Introductions

Monday 5th May 2014

EMBO Practical Course on Computational Molecular Evolution

Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Helenic Center for Marine Research) HCMR

Heraklion, Greece

Aidan Budd, EMBL Heidelberg

What You Get From a Course

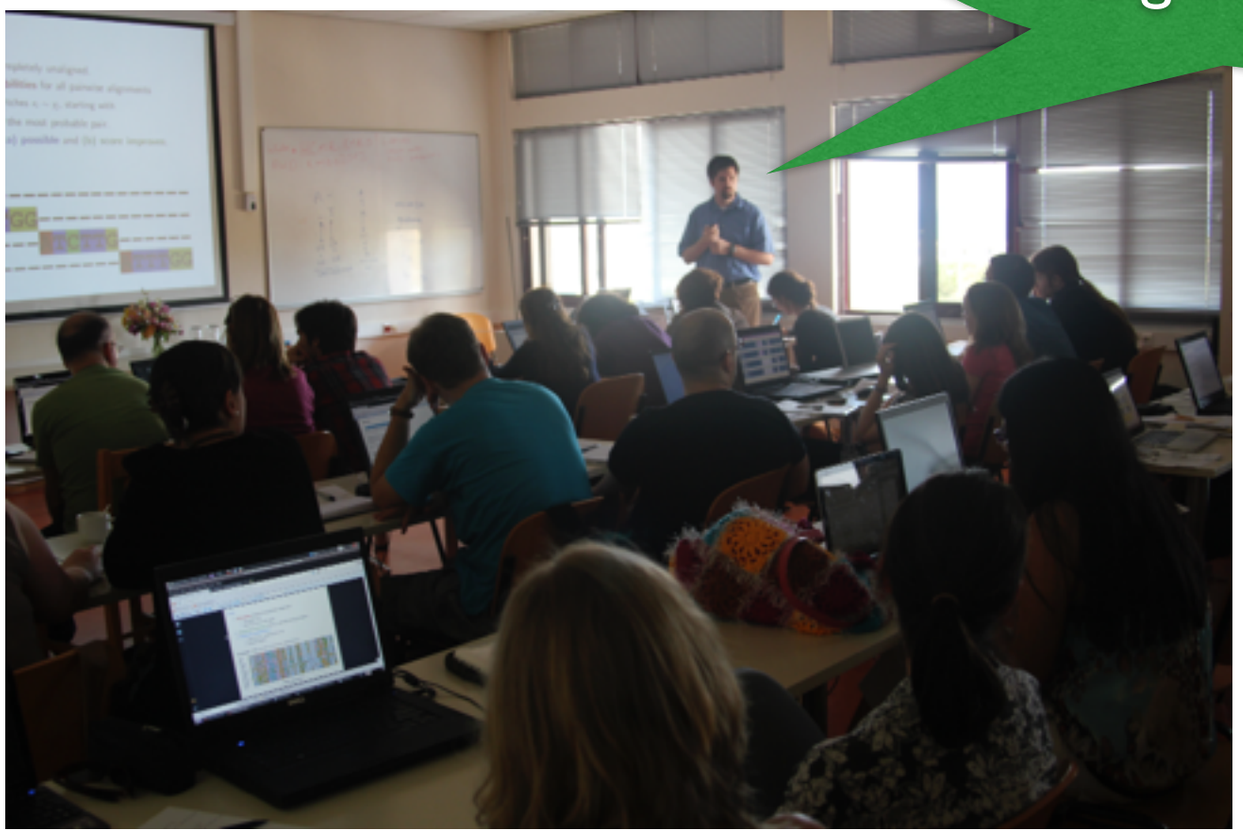
Aidan Budd, EMBL Heidelberg

why participate in a course?

1. to learn

Learning

Presentations and demos



insightful comments about alignments and trees…

Practical exercises and discussions

2. to build useful professional relationships

a. with other trainees

b. with trainers

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both (I estimate, on average) **equally** valuable

so we start with an activity...

to help us getting to know each other…

speed dating

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Speed Dating: Aims

- make it easier to start chatting later in the course
- find people you have things in common with/get on with

Speed Dating: Format

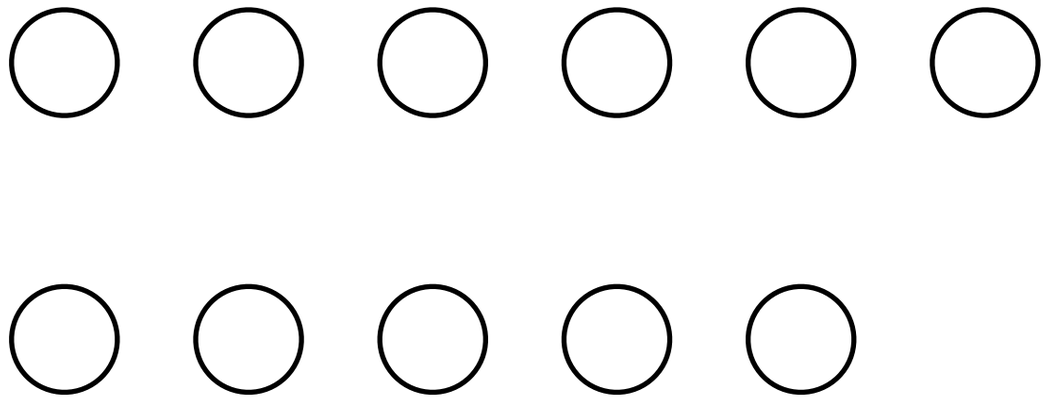
- meet other participants in many 1:1 chats
- tell each other
  - names
  - where you work
  - research topics
  - something people are often surprised to learn about you (e.g. I have three nationalities… am vain enough to choose clothing to fit my eye colour… etc.)
  - try and find someone you know or somewhere you've been that you have in common

Speed Dating: Format

Stand, awkwardly, in two rows

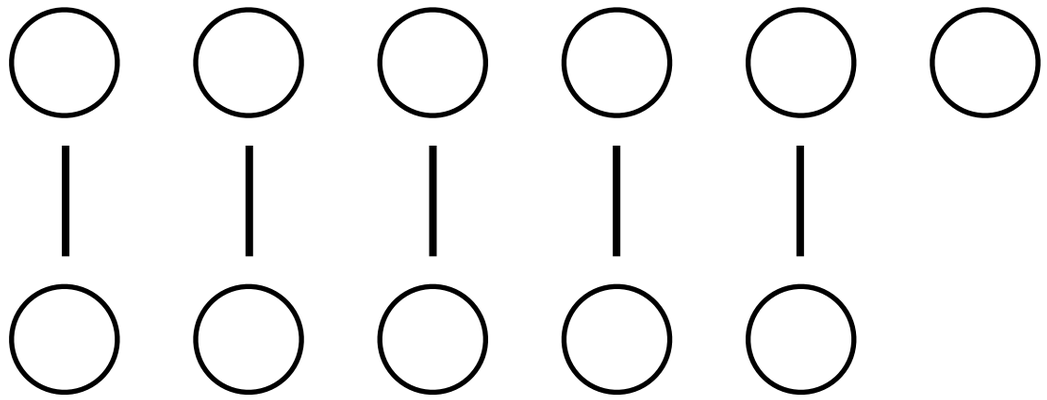
Face one person in the other row

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

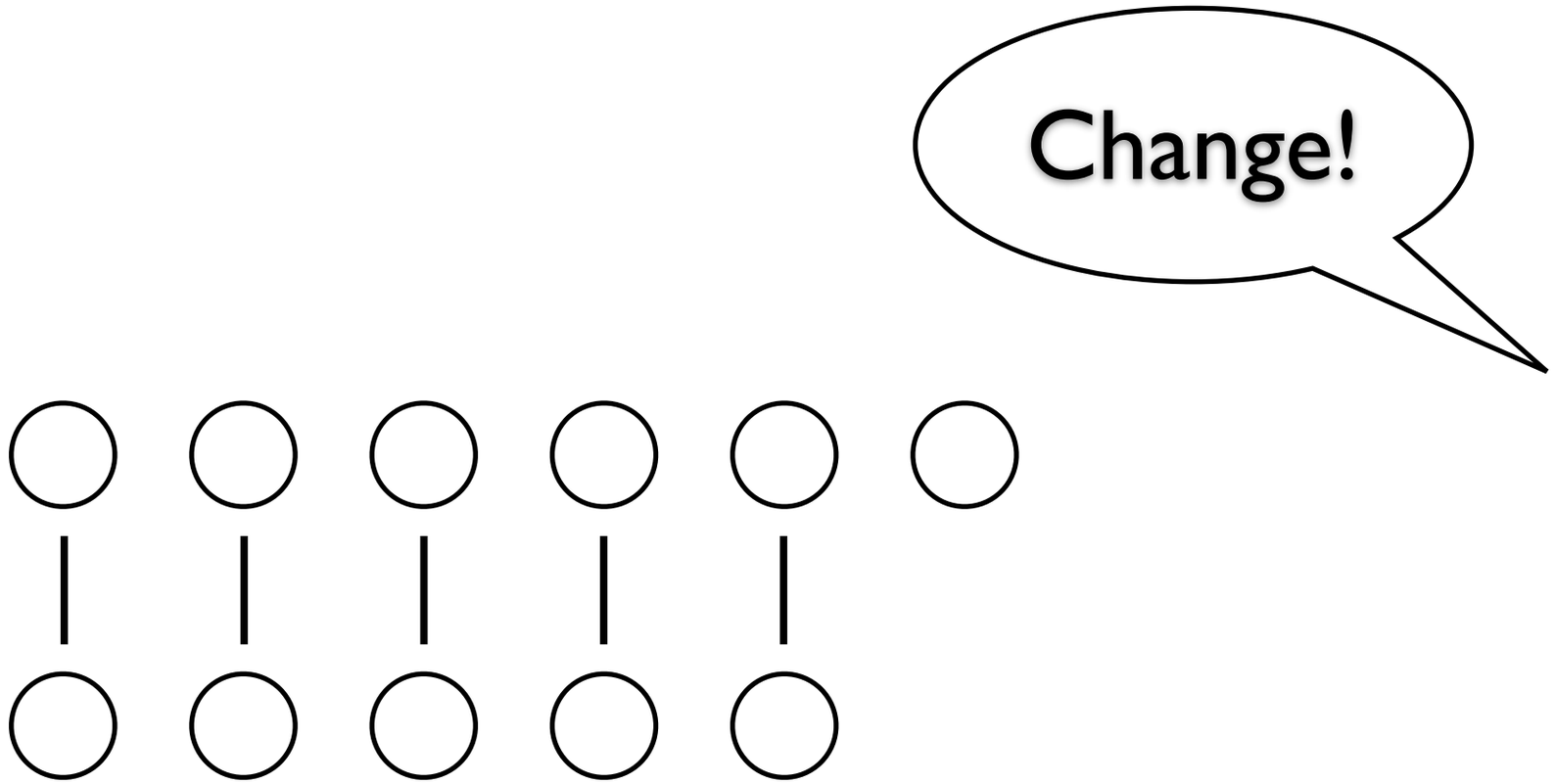


Speed Dating: Format

Chat!



Speed Dating: Format



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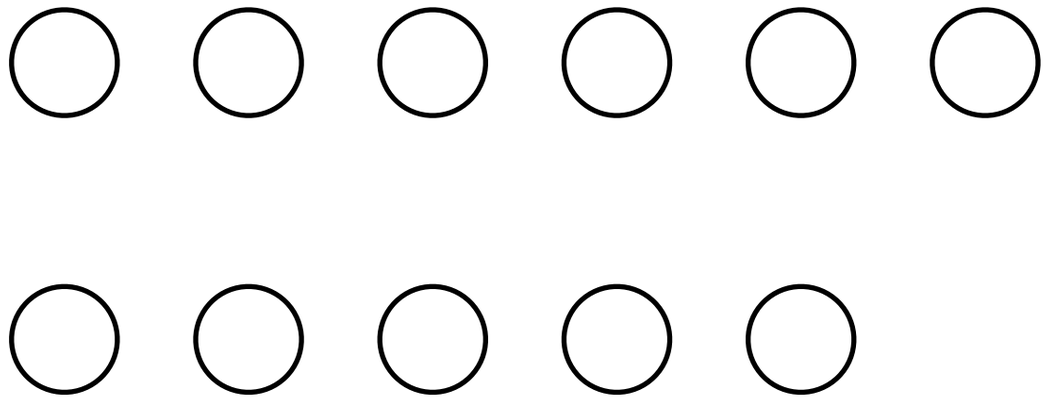
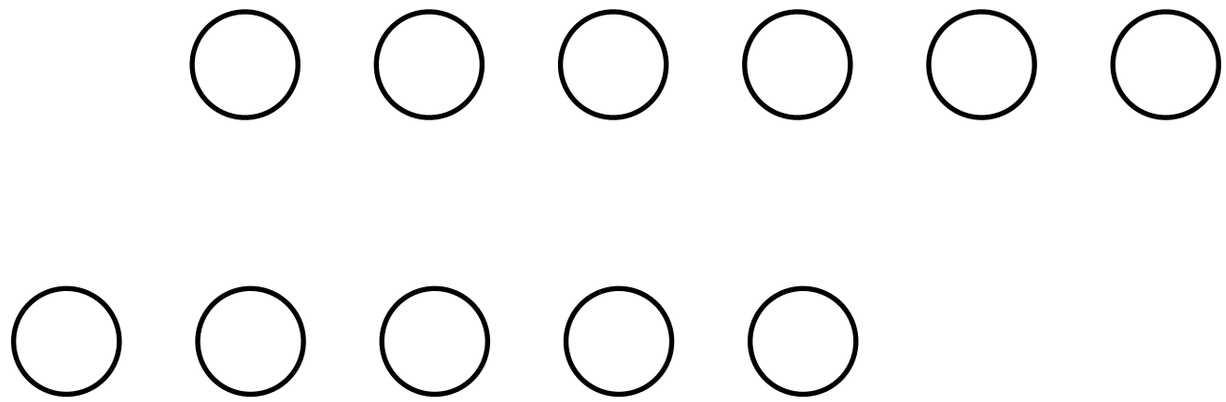
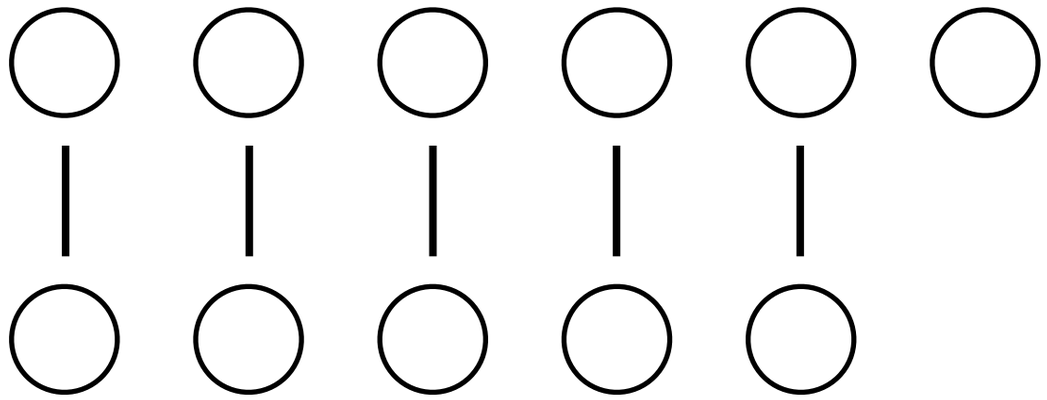


Speed Dating: Format



Speed Dating: Format

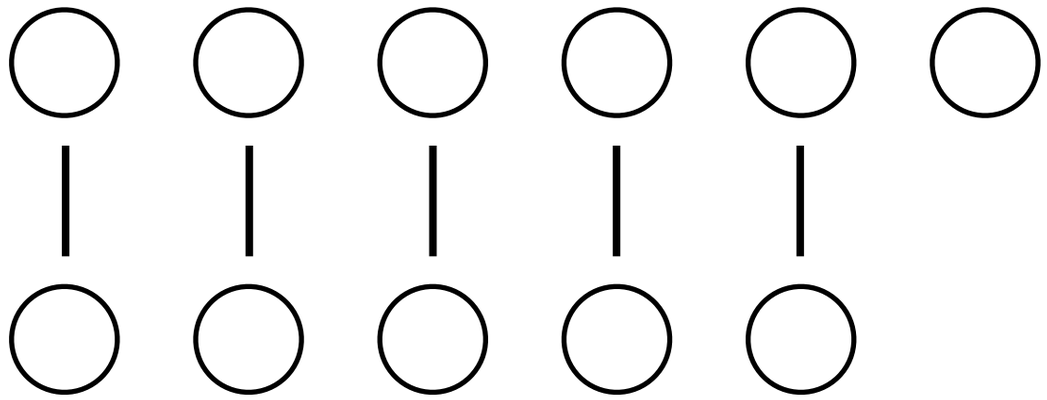
Chat!



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

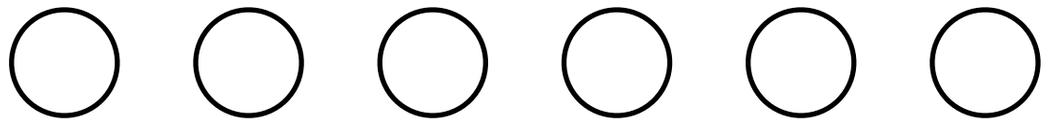
Speed Dating: Format

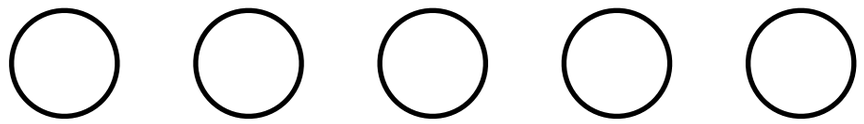
Chat!



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

Speed Dating: Format

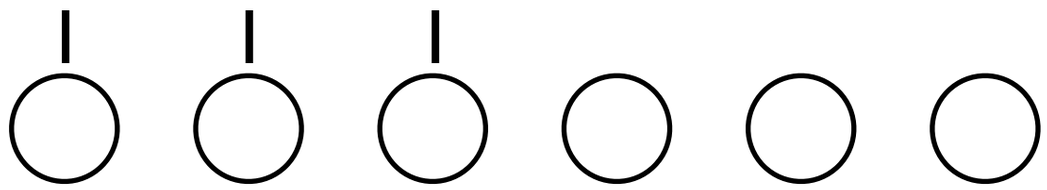


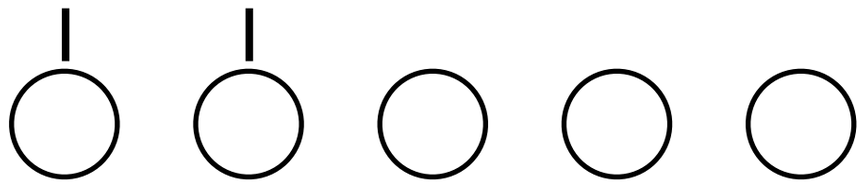


then split each row into two new rows

Speed Dating: Format

Chat!





make two new rows

and start again with the chat…

Session HTML pages

download/git clone from:
https://github.com/aidanbudd/crete2014

“homepage” (links to exercises, instructions, presentations)
homepageInterpretingPhylogeniesCrete2014.html

exercises
interpretingPhylogeniesCrete2014.html

Interpreting Molecular Phylogenetic Trees

Monday 5th May 2014

EMBO Practical Course on Computational Molecular Evolution

Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Helenic Center for Marine Research) HCMR

Heraklion, Greece

Aidan Budd
EMBL Heidelberg, Germany

Laura Emery
EMBL-EBI, Hinxton, UK

Sarah Parks
EMBL-EBI, Hinxton, UK

Introductions and Aims

Aidan Budd, EMBL Heidelberg

Phylogenetics

'phylogenetics' is of Greek origin from the term

- **phyle/phylon** (φυλή/φῦλον), meaning "tribe, race"
- **genetikos** (γενετικός), meaning "relative to birth" from genesis (γένεσις, "birth").

(Wikipedia)

Give me a word, any word, and I show you how the root of that word is Greek

OK, Mr Portokalos, how about the word "Kimono"?

Hah. Kimono.

Hah. Of course. Kimono is come from the Greek word "Himona" which mean winter...

http://www.youtube.com/watch?v=VL9whwwTK6I

http://www.youtube.com/watch?v=2ALrm3nDGXI

(My Big Fat Greek Wedding)

Aims

Often non-trivial to interpret phylogenetic trees appropriately

Understanding better how to interpret such results helps us design better analyses

This session aims to provide:

An overview of terminology and concepts associated with phylogenetic trees

Experience with some commonly-used tools for examining phylogenies

Before we start

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

Why do people
care about phylogenetics?

and

How do people use
phylogenetics?

Aidan Budd, EMBL Heidelberg

Why Should We Care about Phylogenetics?

Useful to consider this question as...

... learning studies show: the more relevant/important a topic is to us...

... the more attention we pay when people talk about it...

... the more effectively we learn about the topic

You all already care, as you need to build/interpret them for your research

By presenting examples of specific applications of phylogenies, hopefully make it **even more relevant/important for you**

...and provides a context for considering **common ways in which phylogenies are used/interpreted**

Why Care About Phylogenies? 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

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

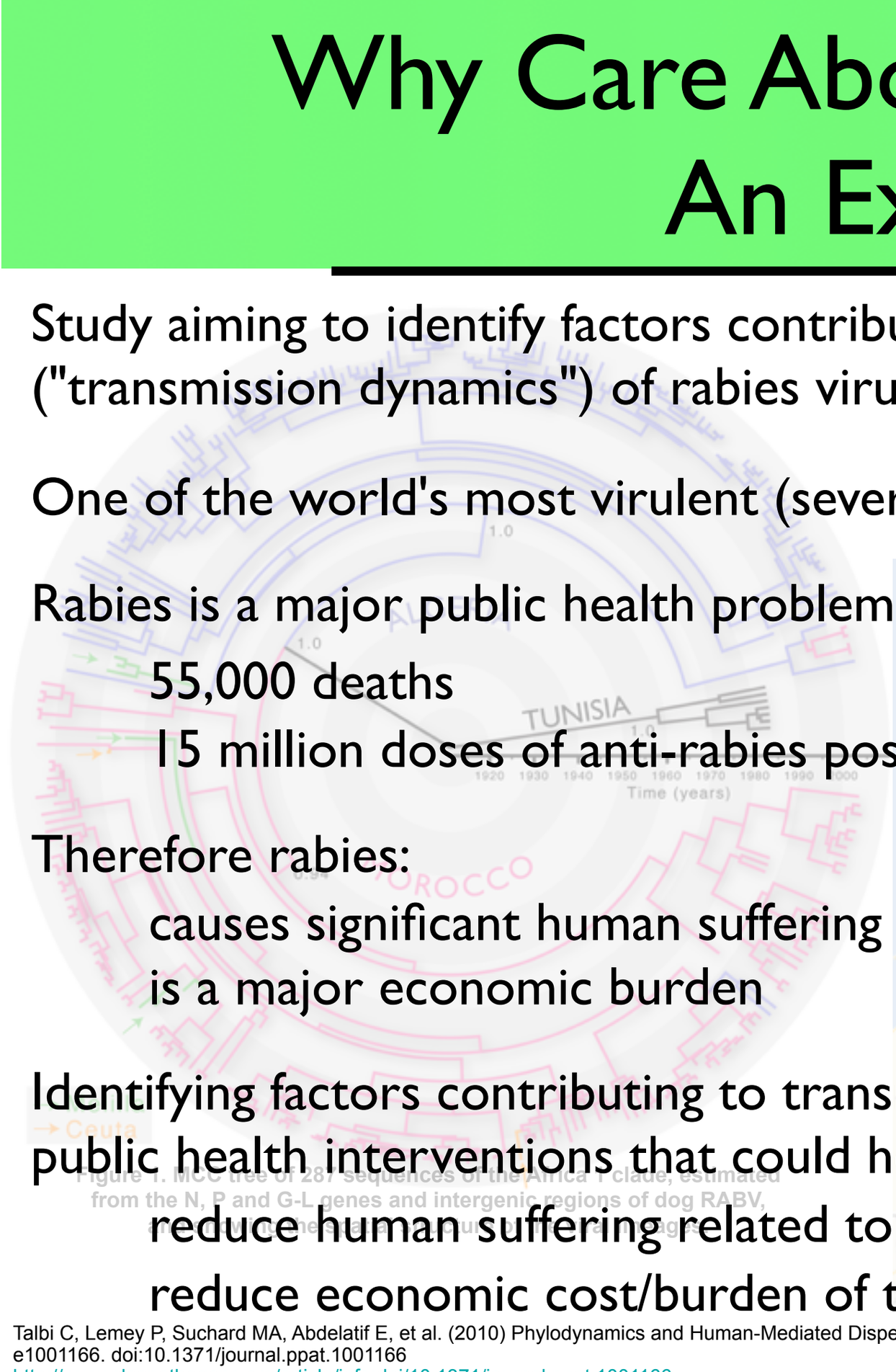
- 55,000 deaths
- 15 million doses of anti-rabies post-exposure prophylaxis administered

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



*autonomous cities of Spain

Talbi C, Lemey P, Suchard MA, Abdelatif E, et al. (2010) Phylodynamics and Human-Mediated Dispersal of a Zoonotic Virus. PLoS Pathog 6(10): e1001166. doi:10.1371/journal.ppat.1001166
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1001166

PLOS | PATHOGENS

Why Care About Phylogenies? An Example

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

Transmission via saliva, particularly via dog bites

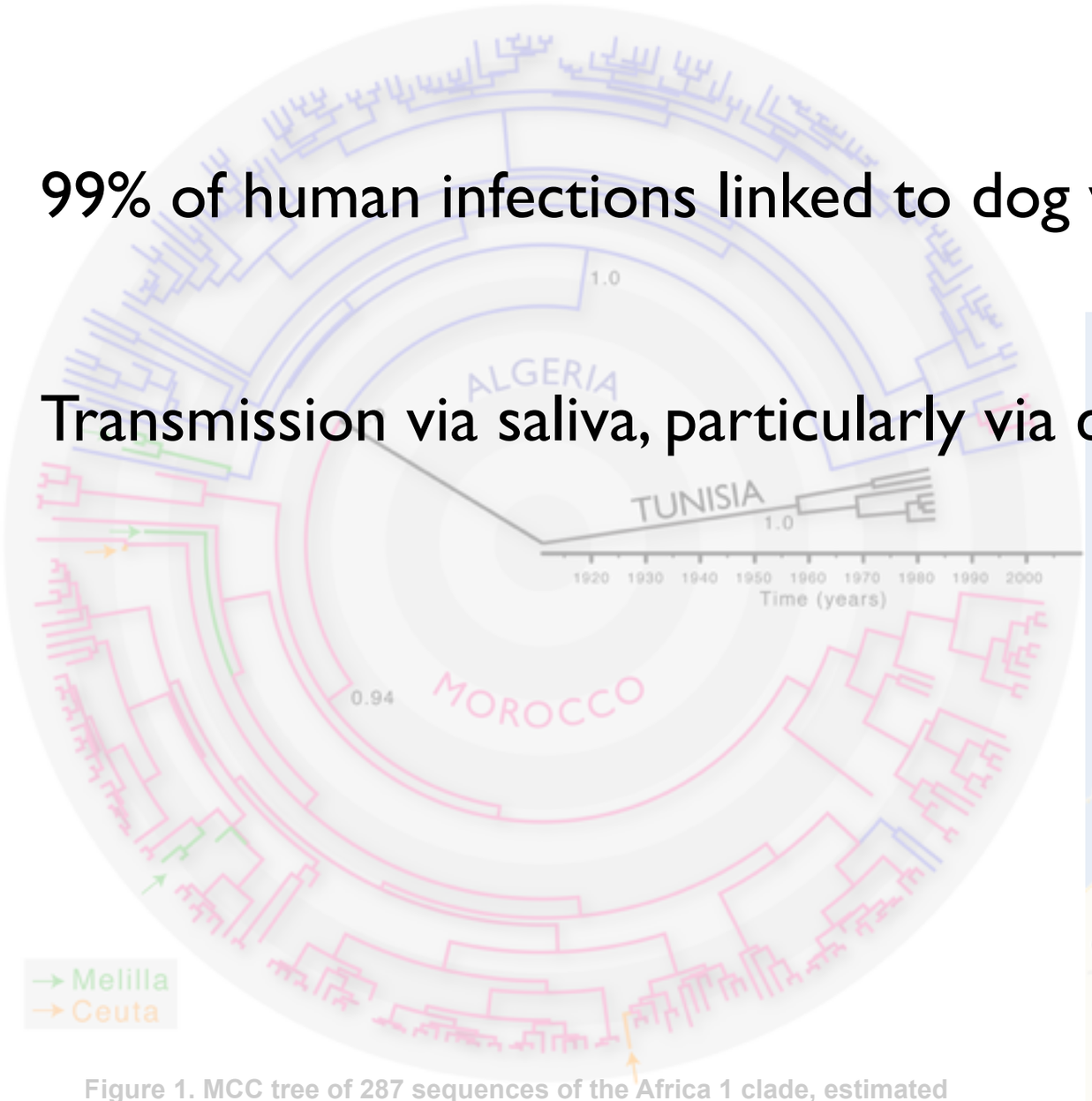


Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.

