

Introductions

Monday 13th - Tuesday 14th April 2015

Wellcome Trust/EMBL-EBI Advanced Course on
Computational Molecular Evolution

Wellcome Trust Genome Campus
Hinxton, UK

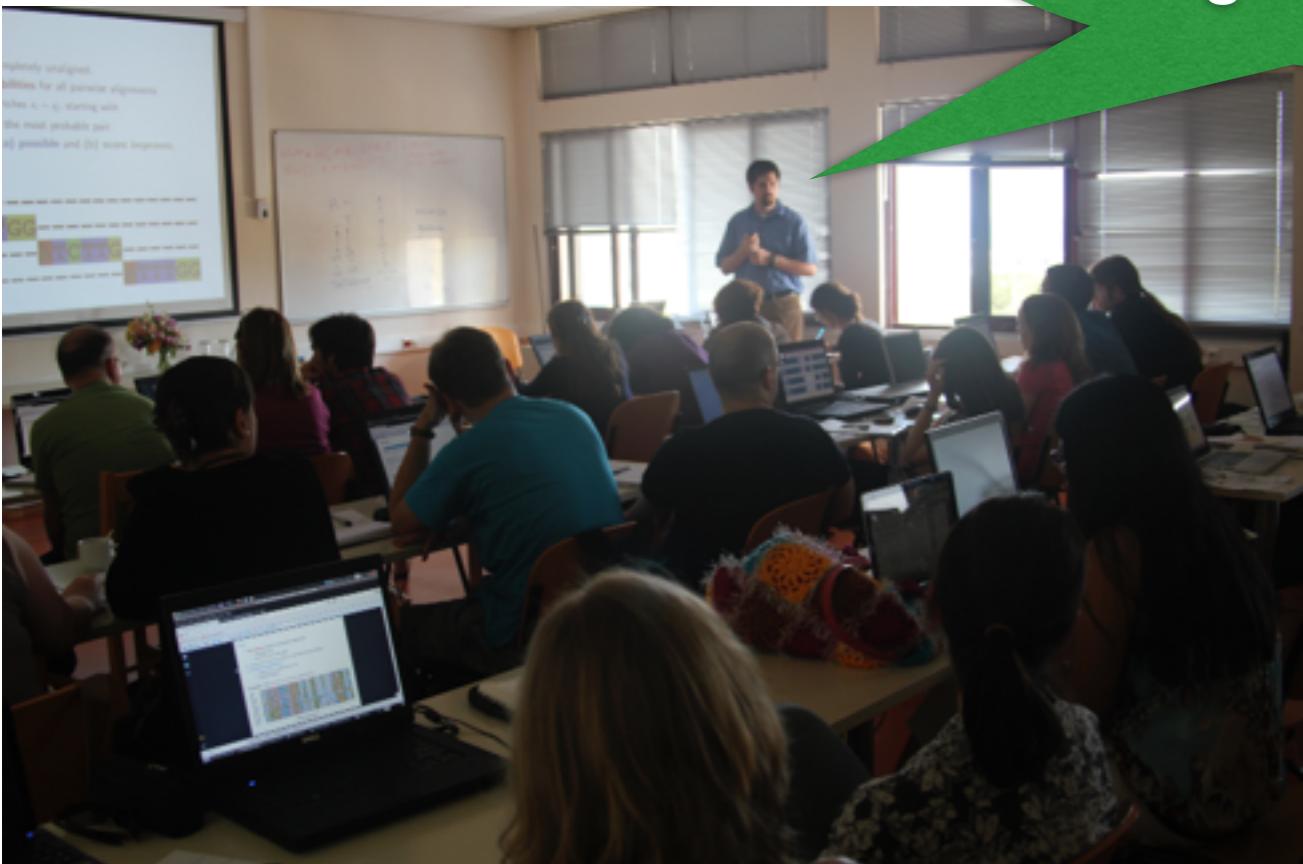
Aidan Budd
EMBL Heidelberg, Germany

What You Get From a Course?

I. knowledge & understanding

Knowledge & Understanding

Presentations and demos



insightful comments about
alignments and trees...



Practical exercises and discussions

2. useful professional relationships

a. with other trainees

b. with trainers



Aidan Budd, EMBL Heidelberg

both (I estimate, on average) similarly valuable

so the message of these first slides...

is that it's not just fun

but also really important

that you make an **effort** during the course

to get to know other trainees and the trainers

so we start with an activity...

to help us get to know each other...

speed dating

Speed Dating:Aims

- facilitate the (for some of us) awkward “introducing ourselves to someone for the first time” by doing lots of them, quickly
- hopefully makes it easier to start chatting later in the course
- quickly find people you have things in common with

Speed Dating: Format

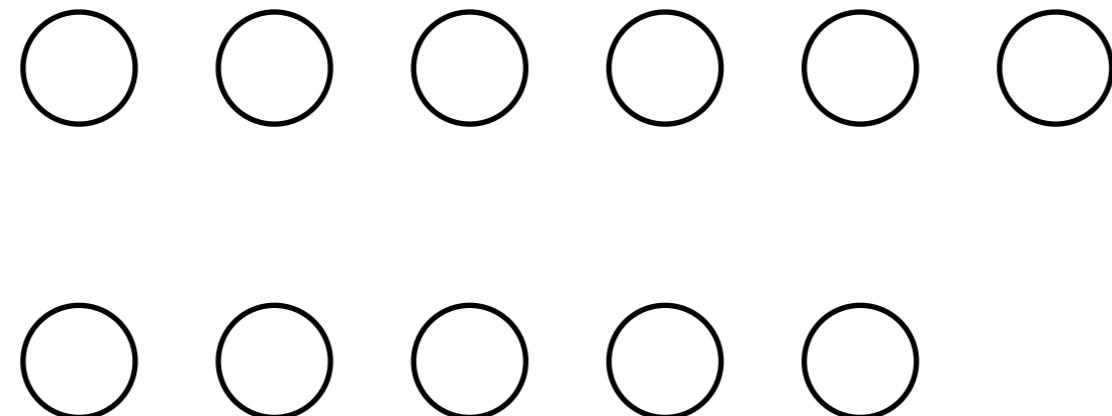
- meet other participants in many 1:1 chats (3-4 minutes)
- tell each other
 - names
 - where you work
 - research topics
 - look for person you both know or place you've both been

Speed Dating: Format

Stand, awkwardly, in two rows

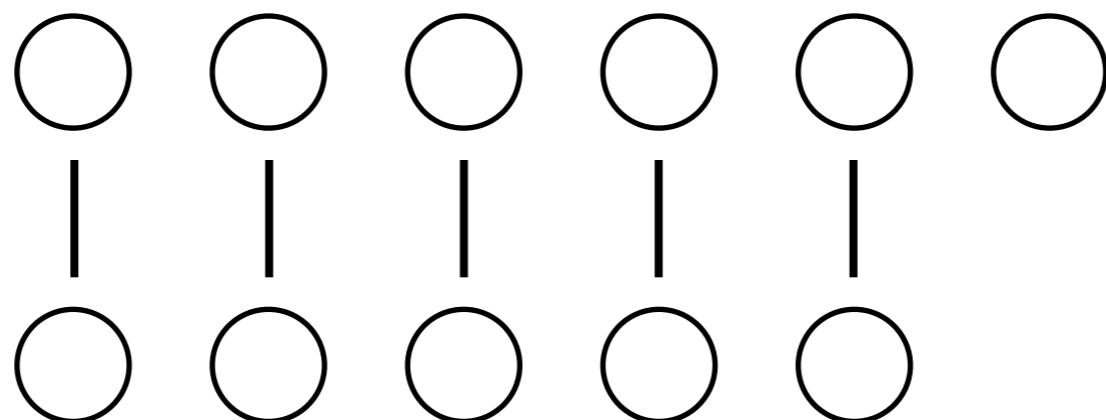
Face one person in the other row

If there's an odd number of you, one person stands alone at one end

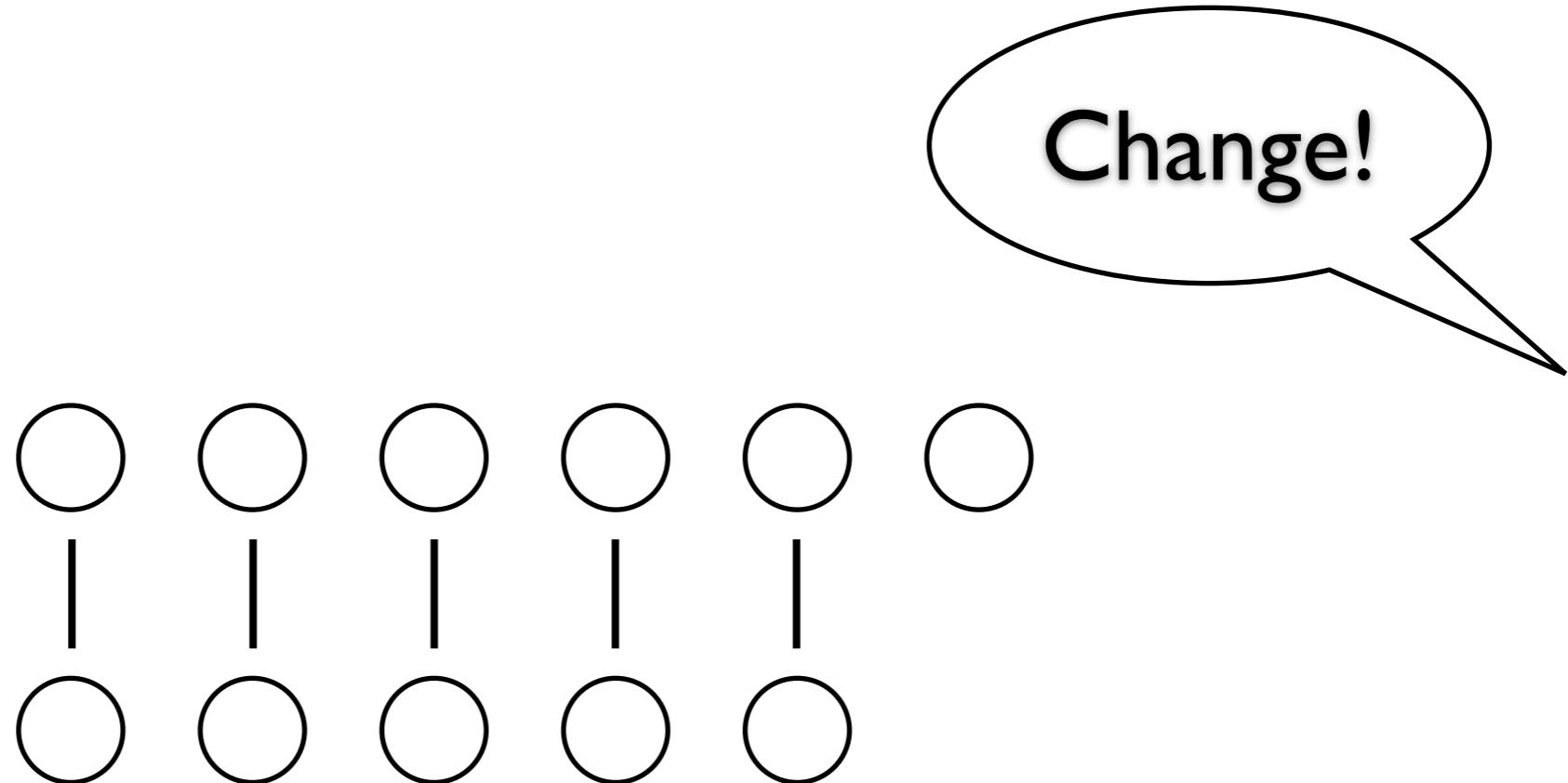


Speed Dating: Format

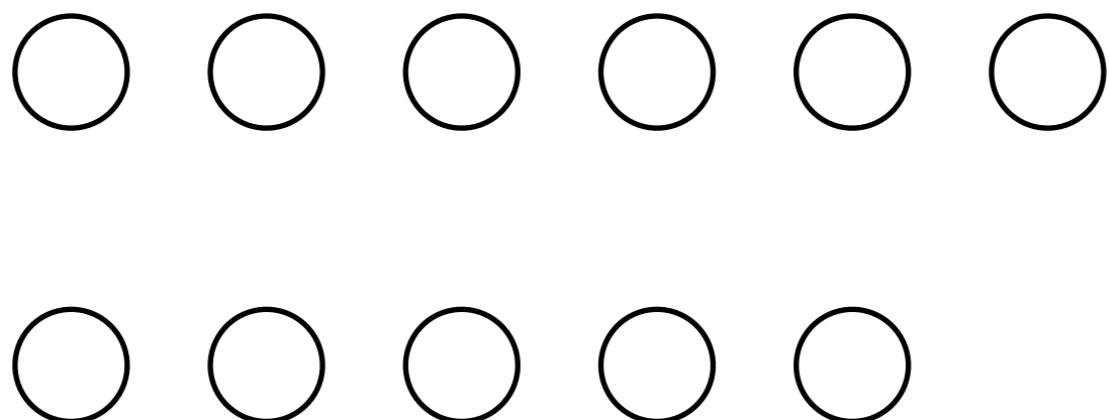
Chat!



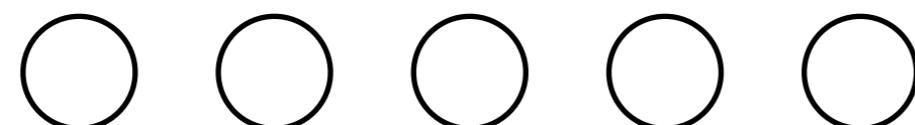
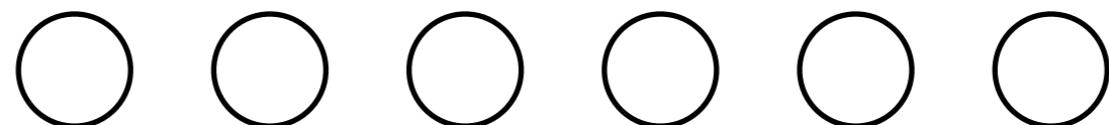
Speed Dating: Format



Speed Dating: Format

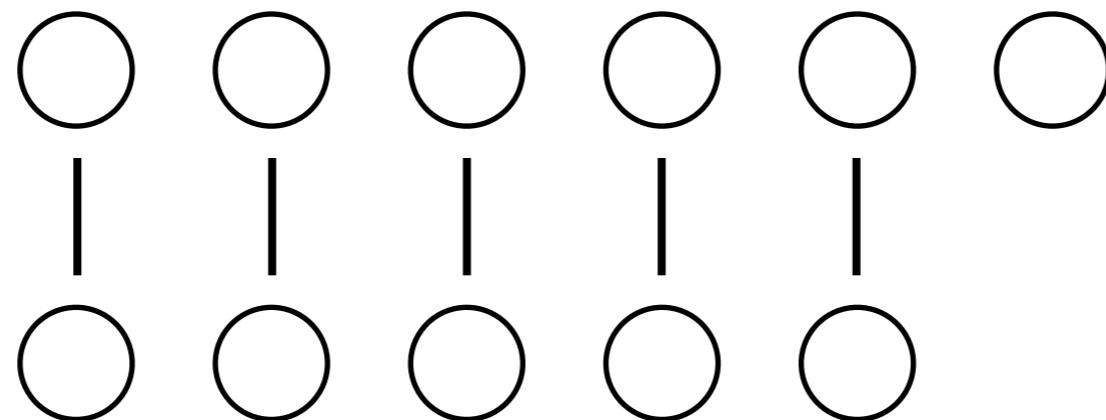


Speed Dating: Format



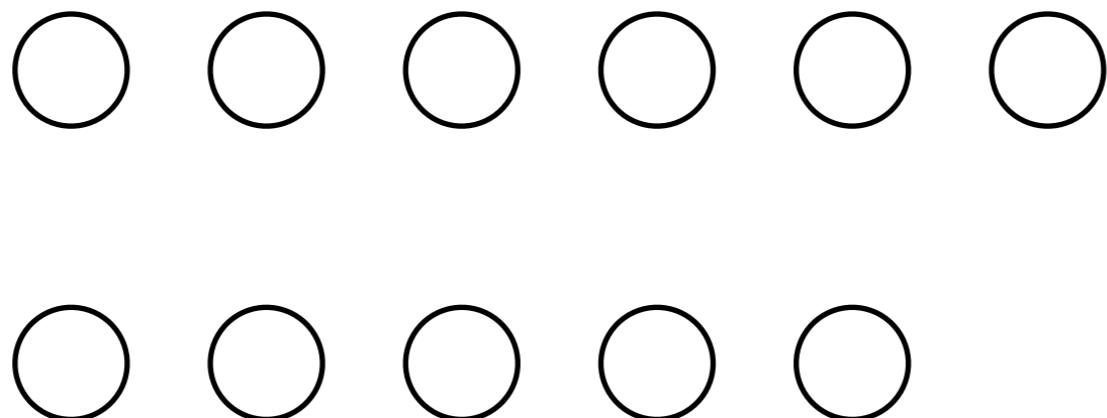
Speed Dating: Format

Chat!



and repeat until you've met everyone in the other row...

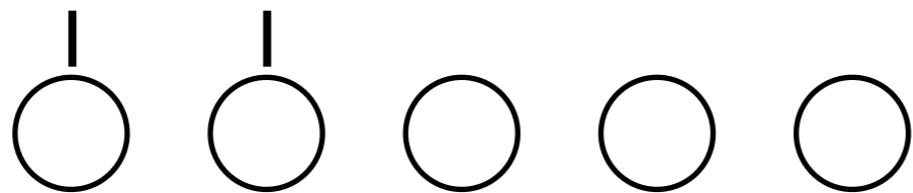
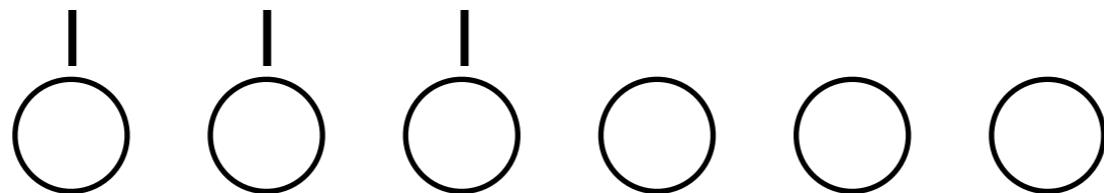
Speed Dating: Format



then split each row into two new rows

Speed Dating: Format

Chat!



make two new rows

and start again with the chat...

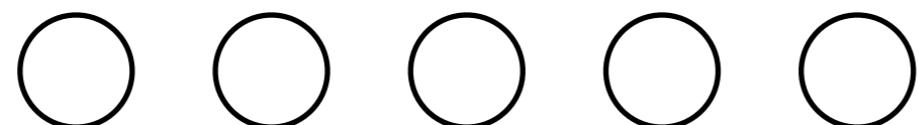
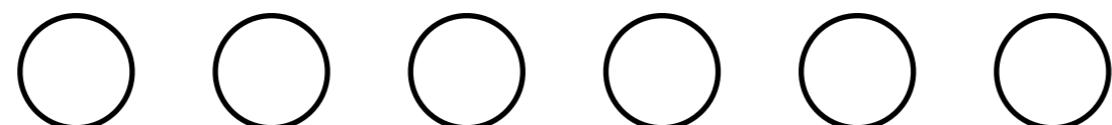
Speed Dating: Format

So... go outside now and form the initial two awkward rows

Face one person in the other row

If there's an odd number of you, one person stands alone at one end

Note - one line stays still, the other **moves...**



Session HTML pages

download zip/git clone from:

<https://github.com/aidanbudd/trainingPhyloIntro>
\$ git clone https://github.com/aidanbudd/trainingPhyloIntro

“homepage” (links to exercises, instructions, presentations)

homepageInterpretingPhylogenies.html

exercises

interpretingPhylogenies.html

Hashtag: #CoME7

Interpreting Molecular Phylogenetic Trees

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Introductions and Aims

Surveying your backgrounds

Who:

... feels comfortable interpreting trees?

(“Oh dear, they’re making statements about relatedness without making any comments about where the root is”)

... feels comfortable estimating trees with ML and Bayesian inference?

(“I’ll just set MrBayes up to run overnight with hotter chains; and I’ll do RAxML again too this time with rapid bootstraps”)

... has a mathematical background?

(“The transition probability matrix can be obtained from the instantaneous rate matrix by exponentiation, and they’ve calculated the matrix exponential using eigen decomposition”)

Surveying your backgrounds

Who:

... has a strong computer science/computational background?
("really having trouble debugging this MC³ implementation")

... collects specimens for their work from the field?
("looked under a hundred rocks today and still can't find a
*Centruroides sculpturatus** for Sally from our lab")

... didn't put their hand up yet?

* according to Wikipedia, this is the Arizona bark scorpion “the most venomous scorpion in North America”. Just sayin’.

Surveying your backgrounds

You're a very diverse group in terms of:

skills, knowledge, experience

what you'll find hard/easy to understand

Working with others e.g. during exercises

helps you learn from each other

helps you get to know each other better

Session Aims

Give an overview of

- common phylogeny terminology, concepts, and tools
- how tools can be linked together to build a ‘pipeline’

Before we start

- Mixture of presentations, demonstrations, discussions, and exercises
- Working together is encouraged
- Please ask questions at any point

How Do We Interpret Molecular Phylogenetic Trees?

An Example

Interpreting Molecular Phylogenetic Trees: An Example

Interpreting together a tree from a published article

Highlights a public health application of phylogenetics

an example of accurate tree estimation having a clear positive impact

Helps explore common features and problems when interpreting trees

Interpreting Molecular Phylogenetic Trees: An Example

Study aiming to identify factors contributing to pattern and rate of transmission ("transmission dynamics") of rabies virus in North Africa

One of the world's most virulent (severe, harmful, infectious) animal diseases

Almost 100% death rate once rabies symptoms start in humans

99% of human infections linked to dog vectors (domestic and wild)

Transmission via saliva, particularly via dog bites

Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.



*autonomous cities of Spain

Interpreting Molecular Phylogenetic Trees: An Example

Rabies is a major public health problem - yearly, worldwide:

55,000 deaths

15,000,000 doses of anti-rabies post-exposure prophylaxis administered

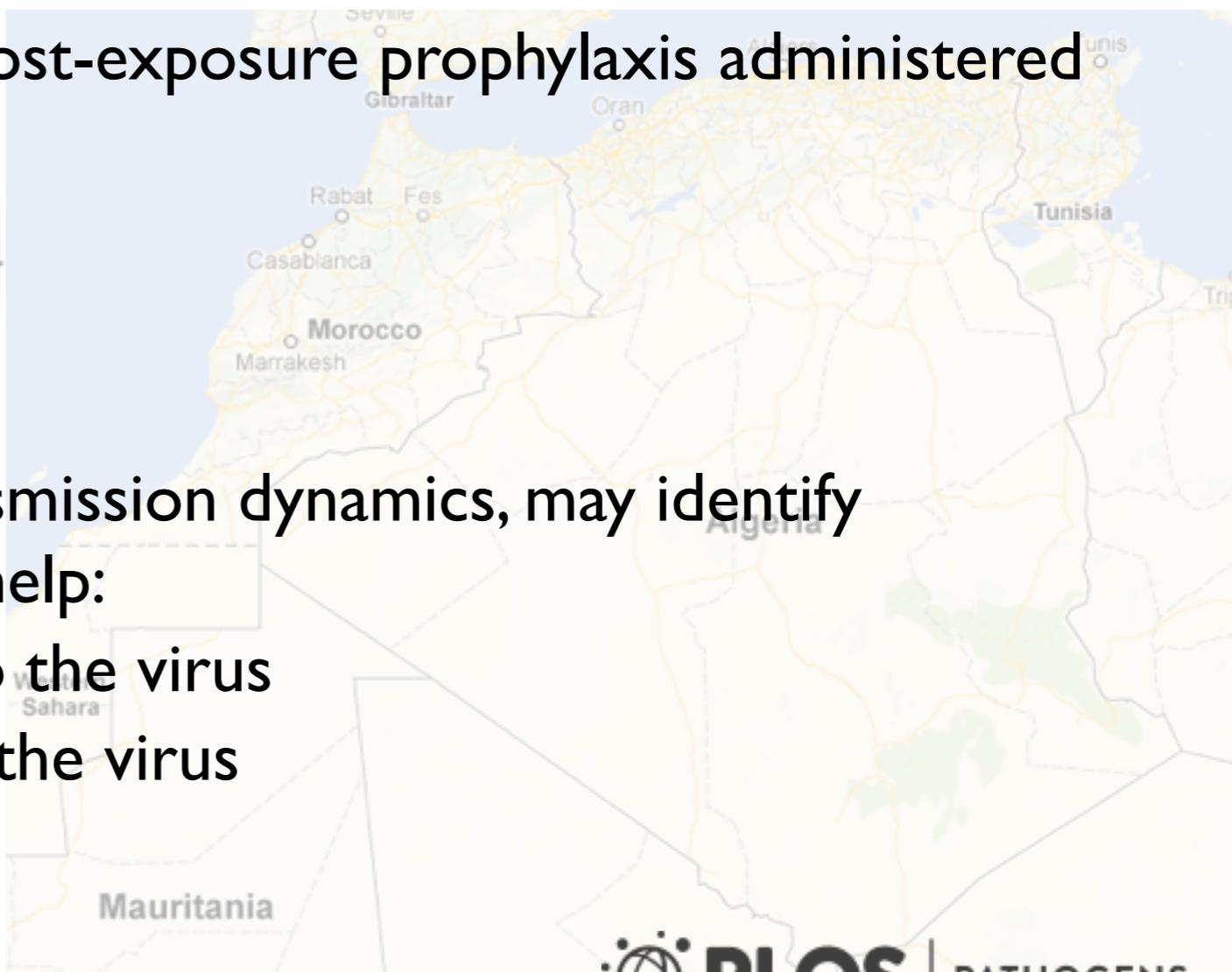
Therefore rabies:

causes significant human suffering
is a major economic burden

Identifying factors contributing to transmission dynamics, may identify public health interventions that could help:

reduce human suffering related to the virus
reduce economic cost/burden of the virus

Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.



