Hashtag: #CoME9

EMBO Practical Course on Computational Molecular Evolution

Sunday 8th–Thursday 19th May 2016

Institute of Marine Biology, Biotechnology and Aquaculture
(IMBBC), Hellenic Centre for Marine Research (HCMR)
Crete, Greece

http://www.ebi.ac.uk/~tamuri/come2016

Welcome!

Aidan Budd, EMBL Heidelberg

TIMETABLE FOR DAY 1

Aidan Budd, EMBL Heidelberg

$ whoami

Aidan Budd, EMBL Heidelberg

8th version of this course

Aidan Budd, EMBL Heidelberg

Support from:

EMBO, HITS (Wellcome), IMBBC, Astir Beach

THANKS!

Margarita Metallinou fee waiver awards:

Thanks to HITS

Aidan Budd, EMBL Heidelberg

Course aims:

- improve understanding of how tools and methods work
- show examples of diverse applications of these approaches
- make you better able to do more appropriate analyses on your data
- give you access to help/advice from many experts
- help you form new professional relationships

Course outline:

1.fundamentals

2.data

3.phylogeny reconstruction

4.hypothesis testing

5.inferences from population data

voluntary “advanced computing” sessions:

1. Markov simulations
2. Pair-wise sequence alignment in R

new this year - an experiment

Logistics

- "comfort breaks"
- WiFi
- keeping on time!
- putting up posters
- feedback
- sign-in sheet
- photos
- social media #EMBOcompmol

Thanks

- Alexis
- Many locals, especially: Eftichia, Cilia, Jacques, Antonis
- Funders
- Trainers
- You!

Antonis Magoulas

Aidan Budd, EMBL Heidelberg

Introductions

Monday 9th May 2016

EMBO Practical Course on Computational Molecular Evolution

Institute of Marine Biology, Biotechnology and Aquaculture
(IMBBC), Hellenic Centre for Marine Research (HCMR)
Crete, Greece

Aidan Budd
EMBL Heidelberg, Germany

What You Get From a Course?

Aidan Budd, EMBL Heidelberg

1. knowledge & understanding

2. useful professional relationships

a. with other trainees

b. with trainers

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

Aidan Budd, EMBL Heidelberg

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)

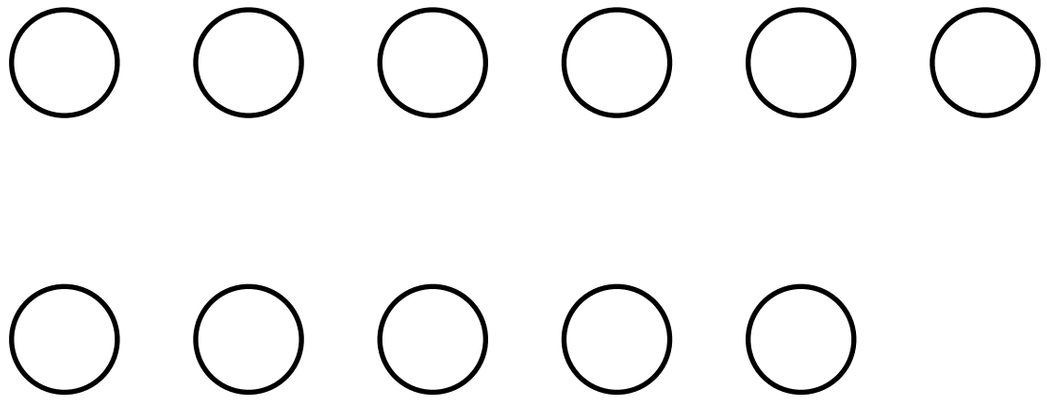
- tell each other
  - names
  - where you work
  - research topics
  - look for person you both know or place you've both been

Speed Dating: Format

Stand, awkwardly, in two rows

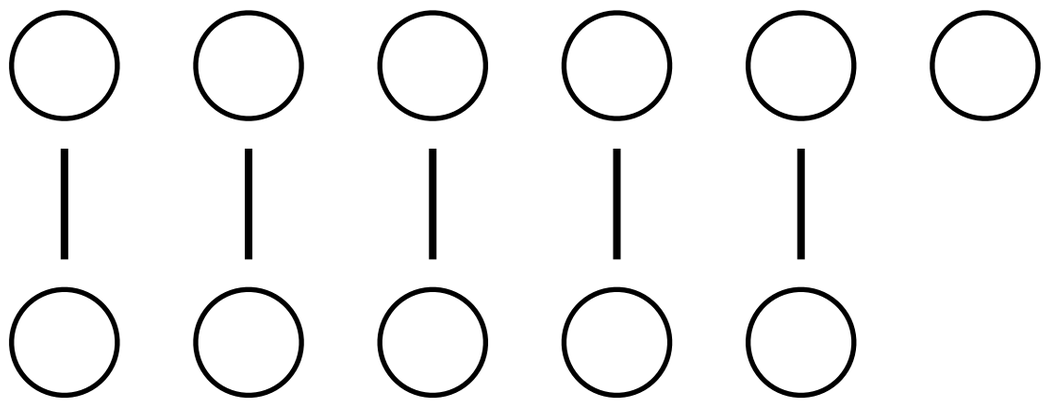
Face one person in the other row

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

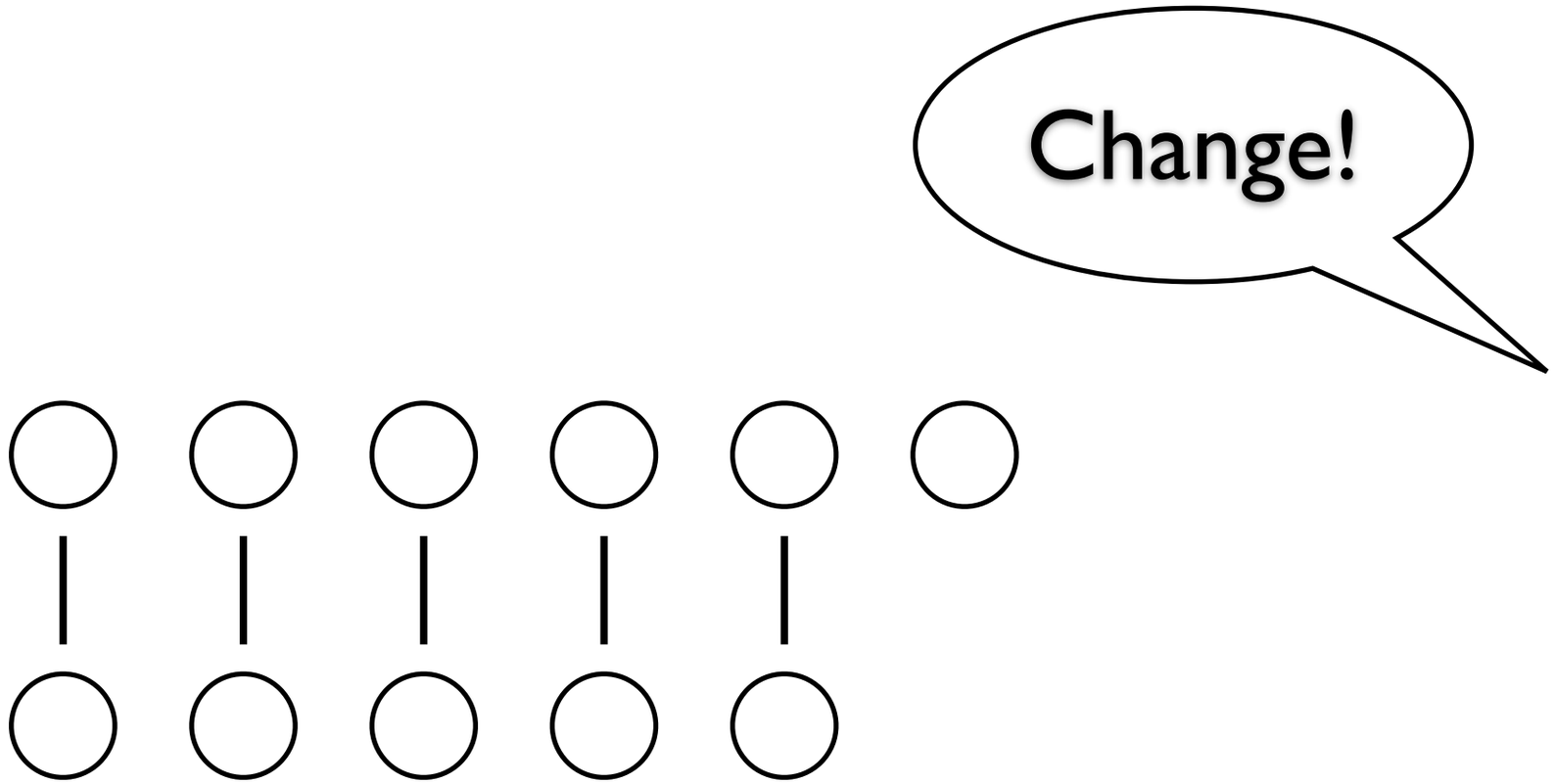


Speed Dating: Format

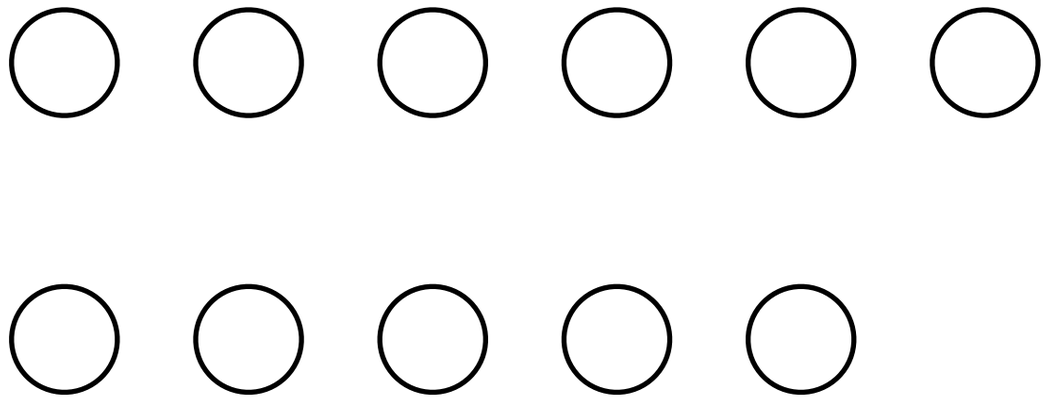
Chat!



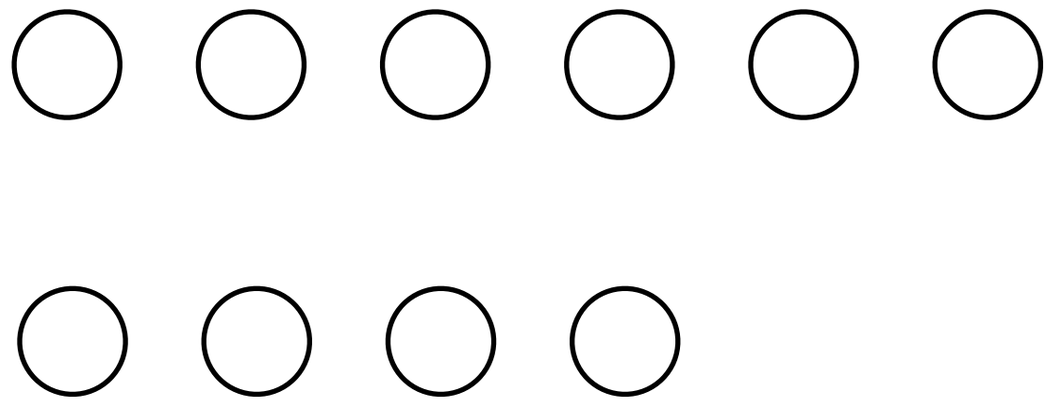
Speed Dating: Format



Speed Dating: Format

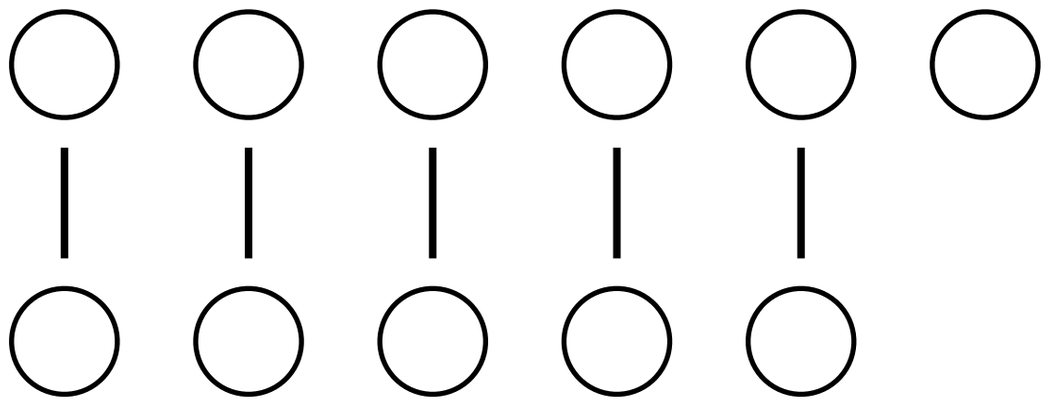


Speed Dating: Format



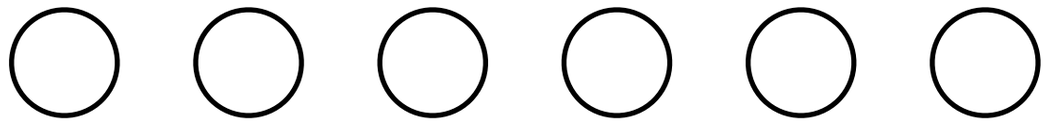
Speed Dating: Format

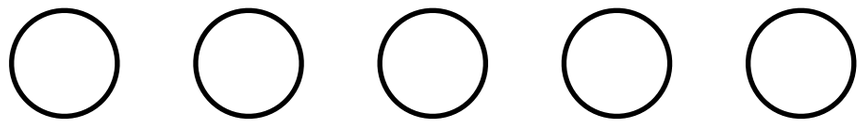
Chat!



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

Speed Dating: Format

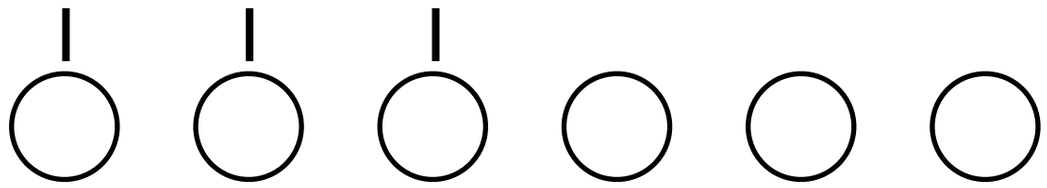


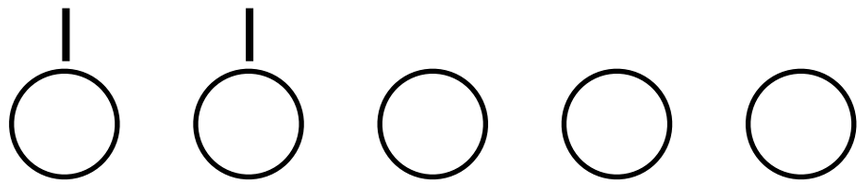


then split each row into two new rows

Speed Dating: Format

Chat!





make two new rows

and start again with the chat…

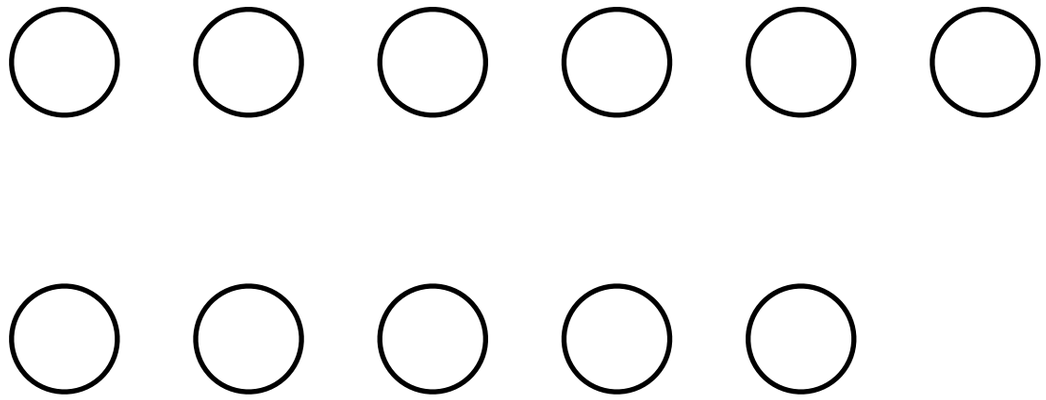
Speed Dating: Format

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: #CoME9

Interpreting Molecular Phylogenetic Trees

Monday 9th May 2016

Aidan Budd
EMBL Heidelberg, Germany

http://www.ebi.ac.uk/~tamuri/come2016

Monday May 9th 2016	
Introduction to course and venue – Chair: Aidan Budd	
09.00–09.30	Introductions – Antonis Magoulas & Aidan Budd
09.30–10.30	Ice breaker with trainee and trainer introductions – Aidan Budd
Interpreting molecular phylogenetic trees (I) – Chair: Aidan Budd	
10.30–11.00	Interpreting phylogenies – Aidan Budd
11.00–11.30	Coffee
Interpreting molecular phylogenetic trees (II) – Chair: Aidan Budd	
11.30–13.00	Interpreting phylogenies – Aidan Budd
13.00–14.30	Lunch
Interpreting molecular phylogenetic trees (III) – Chair: Aidan Budd	
14.30–16.00	Continuous Time Markov Chains – Brian Moore
16.00–16.30	Substitution models – Brian Moore
16.30–17.00	Coffee
Interpreting molecular phylogenetic trees (IV) – Chair: Aidan Budd	
17.00–18.30	Substitution models – Brian Moore
Linux/Unix Introduction (optional) – Chair: Aidan Budd	
18.30–20.00	Presentations and Practical Sessions – Alexey Kozlov
20.30–	Dinner at hotel

How Do We Interpret Molecular Phylogenetic Trees?

An Example

Aidan Budd, EMBL Heidelberg

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

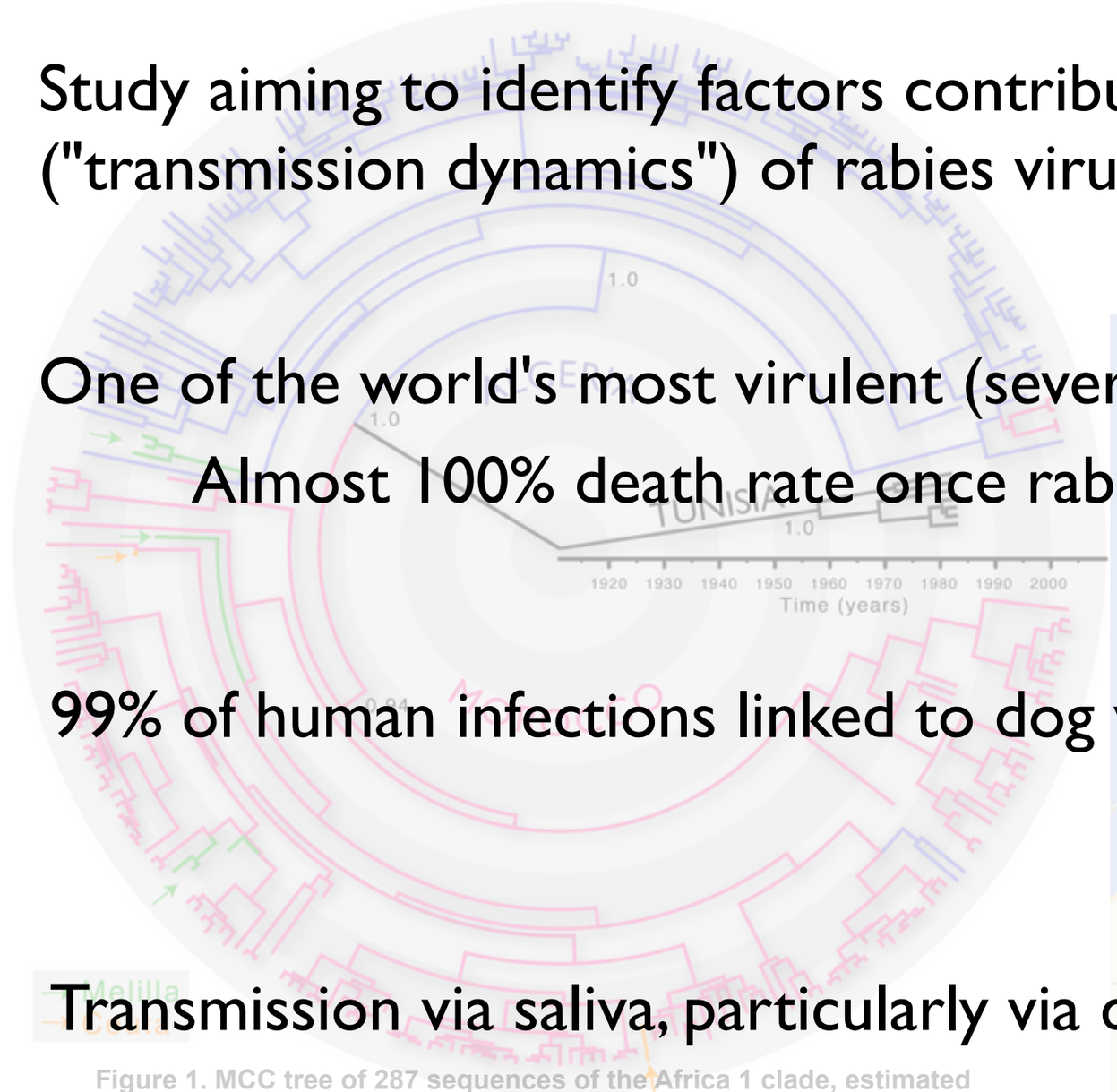


Figure 1. MCC tree of 287 sequences of the Africa 1 clade, estimated from the N, P and G-L genes and intergenic regions of dog RABV, and showing the spatial structure of the viral lineages.

