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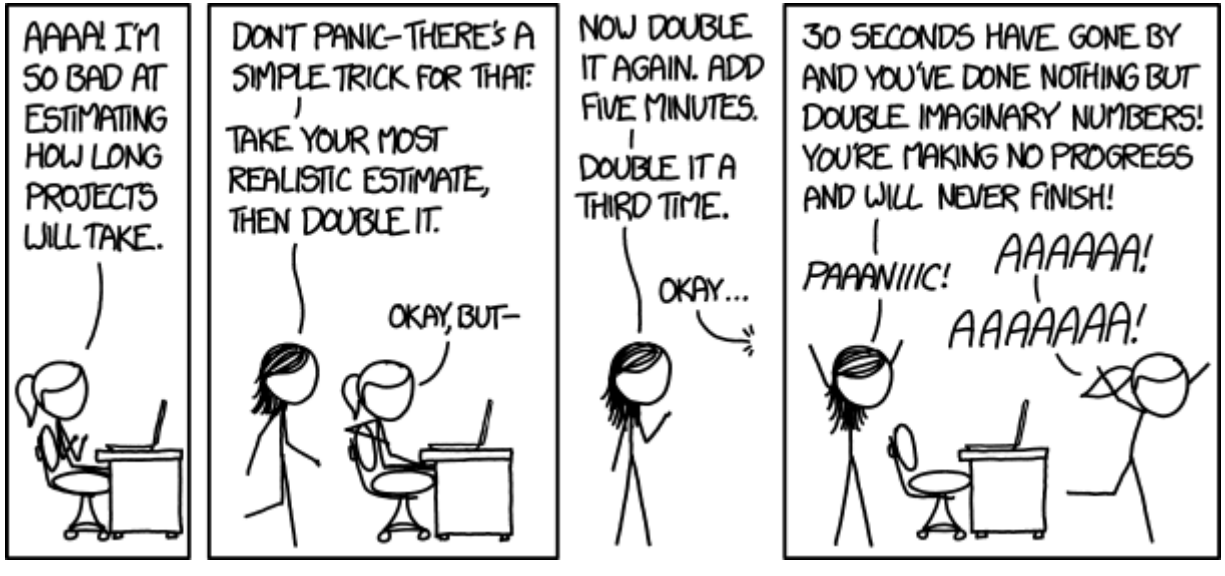
Molecular clocks



Please don't forget to submit your proposal abstracts today!

What is a molecular clock?

- The molecular clock is a hypothesis that evolution at a molecular level (*e.g.*, proteins) occurs at a constant rate.
- Based on linear relationship between the time that species diverged from their common ancestor, and the number of amino acid differences in homologous proteins (*right*).
- The molecular clock does *not* "tick".