Interpreting Molecular Phylogenetic Trees: An Example

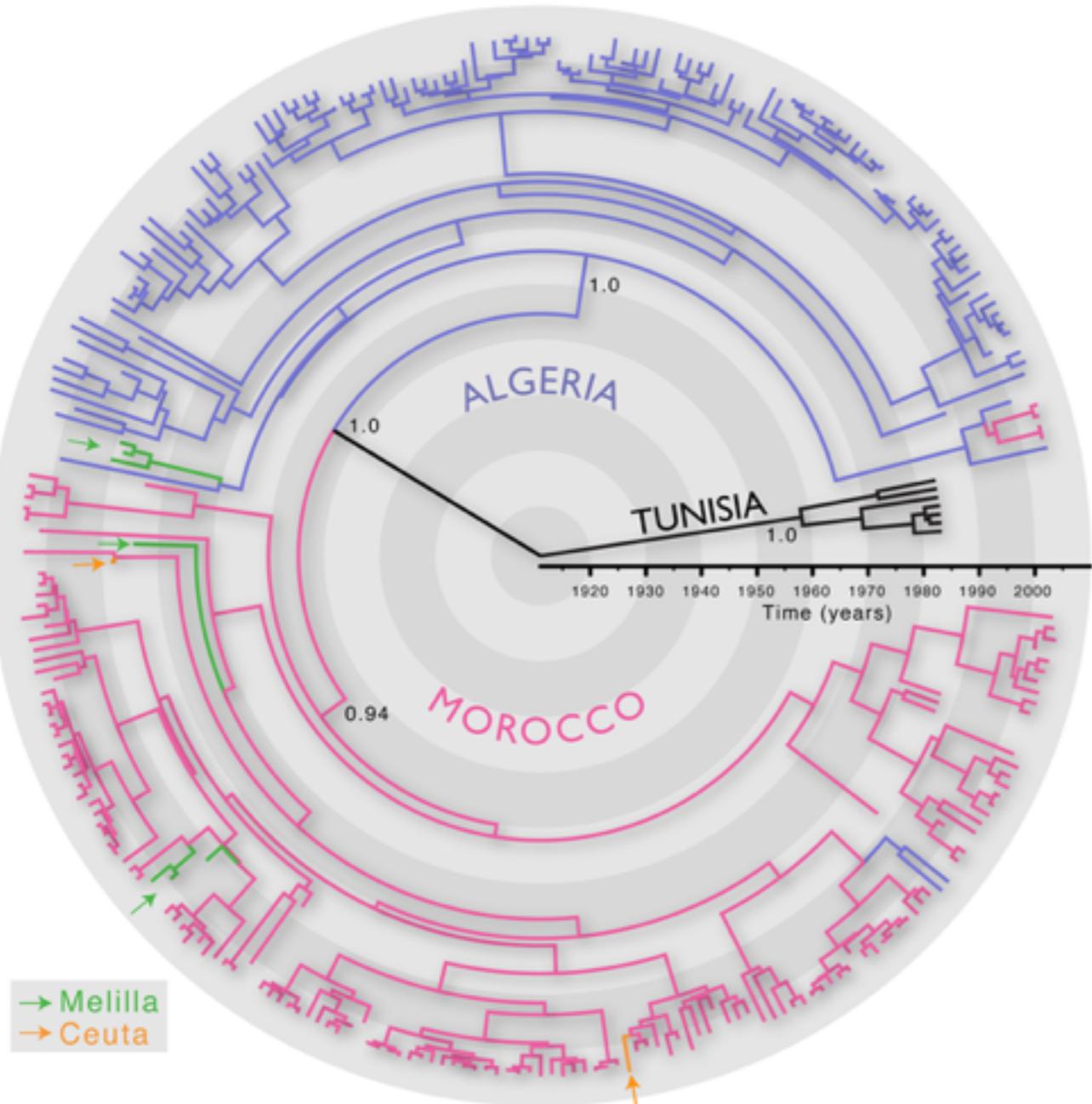
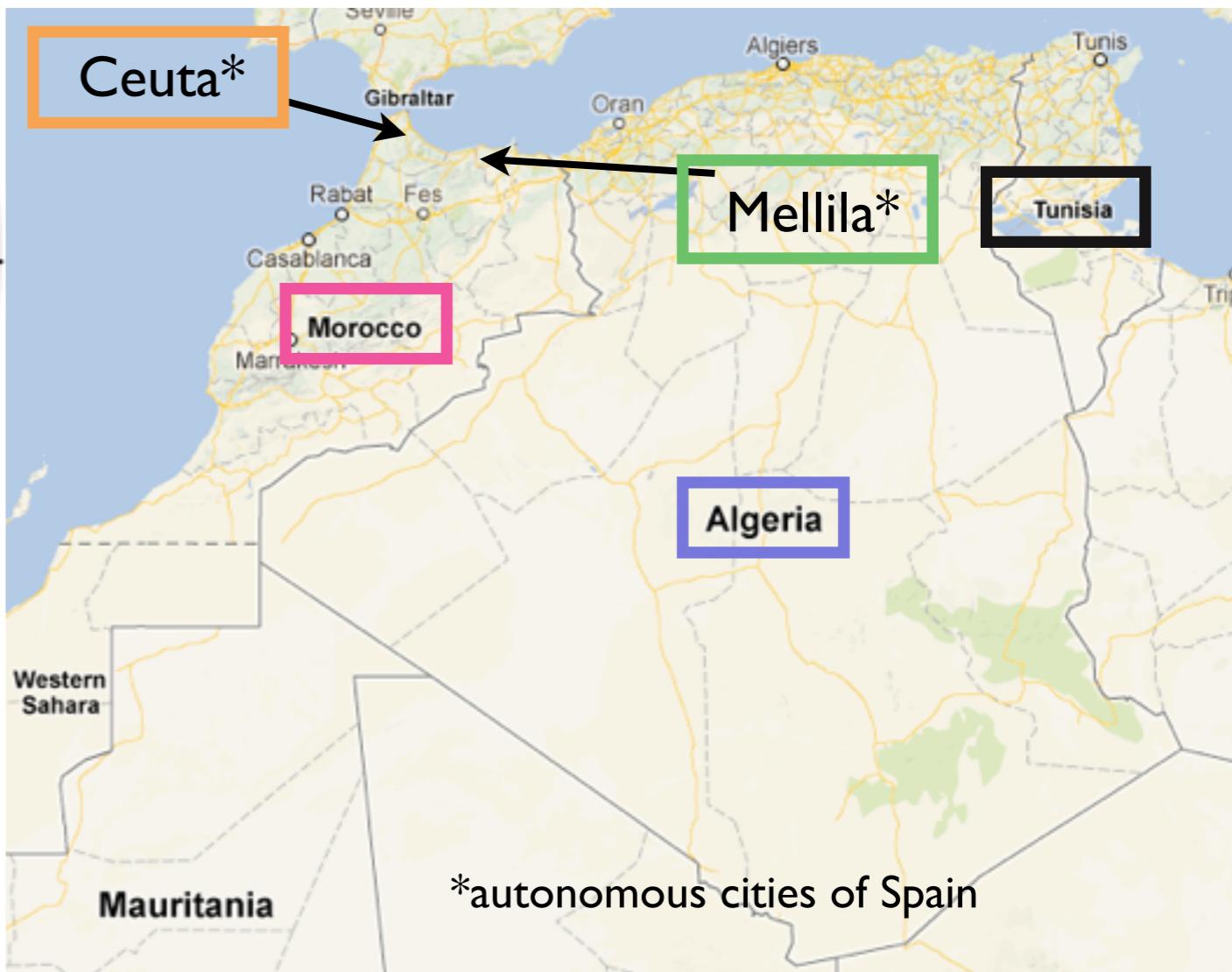


Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.

Phylogeny of rabies virus sampled from North African dogs

Branches coloured according to measured or inferred geographical location



Interpreting Molecular Phylogenetic Trees: An Example

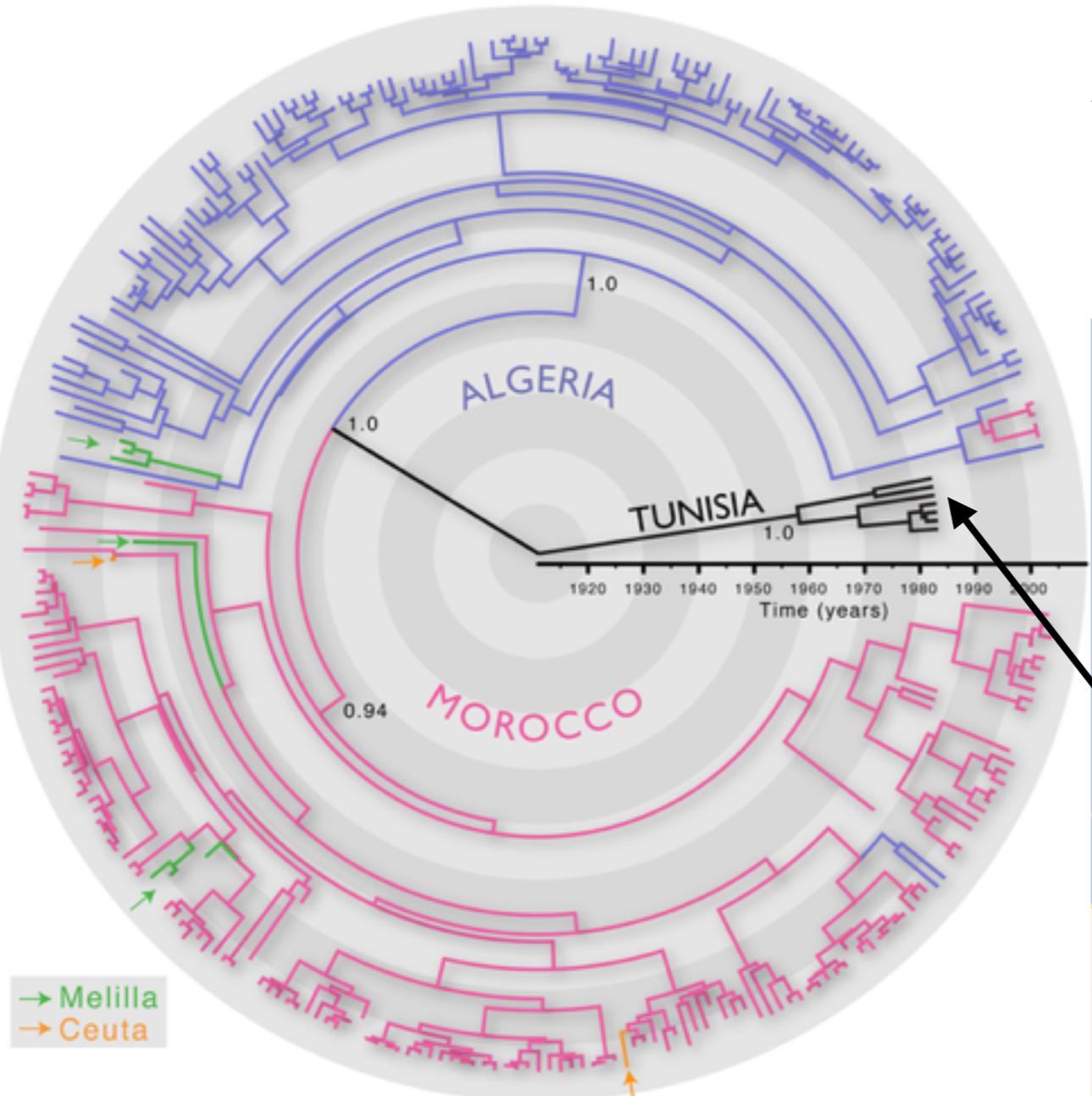
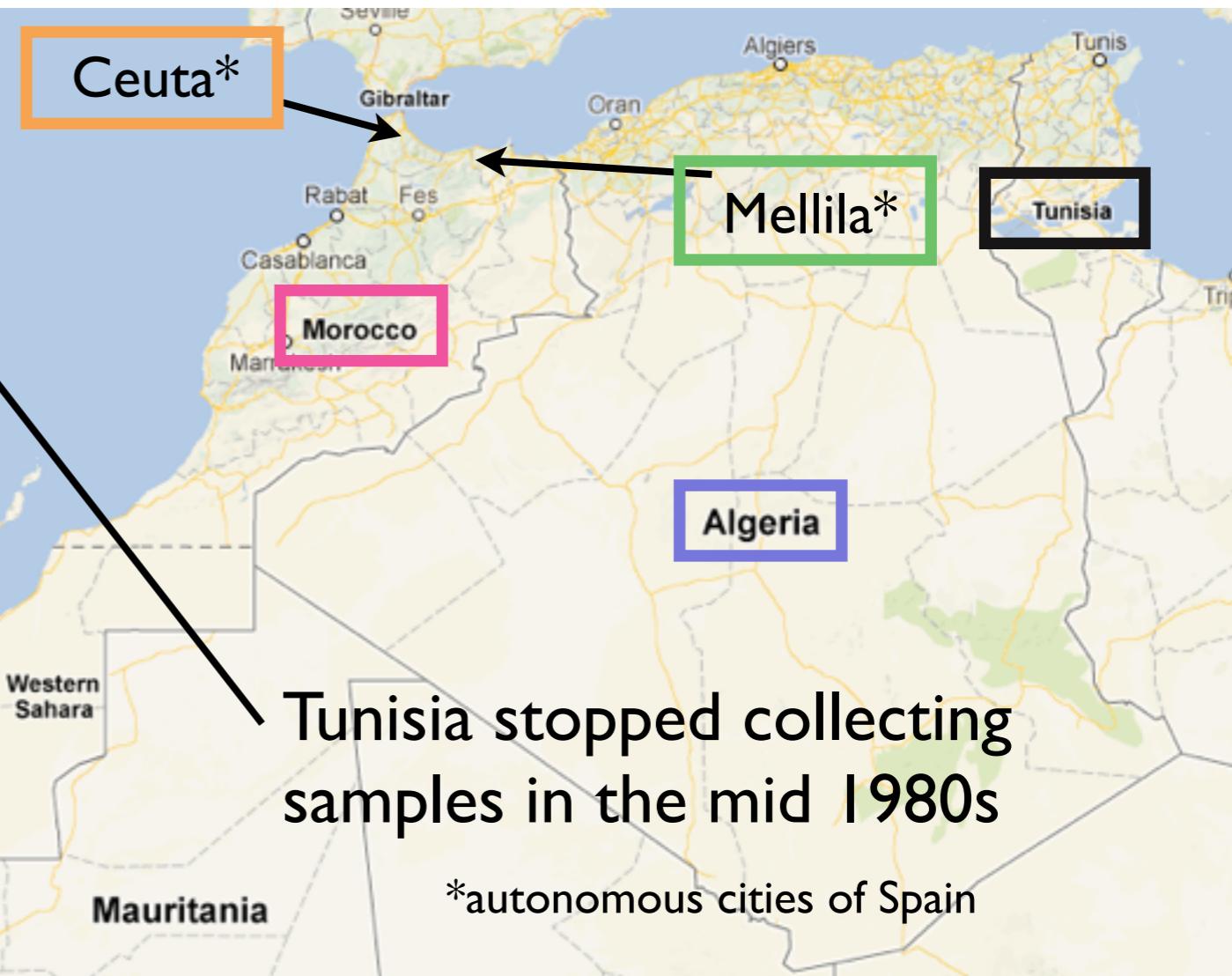


Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.

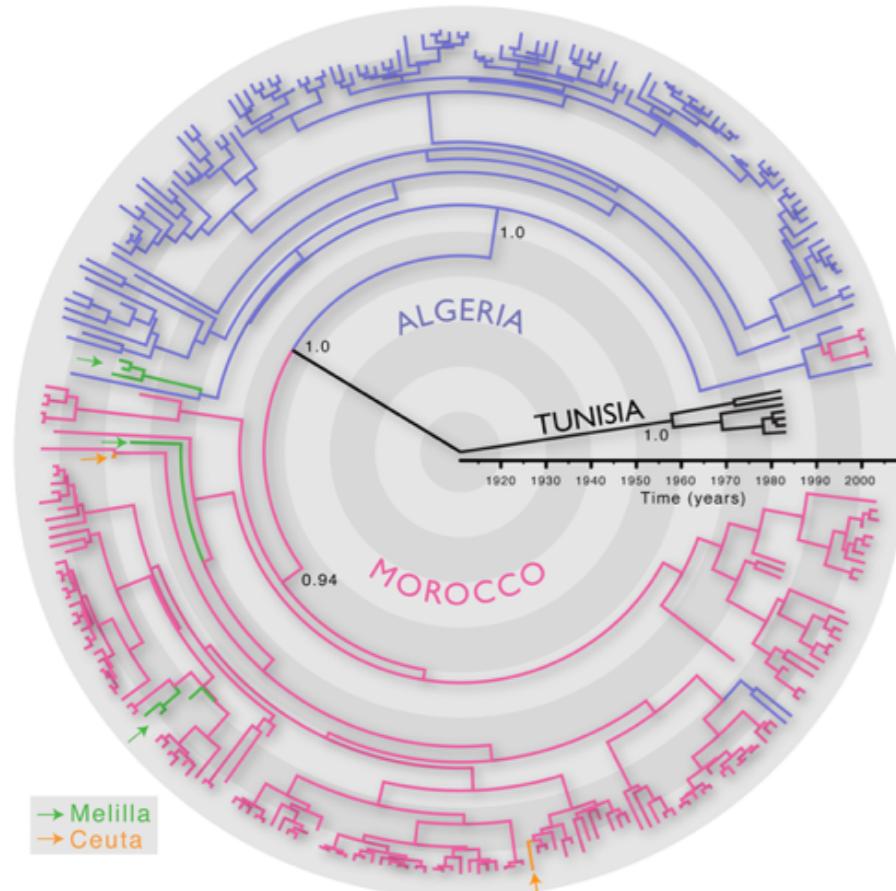
Phylogeny of rabies virus sampled from North African dogs

Branches coloured according to measured or inferred geographical location



*autonomous cities of Spain

Interpreting Molecular Phylogenetic Trees: An Example



Does observing this tree make you consider it
A. more probable
B. less probable
that human activity significantly influences the
dynamics of rabies virus transmission between dogs?

Try and decide, firstly, on your own, without
discussing with your neighbours!

Then we'll take a vote to see what you think

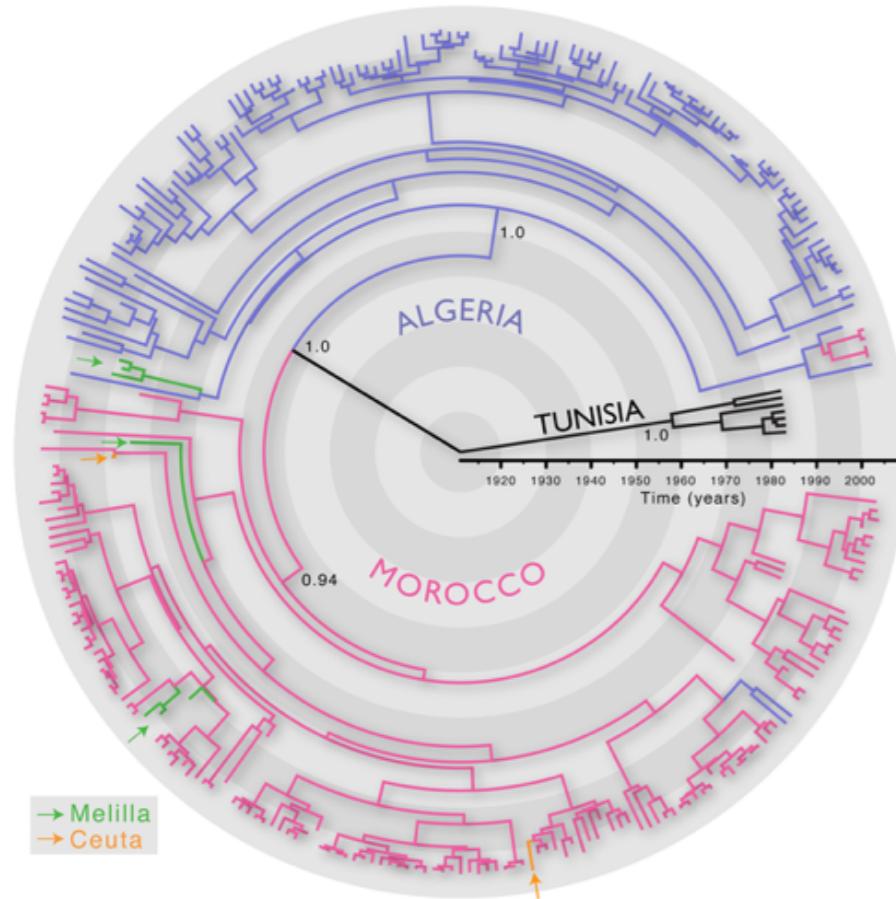
Then discuss the question with your neighbours

Feel uncomfortable that you don't know enough
about the study/data to decide?

Then make (and keep a note of!) reasonable/
possible/plausible assumptions about what you don't
know, then answer assuming these are correct

Don't move to next slide yet!

Interpreting Molecular Phylogenetic Trees: An Example



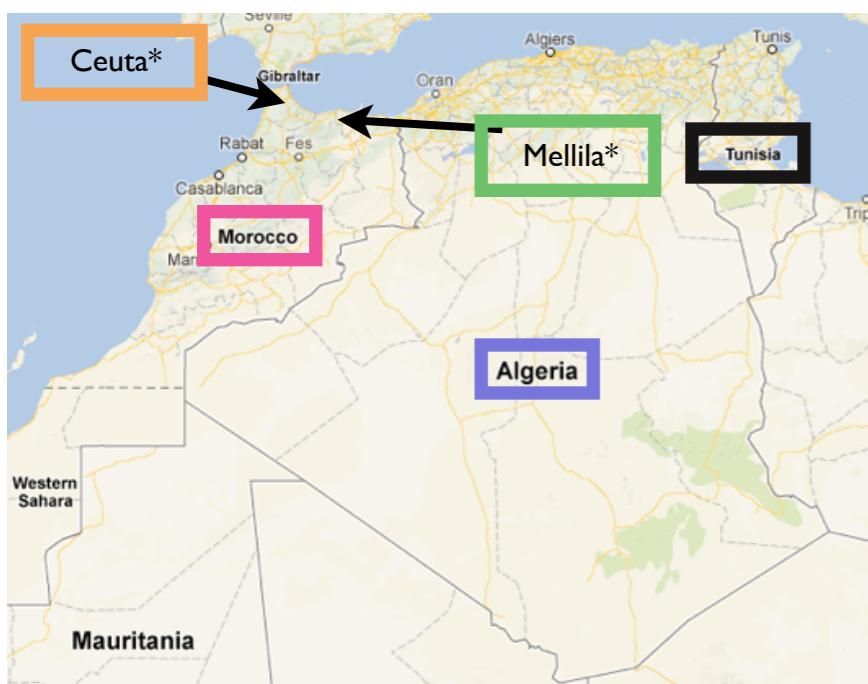
Does observing this tree make you consider it
A. more probable
B. less probable
that human activity significantly influences the
dynamics of rabies virus transmission between dogs?

On the basis of this tree (and several other analyses)
the authors conclude that the data supports a tree that
makes it

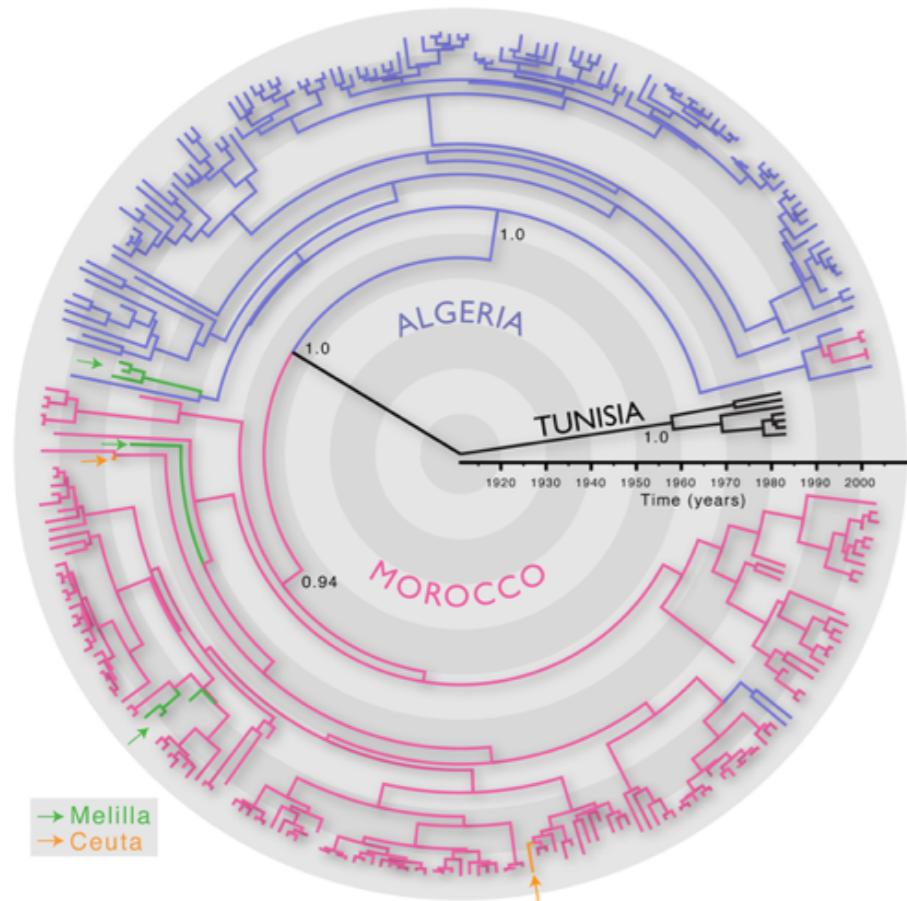
A. more probable
that human activity significantly influences the dynamics
of rabies virus transmission between dogs

seen in the rarity of virus transmission across political (i.e.
at least partially human-activity imposed) borders -

Obvious important implications for public health policy
e.g. suggests that restricting/regulating dog transport may
reduce impact of the virus



Interpreting Molecular Phylogenetic Trees: An Example

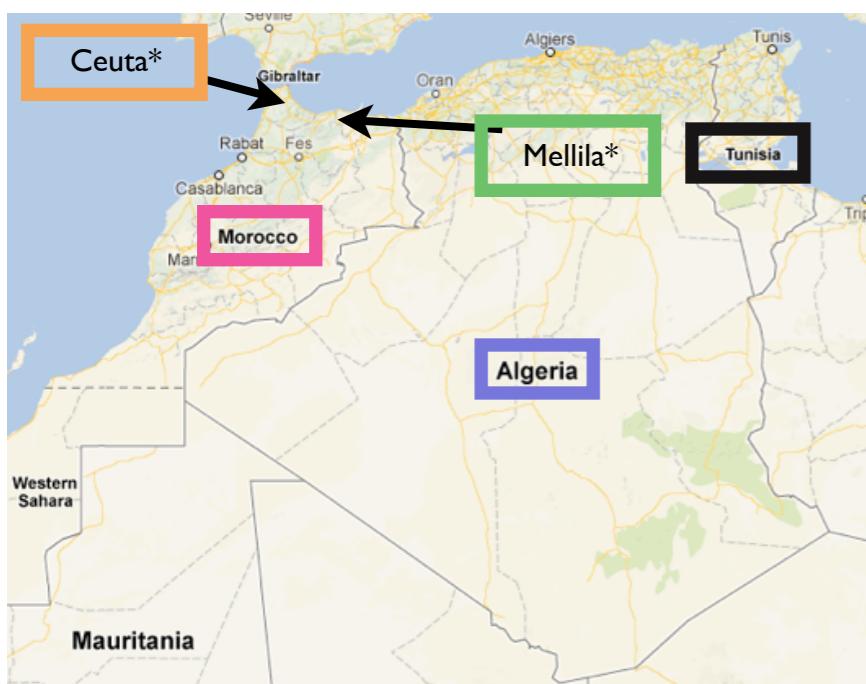


this exercise aimed to highlight that:

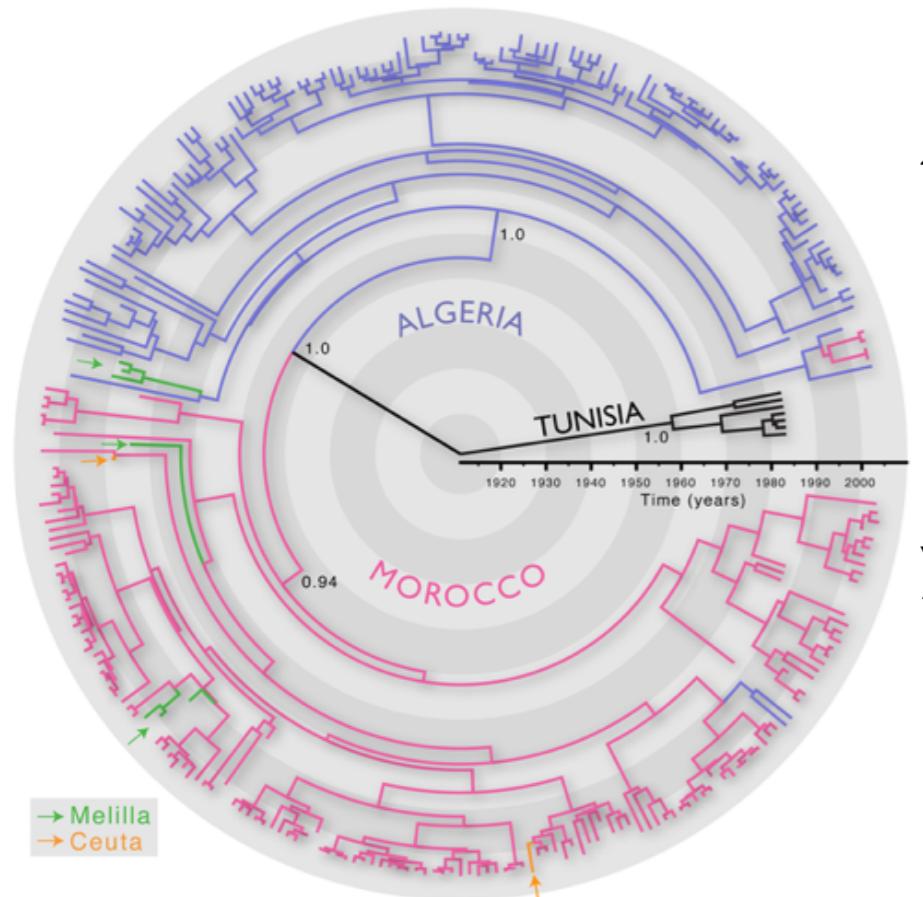
we have to make assumptions/use models to interpret the data - examples of assumptions that could be made while interpreting this tree:

- topology of the tree is correct
- inference of taxon location is correct
- natural geographic (mountains, deserts) do not influence gene flow for the virus

it's useful/important to be aware of what these are and to state them



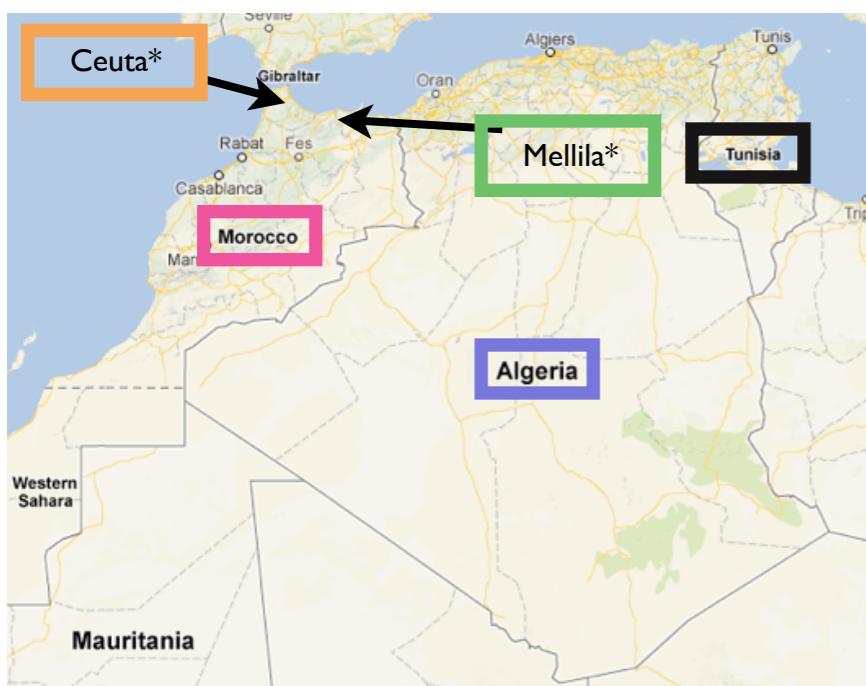
Interpreting Molecular Phylogenetic Trees: An Example



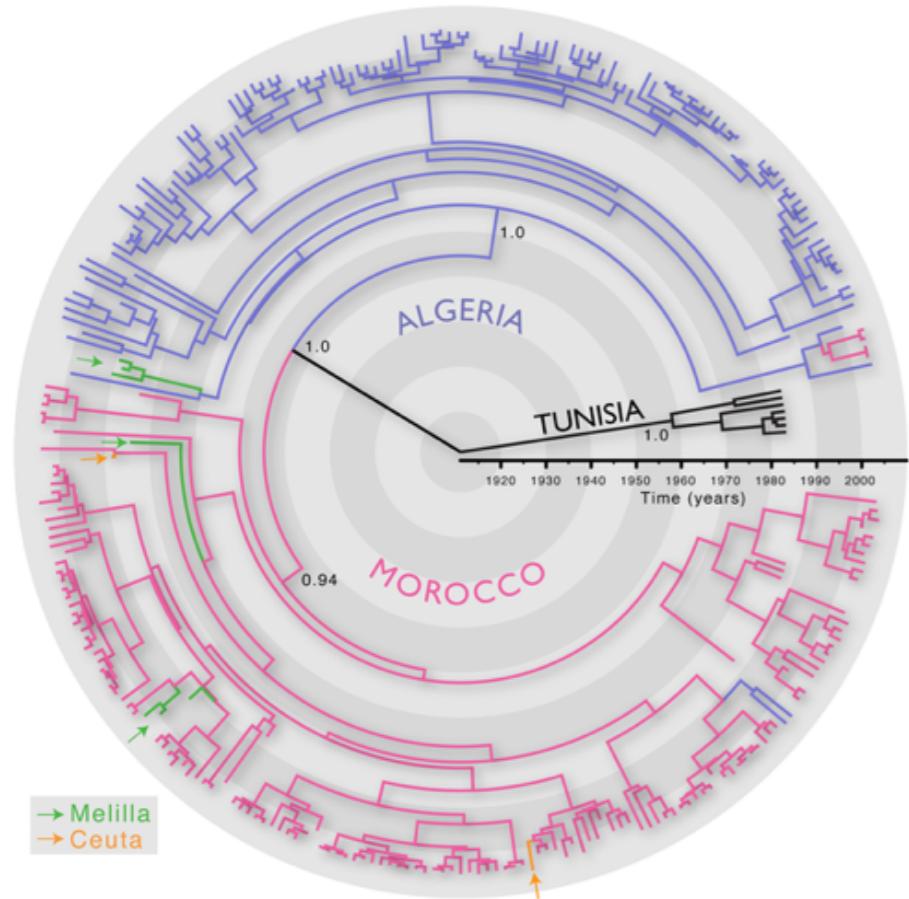
this exercise aimed to highlight that:

it's important to present information (the tree, the sample location) in a way that makes the conclusions you want to draw from the analysis clear/obvious

- it's useful spending time thinking/practicing changing how trees are presented/displayed

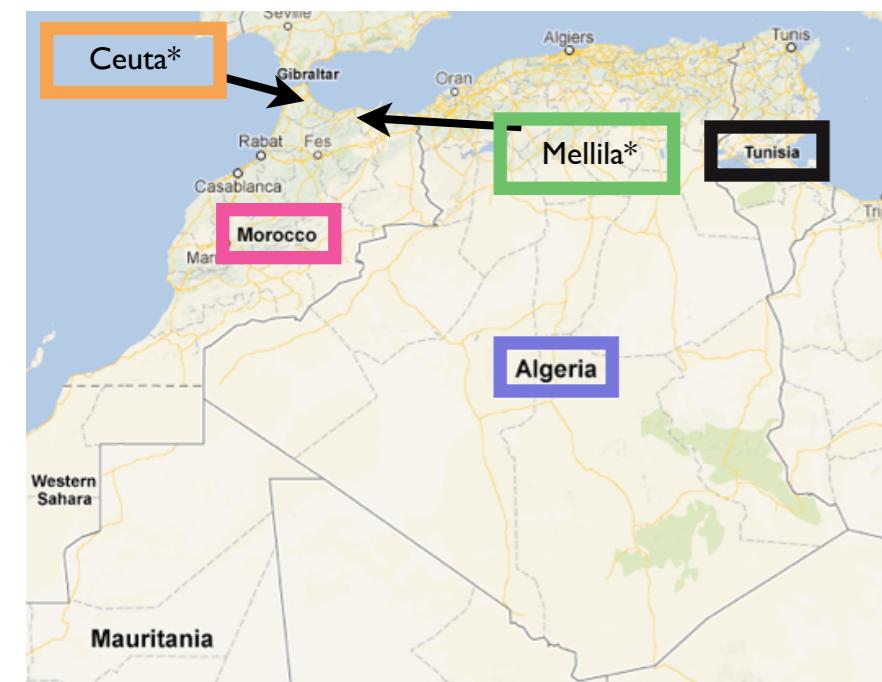


Interpreting Molecular Phylogenetic Trees: An Example



this exercise aimed to highlight that:

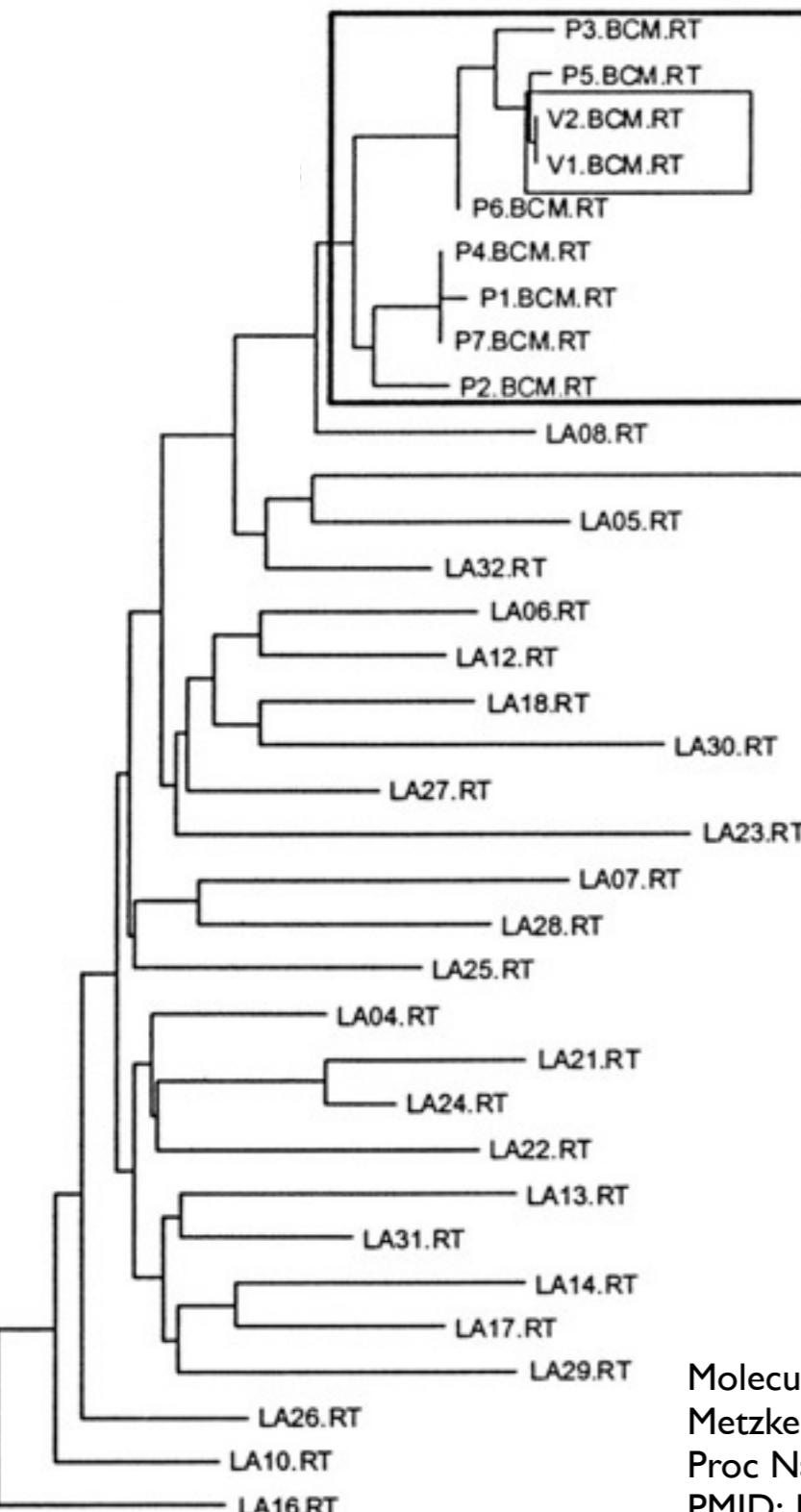
phylogenetic trees can help making important, evidence-based, decisions



Other Applications of Phylogenetics

- Epidemiology
- Forensics
- Selecting conservation targets
- Monitoring trade in illegal organisms
- Bioinformatics tools - in particular:
 - building MSAs
 - predicting function
- Basic evolutionary research
 - characterising processes of evolutionary transformation
 - estimating patterns of transmission of genetic material

Interpreting Molecular Phylogenetic Trees: Another Example



"Louisiana gastroenterologist" (Richard J. Schmidt) accused of attempted second degree murder for allegedly injecting a former lover with blood from one of his HIV+ patients

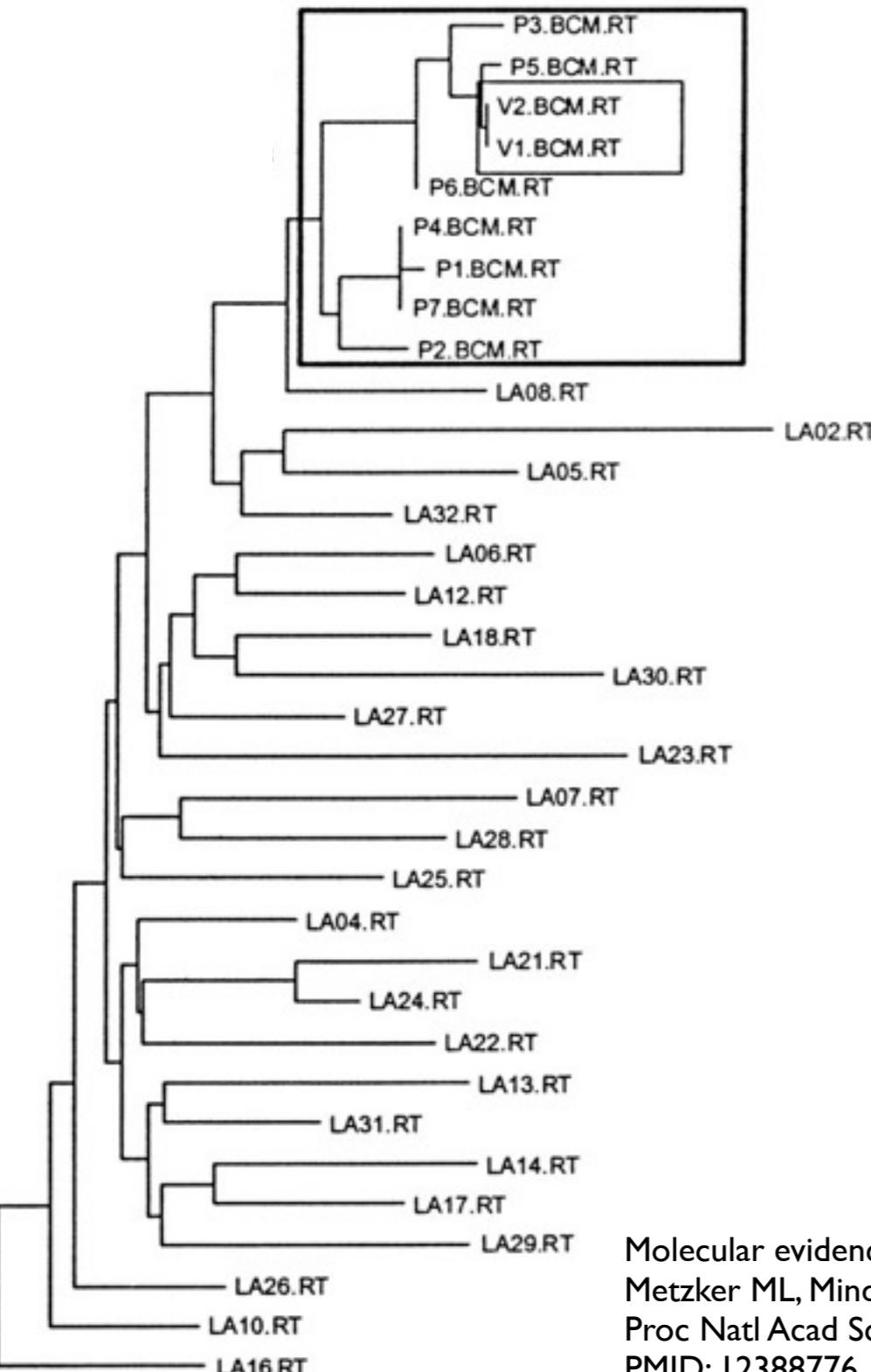
Phylogenetic analyses used as evidence in the trial

Few applications where we care this much (and the accused even more!) that the analysis is done as well as possible

i.e relevant parameters estimated as accurately and precisely as possible

Molecular evidence of HIV-1 transmission in a criminal case.
Metzker ML, Mindell DP, Liu XM, Ptak RG, Gibbs RA, Hillis DM.
Proc Natl Acad Sci U S A. 2002 Oct 29;99(22):14292-7.
PMID: 12388776

Interpreting Molecular Phylogenetic Trees: Another Example



- victim (V)
- gastroenterologist's HIV+ patient (P)
- randomly-selected isolates of contemporary sequences from Louisiana (LA)

Note: several samples taken from patient (7) and victim (2)

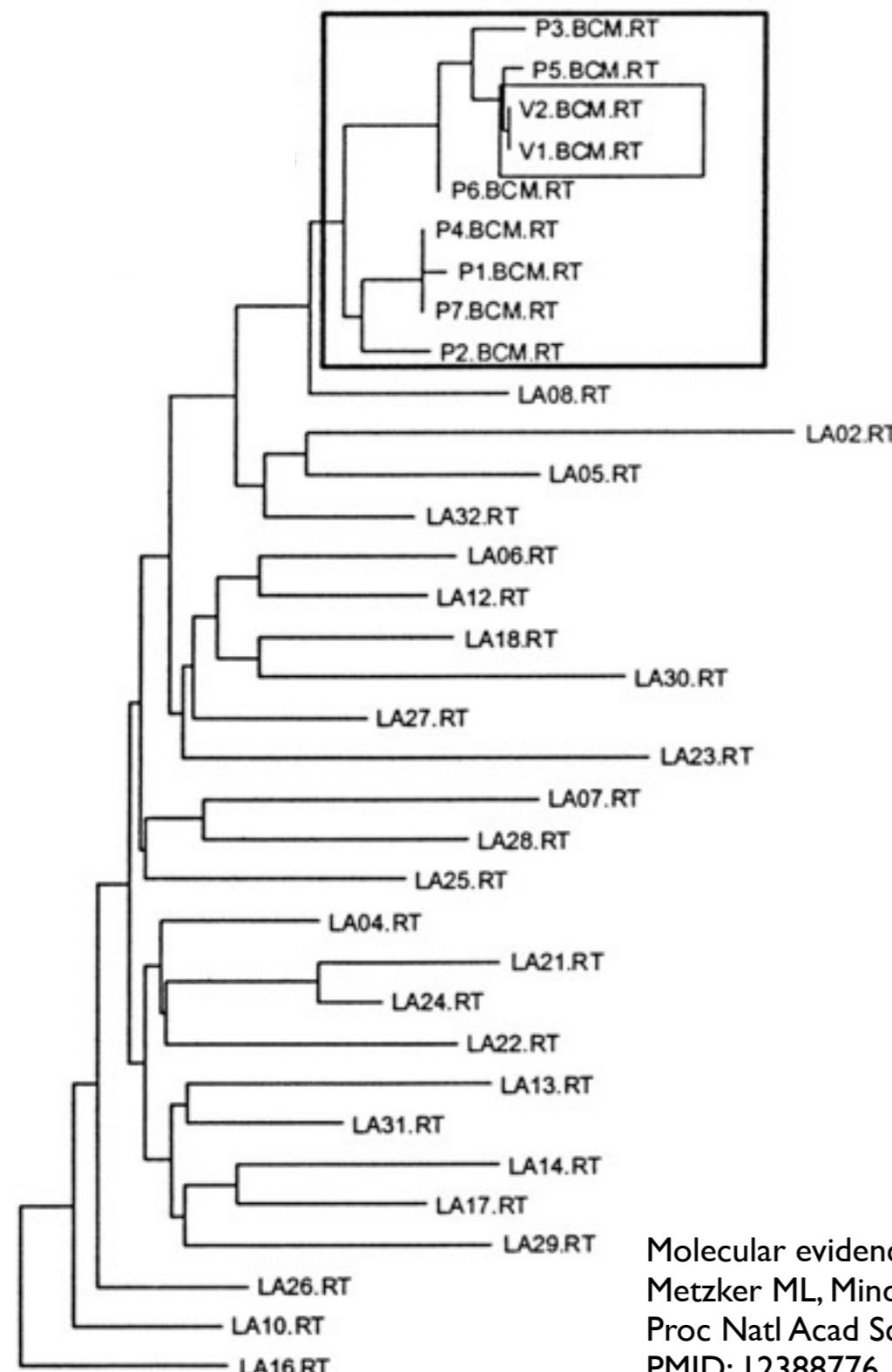
You are a juror on this case....

How/would seeing this phylogeny influence the verdict you would choose in this case?

1. More likely to choose "guilty"
2. More likely to choose "not guilty"
3. Would not influence your choice of verdict

Molecular evidence of HIV-1 transmission in a criminal case.
Metzker ML, Mindell DP, Liu XM, Ptak RG, Gibbs RA, Hillis DM.
Proc Natl Acad Sci U S A. 2002 Oct 29;99(22):14292-7.
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Interpreting Molecular Phylogenetic Trees: Another Example



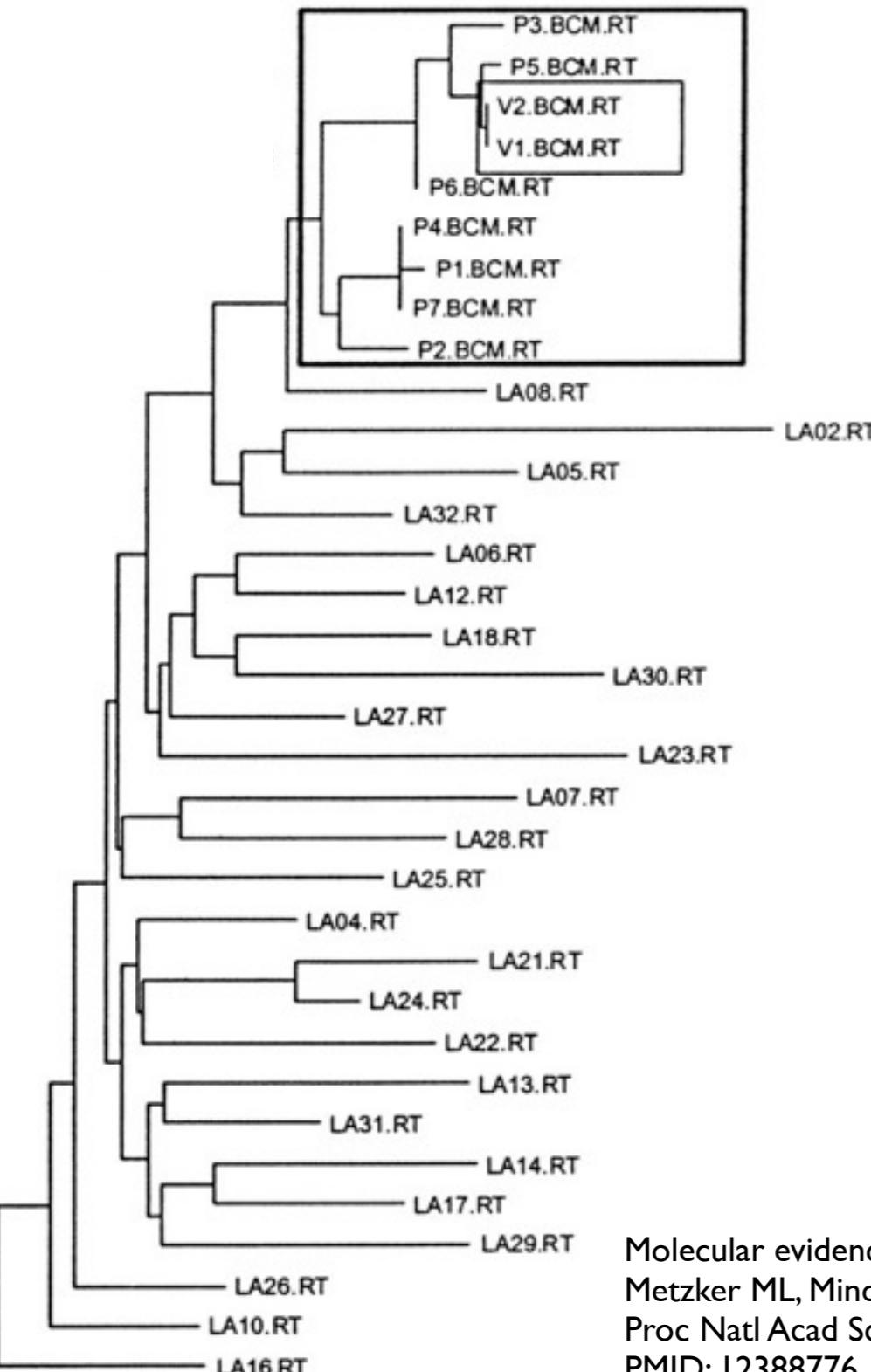
- victim (V)
- gastroenterologist's HIV+ patient (P)
- randomly-selected isolates of contemporary sequences from Louisiana (LA)

Note: several samples taken from patient (7) and victim (2)

You are a **defence attorney for the case...**

What would you ask the expert phylogenetic witness about the analysis to potentially help establish the innocence of the defendant?

Interpreting Molecular Phylogenetic Trees: Another Example



- victim (V)
- gastroenterologist's HIV+ patient (P)
- randomly-selected isolates of contemporary sequences from Louisiana (LA)

Note: several samples taken from patient (7) and victim (2)

You are a **prosecution attorney** for the case...

What would you ask the **expert phylogenetic witness** about the analysis to potentially help establish the **guilt of the defendant?**

Rooted Phylogenies

Terminology and Concepts

Definitions

Phylogeny terminology and concepts "definition" exercise
(see [homepage/InterpretingPhylogenies.html](#))

To begin looking at how we think about phylogenies, we'll explore together how we define several fundamental phylogenetic concepts

Write, on your own, definitions of:

- phylogenetic tree
- branch (of a phylogenetic tree)
- root (of a rooted phylogenetic tree)

Writing forces you to be explicit about what you mean, and can help identify issues you are uncertain about

Then compare your definitions with your neighbour, and write together a consensus definition for each term

Definitions

Phylogeny terminology and concepts "definition" exercise
(see [homepage/InterpretingPhylogenies.html](#))

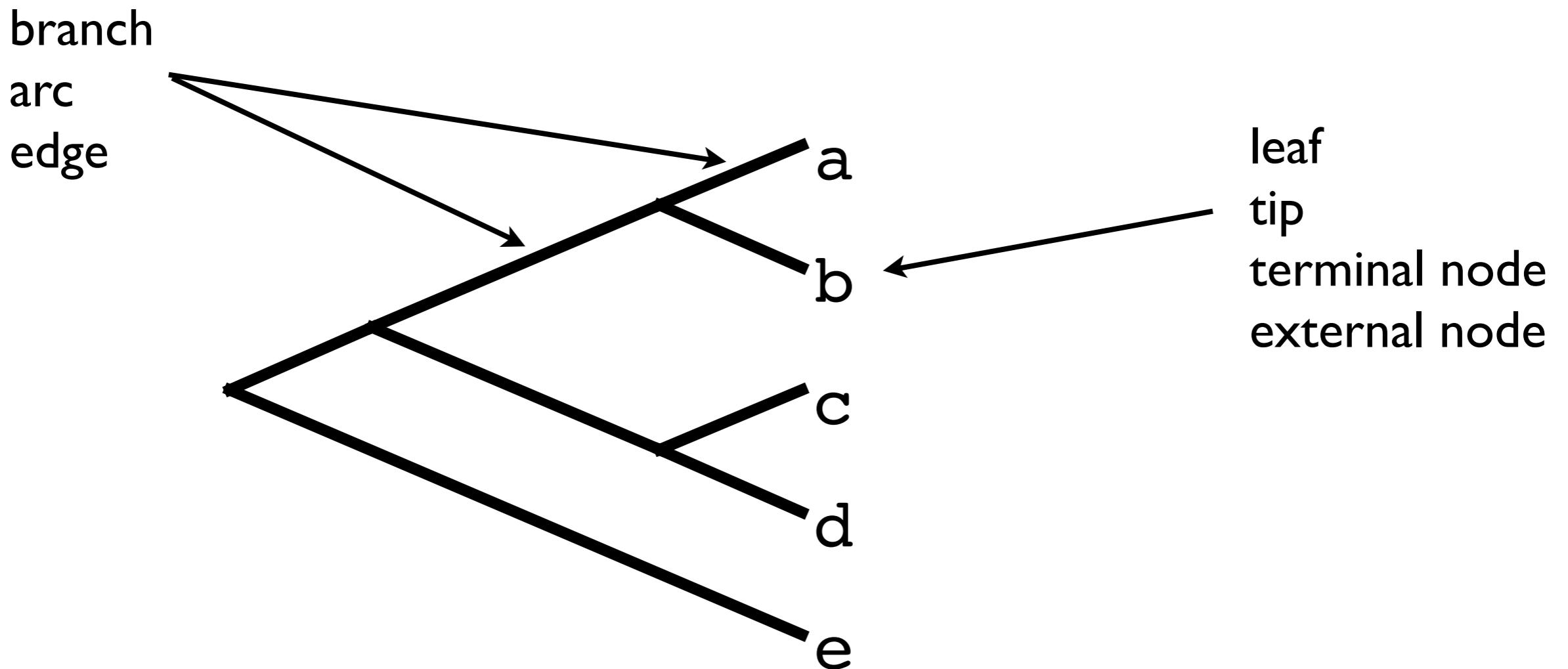
below are some suggested definitions for these terms

phylogenetic tree: A description of a path of transmission of genetic information between a set of taxa.