*autonomous cities of Spain

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

Interpreting Molecular Phylogenetic Trees: An Example

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

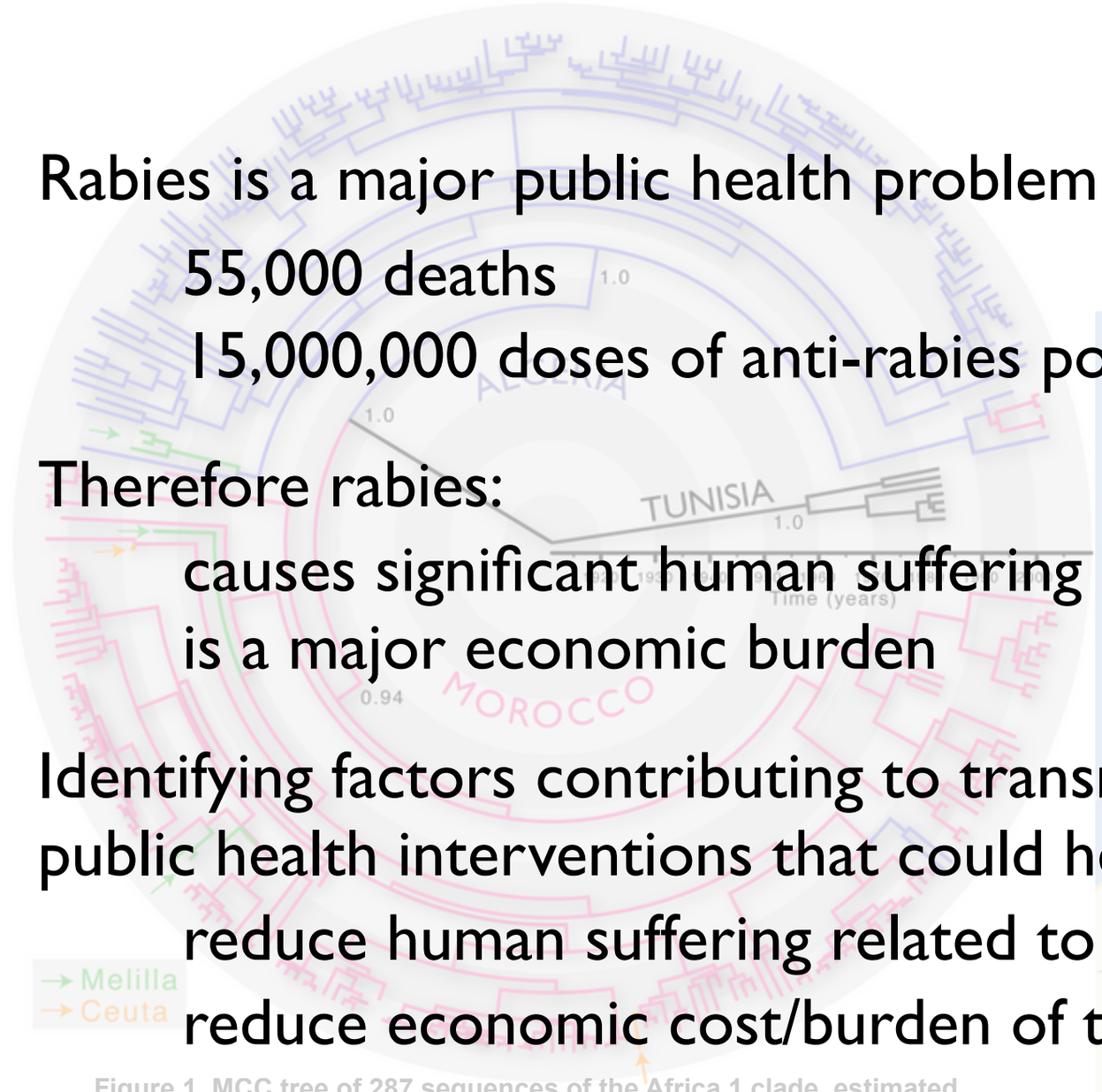
- 55,000 deaths
- 15,000,000 doses of anti-rabies post-exposure prophylaxis administered

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



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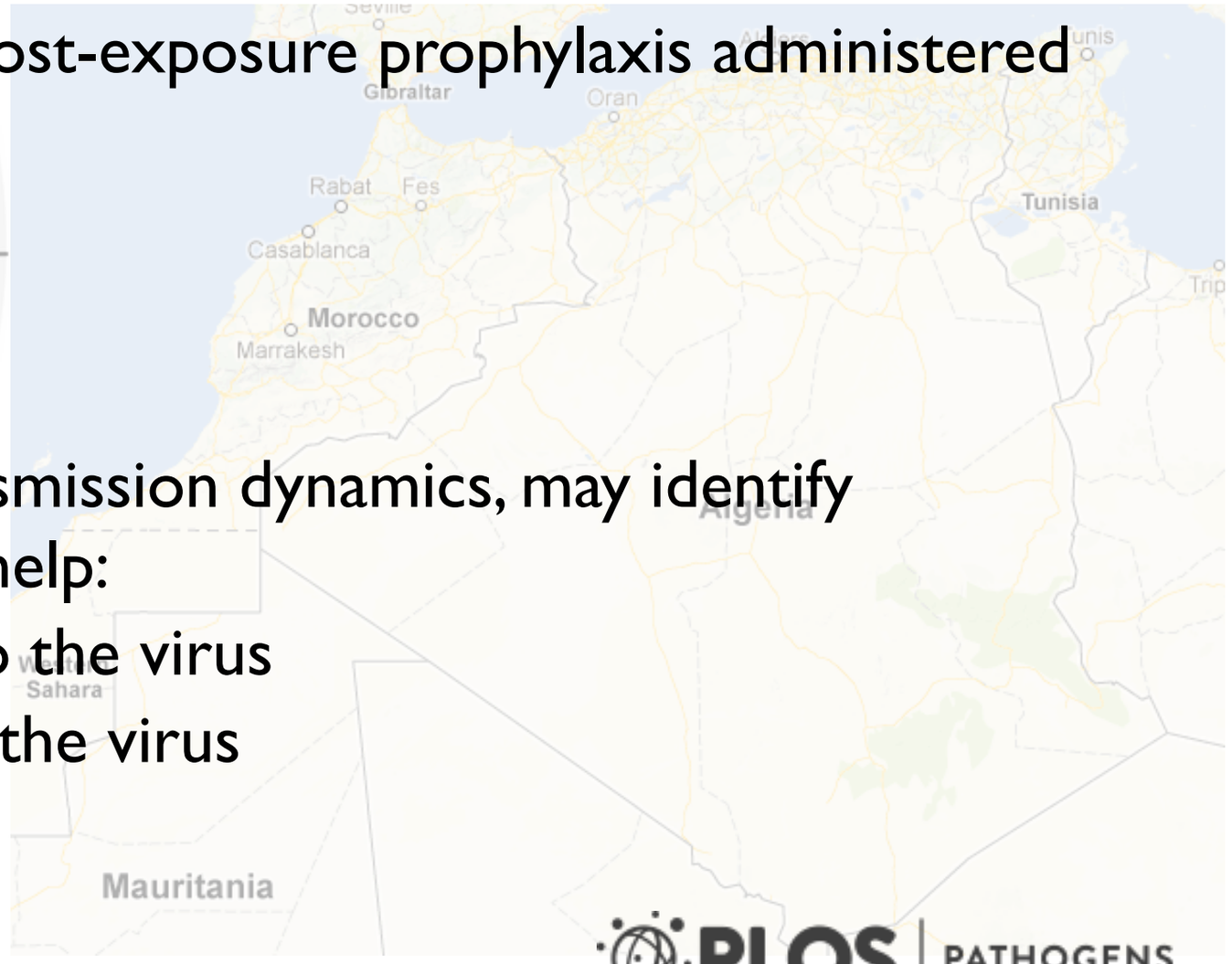

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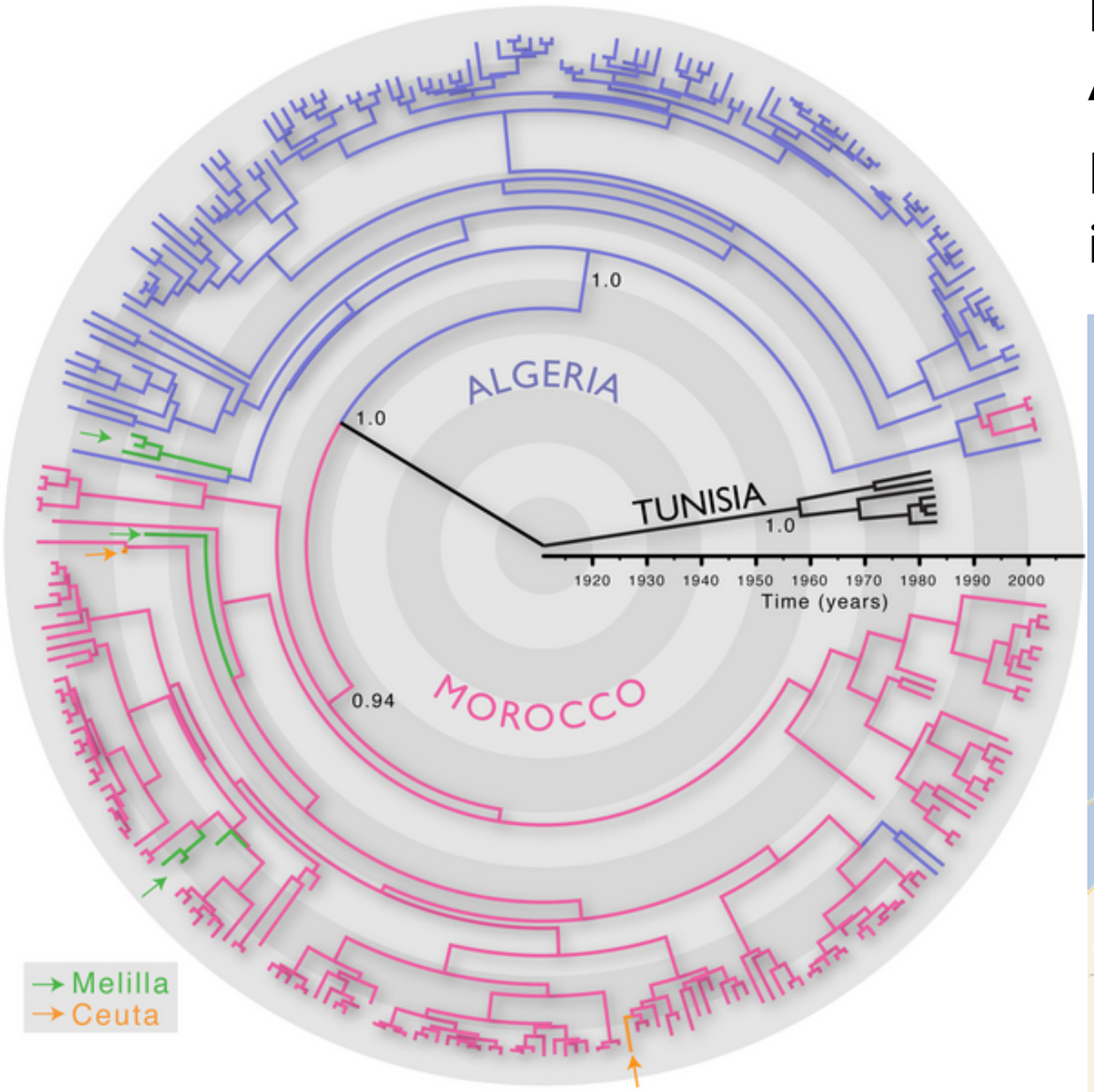

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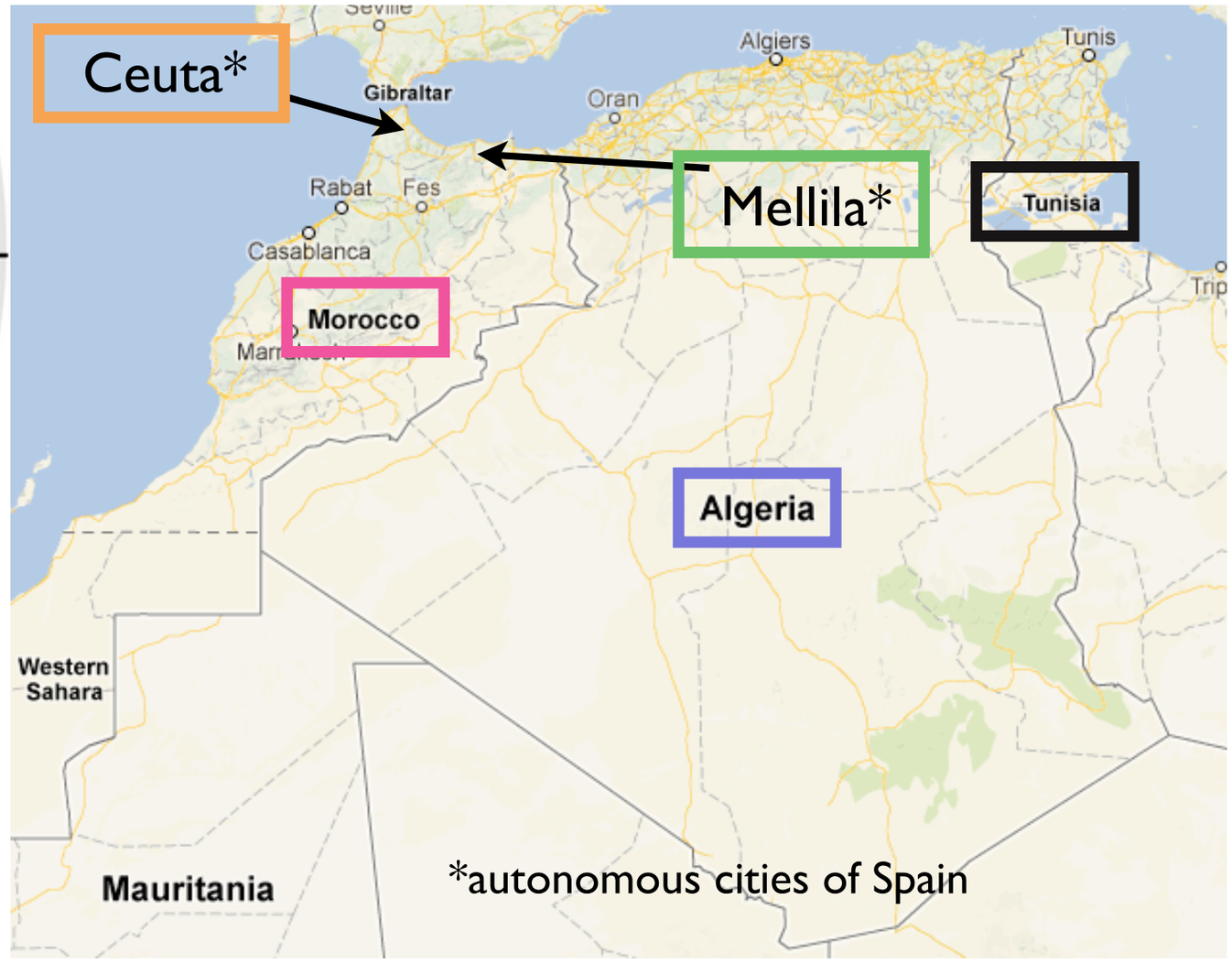
Phylogeny of rabies virus sampled from North African dogs

Branches coloured according to measured or inferred geographical location



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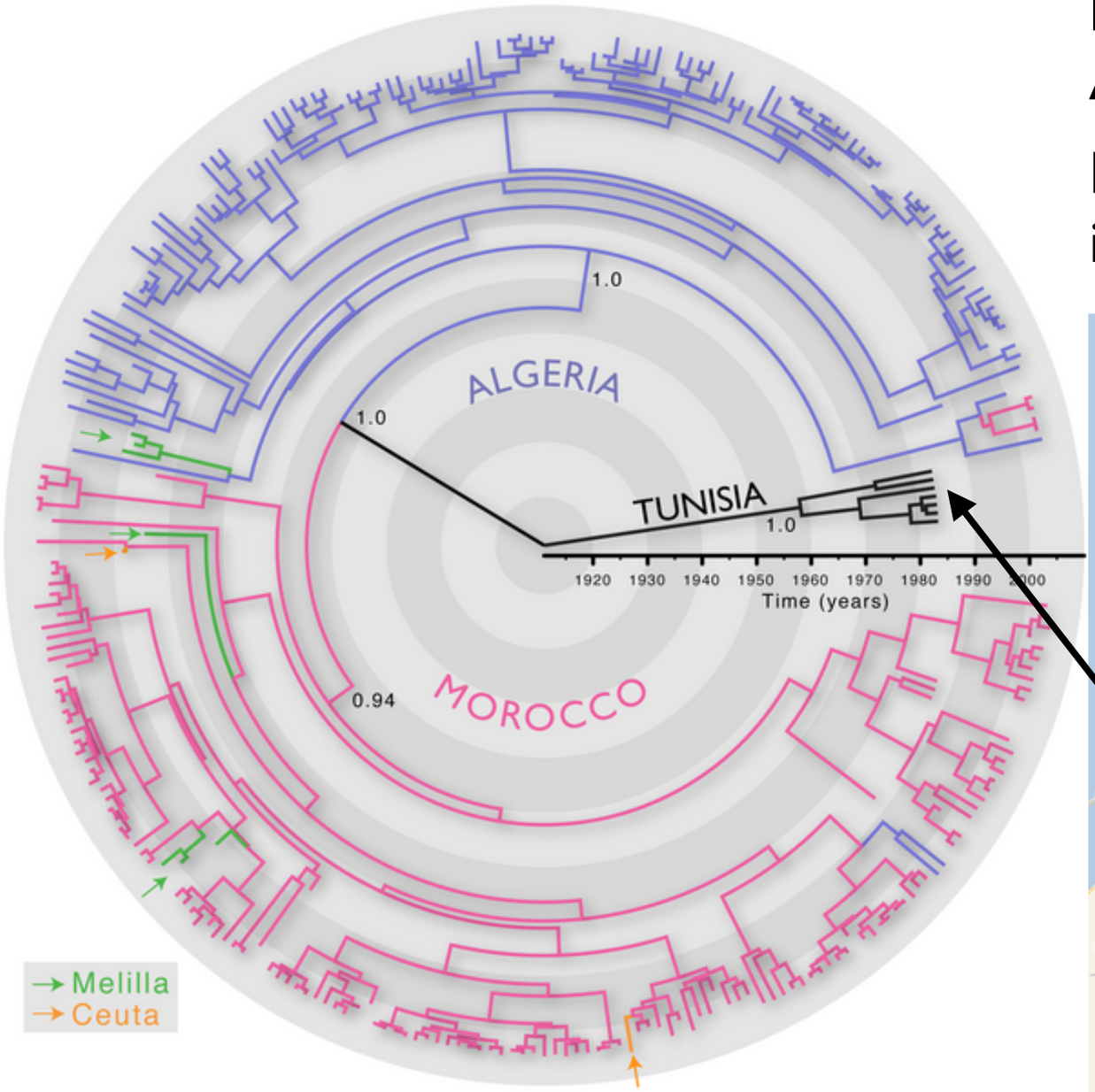
Aidan Budd, EMBL Heidelberg

