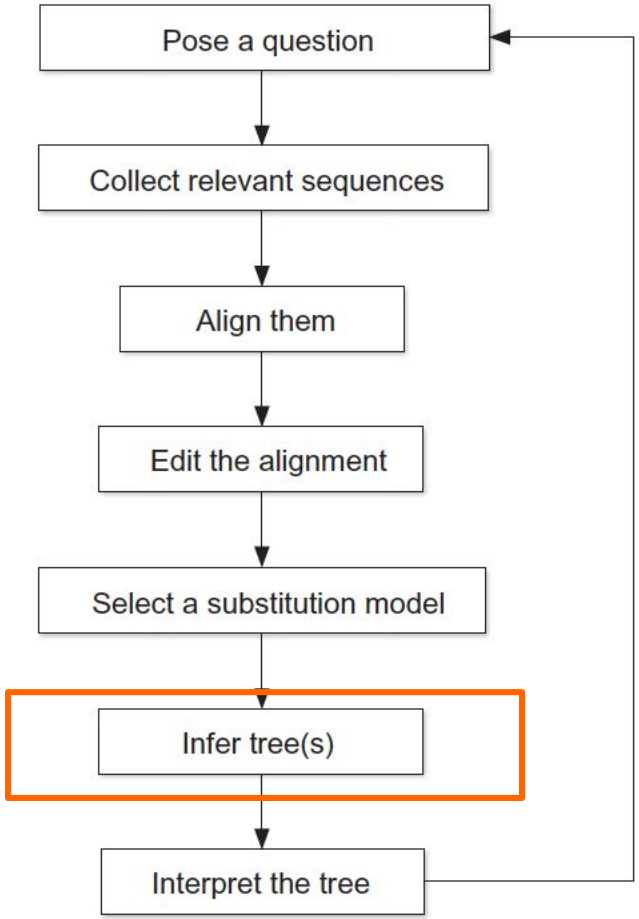
Molecular phylogenetics: a possible flowchart



Is this the best available model?
Is it good enough?

A critical approach to analysis is key: don't fall into the black box!

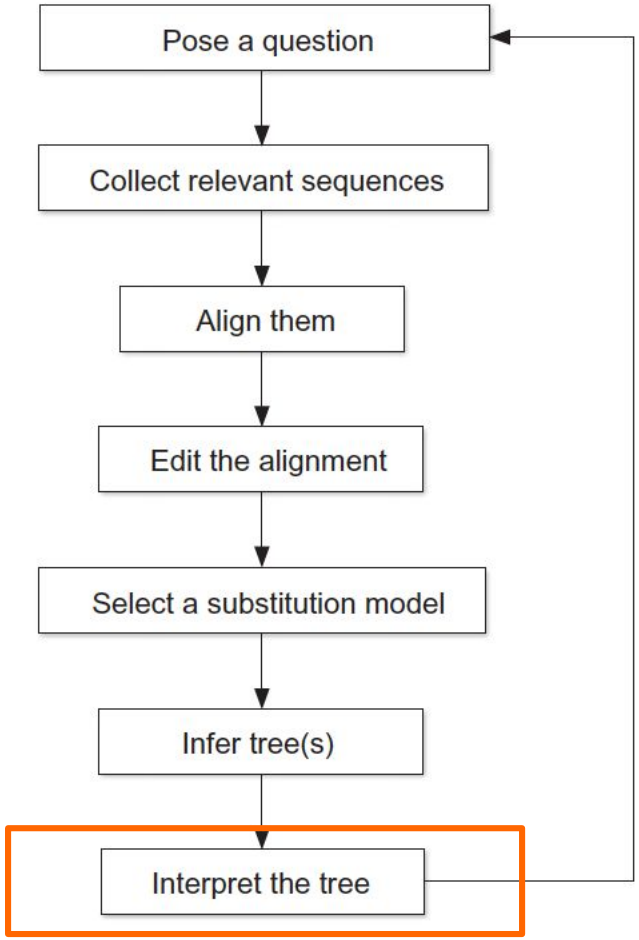
Molecular phylogenetics: a possible flowchart



Are my ML or Bayesian analyses working properly? (Local maxima, convergence)

A critical approach to analysis is key: don't fall into the black box!

Molecular phylogenetics: a possible flowchart



How well-supported are the key branches?

What does the tree mean for my hypothesis?

Can alternative hypotheses be rejected?

A critical approach to analysis is key: don't fall into the black box!

1. Pose a question

- Systematics, classification, character evolution in a clade of interest
- Use phylogenies as part of modelling a broader process

2. Assembling a dataset

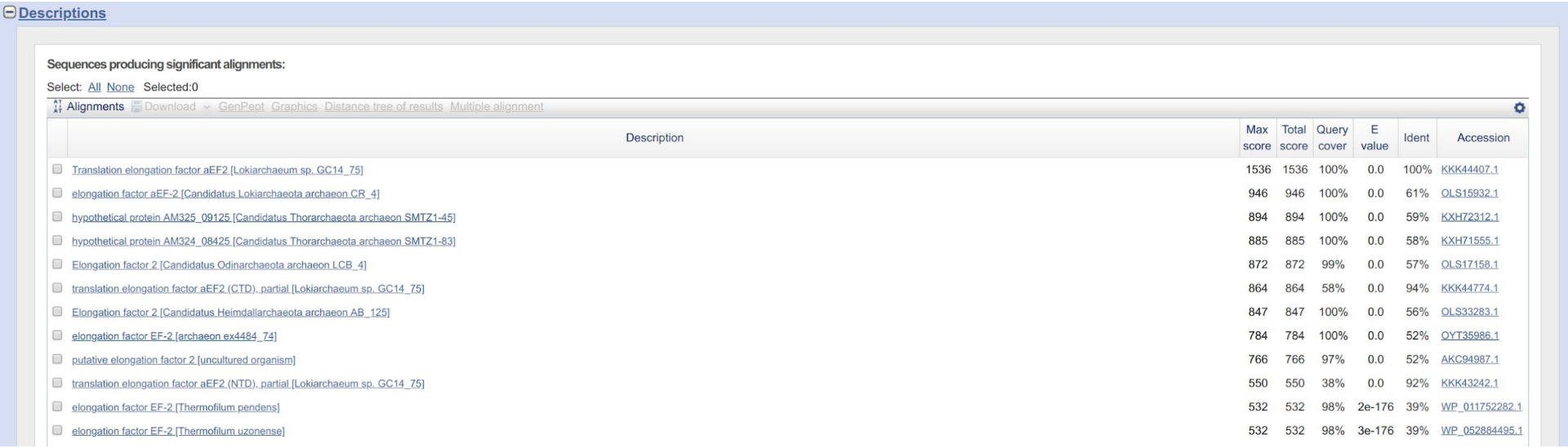
- Where do I get the data?
- Use an existing dataset
- Use your own data
- Use published data to test a new hypothesis (GenBank, Ensembl, PDB, Dryad, FigShare...)

2. Assembling a dataset

- Starting with a query, search sequence databases.
- An iterative process: start broad and refine using initial trees
- Should I include it or not? Do the experiment.

2. Assembling a dataset

- Starting with a query, search sequence databases (BLAST, PSI-BLAST, HMMER).



Descriptions

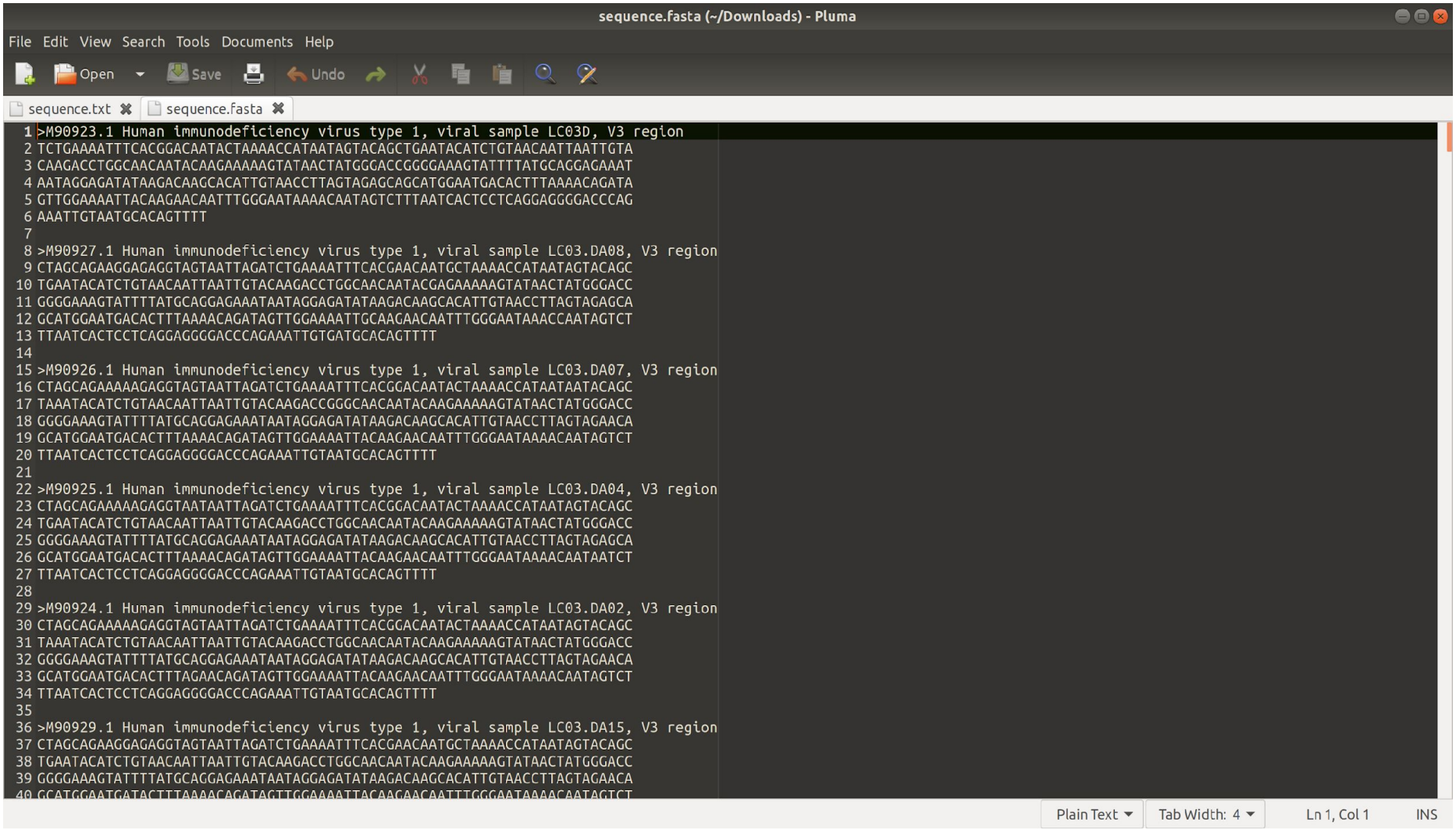
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
Translation elongation factor aEF2 [Lokiarchaeum sp. GC14_75]	1536	1536	100%	0.0	100%	KKK44407.1
elongation factor aEF-2 [Candidatus Lokiarchaeota archaeon CR_4]	946	946	100%	0.0	61%	OLS15932.1
hypothetical protein AM325_09125 [Candidatus Thorarchaeota archaeon SMTZ1-45]	894	894	100%	0.0	59%	KXH72312.1
hypothetical protein AM324_08425 [Candidatus Thorarchaeota archaeon SMTZ1-83]	885	885	100%	0.0	58%	KXH71555.1
Elongation factor 2 [Candidatus Odinarchaeota archaeon LCB_4]	872	872	99%	0.0	57%	OLS17158.1
translation elongation factor aEF2 (CTD), partial [Lokiarchaeum sp. GC14_75]	864	864	58%	0.0	94%	KKK44774.1
Elongation factor 2 [Candidatus Heimdallarchaeota archaeon AB_125]	847	847	100%	0.0	56%	OLS33283.1
elongation factor EF-2 [archaeon ex4484_74]	784	784	100%	0.0	52%	QYT35986.1
putative elongation factor 2 [uncultured organism]	766	766	97%	0.0	52%	AKC94987.1
translation elongation factor aEF2 (NTD), partial [Lokiarchaeum sp. GC14_75]	550	550	38%	0.0	92%	KKK43242.1
elongation factor EF-2 [Thermofilum pendens]	532	532	98%	2e-176	39%	WP_011752282.1
elongation factor EF-2 [Thermofilum uzonense]	532	532	98%	3e-176	39%	WP_052884495.1

2. Assembling a dataset



3. Alignments: hypotheses of homology

ATTGGCG

AGGAG

Sequences!

ATTGGCG

A--GGAG

Alignment!

AGGCG

+TT

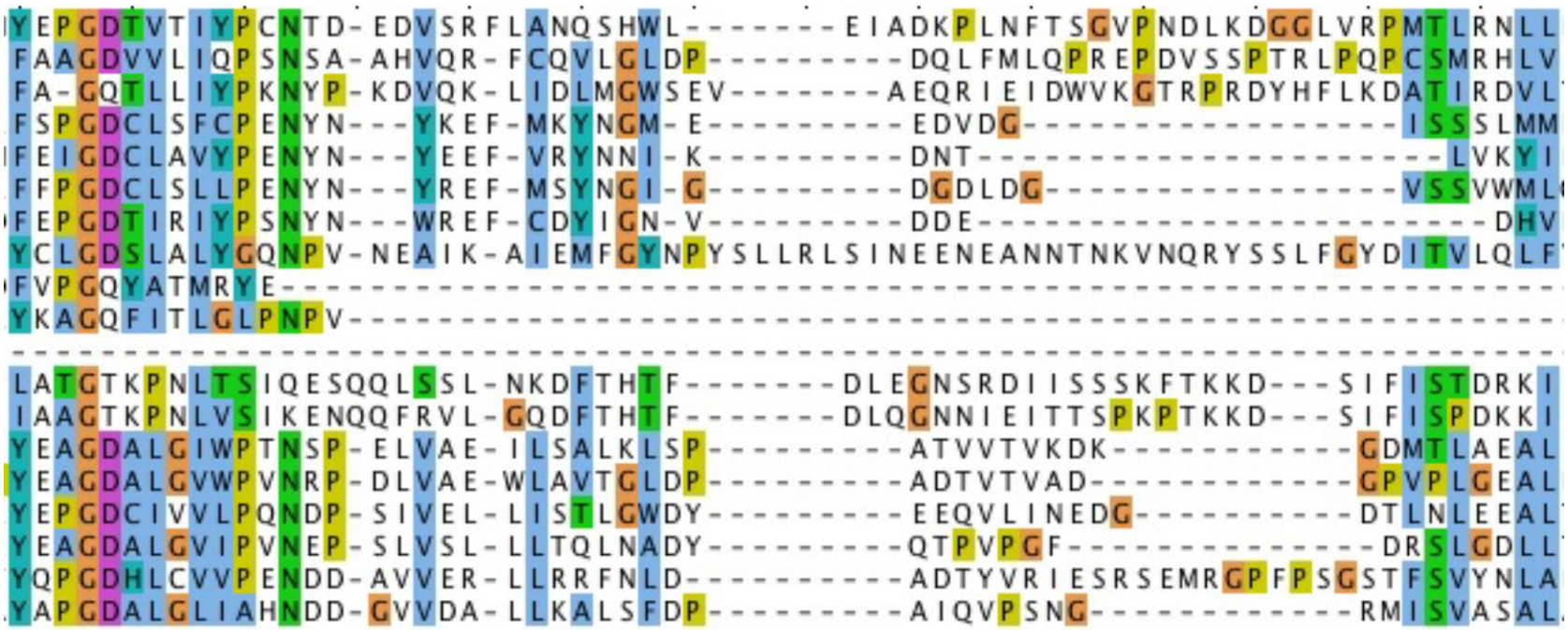
C=>A

ATTGGCG

A--GGAG

Homology!

Some bits are easier to align than others



Homologous!

???

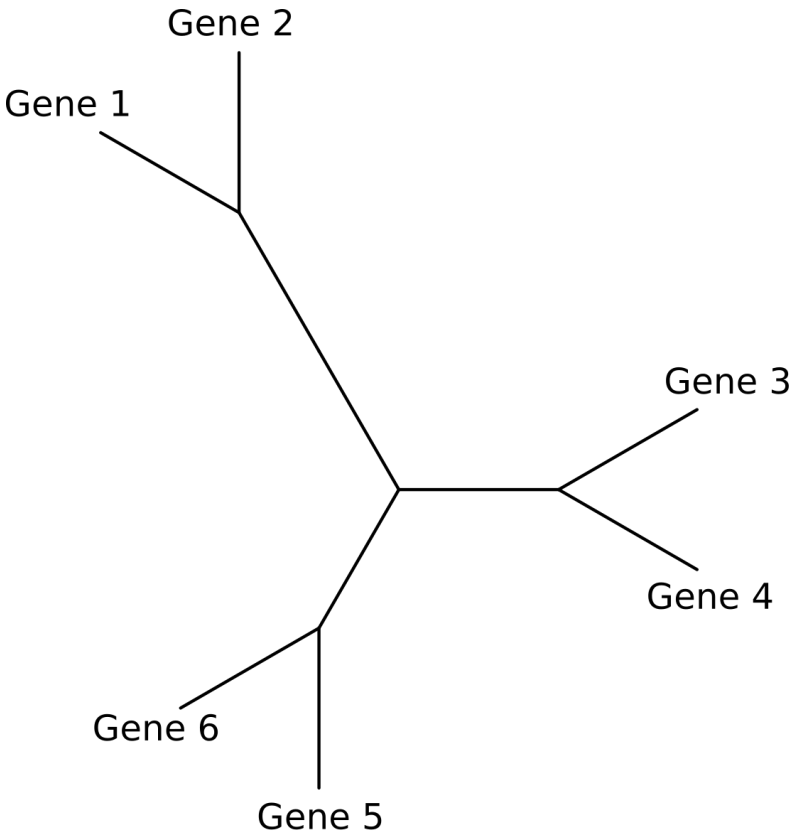
3. Alignments: some considerations

- Tree inference methods assume characters in each alignment column are homologous
- Automated alignment methods often disagree
- The alignment is uncertain, but often fixed in downstream analyses. **It matters!**

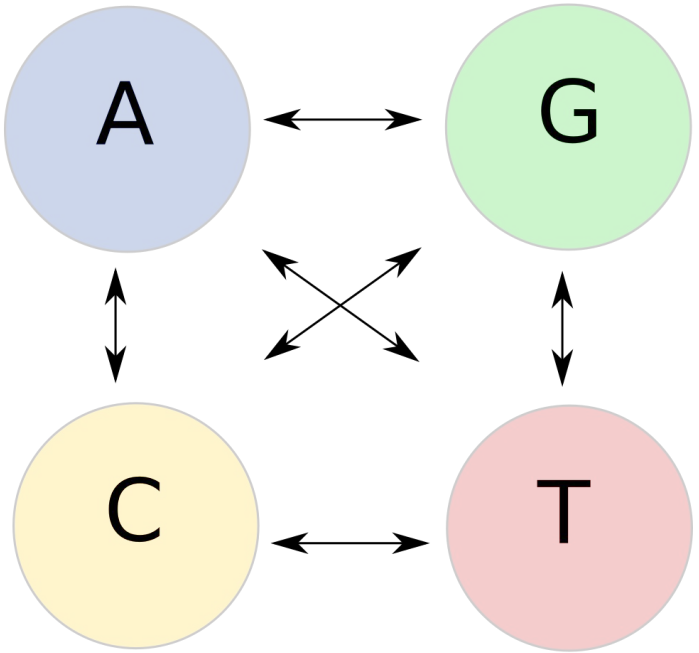
4. Modelling sequence evolution and inferring trees

- Modern phylogenetic analysis is model-based
- Fitting this model to the sequence alignment helps us learn about the parameters of the model (including the tree topology)
- Inference depends on **likelihood**: the probability of the data given the model.