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Why Care About Phylogenies? An Example

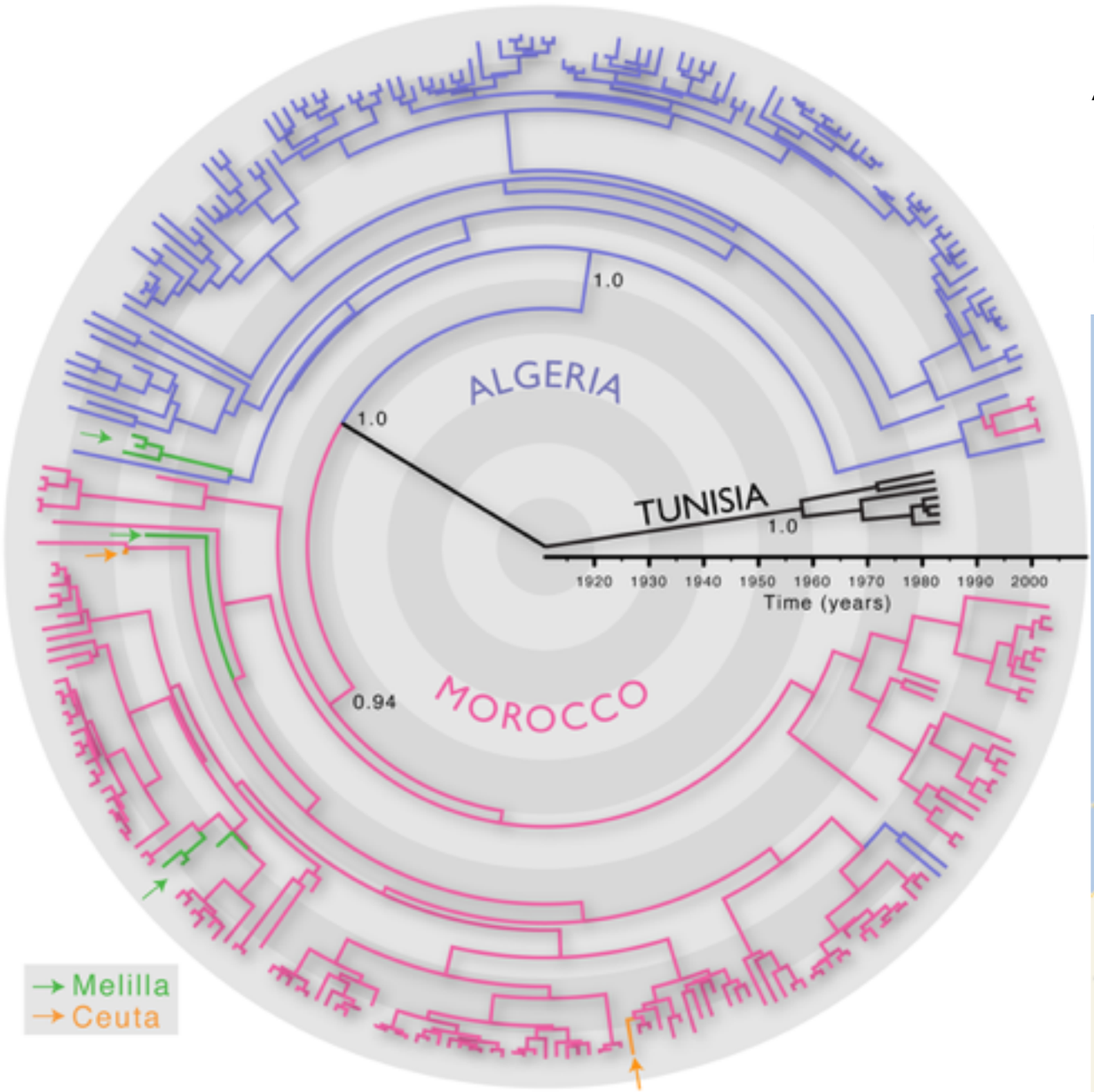
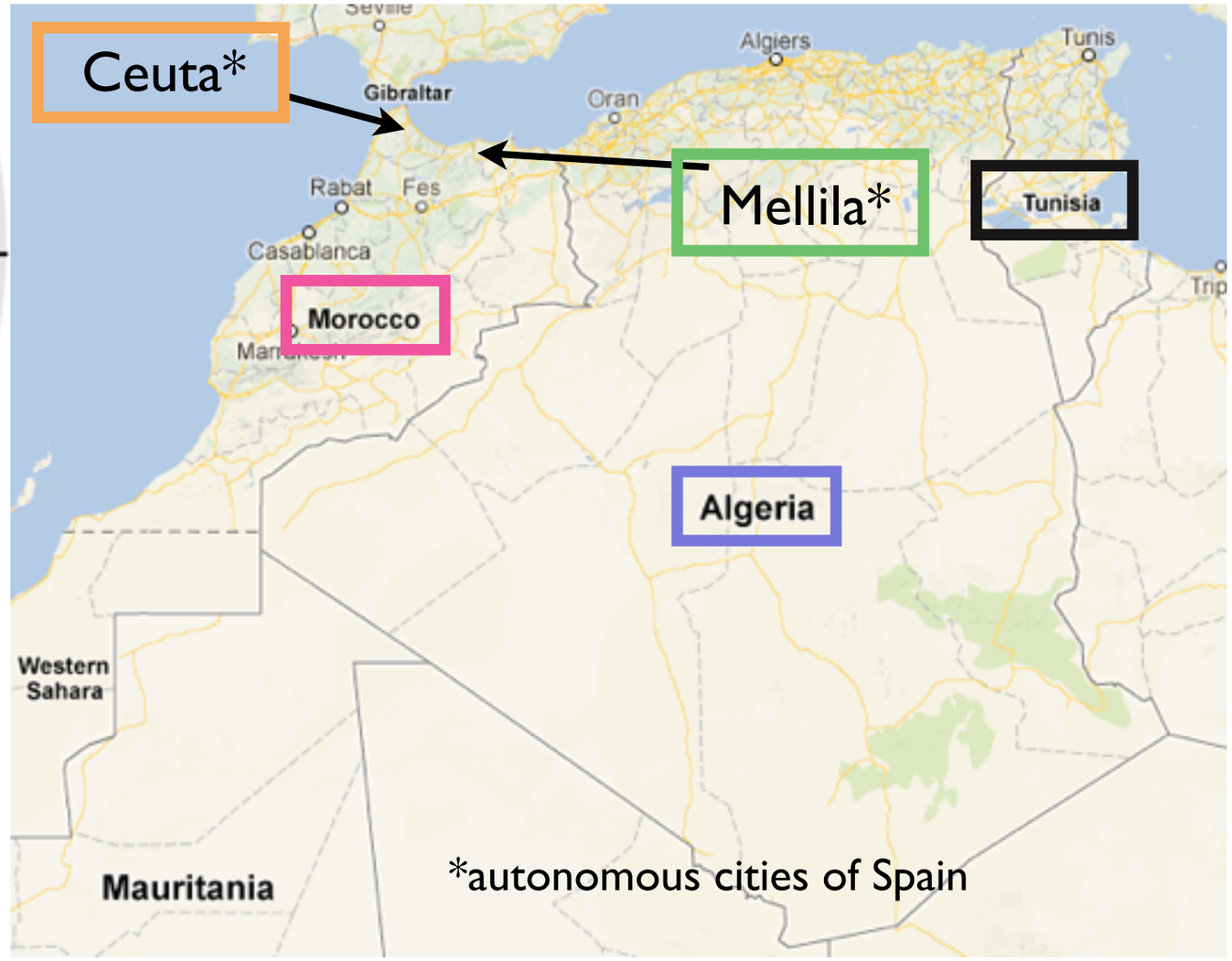


Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.

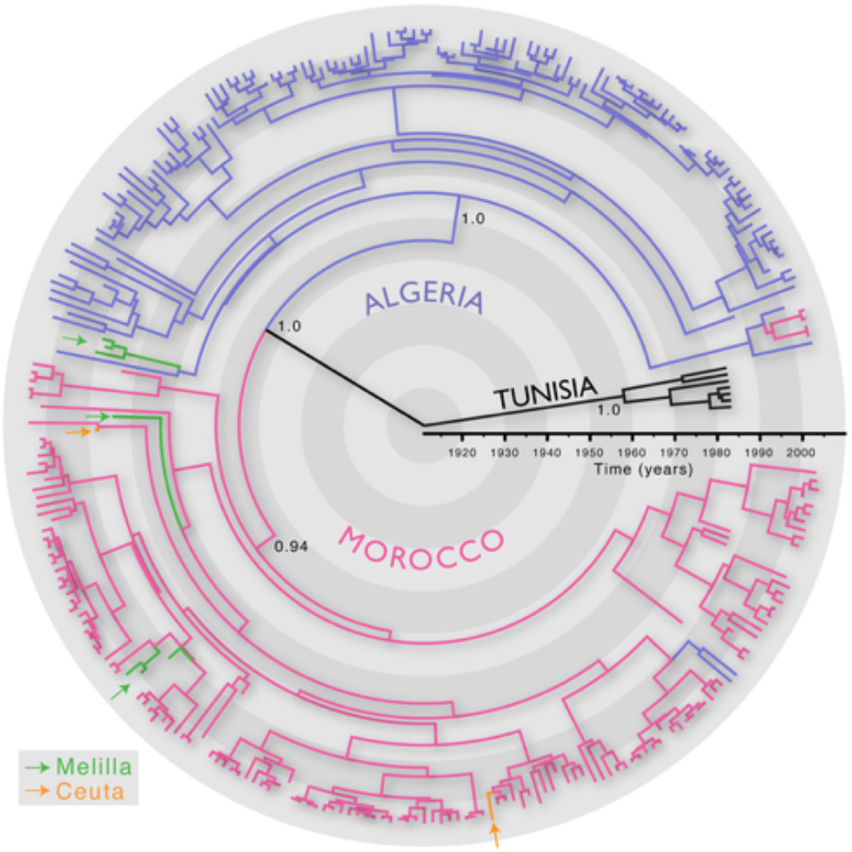
Phylogeny of rabies virus sampled from North African dogs

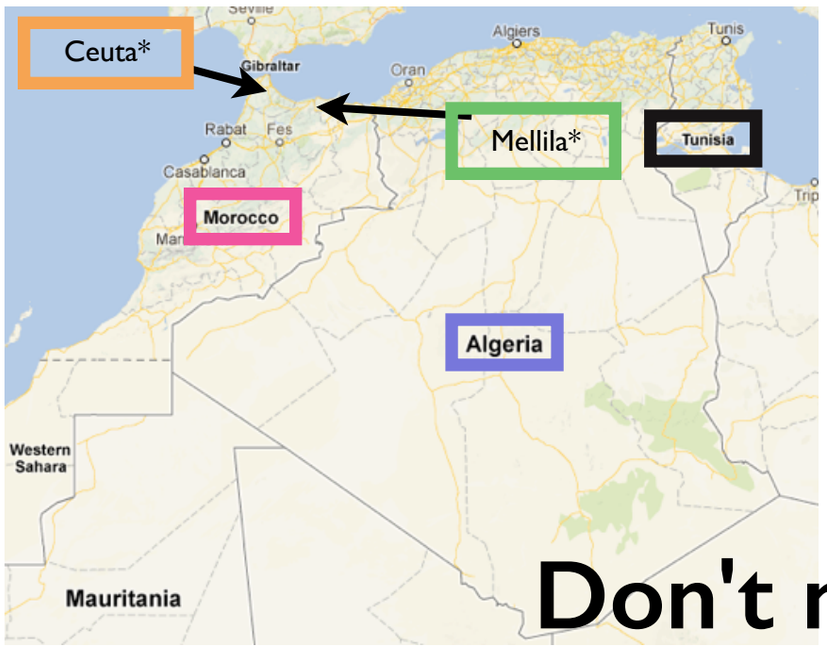
Branches coloured according to measured or inferred geographical location



Talbi C, Lemey P, Suchard MA, Abdelatif E, et al. (2010) Phylodynamics and Human-Mediated Dispersal of a Zoonotic Virus. PLoS Pathog 6(10): e1001166. doi:10.1371/journal.ppat.1001166
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1001166

Why Care About Phylogenies? 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

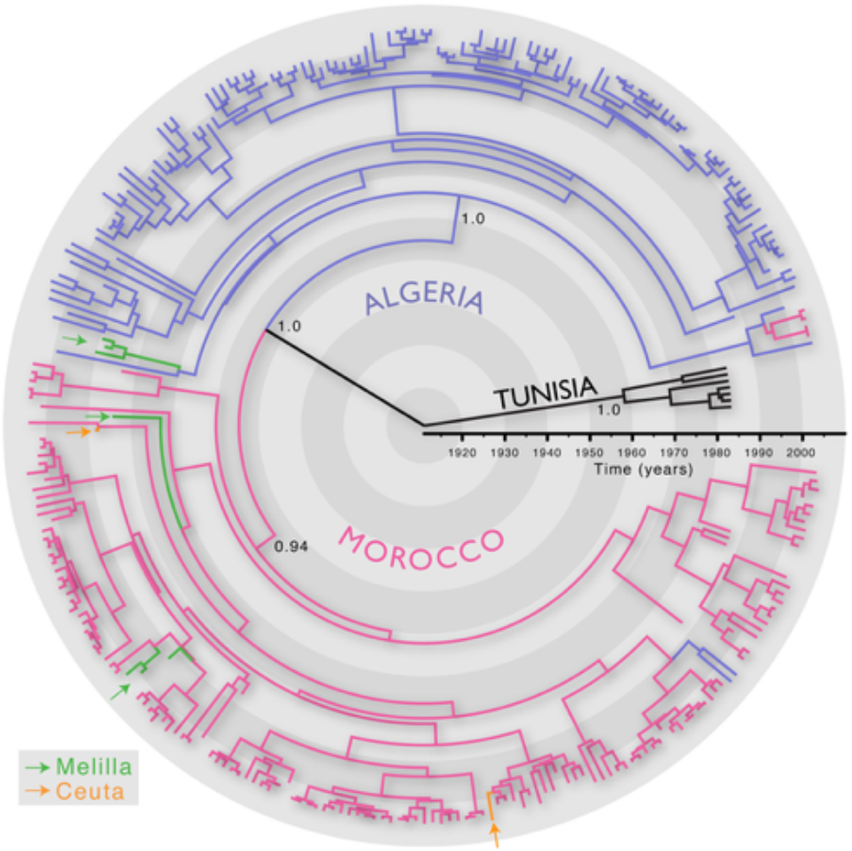
Then we'll take a vote, followed by discussing with your neigbours

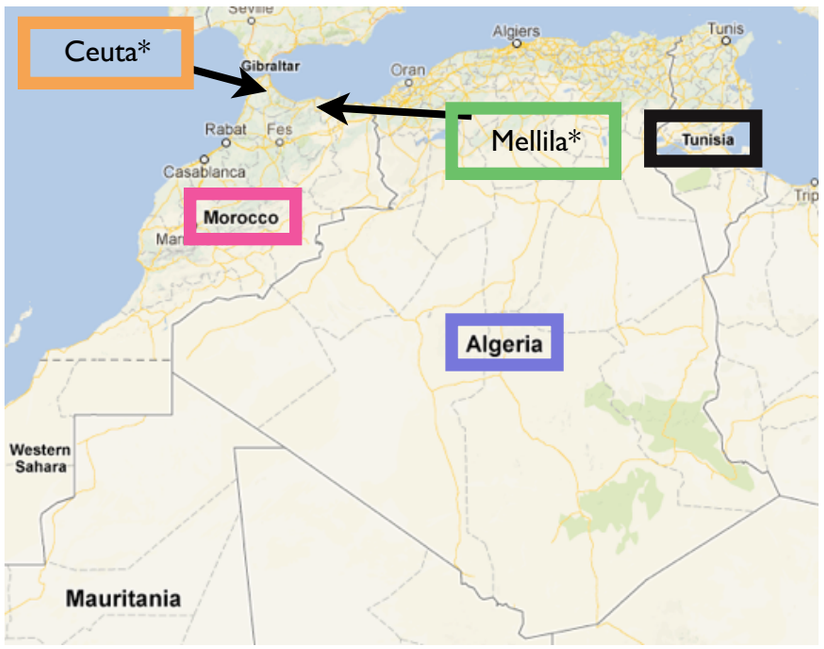
Feel uncomfortable that you don't know enough about the study/data to decide?

Make (and make 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!

Why Care About Phylogenies? 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

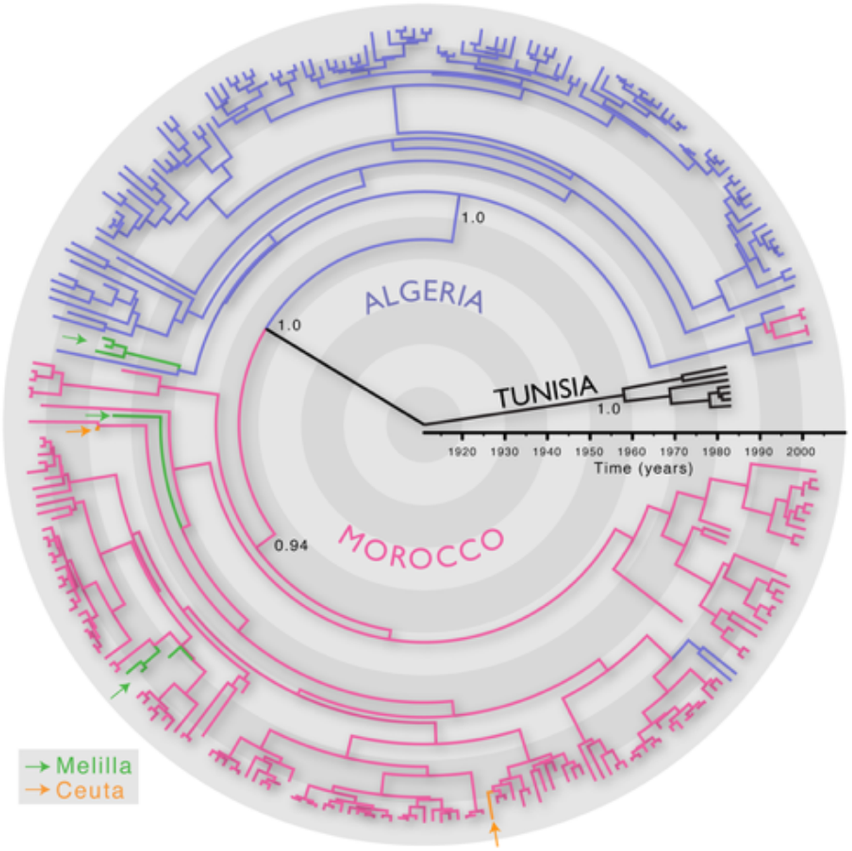
A. more probable

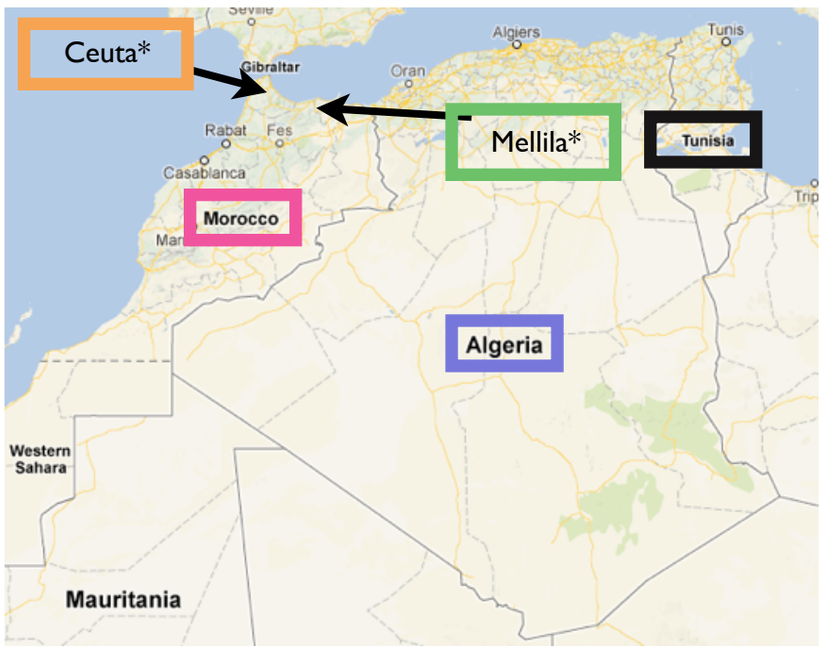
that human activity significantly influences the dynamics of rabies virus transmission between dogs

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

Why Care About Phylogenies? 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**

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

add another slide about common comments/things we learn from the example, and make previous slide less fussy

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

Rooted Phylogenies

Terminology and Concepts

Aidan Budd, EMBL Heidelberg

Definitions

Phylogeny terminology and concepts "definition" exercise
(see homepageInterpretingPhylogeniesCrete2014.html)

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

Try to **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 homepageInterpretingPhylogeniesCrete2014.html)

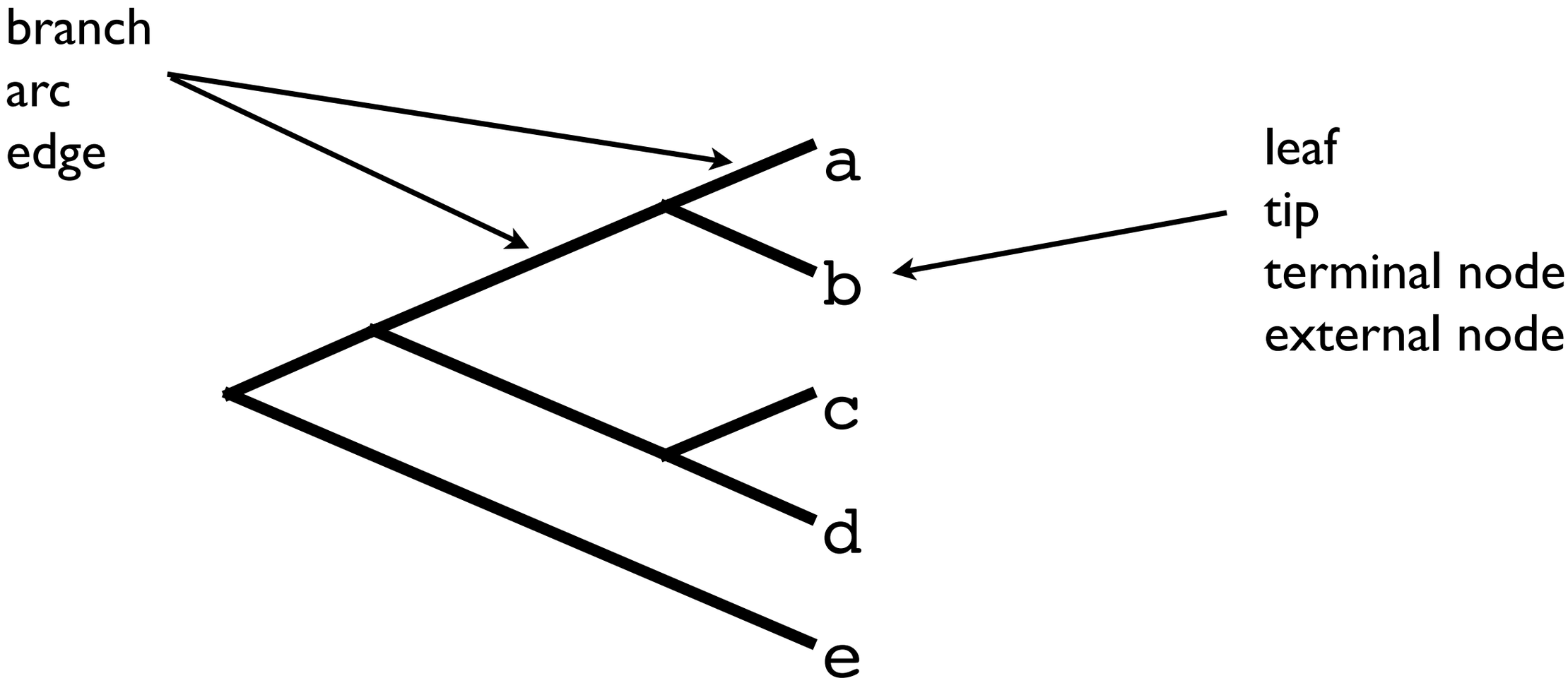
below are some suggested definitions for these terms

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

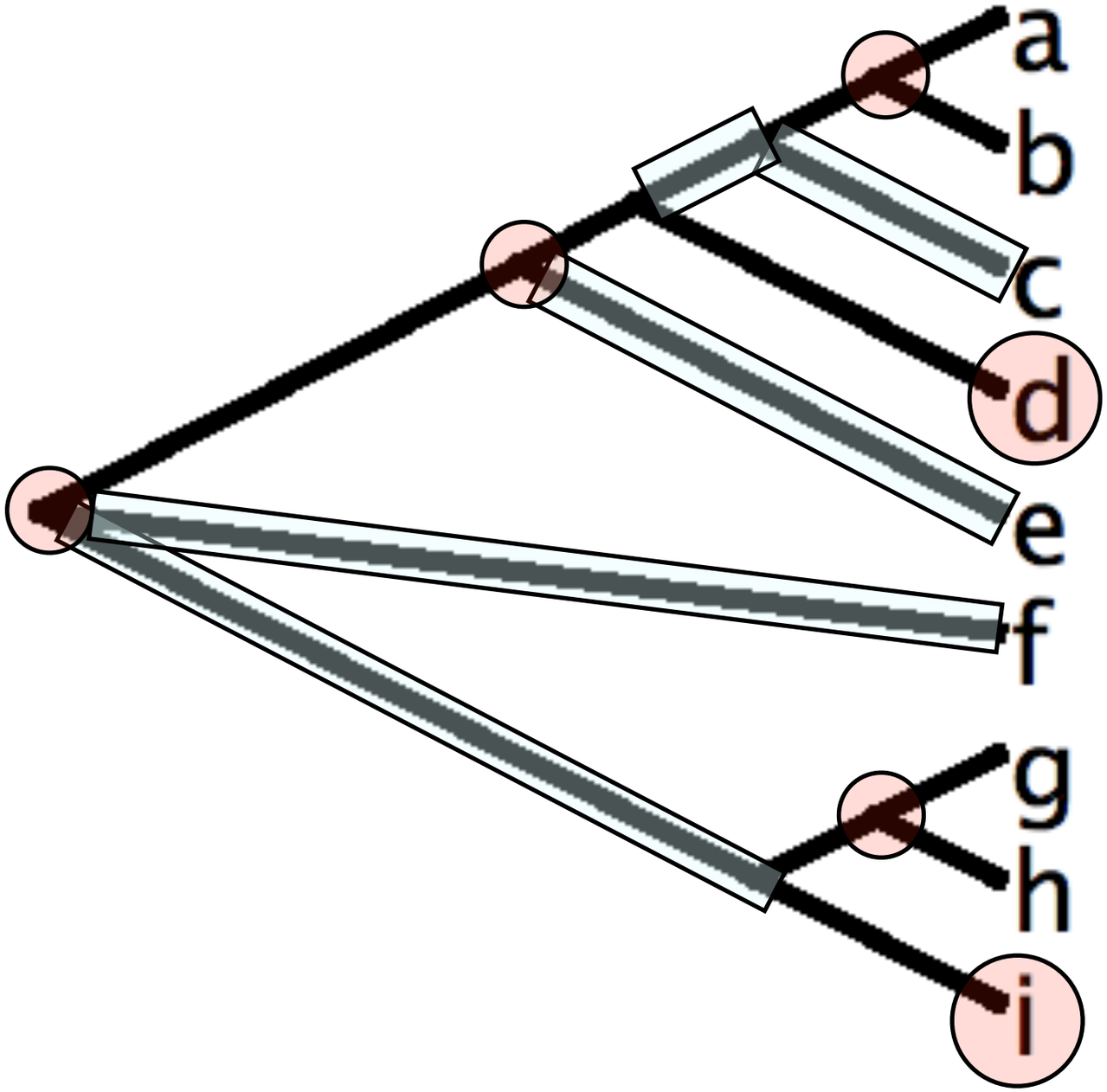
branch (of a phylogenetic tree): Lineages of taxonomic units that link nodes within a phylogenetic tree.

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



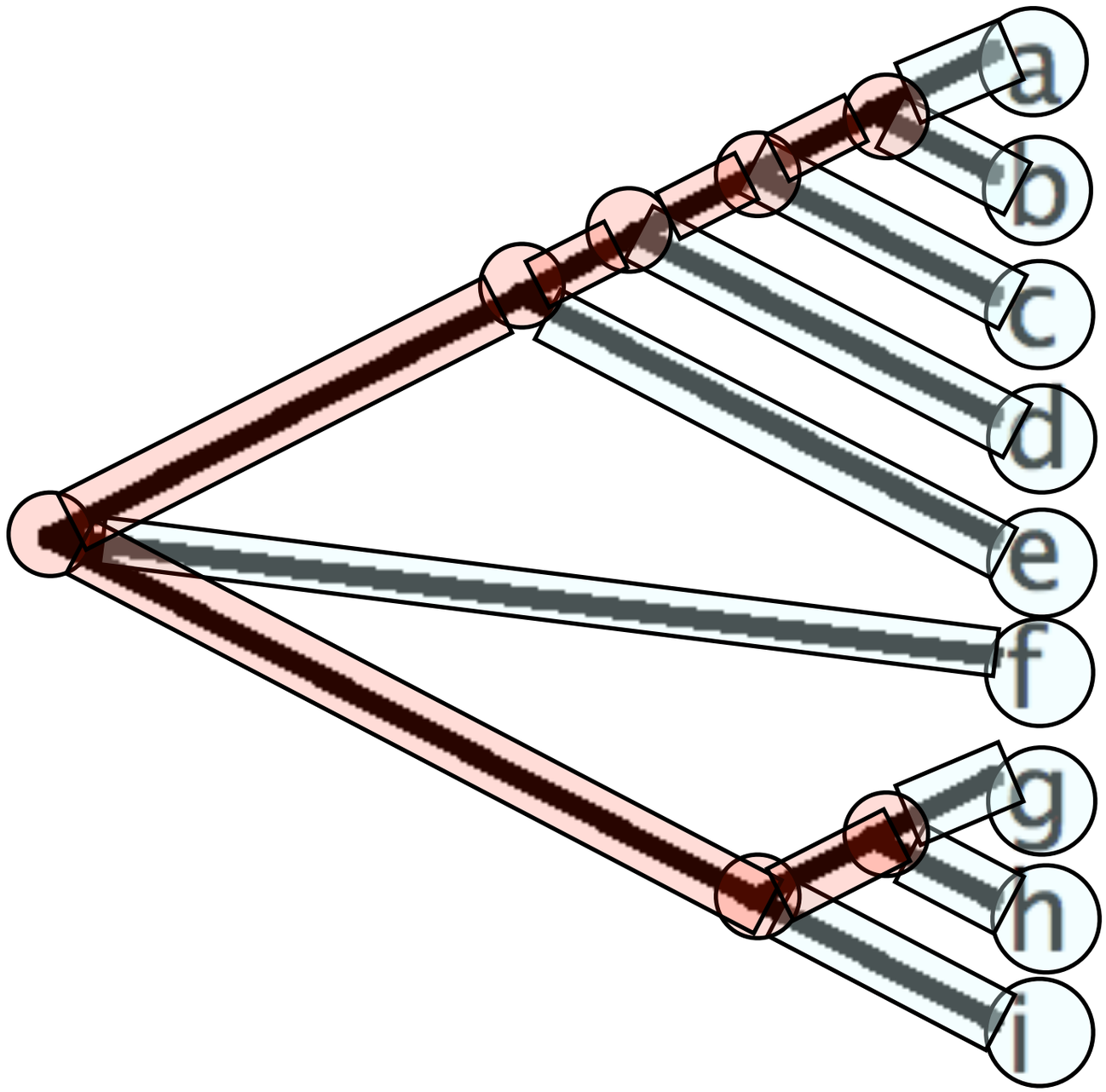
Trees consist of:

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

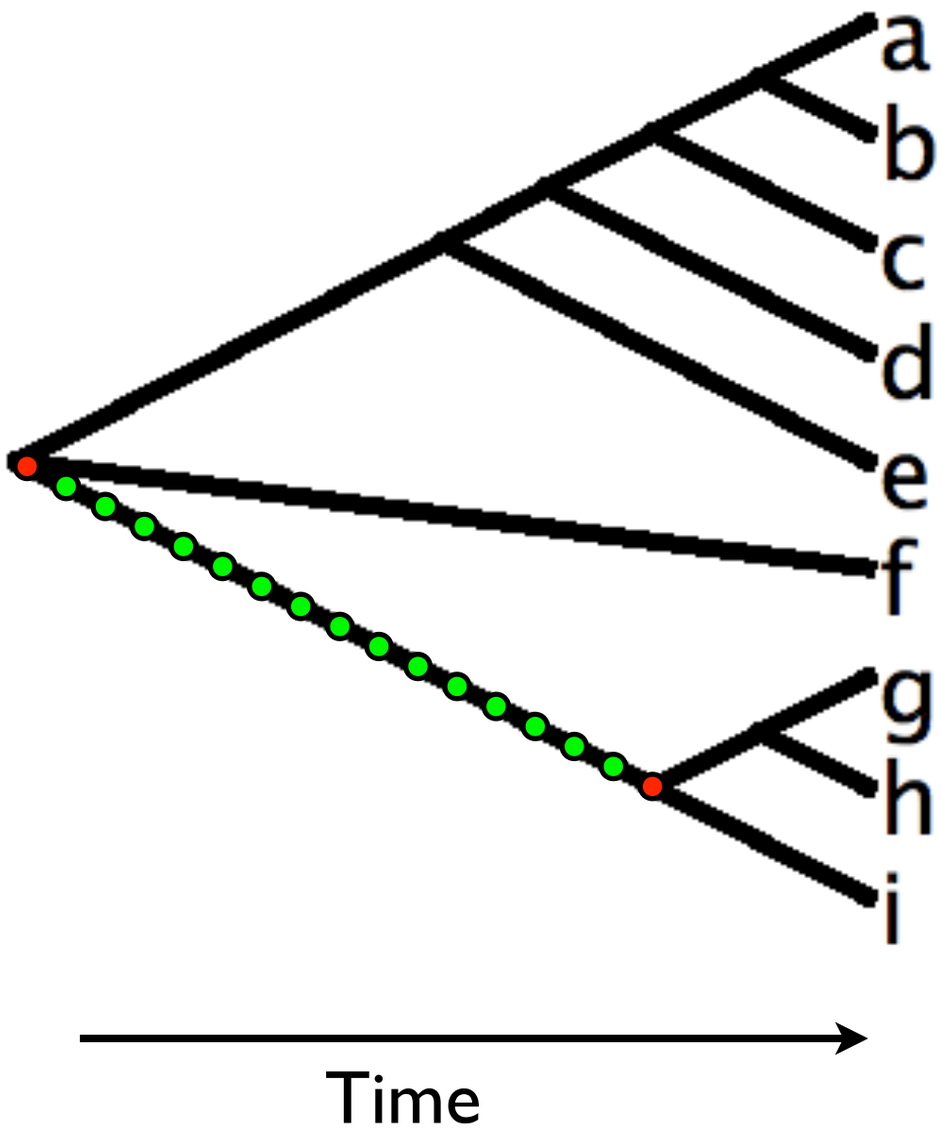
Branch - links two internal nodes

external/terminal

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

Branch - links an external and an internal node

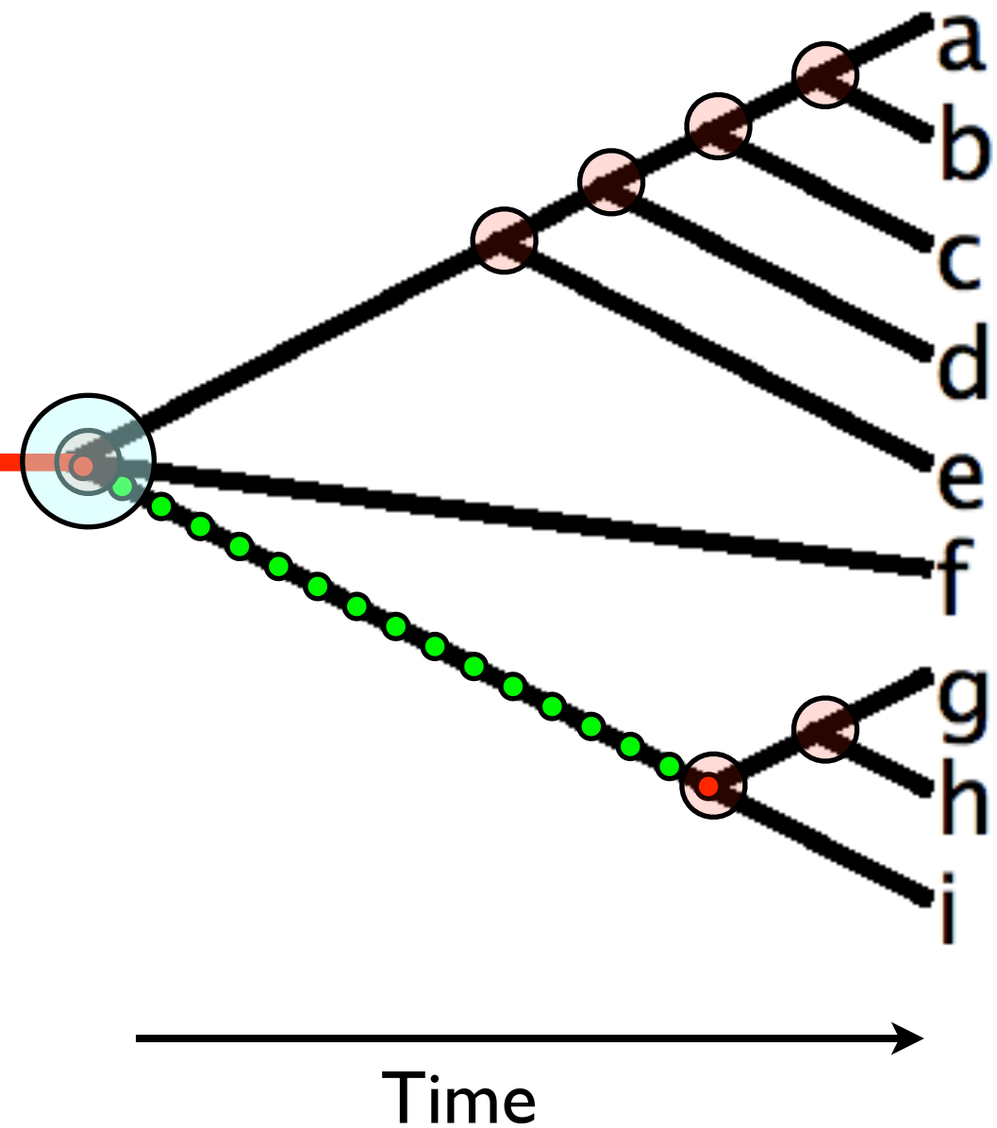
Branches



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

Root (Root Node)

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

Alignments

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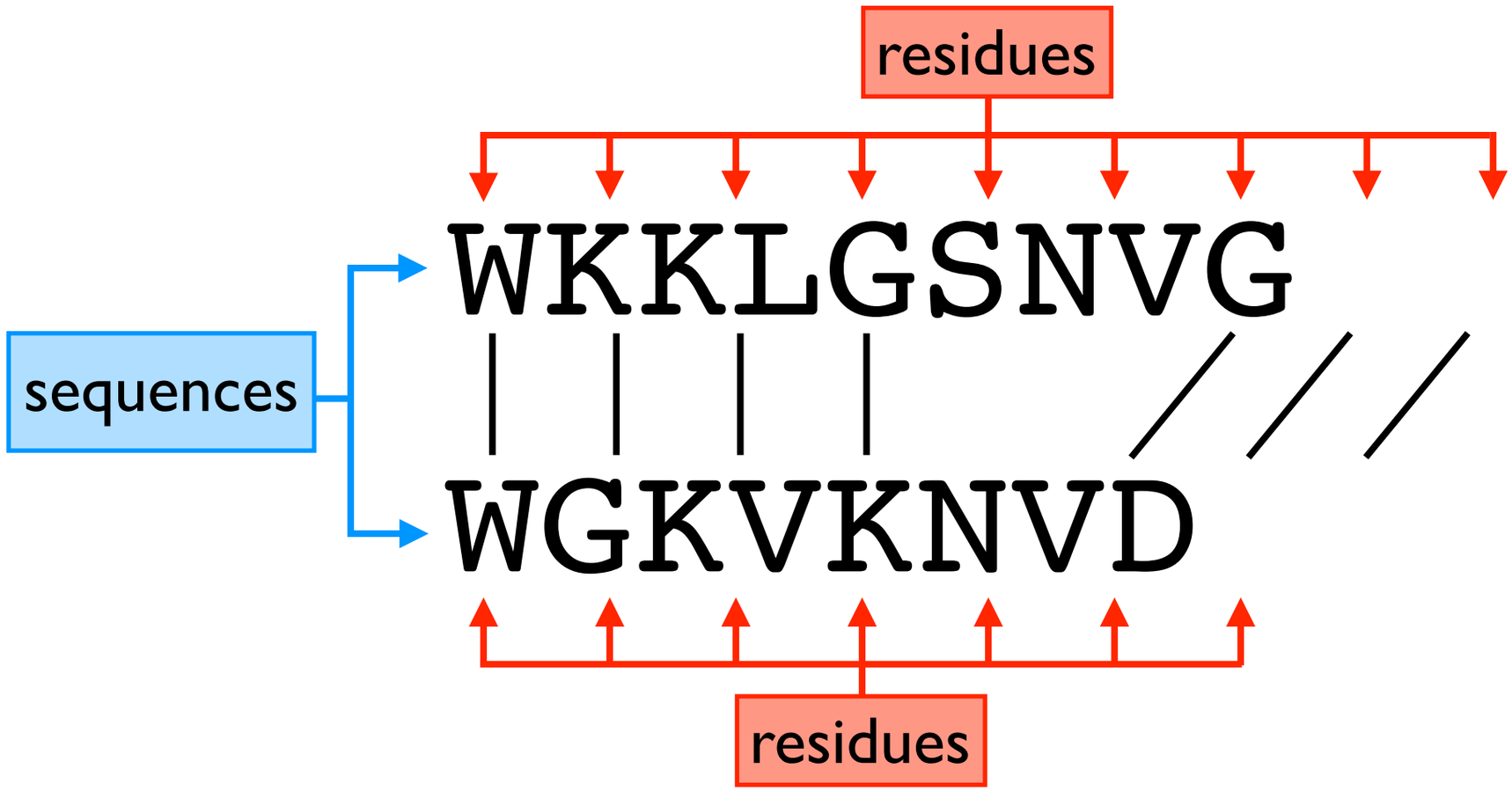
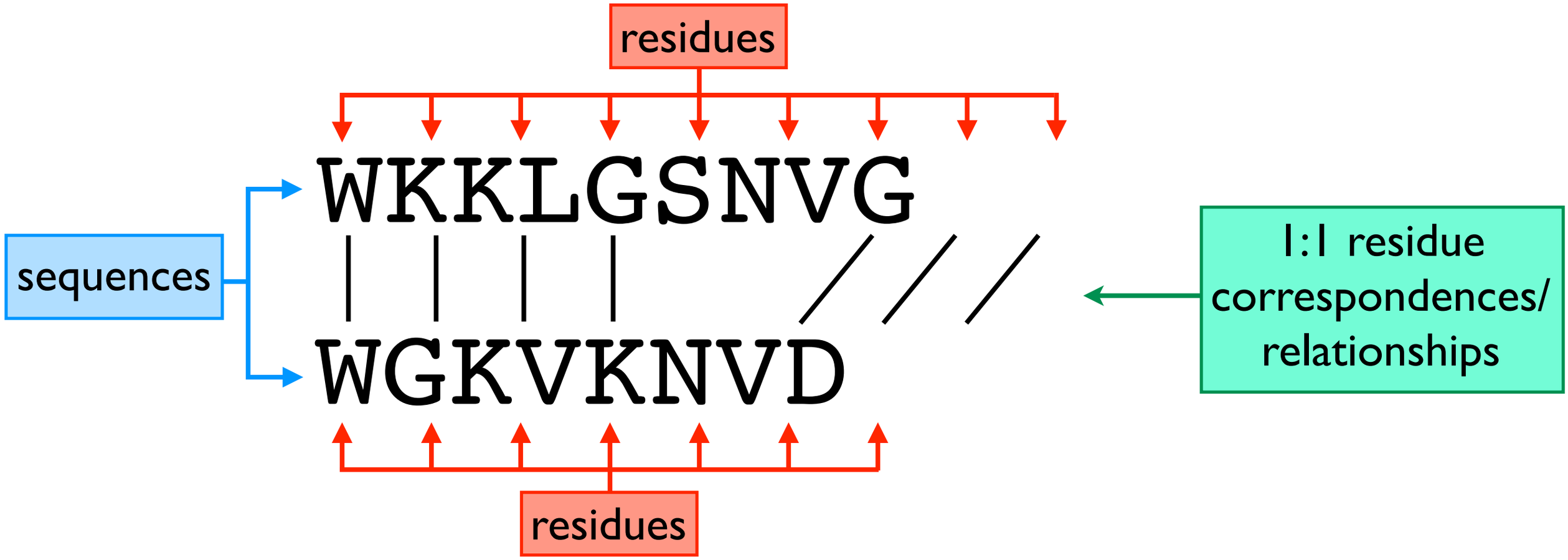
"Anatomy" of a Sequence Alignment



Residues:
Monomers within a polymer (polypeptide or polynucleotide) chain

Sequences:
List of residues in a polymer chain...
...listed in the same order they occur within the polymer

"Anatomy" of a Sequence Alignment

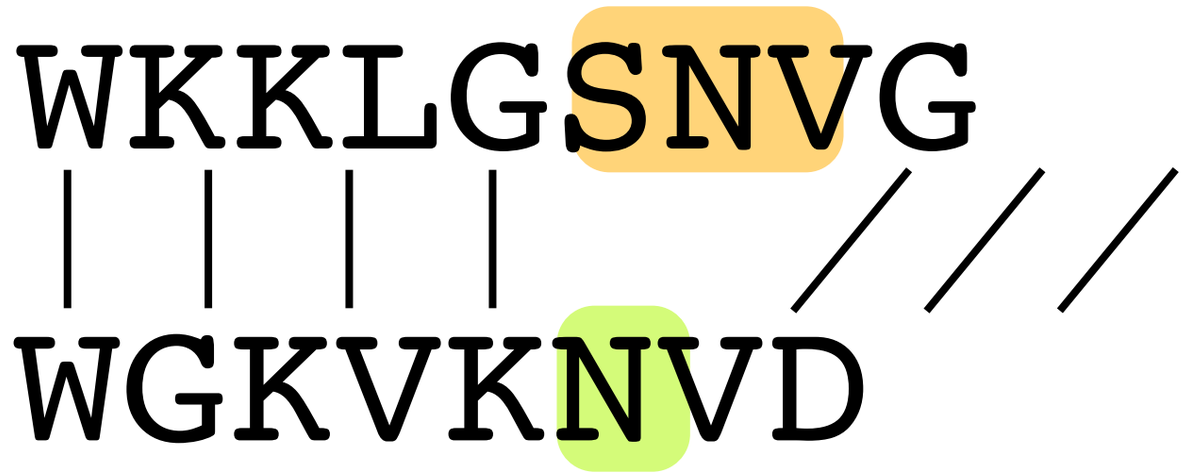


1:1 residue correspondences/relationships

Correspondences between

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

"Anatomy" of a Sequence Alignment



1:1 residue correspondences/ relationships

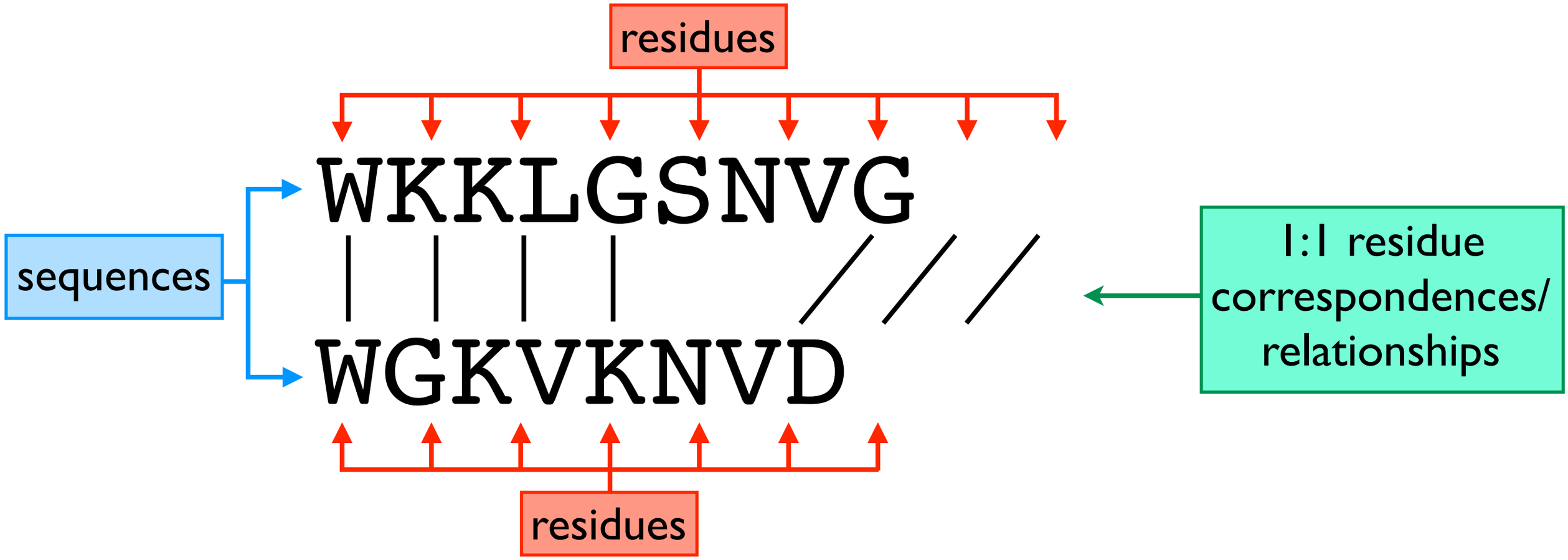
Residue has no equivalent in the top sequence

i.e. no residue in the top sequence has a 1:1 relationship with this residue

Could *perhaps* say there is a "1:2" relationship between this residue and these residues

However, alignments focus on 1:1 relationships

"Anatomy" of a Sequence Alignment

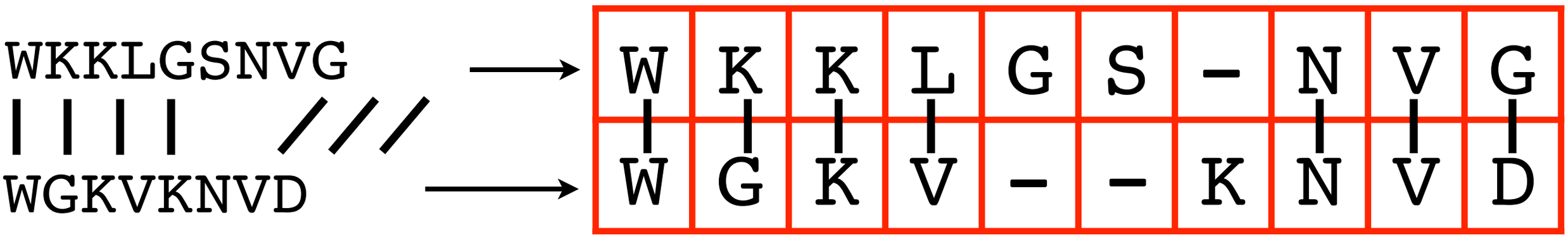


Sequence alignment

A comparison of the residues in two or more sequences...

... describing 1:1 correspondences/relationships/equivalences between residues in different sequences

Sequence Alignment Within a Grid



Often represented using a grid/matrix :

One sequence per row

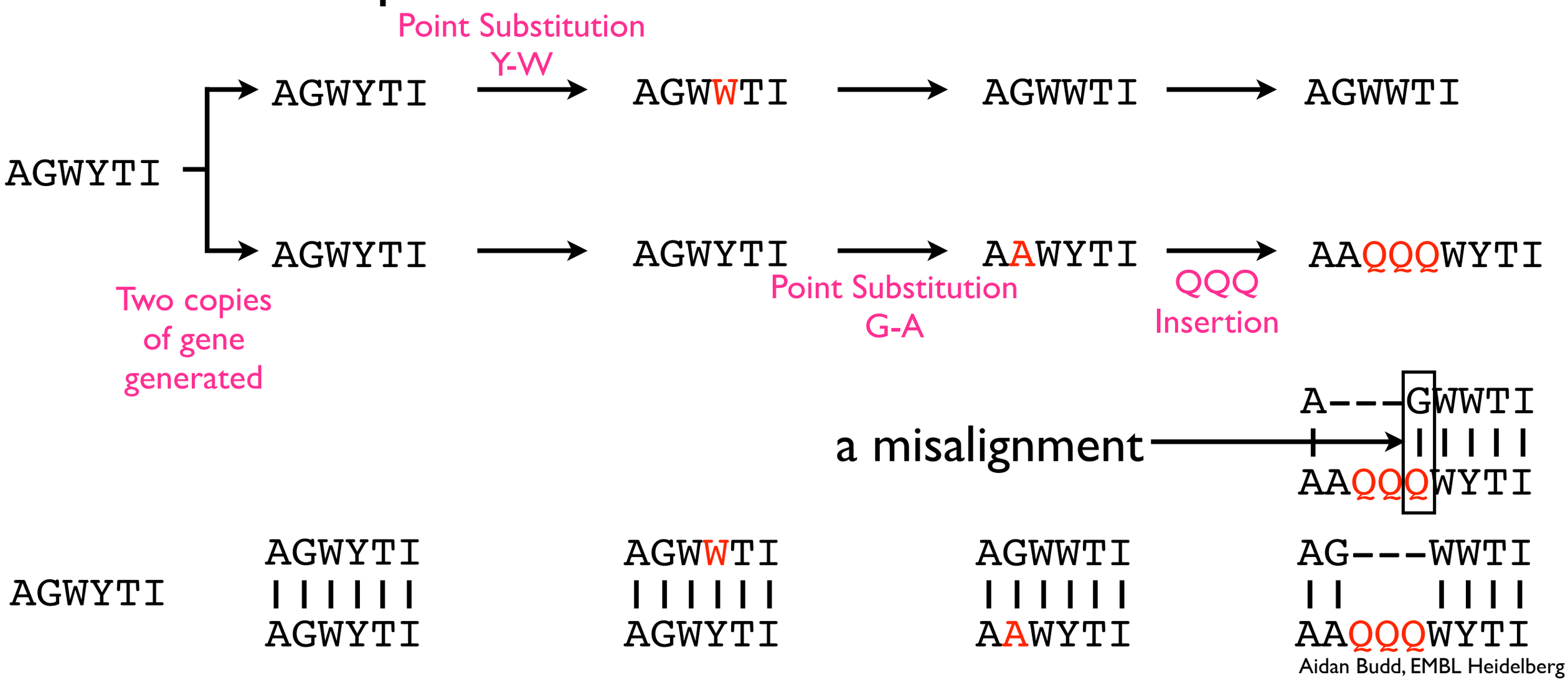
Residues in the same column are 'equivalent'

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

Evolutionary "Equivalence”

Residues are "evolutionarily equivalent" when:

- they are derived from the same residue in an ancestral sequence
- the only mutations experienced during divergence from this ancestral residue were **point substitutions**



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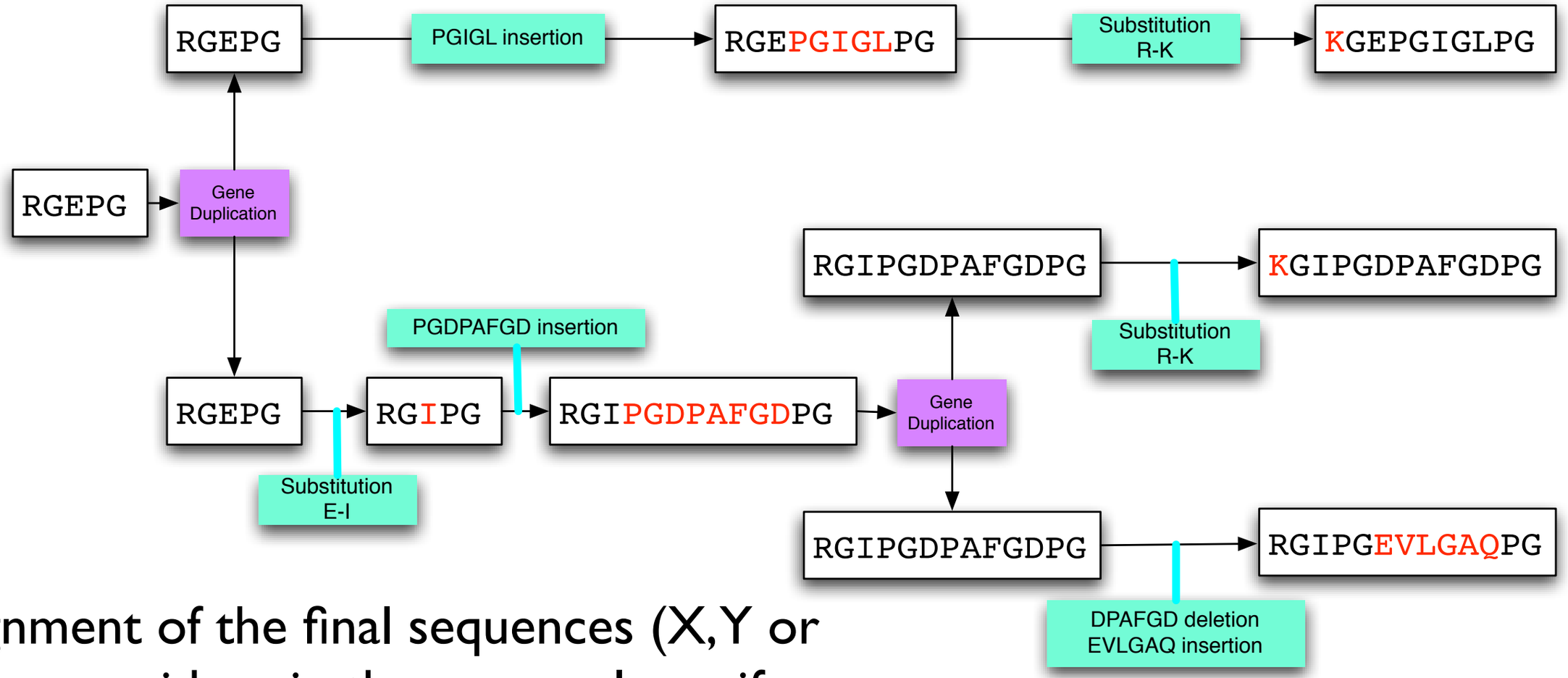
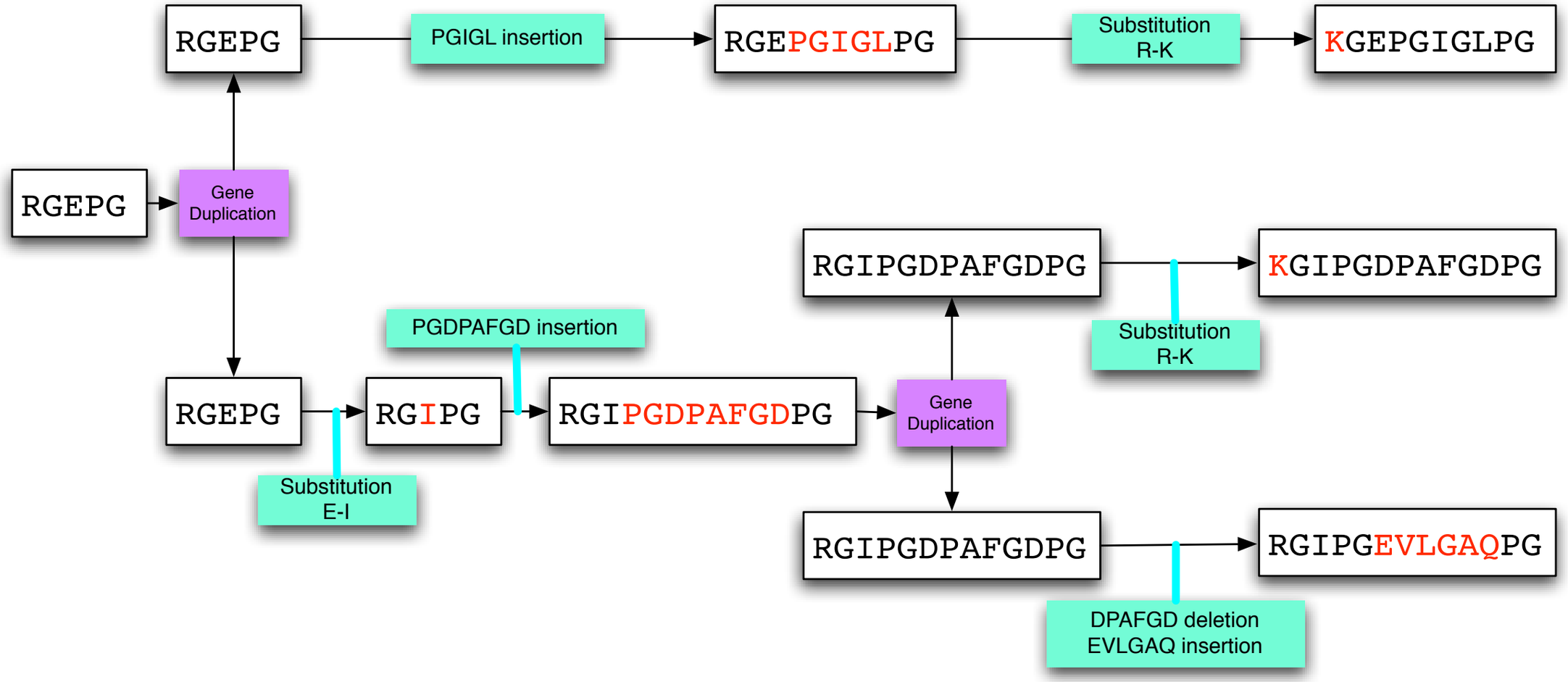
Quiz - Evolutionary Interpretation of Alignments



Which alignment of the final sequences (X, Y or Z) only places residues in the same column if they are related by substitution events?

X	Y	Z
KGEPG---IGLPG	KGEPG------IGL------PG	KGE--------PGIGL------PG
KGIPGDPAFGDPG	KGIPG---------DPAFGDPG	KGIPG-----------DPAFGDPG
RGIPGEVLGAQPG	RGIPGEVLGAQ---------PG	RGIPGEVLGAQ-----------PG

Quiz - Evolutionary Interpretation of Alignments



"True" alignment given history described above

```
KGE--------PGIGL------PG
KGIPG-----------DPAFGDPG
RGIPGEVLGAQ-----------PG
```

PRANK

```
RGIPGEVLGAQPG
KGIPGDPAFGDPG
---KGEPGIGLPG
```

Quiz - Evolutionary Interpretation of Alignments

CLUSTALX

```
K---GEPGIGLPG
KGIPGDPAFGDPG
RGIPGEVLGAQPG
```

MAFFT

```
KGEPG---IGLPG
KGIPGDPAFGDPG
RGIPGEVLGAQPG
```

PRANK

```
RGIPGEVLGAQPG
KGIPGDPAFGDPG
---KGEPGIGLPG
```

Different automatic MSA software gives different results

All are different from the "true" alignment (assuming the scenario of transformation on the previous slide is true)...