Interpreting Molecular Phylogenetic Trees: An Example



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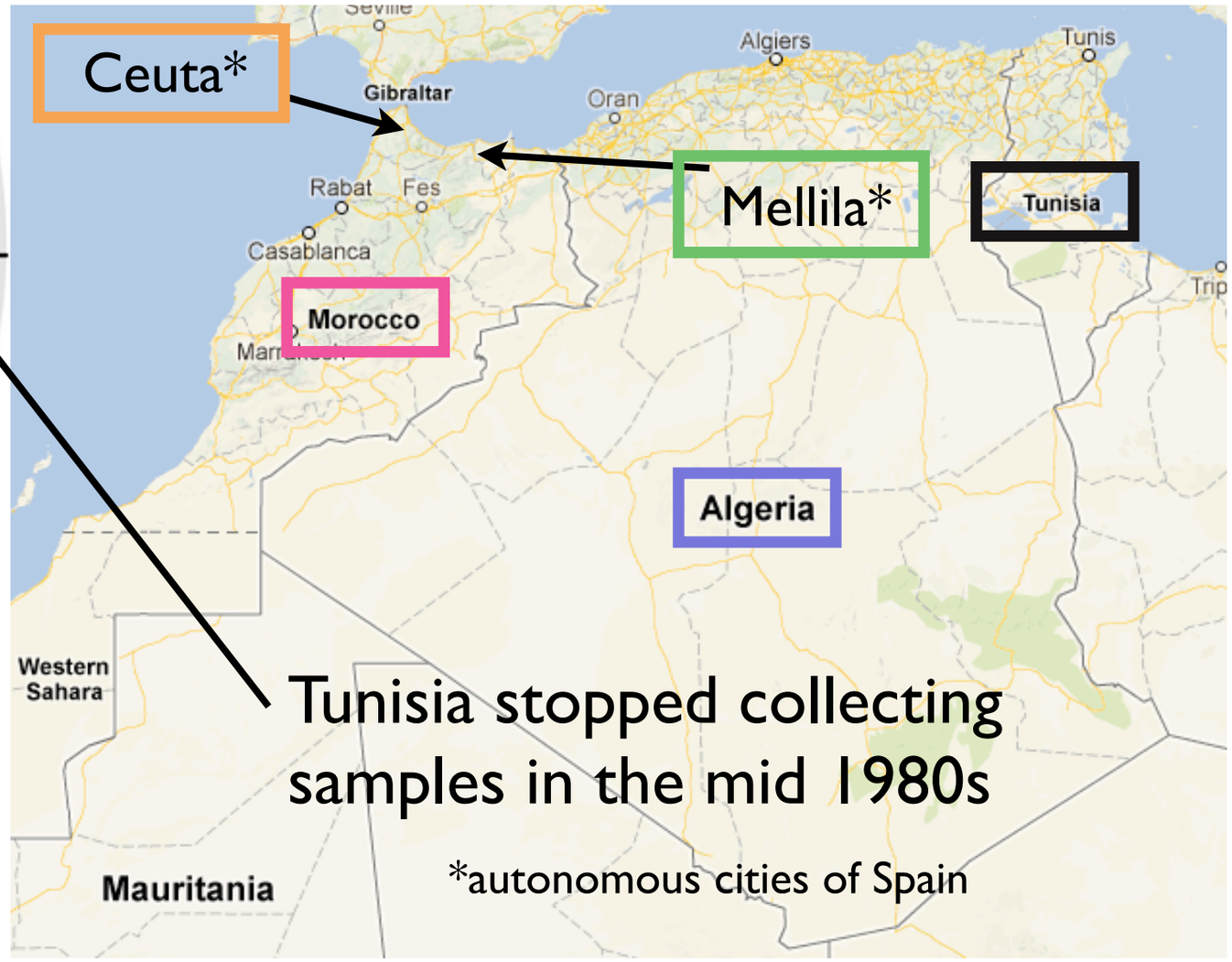

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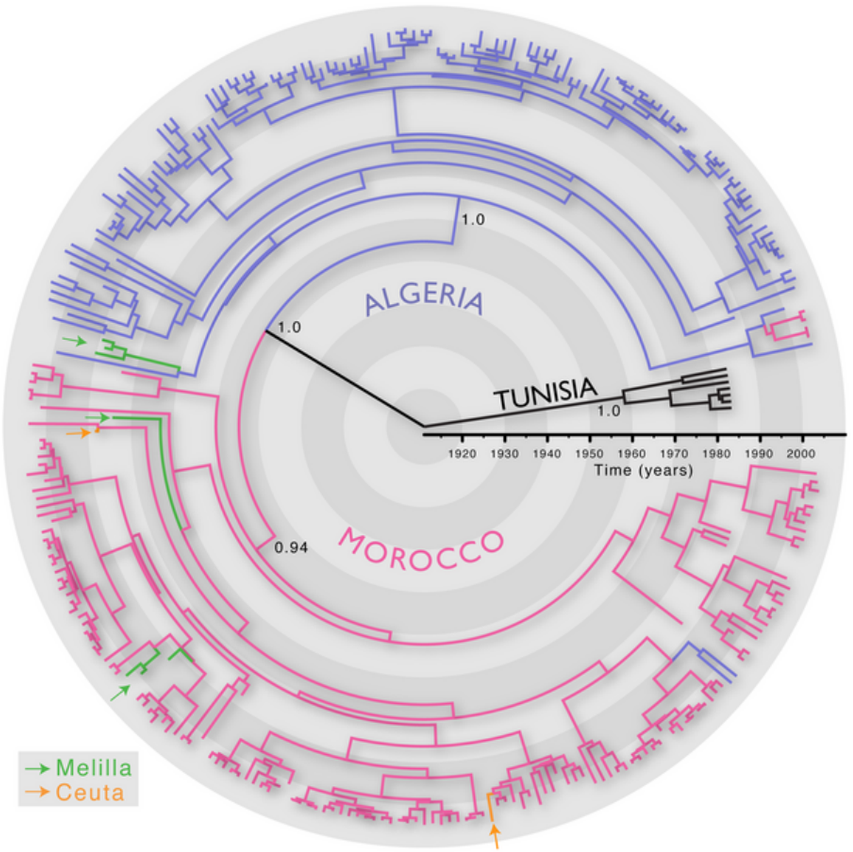
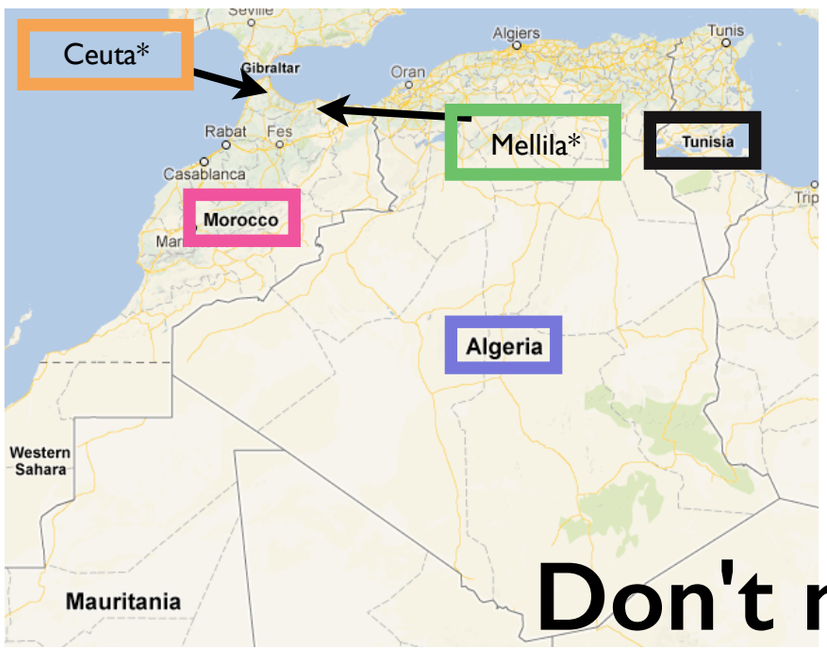
Interpreting Molecular Phylogenetic Trees: An Example





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

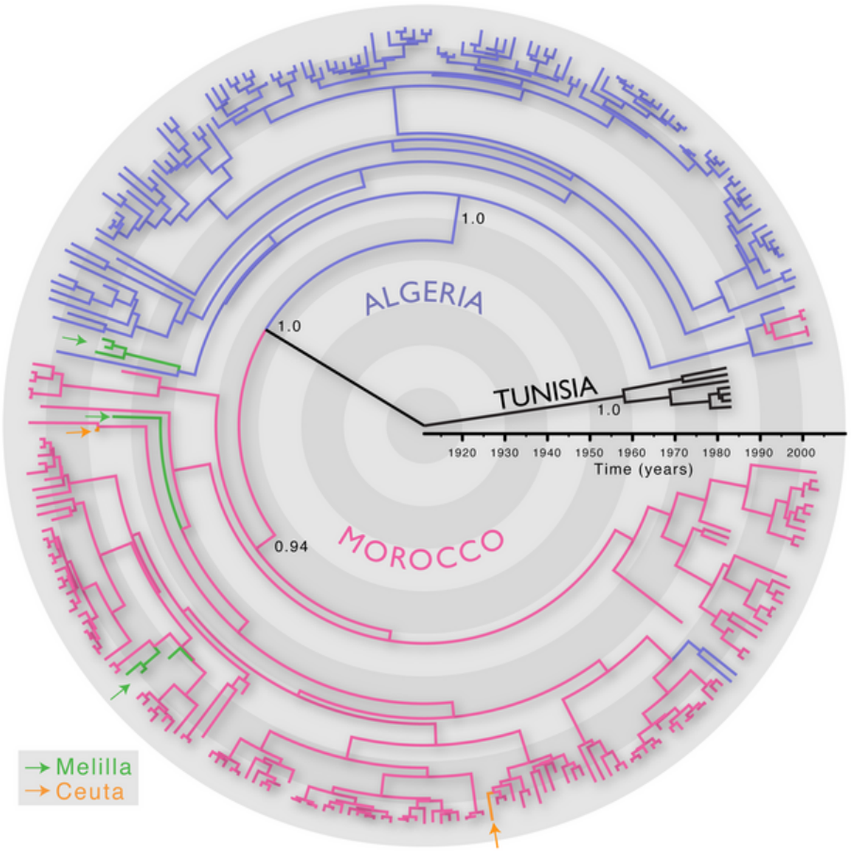
Then discuss the question with your neighbours

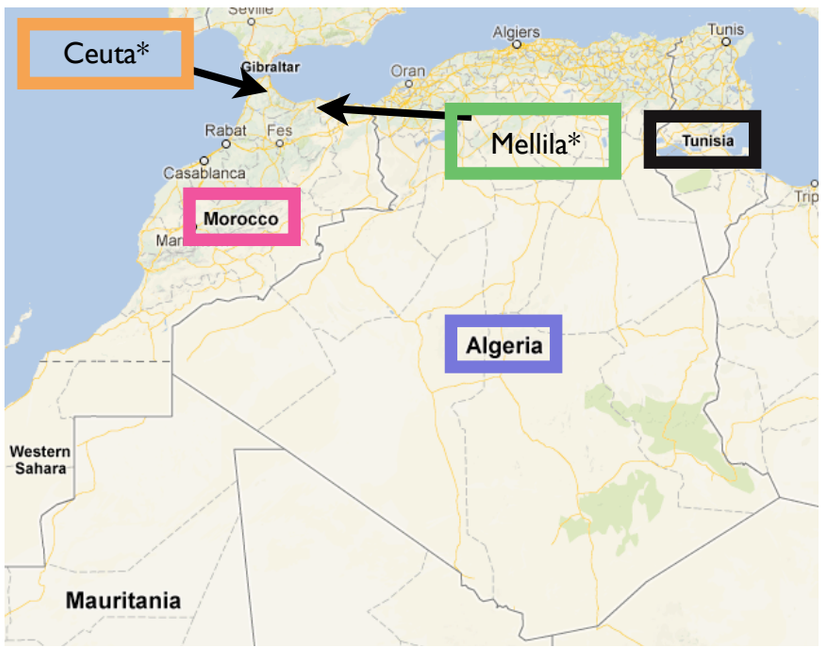
Feel uncomfortable that you don't know enough about the study/data to decide?

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

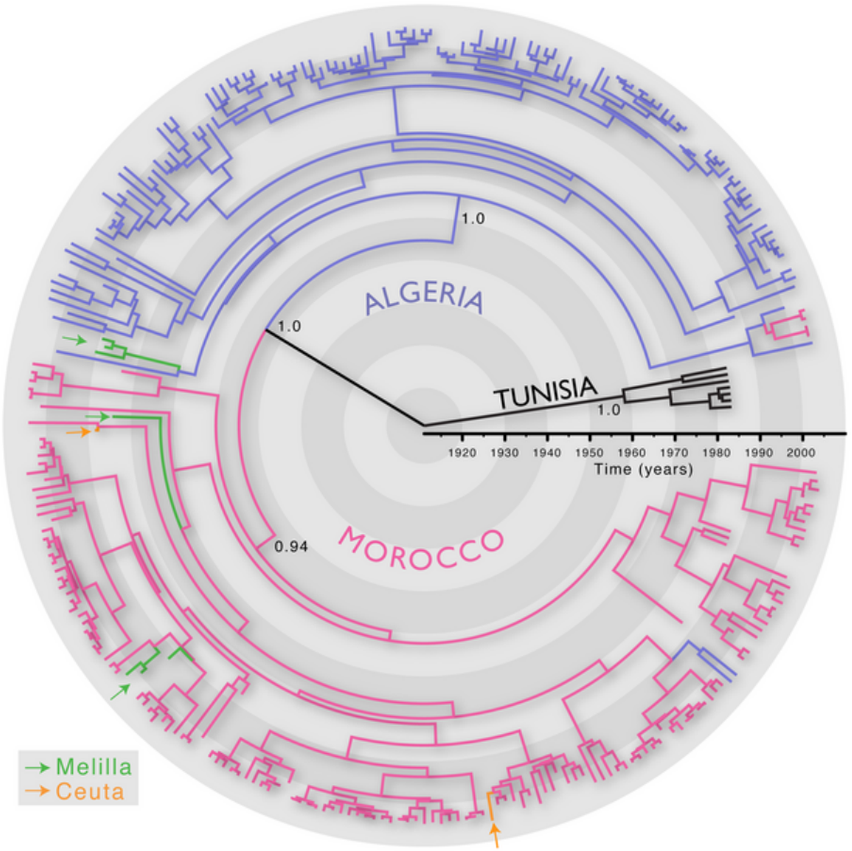
that human activity significantly influences the dynamics of rabies virus transmission between dogs

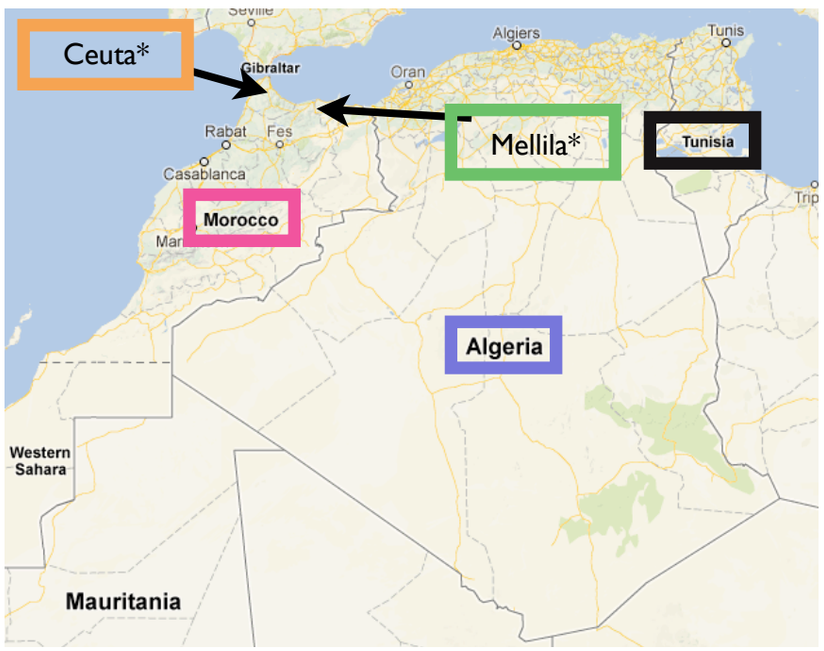
seen in the rarity of virus transmission across political (i.e. at least partially human-activity imposed) borders -

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

Aidan Budd, EMBL Heidelberg

Interpreting Molecular Phylogenetic Trees: An Example





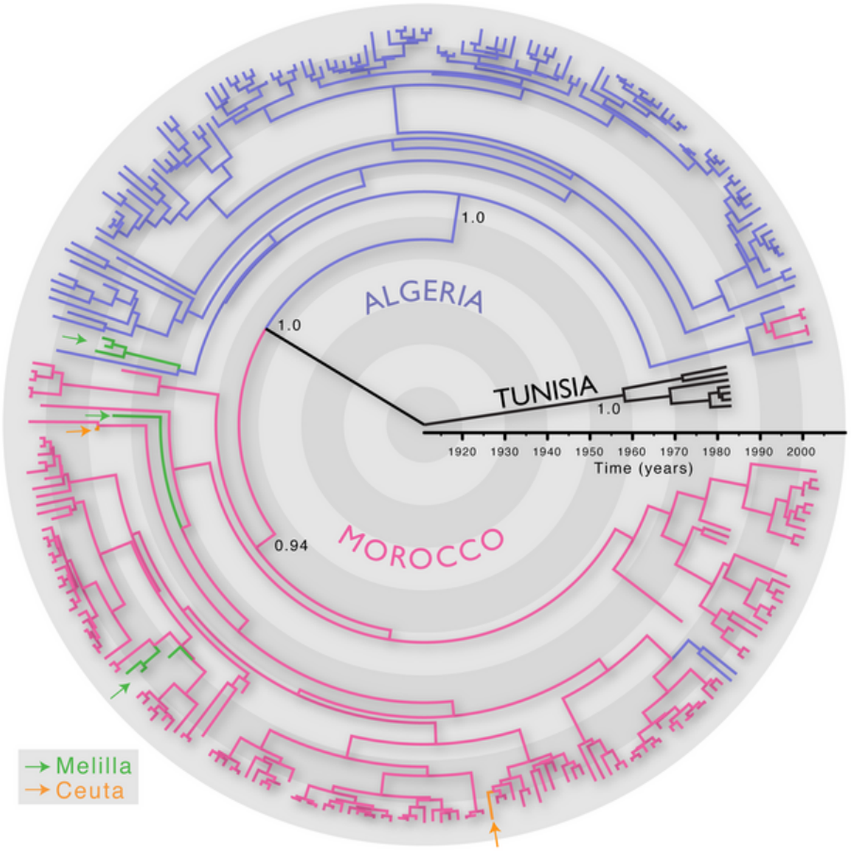
this exercise aimed to highlight that:

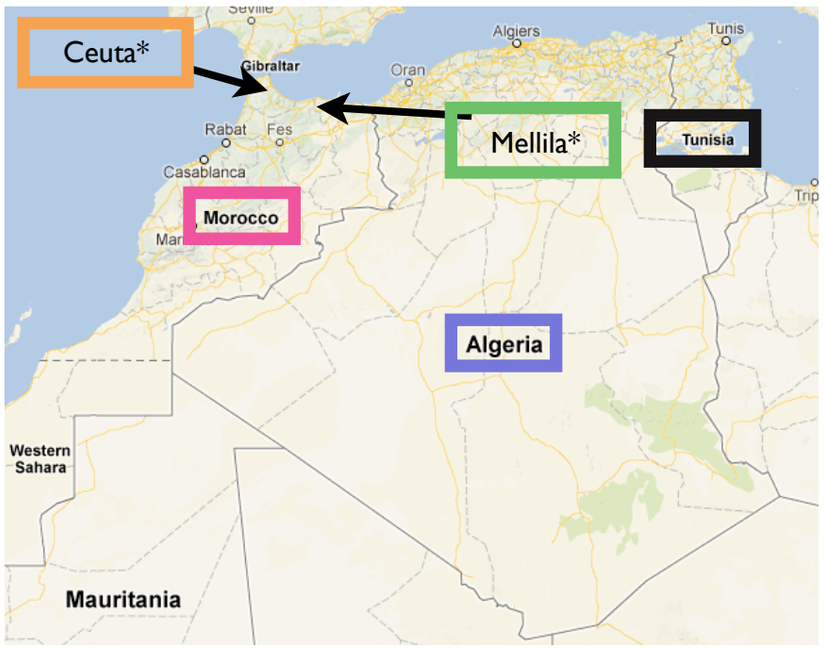
we **have to** make assumptions/use models to interpret the data - examples of assumptions that could be made while interpreting this tree:

- 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





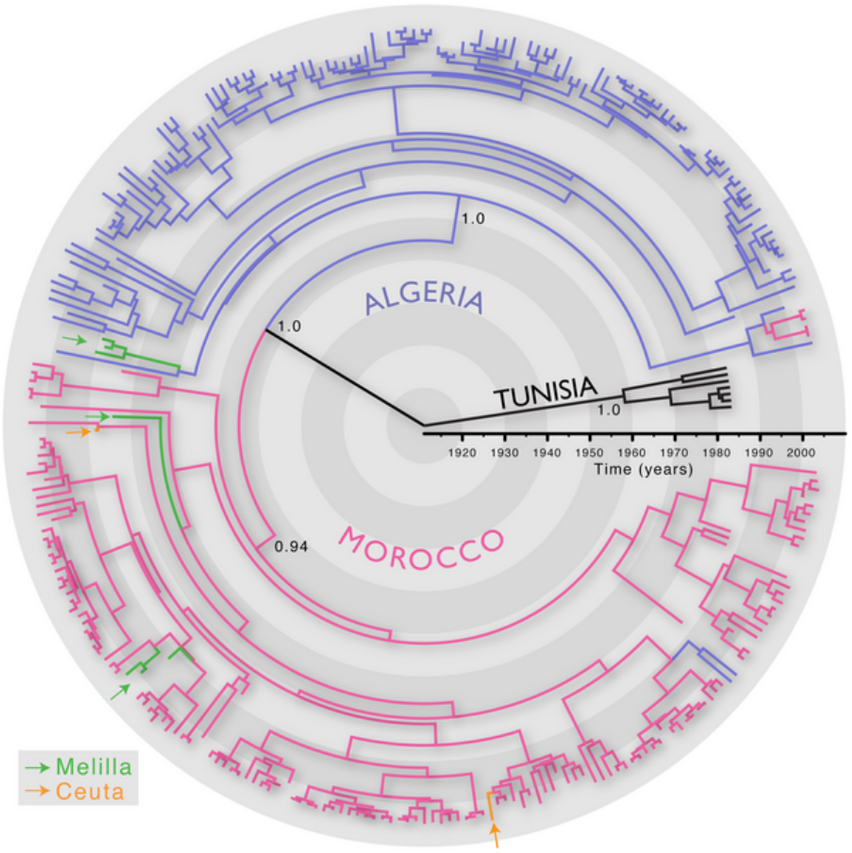
this exercise aimed to highlight that:

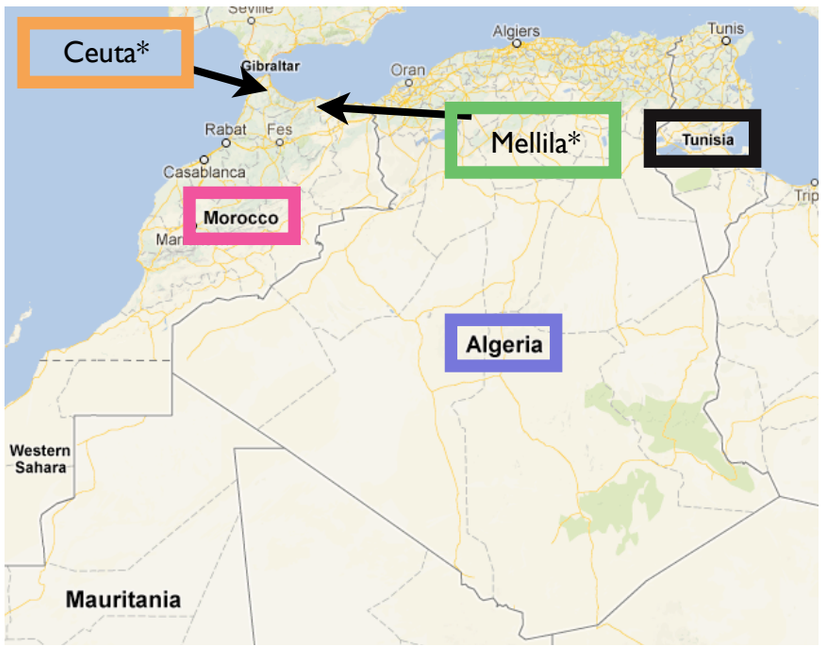
it's important to present information (the tree, the sample location) in a way that makes the conclusions you want to draw from the analysis clear/obvious

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

Aidan Budd, EMBL Heidelberg

Interpreting Molecular Phylogenetic Trees: An Example





this exercise aimed to highlight that:

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

Aidan Budd, EMBL Heidelberg

Interpreting Molecular Phylogenetic Trees: Another Example

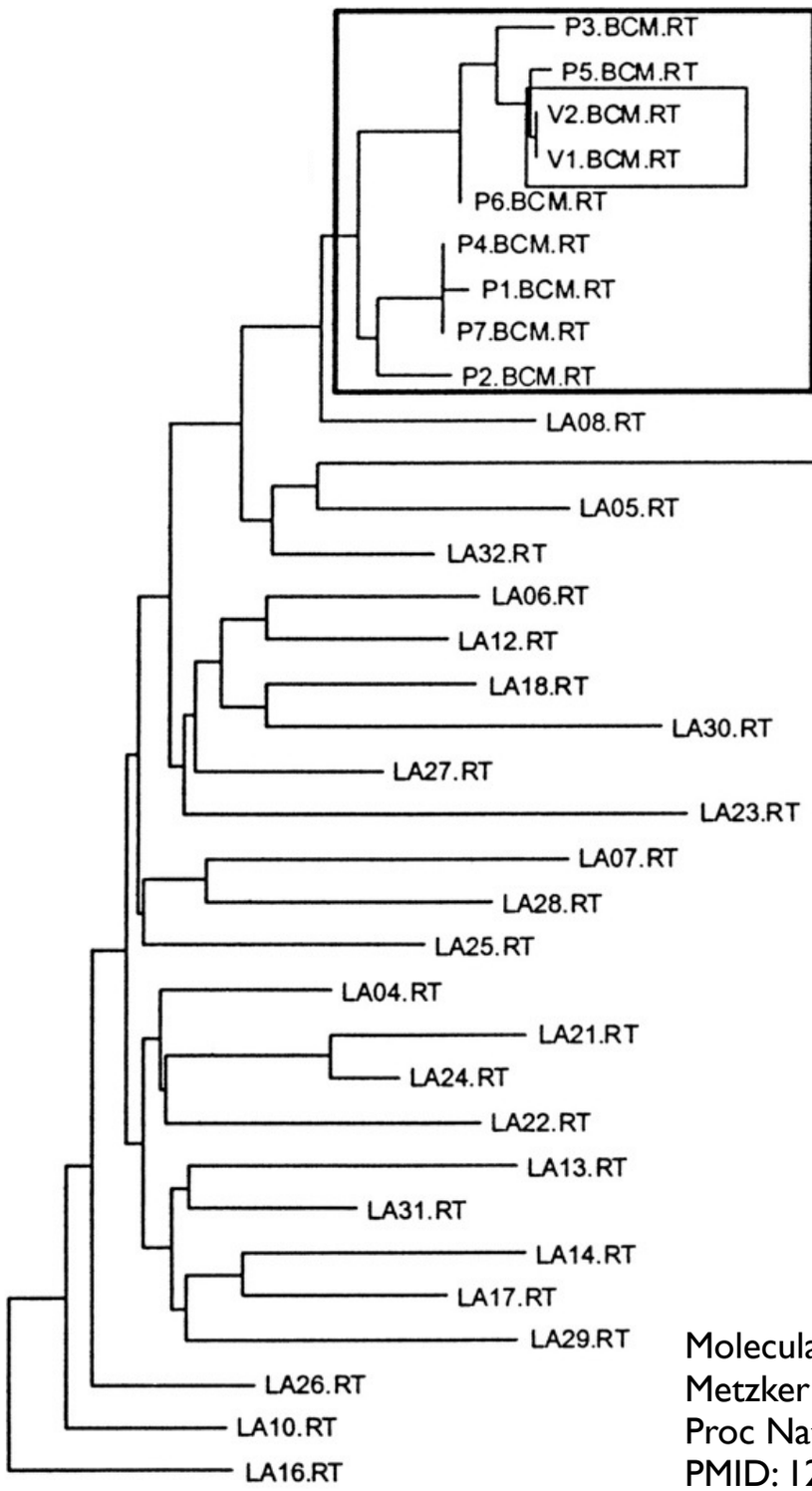
Interpreting together a tree from a published article

Highlights a forensic application of phylogenetics

- an example in which the accuracy with which a tree is estimated has high stakes

Helps explore common features and problems when interpreting trees

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

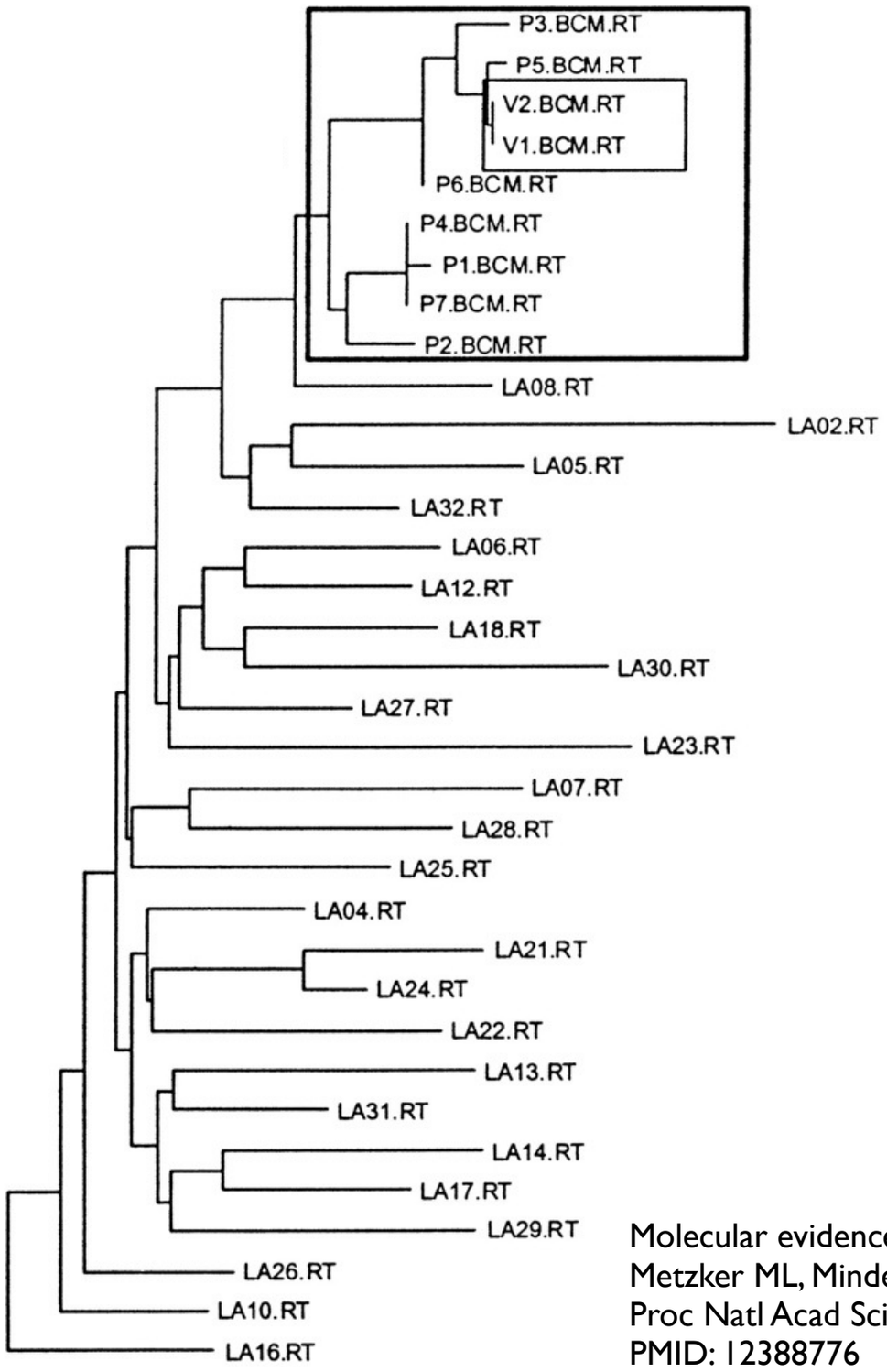
Phylogenetic analyses used as evidence in the trial

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



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

Which questions would you want the witness presenting the tree to be asked…

…to make the data as useful as possible for judging the guilt/non-guilt of the defendant?

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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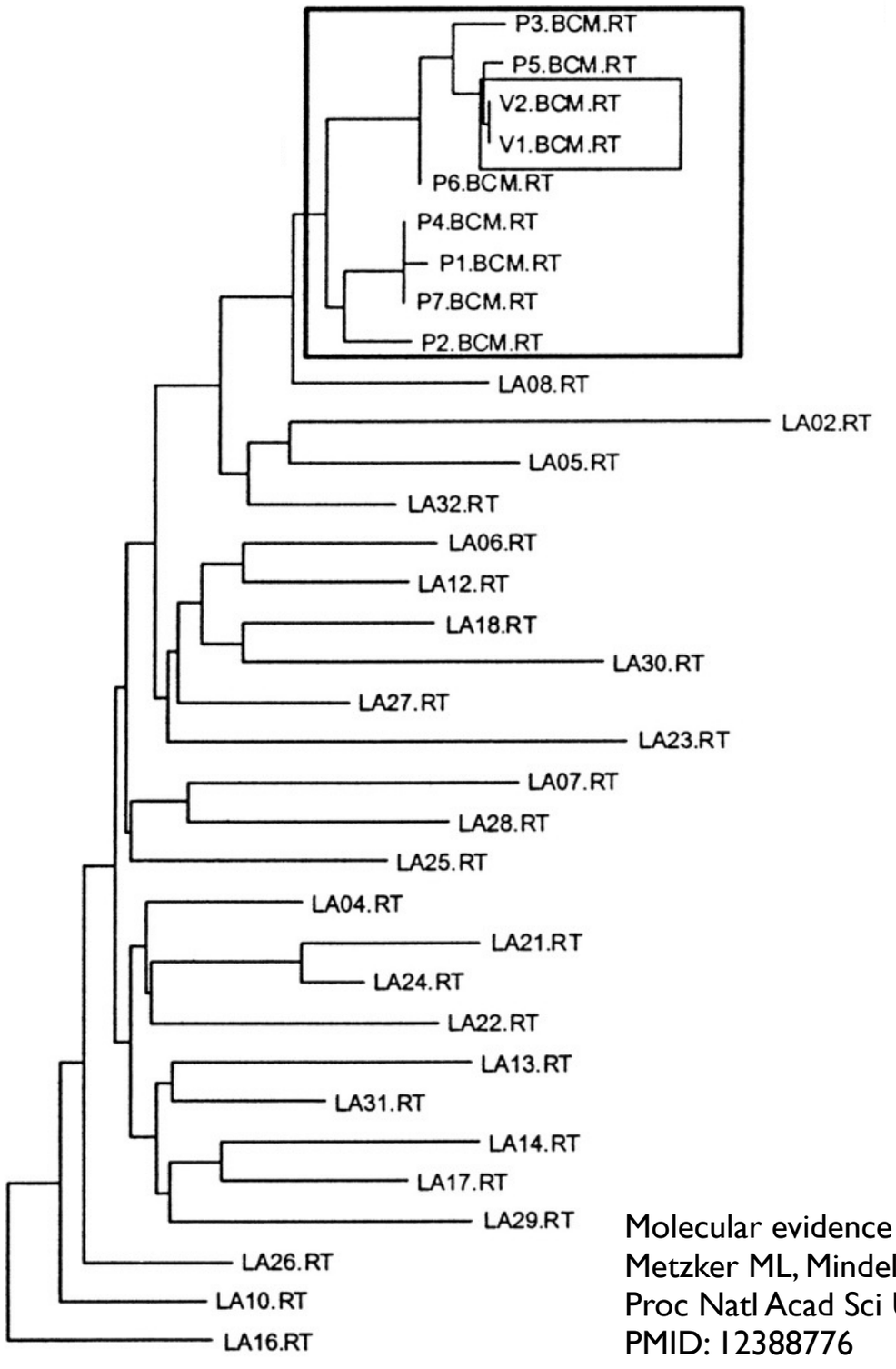
Interpreting Molecular Phylogenetic Trees: Another Example



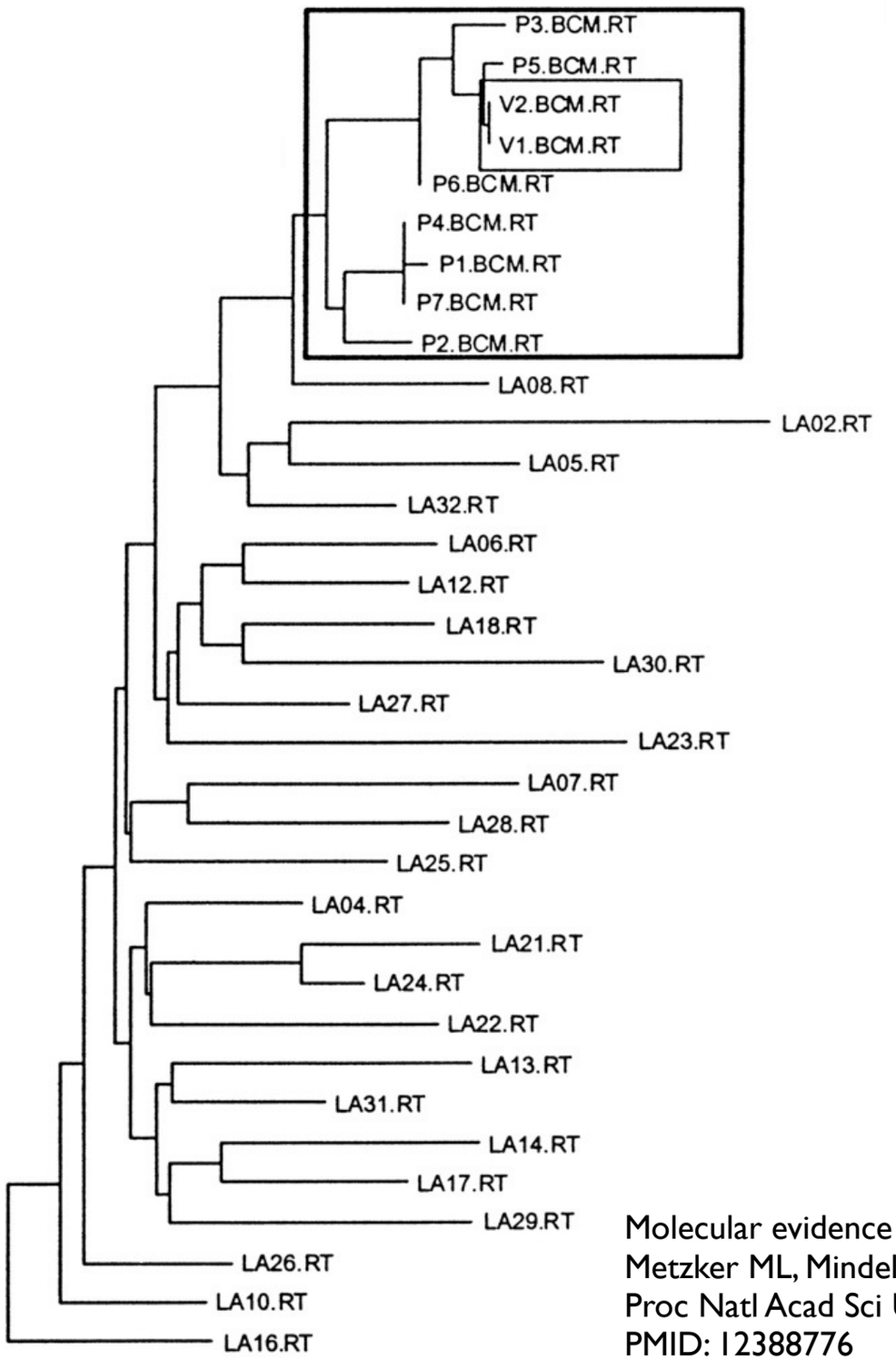
Information about where and how OTUs were sampled impacts how we interpret a tree

The way we label OTUs in a tree affects how quickly and easily we can interpret it - choosing labels to highlight key features of your data relevant to your questions of interest can help make it easier to interpret

Scale bars (when a tree's (branches have a 'length') give important information for our expectations of how accurate we think a tree might be

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



Estimates of random error associated with parameters in the tree is also useful information for assessing how well we think the data supports a given hypothesis

The "quality" of the data (here it's a nucleotide sequence alignment) used to estimate parameters (here we're most interested in the tree topology parameter) also impacts the confidence we have in an parameter value being accurate

Describing parameters we'd expect under different hypotheses (e.g. sketching tree shapes associated with guilt or innocence) can help when interpreting results