A basic model



(a) Phylogenetic tree
(with branch lengths)

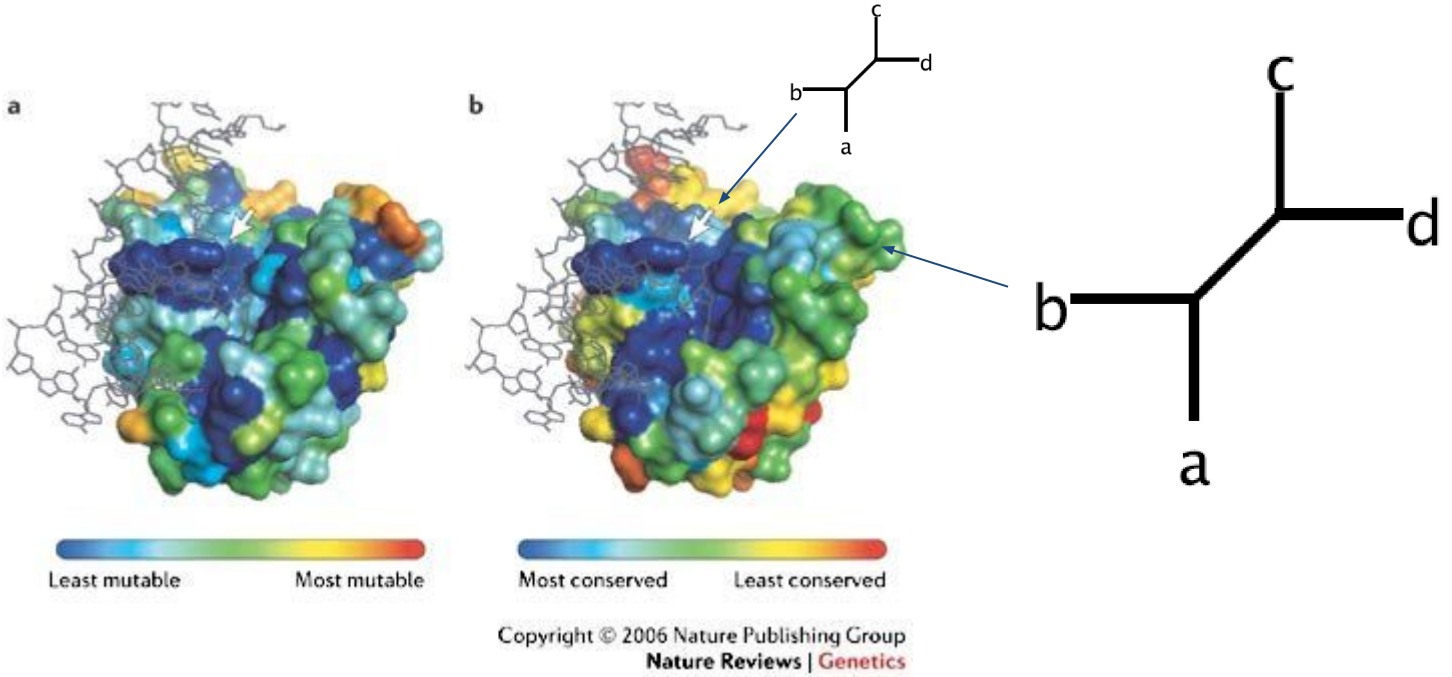


(b) Substitution process
Rates, compositions

Adding complexity (and parameters) to the basic model

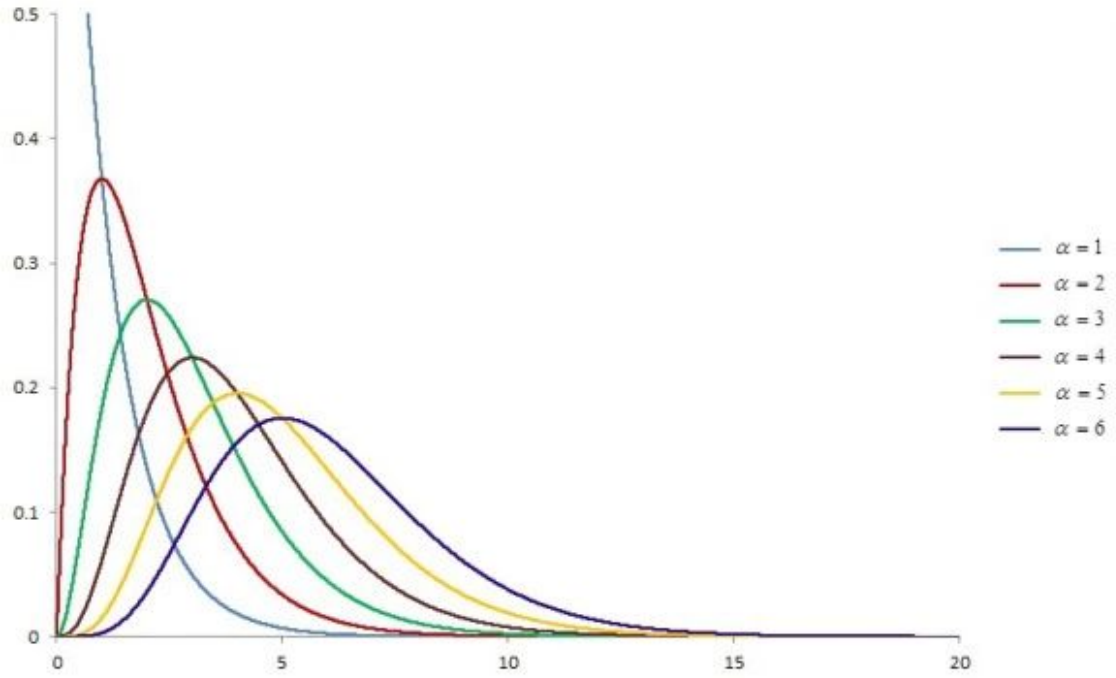
- Different sites evolve at different rates
- Different genes evolve at different rates
- The substitution process might vary across the tree (descent with modification)

Adding complexity: site rates



- Some sites are more important for function than others; they tend to evolve more slowly.
- Model this by having several different rates (tree lengths)

Adding complexity: site rates



- Gamma distribution can take many shapes, depending on a single parameter (alpha). So can model the distribution of rates-across-sites with just one more parameter!
- A ****mixture model****: average the likelihood per site over the possible rates!

Choosing an appropriate model

Likelihood = *P*(alignment | model)

Tree
Branch lengths
Evolutionary rates
Exchangeabilities
etc.

- The evidence we extract from the data totally depends on the model.
- **All models are wrong!**
  ….but some are useful (George Box).

How can we pick a useful model for our data?

Choosing an appropriate model

Likelihood = P(alignment | model)

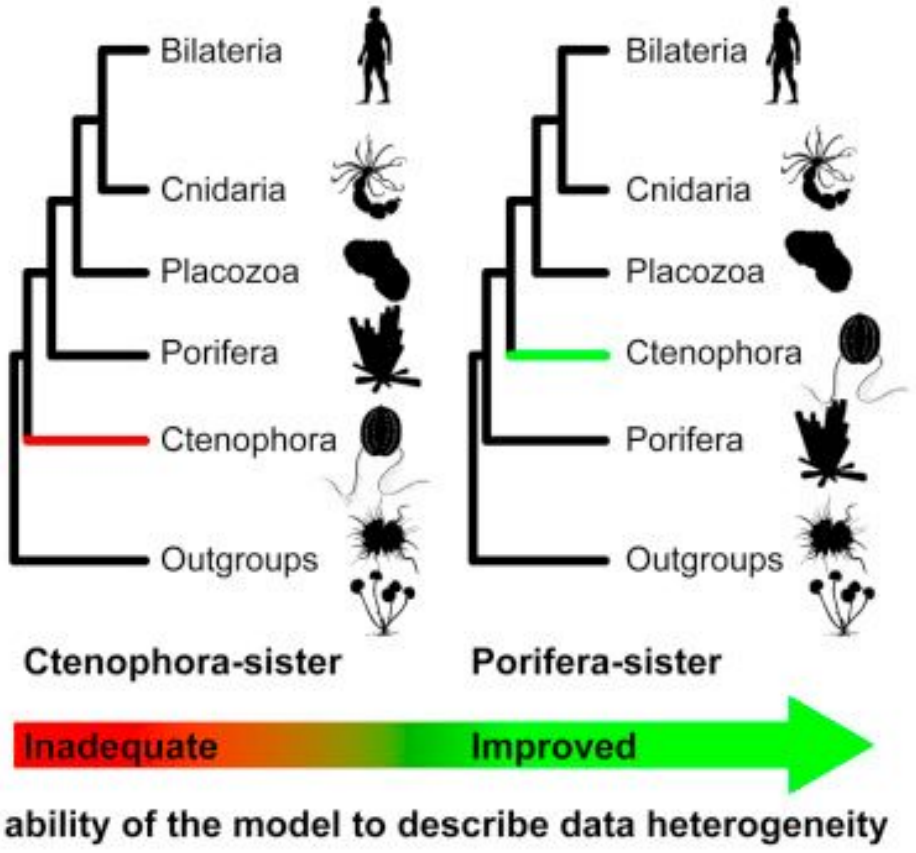
Tree
Branch lengths
Evolutionary rates
Exchangeabilities
etc.

- The likelihood provides a natural way of comparing models.
- Which model makes the data most likely?
- Subtle difficulties of maximum likelihood: model fit is not quite so easy. Use scores that are modifications of the maximum likelihood.

Model selection

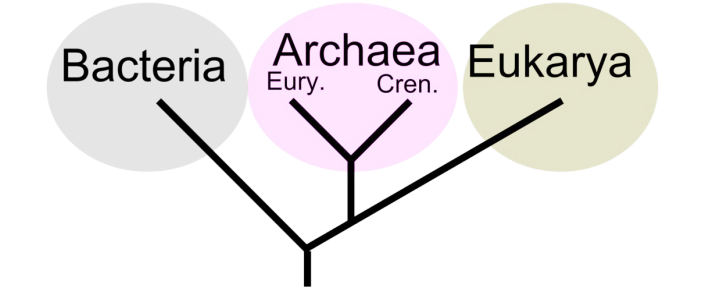
- Phylogeny packages implement a set of candidate models.
- Use statistical tests (AIC, BIC - based on the maximum likelihood) to pick **the best model from that set** for your data.
- It's possible that none of the implemented models is adequate for your data (use simulations?).
- ***Many published analyses are of very poor quality: **be critical and don't fall into this trap.**

Model selection matters: nervous system origins

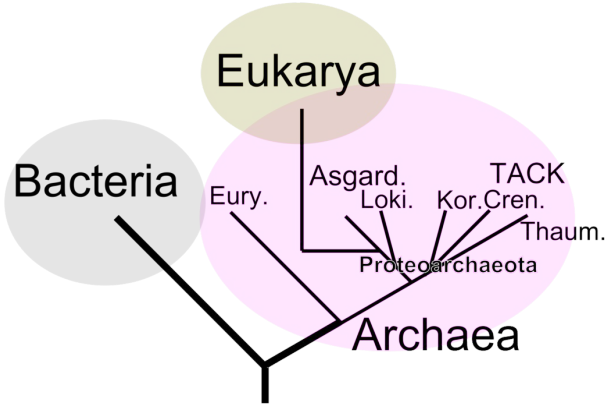


Feuda et al. (2017) *Curr Biol;* Whelan et al. (2017) *Nat Ecol Evol*

Model selection matters: The tree of life



Carl Woese's three domain tree



two domain tree

Cox et al. (2008) PNAS, Williams et al. (2012) PRSB

Fitting models: maximum likelihood vs. Bayesian inference

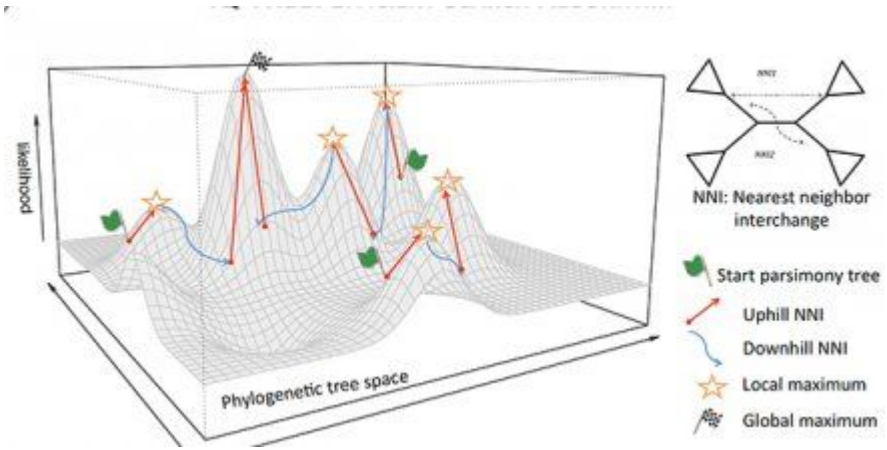
- **To a first approximation, the fit of the model is more important!**

Maximum likelihood

Task: find values for all of the parameters of the model that make the probability of the alignment (likelihood) as high as possible.

ML programs implement (hill climbing) algorithms for joint estimation of these values.

The ML tree is the best point estimate of the true tree, if the model is correct.



Maximum likelihood: assessing support

- ML tree is best estimate of the true tree. But is it much better than any other estimate? (Hypothesis testing, e.g. **AU-test**)
- How certain are we of the relationships on the tree? Are some better supported than others? (**Bootstrapping**)

Maximum likelihood: assessing support with the bootstrap

Bootstrapping is a technique for estimating the confidence interval around a parameter by **resampling and reanalysis** of the original data (alignment).

1. Sample, with replacement, the same number of characters as the original dataset.
2. Repeat tree inference on each of many bootstrap replicates.
3. Assess confidence as the proportion of samples in which each split on the tree appears.

Taxon	1	2	3	4
A	V	W	R	A
B	V	W	R	L
C	I	Y	K	M
D	M	F	K	A

Original dataset (4 characters)

Taxon	1	1	3	2
A	V	V	R	W
B	V	V	R	W
C	I	i	K	Y
D	M	M	K	F

One bootstrap dataset

Maximum likelihood: assessing support with the bootstrap

Bootstrapping is a technique for estimating the confidence interval around a parameter by **resampling and reanalysis** of the original data (alignment).

1. Sample, with replacement, the same number of characters as the original dataset.
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Taxon	1	2	3	4
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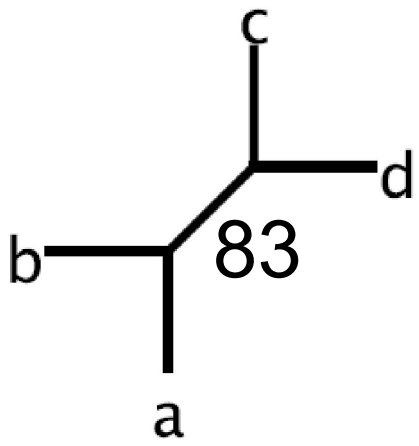
Original dataset (4 characters)



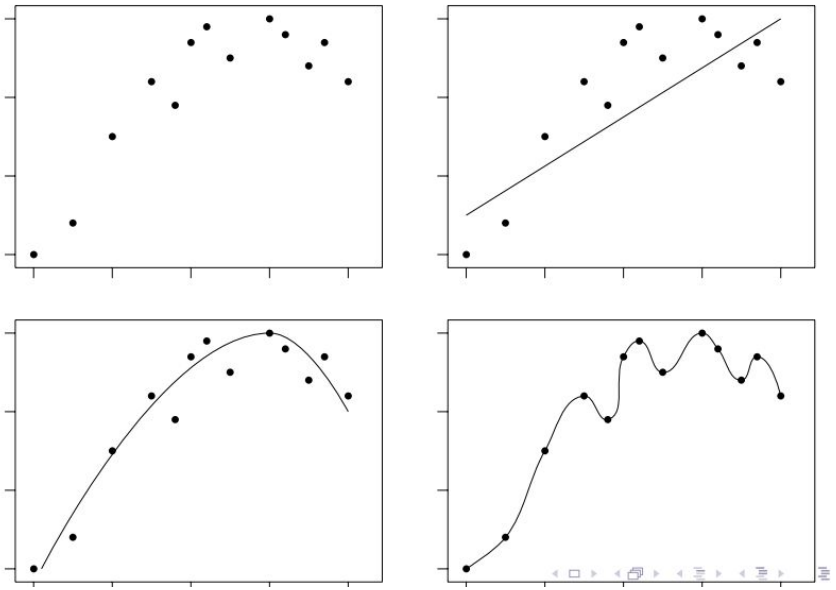
Maximum likelihood: assessing support with the bootstrap

Interpreting bootstrap values:

- ***The bootstrap is not the probability of a split being correct!**
- Early on, some justifications by comparison to bootstrapping confidence intervals in other settings (v**ariance of bootstrap trees around ML tree ~ variance of ML trees around true tree**)
- These days, mostly thought of as a way of getting at the robustness of the signal for relationships in the alignment.
- Values of >70 usually taken as being somewhat reliable.

Maximum likelihood: the problem of model complexity

- Since we always find the optimal values for all parameters in the model, adding parameters will not make the fit to the data worse! (So, can't directly compare the maximum likelihoods)
- We run the risk of overparameterisation (too many, or useless, parameters). This interferes with our ability to generalise/predict from the model!



Maximum likelihood: the problem of model complexity

To address this problem, likelihood can be penalized by the number of model parameters when comparing models, e.g. with the Akaike Information Criterion:

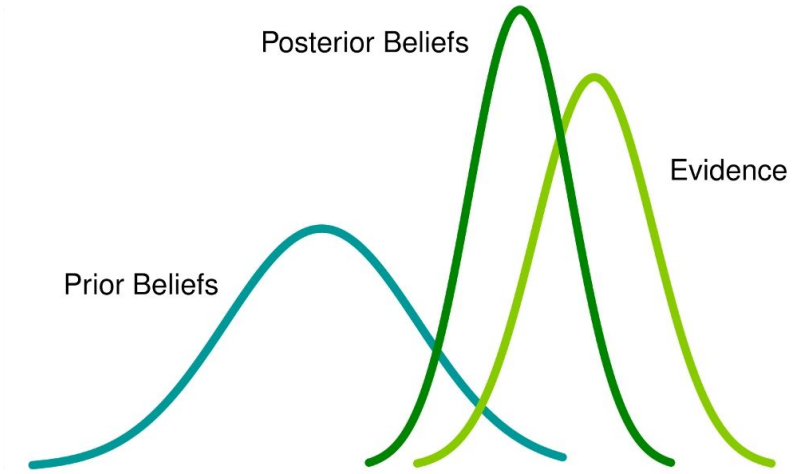
AIC = 2k - 2ln(ML)

[k = number of parameters; ln(ML) = log of maximum likelihood.
Choose smallest AIC!