... because that scenario is very unlikely under the models of evolutionary transformation incorporated within these tools

X

```
KGEPG---IGLPG
KGIPGDPAFGDPG
RGIPGEVLGAQPG
```

Y

```
KGEPG------IGL------PG
KGIPG---------DPAFGDPG
RGIPGEVLGAQ---------PG
```

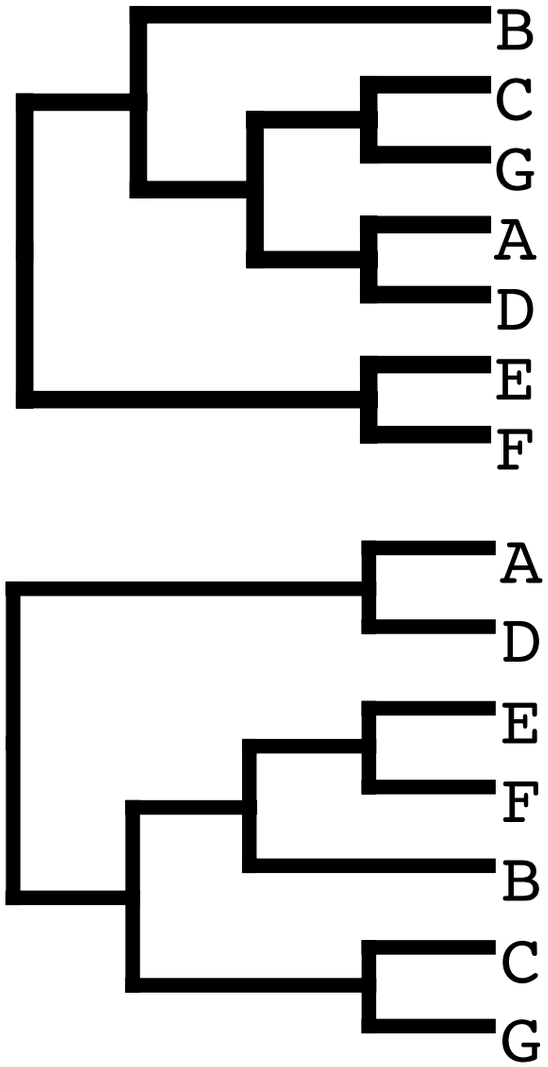
Z

```
KGE--------PGIGL------PG
KGIPG-----------DPAFGDPG
RGIPGEVLGAQ-----------PG
```

Branch Lengths

Aidan Budd, EMBL Heidelberg

Unscaled Trees



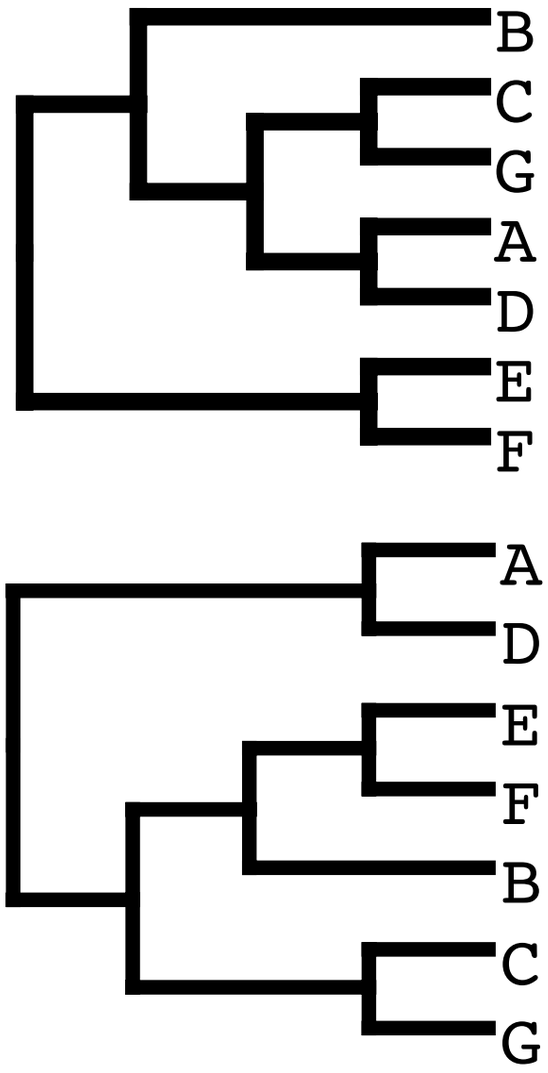
Same **unscaled** unrooted tree

Branch lengths provide no information

Branch lengths usually chosen to align OTU labels

Re-rooting the tree typically changes the choice of branch lengths

Scaled Trees



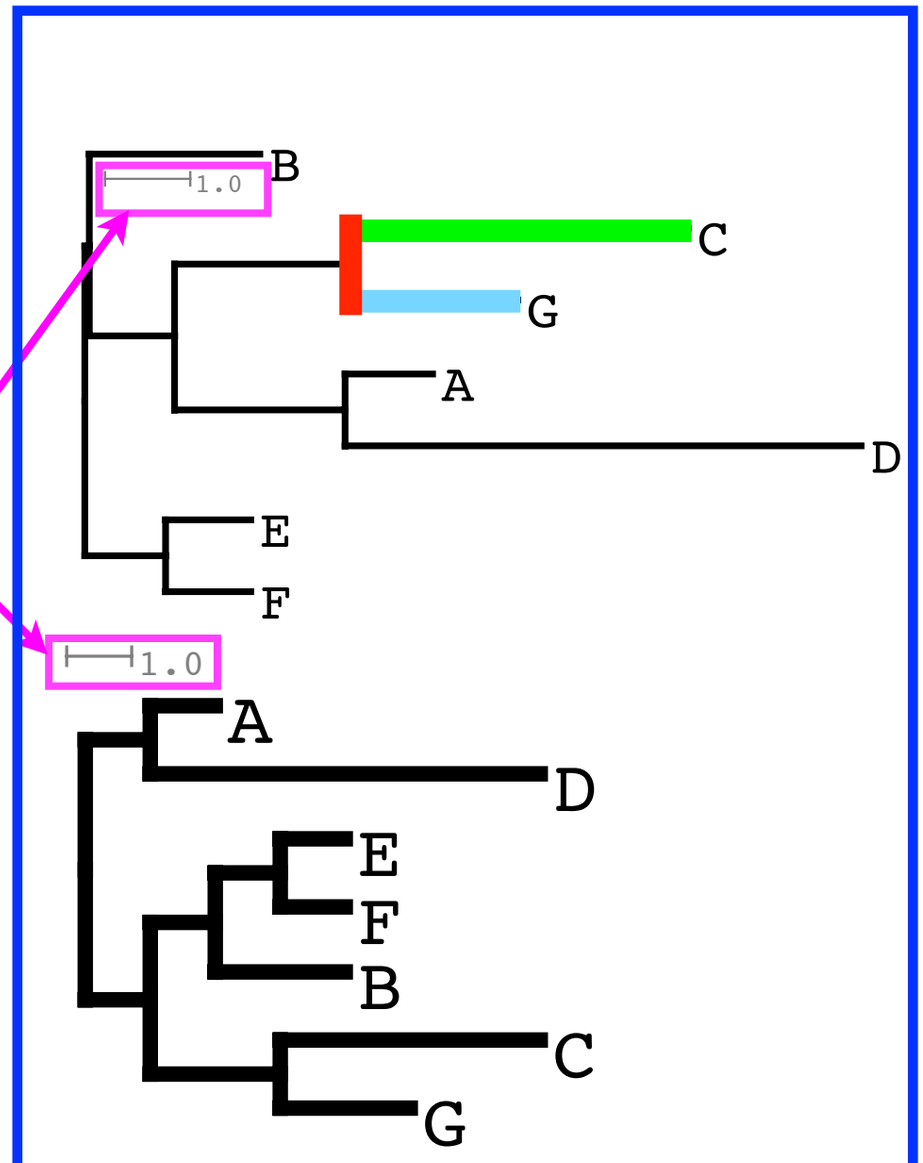
Same **unscaled** unrooted tree

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!)



Same **scaled** unrooted tree

Branch Lengths

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

```
SeqA  IKTILKWWSP
SeqB  IKTIVKWDSP
```

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**

Branch Lengths

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

SeqA IKTILKWWSP
SeqB IKTIVKWDSP

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**

More Rooted Tree Terminology

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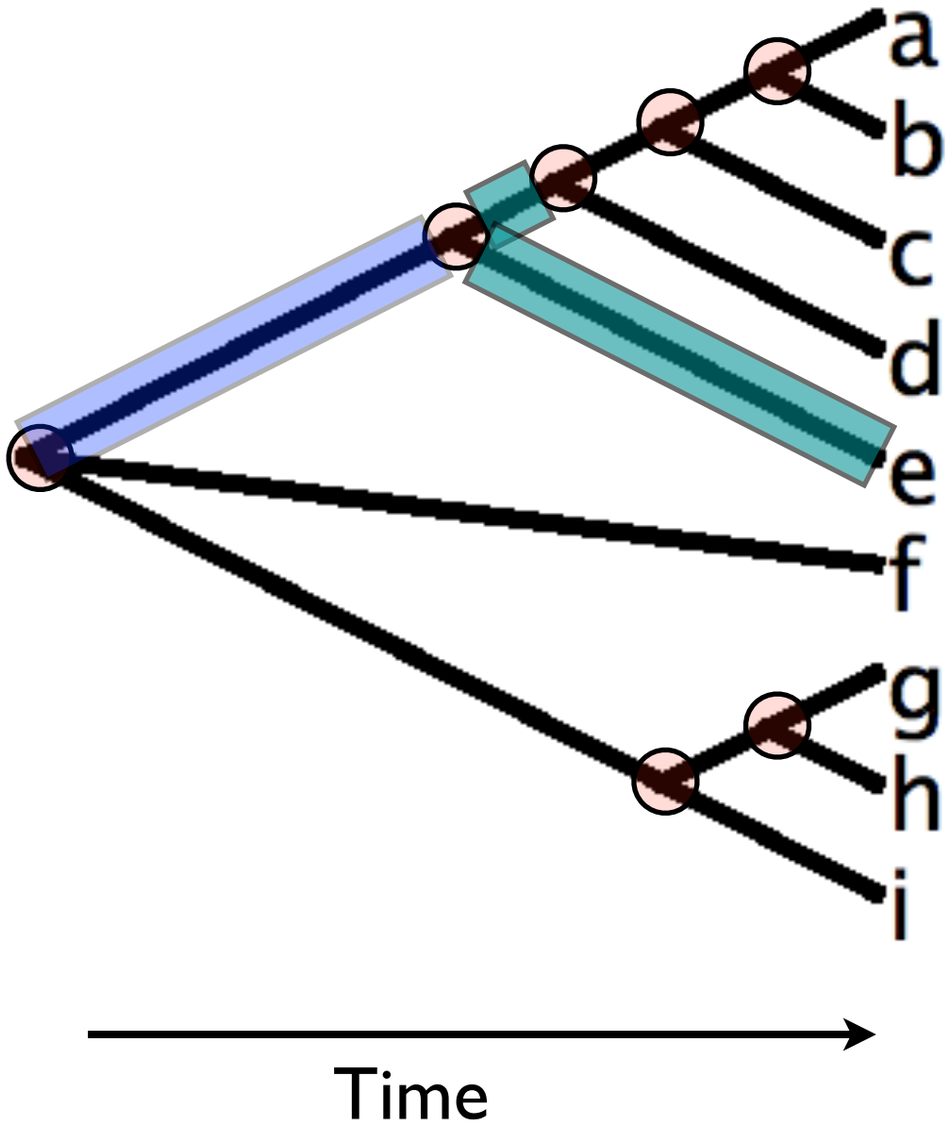
Parent/Daughter Branches

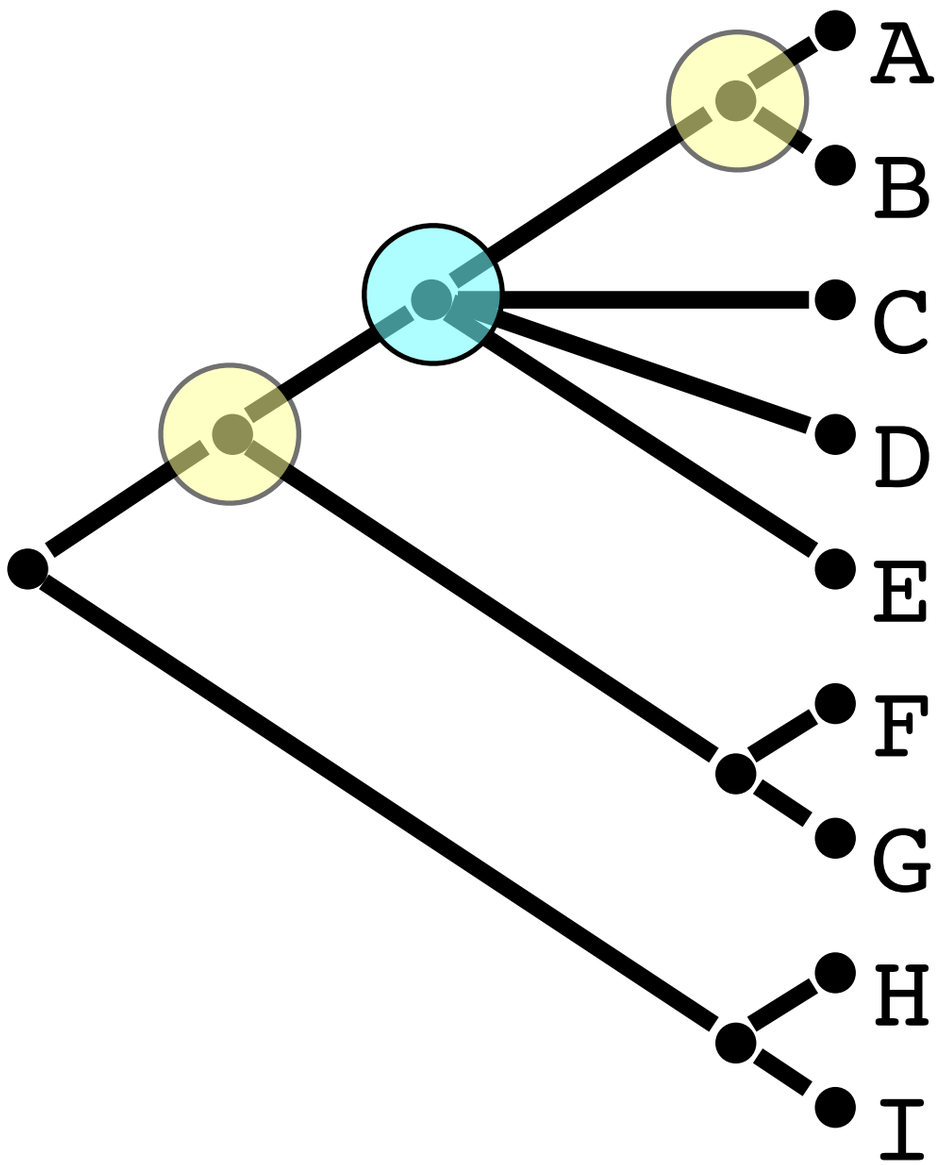


parental/ancestral lineages/branches

diverge into

multiple **daughter** lineages/branches

Polytomies



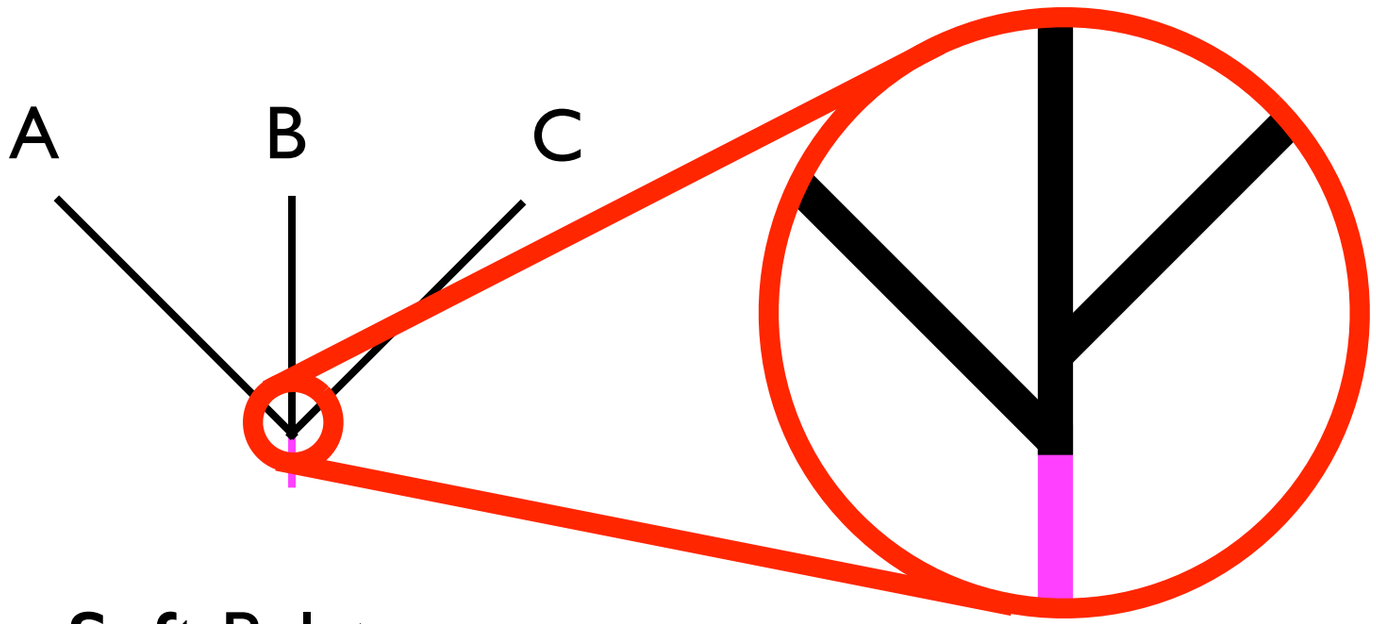
Polytomies

Internal nodes associated with more than two daughter branches

Internal nodes with **two daughter branches** are **bifurcations**

How many bifurcations on the tree? (a) **4** (b) **5** (c) **6**

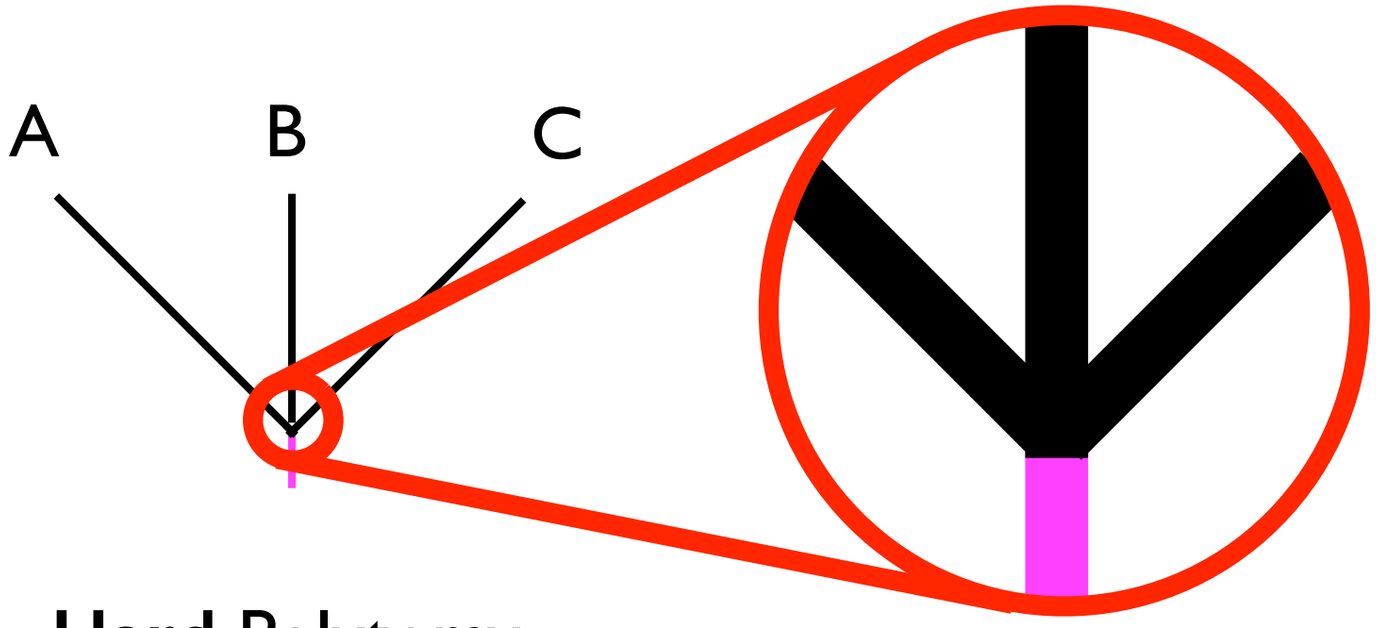
Interpreting Polytomies



Soft Polytomy

Lineages only bifurcate - internal lineages so short that no identifiable change/evolution occurred along them

Thus true pattern of lineage divergence cannot be resolved



Hard Polytomy

Ancestral lineage diverged into 3+ lineages simultaneously

NB: Some software only accepts bifurcating trees

Relatedness

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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

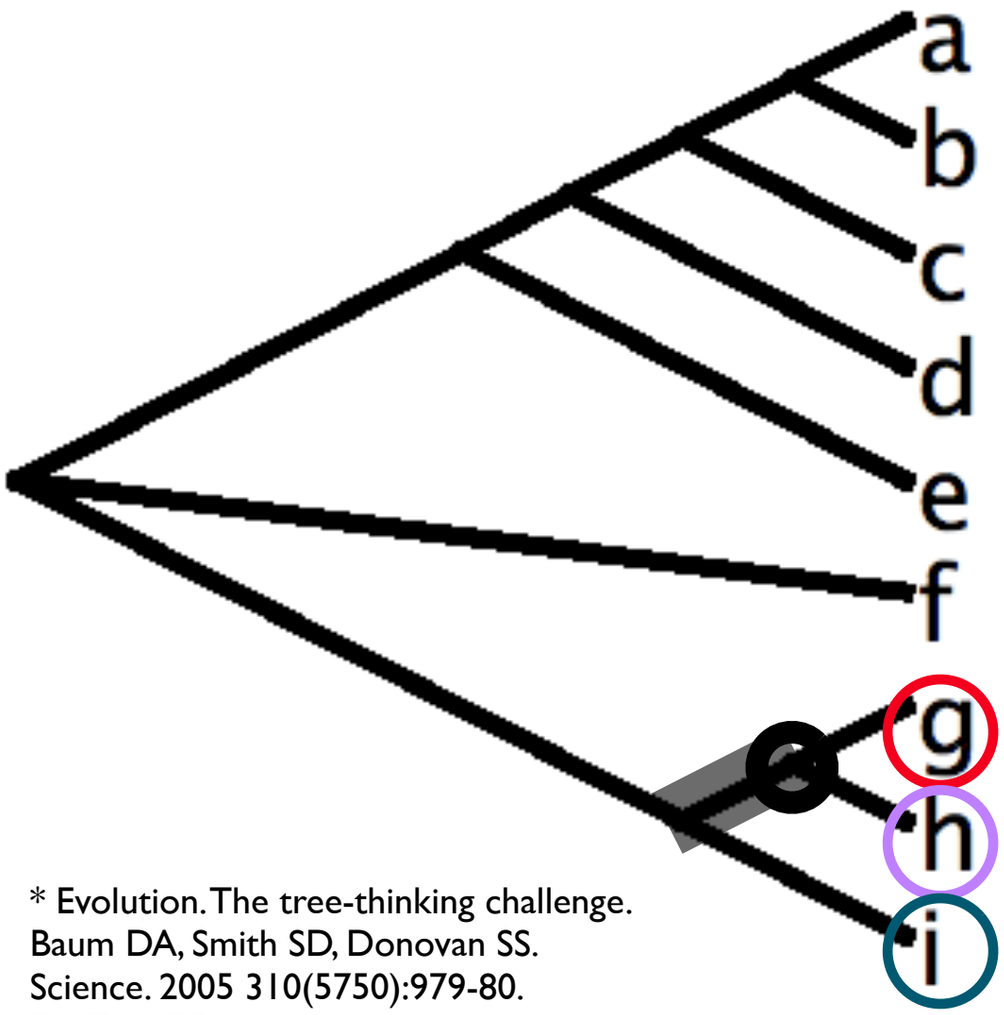
As many trees are estimated to inform ideas about patterns of relatedness, we need a clear understanding of how the term is used in this context

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

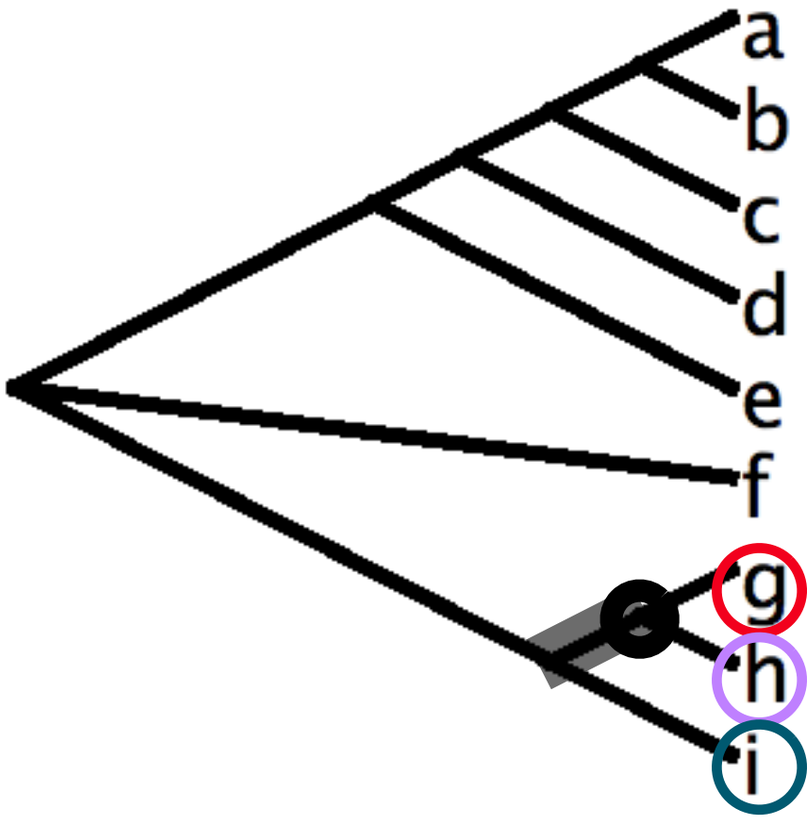
is associated with the extent of ancestry (i.e. the number of ancestors) taxa share with each other

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

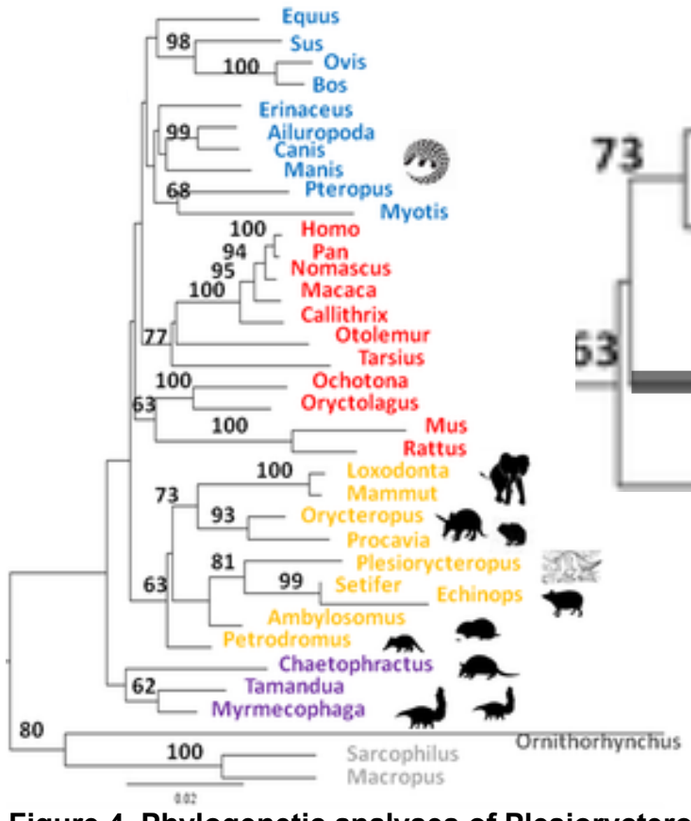
Relatedness
(in the context of phylogenetic trees)

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

g is **more closely related** to **h** than **g** is to **i**



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



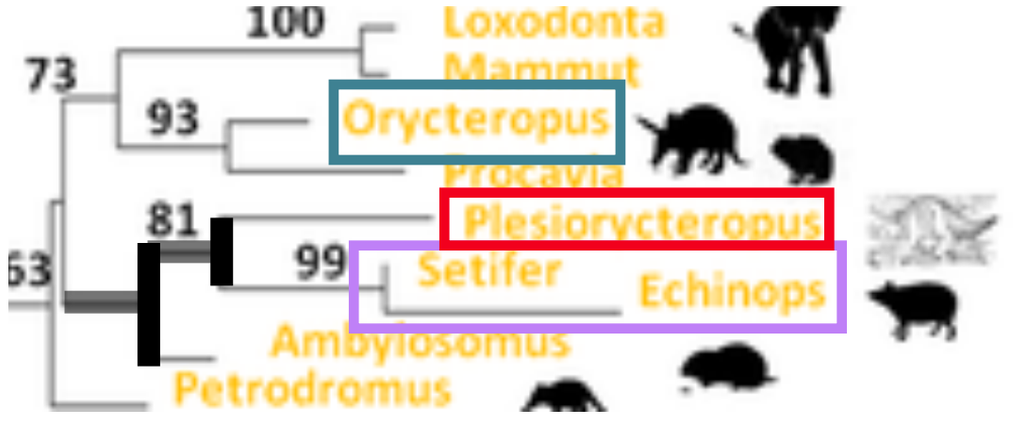


Figure 4. Phylogenetic analyses of Plesiorycteropus collagen (I) sequences obtained by LC-MS in comparison to previously postulated closest relatives.