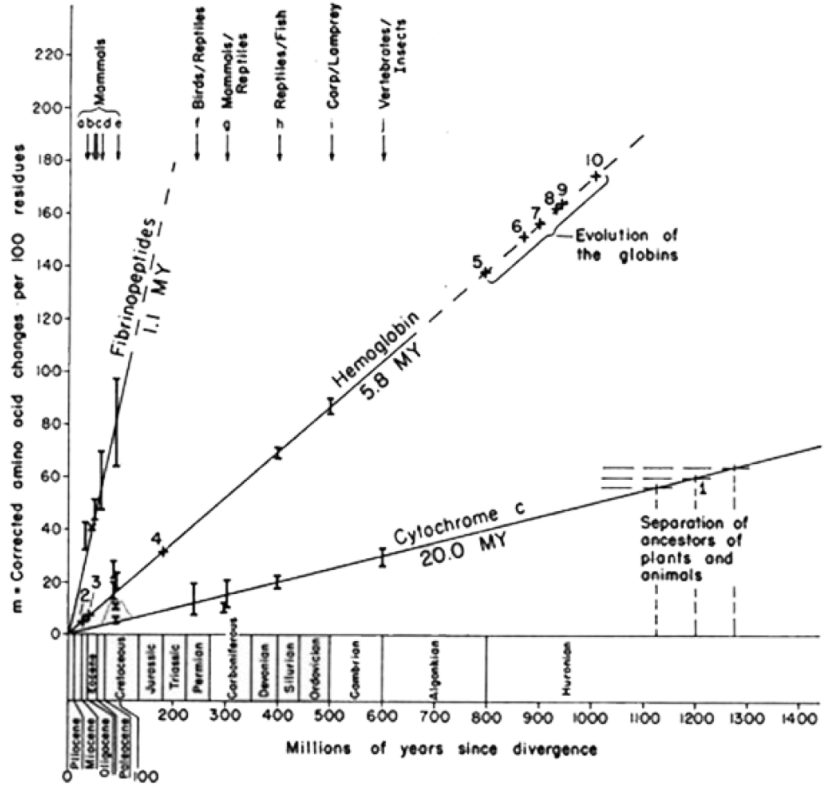


Figure from RE Dickerson (1971) J Mol Evol 1: 26.

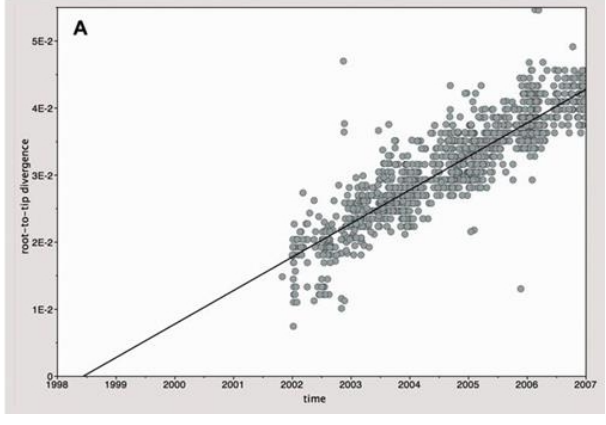
Neutral theory

- The neutral theory: "the great majority of evolutionary changes at the molecular level [...] are caused not by Darwinian selection but by random drift"
- Without selection, nucleotide substitutions should be a random process at a constant rate.
- Today, this model is denoted the "strict" clock and is seldom supported.

Motoo Kimura

Testing for a clock

- If a clock exists, then sequences should become more distant from the common ancestor as a *linear trend* over time.

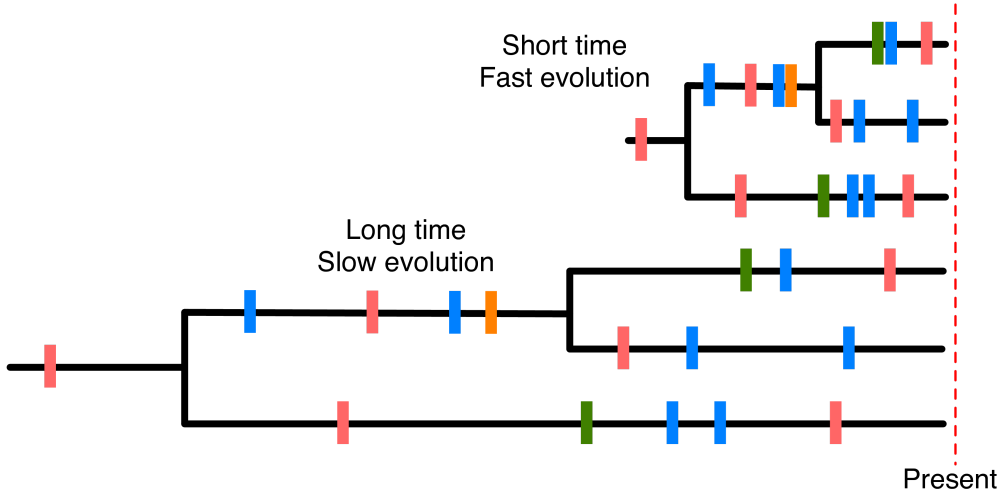


- (above) Plot of total branch length between root and tip, against sample collection date of tip, for influenza A virus (Rambaut *et al.* 2015 Virus Evolution).