E.g.
ln(ML) = -17456
-2ln(ML) = 34912

AIC = 34912 + 2(number of parameters)
Smallest AIC: best ML with fewest parameters.

Bayesian inference



Conceptualise the analysis as an **updating** of our prior beliefs, based on new **evidence**.

Our beliefs about parameters are expressed as **distributions**.

The **evidence = likelihood** of the data (as in ML).

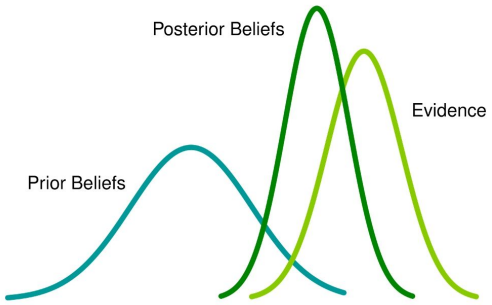
This seems to follow human reasoning.

Bayes theorem

$$P(H|D) = \frac{P(H)\,P(D|H)}{P(D)}$$

P(H|D): Probability of Hypothesis (tree, model…) given Data (alignment)

P(H): Prior probability of Hypothesis
P(D|H): Probability of Data given Hypothesis (**the likelihood!**)
P(D): Prior probability of the Data



Prior beliefs are a central part of logical reasoning

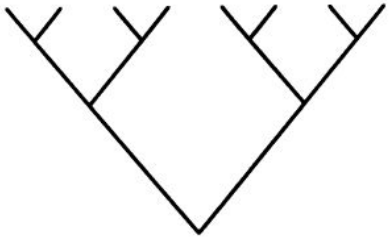
$$P(H|D) = \frac{P(H)\,P(D|H)}{P(D)}$$

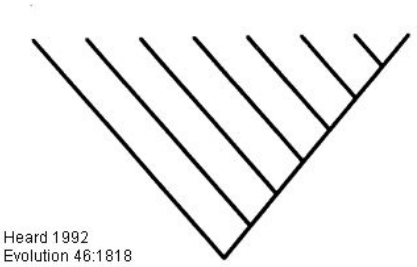
- 1 out of 100 people in the population at large have disease X.
- A **diagnostic test** exists: it has a false negative rate of 0% and a false positive rate of 10%.
- As part of a routine checkup, Billy takes the test and it is positive.
- What is the probability that Billy has disease X?

How can we have prior beliefs about e.g. the tree?

- We do: e.g. which of these trees is more probable, all else being equal?

- We usually want to use “weak” (flat) priors
- Some priors are fairly strong (e.g. on branch lengths)
- Some priors are extremely strong (e.g. node ages in molecular clock)





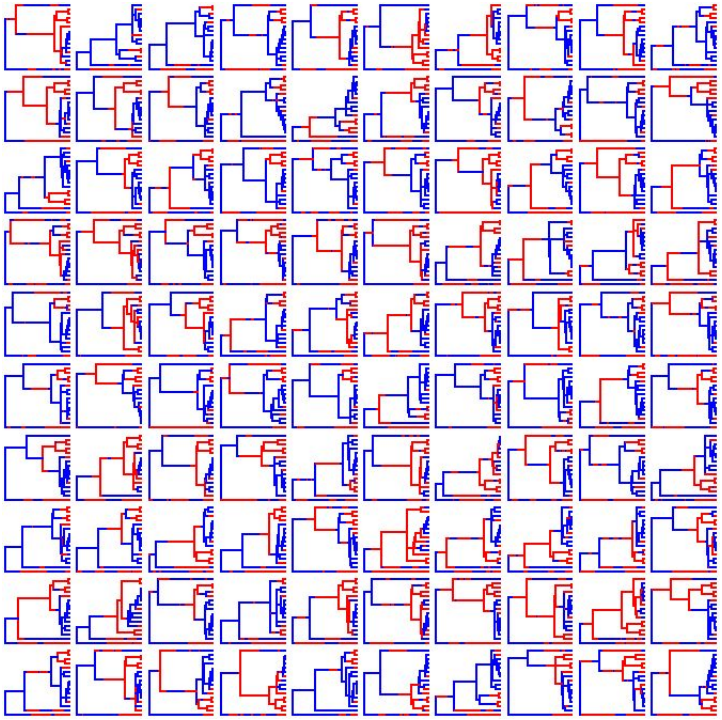
Heard 1992
Evolution 46:1818

Indisputable superiority of Bayesian approach (1): quantifying support

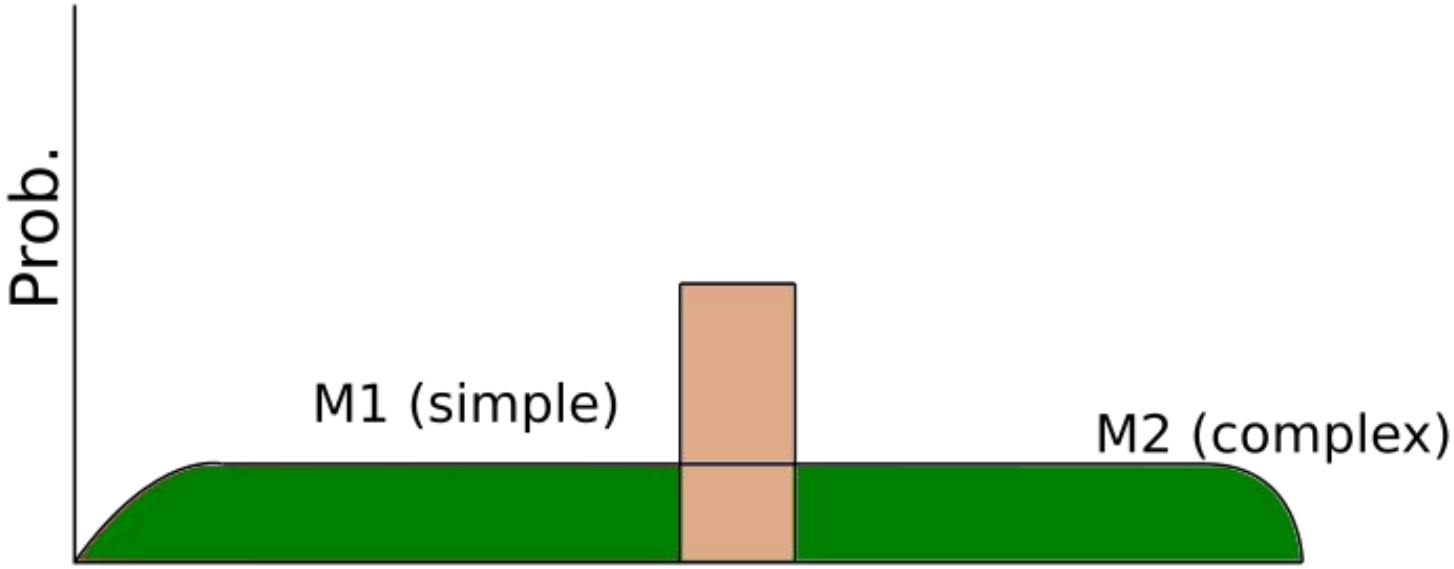
We have a prior distribution on trees, updated by the evidence, to give a posterior **distribution** of trees: a natural measure of uncertainty.

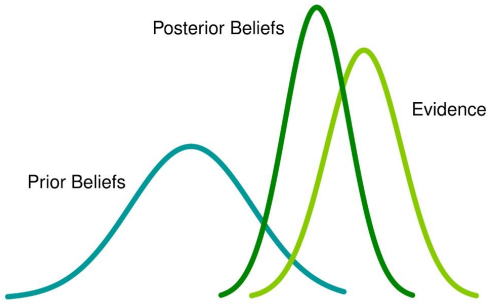
No need for bootstrapping.

Has a direct interpretation: the **frequency of a tree (or a split) in the posterior distribution is the probability that it is true**, under the model.



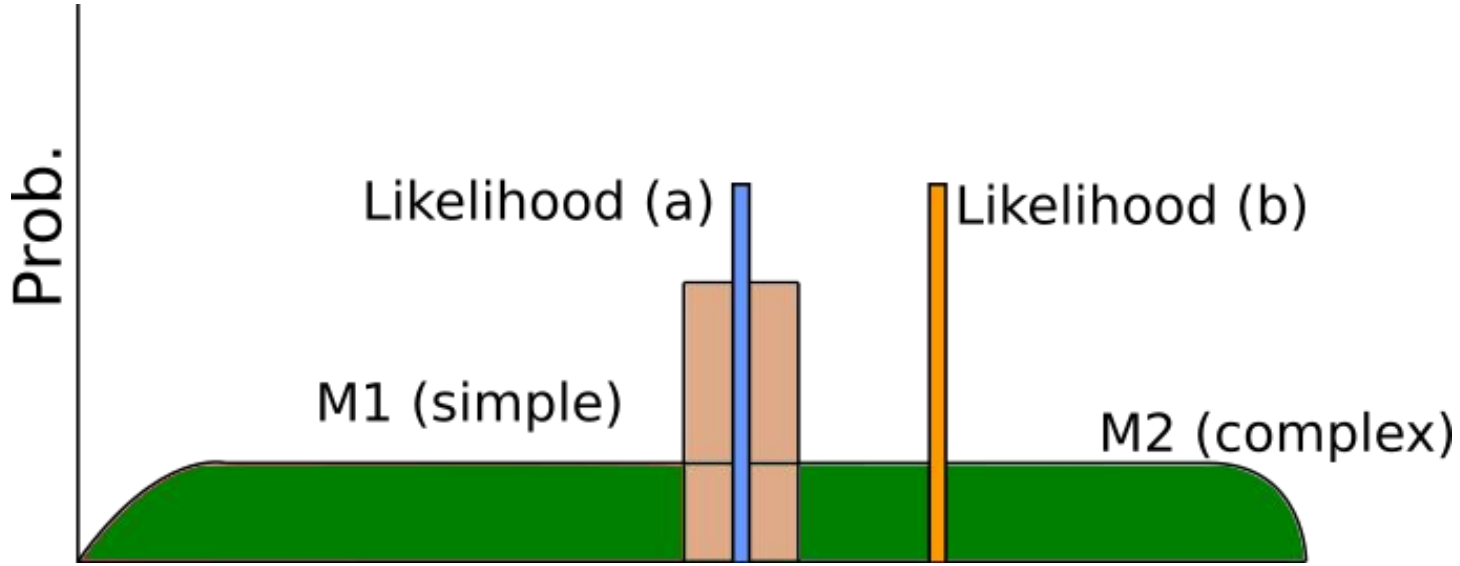
Indisputable superiority of Bayesian approach (2): natural incorporation of model complexity

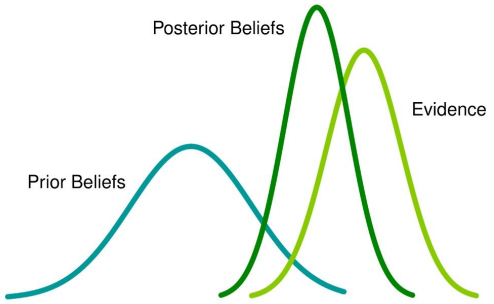




Complex models predict a wider range of outcomes *a priori*, but P(outcomes) must sum to 1.

Indisputable superiority of Bayesian approach (2): natural incorporation of model complexity

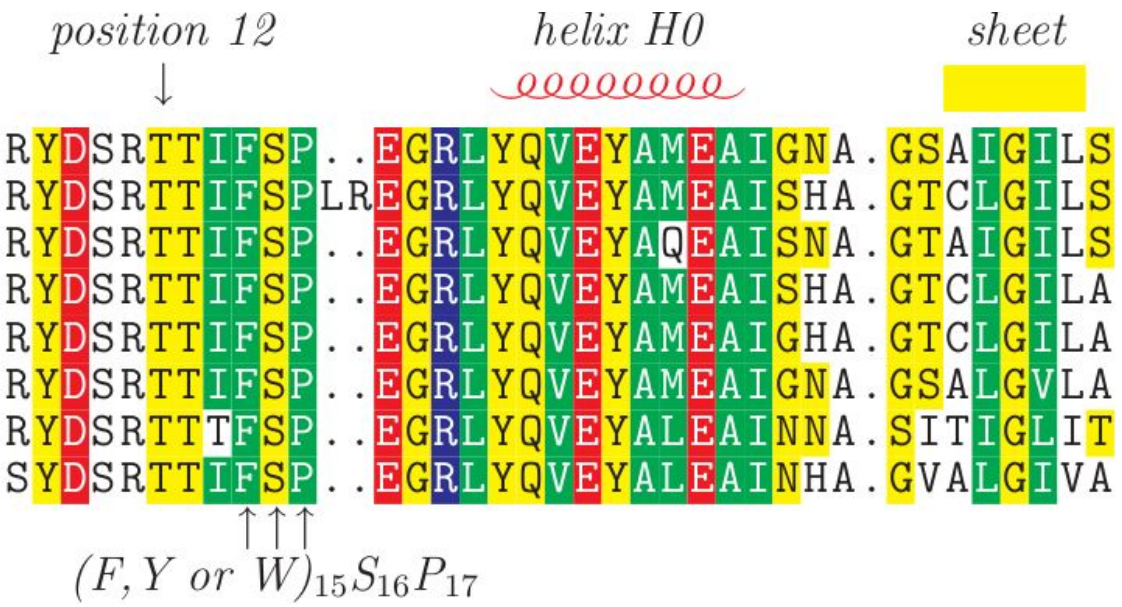




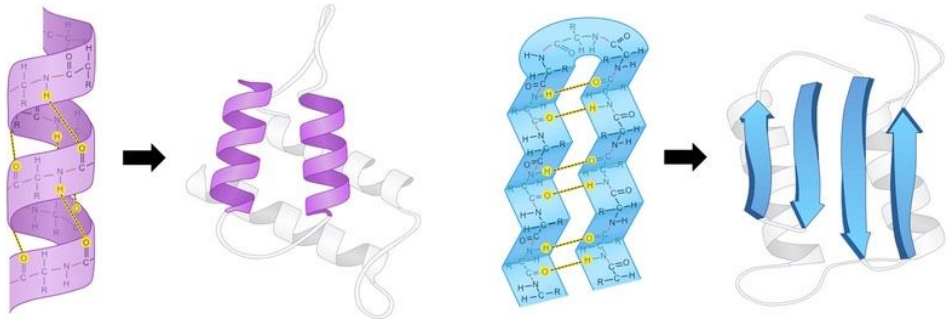
Complex models predict a wider range of outcomes *a priori*, but P(outcomes) must sum to 1!

Priors provide a natural penalty for model complexity.

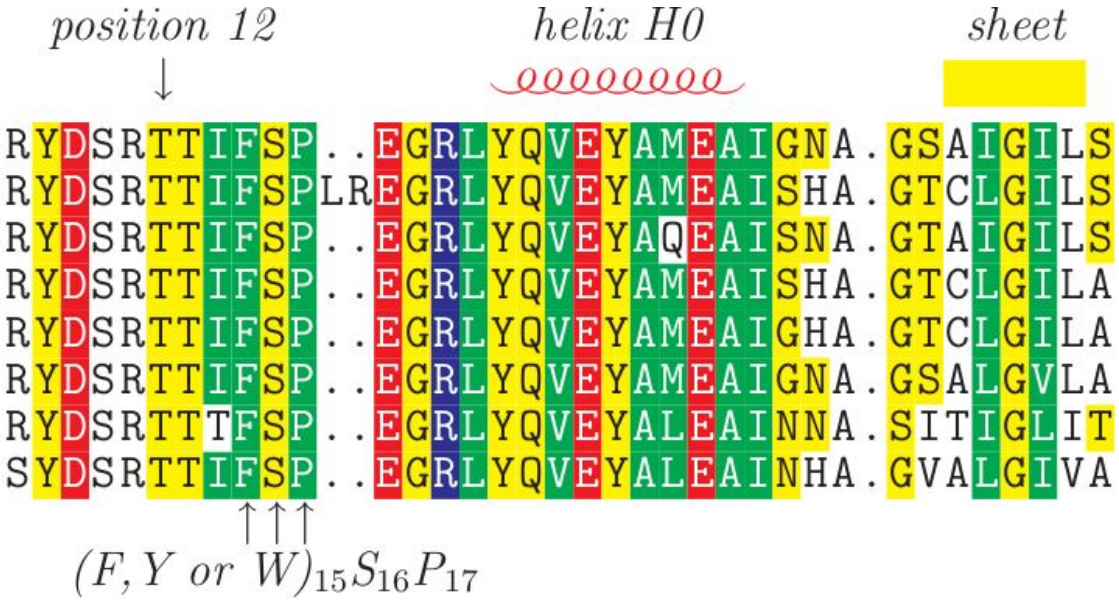
A complex model: site-specific sequence compositions

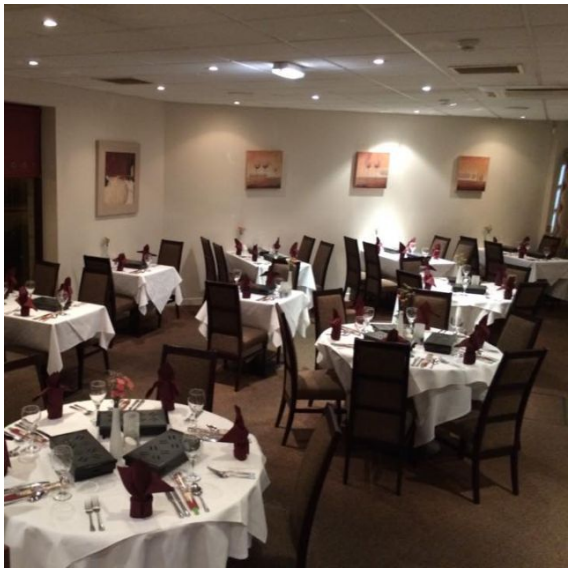


How the hell to model this?
How many different compositions are there?
How are sites allocated to compositions?



The Dirichlet process: a prior distribution *on discrete distributions*





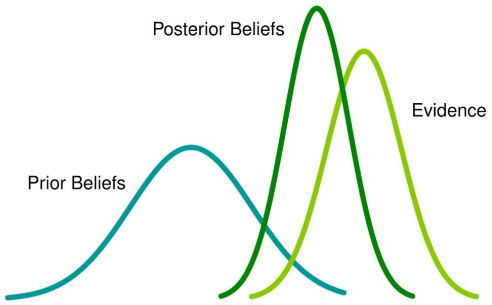
We can draw sets of composition "centroids" (vectors of e.g. 20 proportions) from a Dirichlet process prior.

- The result is an ***__infinite mixture model__***: we average over an unknown, but possibly very large, number of different compositions!
- This will usually have uncountably many parameters: can't compute BIC or similar (but prior puts constraints on the draws via a concentration parameter).
- **Usually fits the real data very well.** Can't be done with ML.

Sampling from P(H|D) using MCMC

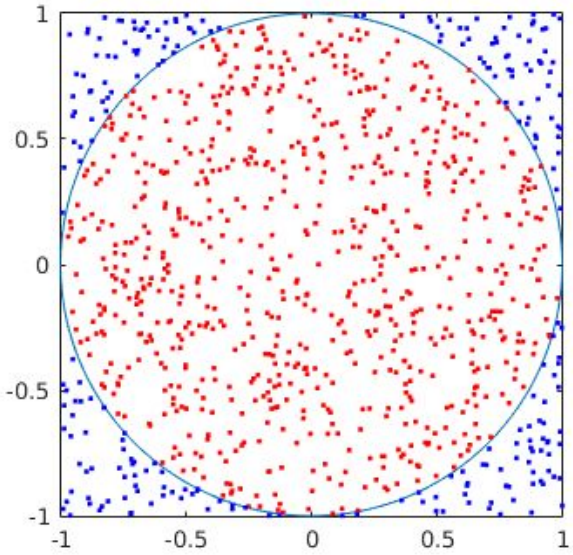
$$P(H|D) = \frac{P(H)\,P(D|H)}{P(D)}$$

Uh oh!