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

* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
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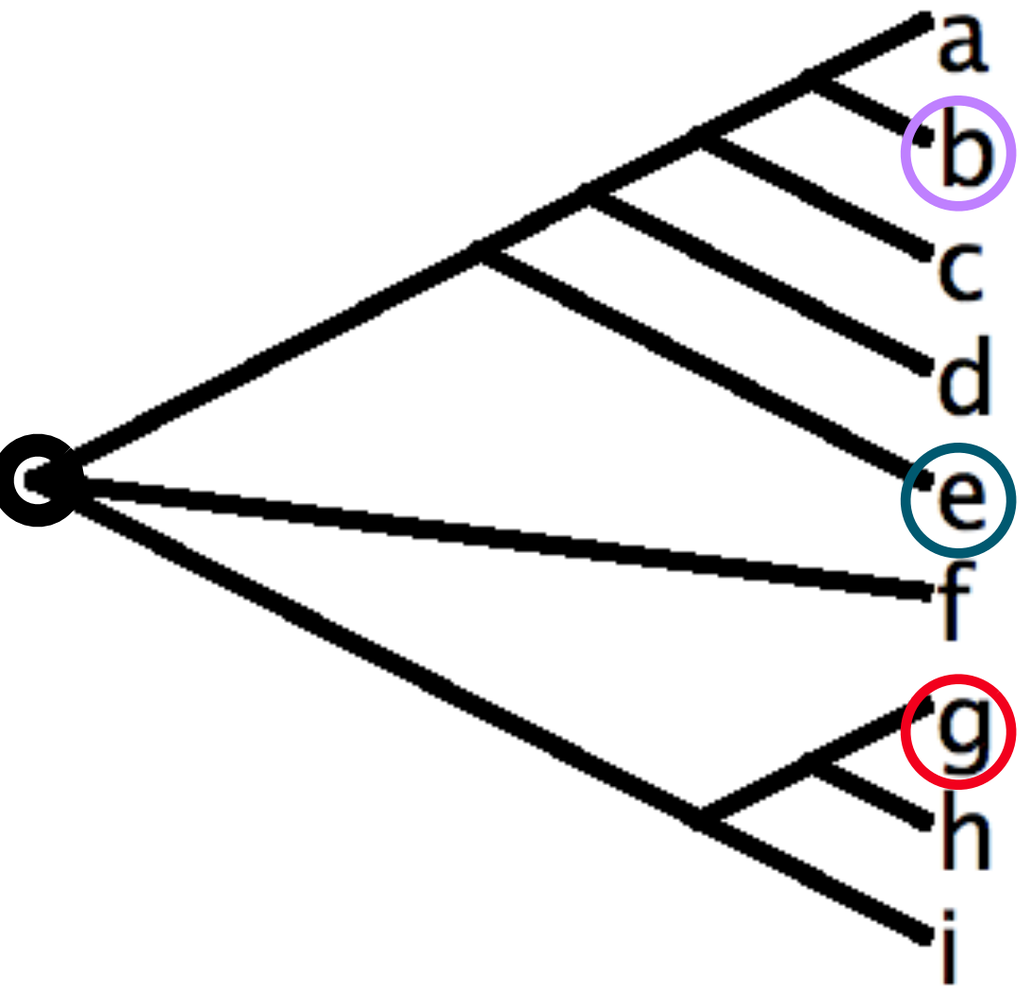
Relatedness
(in the context of phylogenetic trees)



of all the OTUs represented in this tree

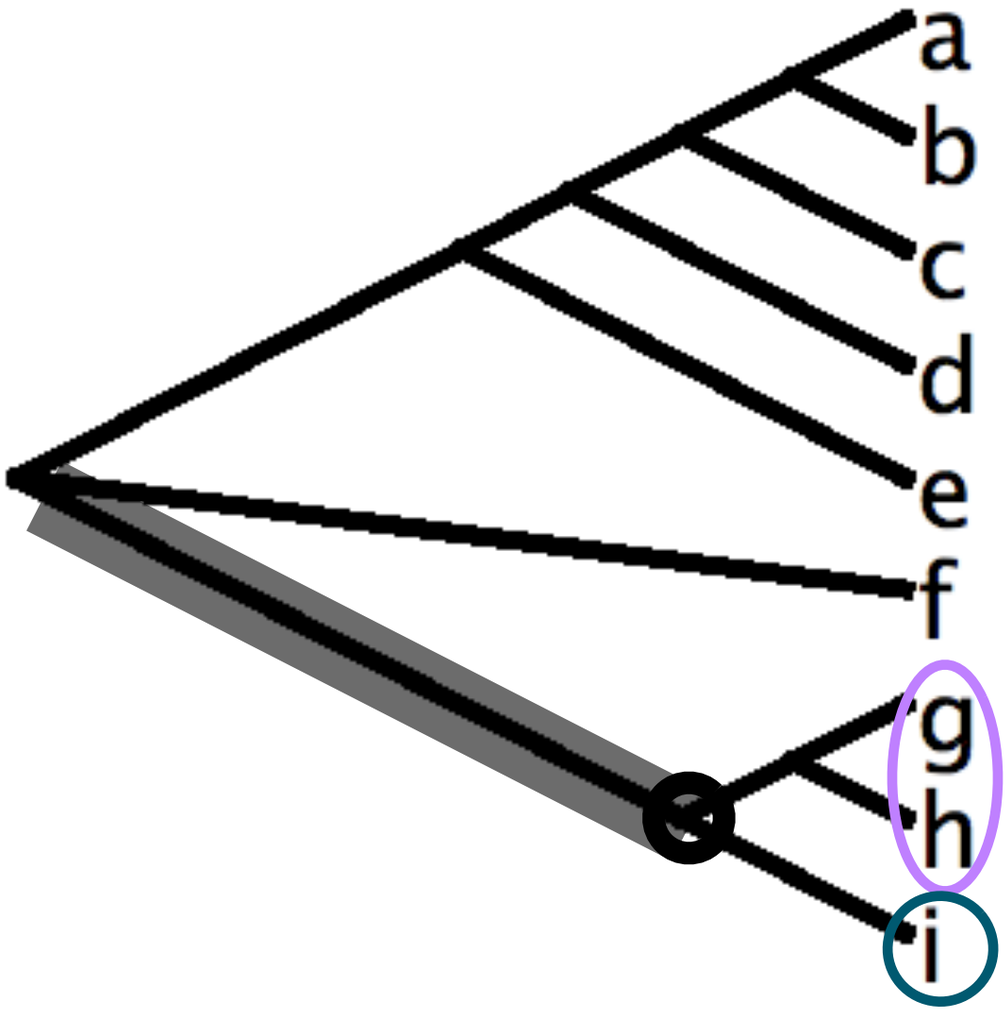
g is **equally distantly related** to **b** as it is to **e**

because **g** shares **the same** most recent common ancestor with **b** as it does with **e**

"the two species are equally distantly related to *S. cerevisiae*."

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)



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.

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**)

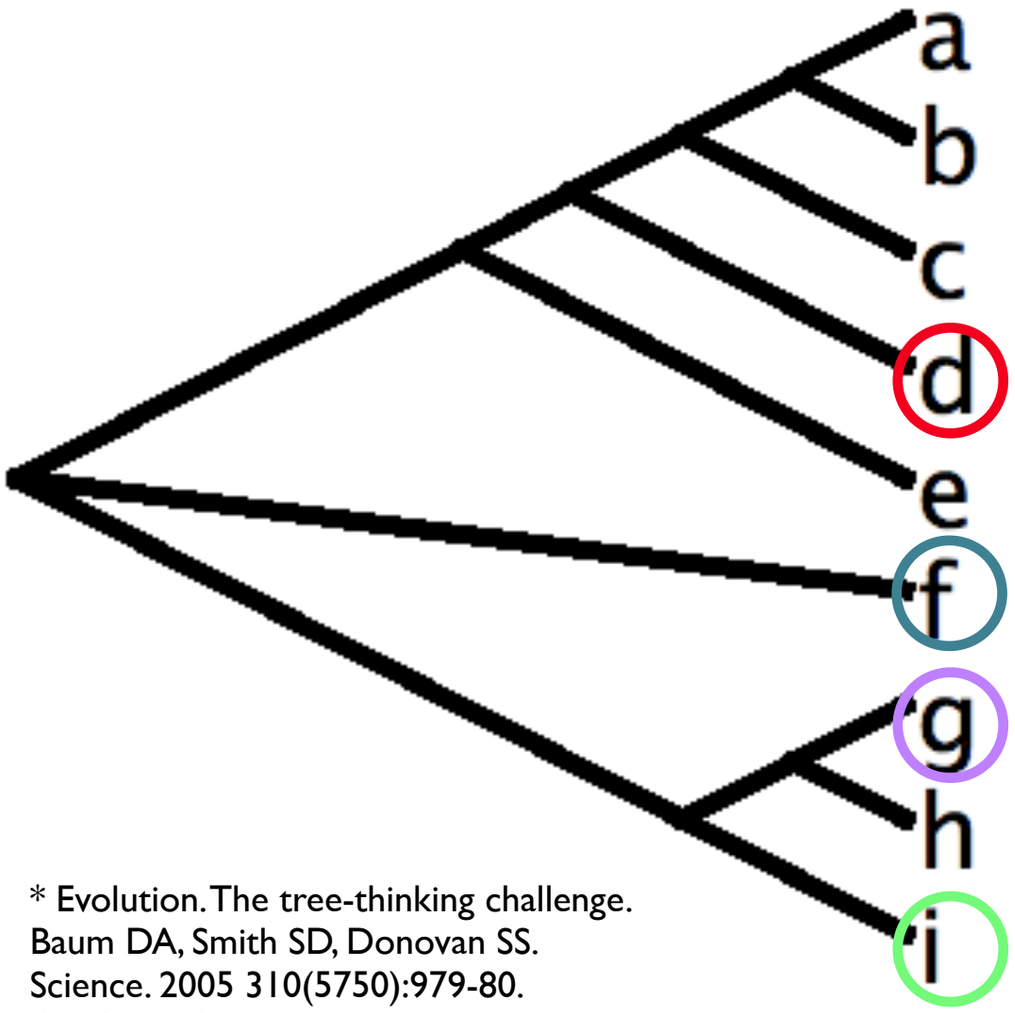
because **i** shares common ancestors with **g** and **h** that it does not share with any other OTUs in the tree

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

Aidan Budd, EMBL Heidelberg

Relatedness
(in the context of phylogenetic trees)

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



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

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

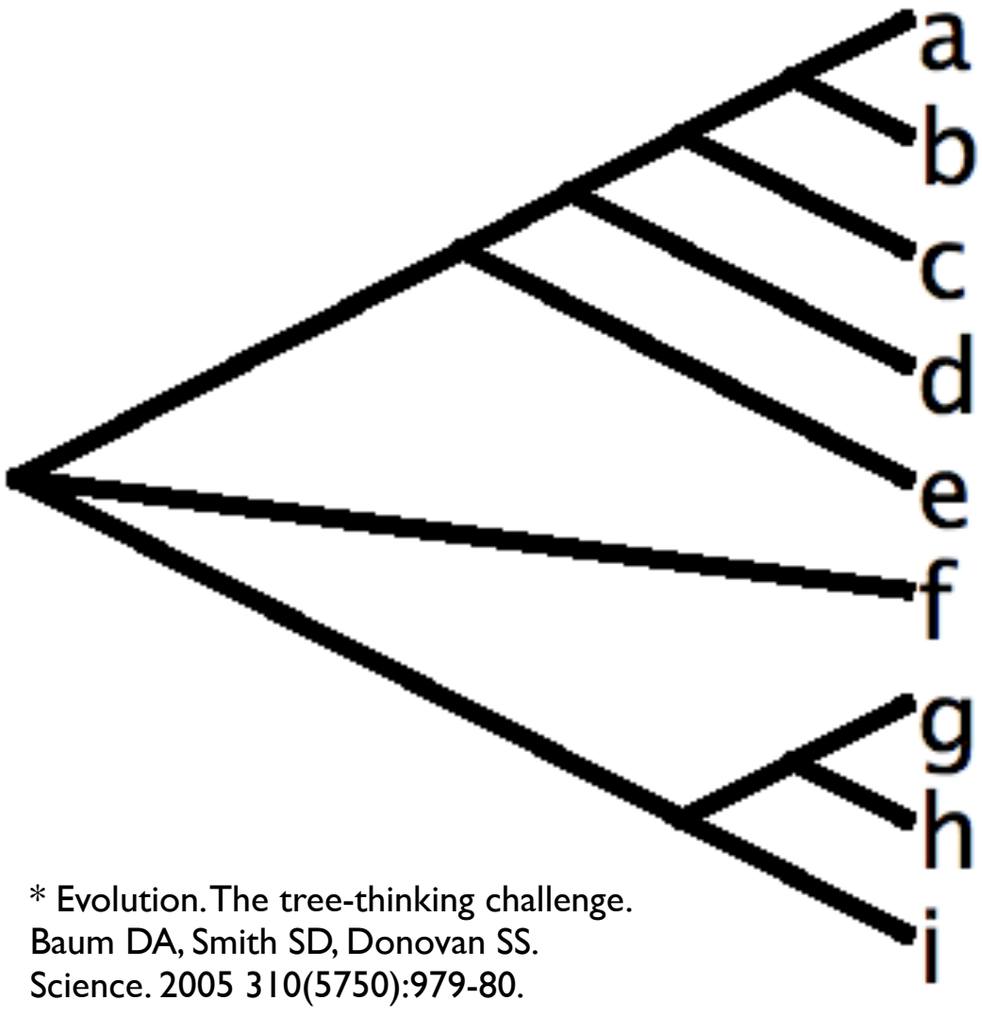
1. d is **more closely related** to g than to f or i

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

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

Relatedness
(in the context of phylogenetic trees)

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

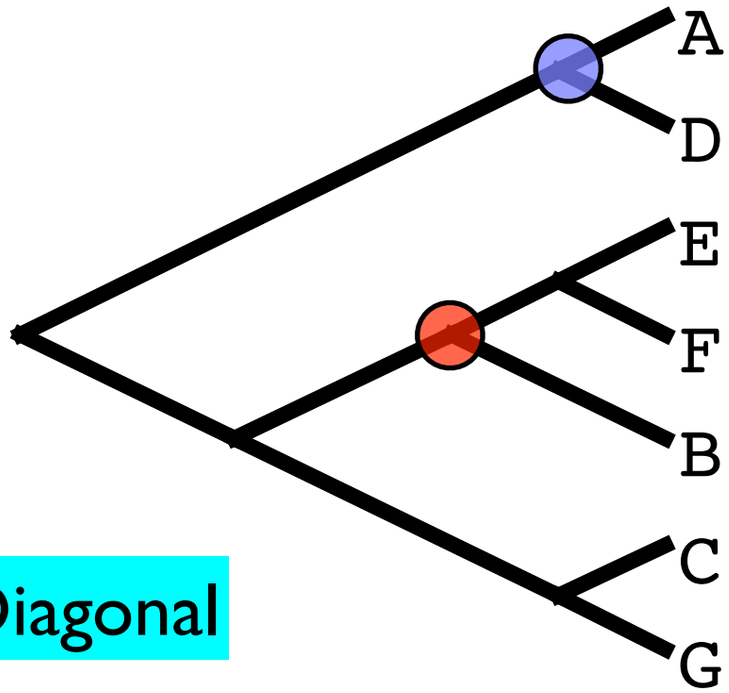


* Evolution. The tree-thinking challenge.
Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

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

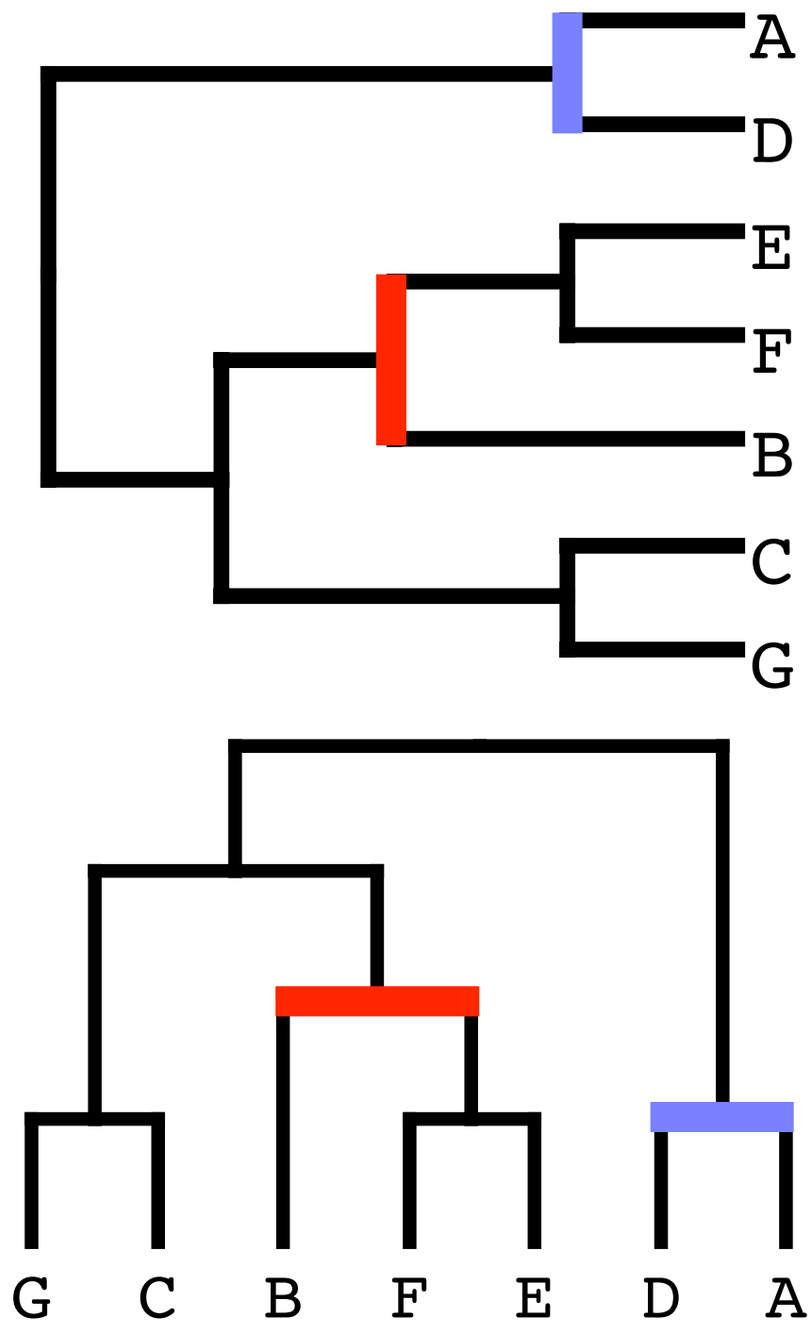
Tree Representations



Diagonal

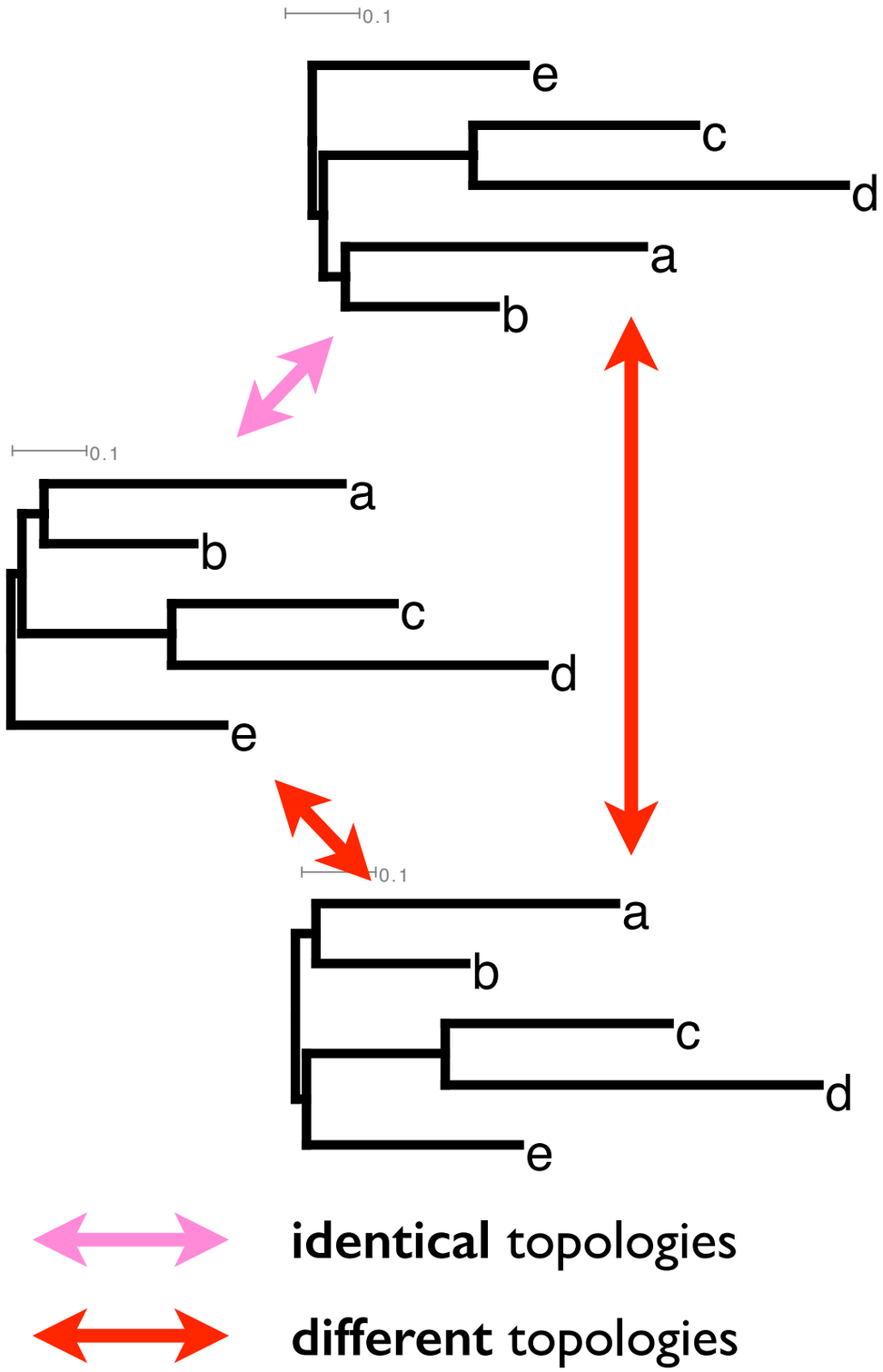
Most rooted tree figures use a "rectangular" rather than a "diagonal" representation

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



Rectangular

Tree Topology



identical topologies

different topologies

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

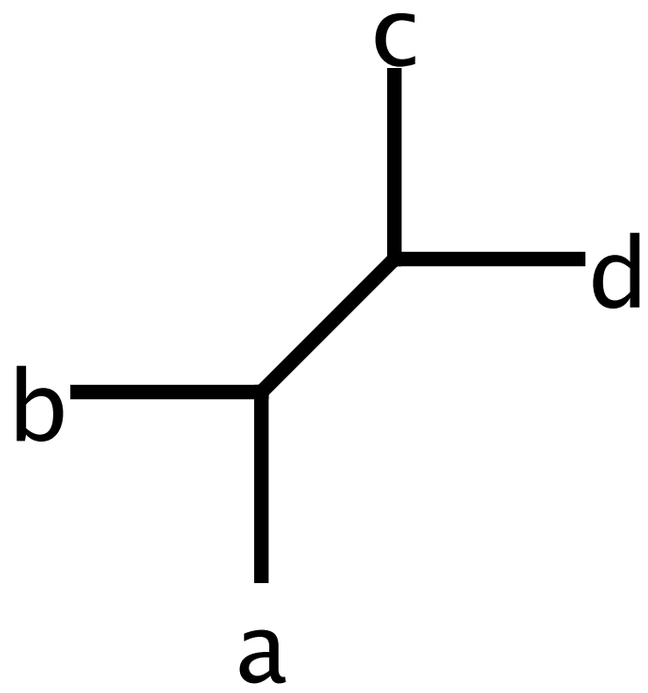
Unrooted Phylogenies

Aidan Budd, EMBL Heidelberg

Unrooted Trees

There's no root on the tree...

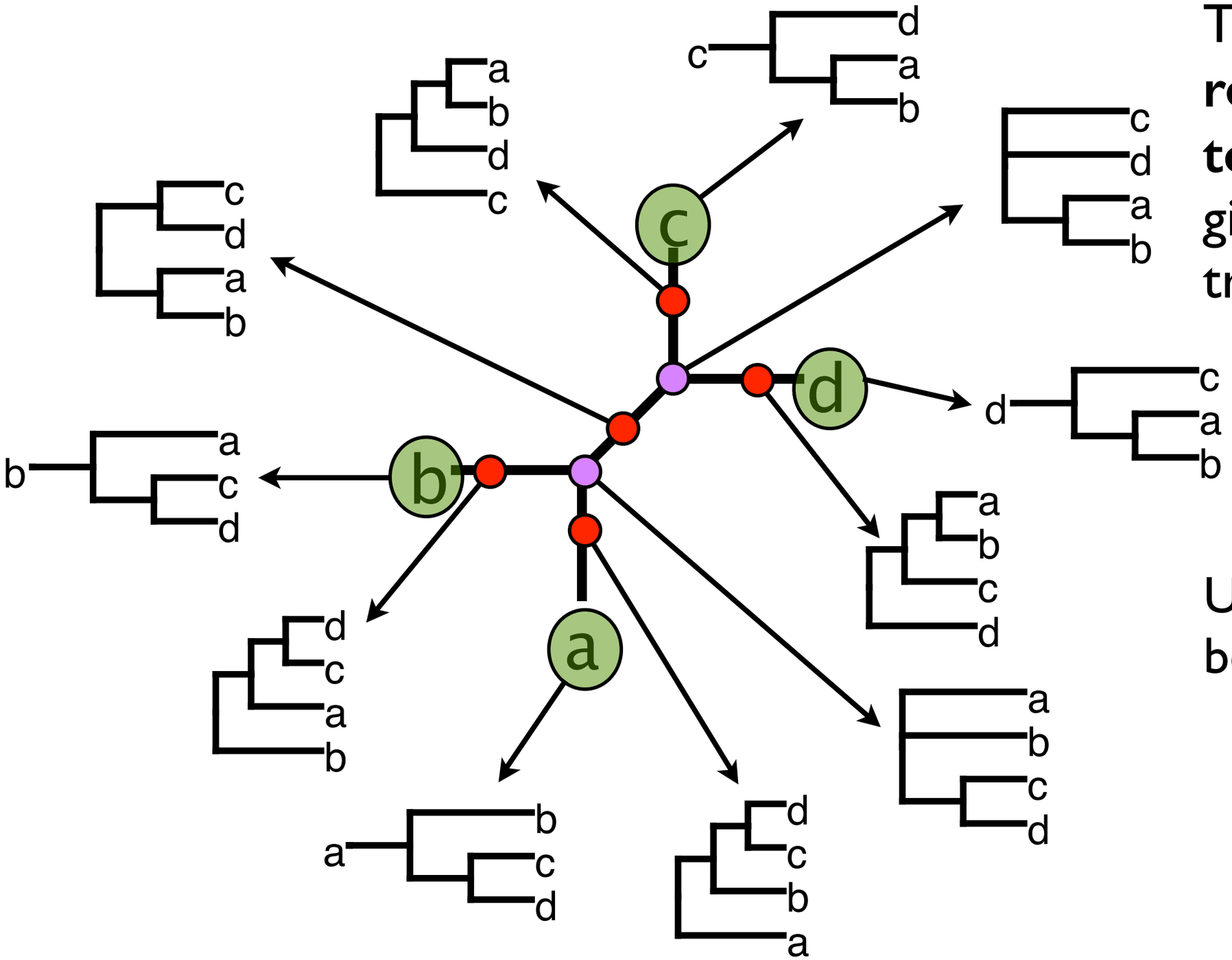
... which is usually interpreted as meaning that these taxa are related by a rooted tree **but** we don't know where the root is



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:

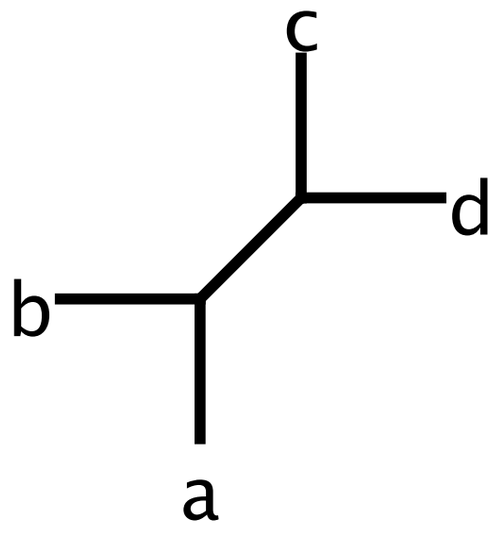
- branches
- interior nodes
- terminal nodes

Quiz

Which of these statements is true, given the unrooted tree topology shown below?

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**



None of these is true, given this unrooted tree topology, under all possible rootings of the tree

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

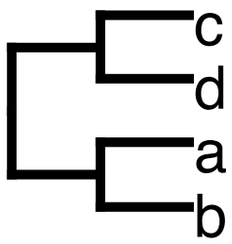
And, while 3. is true for some of the rooted trues, in others it is not

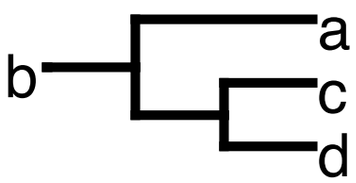
Draw the set of rooted tree topologies in which statement 3. is:

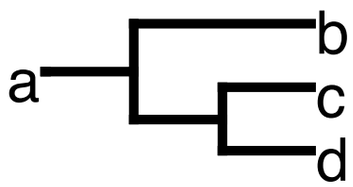
- true
- false

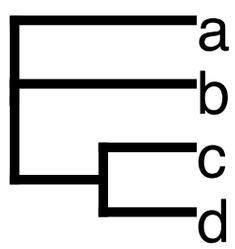
Quiz

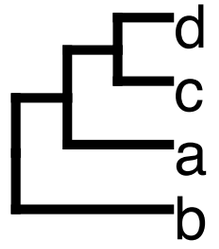
d more closely related to **c** than it is to **a** or **b**

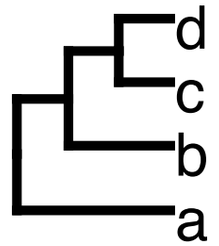




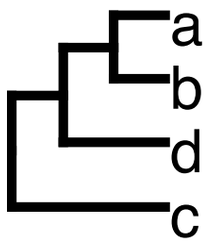


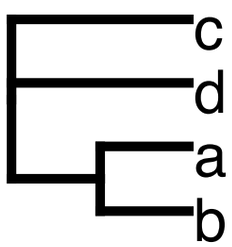


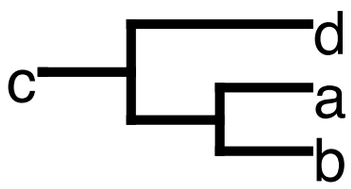


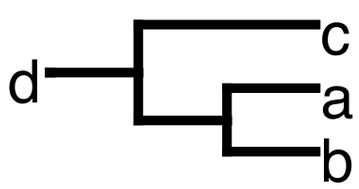


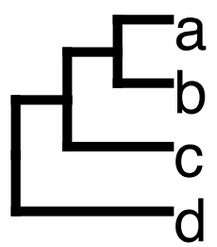
d not more closely related to **c** than it is to **a** or **b**





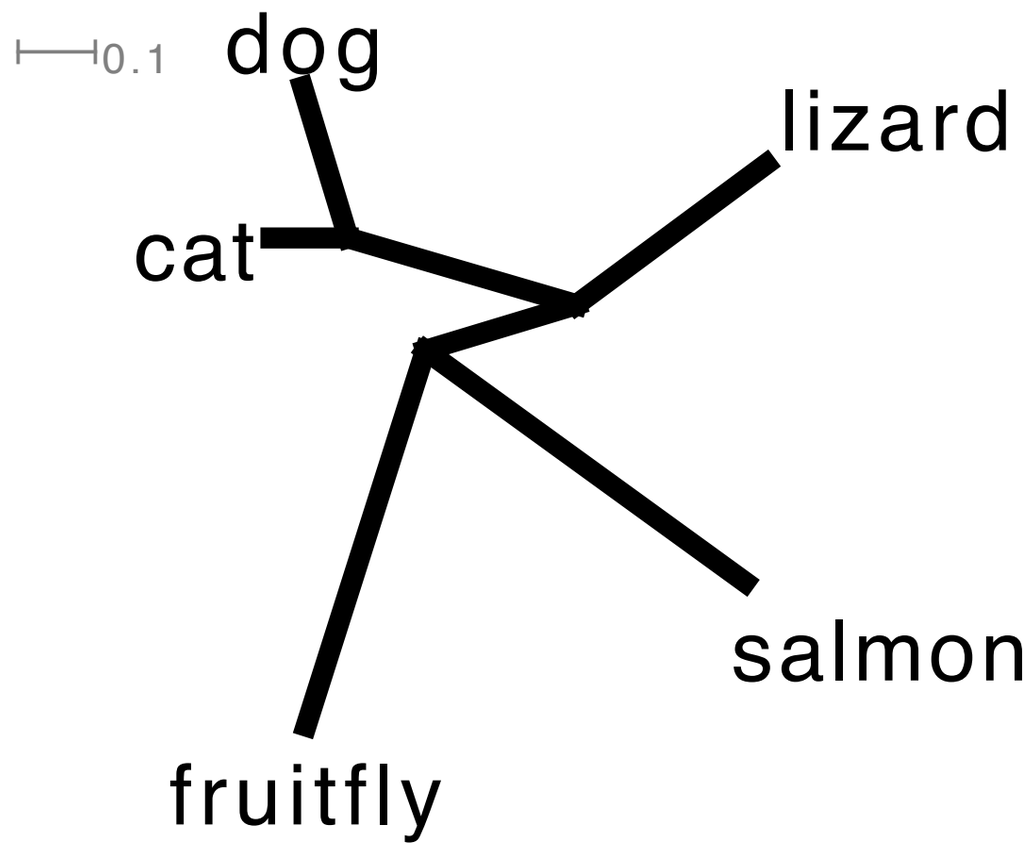






Unrooted ⟶ Rooted

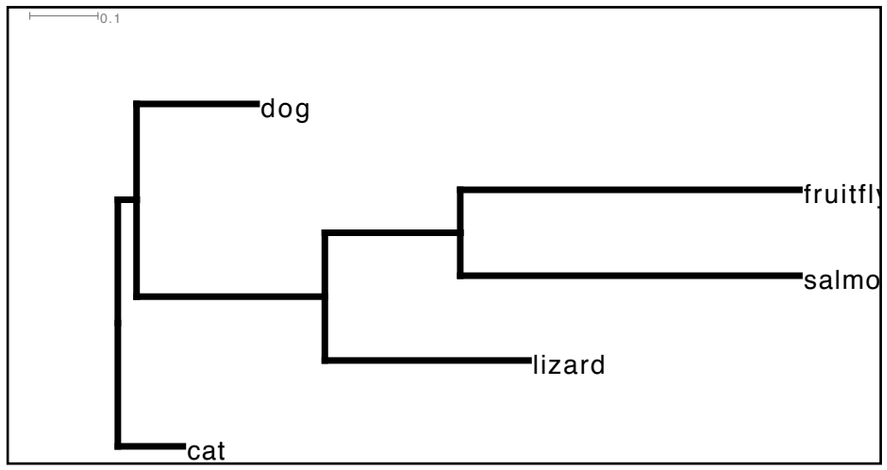
This **unrooted** tree can be rooted to yield several different **rooted topologies**.