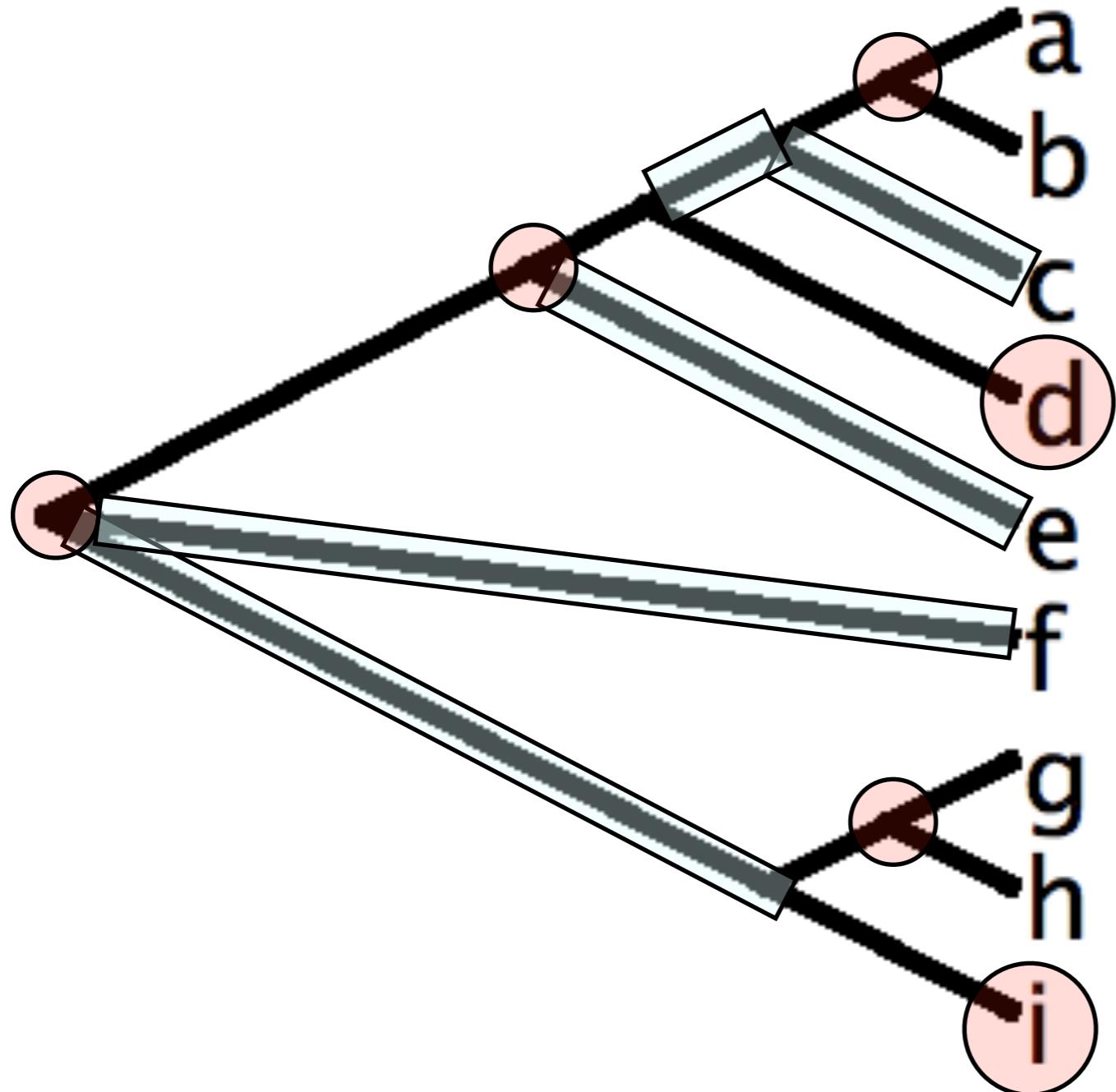
branch (of a phylogenetic tree): Lineages of taxonomic units that link nodes within a phylogenetic tree.

root (of a rooted phylogenetic tree): In a rooted tree, the node that represents the most recent common ancestor taxon of all other taxa in the tree.

Alternative Tree-Related Terminologies



Trees: Branches and Nodes



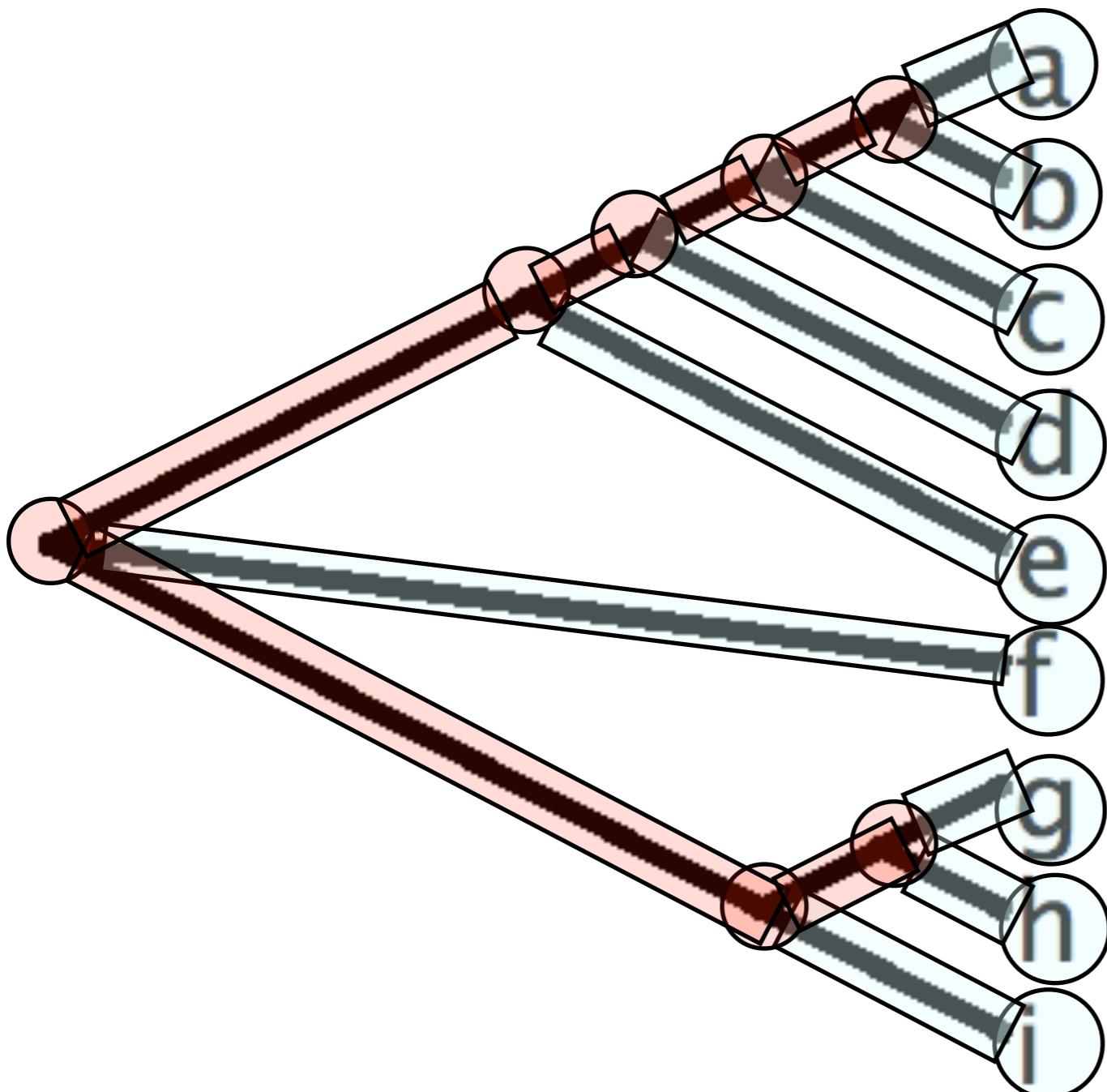
Trees consist of:

branches

nodes (ends of branches)

Internal/External Nodes/Branches

Branches and Nodes are either:



internal/interior

Node - at the intersection of two or more branches

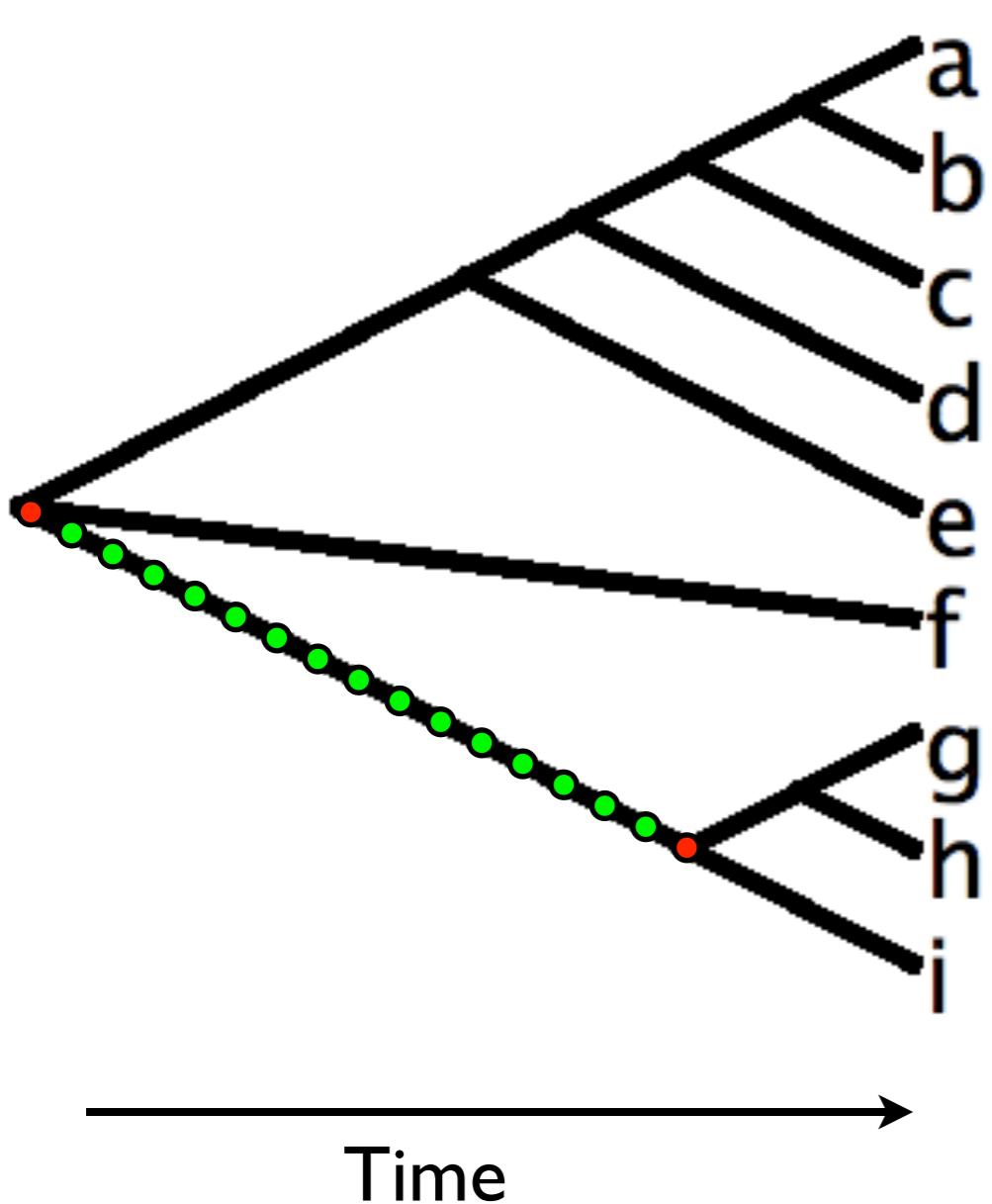
Branch - links two internal nodes

external/terminal

Node - associated with an extant sequence/OTU (operational taxonomic unit)

Branch - links an external and an internal node

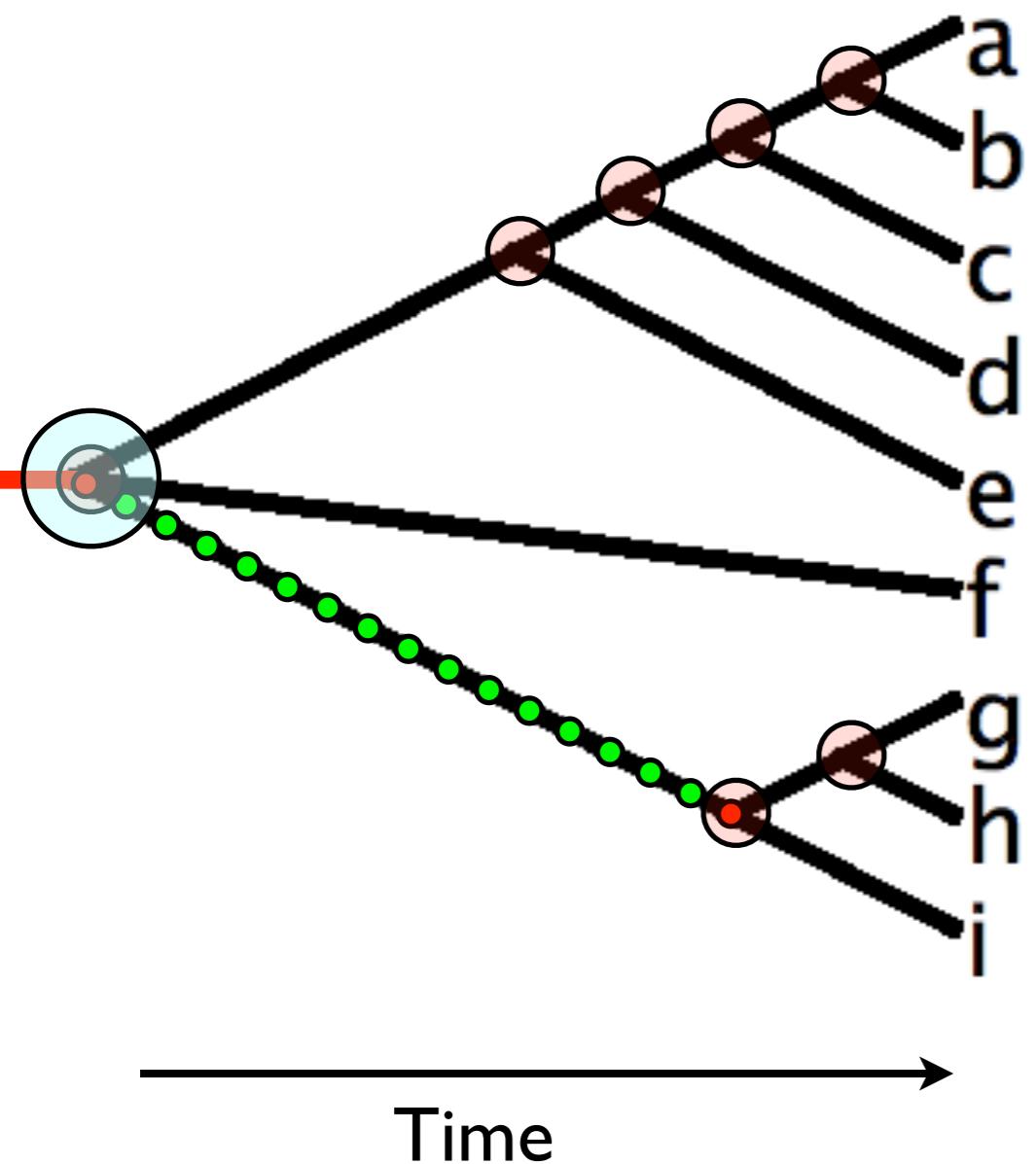
Branches



Branches

- represent successive generations of “taxa”
- ‘later’ taxa have “earlier” taxa as their ancestors
- i.e. a lineage
- time flows from the base of the tree to the tips

Internal Nodes



Internal Nodes

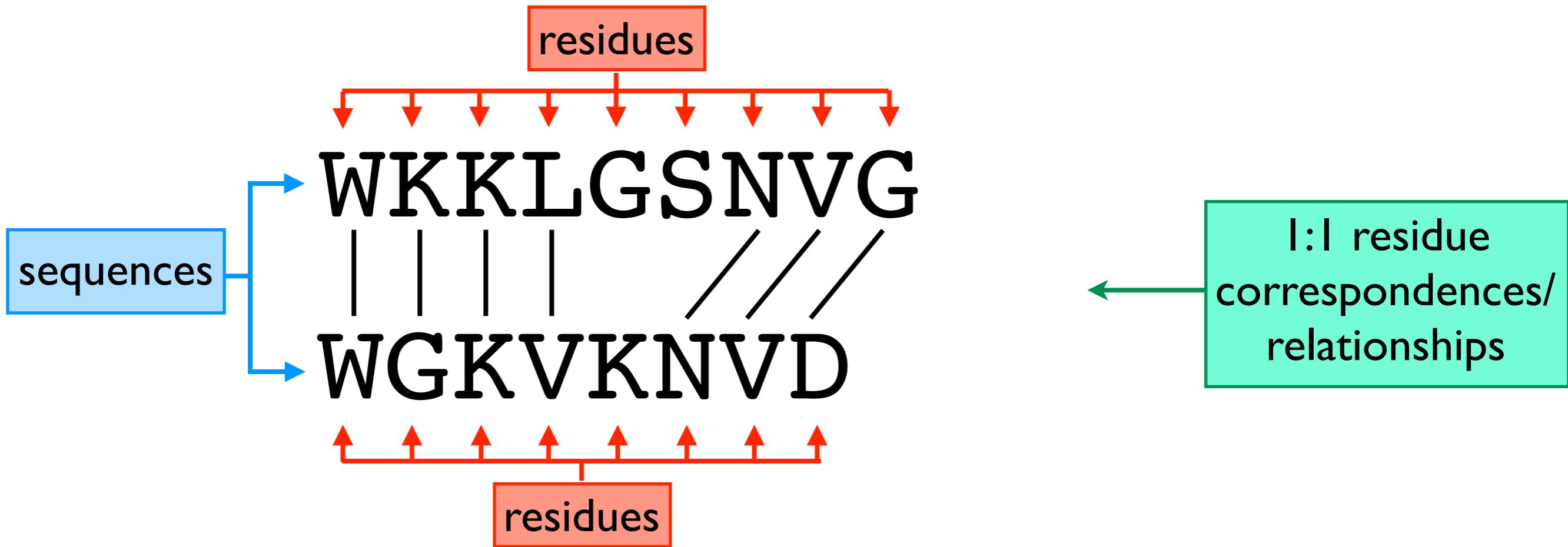
- represent hypothetical ancestral taxa/sequences/organisms
- i.e. HTUs - hypothetical taxonomic units
- i.e. their state/existence is inferred or hypothesised, not measured directly

Root (Root Node)

- A "special" internal node
- The most recent common ancestor of all OTUs
- Usually implies many other **less recent common ancestors**

Alignments

"Anatomy" of a Sequence Alignment



I:I residue correspondences/relationships

Correspondences between

- a single residue in one sequence and
- a single residue in another sequence

"Anatomy" of a Sequence Alignment



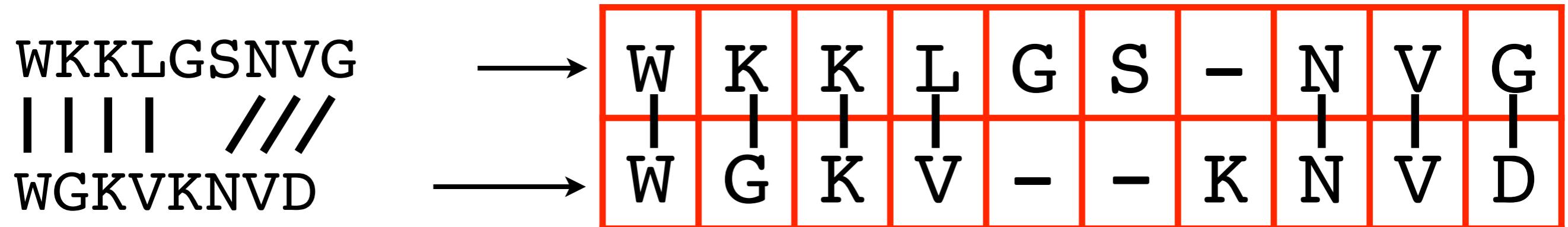
I:I residue correspondences/relationships

Residue has no link to a residue in the top sequence
i.e. no residue in the top sequence has a I:I relationship with this residue

Could perhaps say there is a "I:2" relationship between this residue and these residues

However, alignments focus on I:I relationships

Sequence Alignment Within a Grid



Often represented using a **grid/matrix**:

One sequence per row

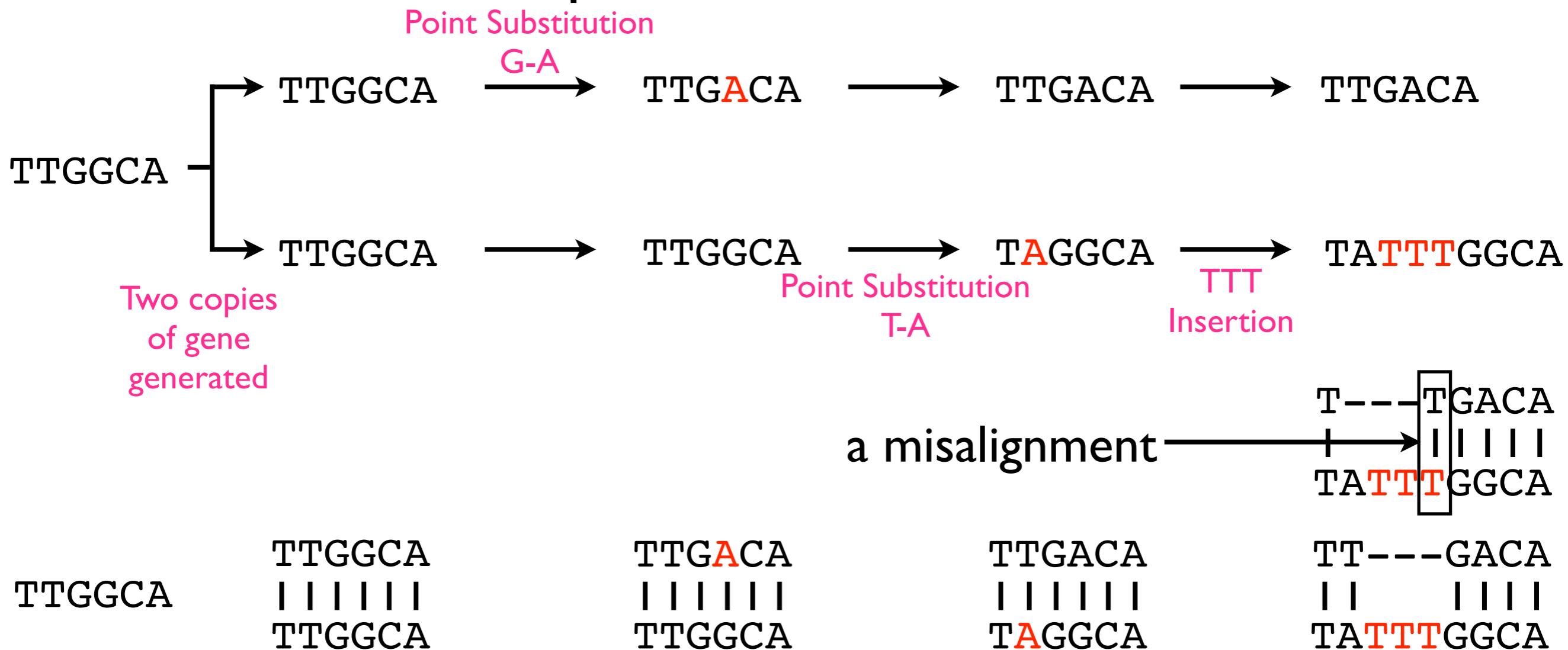
Residues in the same column are 'linked' ('equivalent'? 'homologous'?)

Gap characters (usually "-") indicate that the sequence contains no residues 'equivalent' to other residues in that column

Evolutionary "Equivalence"

Residues are "evolutionarily linked" when:

- you (believe) their evolutionary history can be traced back to the same residue in an (inferred) ancestral sequence...
- ... such that the only changes experienced during divergence from this ancestral residue were **point substitutions**



Branch Lengths

Branch Lengths

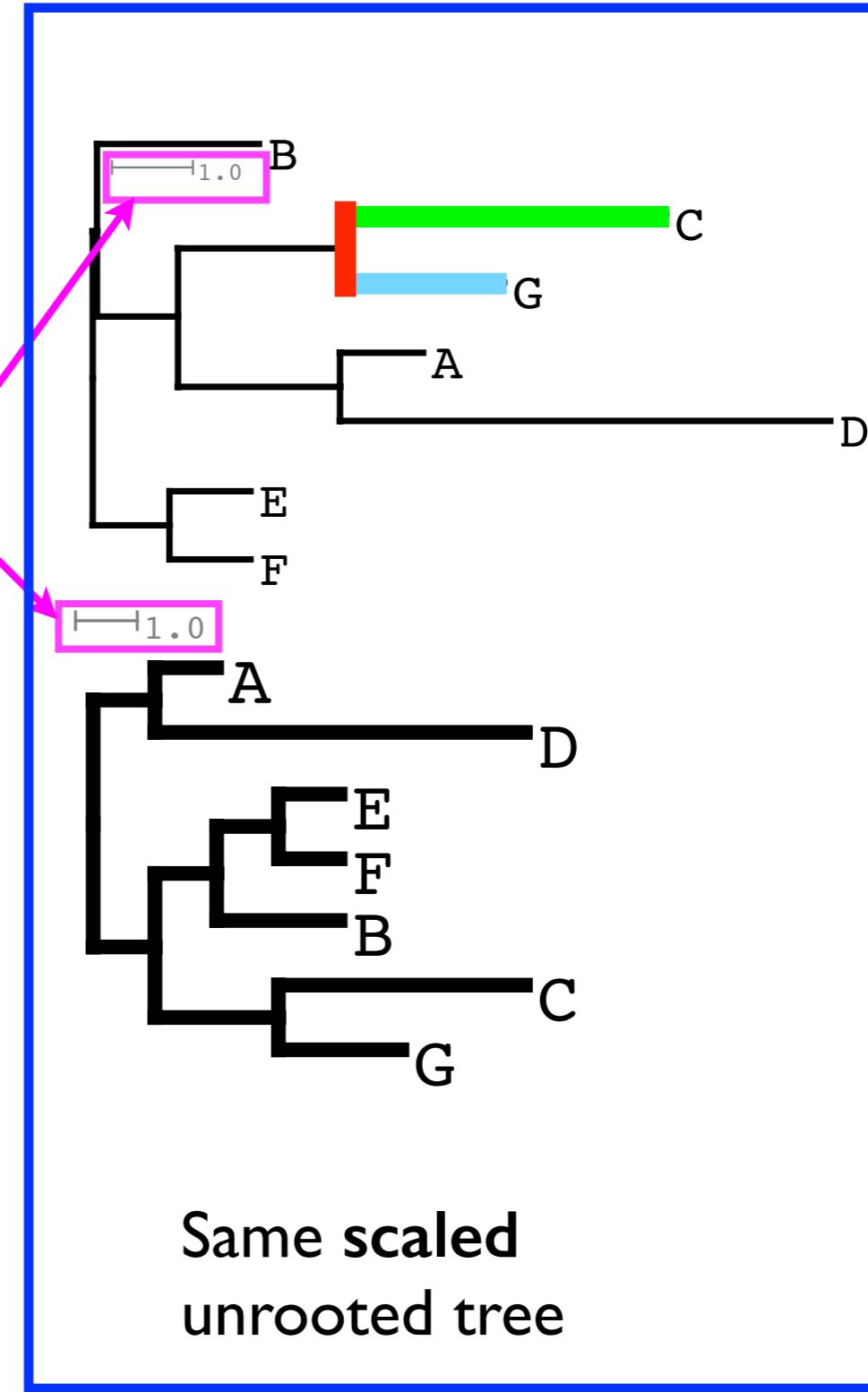
Branch length usually represents some measure of the difference/distance between TUs at ends of the branch

Tree should be presented together with a scale bar

For rectangular trees, “node lines” are NOT branches! Their length provides no indication of intertaxa difference/distance!

i.e. distance between taxa C and G is the sum of the green and cyan lines (it does NOT include the length of the red line!)