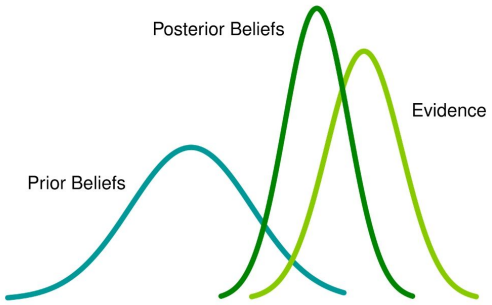


- We can't analytically find P(H|D) in the phylogenetic case because P(D) is rather difficult to calculate (average over all trees, branch lengths, other model parameters…)
- We use numerical methods (Markov Chain Monte Carlo) to sample from P(H|D); with enough samples, we obtain a reasonable approximation of P(H|D).

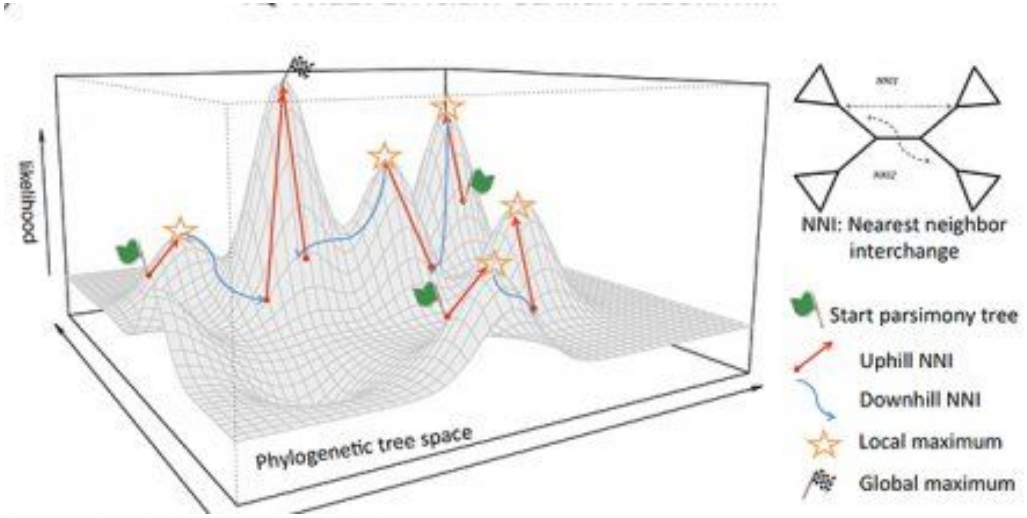
Sampling from P(H|D) using MCMC

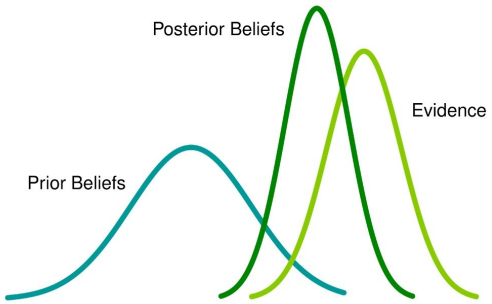


How could we estimate the area of this circle, without knowing much about geometry?



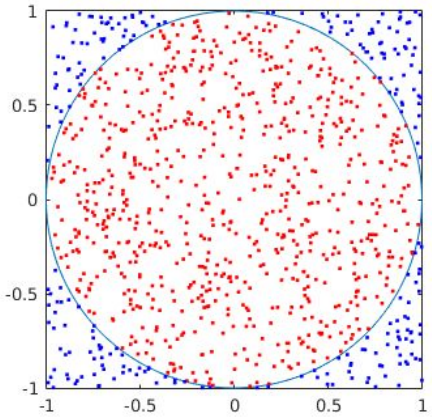
Sampling from P(H|D) using MCMC

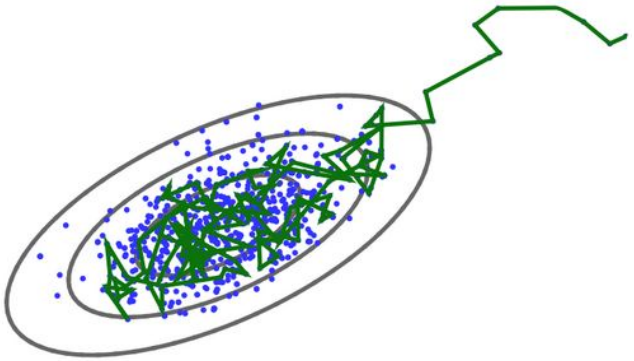


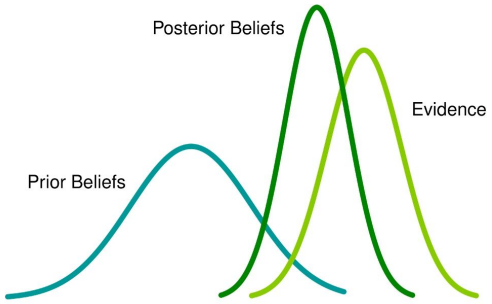


What about a more complex, multidimensional shape (like a probability distribution!)?

Sampling from P(H|D) using MCMC







- We draw samples from P(H|D) using a biased random walk, such that histograms of the samples/set of trees drawn will approximate the distribution.
- We need to sample in a biased way because P(H|D) has many dimensions, and the region(s) of credible values with P > 0 is quite small.
- Can mostly sample from high-probability regions by biasing the “walk” using the ratio of P(D|H)P(H) at the current and proposed region to decide whether to jump or not.
- **NB: We sample to estimate the whole density: NOT just the maximum likelihood!**

Analysis with ML and Bayesian methods: practical considerations

(Methods have a lot in common; either is often OK, and better than alternatives).

Maximum likelihood:

+ Very efficient implementations (scale to 100s or 1000s of sequences, 10,000s of alignment sites)
+ Fitting the models usually a bit less involved (but: local maxima)
- Support values hard to interpret
- Limited to simple models

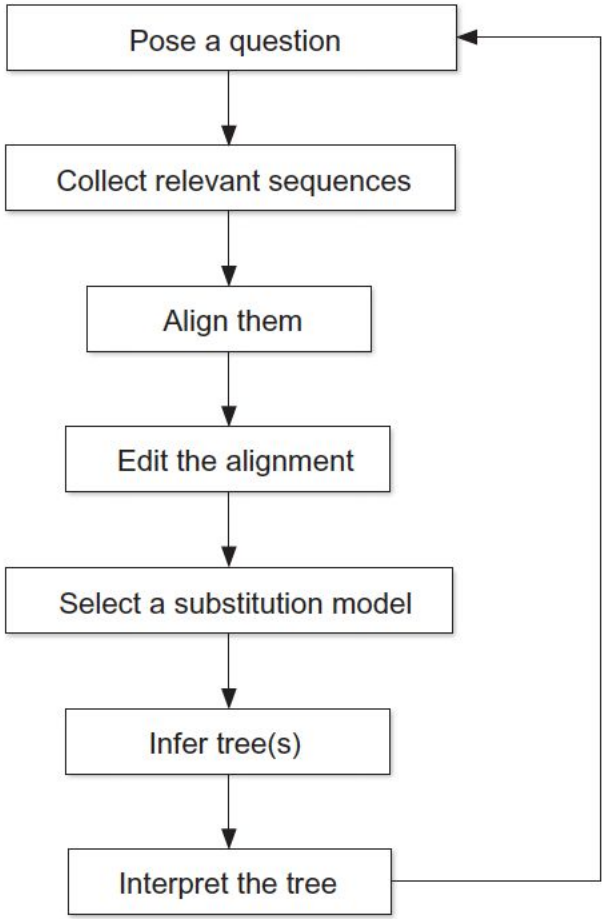
Bayesian:

+ A principled framework for inference (...)
+ Natural way to fit more complex models (best-fitting models are all Bayesian-only)
+ Natural support values
- Getting MCMC to work can be difficult
- Don’t scale to very large datasets

Fitting phylogenetic models: summary

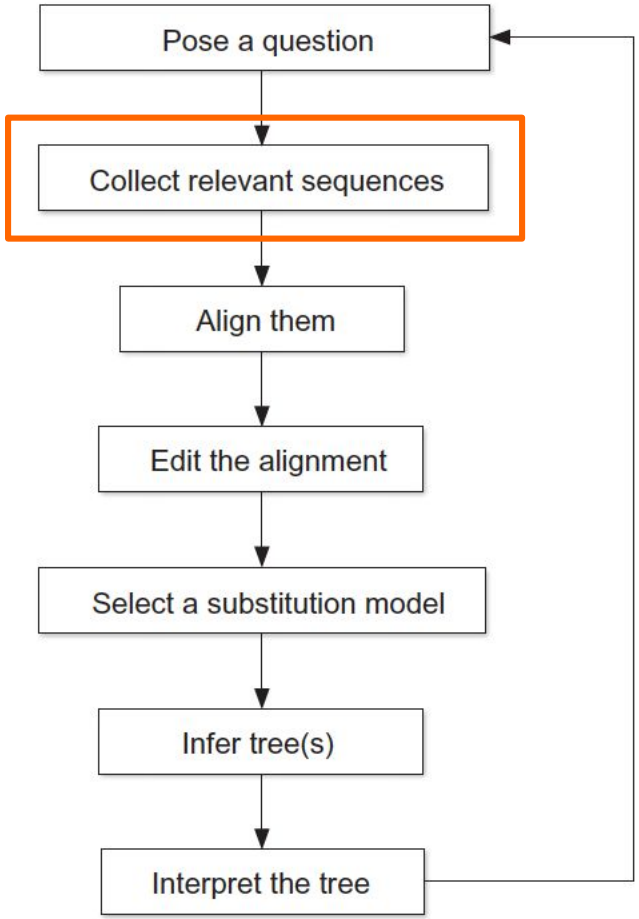
- Phylogenetic trees are statistical inferences
- The inferences are based on a model: it's important, so do experiments!
- Quantifying the uncertainty in our inferences is critically important for interpretation
- ML & Bayesian methods both provide a framework for assessing support

Molecular phylogenetics: a possible flowchart



A critical approach to analysis is key: don't fall into the black box!

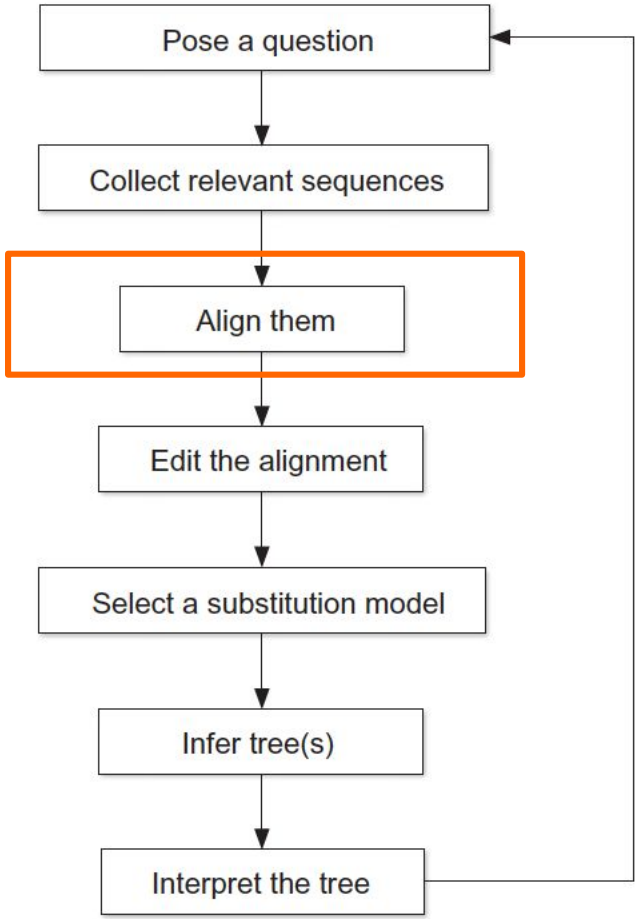
Molecular phylogenetics: a possible flowchart



Did I miss any important ones?

A critical approach to analysis is key: don't fall into the black box!

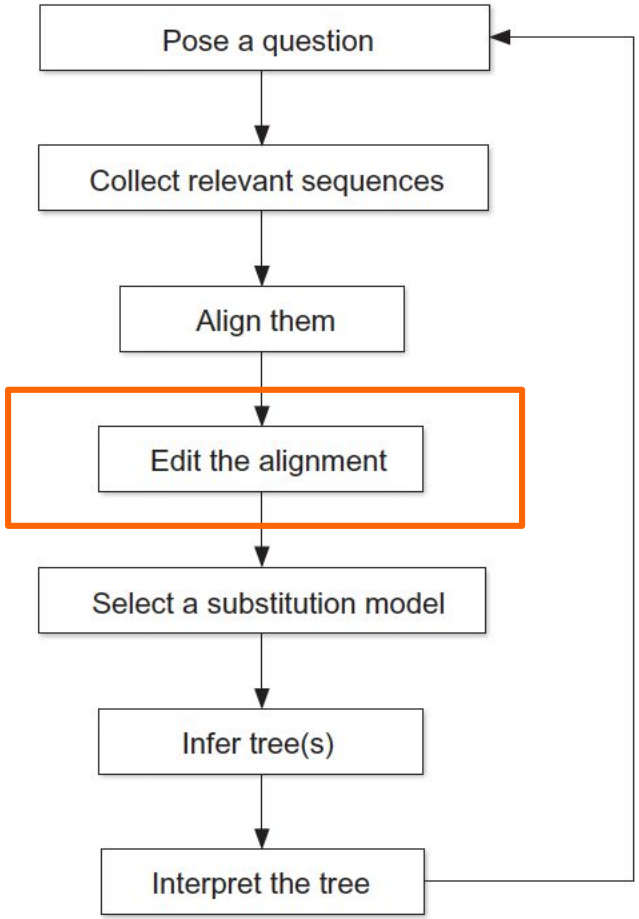
Molecular phylogenetics: a possible flowchart



How certain is my alignment?
Would other alignments give a different tree?

A critical approach to analysis is key: don't fall into the black box!

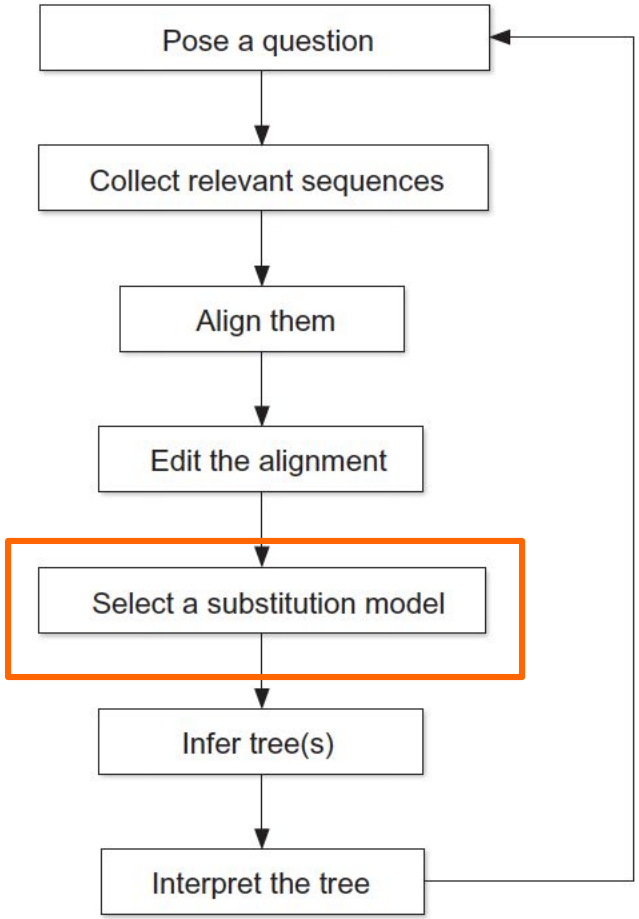
Molecular phylogenetics: a possible flowchart



Which bits, if any, should I remove?

A critical approach to analysis is key: don't fall into the black box!

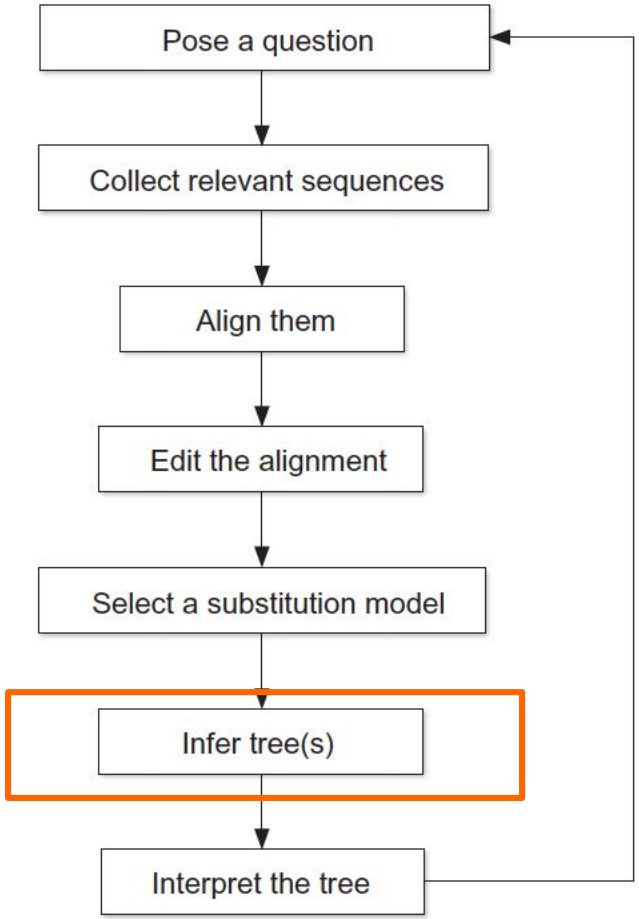
Molecular phylogenetics: a possible flowchart



Is this the best available model?
Is it good enough?

A critical approach to analysis is key: don't fall into the black box!