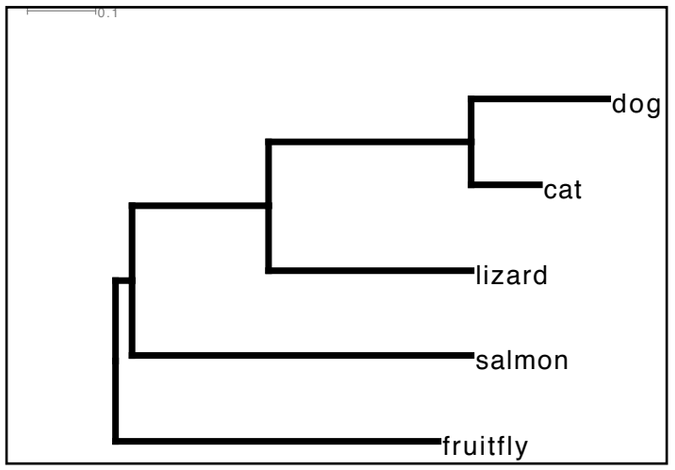


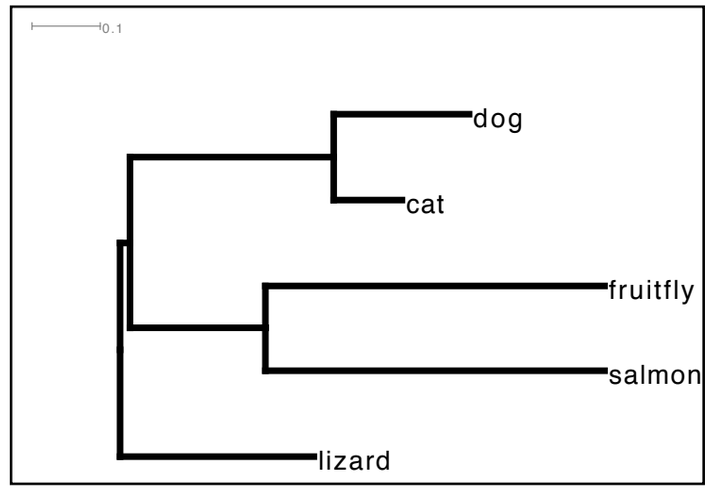
How many different rooted topologies exist by placing the root on a/an:

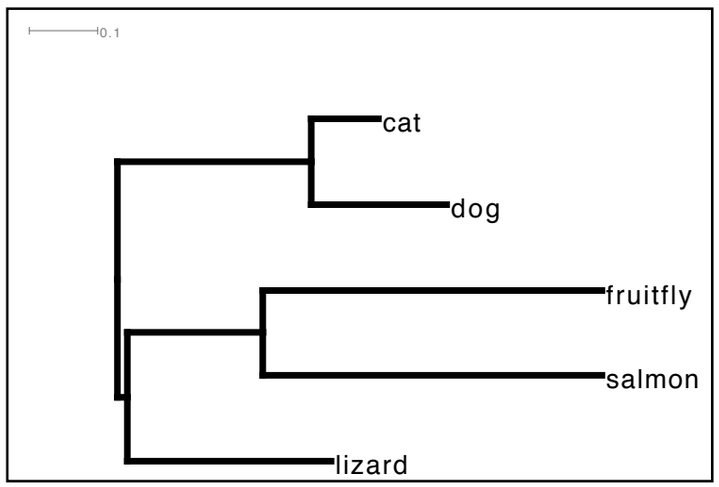
- branch? Draw all of these.
- terminal node? Draw one of these.
- internal node? Draw one of these.

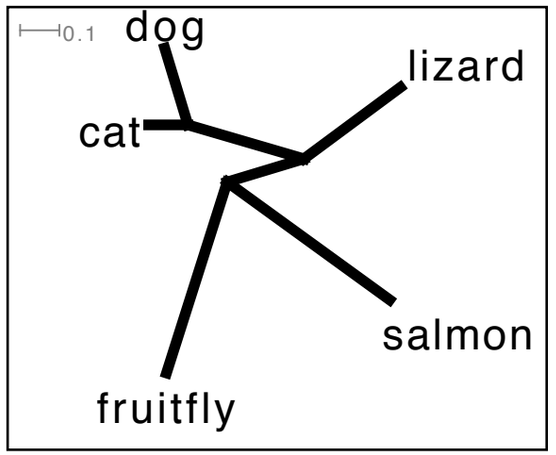
All Topologies Rooted on a Branch of the Unrooted Tree

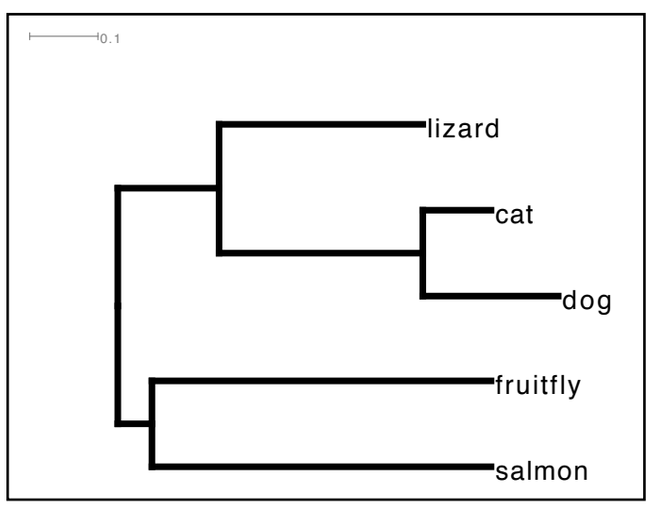


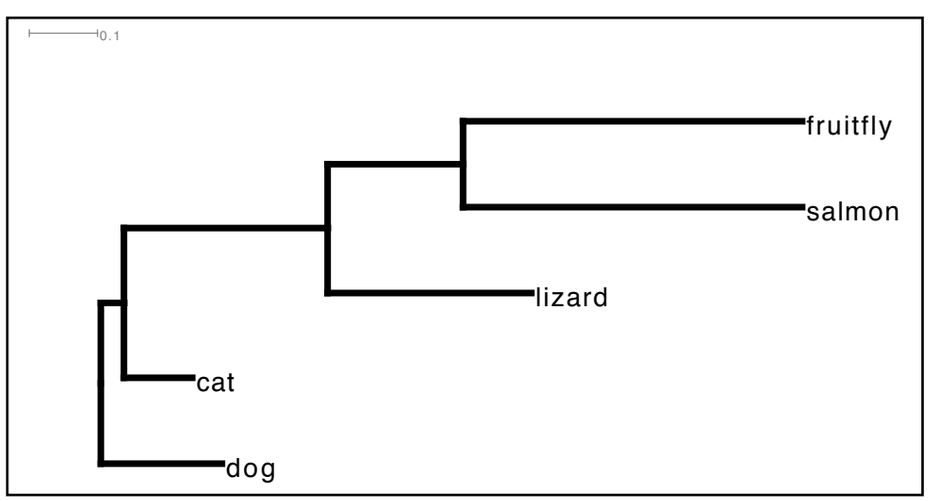


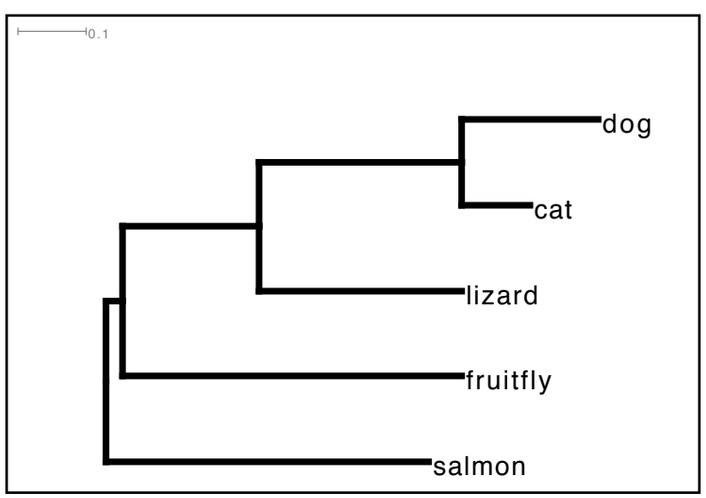












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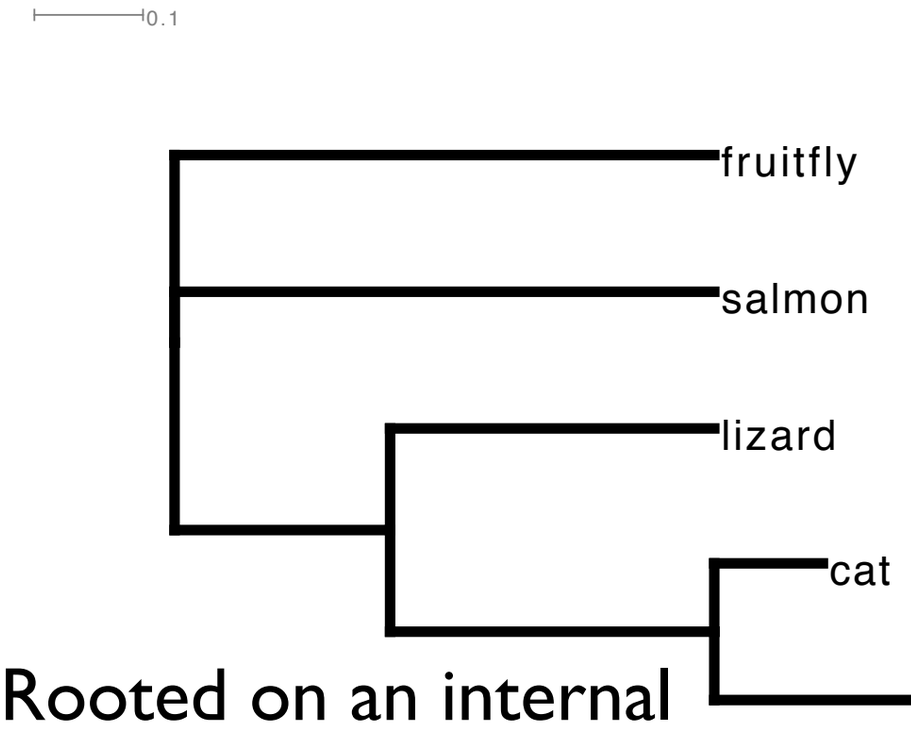
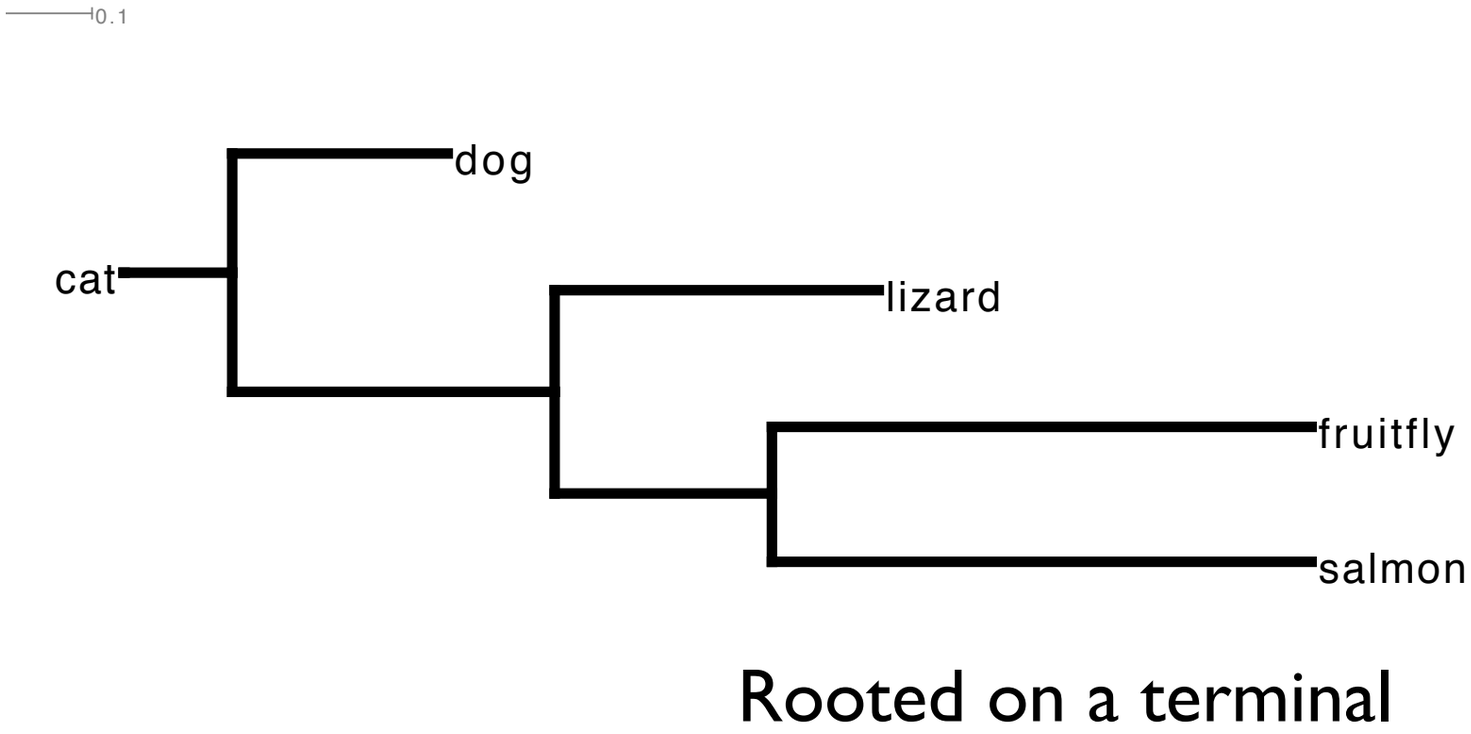
Roots on Terminal and Internal Nodes

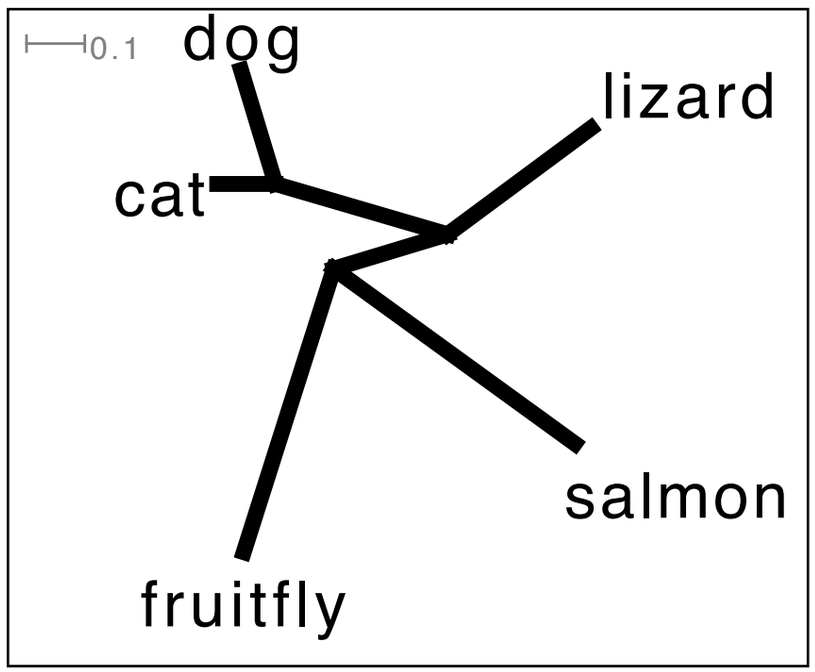


Rooted on an internal node of unrooted tree

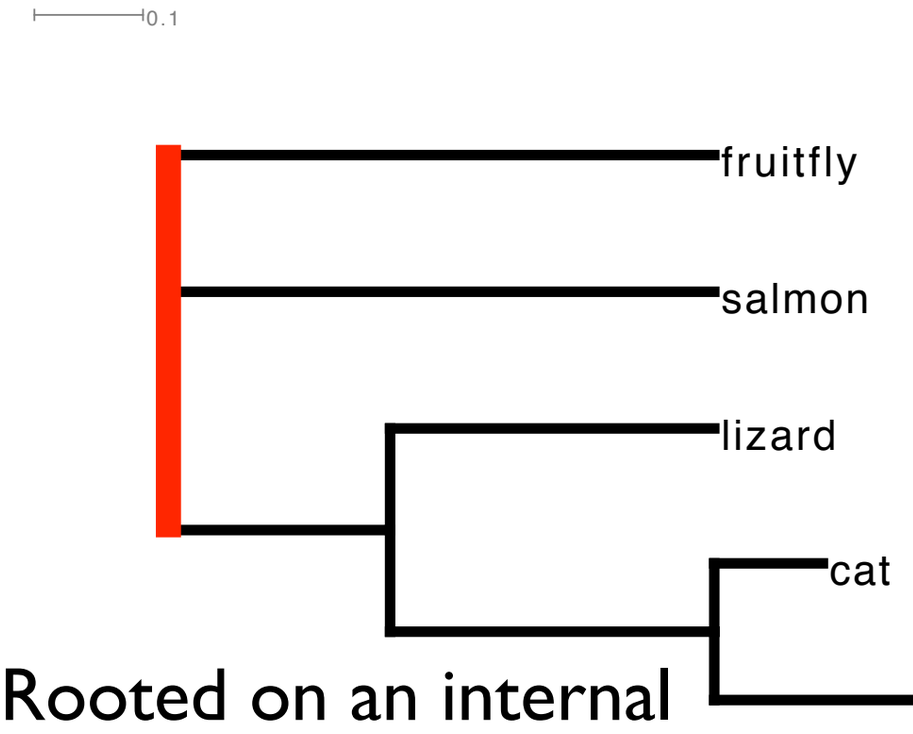


Rooted on a terminal node of unrooted tree

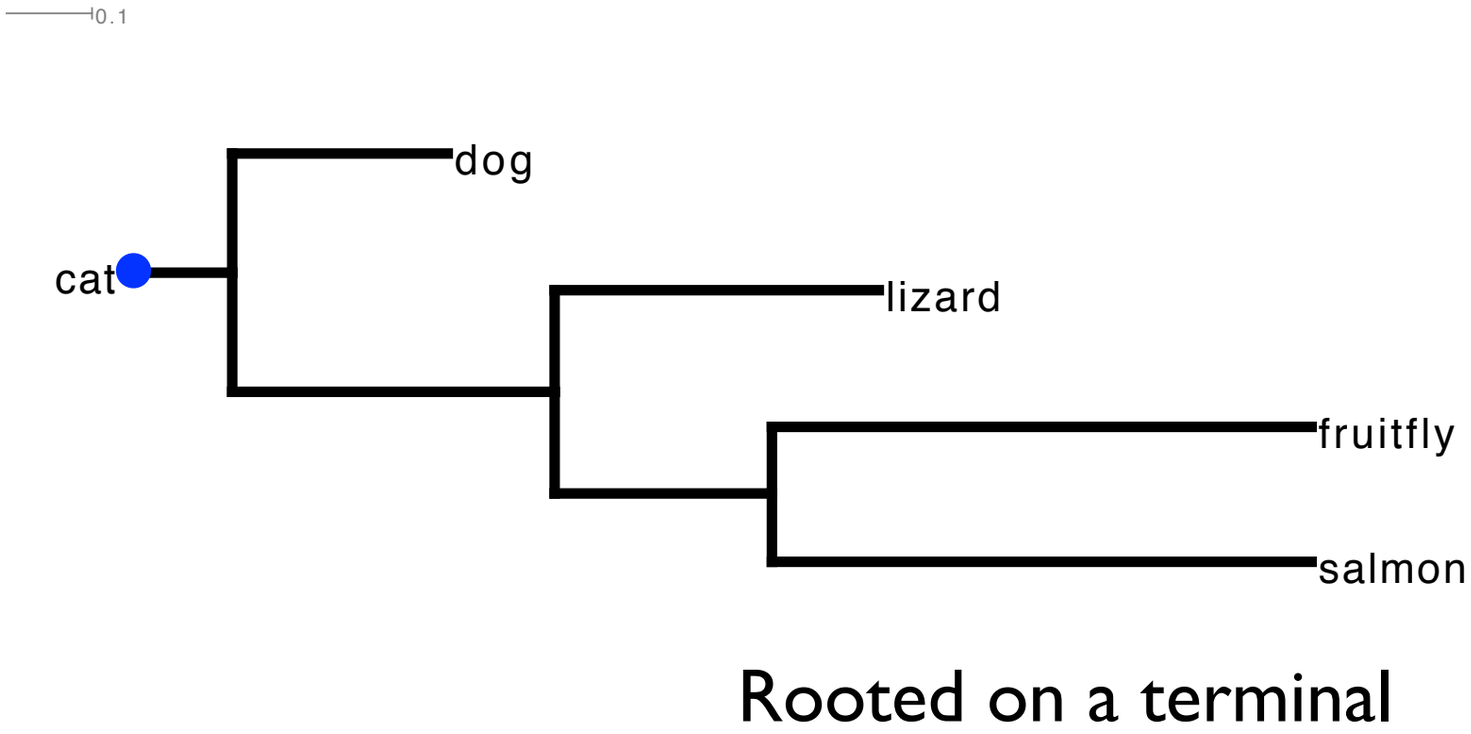
On the unrooted tree image to the right, label the two nodes on which the two above trees are rooted



Roots on Terminal and Internal Nodes

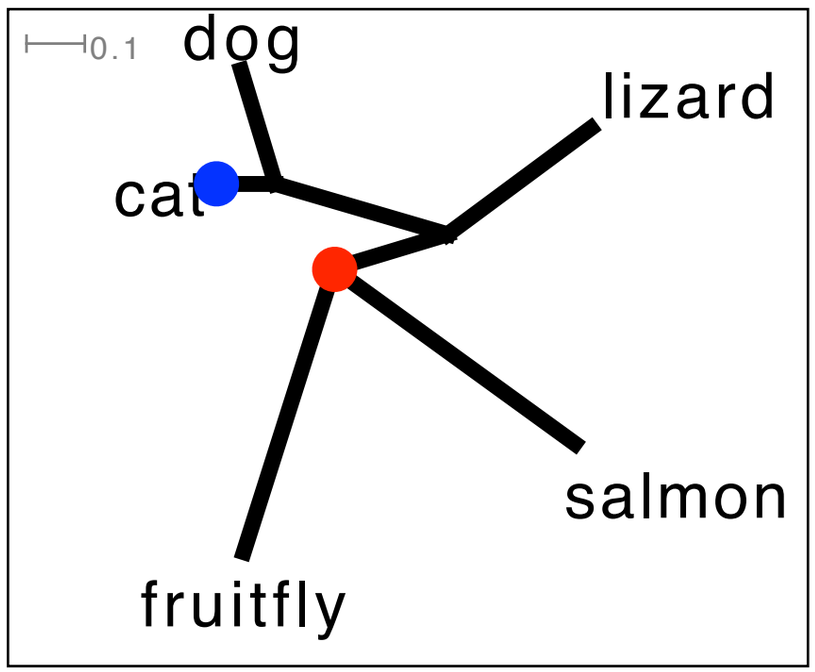


Rooted on an internal node of unrooted tree



Rooted on a terminal node of unrooted tree

On the unrooted tree image to the right, label the two nodes on which the two above trees are rooted



Aidan Budd, EMBL Heidelberg

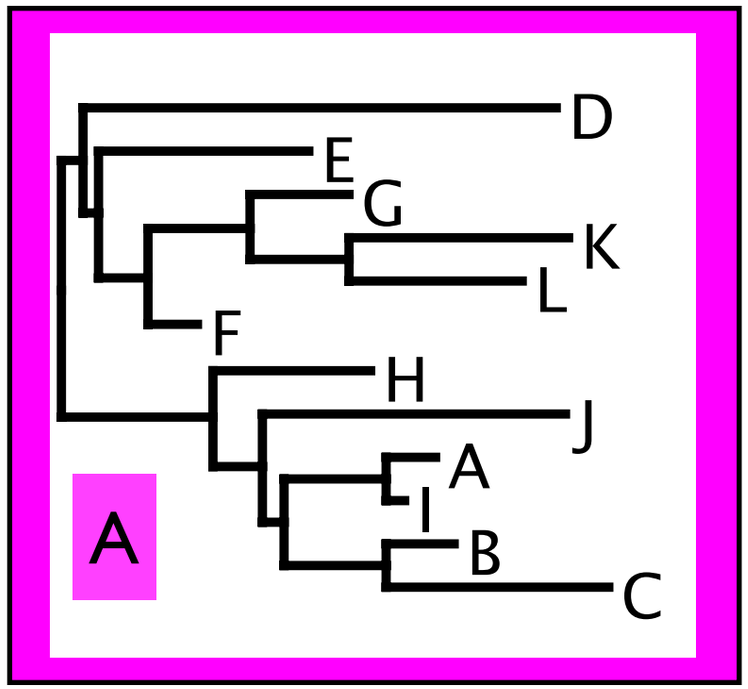
NEWICK format and tree visualisation

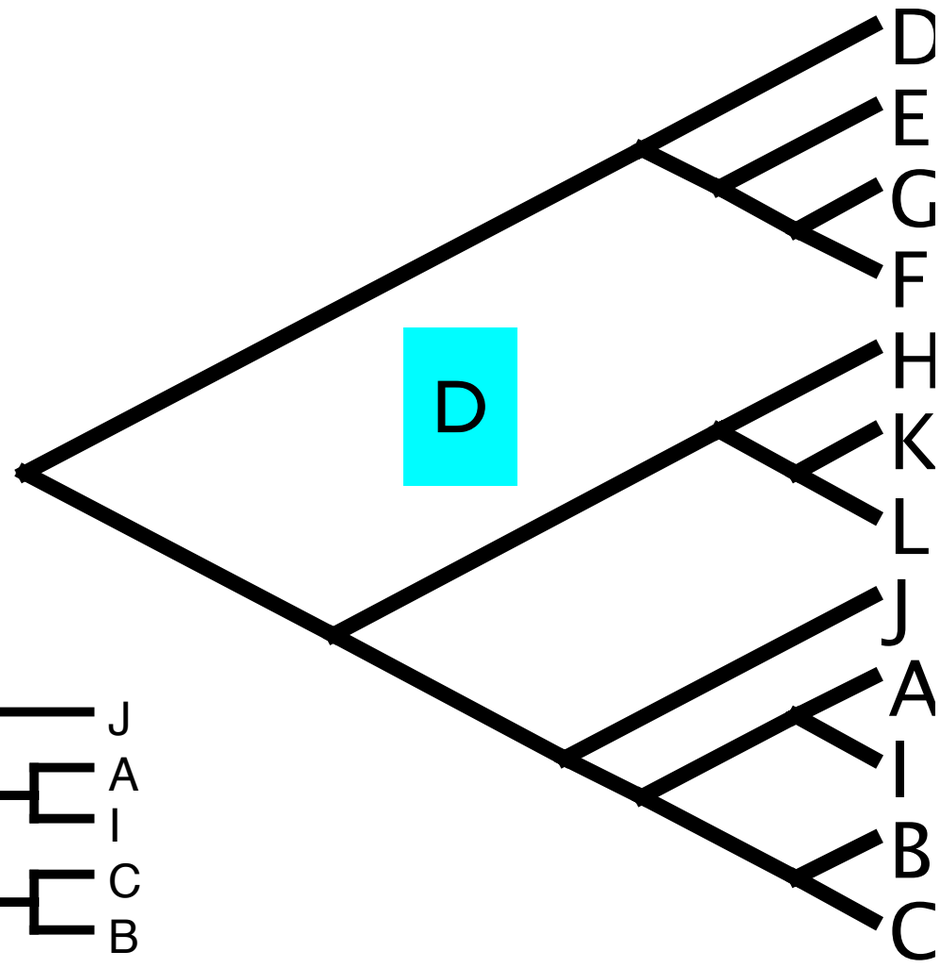
Laura Emery

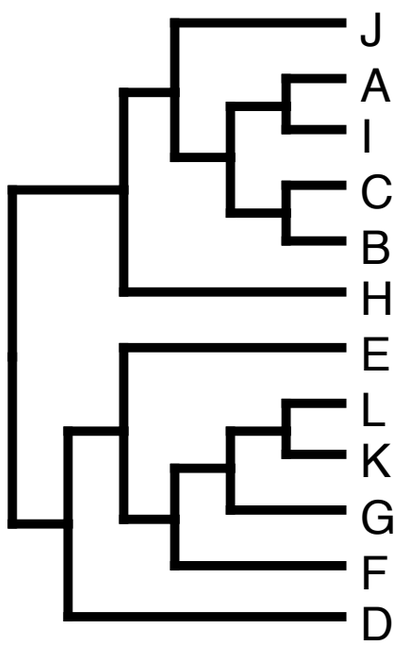
Aidan Budd, EMBL Heidelberg

Quiz: recognise identical topologies

Which of the trees has the same TOPOLOGY and ROOT as tree A?