Clocks and trees

- When we reconstruct a tree by fitting a model of evolution, the lengths of branches are measured in units of *expected numbers of substitutions*.
- This weird unit exists because most models are scaled to $\mu \times t$ (the rate of evolution multiplied by time).
- We say that the rate and time are *confounded* - it is impossible to estimate one parameter without knowing the true value of the other.

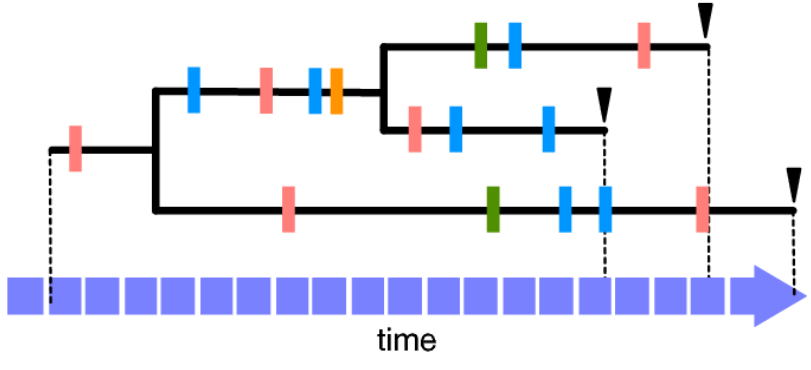
Confound it!



- A tree can be stretched back in time ($\uparrow t$) and explain the data with *exactly the same likelihood* if we decrease the rate ($\downarrow \mu$).
- Conversely, if we compress the tree forward in time ($\downarrow ts$), we obtain the same likelihood by speeding up the rate ($\uparrow \mu$).

Estimating the clock

- For infectious diseases, measurable evolution can occur on a time scale of weeks.
- This means that a branch of the tree can "grow" in the time between sampling different infections.



- We can use sampling times to "pin down" the tree and prevent the free-scaling problem.

Rooting the tree

- If we are directly measuring *time*, then we must impose a direction of time's flow on the tree.
- This means that we must locate the tree's *root* (the earliest point in time).
- Rooting trees is difficult! By definition, it is the point that is the most distant in time — it cannot be directly observed.

Outgroup rooting

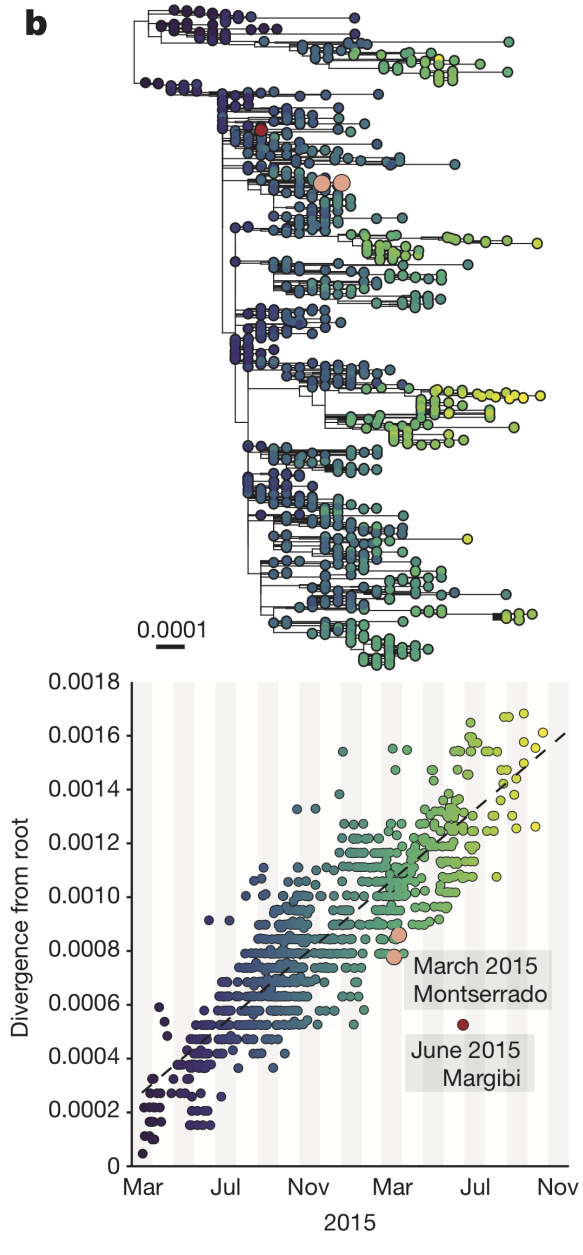
- An *outgroup* is a species or infection that is not closely related to the sample set.
- We place the root at the point where the branch leading to the outgroup intersects the tree.
- If the outgroup is too *close*, then the root is too influenced by sampling bias (our choice of outgroup).
- If the outgroup is too *distant*, then where it intersects the tree is subject to random variation.