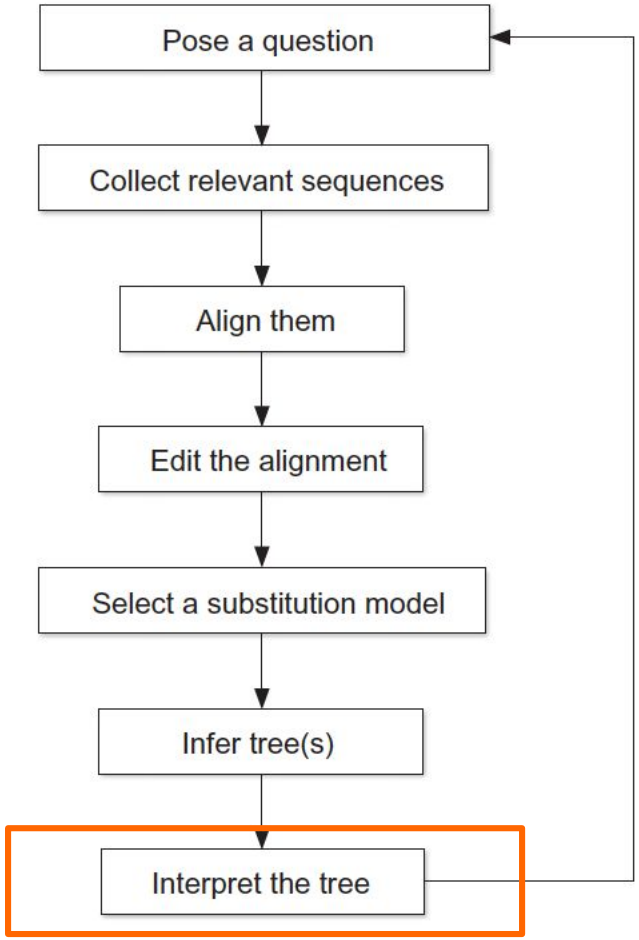
Molecular phylogenetics: a possible flowchart



Are my ML or Bayesian analyses working properly? (Local maxima, convergence)

A critical approach to analysis is key: don't fall into the black box!

Molecular phylogenetics: a possible flowchart



How well-supported are the key branches?

What does the tree mean for my hypothesis?

Can alternative hypotheses be rejected?

A critical approach to analysis is key: don't fall into the black box!

(Brief, crappy) introduction to the molecular clock

The molecular clock

- **Genetic differences accumulate over time**
- **The amount of divergence between species is roughly proportional to the age of their last common ancestor**

Zuckerkandl & Pauling (1962)

The molecular clock

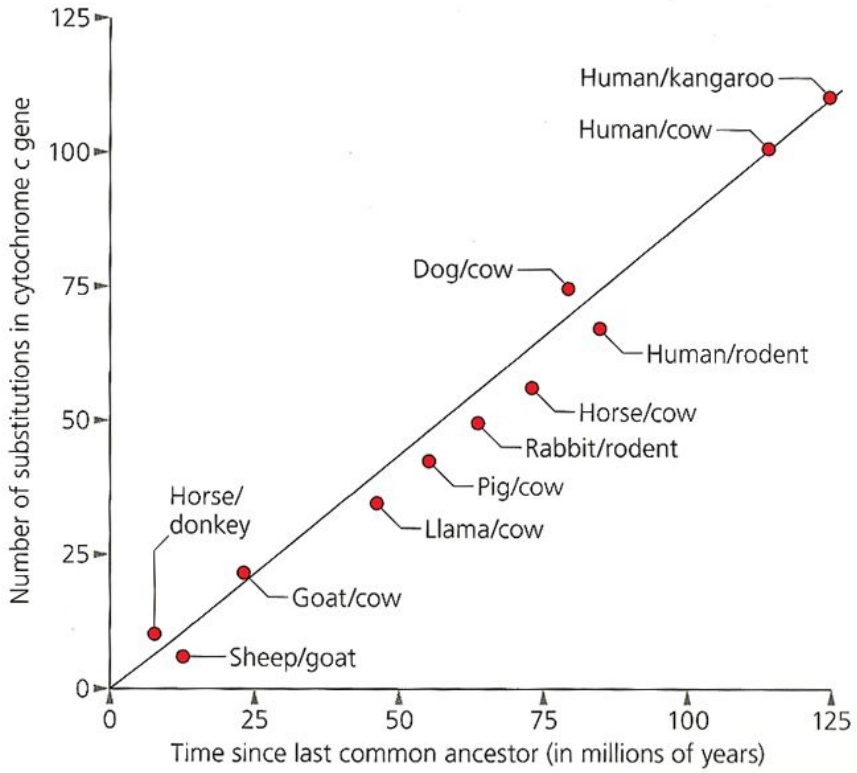
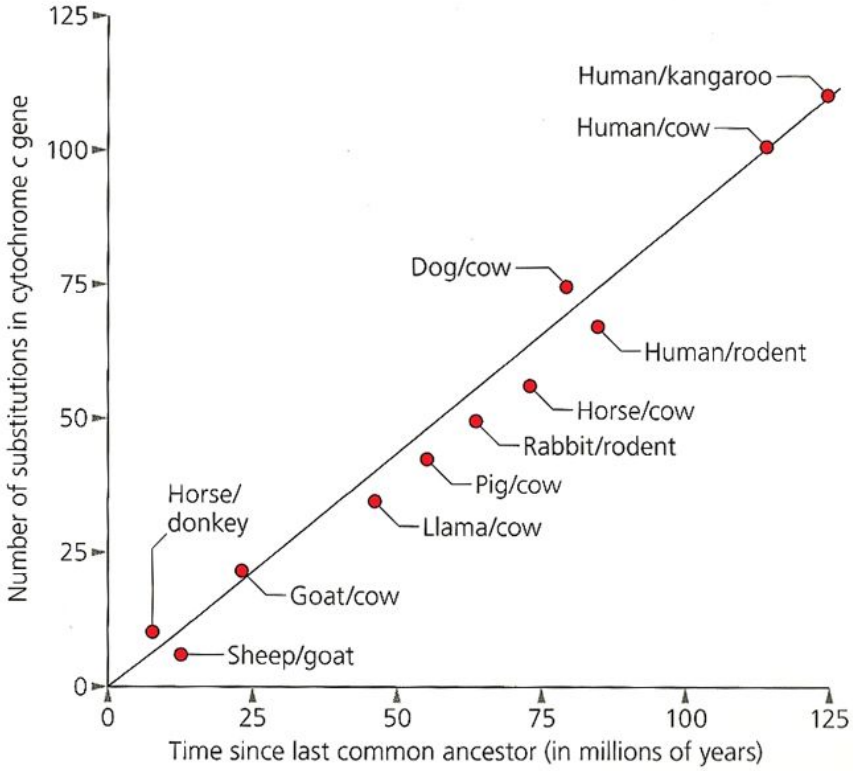


Figure 7.8 DNA mutates at a roughly clock-like rate. This graph shows how distantly related pairs of species have a large number of different substitutions in the cytochrome c gene. (Adapted from Moore and Moore, 2004)

Moore and Moore (2004)

Quiz: why is there a molecular clock?



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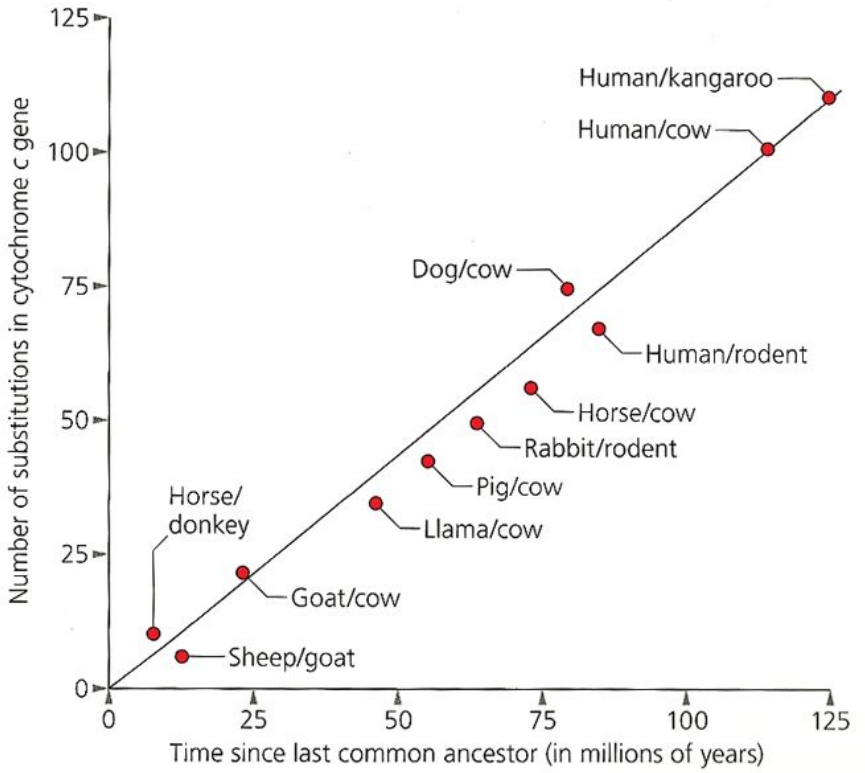
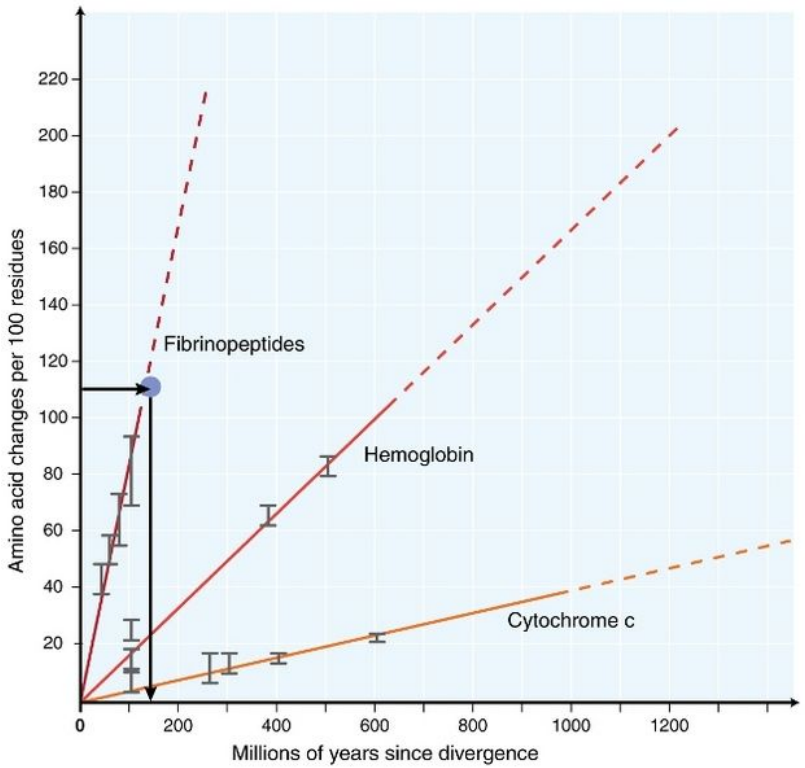


Figure 7.8 DNA mutates at a roughly clock-like rate. This graph shows how distantly related pairs of species have a large number of different substitutions in the cytochrome c gene. (Adapted from Moore and Moore, 2004)

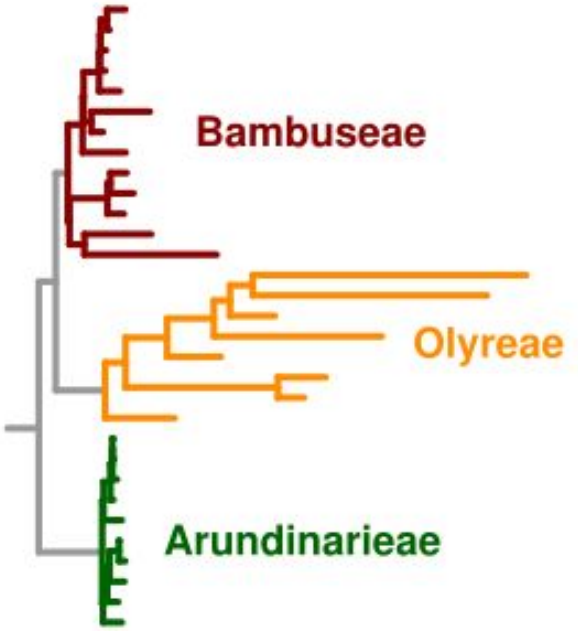
- **Selective neutrality (substitution rate = mutation rate)**
- **Pragmatic, long-term average?**

Moore and Moore (2004)

Variation in the molecular clock



Across genes

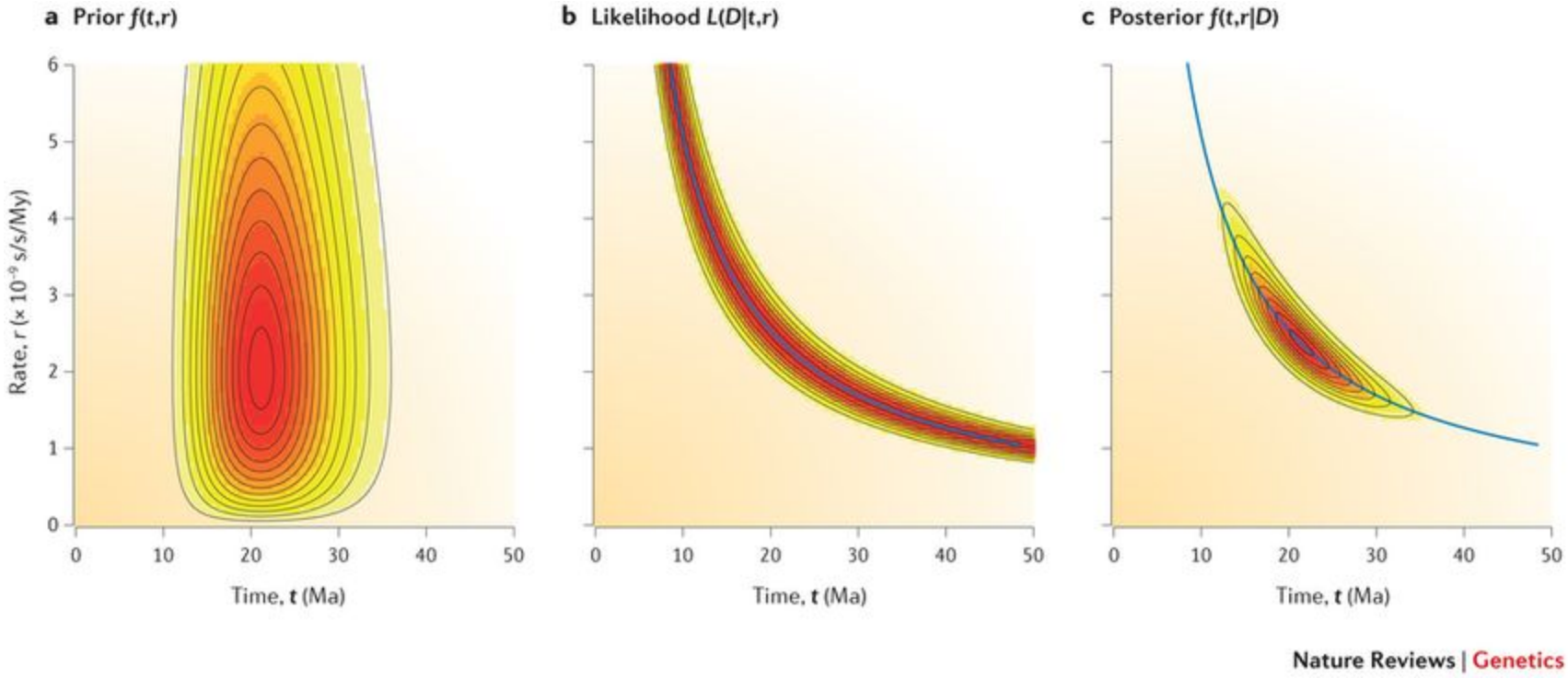


Across species

No strict clock, but just relax...

- Clock ticks at different rates among:
  - sites in a molecule
  - genes
  - regions of genomes
  - genomes in the same cell
  - taxonomic groups
- Bayesian methods allow these assumptions to be relaxed, some dating information to be extracted.

Inference under a relaxed molecular clock



A very natural way to combine fossil calibrations and sequence data.

But, note the different role of the prior!

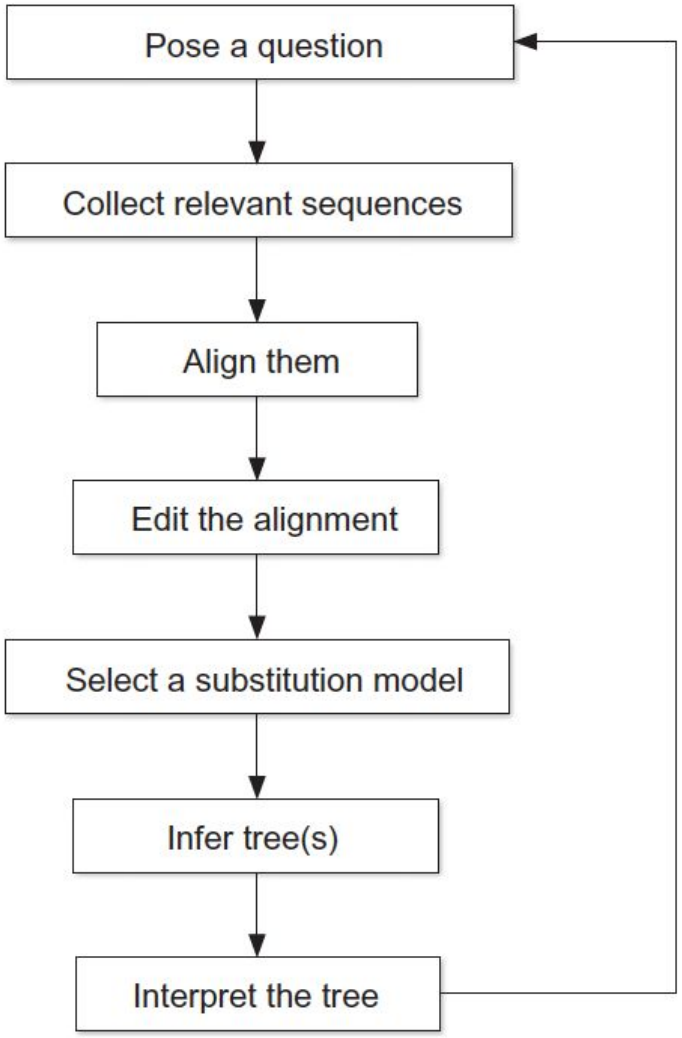
Donoghue and Yang

Phylogenetics: some key questions

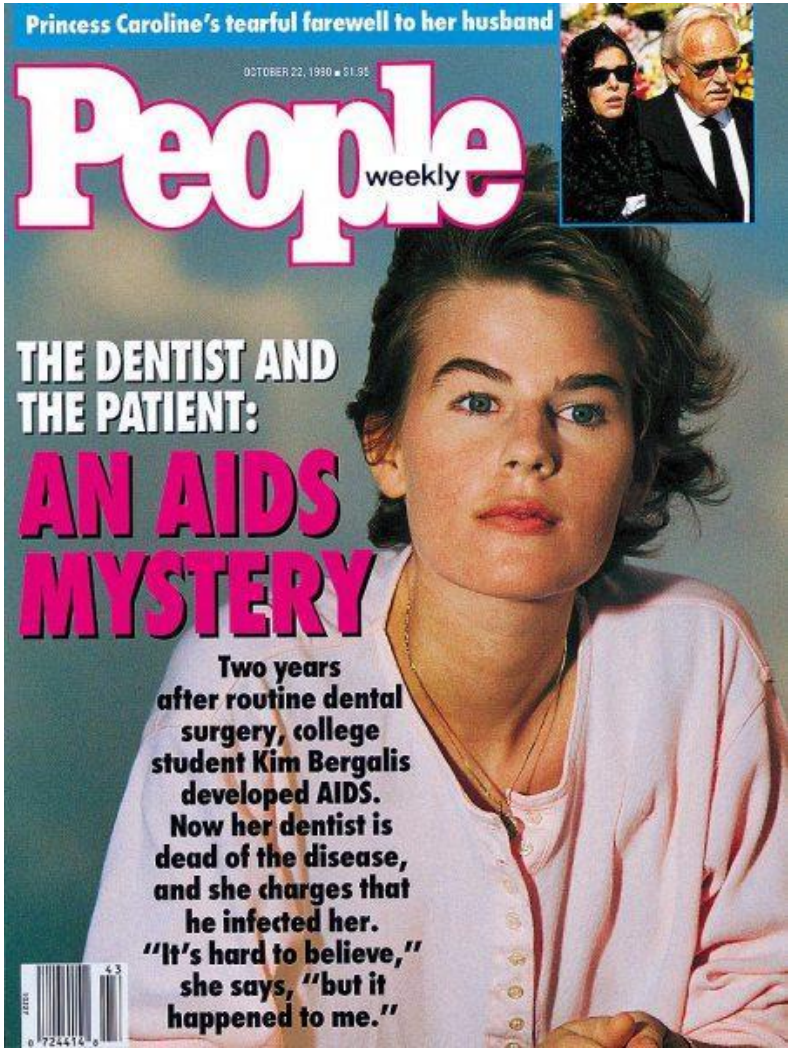
- Did I analyse all the relevant data?
- Does the model fit the data?
- How well-resolved is the tree? Can alternative hypotheses be rejected?
- What do these analyses mean for my question?