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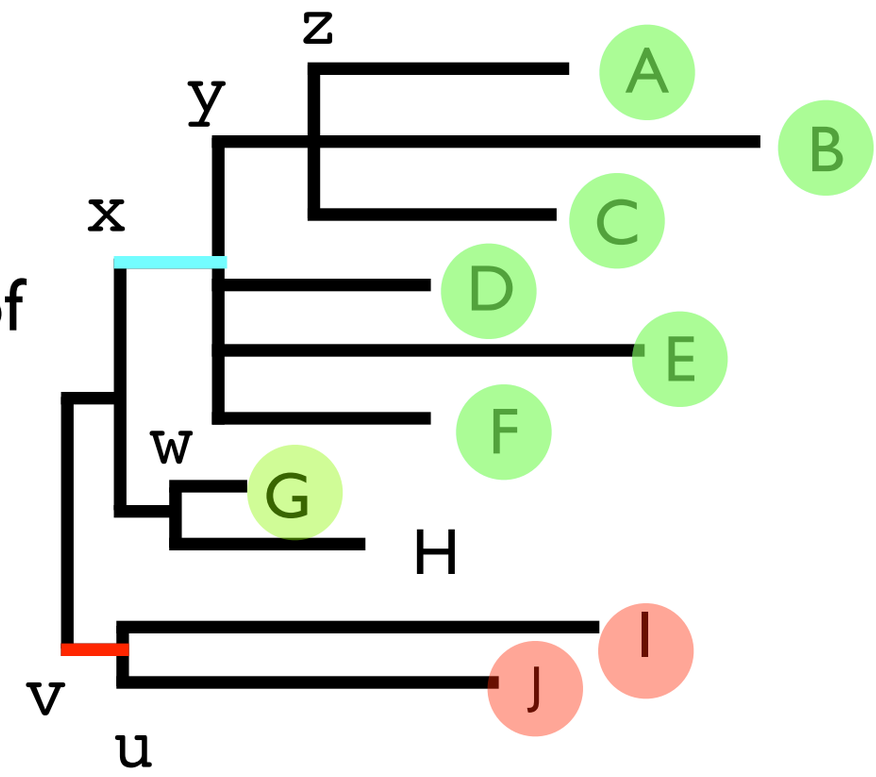
Clades

Clade:

A set of OTUs that includes **all descendants** of a given internal ancestral/internal branch



Clades:

Branch xy specifies the clade ABCDEF

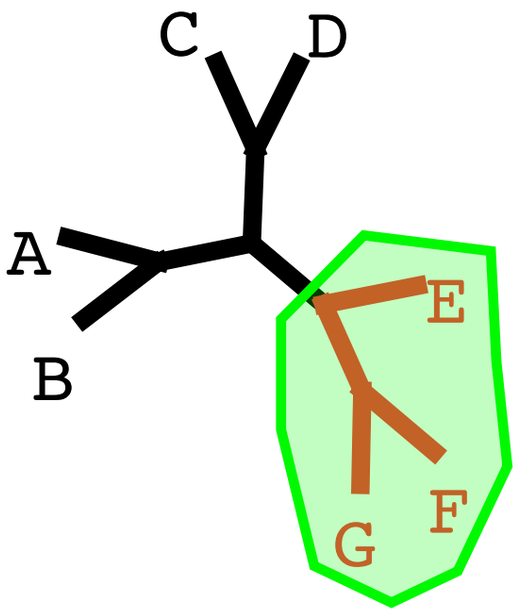
IJ is a clade as there is a branch vu for which has only IJ as its descendants

~~**Clades:**~~

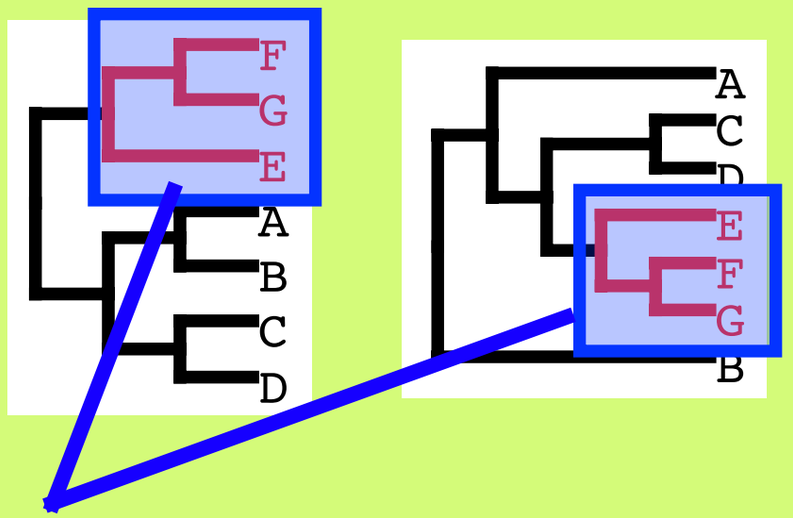
ABCDEFG - no branch has ALL and ONLY these taxa as descendants

Clans

Group of OTUs are a **clan** if there is at least one rooted phylogeny where they form a clade.



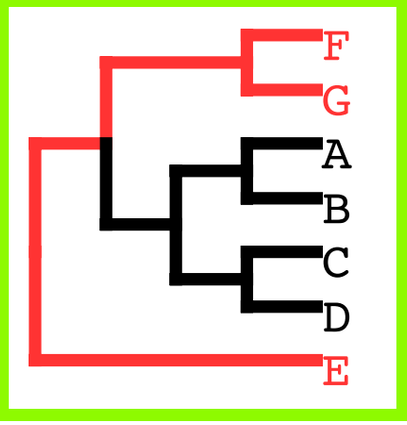
Of clades and clans: terms for phylogenetic relationships in unrooted trees.
Wilkinson M, McInerney JO, Hirt RP, Foster PG, Embley TM.
Trends Ecol Evol. 2007 Mar;22(3):114-5.
PMID: 17239486

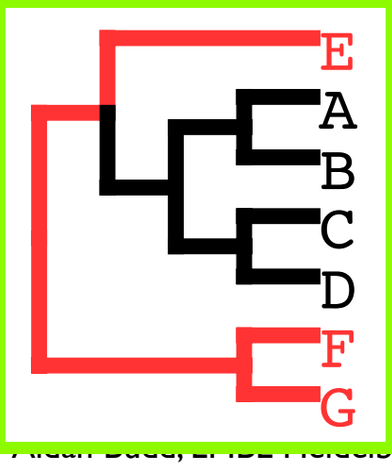


clades

therefore EFG form a **clan**

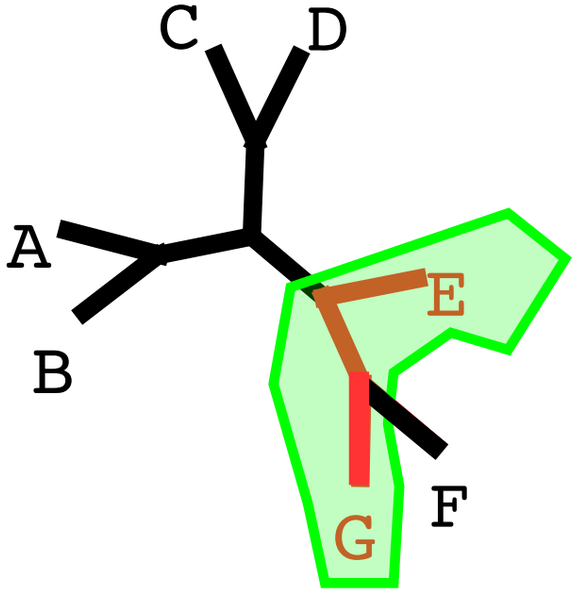
However! Under some rootings EFG **does not** form a clade

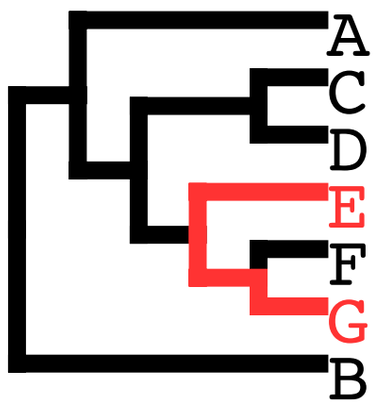


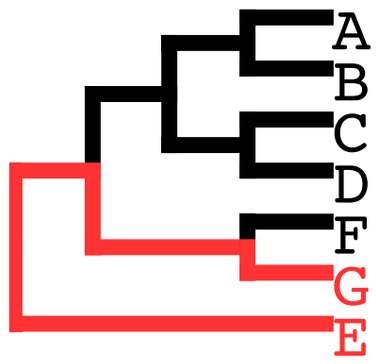


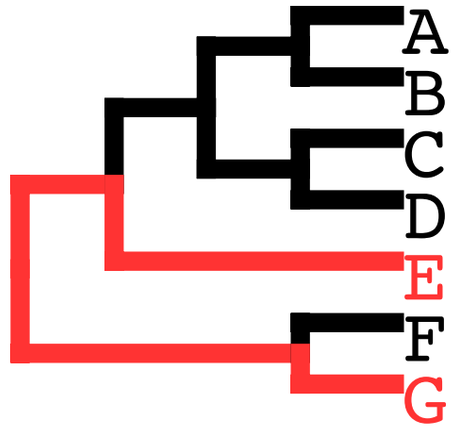
Clans

Group of OTUs are a **clan** if there is at least one rooted phylogeny where they form a monophyletic group/clade.









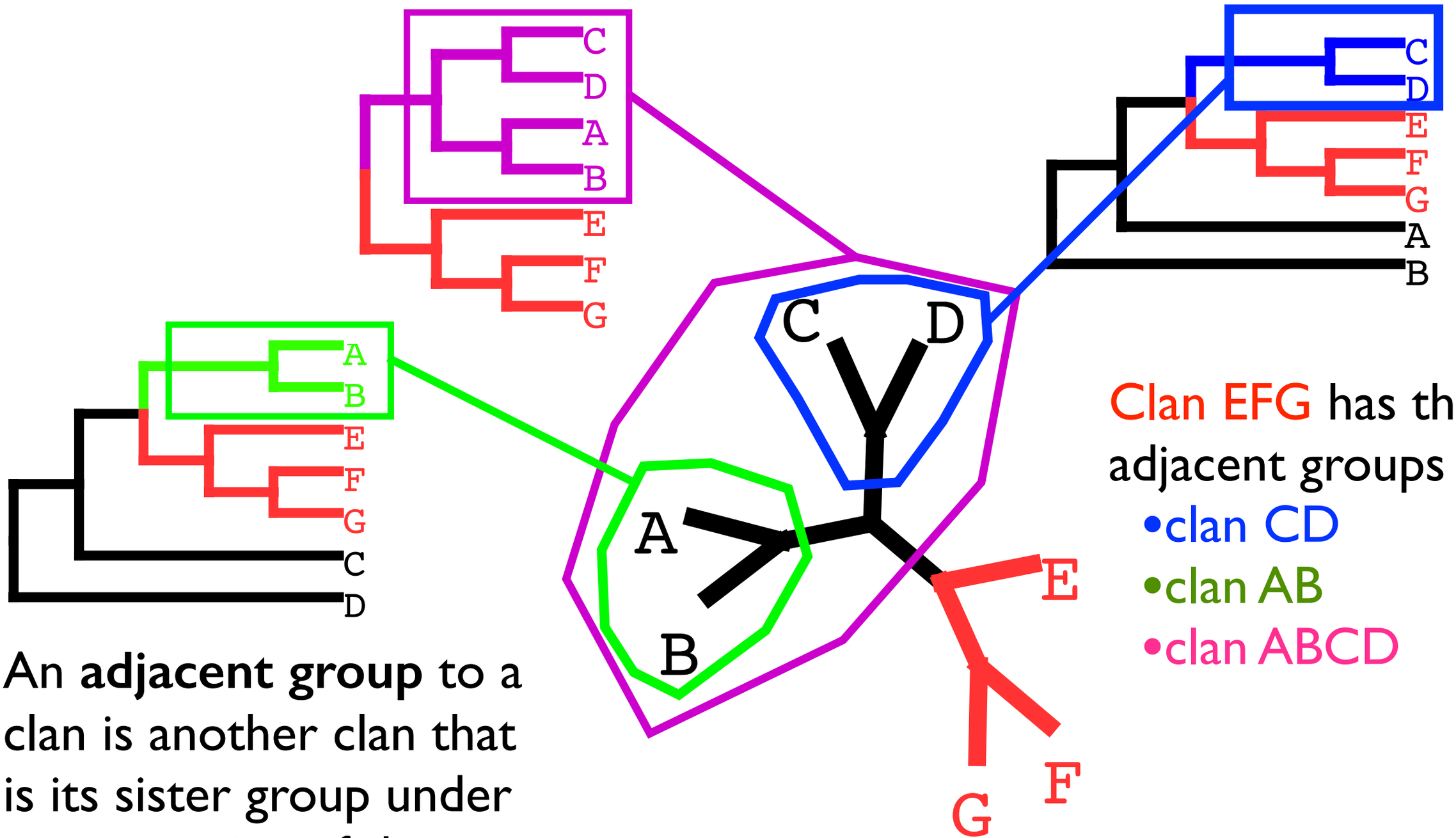
...

NO rooted trees place EG in a monophyletic group

Therefore EG **is not** a clan

Of clades and clans: terms for phylogenetic relationships in unrooted trees.
Wilkinson M, McInerney JO, Hirt RP, Foster PG, Embley TM.
Trends Ecol Evol. 2007 Mar;22(3):114-5.
PMID: 17239486

Adjacent Groups



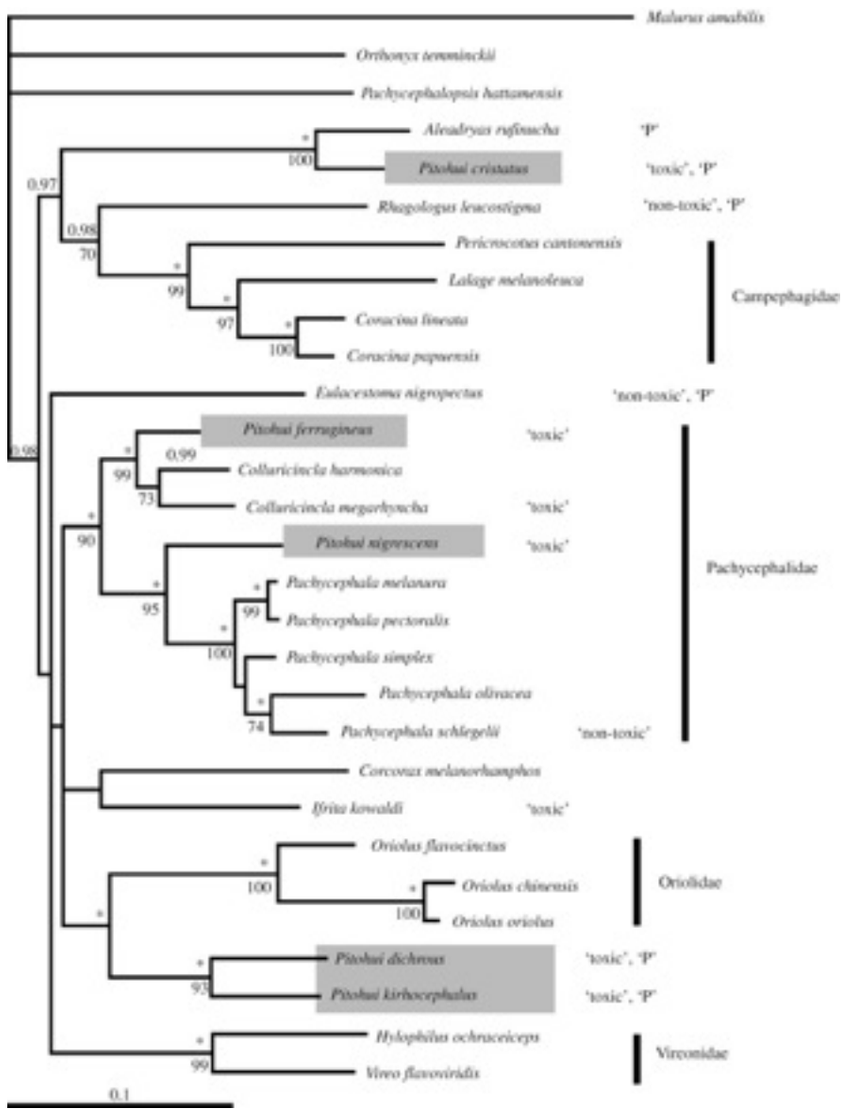
An **adjacent group** to a clan is another clan that is its sister group under some rooting of the tree

Clan EFG has three adjacent groups

- clan CD
- clan AB
- clan ABCD

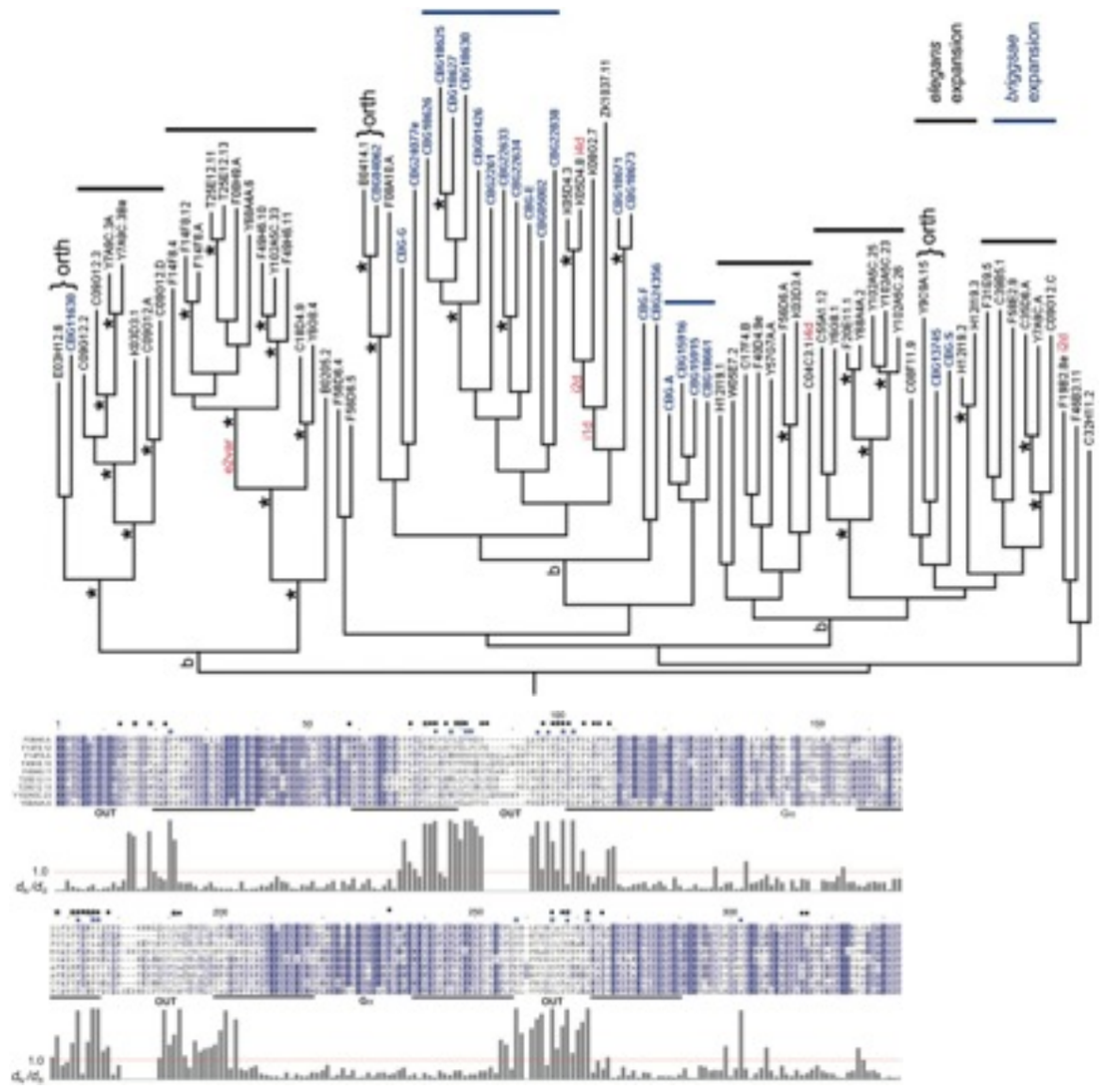
Of clades and clans: terms for phylogenetic relationships in unrooted trees.
Wilkinson M, McInerney JO, Hirt RP, Foster PG, Embley TM.
Trends Ecol Evol. 2007 Mar;22(3):114-5.
PMID: 17239486

Unrooted Trees are Sometimes Sufficient



Under all rootings, poisonous members of the order are non-monophyletic

Polyphyletic origin of toxic Pitohui birds suggests widespread occurrence of toxicity in corvoid birds.
Jønsson KA, Bowie RC, Norman JA, Christidis L, Fjeldså J.
Biol Lett. 2008 Feb 23;4(1):71-4.
PMID: 18055416



Sites of positive selection identified using reversible models of codon substitution

Adaptive evolution in the SRZ chemoreceptor families of Caenorhabditis elegans and Caenorhabditis briggsae.
Thomas JH, Kelley JL, Robertson HM, Ly K, Swanson WJ.
Proc Natl Acad Sci U S A. 2005 Mar 22;102(12):4476-81
PMID: 15761060

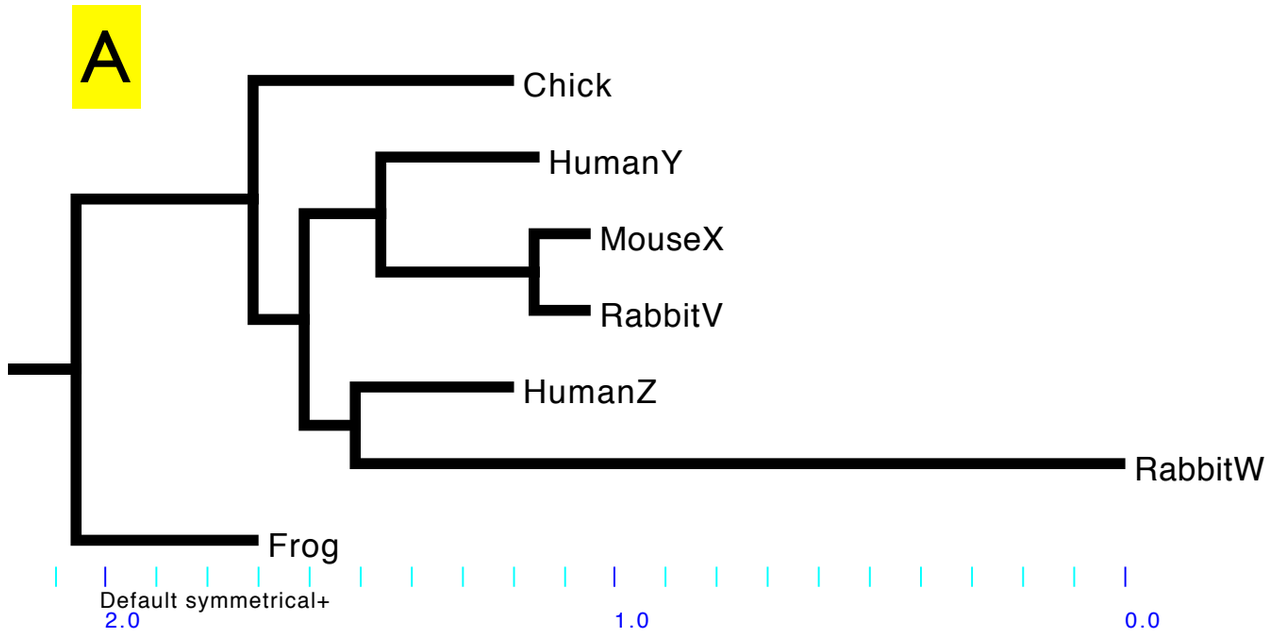
Mathematical Models of Sequence Evolution

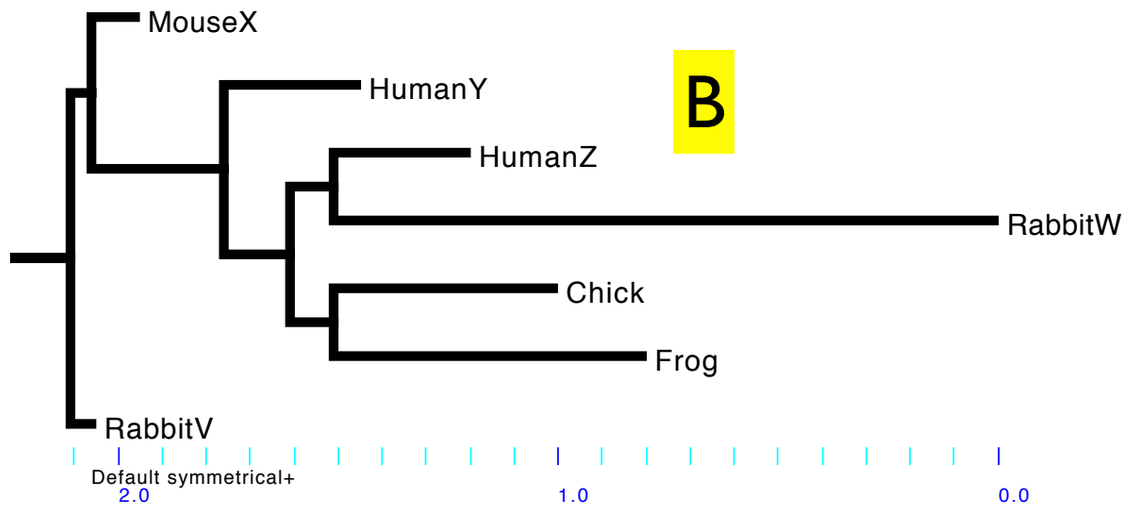
Sarah Parks

Rooting Trees/Reconciling Gene/ Species Trees

Aidan Budd, EMBL Heidelberg

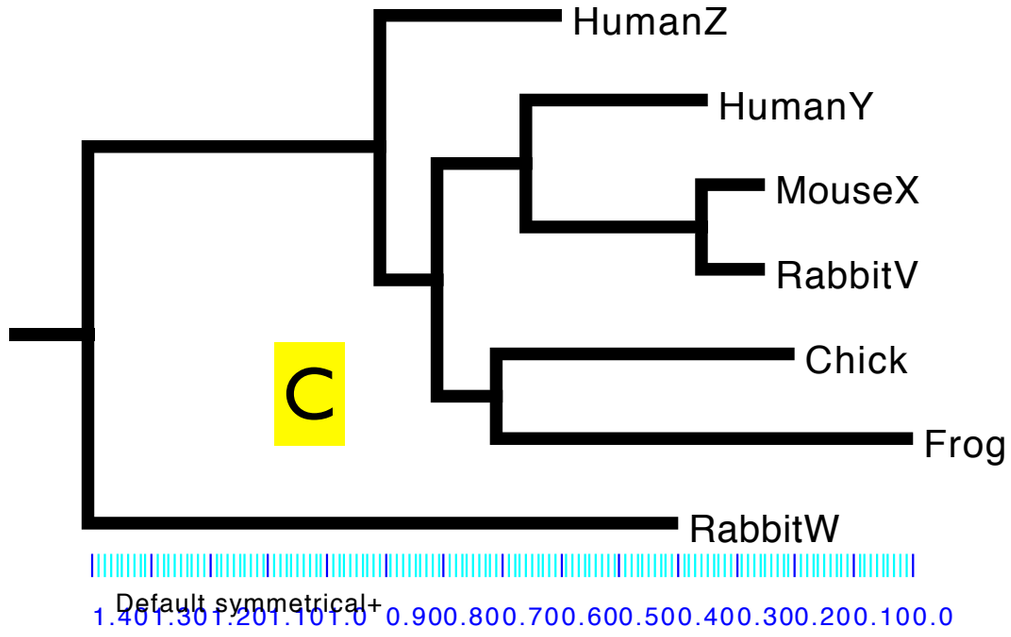
Where does the root go?





A B and C are all from the same unrooted tree

Which rooted tree has the root in the most plausible position?



Example Phylogeny Estimation Workflow

Aidan Budd, EMBL Heidelberg

Phylogenetic Workflows

- Every analysis is different
- There is no “one size fits all” approach
- There are, however, common “phases”/“stages” to many analysis
- We present here **example** analyses to highlight these phases
- Two examples workflows are given
- The first focuses more on common concepts
- The second more on common pragmatic/practical aspects of analyses

Example Phylogeny Estimation Workflow

1. Focusing on concepts

Aidan Budd, EMBL Heidelberg

Statistical Estimation of Phylogeny: An Outline

Statistical paradigm	Statistical phylogenetic paradigm
pose substantive question	what if the phylogeny of a group of organisms?
develop stochastic model with parameters that, if known, would answer the question.	develop phylogenetic model with tree (and branch lengths) and a Markov model describing how traits change over tree.
collect observations that are informative about model parameters.	construct a data matrix (e.g., of DNA sequences) sampled from the group of organisms.
find the best estimate of parameters conditioned on the observations at hand using some criterion.	find the best estimate of phylogeny using maximum likelihood criterion or Bayesian inference criterion.