C ————— G



Branch Lengths

Often an ESTIMATE of the EXPECTED/AVERAGE number of substitutions per site between two sequences

SeqA	I	K	T	I	I	L	K	W	W	S	P
SeqB	I	K	T	I	V	K	W	D	S	P	

If we assume:

- All identical residues between two sequences have not experienced substitutions
- All different residues have experienced one substitution

Mean/Average No. Substitutions = $2/10 = 0.2$

SeqA —————^{0.2}———— SeqB

Branch Lengths

Often an ESTIMATE of the EXPECTED/AVERAGE number of substitutions per site between two sequences

SeqA	I	K	T	I	I	L	K	W	W	S	P
SeqB	I	K	T	I	V	K	W	D	S	P	

Branch-length estimate depends on SUBSTITUTION MODEL

Further assumptions of this model

- All alignment positions/residues evolve (are substituted at) the same rate
- All residues substitute to all other residues at the same rate i.e. A->G at same frequency as A->W

SeqA ————— 0.2 SeqB

Laura's session on tree visualisation

Relatedness

Relatedness (in the context of phylogenetic trees)

Inferring patterns of relatedness is often one of the main aims of evolutionary tree estimation.

"relatedness" in this context has a specific meaning, as exemplified here:

*"the more recently species share a common ancestor, the more closely related they are" **

As "relatedness" has other meanings in other contexts, there can be some confusion about it's meaning in a **phylogenetic** context

As many trees are estimated to inform ideas about patterns of relatedness, we need a clear understanding of how the term is used in this context

Thus, in the next slides, we will look at several examples of how the word is used when describing phylogenetic relationships

* Evolution. The tree-thinking challenge.

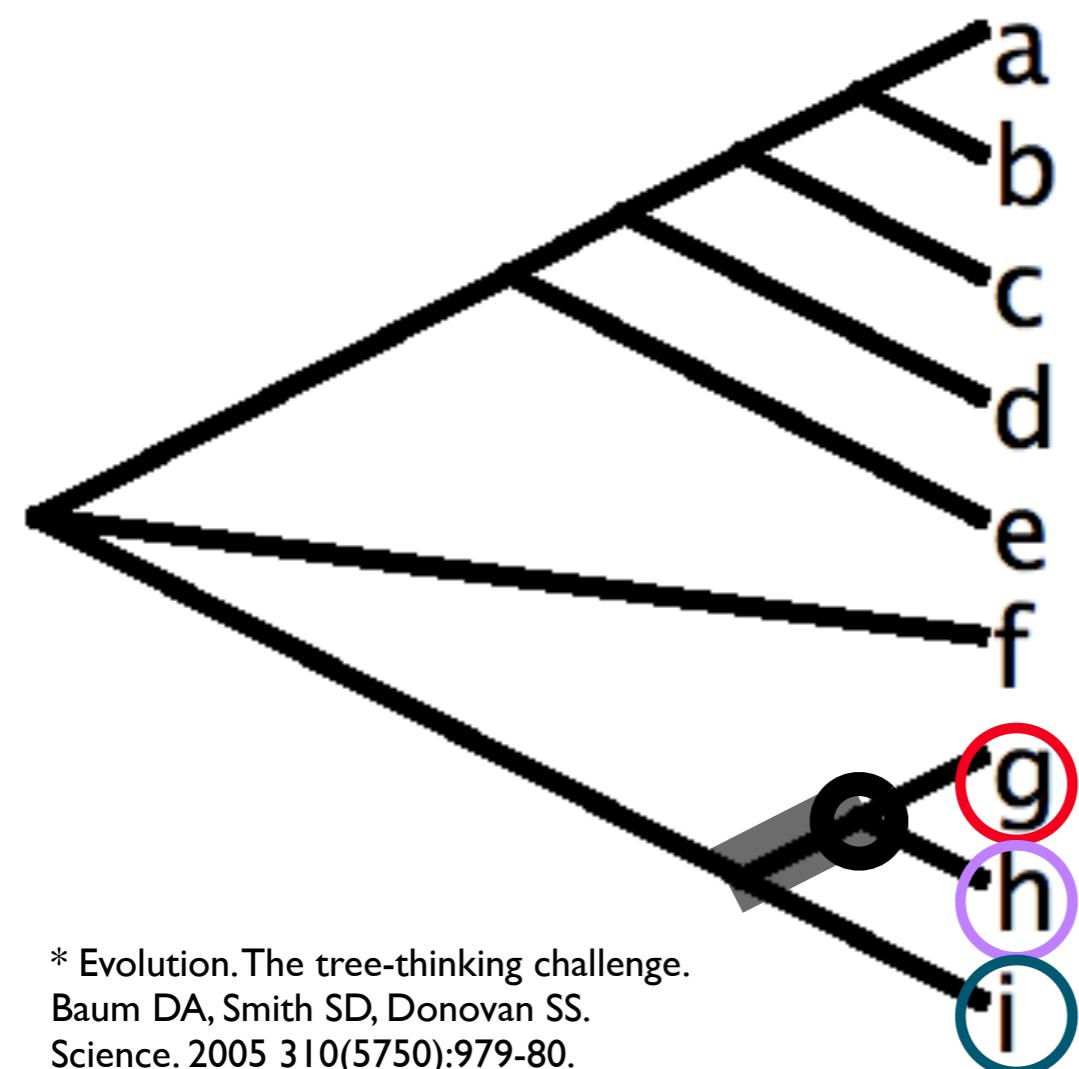
Baum DA, Smith SD, Donovan SS.

Science. 2005 310(5750):979-80.

PMID: 16284166

Relatedness (in the context of phylogenetic trees)

"the more recently species share a common ancestor, the more closely related they are" *

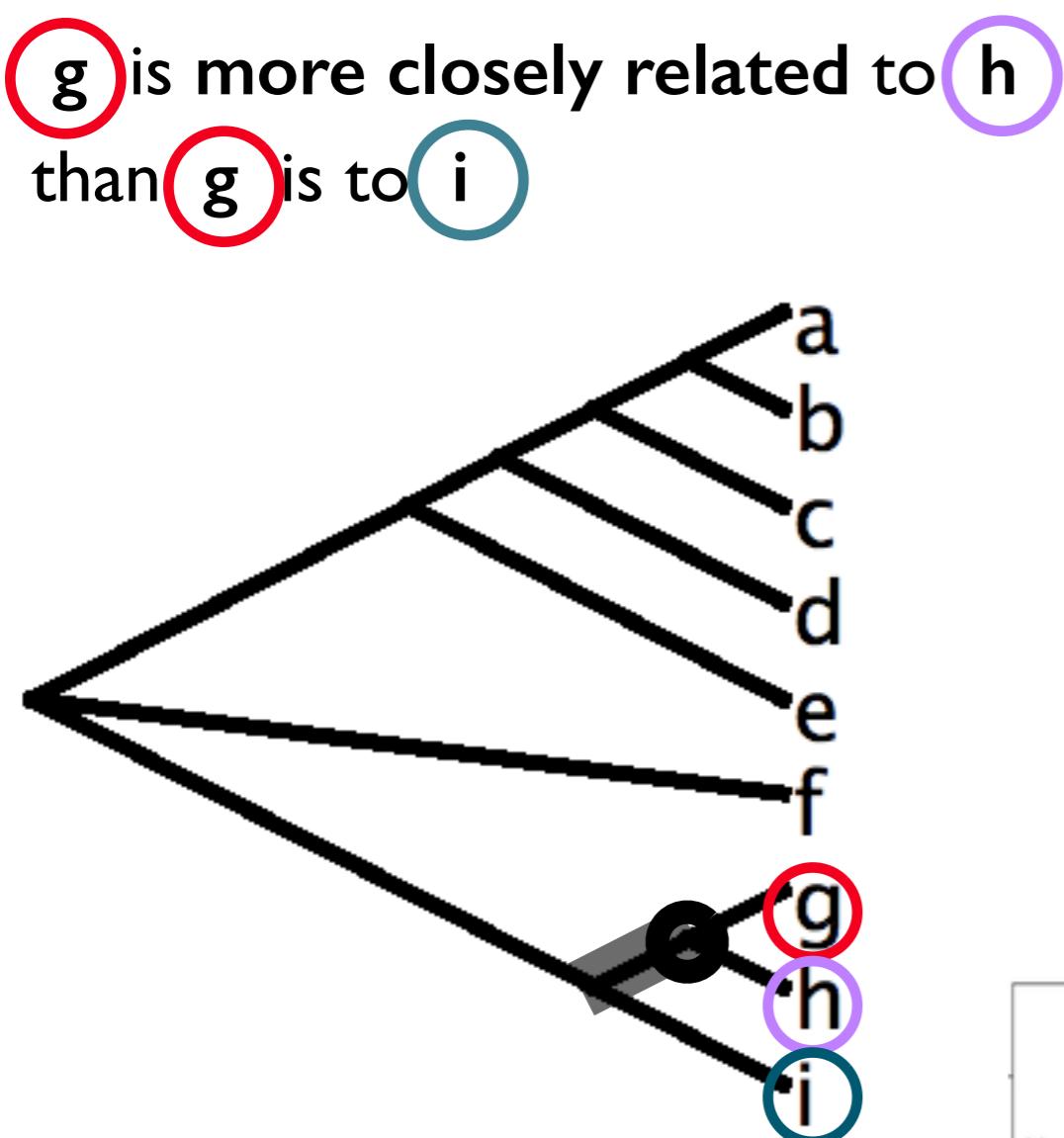


g is more closely related to h
than g is to i
because g and h share common ancestors
that neither share with i
i.e. degree of relatedness
is associated with the extent of ancestry
(i.e. the number of ancestors) taxa share
with each other

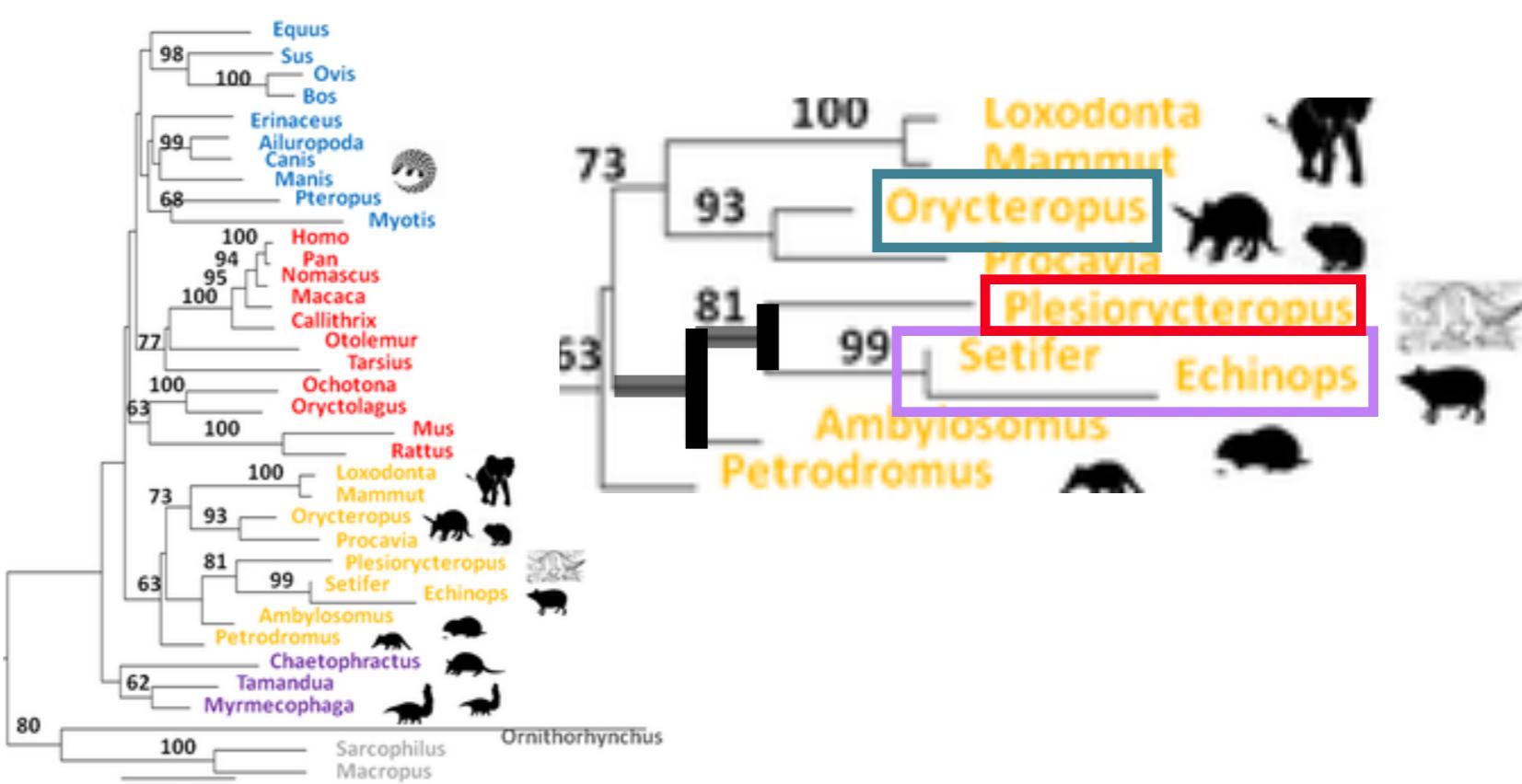
* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

Relatedness (in the context of phylogenetic trees)

"the more recently species share a common ancestor, the more closely related they are" *



... Plesiorycteropus is more closely related to tenrecoids than to tubulidentates ..



* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

Figure 4. Phylogenetic analyses of Plesiorycteropus collagen (I) sequences obtained by LC-MS in comparison to previously postulated closest relatives.

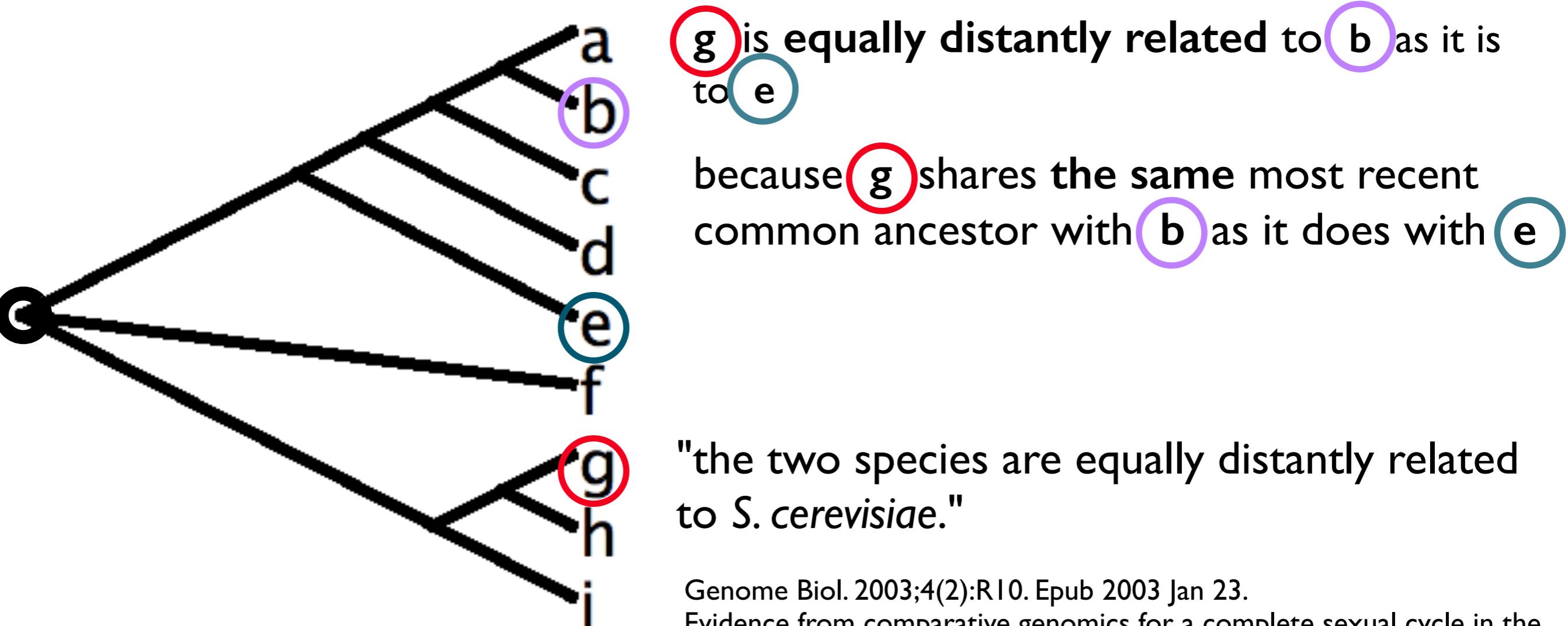
Buckley M (2013) A Molecular Phylogeny of Plesiorycteropus Reassigns the Extinct Mammalian Order 'Bibymalagasia'. PLoS ONE 8(3): e59614. doi:10.1371/journal.pone.0059614

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0059614>

Aidan Budd, EMBL Heidelberg

Relatedness (in the context of phylogenetic trees)

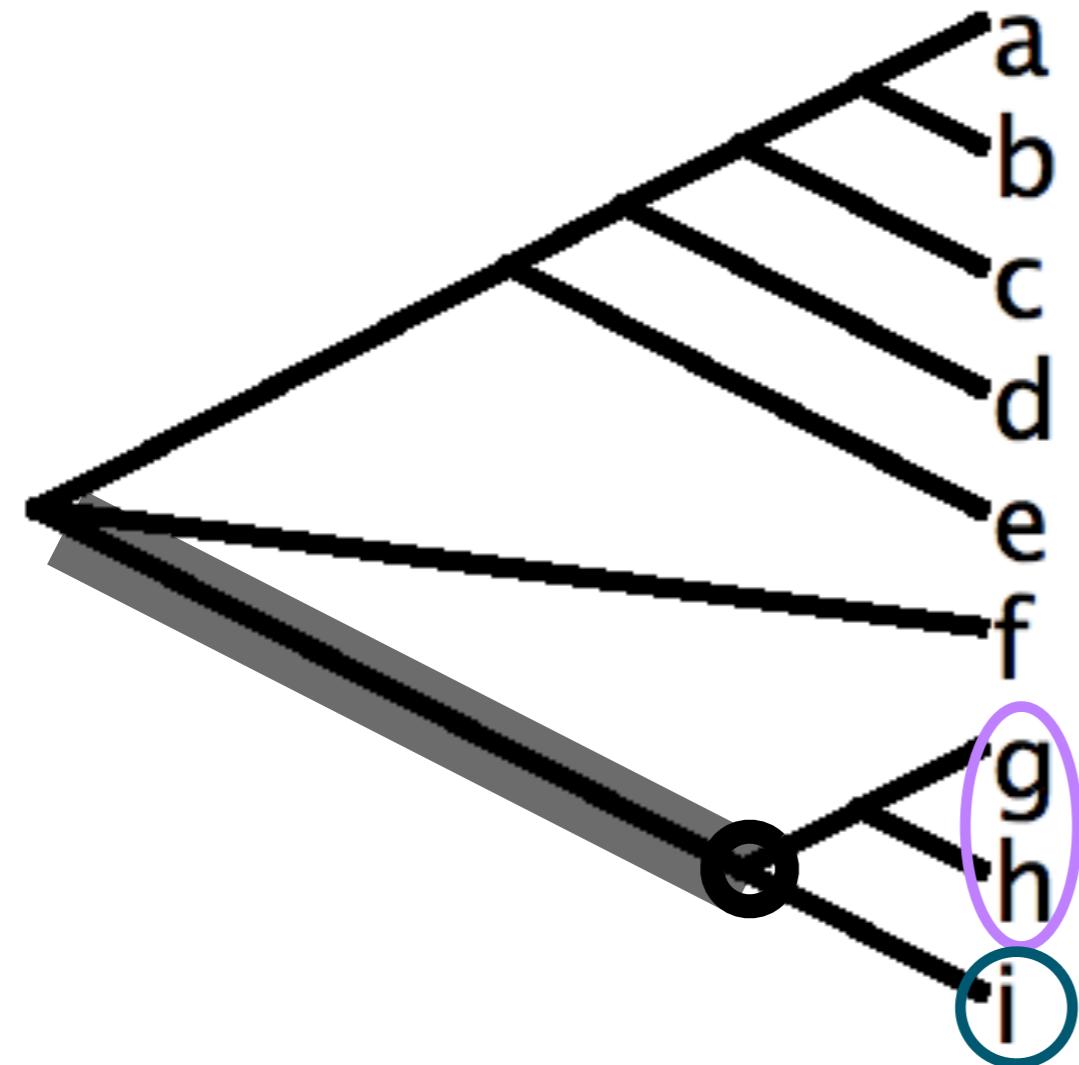
of all the OTUs represented in this tree



Genome Biol. 2003;4(2):R10. Epub 2003 Jan 23.
Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast *Candida glabrata*.
Wong S, Fares MA, Zimmermann W, Butler G, Wolfe KH.

Relatedness (in the context of phylogenetic trees)

of all the OTUs represented in this tree



i is most closely related to g and h
(i.e. i is the *sister group* of g and h... which is equivalent to saying g and h are the sister group of i)

because i shares common ancestors with g and h that it does not share with any other OTUs in the tree

"PEPV was confirmed to [...] be most closely related to Turkeypox virus (TKPV), Ostrichpox virus (OSPV) and Pigeonpox virus (PGPV)."

Virol J. 2009 May 8;6:52. doi: 10.1186/1743-422X-6-52.

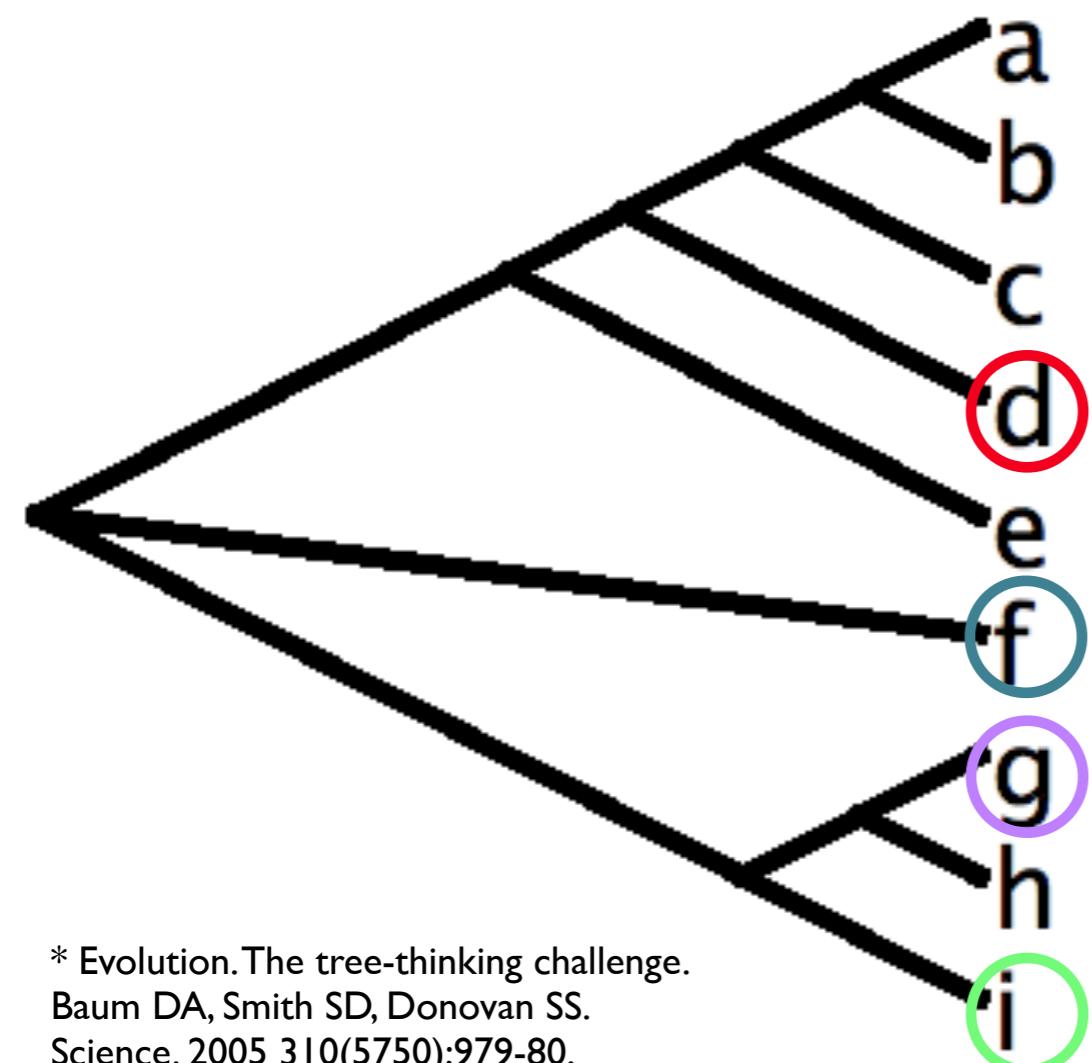
Phylogenetic analysis of three genes of Penguinpox virus corresponding to Vaccinia virus G8R (VLTF-1), A3L (P4b) and H3L reveals that it is most closely related to Turkeypox virus, Ostrichpox virus and Pigeonpox virus.

Carulei O, Douglass N, Williamson AL.

Relatedness (in the context of phylogenetic trees)

"the more recently species share a common ancestor, the more closely related they are" *

Which of the following statements is correct, given this tree?