Molecular phylogenetics practical

Practical: A first phylogenetic analysis



Dentist-patient transmission of HIV?



In the early 90s, a dentist was accused of infecting several of his patients with HIV during surgical procedures.

After a "low-risk" patient was diagnosed with HIV, other patients were screened. 10 had HIV.

Did the dentist infect them? We will do a phylogenetic analysis to evaluate these claims.

The data

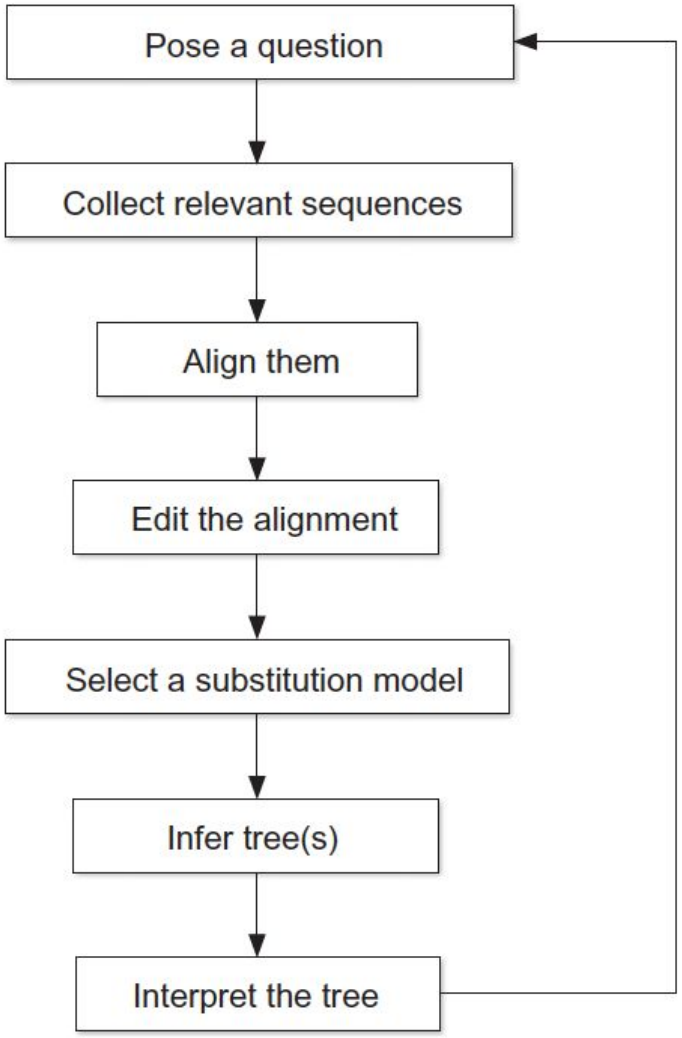
Molecular Epidemiology of HIV Transmission in a Dental Practice

Chin-Yih Ou, Carol A. Ciesielski, Gerald Myers, Claudiu I. Bandea, Chi-Cheng Luo, Bette T. M. Korber, James I. Mullins, Gerald Schochetman, Ruth L. Berkelman, A. Nikki Economou, John J. Witte, Lawrence J. Furman, Glen A. Satten, Kersti A. MacInnes, James W. Curran, Harold W. Jaffe, Laboratory Investigation Group,* Epidemiologic Investigation Group†

HIV *env* sequences from:

- The dentist
- His patients
- Local controls

The workflow



Q: Are patient sequences *descended* from the dentist's sequences?

Let's use NCBI…

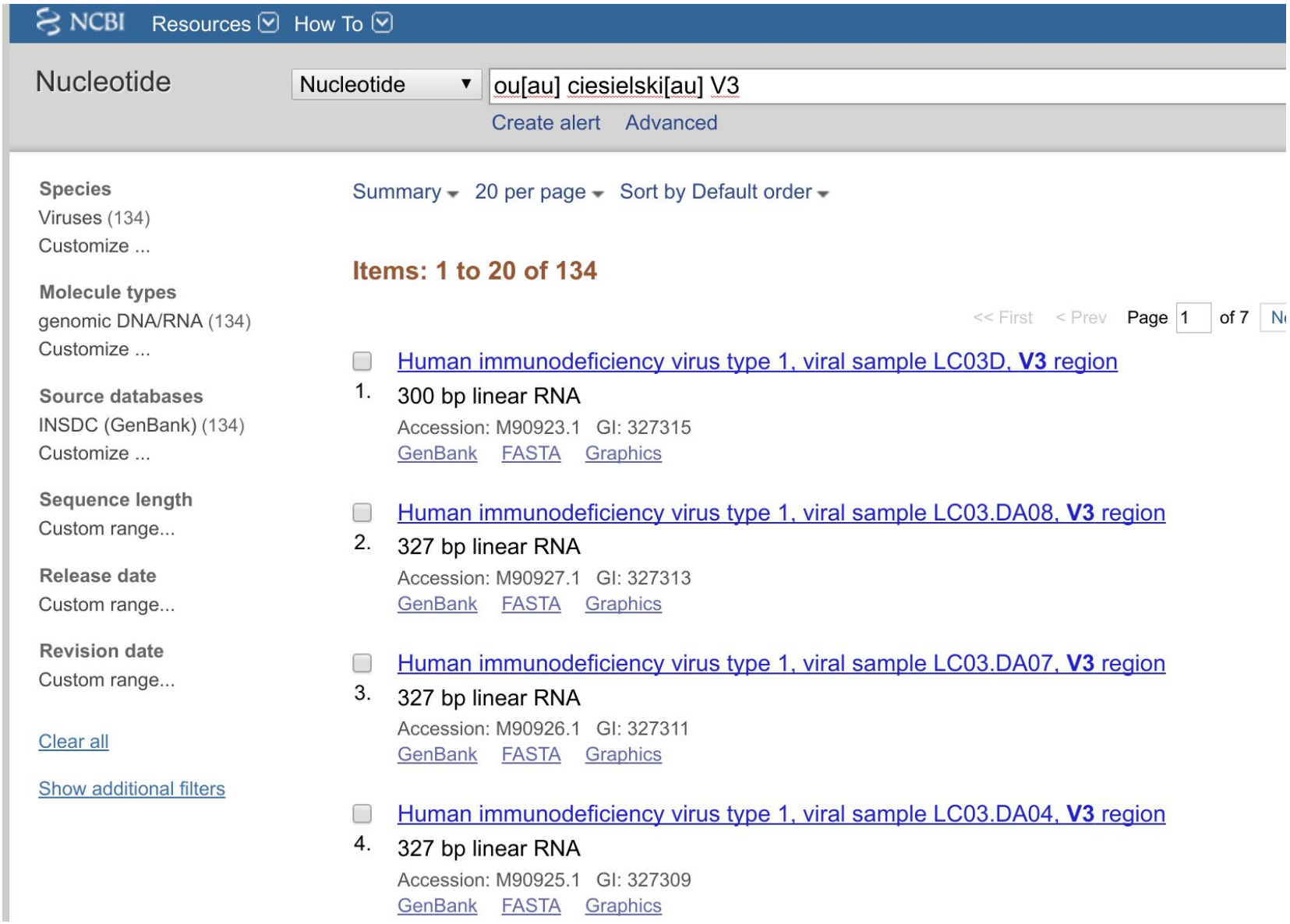
We'll use mafft.

***

We'll use the best-fitting model in IQ-Tree...

Does the phylogeny support the claims?

How to retrieve the data?

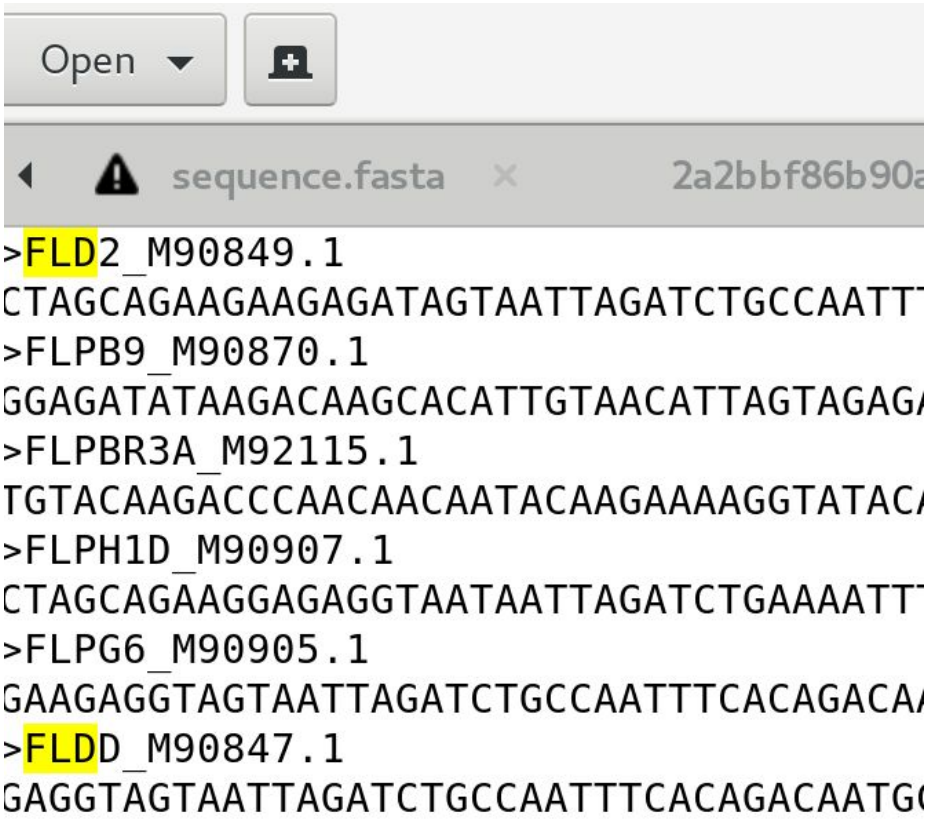


How to retrieve the data?

- **Dentist sequences: FLD1,2,4,5,7,8**
- **Patient sequences: FLP[A-H]: take 3 isolates/patient**
- **Local controls (LCXX, FLQ): take at least 10.**

The workflow

- **Download the sequences in FASTA format, save in a text file.**
- **Rename with**
- **sensible tags**
- **FASTA format:**



Some useful commands

Alignment:

```
mafft --auto mySequences > myAlignment
```

View the alignment in `belvu/Jalview`.

Tree inference (black box):

```
iqtree -s myAlignment -m MFP -bb 1000
```

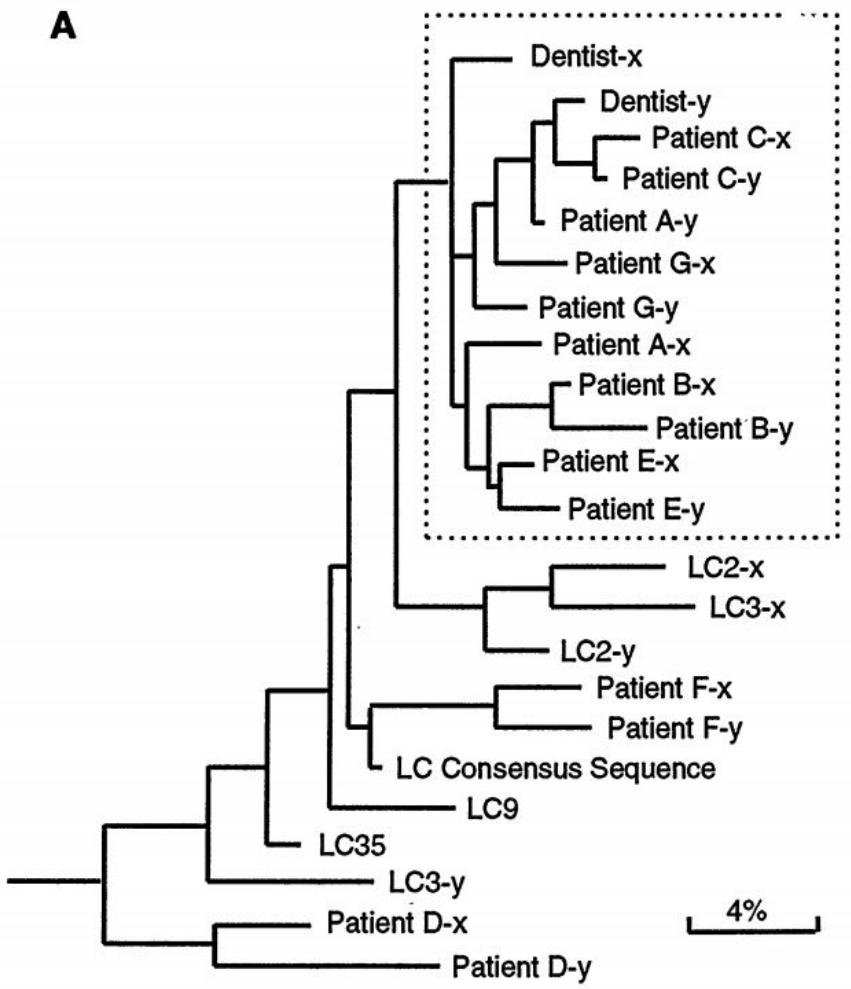
Questions

1. Did the patients get their viruses from the dentist?
2. How much would you bet on it?
3. Why include local controls in the analysis?

For a bonus practical, see:

`https://github.com/Tancata/bioinf_workshop`

Ou et al. (1992)


A
Dentist-x
Dentist-y
Patient C-x
Patient C-y
Patient A-y
Patient G-x
Patient G-y
Patient A-x
Patient B-x
Patient B-y
Patient E-x
Patient E-y
LC2-x
LC3-x
LC2-y
Patient F-x
Patient F-y
LC Consensus Sequence
LC9
LC35
LC3-y
Patient D-x
Patient D-y
4%

Some properties of the patients

Table 1. Dental cohort clinical information and HIV nucleotide variation in the C2-V3 domain of the envelope gene.

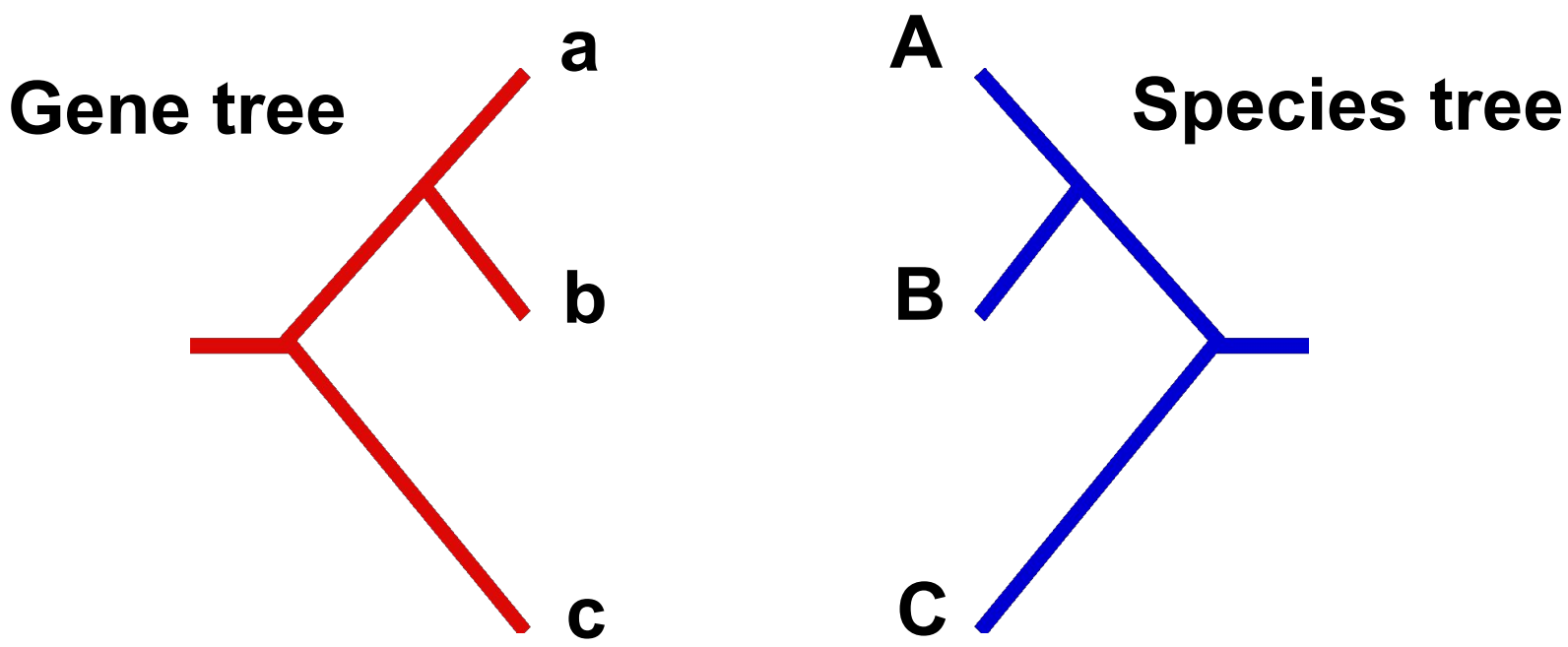
Person	Sex	Known risk factor	Clinical status*	M13 clones (no.)	Intraperson variation† (%)	Interperson variation† (%)	
						To dentist	To 30 LCs‡
Dentist	M	Yes	AIDS	6	3.3 (0.8–5.4)		11.0 (5.8–16.0)
Patient A	F	No	AIDS	6	2.0 (0.0–4.5)	3.4 (0.8–6.2)	10.9 (5.4–14.8)
Patient B	F	No	Asymptomatic (CD4 = 222/μl)	12	1.9 (0.4–3.7)	4.4 (2.1–7.0)	11.2 (6.2–16.5)
Patient C	M	No§	Asymptomatic (CD4 = <50/μl)	5	1.2 (0.4–1.6)	3.4 (2.1–4.9)	11.1 (7.0–15.6)
Patient E	F	No	Asymptomatic (CD4 = 567/μl)	6	2.1 (0.4–3.7)	3.4 (1.2–6.6)	10.8 (5.8–14.8)
Patient G	M	No	Asymptomatic (CD4 = 400/μl)	5	2.8 (1.6–3.7)	4.9 (2.9–7.0)	11.8 (6.2–16.9)
Patient D	M	Yes	AIDS	5	7.5 (0.0–9.9)	13.6 (11.5–15.6)	13.1 (7.8–17.3)
Patient F	M	Yes	Asymptomatic (CD4 = 253/μl)	6	3.0 (0.8–5.8)	10.7 (8.2–13.6)	11.9 (7.0–17.3)

Introduction to phylogenomics

Phylogenetic analysis of genome data

- **These days, data collection is at the genome scale (genomes, transcriptomes, genome-wide variation): not just one "marker" gene, but all of them**
- **Far more evolutionary information than from single genes, but we need to consider additional evolutionary processes (gene duplications, etc.)**
- **More data is better, but only if it is analysed properly!**

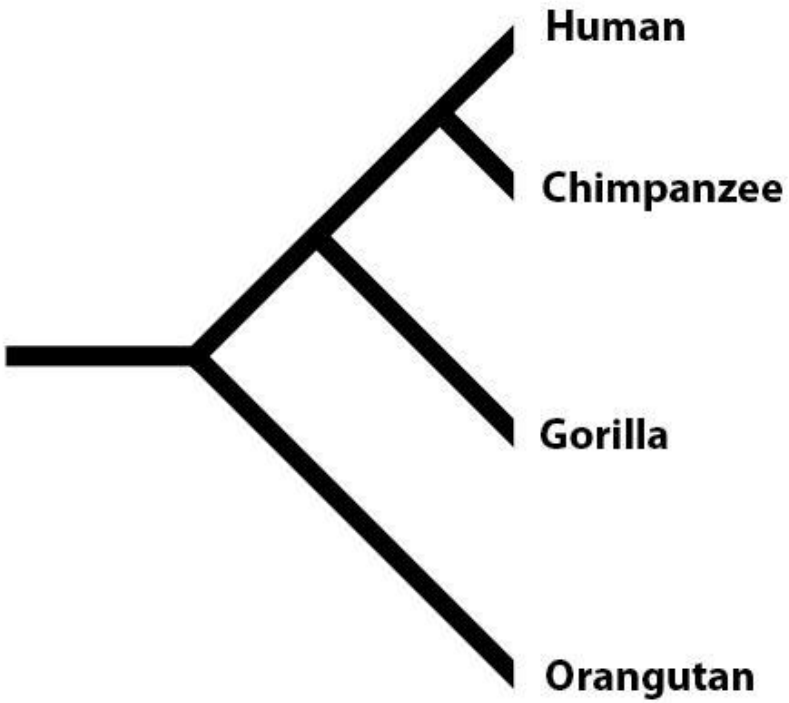
Gene trees and species trees



We often assume that gene trees give us species trees:
but different gene trees often disagree!