Huelsenbook

Brian R. Moore, UC Davis

Aidan Budd, EMBL Heidelberg

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)
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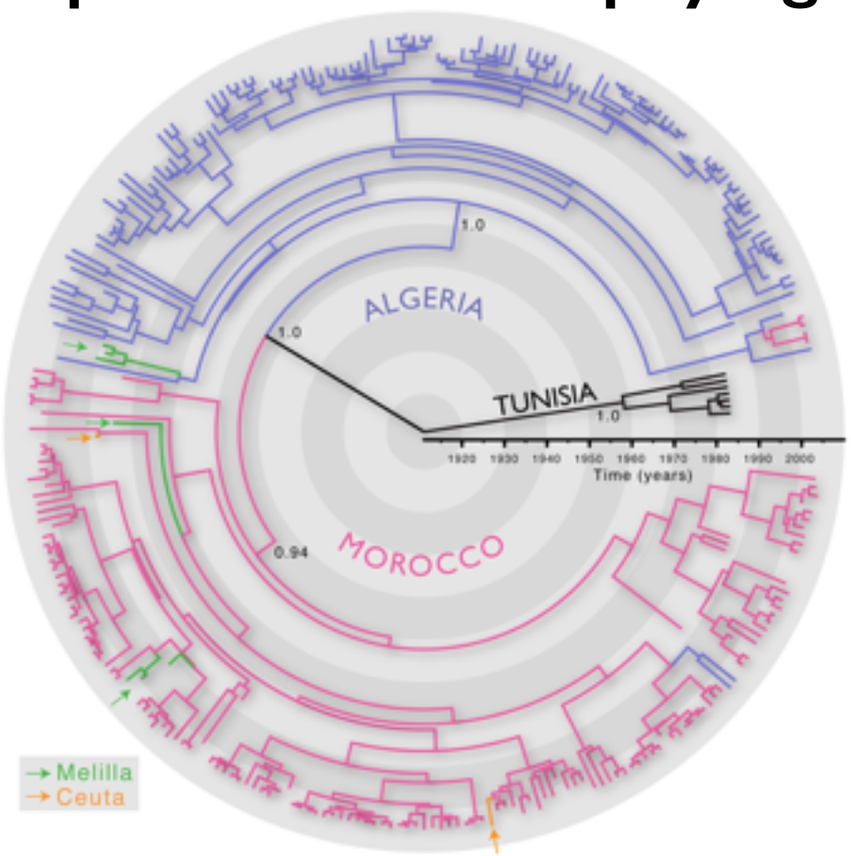
Example Phylogeny Estimation Workflow

1. 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**



In this case, we looked earlier at how the topology parameter (i.e. set of "relatedness statements" estimated from the data) of a phylogenetic model the evolution of dog rabies viruses from north Africa informs our belief in the significance of certain factors in the spread of the virus

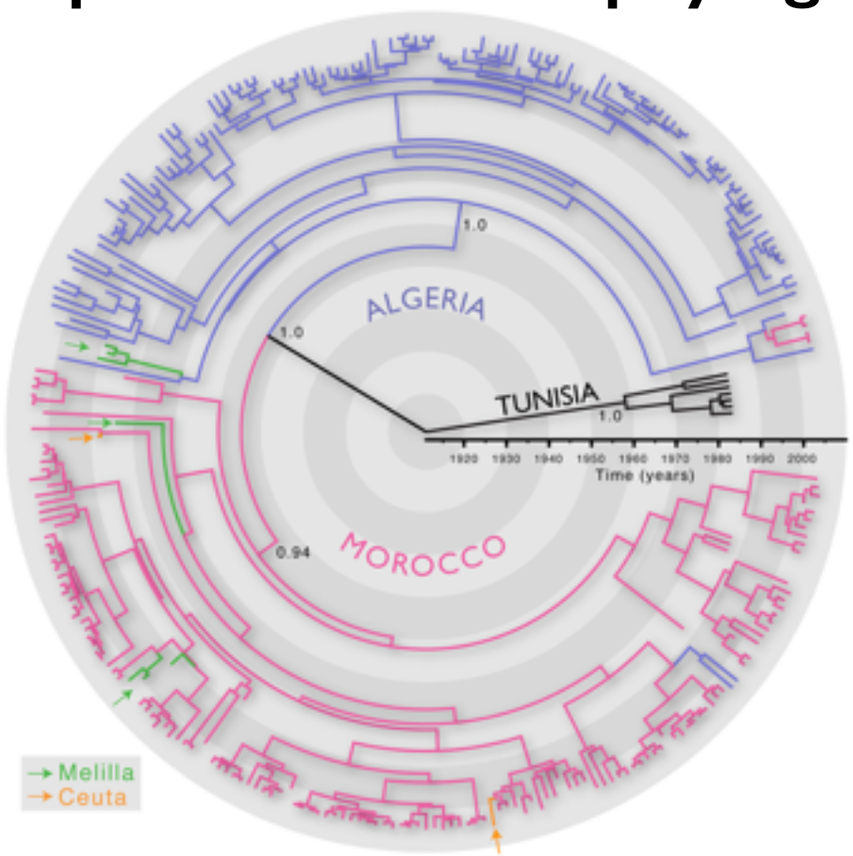
Example Phylogeny Estimation Workflow

1. 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

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

Olivier Gascuel – Phylogenetic models – ISCB-ASBCB Casablanca 2013

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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

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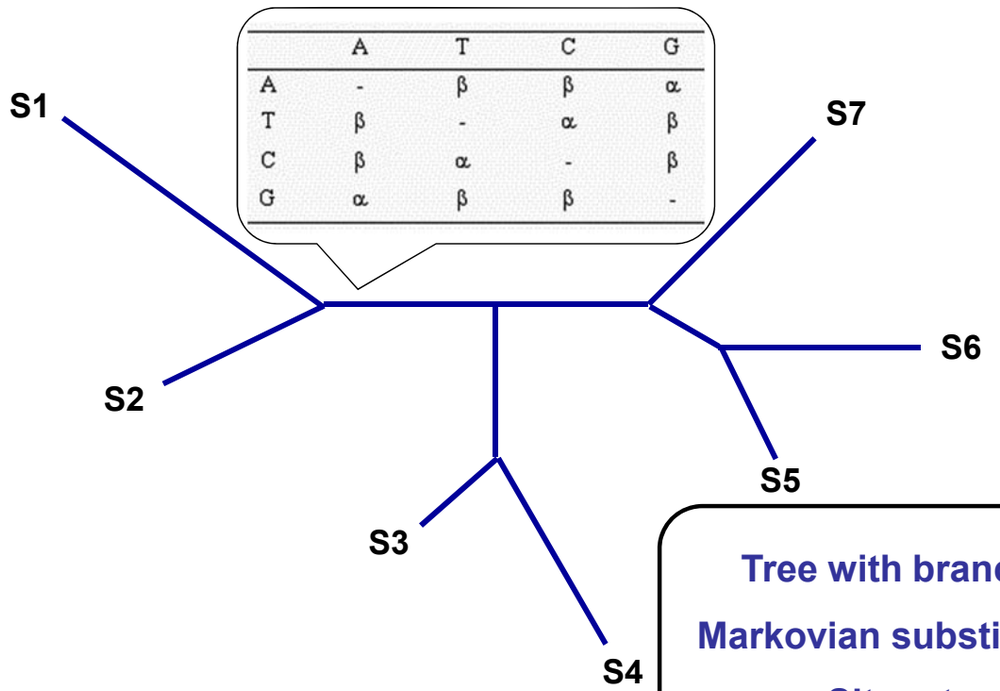
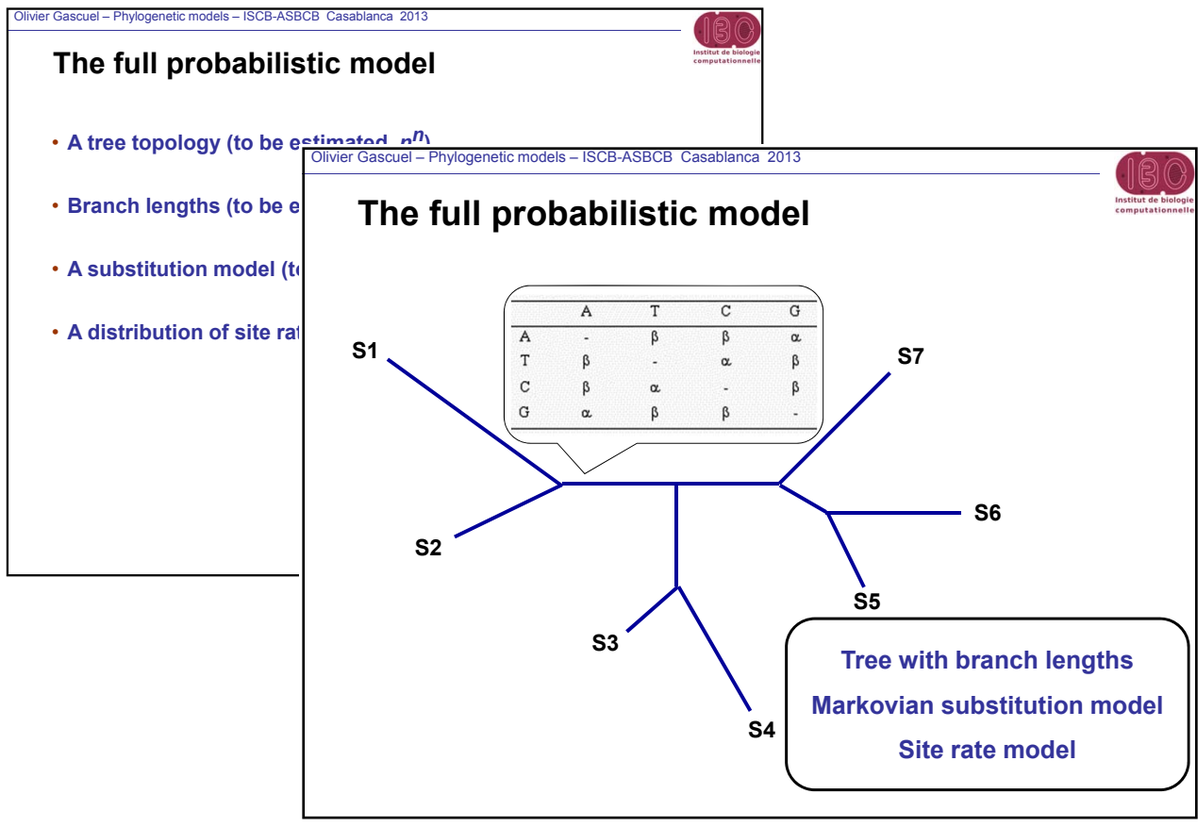
The full probabilistic model



Thanks to Olivier Gascuel for these slides

Aidan Budd, EMBL Heidelberg

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

But could also be other parameters e.g. identifying positive selection (omega parameter in certain codon-based substitution models above a particular value) that are of most interest

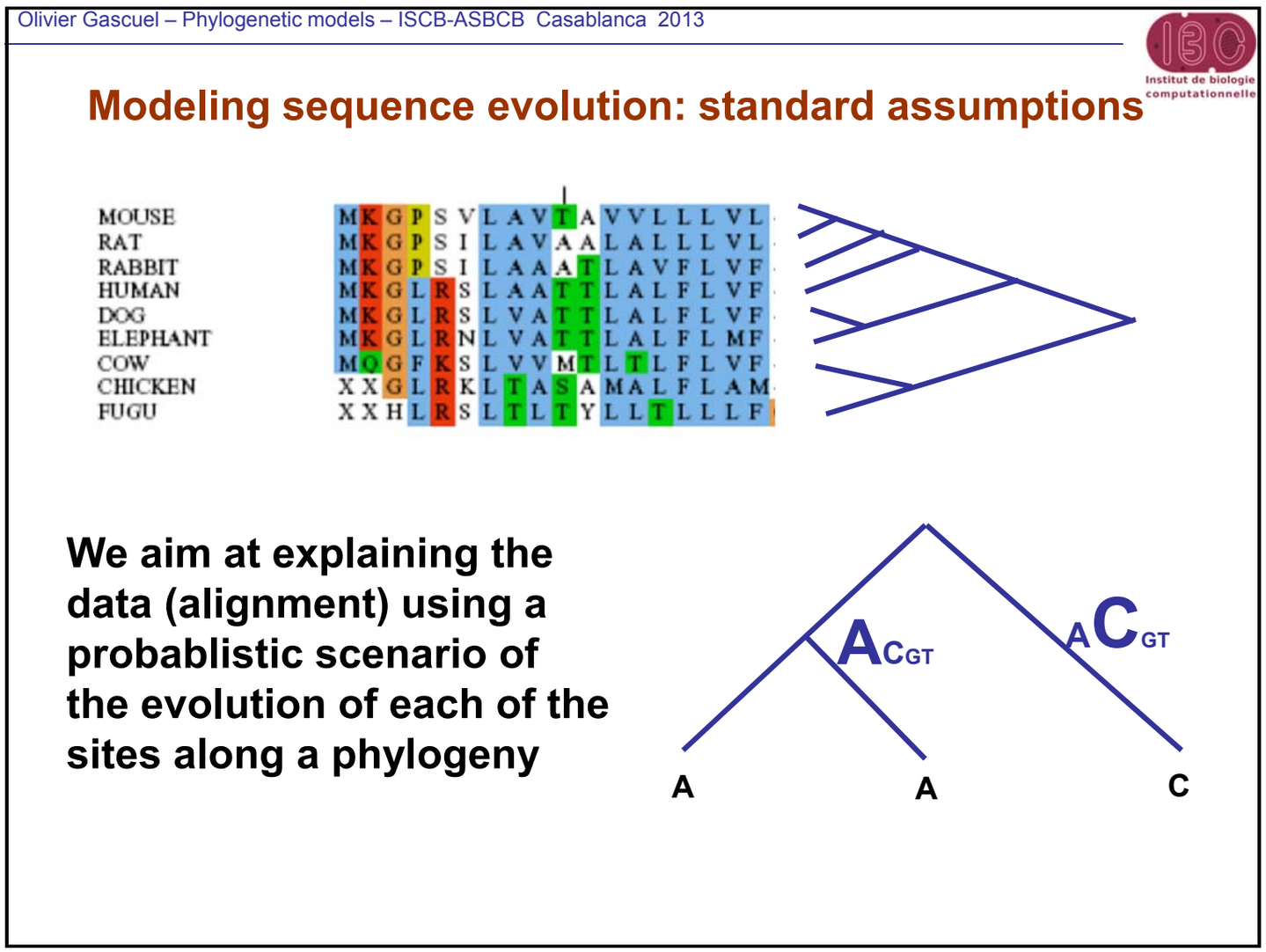
Thanks to Olivier Gascuel for these slides

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)

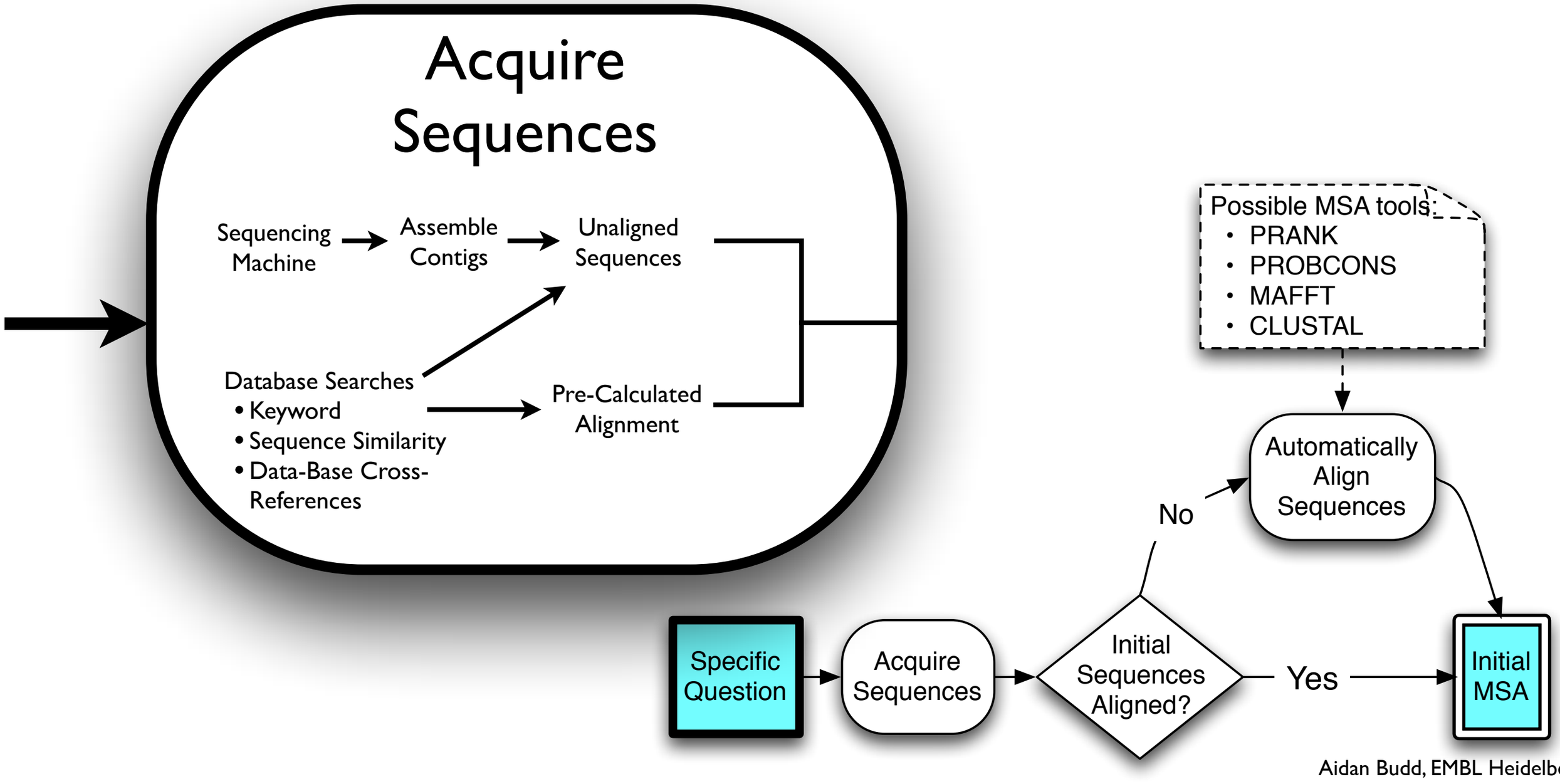


e.g. build a multiple sequences alignment of north African dog rabies sequences

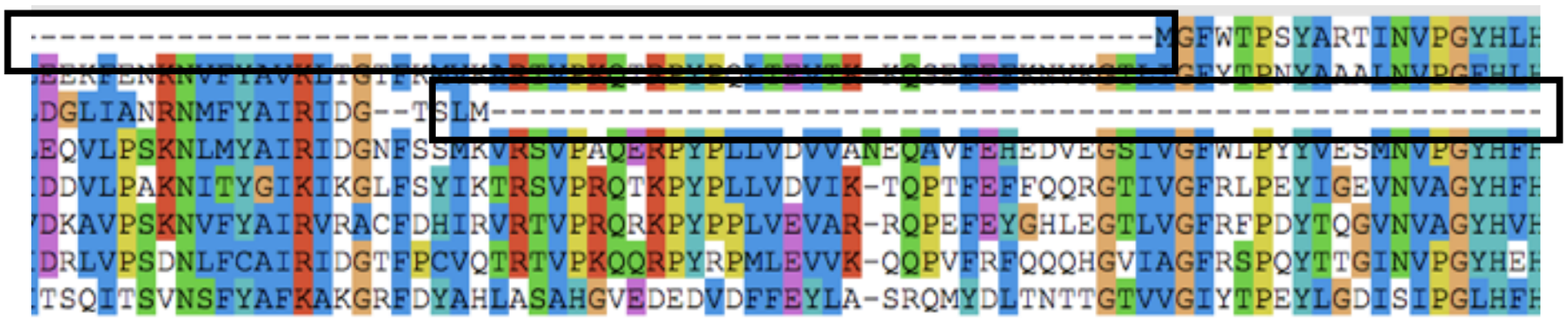
Thanks to Olivier Gascuel for this slide

Example Phylogeny Estimation Workflow

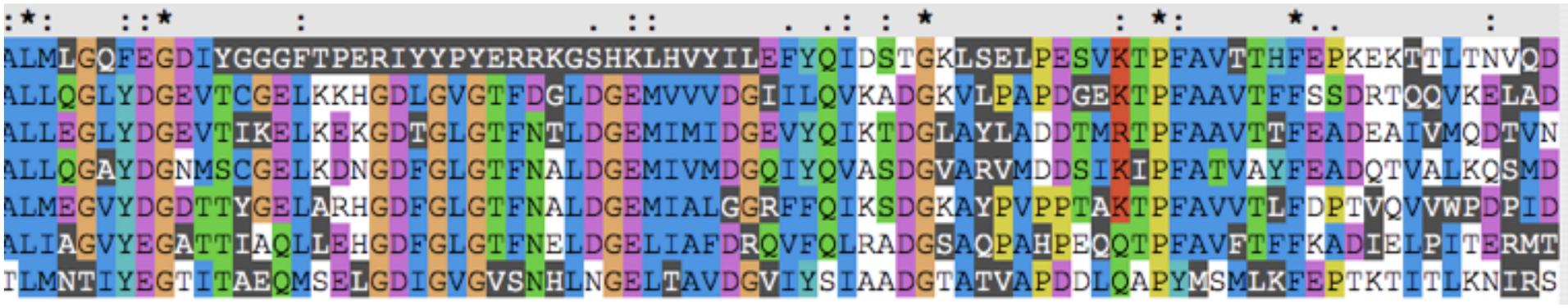
3. Collect observations informative about the model parameter(s)



Example Phylogeny Estimation Workflow
Unusual Sequences



Short/fragmented sequences



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

Unusual pattern of "conservation"

Example Phylogeny Estimation Workflow

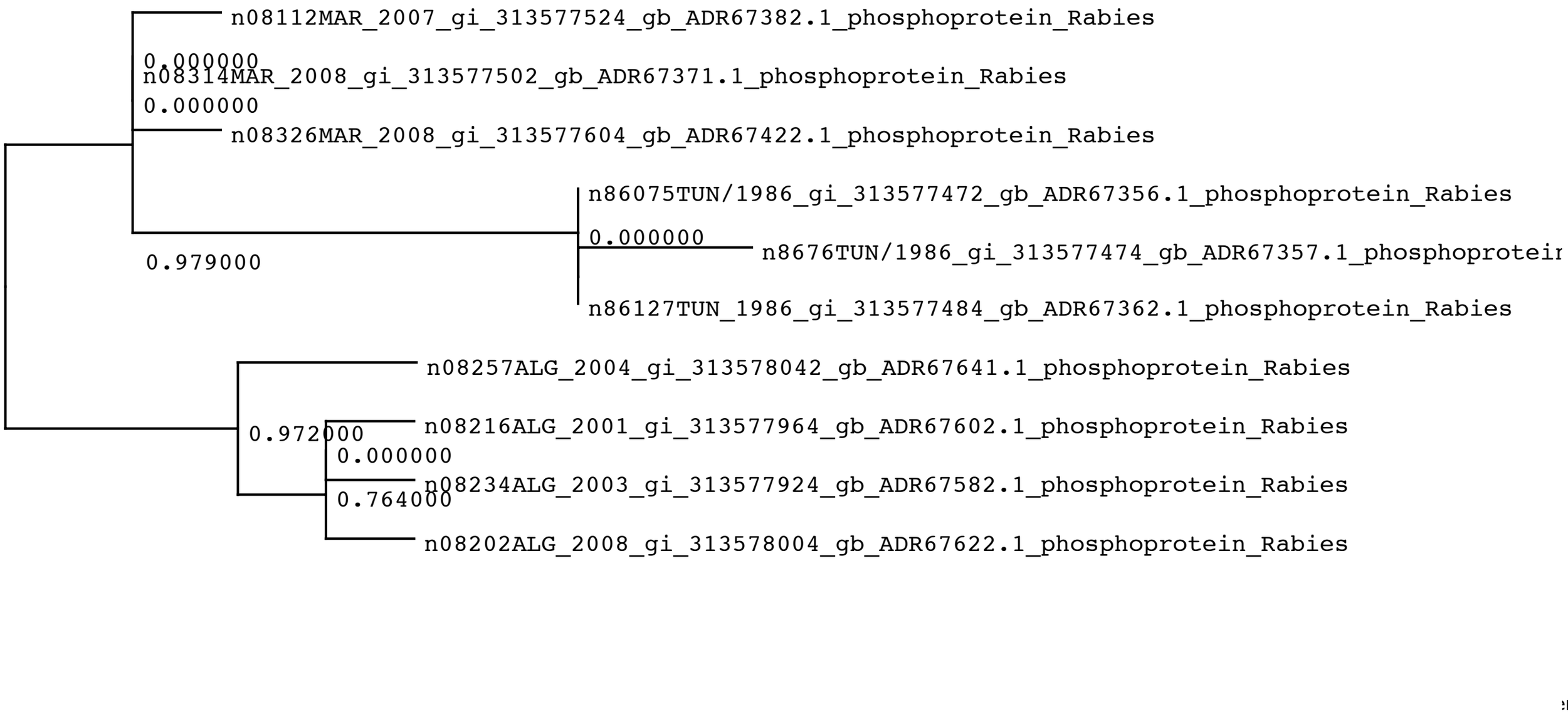
1. Pose a substantive question
2. Build a model involving parameters that, if known, could answer the question
3. Collect observations informative about the model parameter(s)
4. Find best estimate(s) of the parameter(s), conditioned on these observations
5. Estimate sampling/random error associated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

Example Phylogeny Estimation Workflow

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



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

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

described in this HTML document **interpretingPhylogeniesCrete2014.html**

Example Phylogeny Estimation Workflow

1. Focusing on practical/pragmatic issues

Aidan Budd, EMBL Heidelberg

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

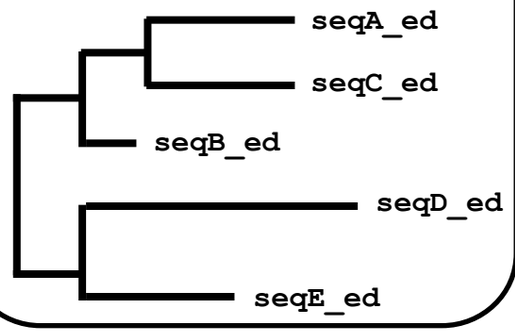
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

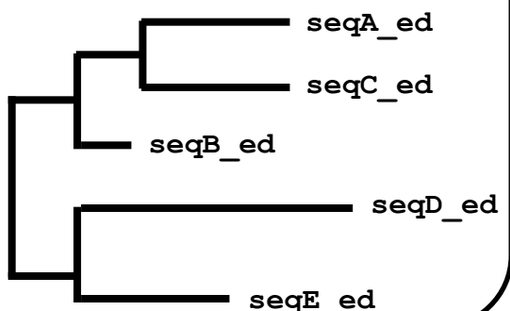
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

1. Choose a question: **me, today, using your brain**

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed AGTCGGTGATCCA
seqB_ed TCGGTGATCCA
seqC_ed AGTCGGTGAT
seqD_ed AGTCGTTGATCCA
seqE_ed GTCAGTGATCC
```

4. Align sequences

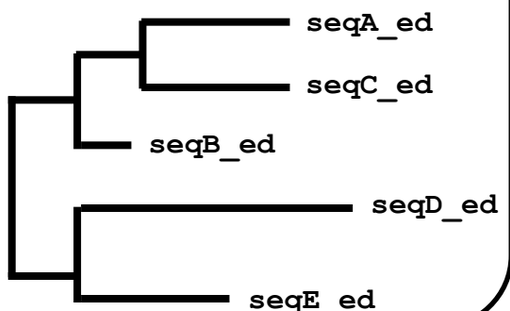
```
seqA_ed AGTCGGTGATCCA
seqB_ed --TCGGTGATCCA
seqC_ed AGTCGGTGAT---
seqD_ed AGTCGTTGATCCA
seqE_ed -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

2. Collect sequences: **Stephen, DATES, BLAST, XXXXX**

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

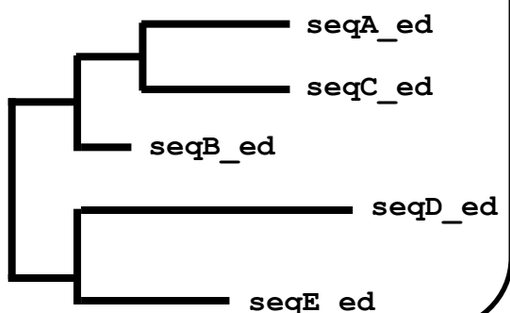
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

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

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

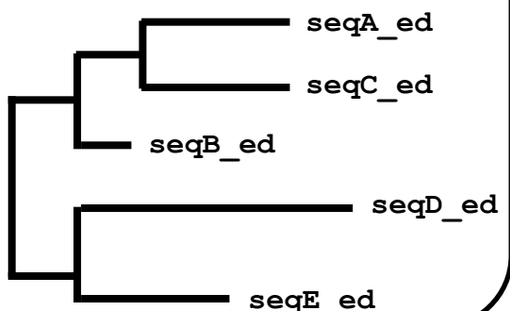
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

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

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA! AGTCGGTGATCCA
seqB! TCGGTGATCCA
seqC! AGTCGGTGAT
seqD! AGTCGTTGATCCA
seqE! GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed AGTCGGTGATCCA
seqB_ed TCGGTGATCCA
seqC_ed AGTCGGTGAT
seqD_ed AGTCGTTGATCCA
seqE_ed GTCAGTGATCC
```

4. Align sequences

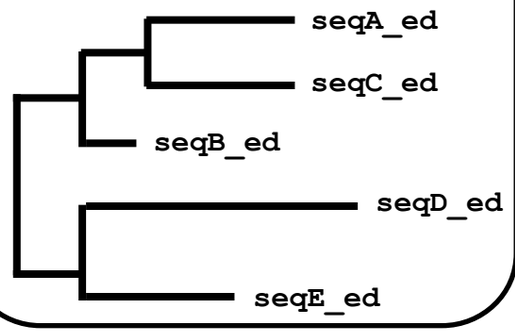
```
seqA_ed AGTCGGTGATCCA
seqB_ed --TCGGTGATCCA
seqC_ed AGTCGGTGAT---
seqD_ed AGTCGTTGATCCA
seqE_ed -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

5. Model selection: **Nick and Ziheng, DATE, PAML, jModelTest**

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

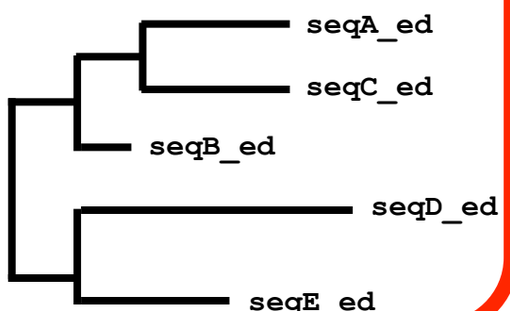
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

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

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA! AGTCGGTGATCCA
seqB! TCGGTGATCCA
seqC! AGTCGGTGAT
seqD! AGTCGTTGATCCA
seqE! GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed AGTCGGTGATCCA
seqB_ed TCGGTGATCCA
seqC_ed AGTCGGTGAT
seqD_ed AGTCGTTGATCCA
seqE_ed GTCAGTGATCC
```

4. Align sequences

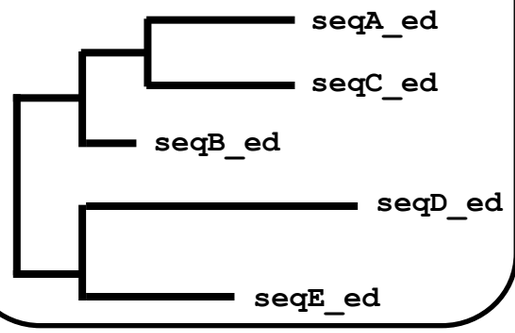
```
seqA_ed AGTCGGTGATCCA
seqB_ed --TCGGTGATCCA
seqC_ed AGTCGGTGAT---
seqD_ed AGTCGTTGATCCA
seqE_ed -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

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

Example Phylogeny Estimation Workflow

1. Choose a question

?

2. Collect sequences

```
seqA!  AGTCGGTGATCCA
seqB!  TCGGTGATCCA
seqC!  AGTCGGTGAT
seqD!  AGTCGTTGATCCA
seqE!  GTCAGTGATCC
```

3. Edit sequence identifiers

```
seqA_ed  AGTCGGTGATCCA
seqB_ed  TCGGTGATCCA
seqC_ed  AGTCGGTGAT
seqD_ed  AGTCGTTGATCCA
seqE_ed  GTCAGTGATCC
```

4. Align sequences

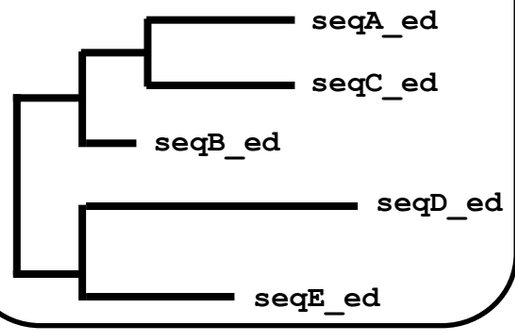
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

5. Model selection

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta\pi_T & \beta\pi_C & \alpha\pi_G \\ \beta\pi_A & \cdot & \alpha\pi_C & \beta\pi_G \\ \beta\pi_A & \alpha\pi_T & \cdot & \beta\pi_G \\ \alpha\pi_A & \beta\pi_T & \beta\pi_C & \cdot \end{bmatrix}$$

$$\mathbf{Q} = \begin{bmatrix} \cdot & \beta & \beta & \alpha \\ \beta & \cdot & \alpha & \beta \\ \beta & \alpha & \cdot & \beta \\ \alpha & \beta & \beta & \cdot \end{bmatrix} \qquad \mathbf{Q} = \begin{bmatrix} \cdot & \mu\pi_T & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \cdot & \mu\pi_C & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \cdot & \mu\pi_G \\ \mu\pi_A & \mu\pi_T & \mu\pi_C & \cdot \end{bmatrix}$$

6. Phylogeny estimation



7. Algorithm diagnosis

8. Answer question/ hypothesis testing

8. Answer question/hypothesis testing: **Nick, Ziheng, Maria, Brian, Olivier, DATES, aLRTs, AIC, Bayes factors**

1. Write down a substantive question that can be informed by estimating the value of parameter(s) of a phylogenetic model
2. Collect an appropriate set of sequences
3. Edit sequence identifiers to be unique, compatible with analysis tools, and meaningful (in the context of allowing us to easily answer our question(s) of interest when examining resulting phylogenetic trees and other results)
4. Align sequences
5. Model Selection
6. Phylogeny estimation
7. Algorithm Diagnosis
8. Examine the results of your analyses and address your substantial question of interest