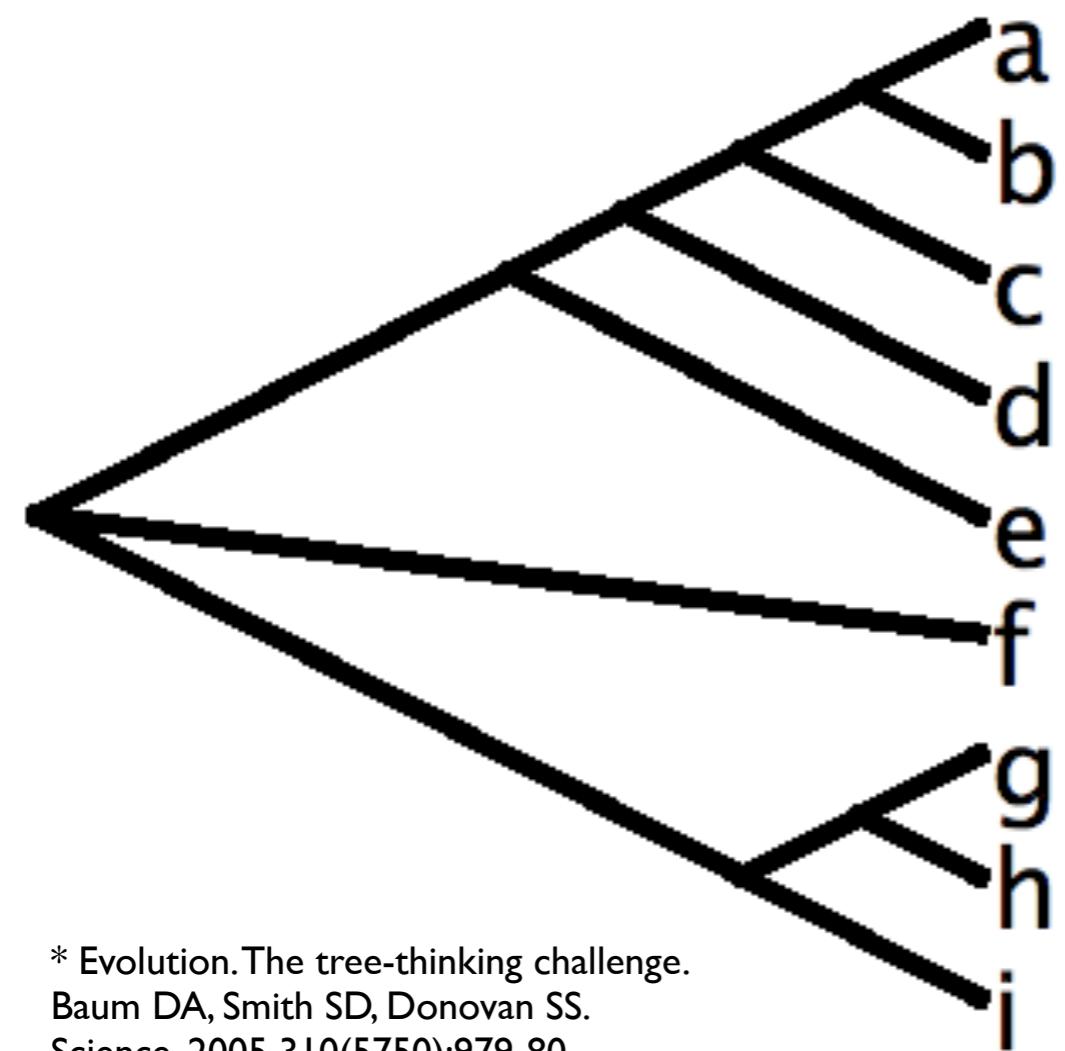
1. **d** is more closely related to than to **f** or **i** g
2. **d** is more closely related to than to **g** or **i** f
3. **d** is more closely related to than to **g** or **f** i

* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

Relatedness (in the context of phylogenetic trees)

"the more recently species share a common ancestor, the more closely related they are" *

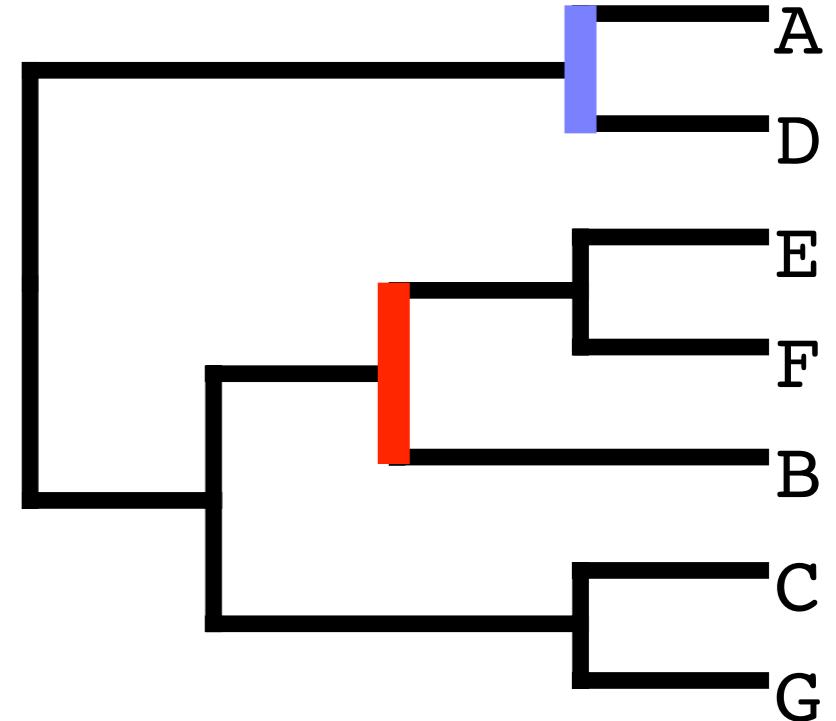
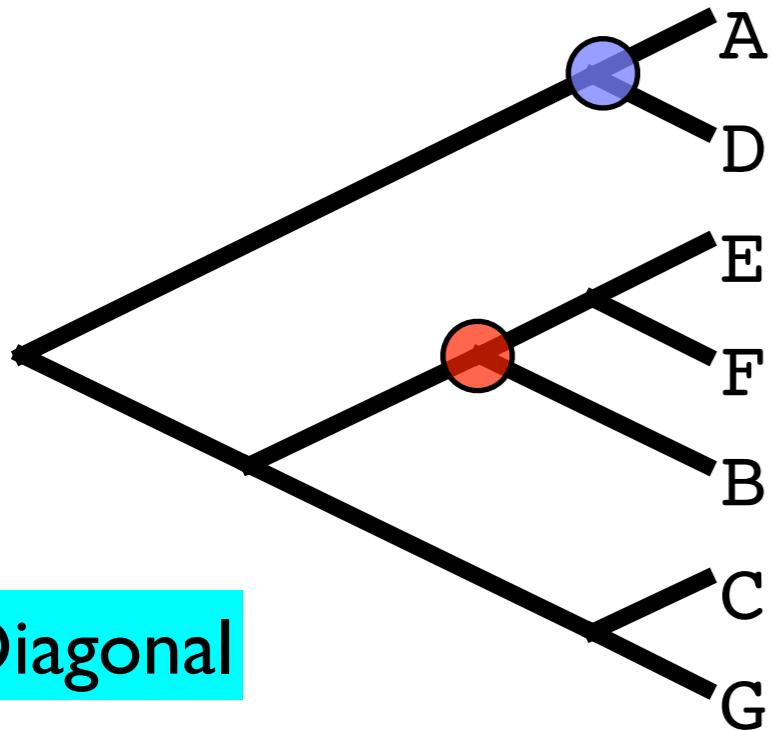
Why spend so much time discussing "relatedness" with you?



Many analyses aim to test whether particular "relatedness" statements are supported by the data - thus crucial that the statements are understood correctly, which is not always easy

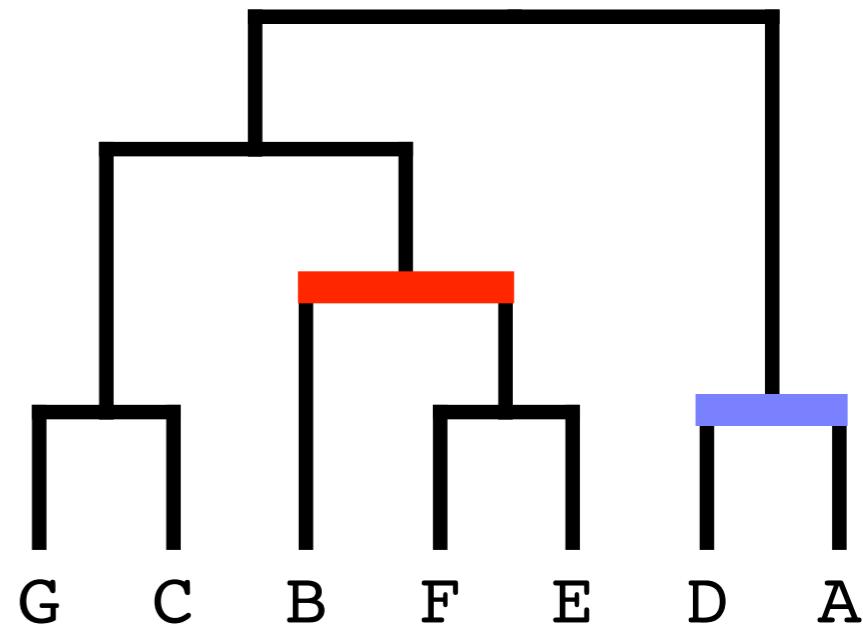
* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

Tree Representations



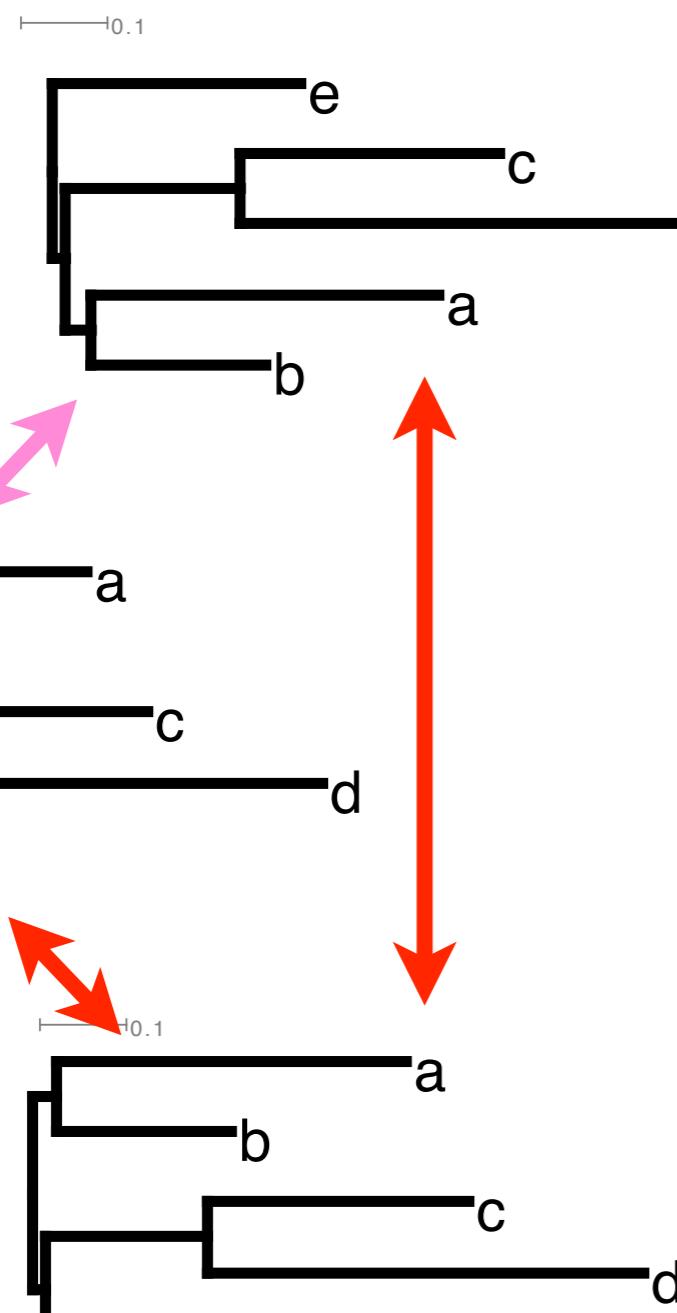
Most rooted tree figures use a “rectangular” rather than a “diagonal” representation

Rectangular trees represent internal nodes with lines perpendicular to lines representing the branches



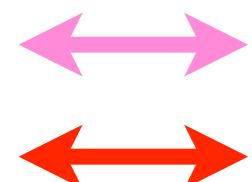
Rectangular

Tree Topology



Trees with **identical topologies**...
... describe the same set of "relatedness statements" between taxa
i.e. any (true!) statement such as
"c is more closely related to a than c is to e"
is true for all trees with identical topologies

Trees with **different topologies**...
... describe different sets of "relatedness statements" between taxa

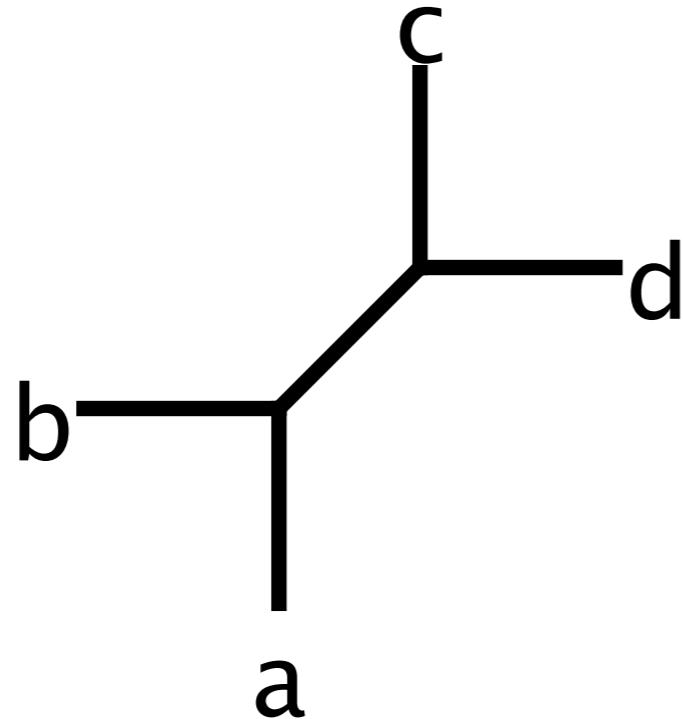


Unrooted Phylogenies

Unrooted Trees

There's no root on the tree...

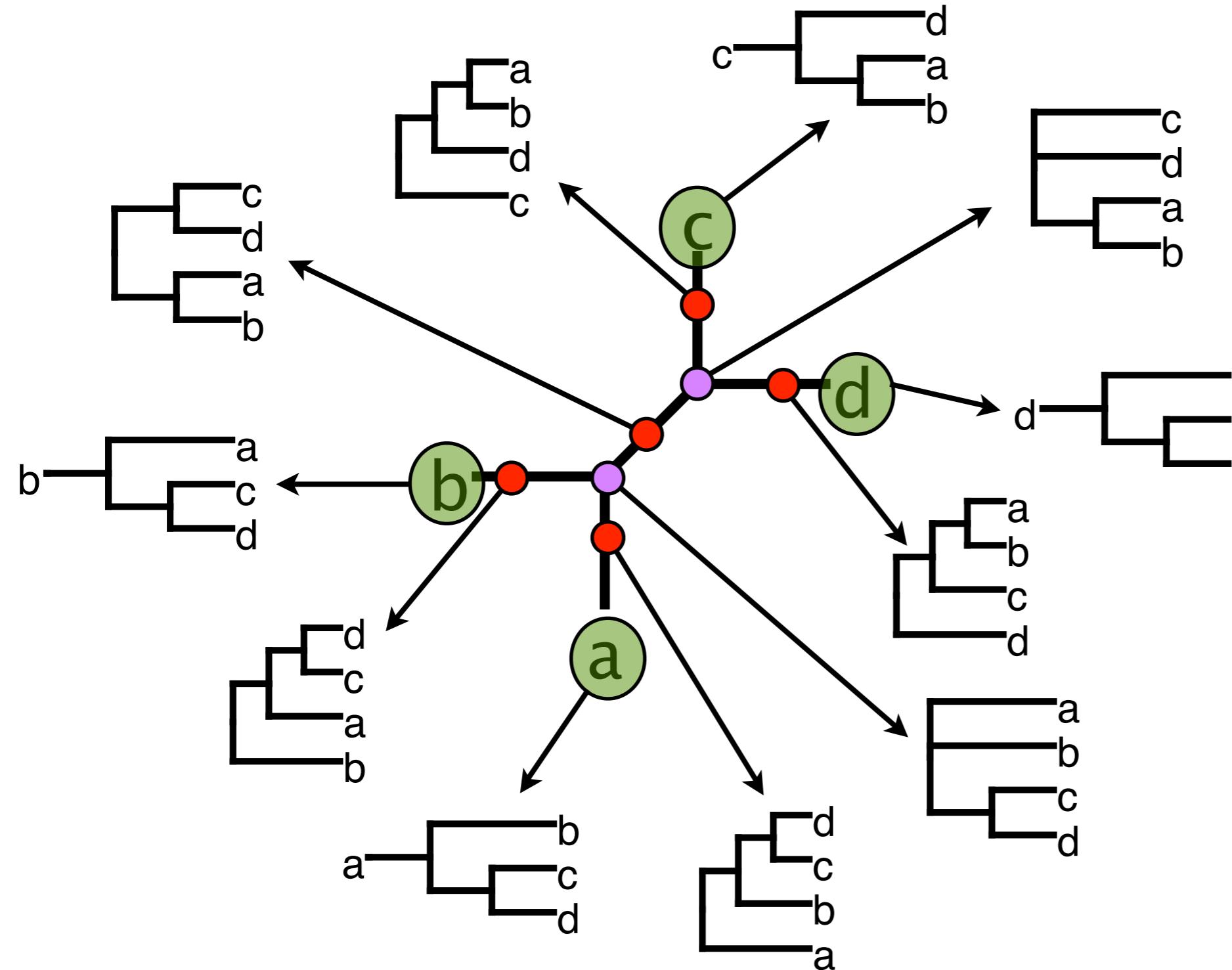
...which is usually interpreted as meaning that these taxa are related by a rooted tree but we don't know where the root is



Many applications of phylogenies require a rooted tree

But many tree estimation tools yield only unrooted trees!

Unrooted → Rooted



There are multiple **rooted tree topologies** for a given unrooted tree topology

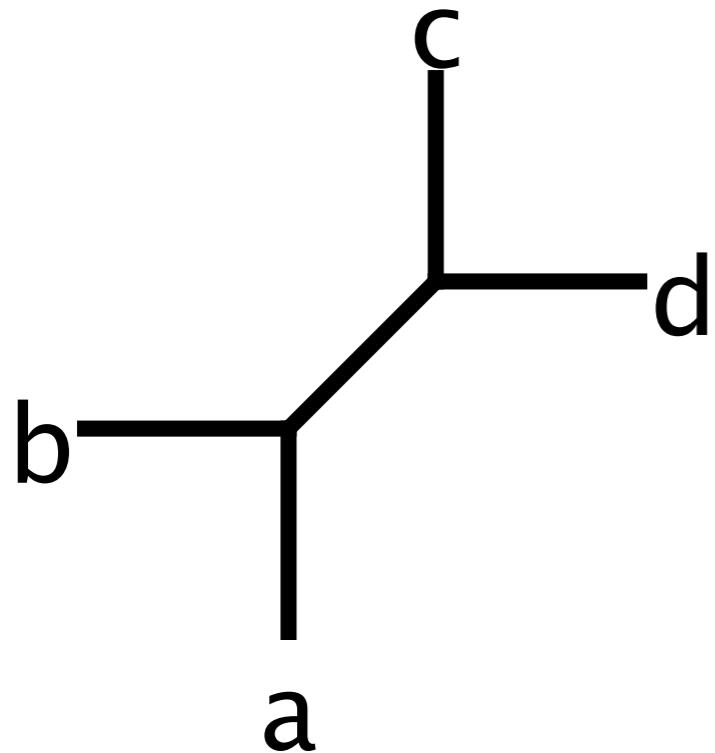
Unrooted trees can be rooted on their:

- **branches**
- **interior nodes**
- **terminal nodes**

Quiz

Assume we estimate an unrooted tree and have no additional data to infer the location of the root

In this case, which (if any) of the following statements would we be justified in making about the pattern of relatedness of the taxa shown in the tree?

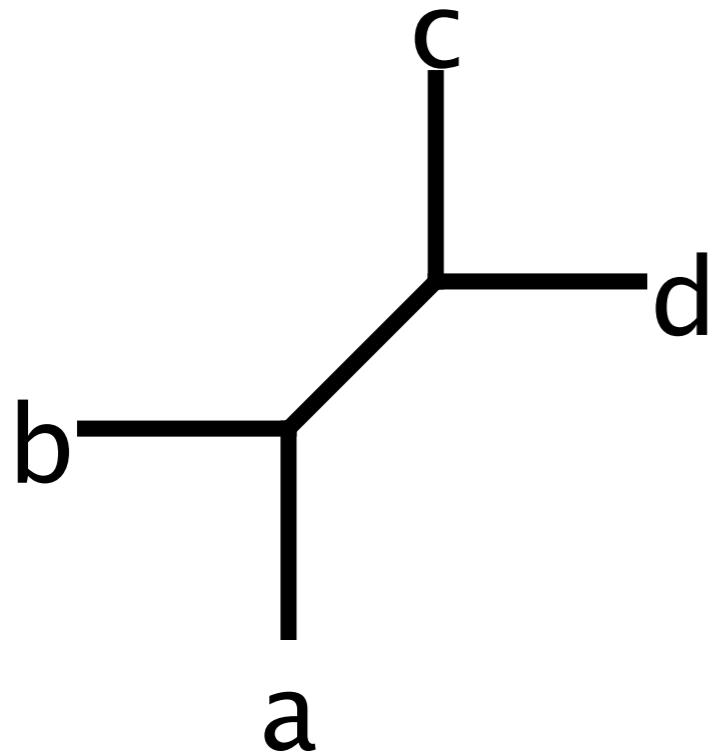


- d more closely related to:**
1. **a** than it is to **b** or **c**
 2. **b** than it is to **a** or **c**
 3. **c** than it is to **a** or **b**

Quiz

We aren't justified in making any of these statements, as the tree is unrooted, and none of them is true under all possible footings of the tree

Indeed, no rooted topology contains the relationships described in 1. and 2.



And, while 3. is true for some of the rooted trues, in others it is not

Draw the set of rooted tree topologies in which statement 3. is:

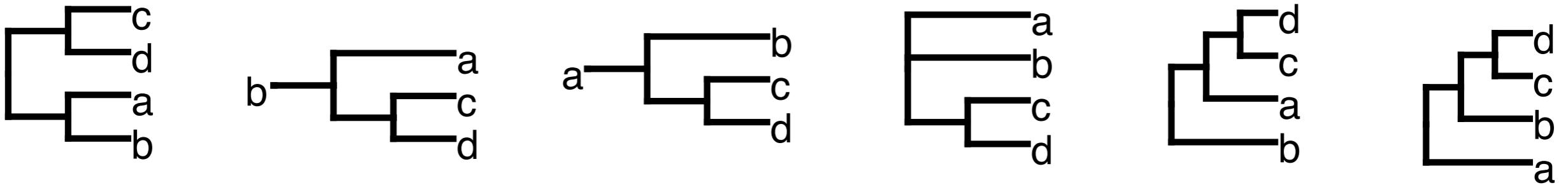
- true
- false

d more closely related to:

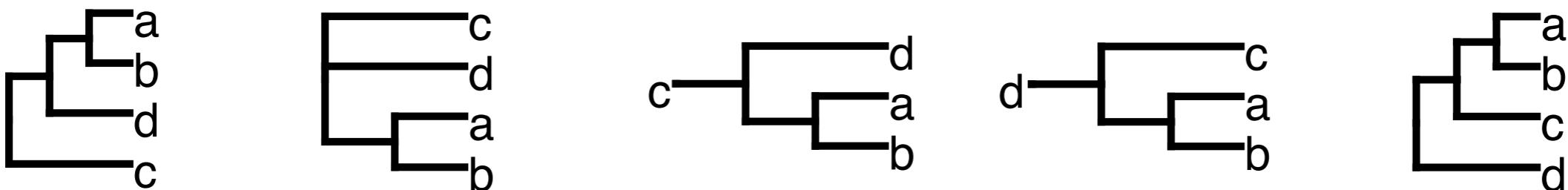
1. **a** than it is to **b** or **c**
2. **b** than it is to **a** or **c**
3. **c** than it is to **a** or **b**

Quiz

d more closely related to c than it is to a or b



d **not** more closely related to c than it is to a or b



Mathematical Models of Sequence Evolution

Kevin Gori

Example Phylogeny Estimation Workflow

Phylogenetic Workflows

Every analysis is different - no “one size fits all” approach!

But there are concepts/features/tasks/tools common to many analyses

We present **example** analyses to highlight some of these

We'll look at similar workflows/analyses from two different perspectives

- considering a ‘standard’ phylogenetic analyses as a ‘standard’ statistical analyses
- highlighting tasks, tools, and concepts found in many molecular phylogenetic analyses

Example Phylogeny Estimation Workflow

I. phylogenetic analysis as a standard
statistical analysis

Statistical Estimation of Phylogeny: An Outline

Statistical paradigm

pose substantive question

develop stochastic model with parameters that, if known, would answer the question.

collect observations that are informative about model parameters.

find the best estimate of parameters conditioned on the observations at hand using some criterion.

Statistical phylogenetic paradigm

what if the phylogeny of a group of organisms?

develop phylogenetic model with tree (and branch lengths) and a Markov model describing how traits change over tree.

construct a data matrix (e.g., of DNA sequences) sampled from the group of organisms.

find the best estimate of phylogeny using maximum likelihood criterion or Bayesian inference criterion.

Huelsenbook

Brian R. Moore, UC Davis

Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate [this formulation of the problem inspired by Brian R Moore's slides - thanks Brian!]
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
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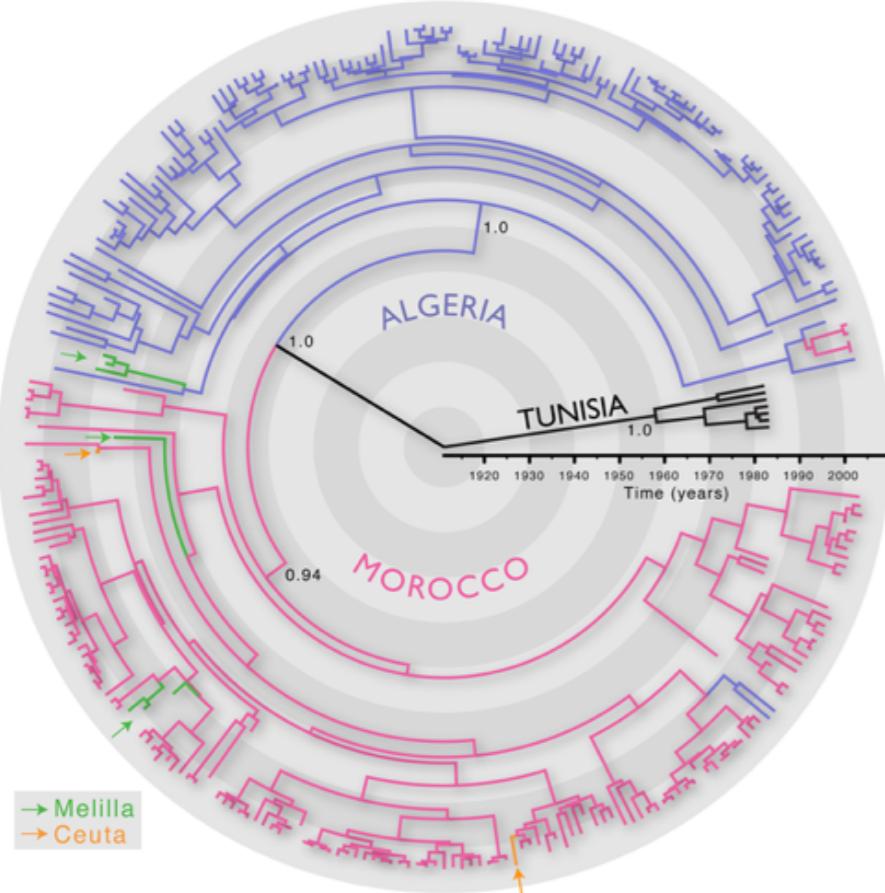
Example Phylogeny Estimation Workflow

I. Pose a substantive question

For example:

Can we identify factors promoting rabies virus transmission that could be addressed via public-health measures?