(and that's OK...)

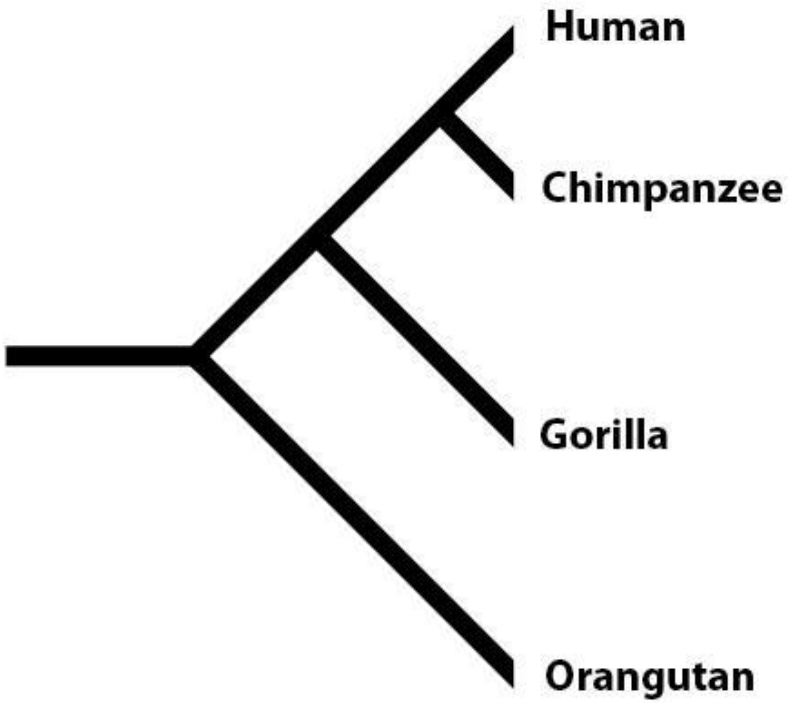
Human-Chimp-Gorilla



- **~15% Human genome more similar to Gorilla than Chimp**
- **~15% Chimp genome more similar to Gorilla than Human**

Scally et al. (2012)

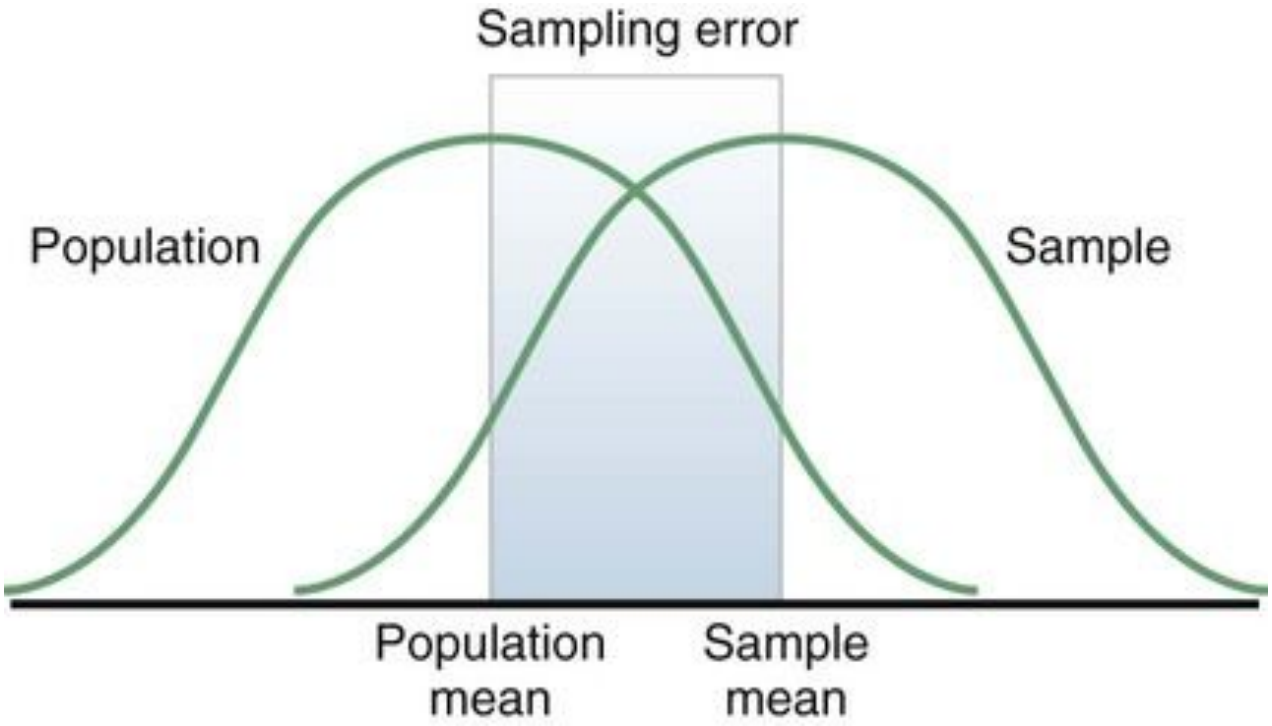
Quiz: why?



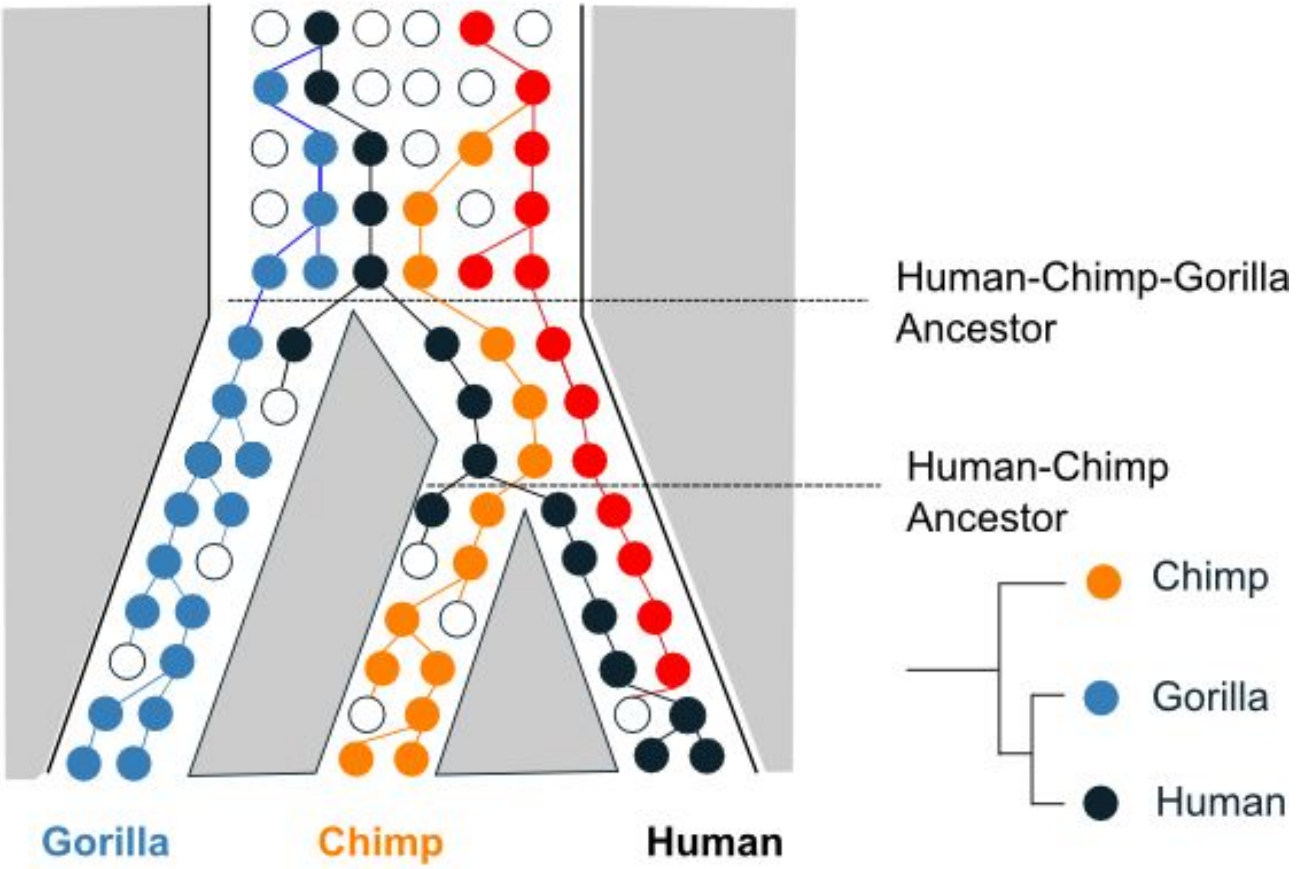
- **~15% Human genome more similar to Gorilla than Chimp**
- **~15% Chimp genome more similar to Gorilla than Human**

Scally et al. (2012)

Causes of disagreement (1): stochastic/sampling error



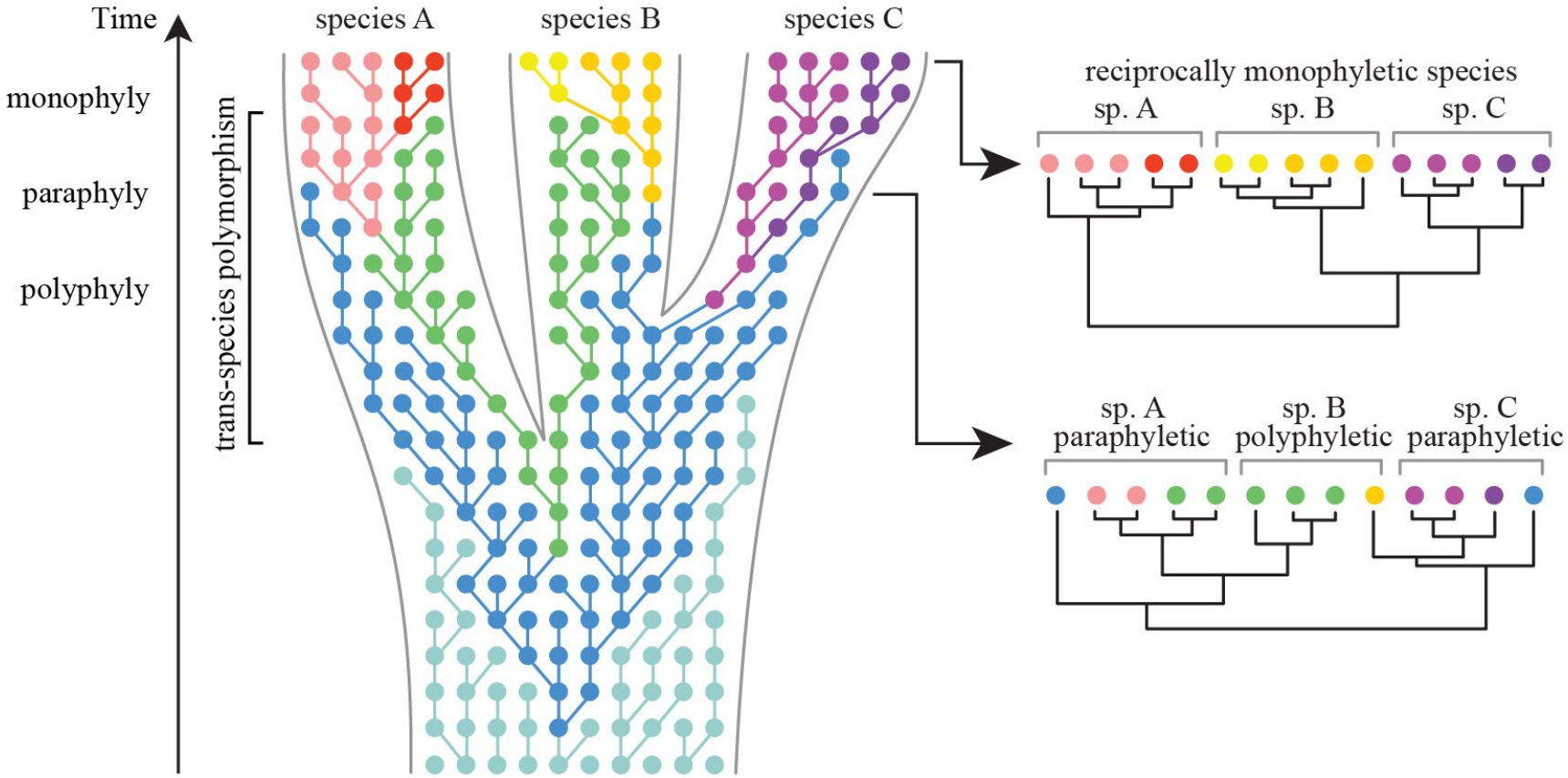
Causes of disagreement (2): genuinely different gene histories



Incomplete lineage sorting

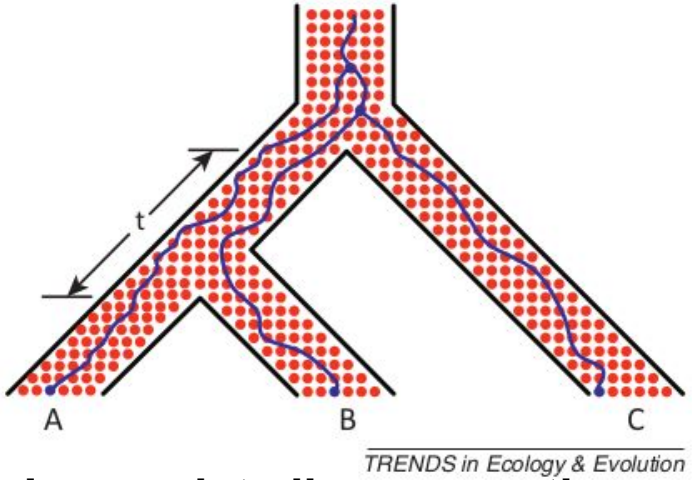
Genome-level evolution

- **An extra layer in our models of molecular evolution: genes (and alleles) evolve within the species tree**

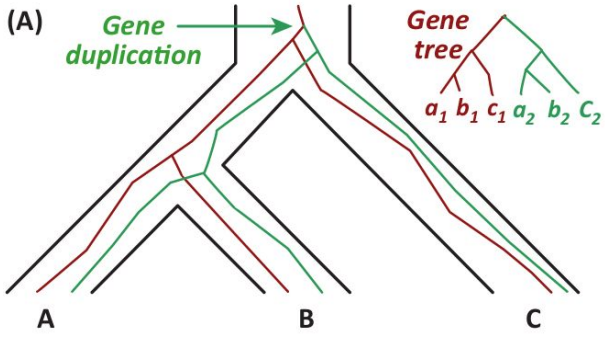


Leliaert et al. (2014)

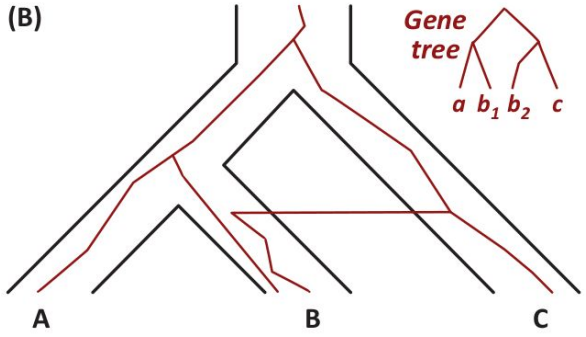
Genome-level evolution on short and long timescales



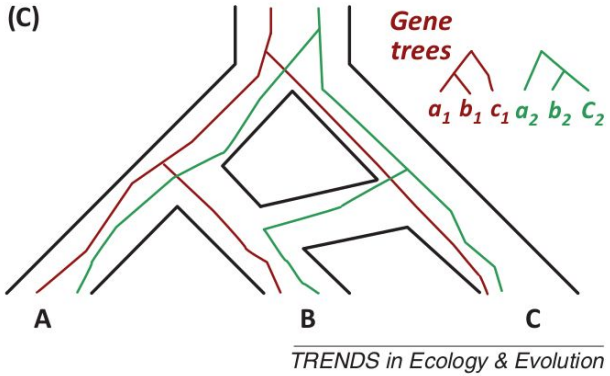
Incomplete lineage sorting



Gene duplication



Horizontal transfer



Hybridization

Nakleh (2013)

Genome evolution on short and long timescales

- **Current methods for modelling genome evolution lie at the interface between population genetics and phylogenetics:**

- **Short timescales: incomplete lineage sorting, hybridization**
- **Long timescales: gene duplications, losses, transfers**

- **This is due to model limitations, not a disconnect in the underlying biology.**