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

Relatedness

Aidan Budd, EMBL Heidelberg

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

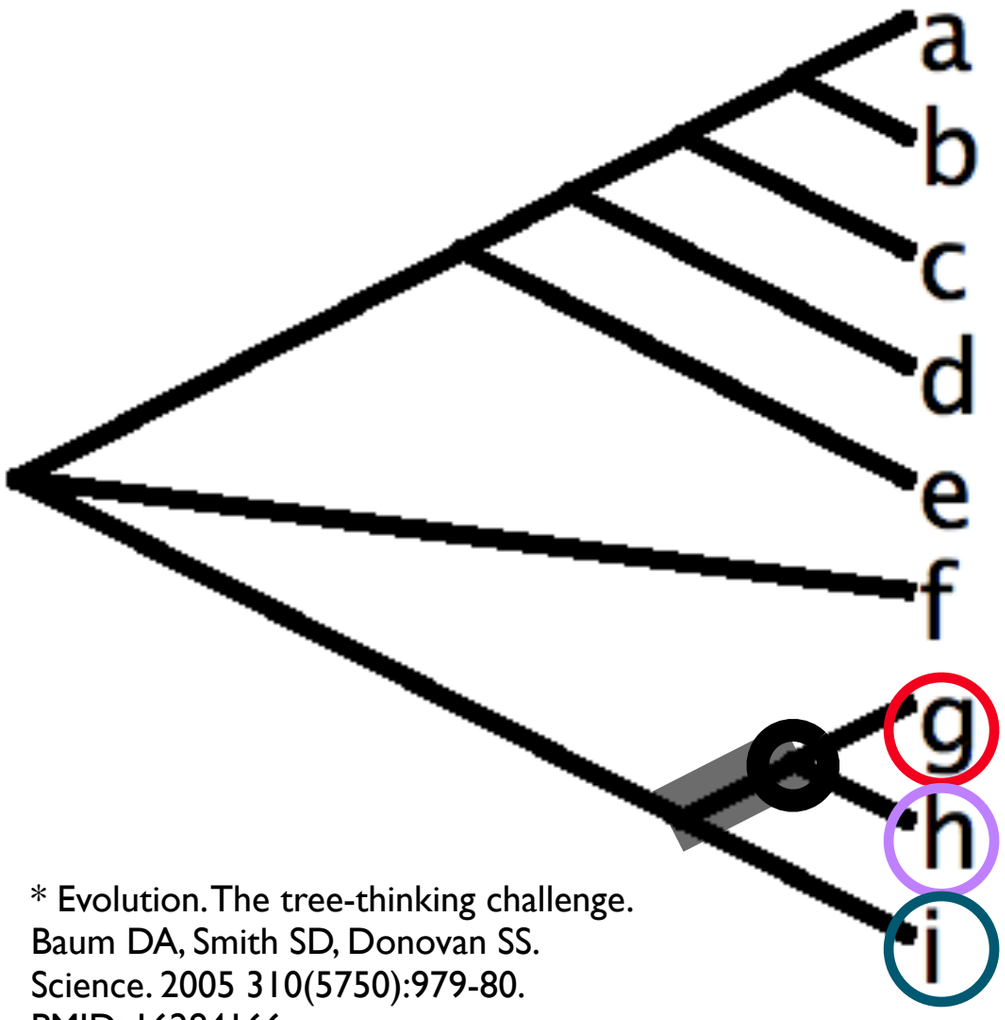
As many trees are estimated to inform ideas about patterns of relatedness, we need a clear understanding of how the term is used in this context

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

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Relatedness
(in the context of phylogenetic trees)

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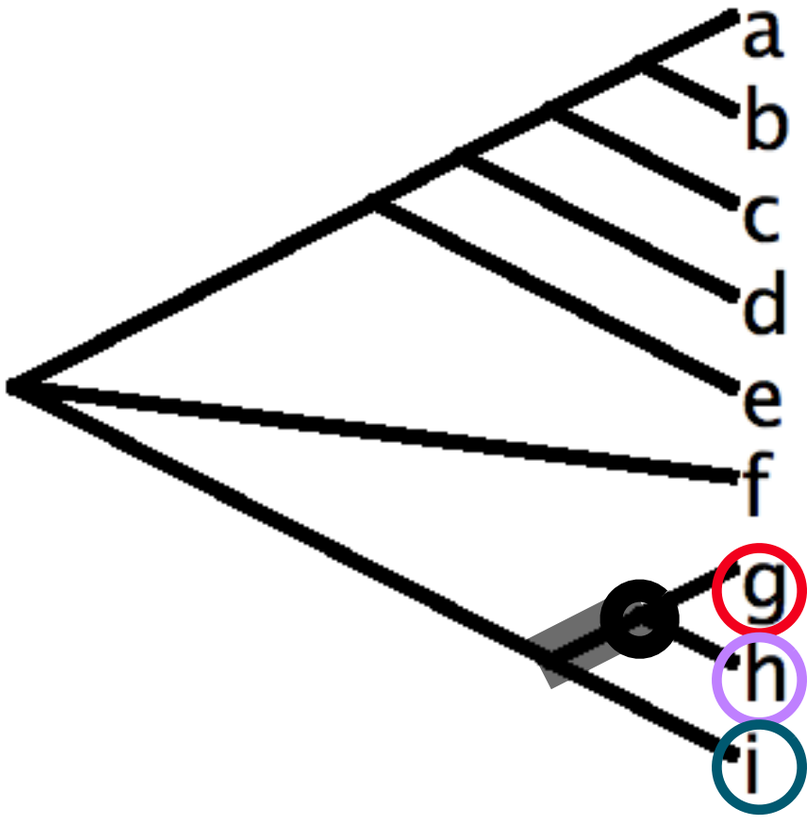
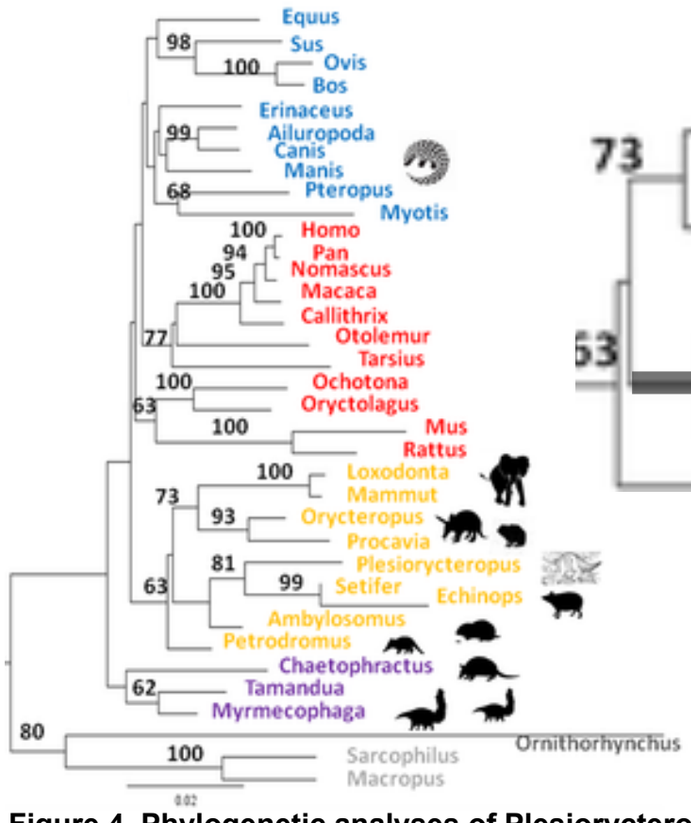


"... Plesiorycteropus is **more closely related** to tenrecoids than to tubulidentates .."



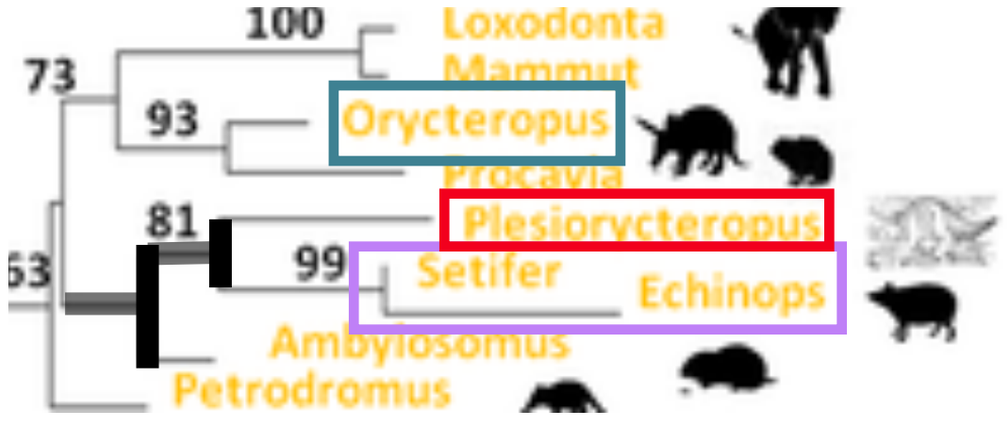


Figure 4. Phylogenetic analyses of Plesiorycteropus collagen (I) sequences obtained by LC-MS in comparison to previously postulated closest relatives.

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

Aidan Budd, EMBL Heidelberg

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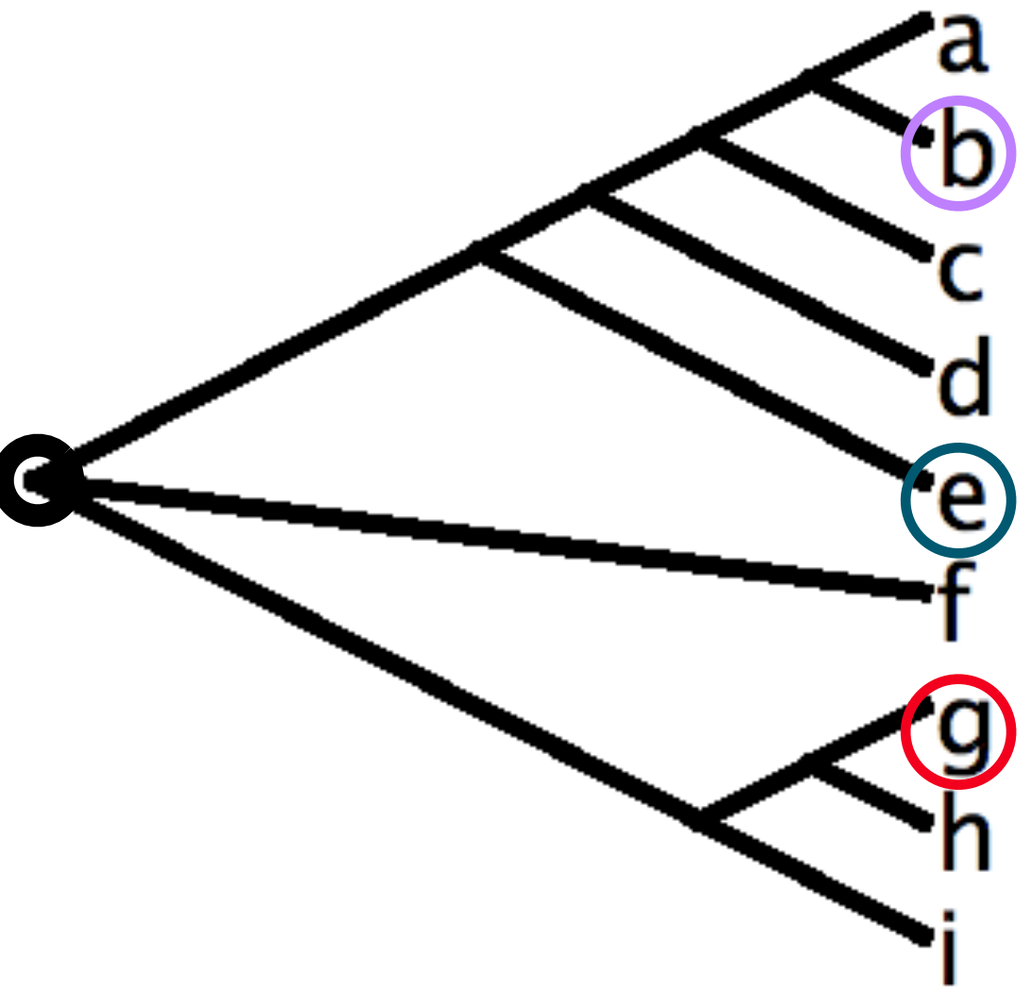
Relatedness
(in the context of phylogenetic trees)



of all the OTUs represented in this tree

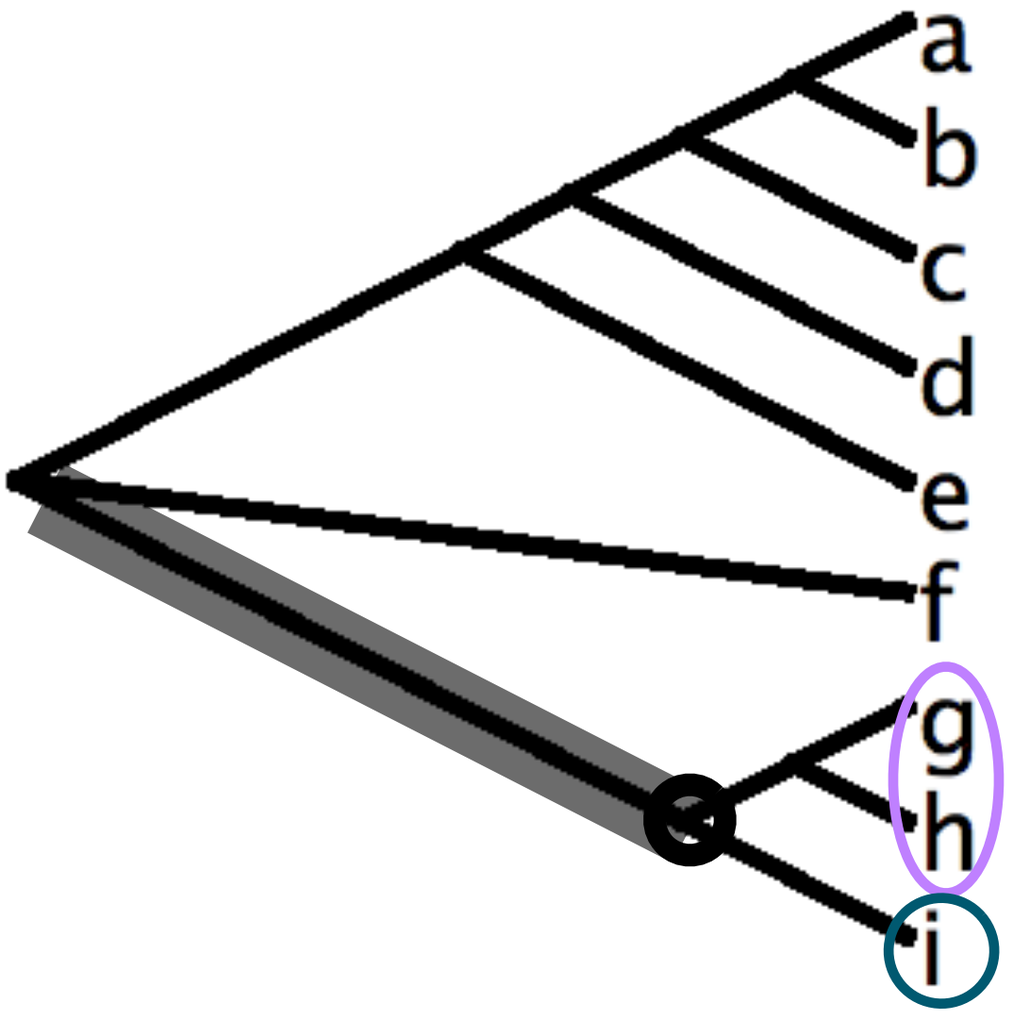
g is **equally distantly related** to **b** as it is to **e**

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**)

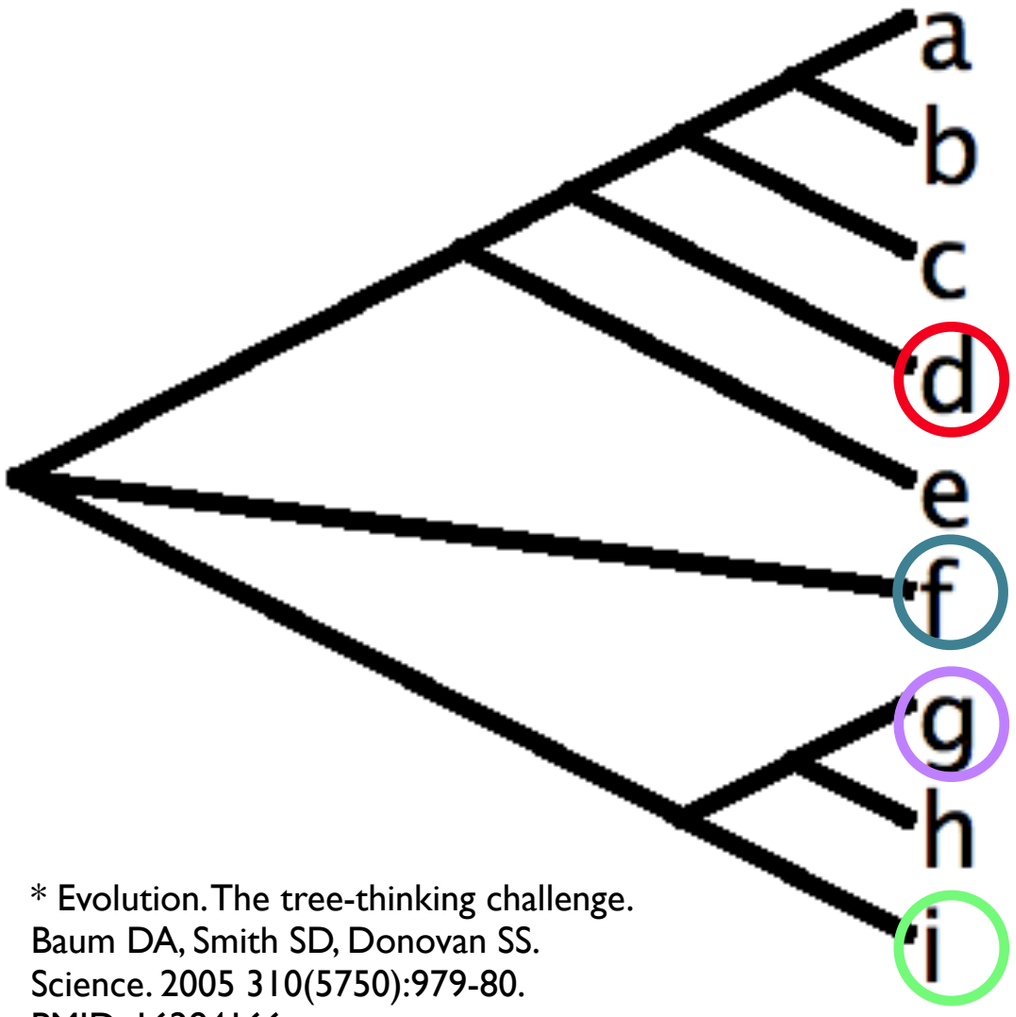
because **i** shares common ancestors with **g** and **h** that it does not share with any other OTUs in the tree

"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" *



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Baum DA, Smith SD, Donovan SS.
Science. 2005 310(5750):979-80.
PMID: 16284166

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

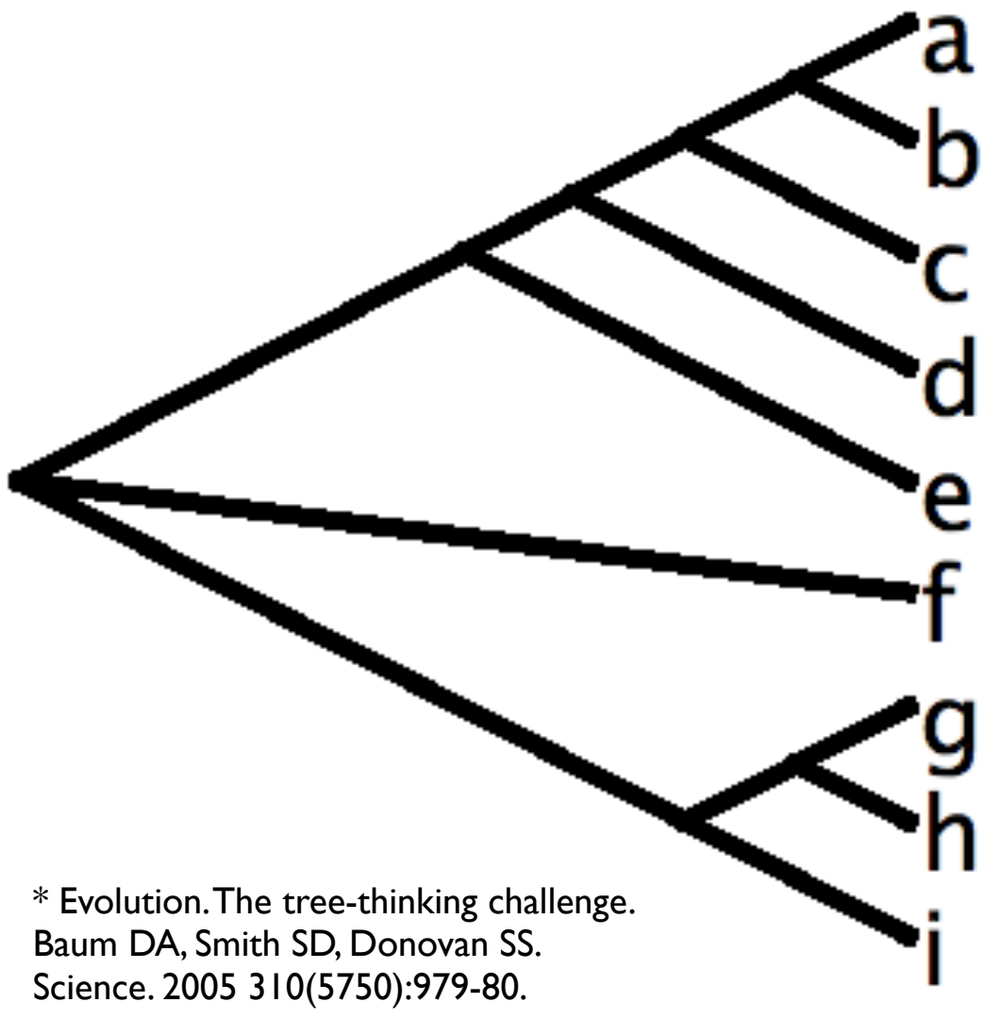
1. d is **more closely related** to g than to f or i

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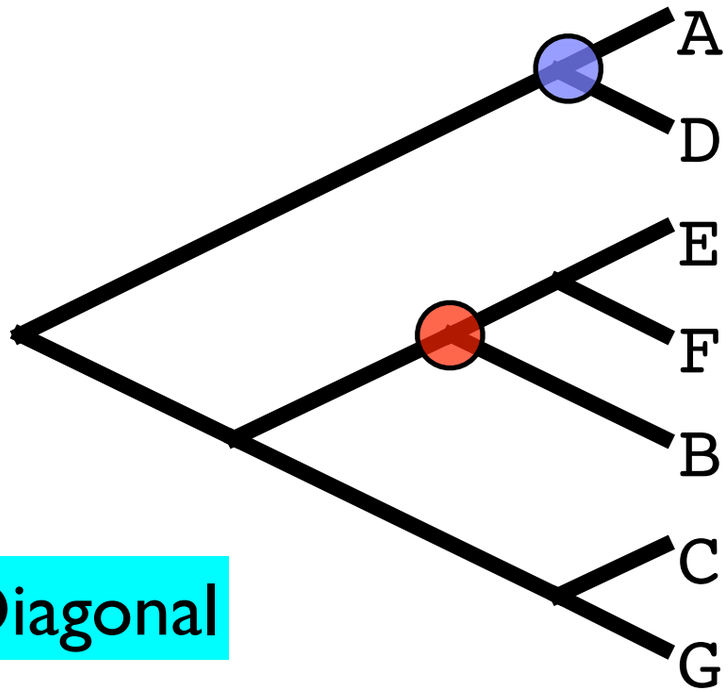
Why spend so much time discussing "relatedness" with you?