Crucially: a substantive question such that **knowledge** (or rather estimation) of parameters in a phylogenetic model can inform our answer



We saw earlier how our estimate of the **topology parameter** of this phylogenetic analysis of dog rabies viruses informs our answer to this question

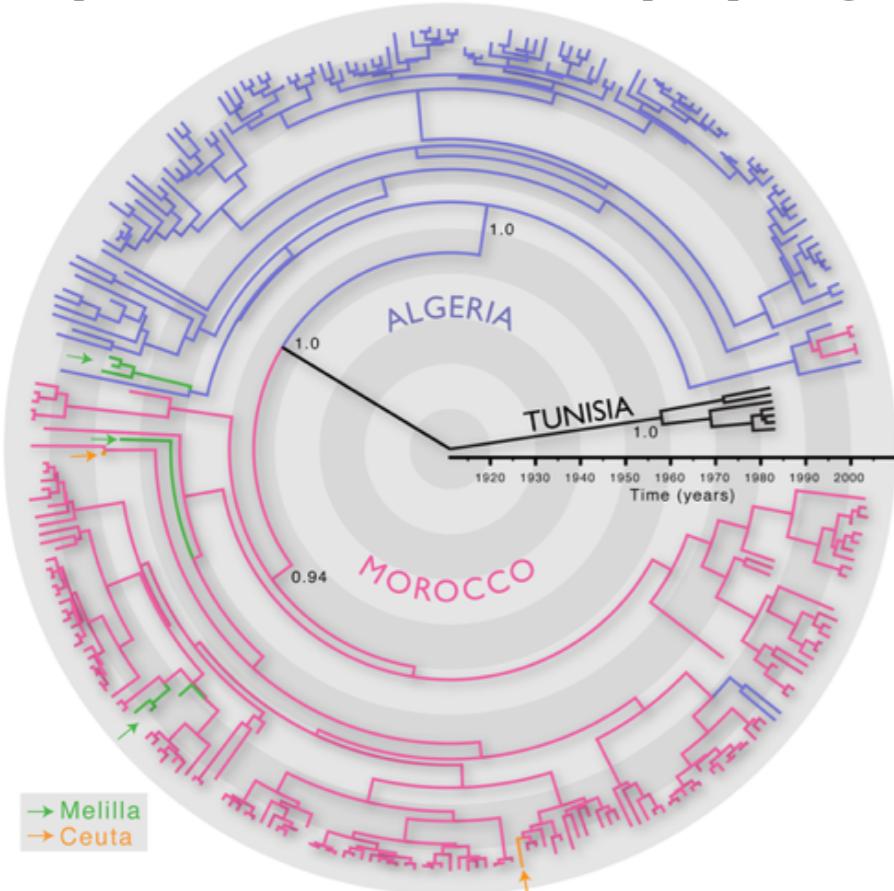
Example Phylogeny Estimation Workflow

I. Pose a substantive question

For example:

Can we identify factors promoting rabies virus transmission that could be addressed via public-health measures?

Crucially: a substantive question such that **knowledge** (or rather estimation) of parameters in a phylogenetic model can inform our answer



Reformulating/recasting the question in terms of such parameters can help guide our analysis (e.g. helping us decide which data to collect)

For example, in this case:

Are virus samples that are closely located, but in different countries, relatively closely or distantly related to each other?

Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

2. Build a model involving parameters that, if known, could answer the question

Olivier Gascuel – Phylogenetic models – ISCB-ASBCB Casablanca 2013



The full probabilistic model

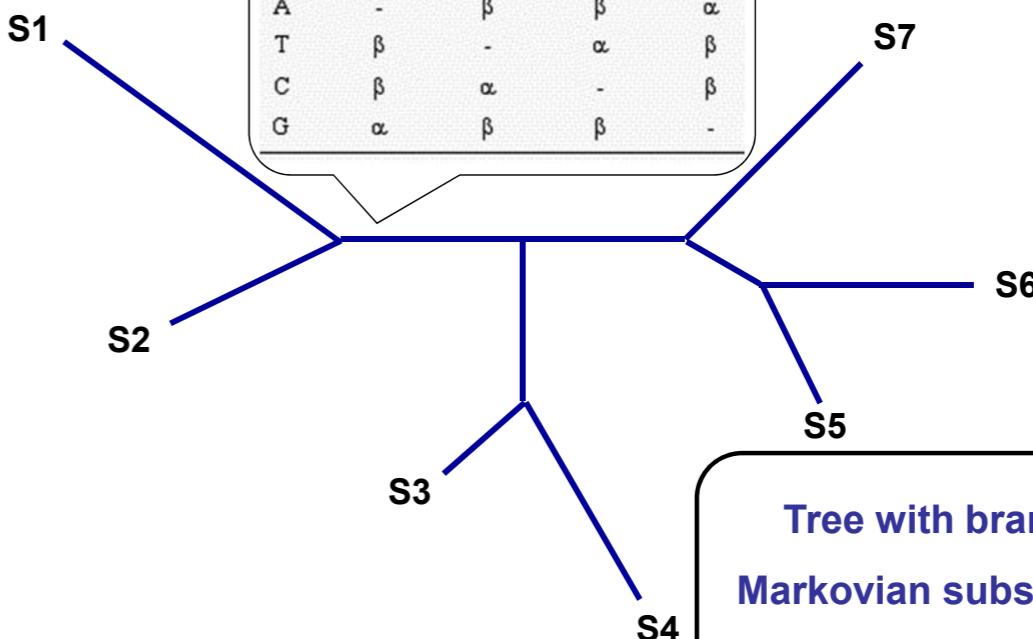
- A tree topology (to be estimated, n^n)
- Branch lengths (to be estimated, $2n-3$)
- A substitution model (to be (partly) estimated, 1, 3, 4, ...208 ...)
- A distribution of site rates (to be estimated, 1, 2, ...)

Olivier Gascuel – Phylogenetic models – ISCB-ASBCB Casablanca 2013



The full probabilistic model

	A	T	C	G
A	-	β	β	α
T	β	-	α	β
C	β	α	-	β
G	α	β	β	-



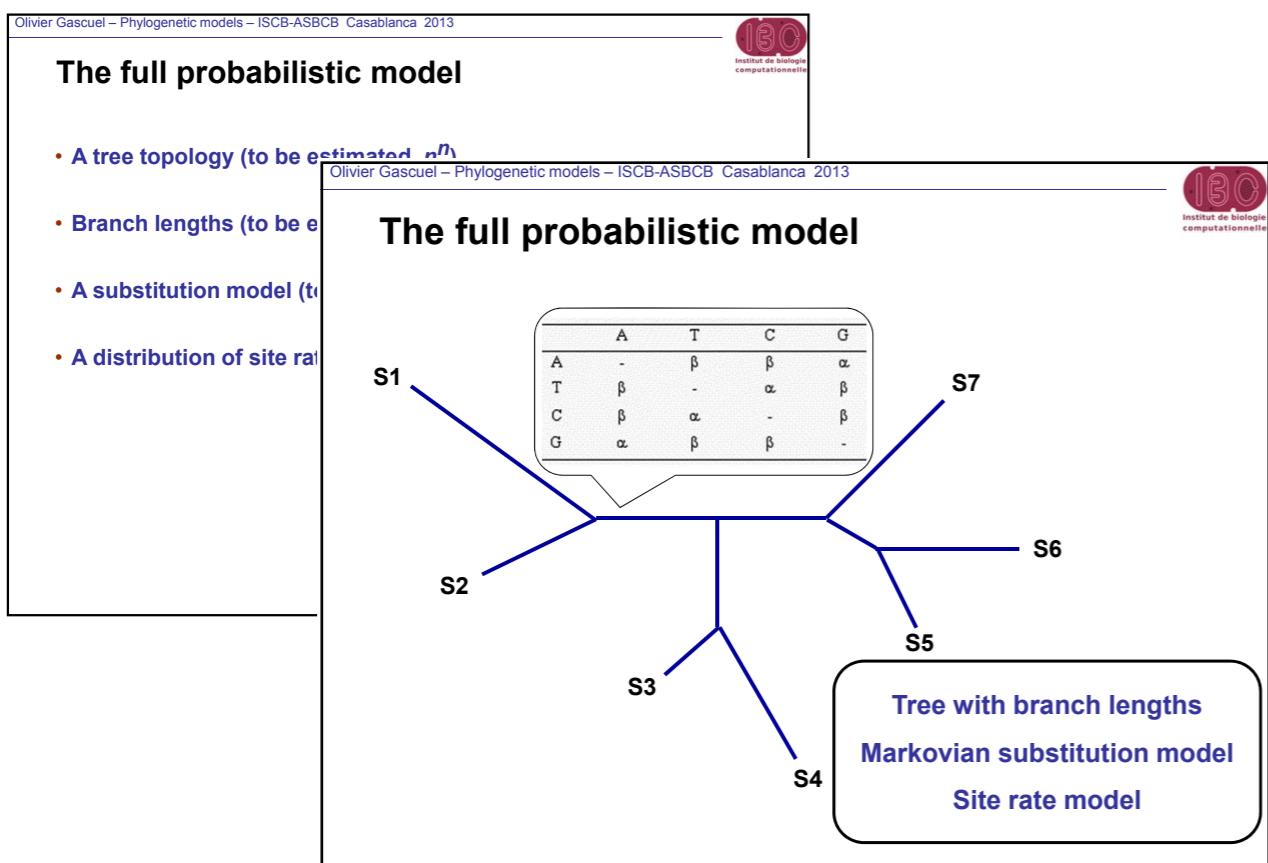
Tree with branch lengths
Markovian substitution model
Site rate model

Thanks to Olivier Gascuel for these slides

Aidan Budd, EMBL Heidelberg

Example Phylogeny Estimation Workflow

2. Build a model involving parameters that, if known, could answer the question



For north African rabies analysis, a parameter of interest was the rooted tree topology

Sometimes, however, it's not the topology, but some other parameters e.g. identifying positive selection (omega parameter in certain codon-based substitution models above a particular value) that are of most interest

Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. **Collect observations informative about the model parameter(s)**
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

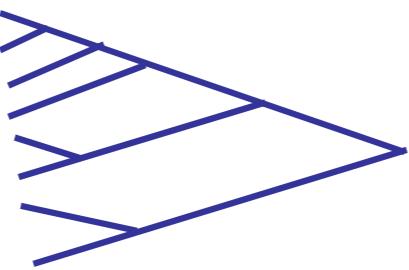
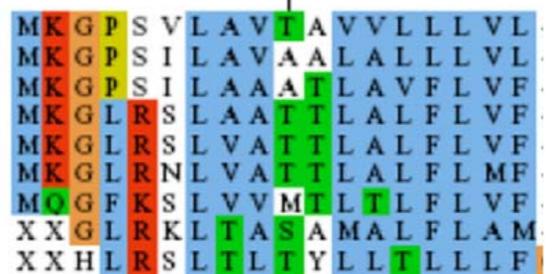
3. Collect observations informative about the model parameter(s)

Olivier Gascuel – Phylogenetic models – ISCB-ASBCB Casablanca 2013



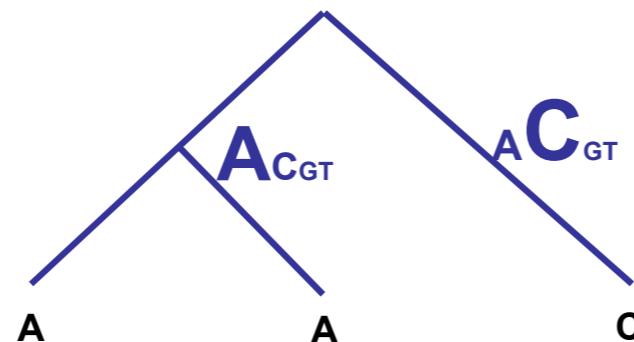
Modeling sequence evolution: standard assumptions

MOUSE
RAT
RABBIT
HUMAN
DOG
ELEPHANT
COW
CHICKEN
FUGU



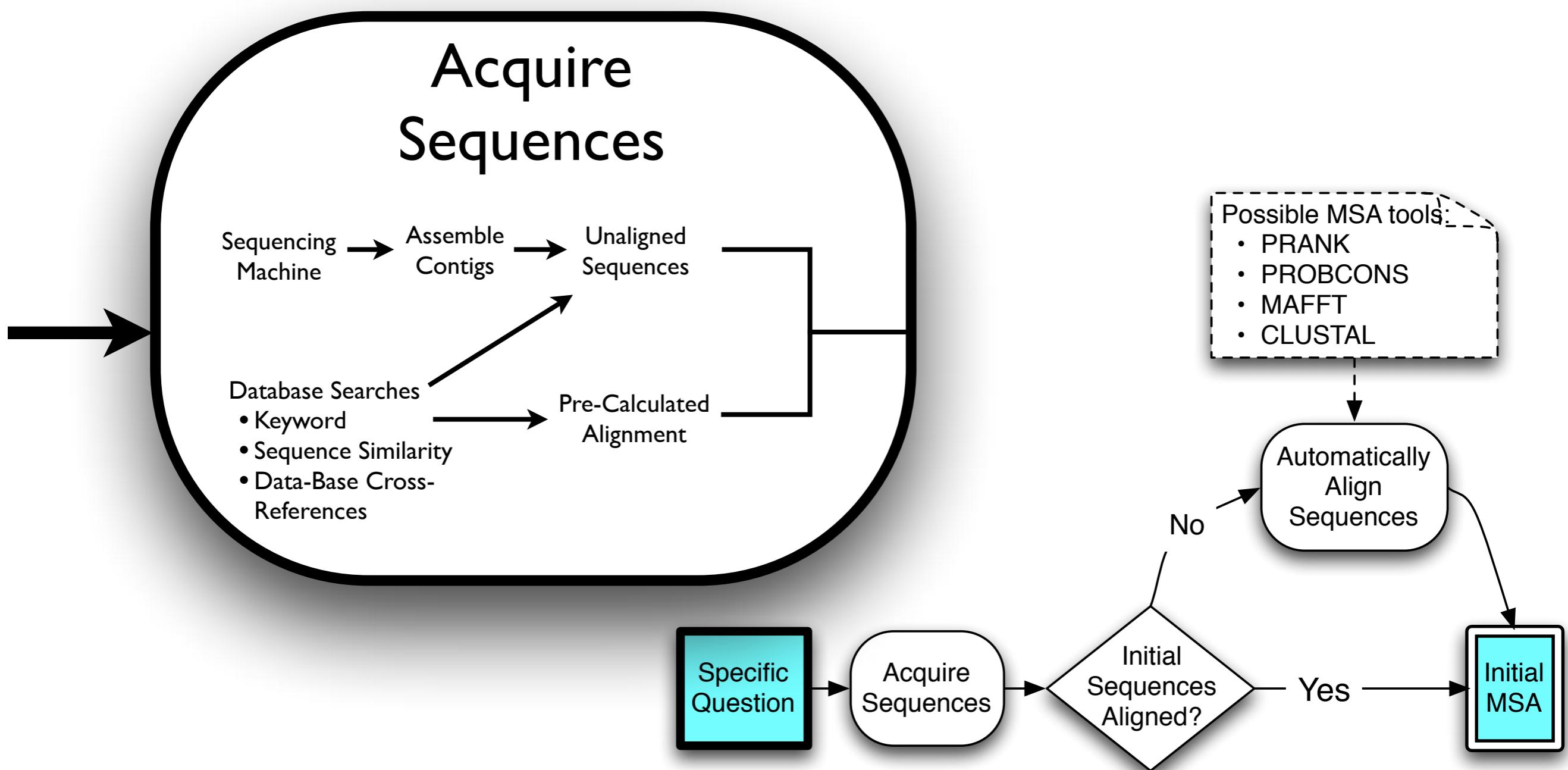
e.g. build a multiple sequences alignment of north African dog rabies sequences

We aim at explaining the data (alignment) using a probabilistic scenario of the evolution of each of the sites along a phylogeny



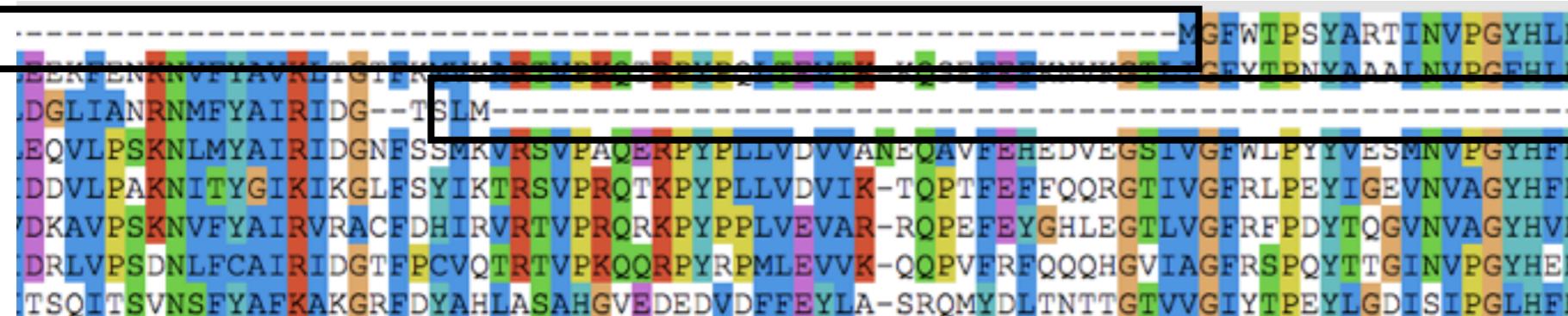
Example Phylogeny Estimation Workflow

3. Collect observations informative about the model parameter(s)

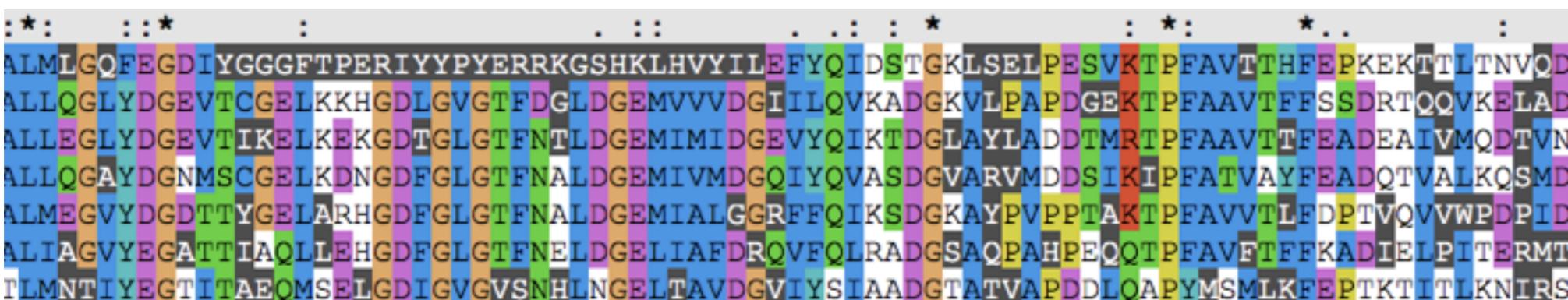


Example Phylogeny Estimation Workflow

Unusual Sequences



Short/fragmented sequences



With CLUSTALX “”Quality”->”Show Low-Scoring Segments” switched on

Unusual pattern of "conservation"

Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate

In upcoming demonstration:

- Choose a substitution model
- Estimate a phylogeny using this model, with aLRT

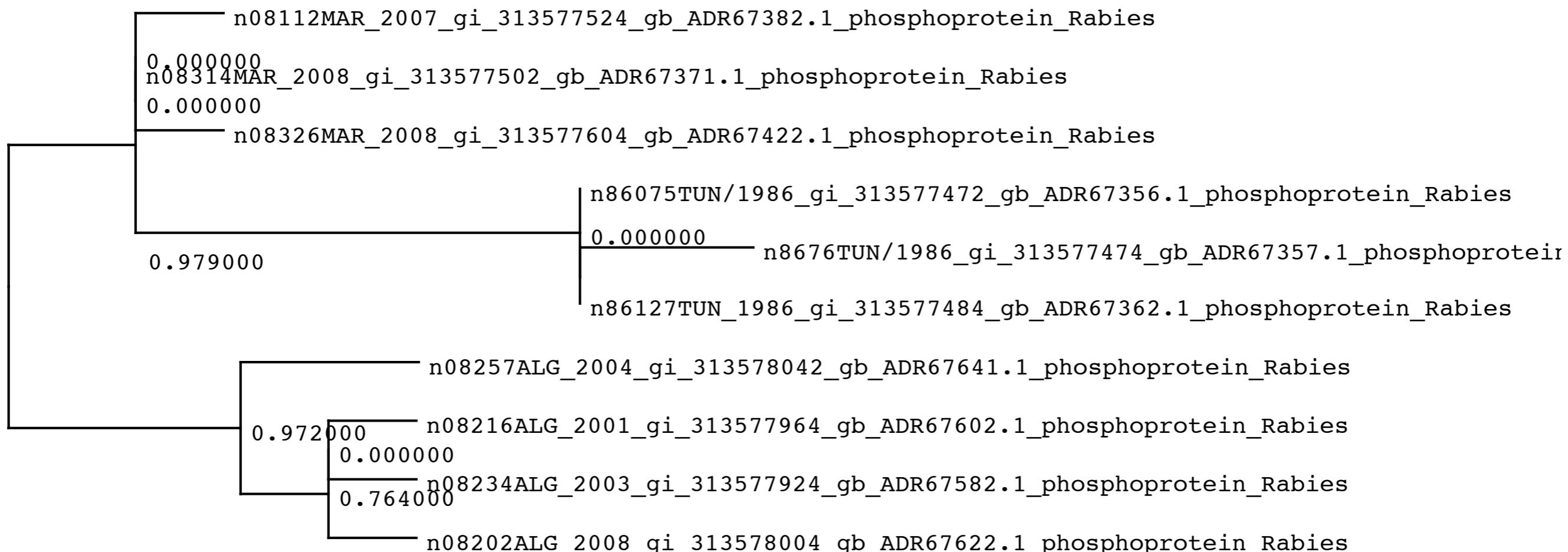
Example Phylogeny Estimation Workflow

1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated with parameter estimate
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

6. Answer your question using these parameter estimates

H₀.0010



Example Phylogeny Estimation Workflow

Demo and Exercises

We'll follow a demonstration, and you'll have a chance to try this kind of phylogenetic workflow yourself, using the section:

“phylogenetic analysis as a standard statistical analysis”

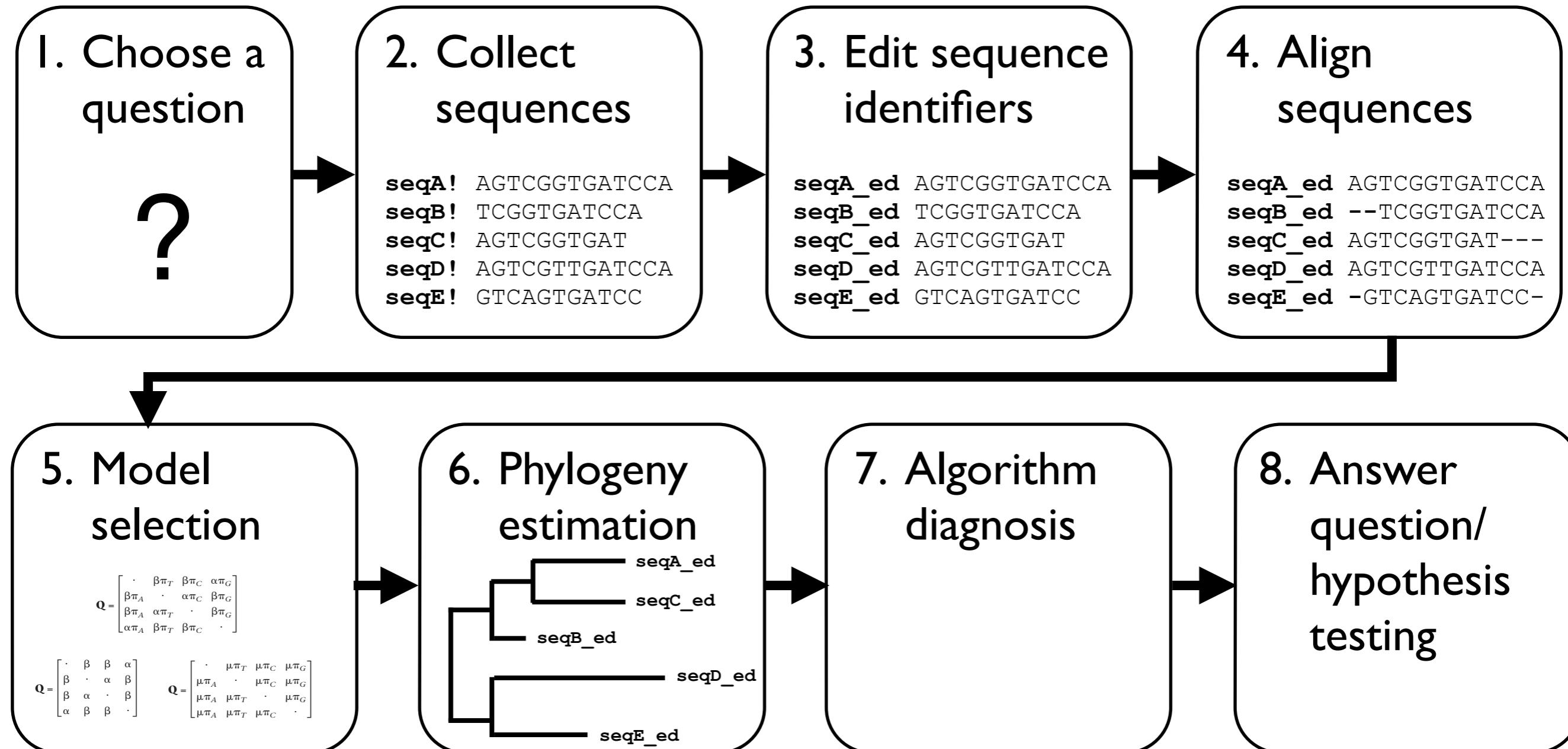
- **demonstration** with North African dog rabies viruses
- **exercise** with Louisiana gastroenterologist example

described in this HTML document [interpretingPhylogenies.html](#)