-

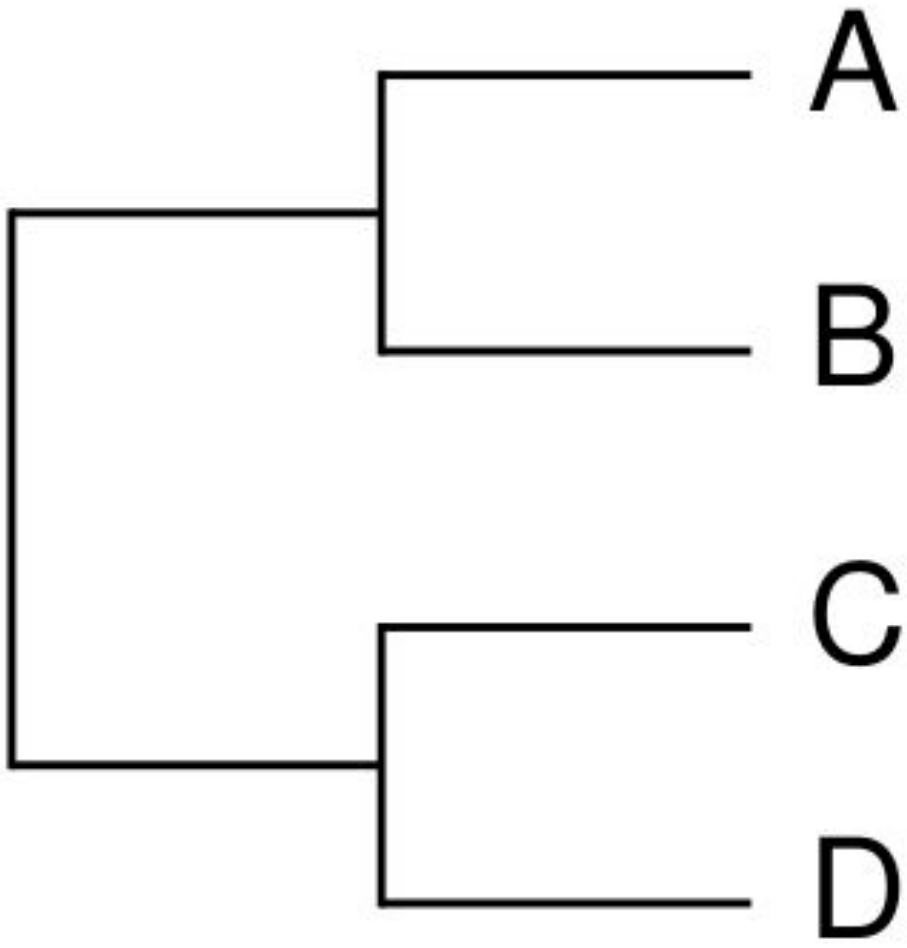
Disagreements between gene trees and species trees

- **Whole-genome data are great**
  - **More information to resolve evolutionary relationships**
- **But, trees inferred from different genes don't always agree**
  - **Stochastic error**
  - **Various biological processes**

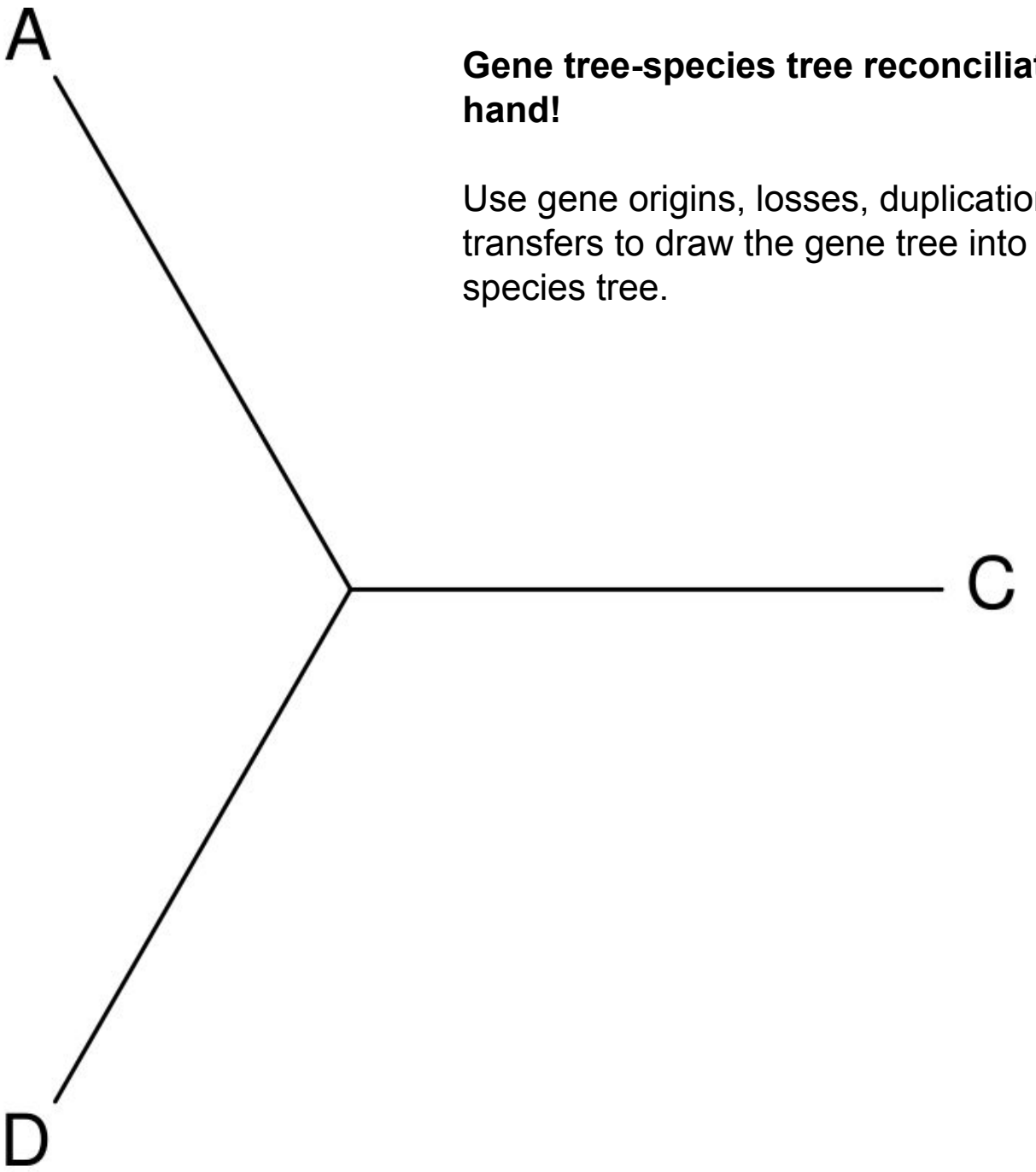
Is a single tree appropriate?

- **Disagreement between gene trees and species trees**
  - **Stochastic error**
  - **Recombination, sex, incomplete lineage sorting**
  - **Horizontal gene transfer**
  - **Mistaken orthology, paralogy**
- **Alternative methods in these cases:**
  - **Multispecies coalescent (Beast, ASTRAL, RevBayes...)**
  - **Gene tree-species tree reconciliation (guenomu, ALE, ...)**
  - **Similarity networks**

Games with species and gene trees

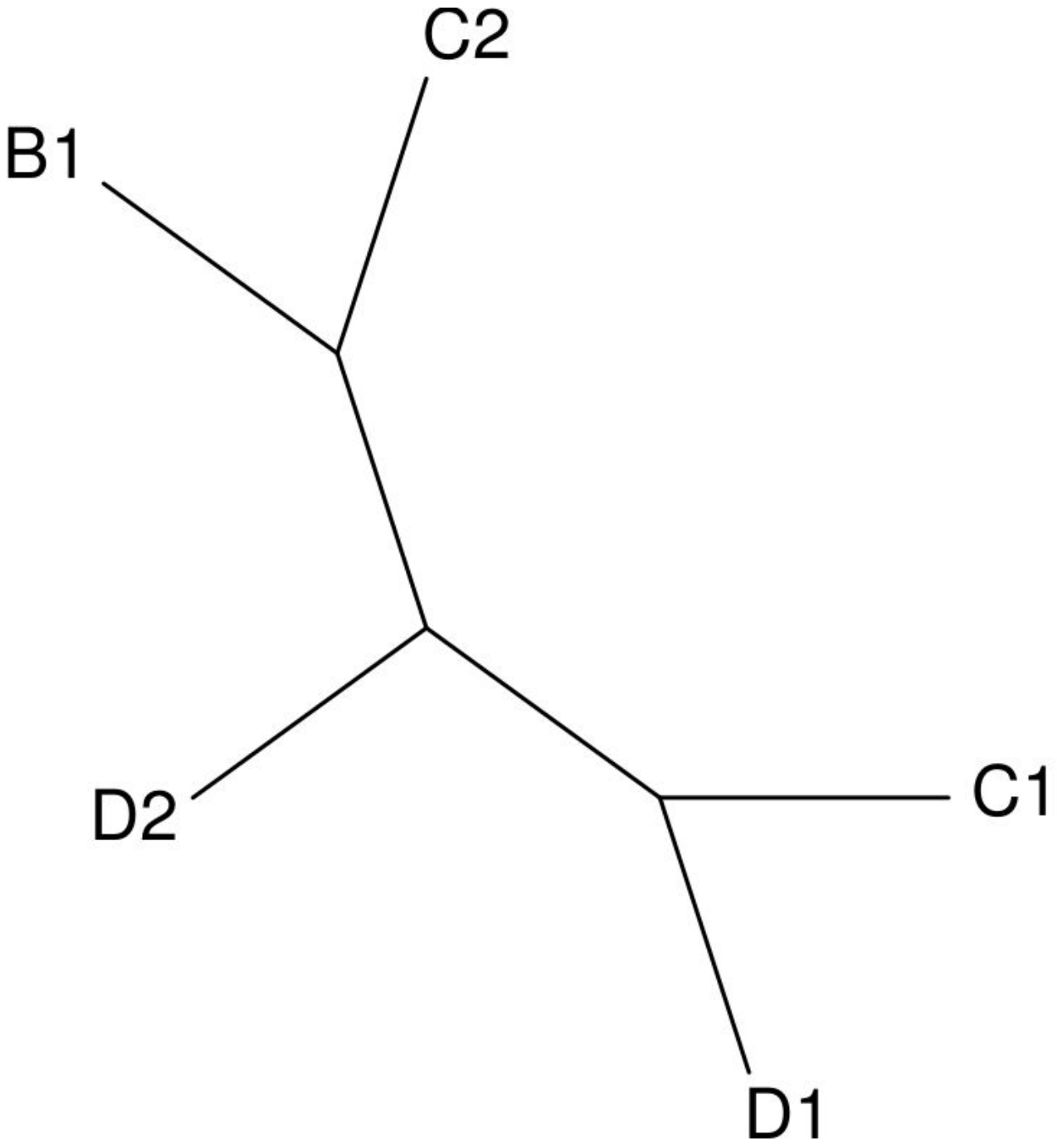


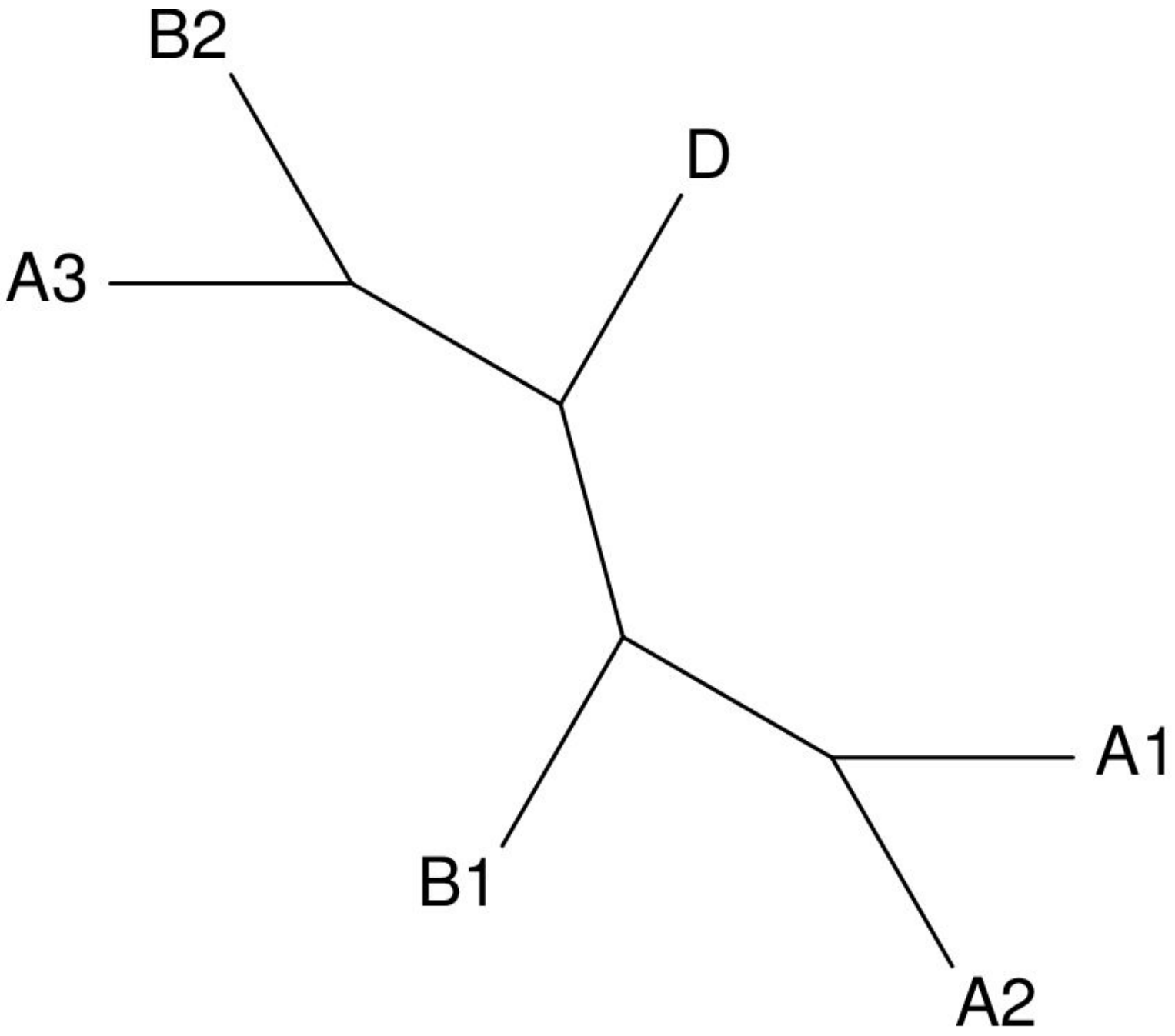
A rooted species tree, assumed known.

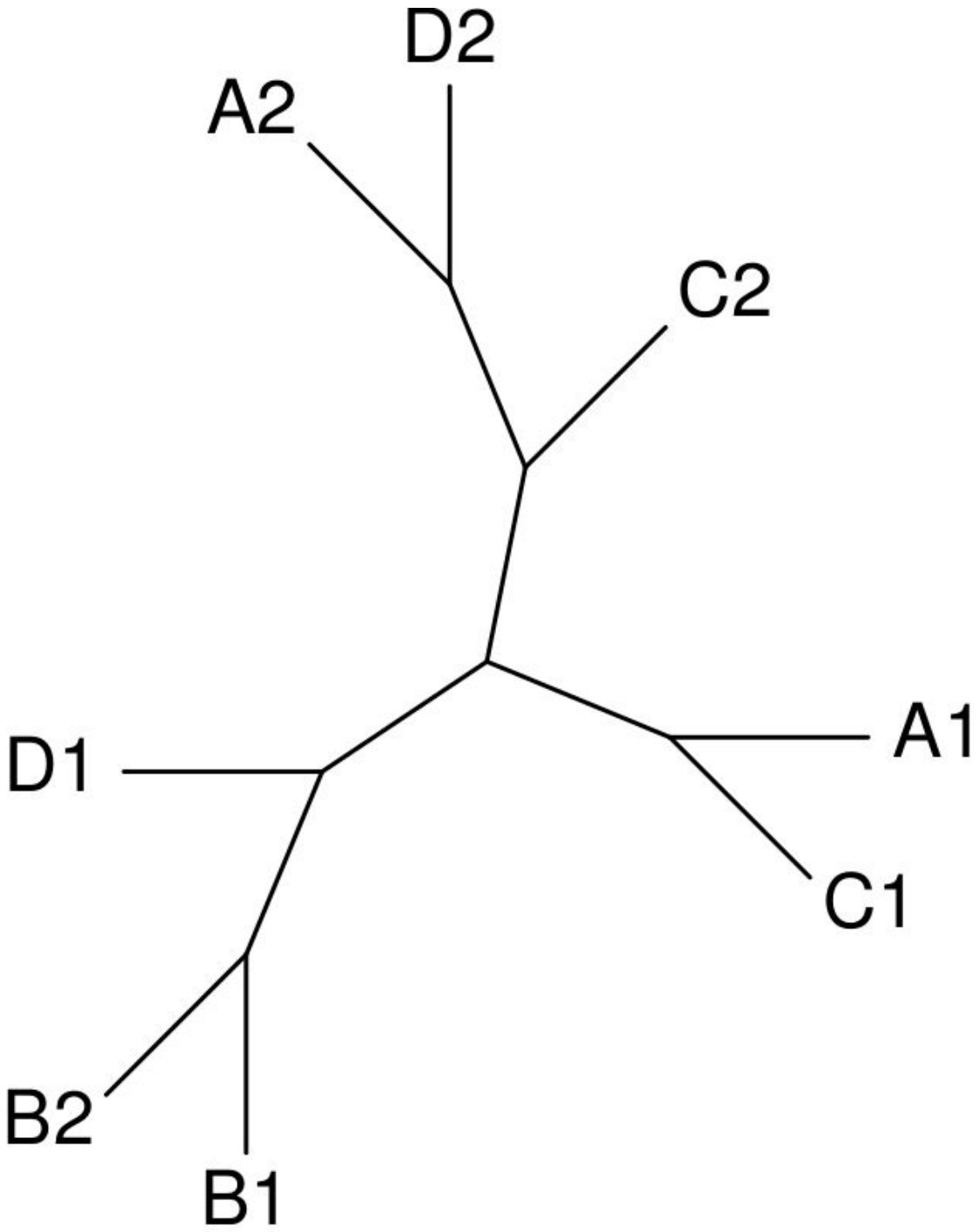


Gene tree-species tree reconciliation: by hand!

Use gene origins, losses, duplications and transfers to draw the gene tree into the species tree.


C2
B1
D2
C1
D1


B2
D
A3
A1
B1
A2


D2
A2
C2
A1
D1
C1
B2
B1

Disagreements among trees express information

- Several reconciliations may seem plausible
- Rates of gene duplications, losses, transfers can be inferred
- Would a different species tree make reconciliations easier?

Some further reading

Papers:

- Yang and Rannala (2008) “Molecular phylogenetics: principles and practice”. *Nat Rev Genet*
- Holder and Lewis (2003) “Phylogeny estimation: traditional and Bayesian approaches.” *Nat Rev Genet*

Books:

- Bromham L. “An introduction to molecular evolution and phylogenetics.”
- Yang Z. “Computational molecular evolution: a statistical approach.”