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

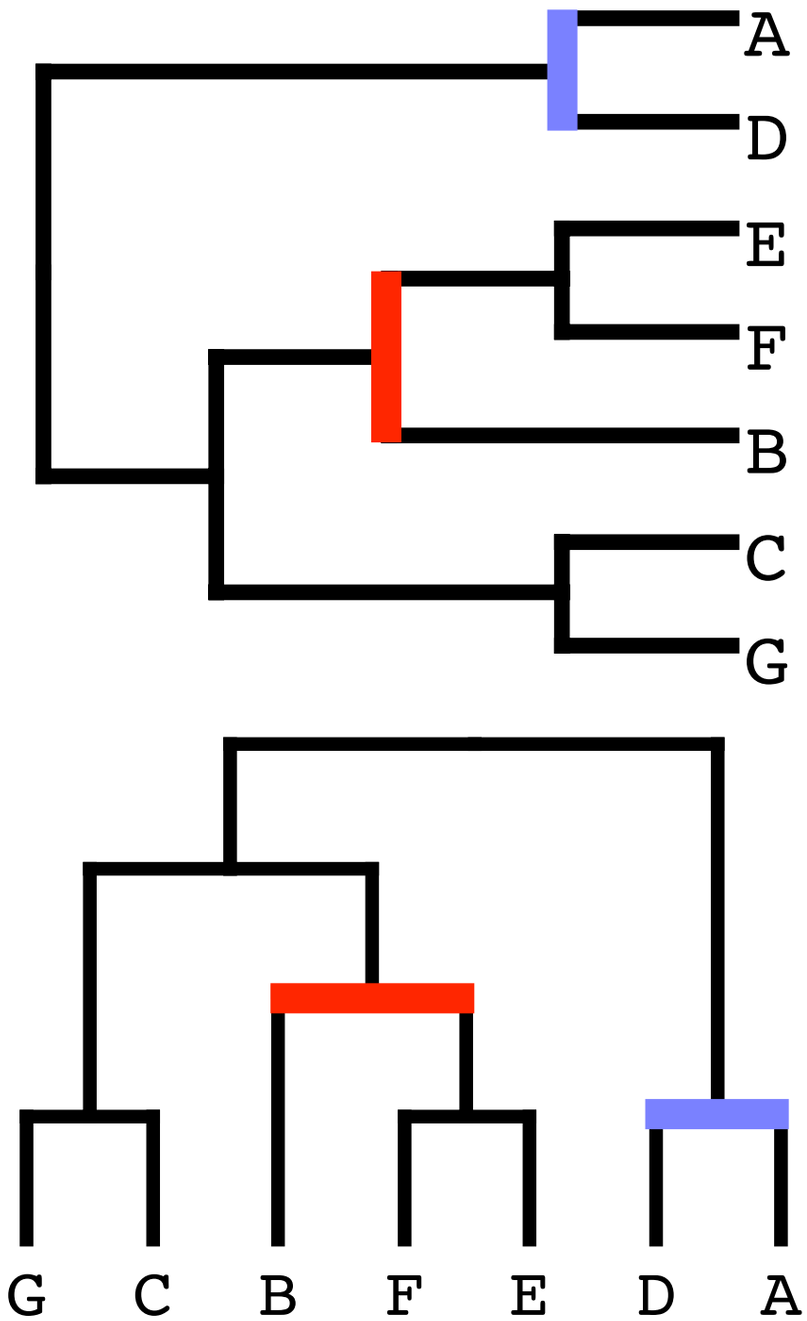
Tree Representations



Diagonal

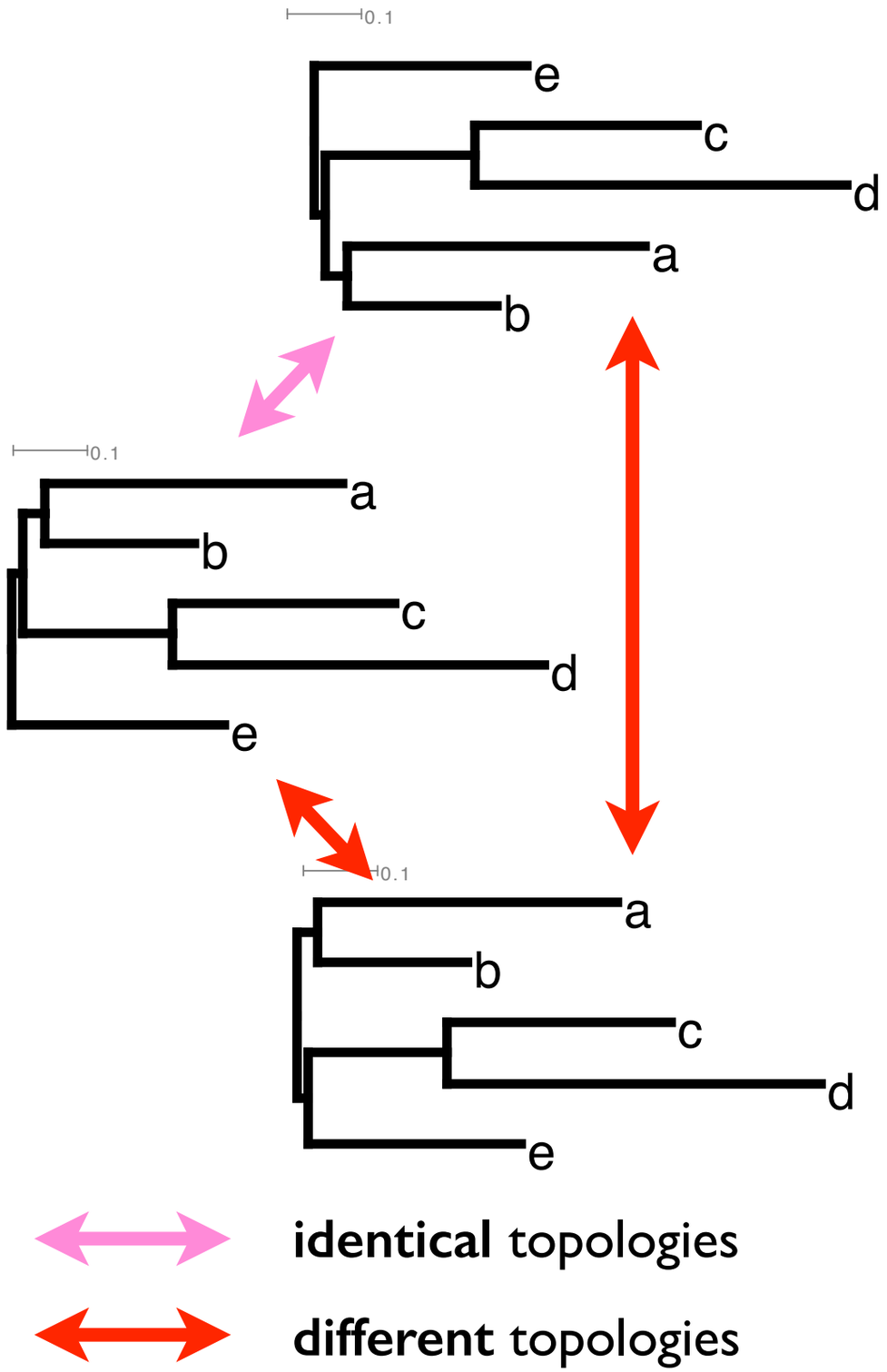
Most rooted tree figures use a "rectangular" rather than a "diagonal" representation

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



Rectangular

Tree Topology



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

identical topologies

different topologies

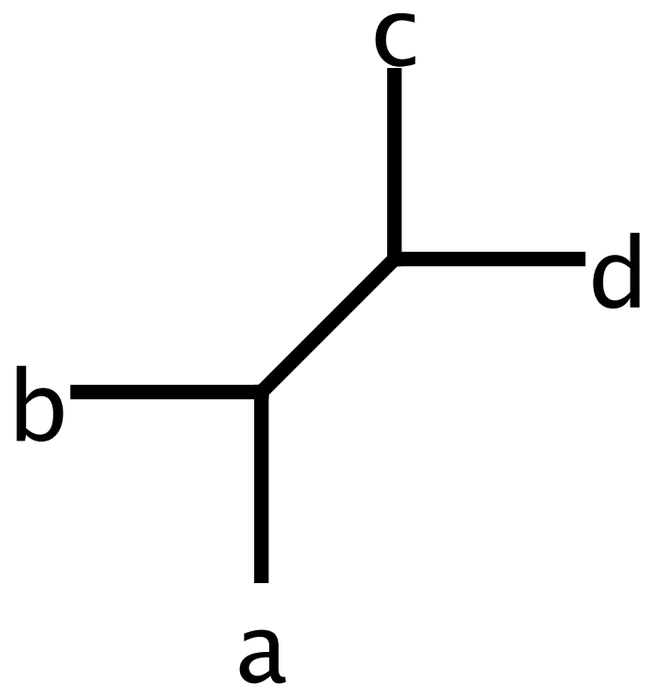
Unrooted Phylogenies

Aidan Budd, EMBL Heidelberg

Unrooted Trees

There's no root on the tree...

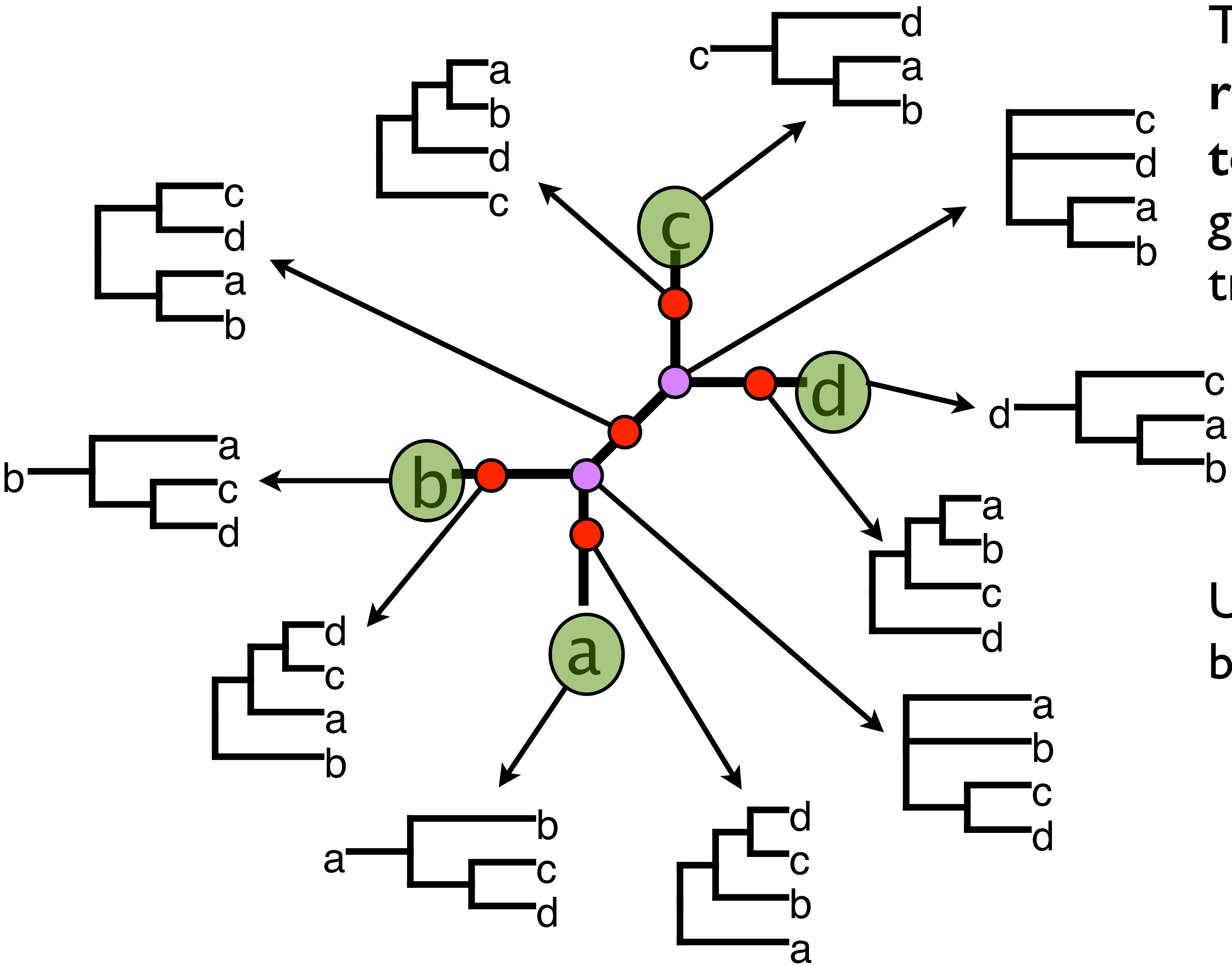
... which is usually interpreted as meaning that these taxa are related by a rooted tree **but** we don't know where the root is



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

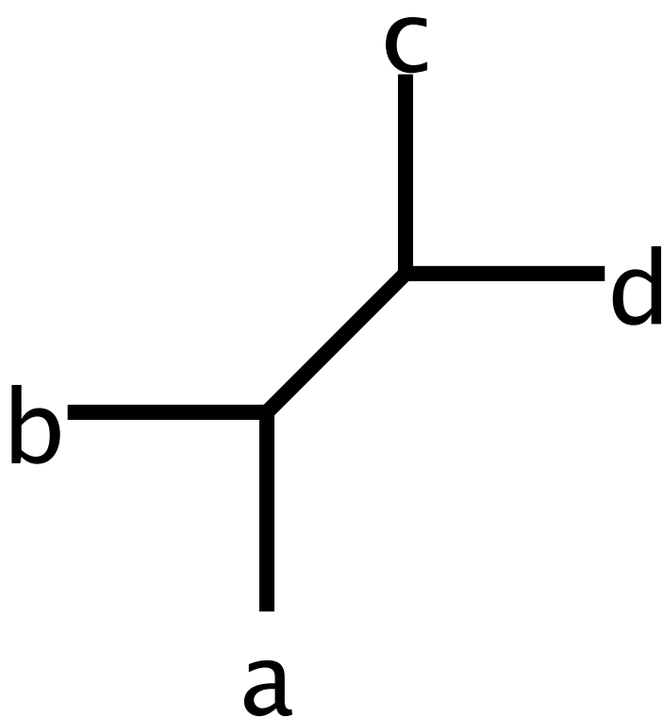
Unrooted trees can be rooted on their:

- branches
- interior nodes
- terminal nodes

Quiz

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

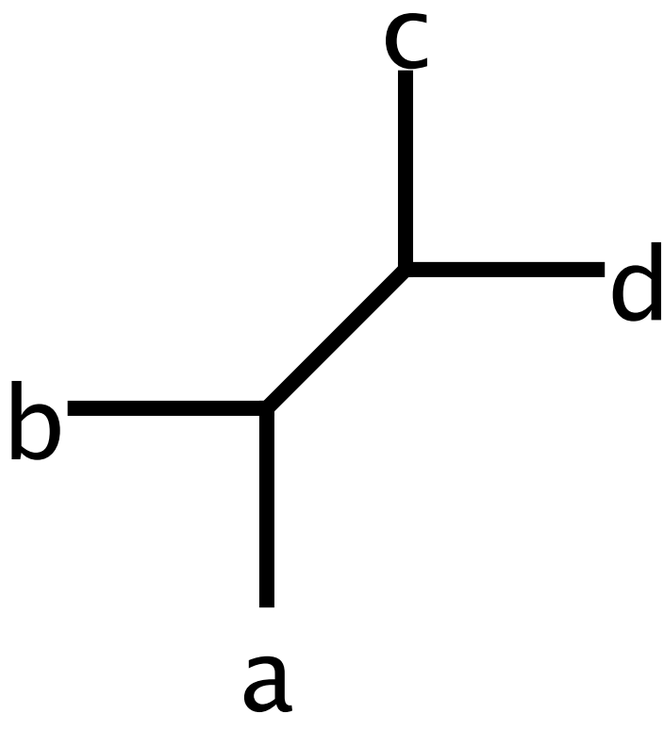
We aren't justified in making any of these statements, as the tree is unrooted, and none of them is true under all possible footings of the tree

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

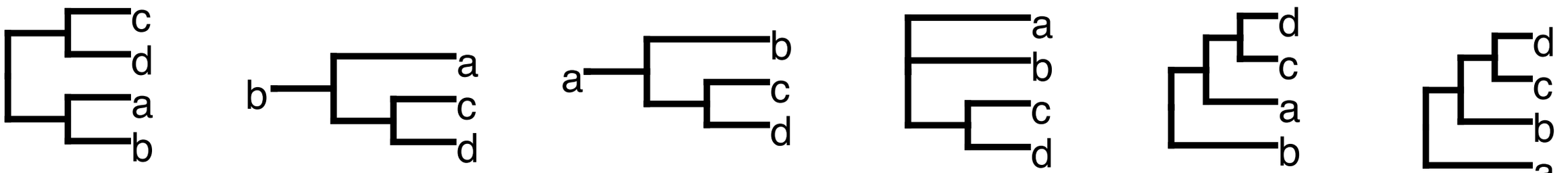


d more closely related to:

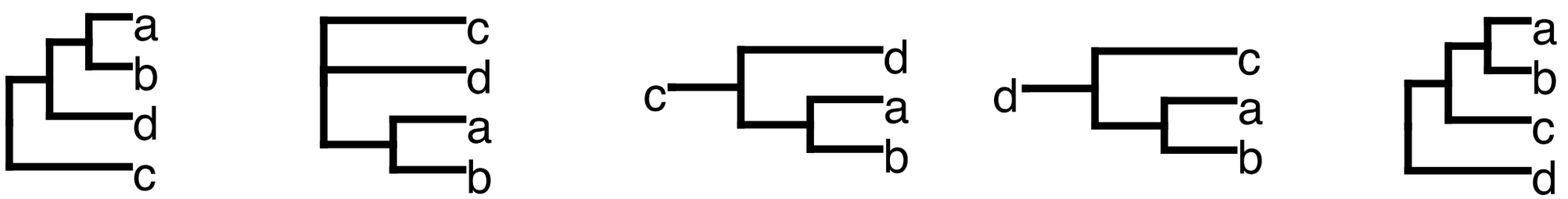
1. **a** than it is to **b** or **c**
2. **b** than it is to **a** or **c**
3. **c** than it is to **a** or **b**