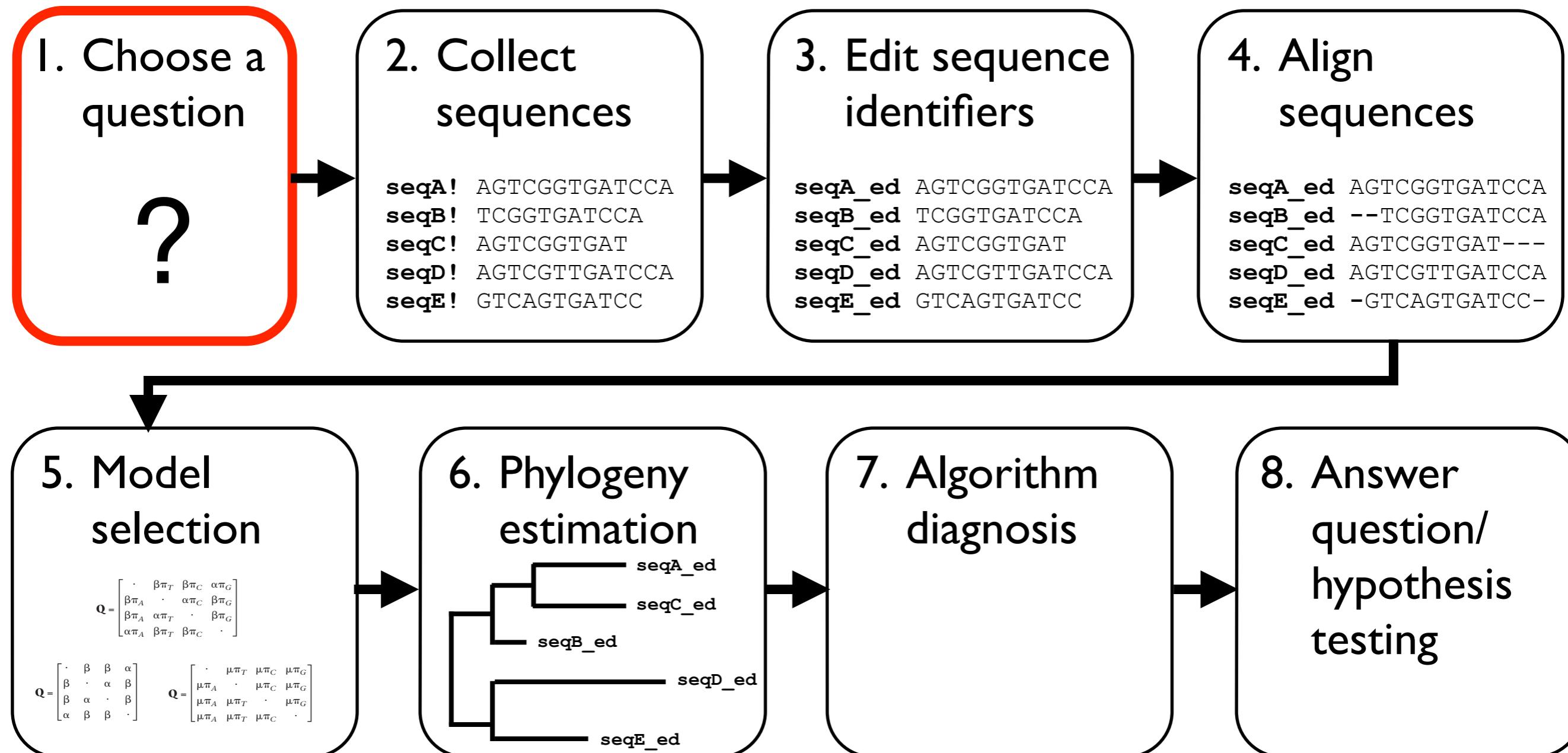
Example Phylogeny Estimation Workflow

2. Common tasks and concepts found in
many phylogenetic analyses

Example Phylogeny Estimation Workflow

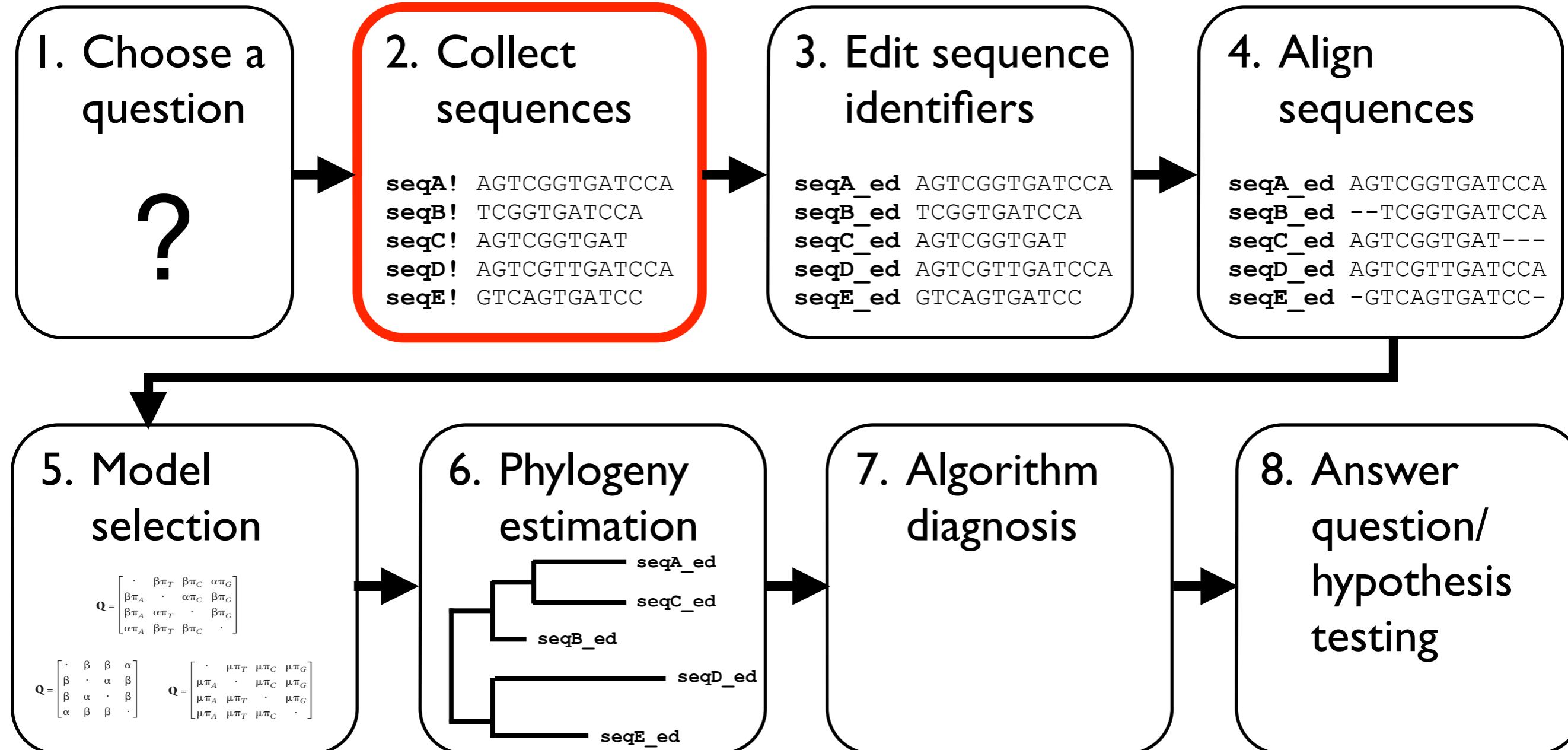


Example Phylogeny Estimation Workflow



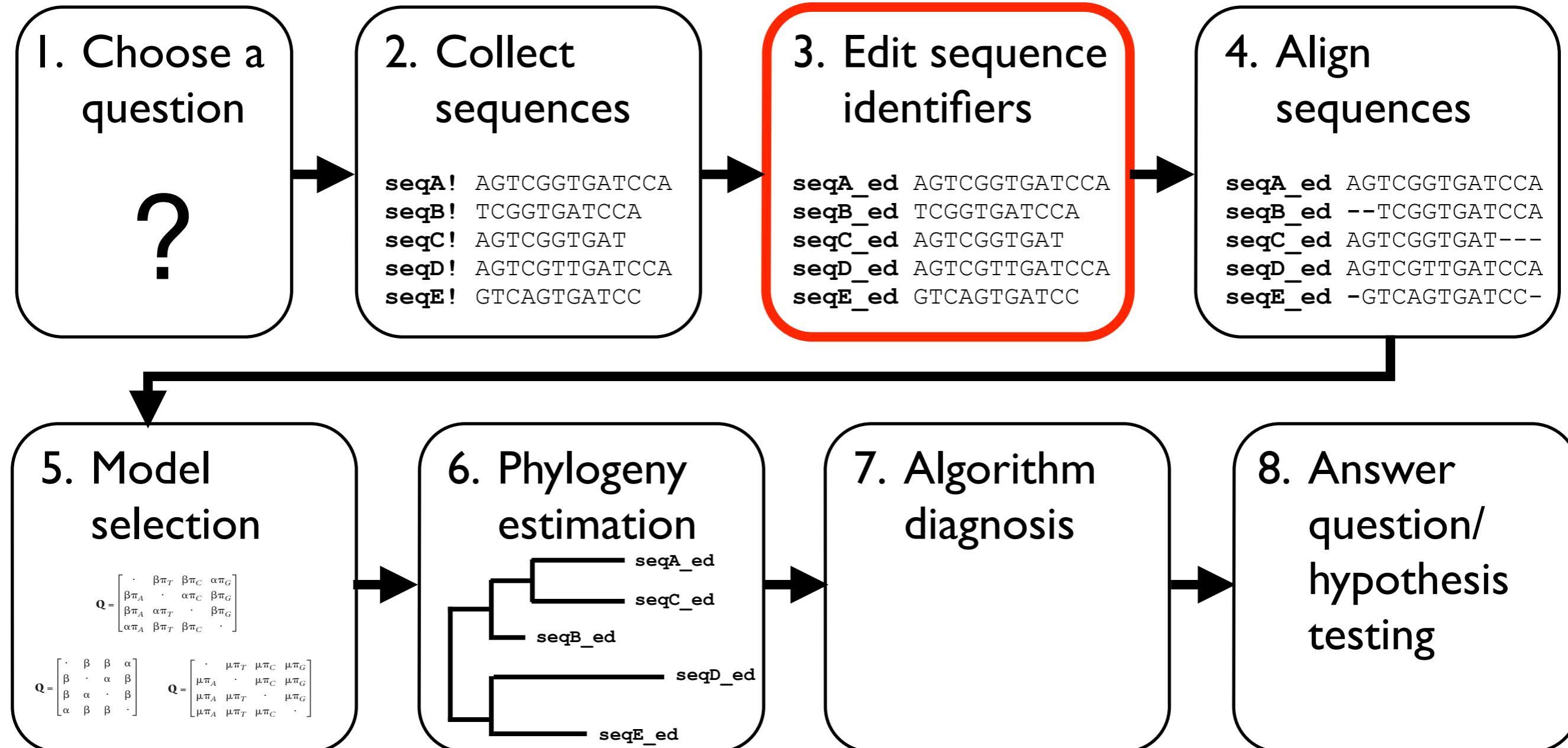
I. Choose a question: me, today, using your brain

Example Phylogeny Estimation Workflow



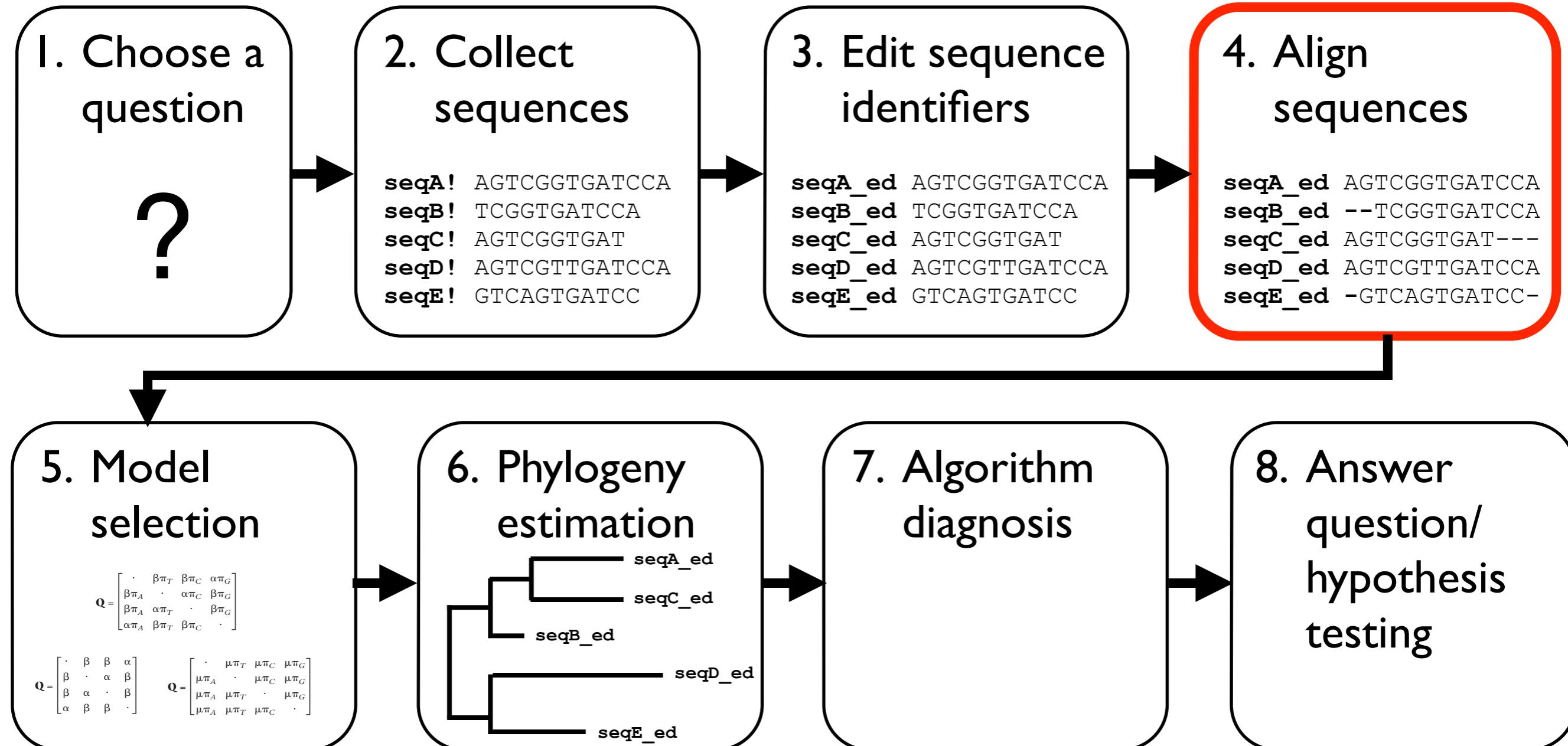
2. Collect sequences: Stephen, BLAST, MCL, etc.

Example Phylogeny Estimation Workflow



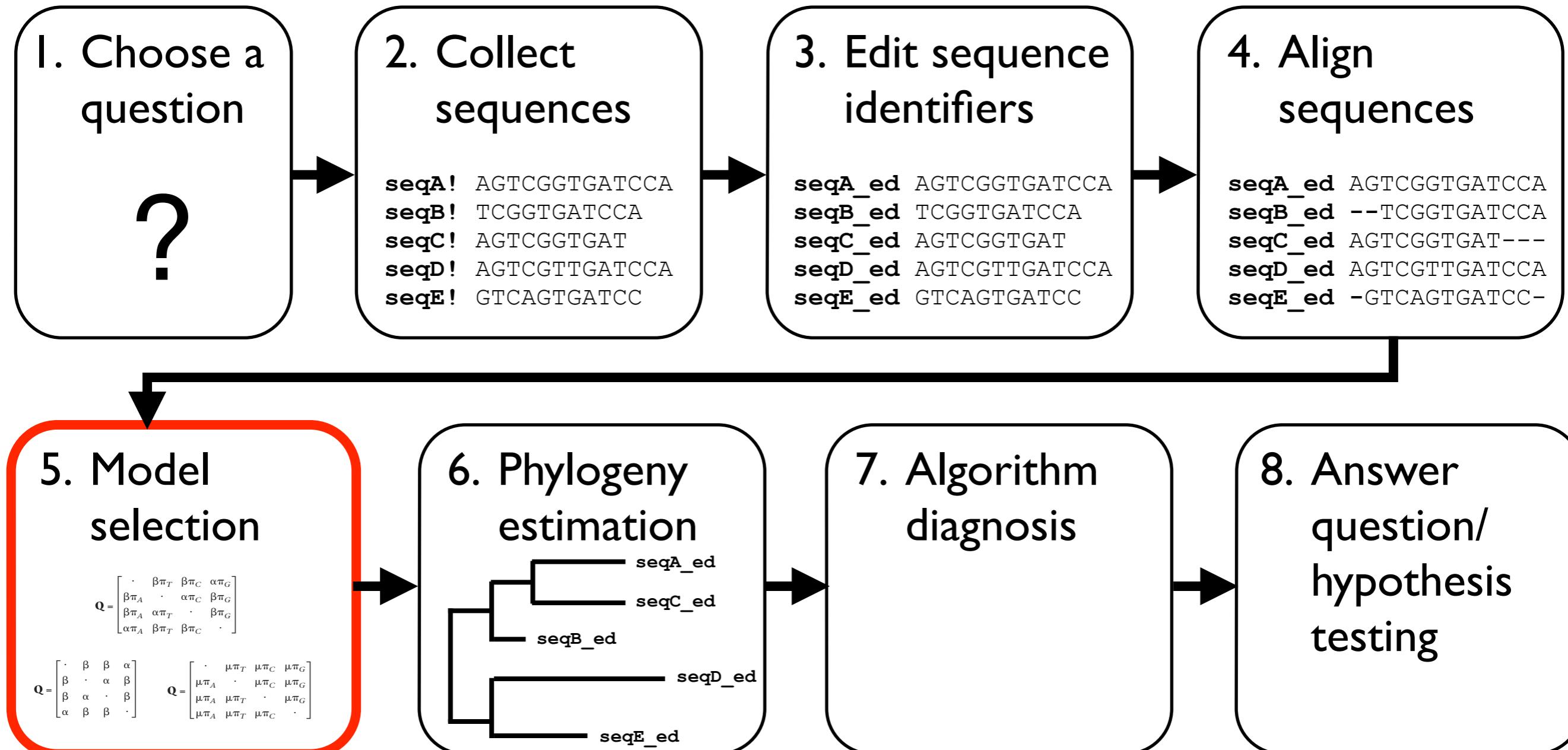
3. Edit sequence identifiers: me, today, text editors or scripting tools

Example Phylogeny Estimation Workflow



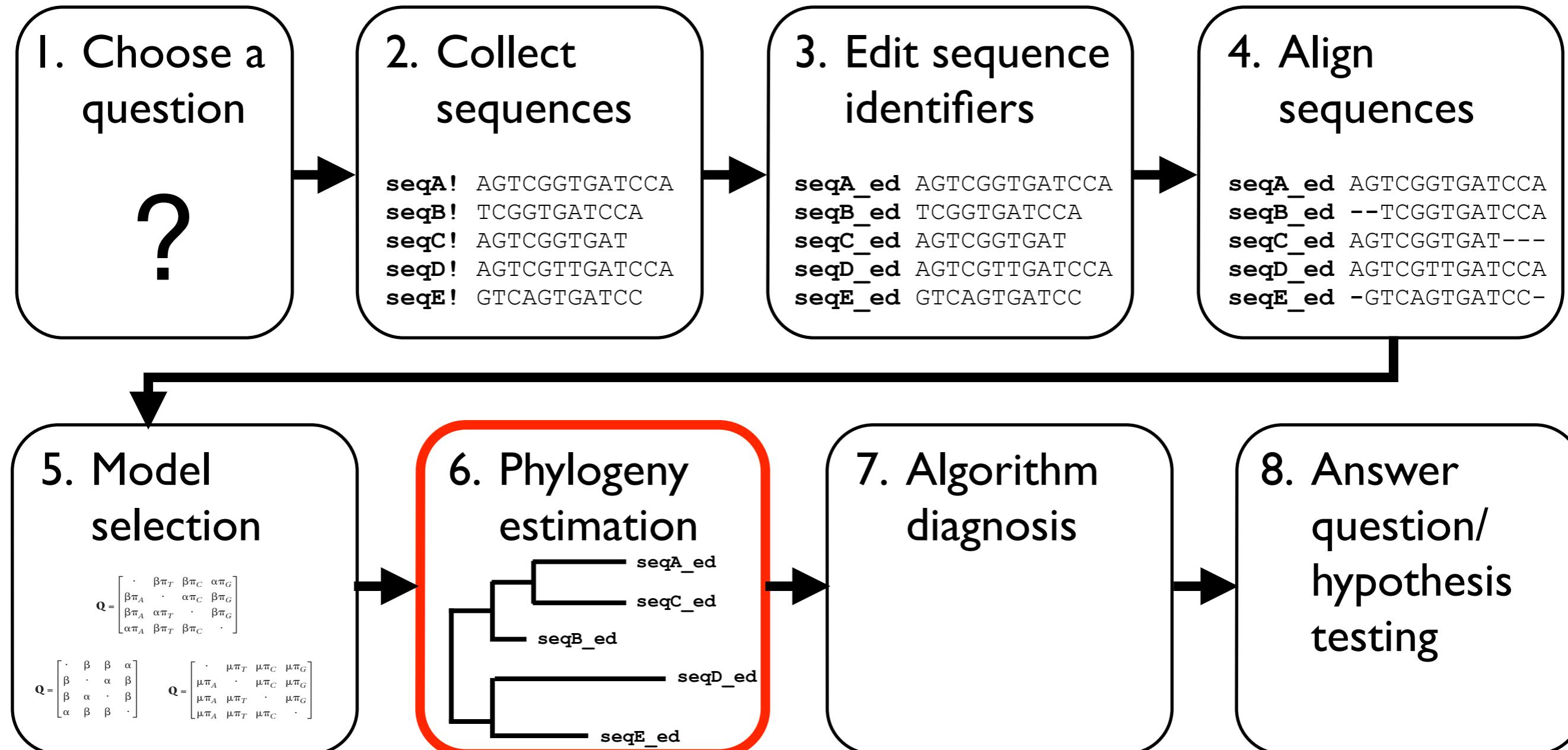
4. Align sequences: Ben, BaliPhy Prank, muscle, Probcons, mafft etc.

Example Phylogeny Estimation Workflow



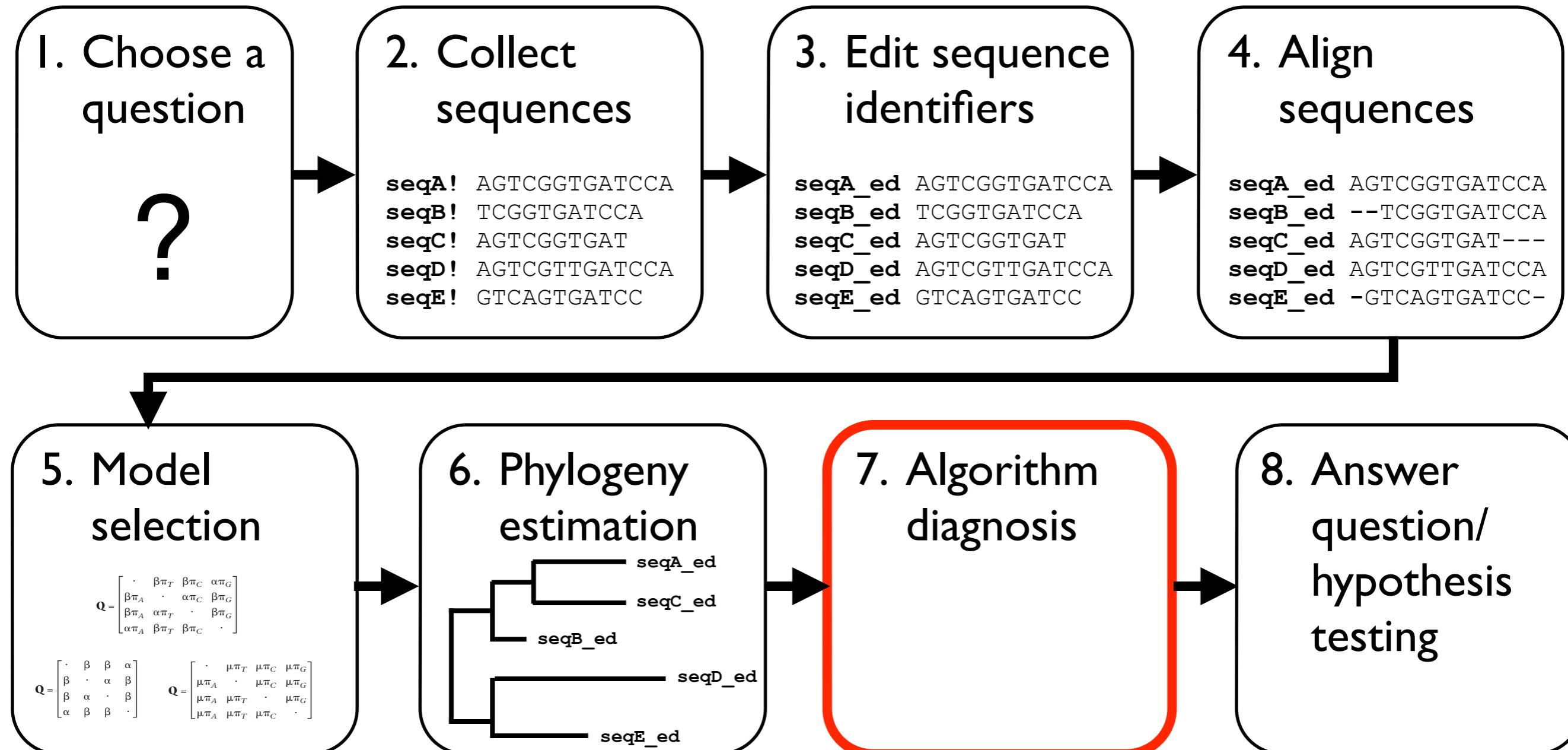
5. Model selection: Maria and Asif, PAML, jModelTest

Example Phylogeny Estimation Workflow



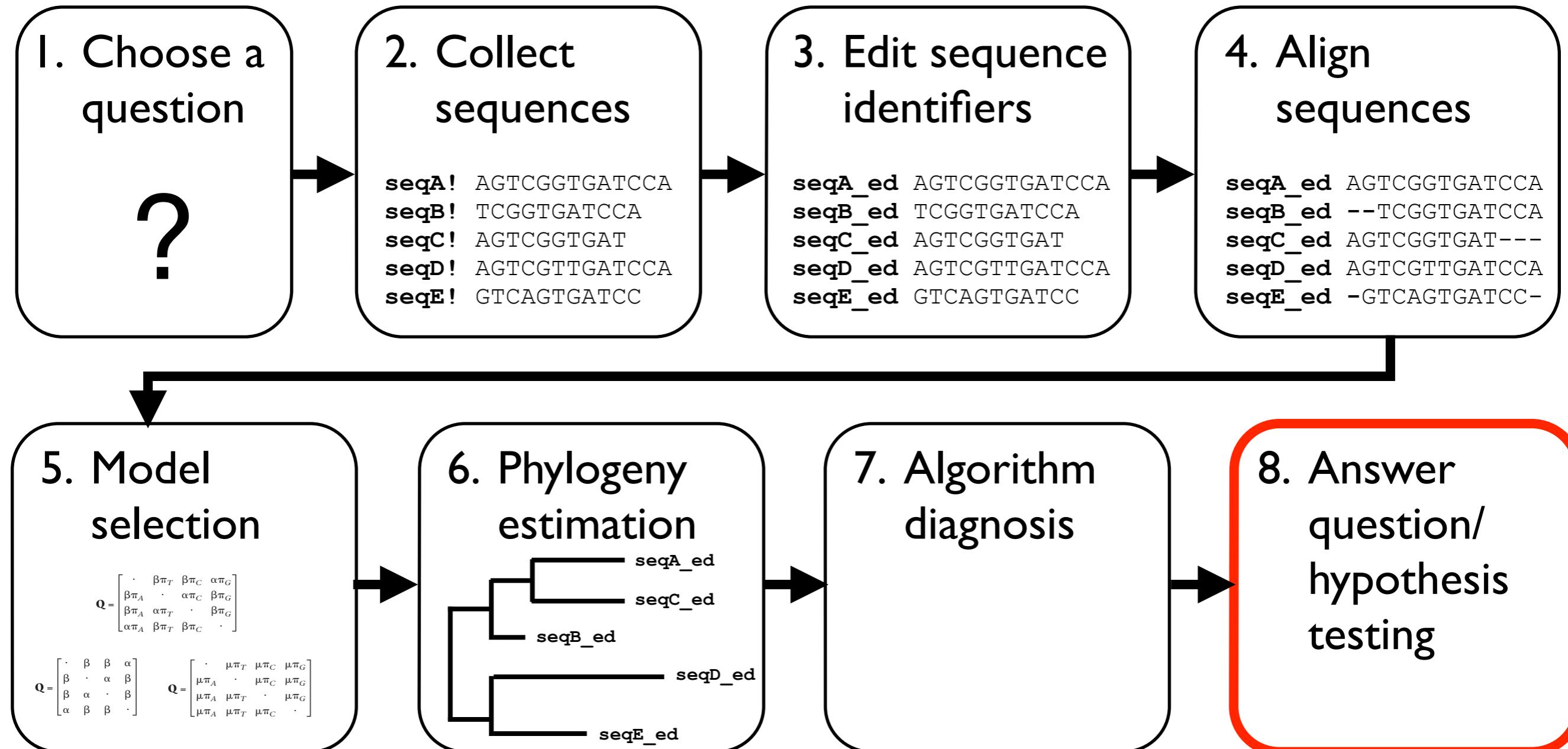
6. Phylogeny estimation: **Maria**, **Brian**, **Olivier**, **RAXML**, **PhyML**, etc.

Example Phylogeny Estimation Workflow



7. Algorithm diagnosis: Maria, Brian, Olivier, Tracer, AWTY, etc.

Example Phylogeny Estimation Workflow



8. Answer question/hypothesis testing: Asif, Nick, Tracy, Jeff, Maria, Brian, Olivier, aLRTs, AIC, Bayes factors

Example Phylogeny Estimation Workflow

Demo and Exercises

We'll follow a demonstration, and you'll have a chance to try this kind of phylogenetic workflow yourself, using the

- "Pragmatic" **demonstration** with North African dog rabies viruses
- "Pragmatic" **exercise** with Louisiana gastroenterologist example

described in this HTML document **interpretingPhylogenies.html**

Brief overview of the course:

Introduction to viewing, interpreting, estimating trees
yesterday and today; Adrian, Kevin, me, Laura

Constructing a dataset for further phylogenetic analysis (collecting sequences, clustering, aligning them)
today and tomorrow; Stephen and Ben

Estimating phylogenies

Thursday - Sunday; Brian, Olivier, Maria, Alexis

Hypothesis testing

Monday - Tuesday; Maria and Asif

Estimating divergence times

Wednesday; Jeff and Tracy (also, earlier, Tanja's presentation)

Using statistical phylogenetic approaches to make inferences from population data

Wednesday - Thursday; Ziheng, Adam, Bruce

Brief overview of the course:

Also:

Using modern sequencing methods for phylogenetics (Adam, Alexis)

Keynote presentations (Martin, John, Tanja)

Using parallel computing for phylogenetics (Alexis, Paschalia)