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**



Example Phylogeny Estimation Workflow

Aidan Budd, EMBL Heidelberg

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

1. phylogenetic analysis as a standard statistical analysis

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
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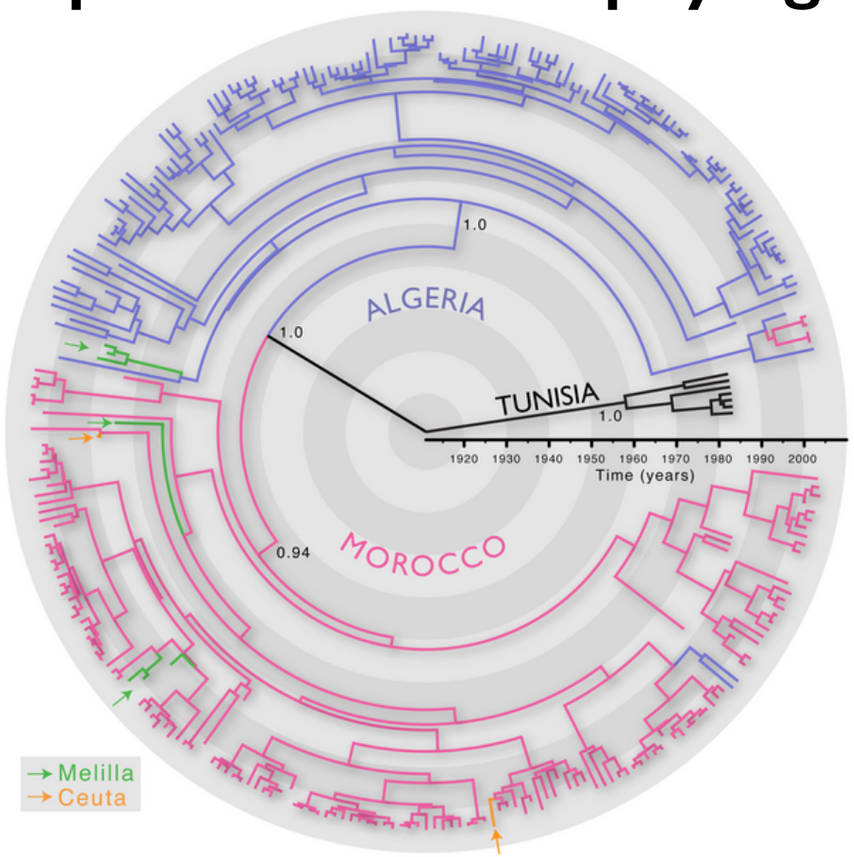
Example Phylogeny Estimation Workflow

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**



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

1. Pose a substantive question

For example:

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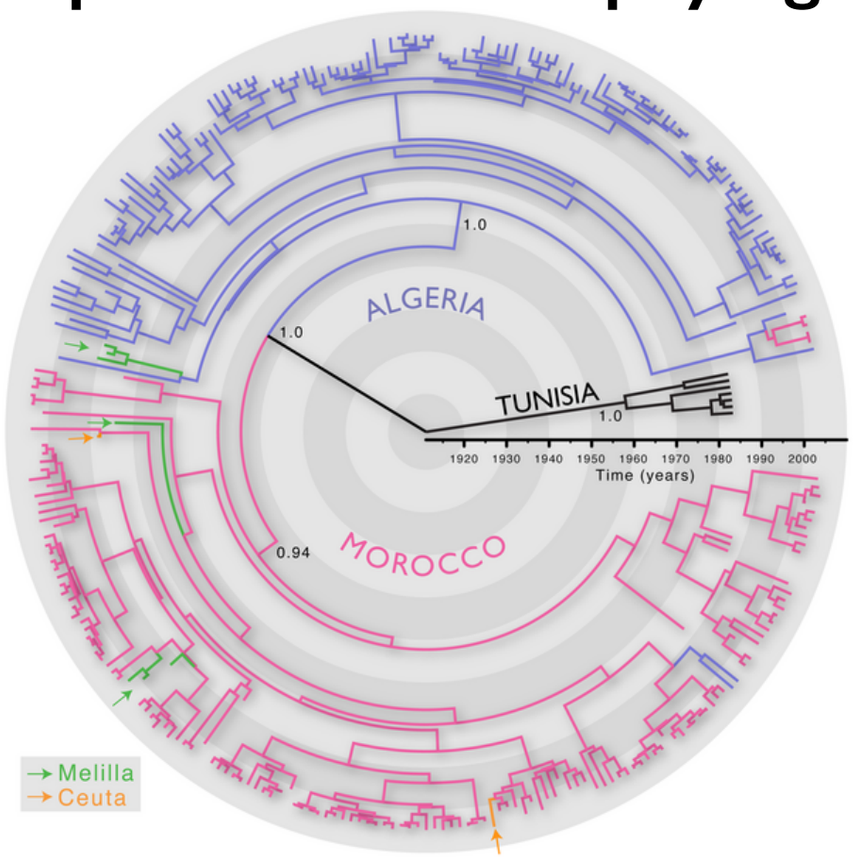
Crucially: a substantive question such that **knowledge** (or rather estimation) **of parameters in a phylogenetic model can inform our answer**



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**?

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)
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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

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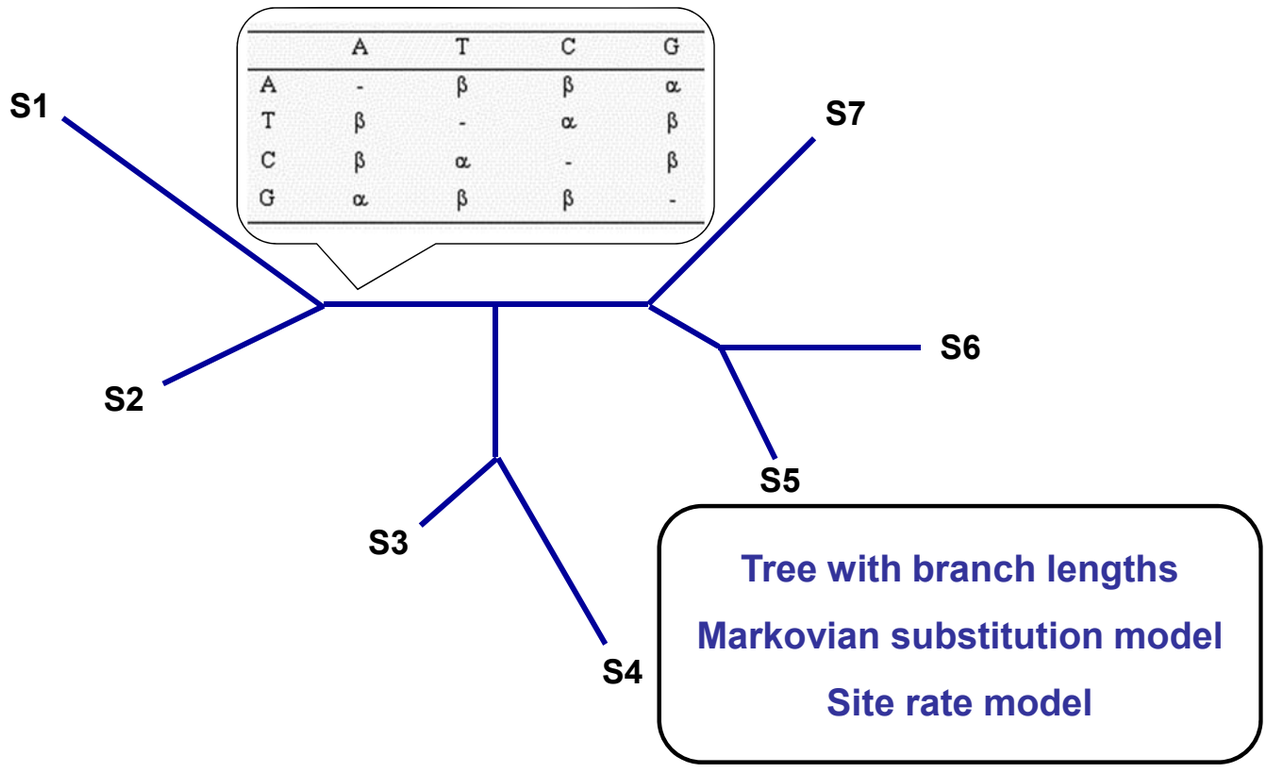
The full probabilistic model

- 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

Institut de biologie computationnelle

The full probabilistic model

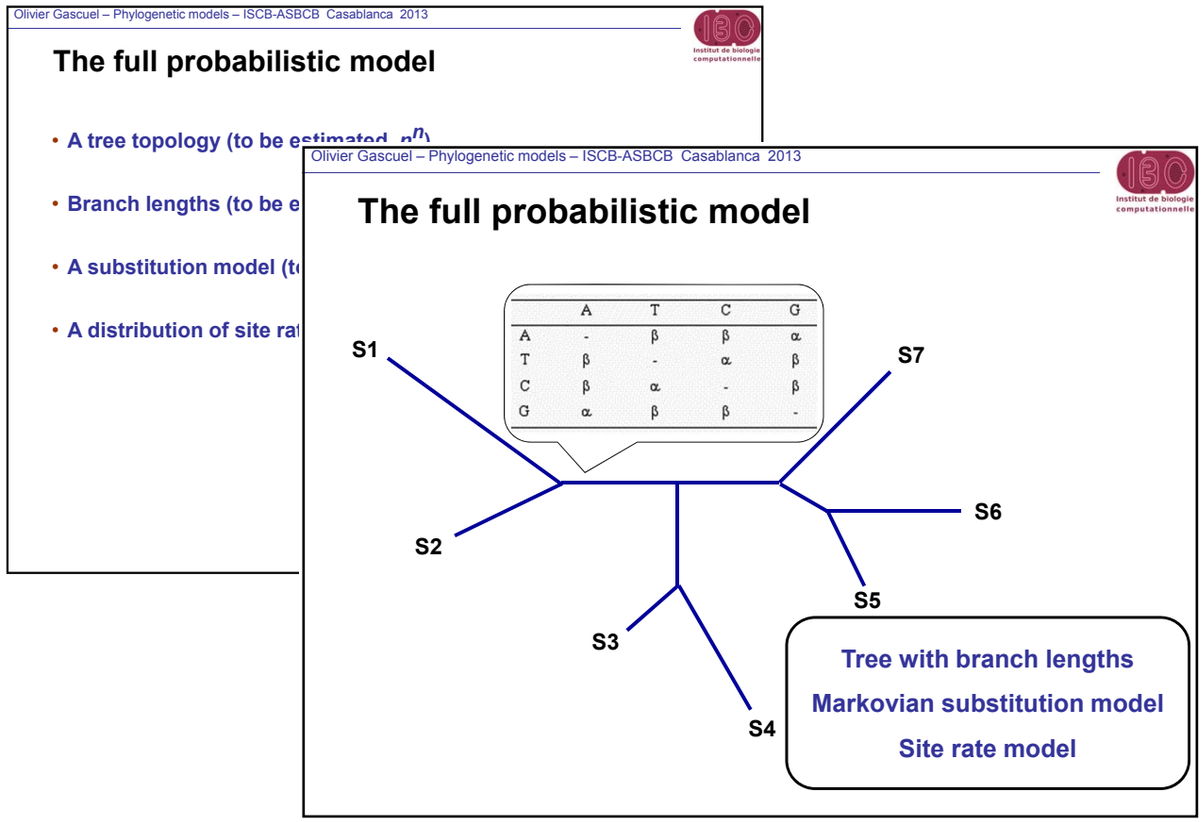


Thanks to Olivier Gascuel for these slides

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

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)
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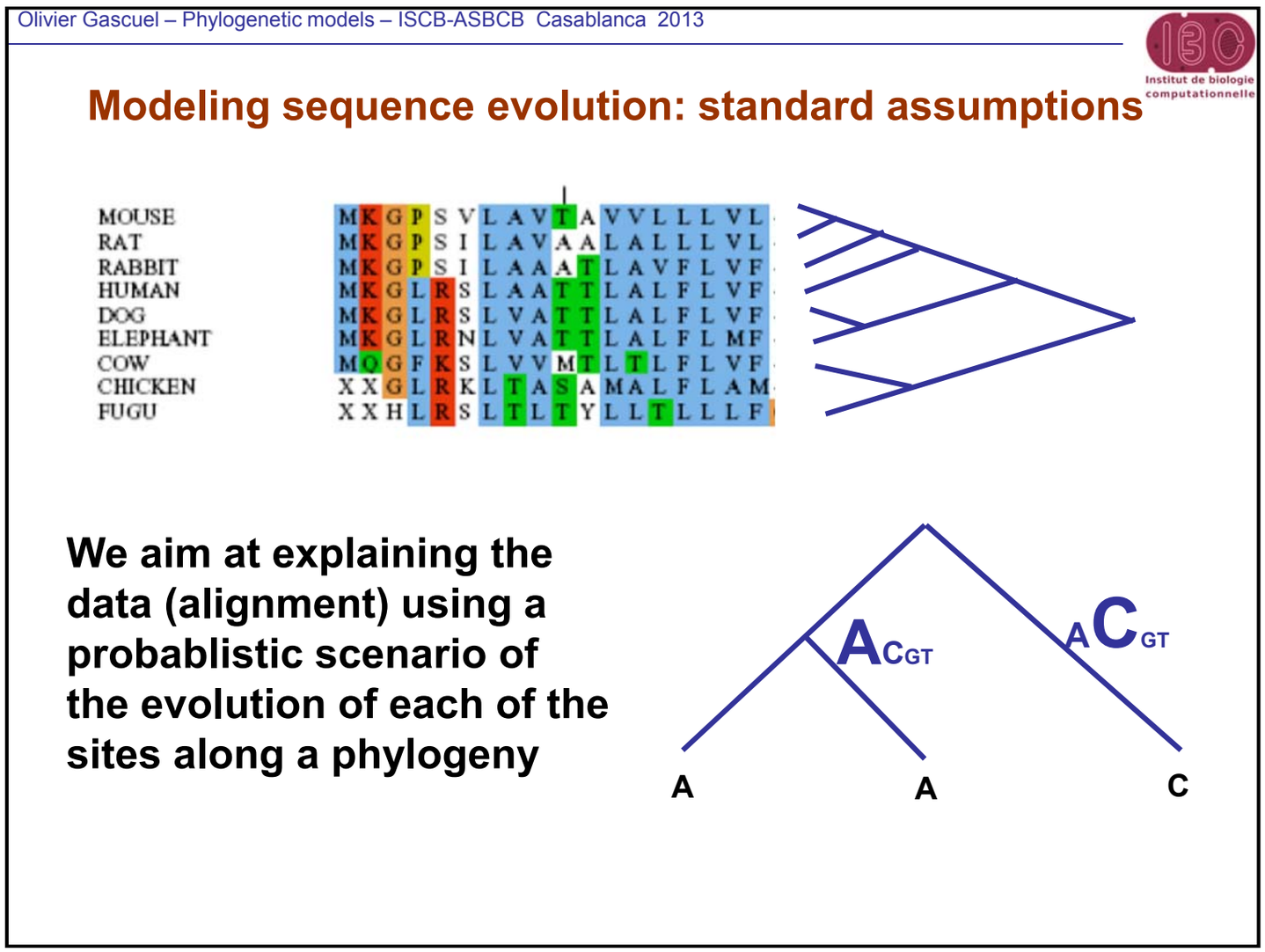
6. Answer your question using these parameter estimates

Example Phylogeny Estimation Workflow

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

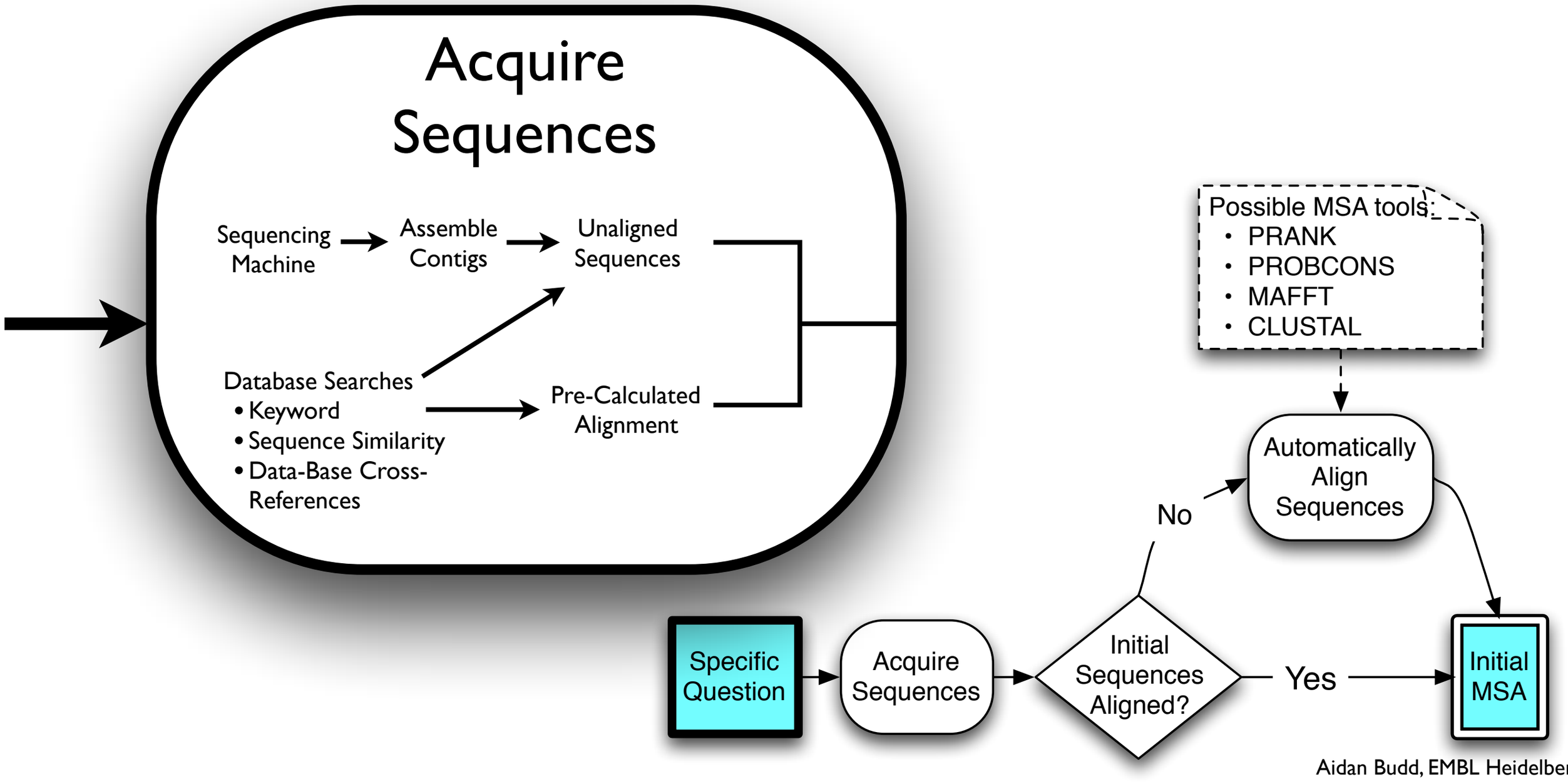


e.g. build a multiple sequences alignment of north African dog rabies sequences

Thanks to Olivier Gascuel for this slide

Example Phylogeny Estimation Workflow

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



Example Phylogeny Estimation Workflow

1. Pose a substantive question
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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
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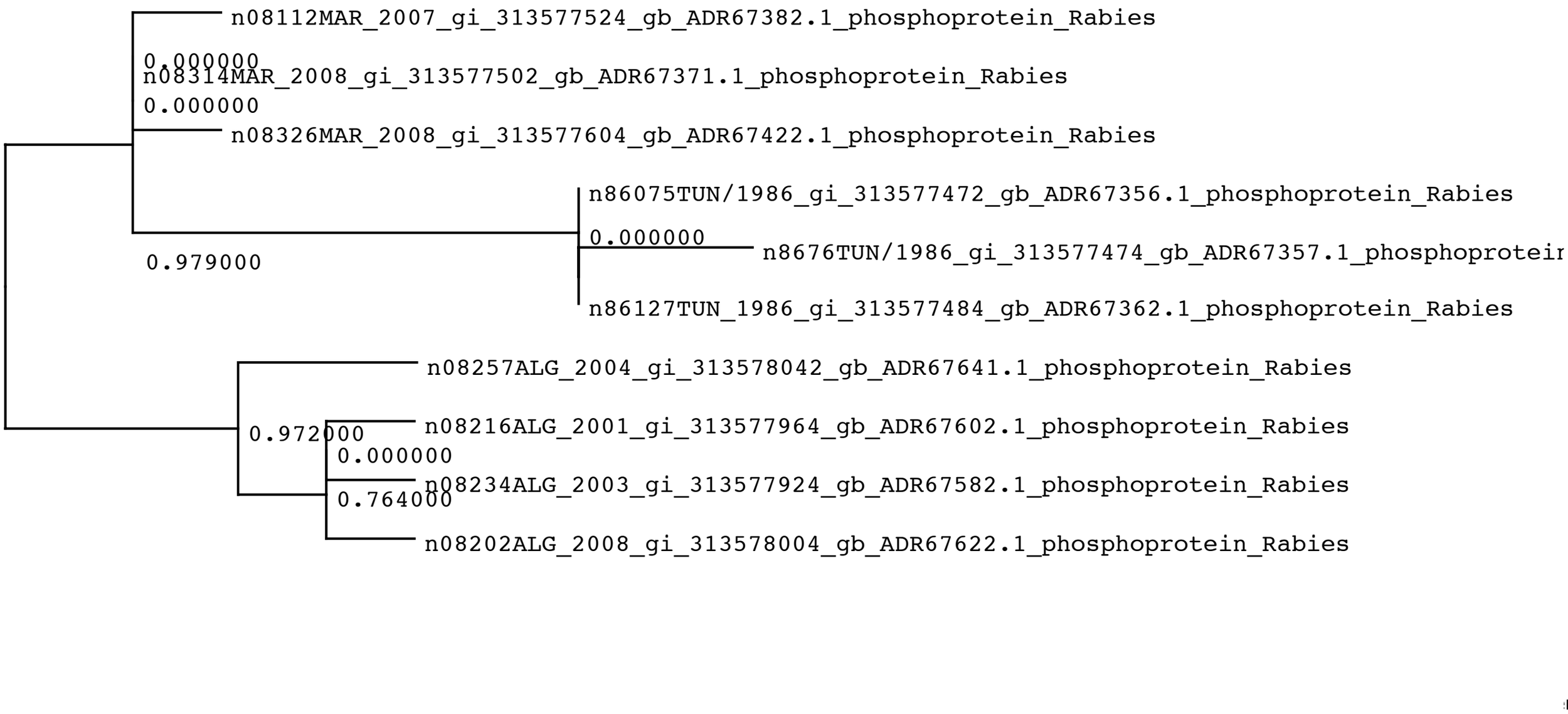
6. Answer your question using these parameter estimates

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 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

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

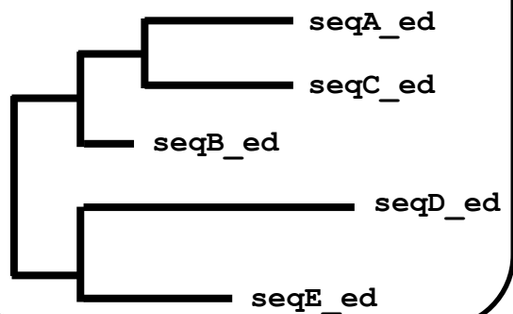
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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

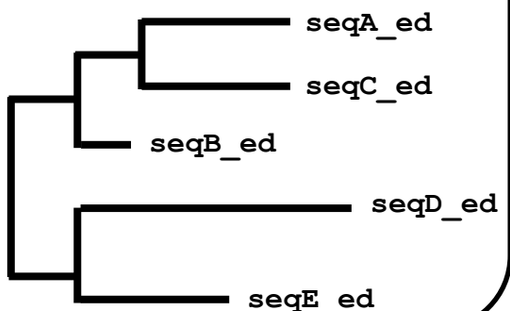
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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

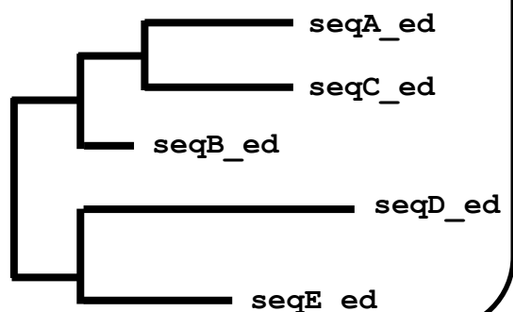
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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, BLAST, MCL, 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

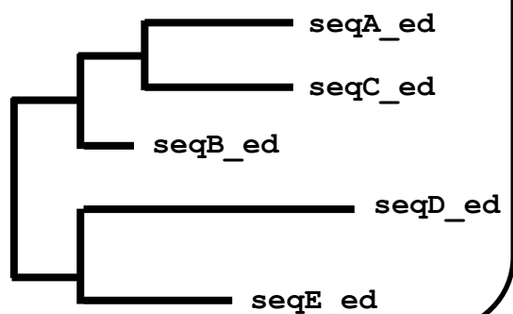
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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

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

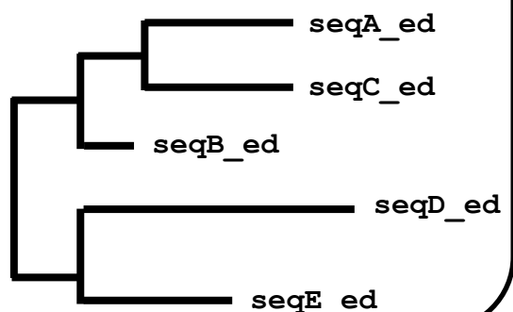
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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, 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

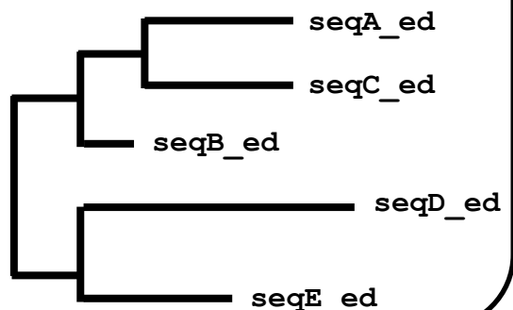
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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: **Maria and Asif, 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

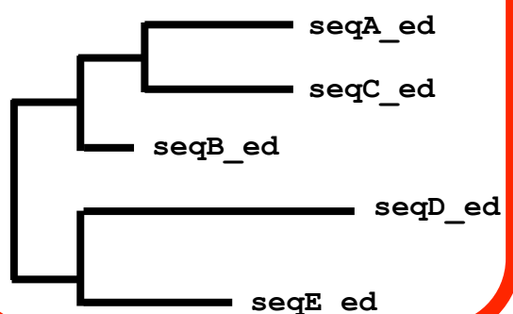
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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, 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

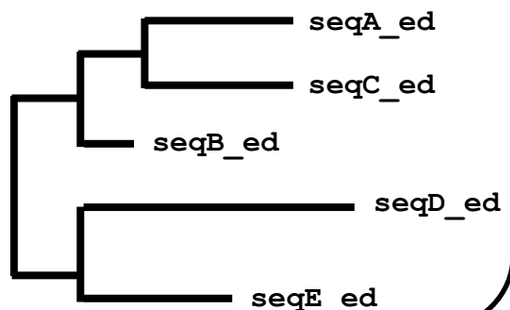
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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, 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

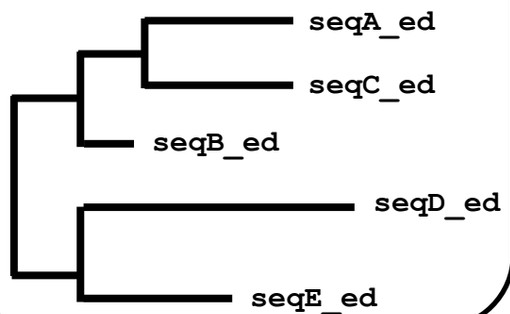
```
seqA_ed  AGTCGGTGATCCA
seqB_ed  --TCGGTGATCCA
seqC_ed  AGTCGGTGAT---
seqD_ed  AGTCGTTGATCCA
seqE_ed  -GTCAGTGATCC-
```

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: **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

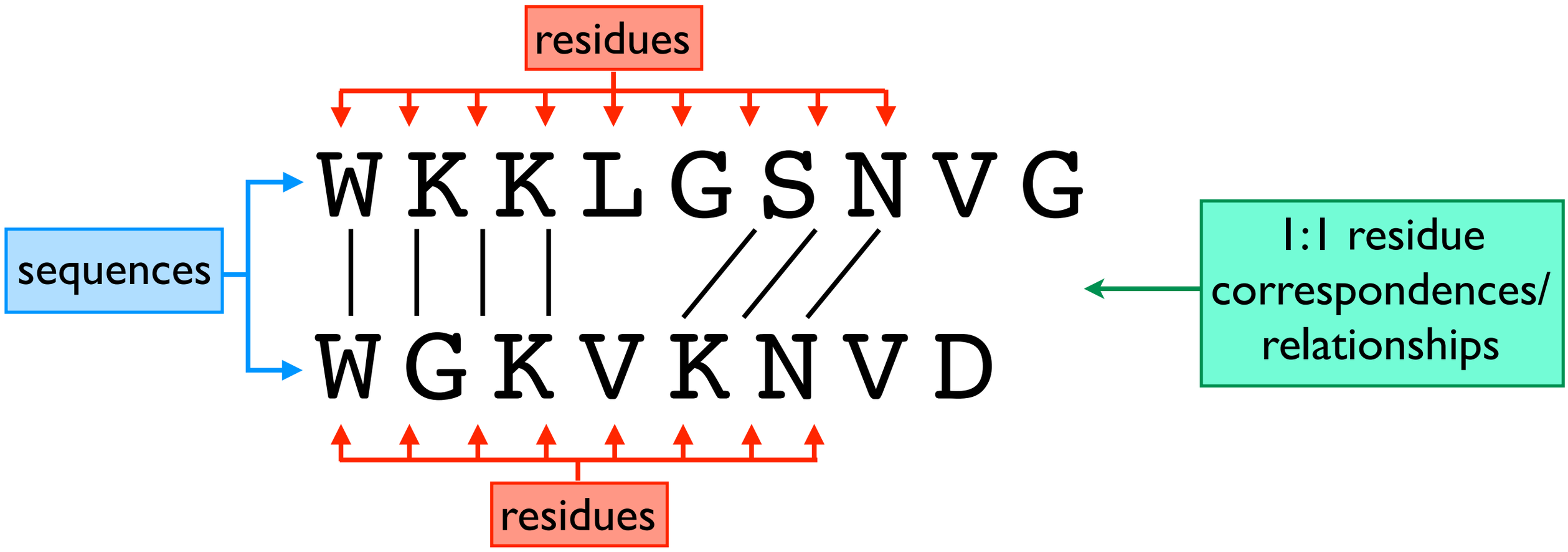
- "Pragmatic" **demonstration** with North African dog rabies viruses
- "Pragmatic" **exercise** with Louisiana gastroenterologist example

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

Alignments

Aidan Budd, EMBL Heidelberg

"Anatomy" of a Sequence Alignment

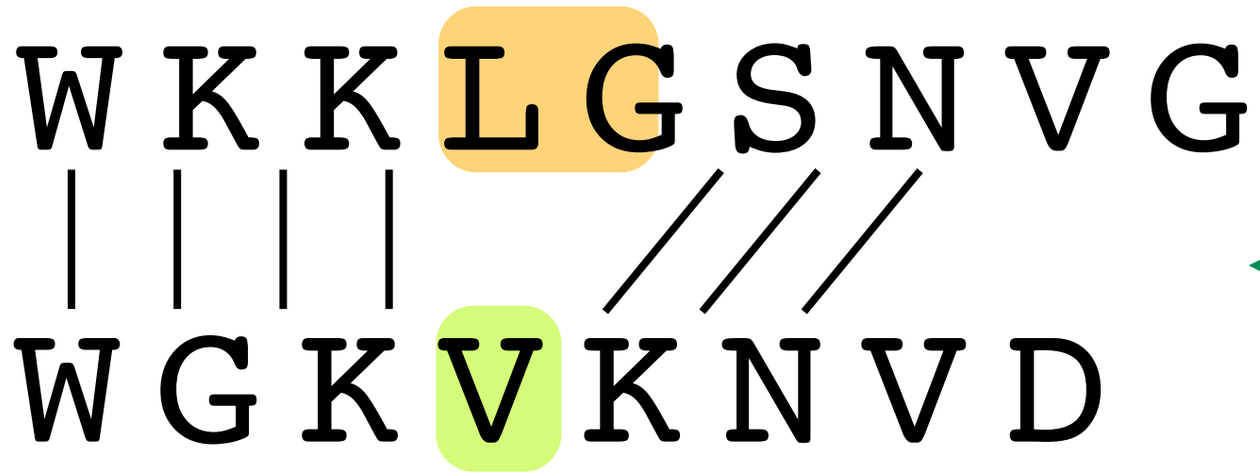


1:1 residue correspondences/relationships

Correspondences between

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

"Anatomy" of a Sequence Alignment



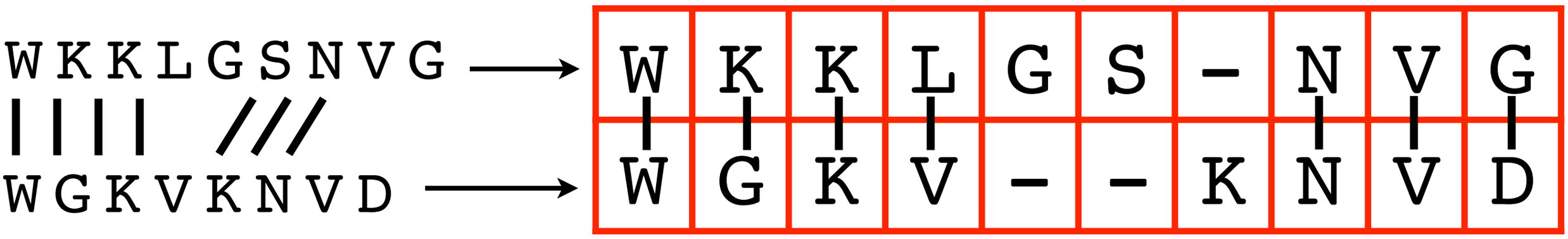
1:1 residue correspondences/ relationships

Residue has no link to a residue 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

Sequence Alignment Within a Grid



Often represented using a grid/matrix :

One sequence per row

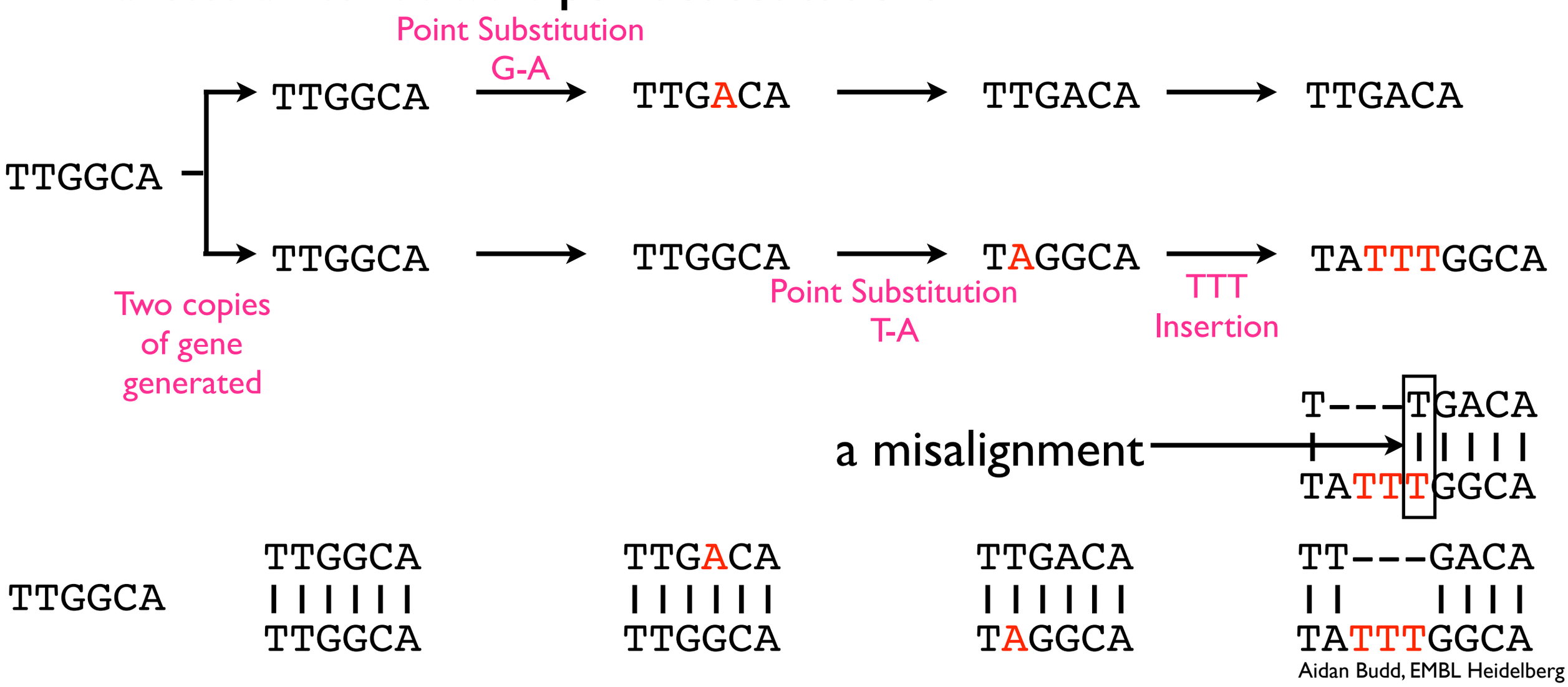
Residues in the same column are ‘linked’ (‘equivalent’? ‘homologous’?)

Gap characters (usually "-") assert 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

Aidan Budd, EMBL Heidelberg

Branch Lengths

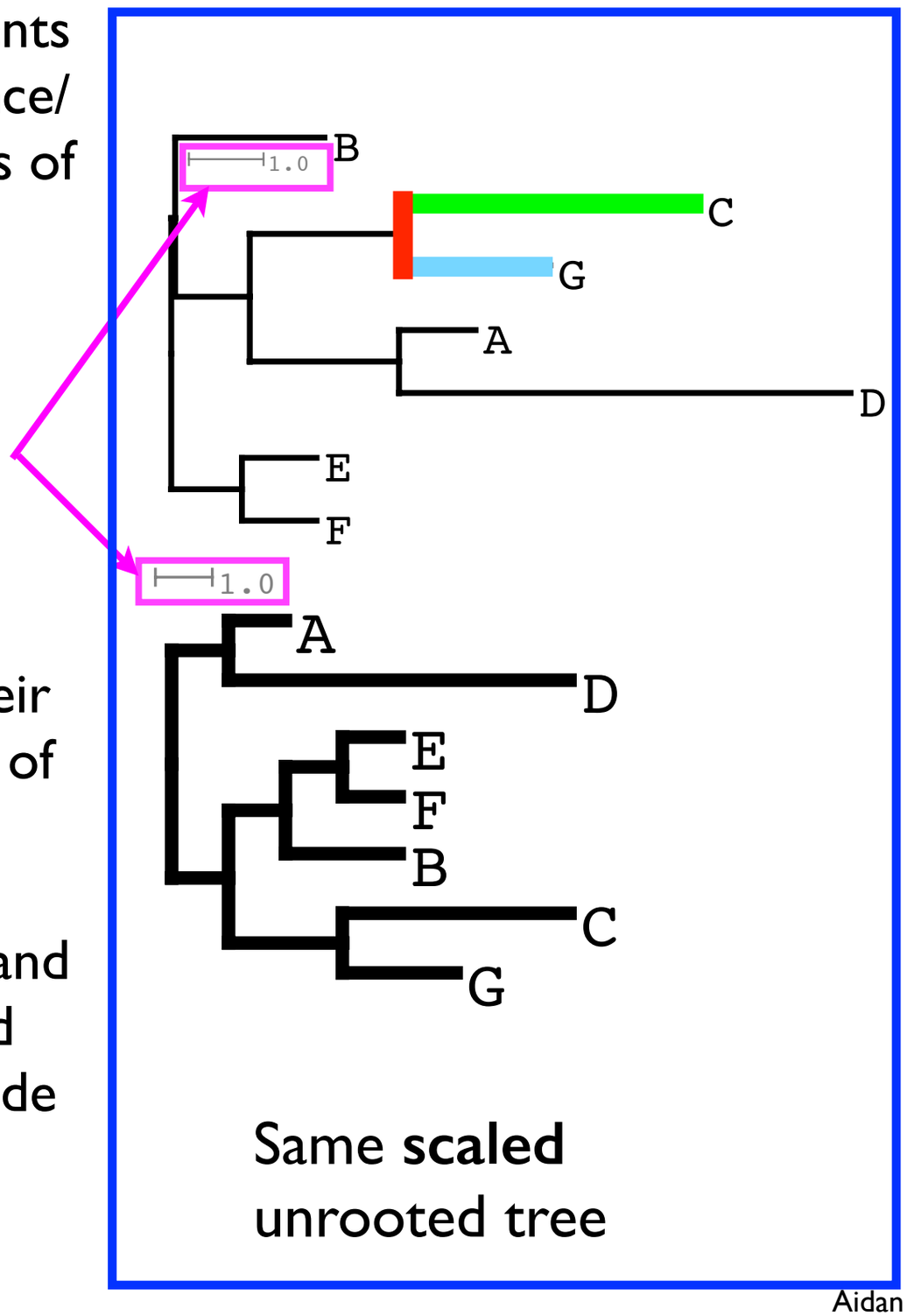
Branch length usually represents some measure of the difference/ distance between TUs at ends of the branch

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 IKTI**L**KW**W**SP
SeqB IKTI**V**KW**D**SP

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

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

SeqA IKTI**L**KW**W**SP
SeqB IKTI**V**KW**D**SP

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**