Choosing an outgroup

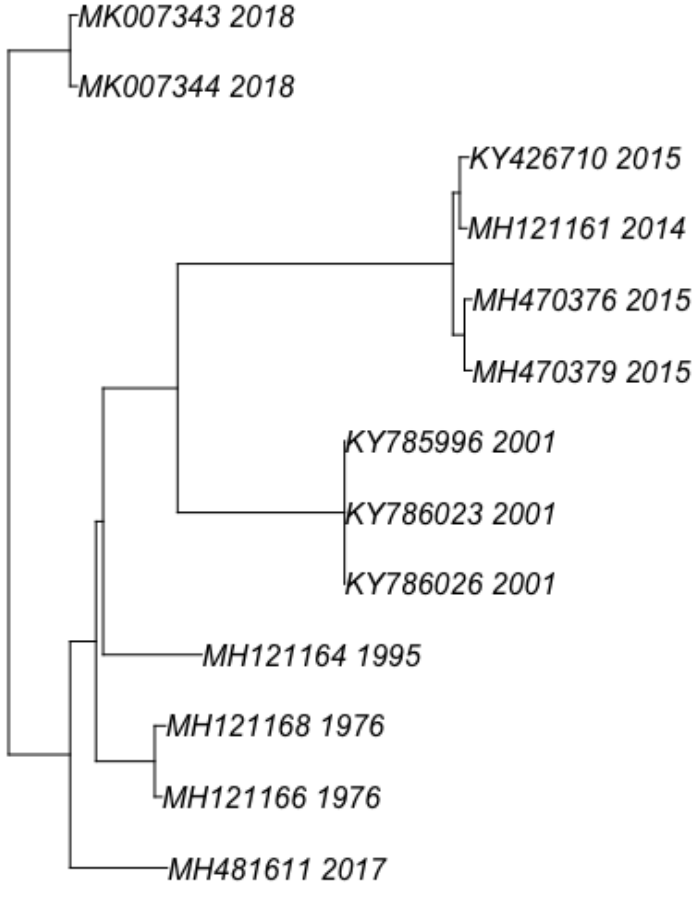
- There is no objective framework to select an "optimal" outgroup.
- We often reach an informal consensus about which outgroup to use, without any other rationale.
- *e.g.*, HIV-1 subtype D is an outgroup for HIV-1.
- If an early isolate of the pathogen is available, it is sometimes used as an outgroup.
- *e.g.*, West Nile virus isolate B956 (isolated in 1940).
- **Use different outgroups to see how robust your conclusions are to choice of outgroup!**

Root-to-tip regression

- We can simultaneously estimate the clock (rate of evolution) *and* locate the root.
- Try different placements of the root and look at the plot that results.
- The "best" root should give the cleanest (positive) linear trend.
- (*right*) Phylogeny and root-to-tip plot for Ebolavirus isolates from 2013-2016 epidemic in West Africa.



"Bad" root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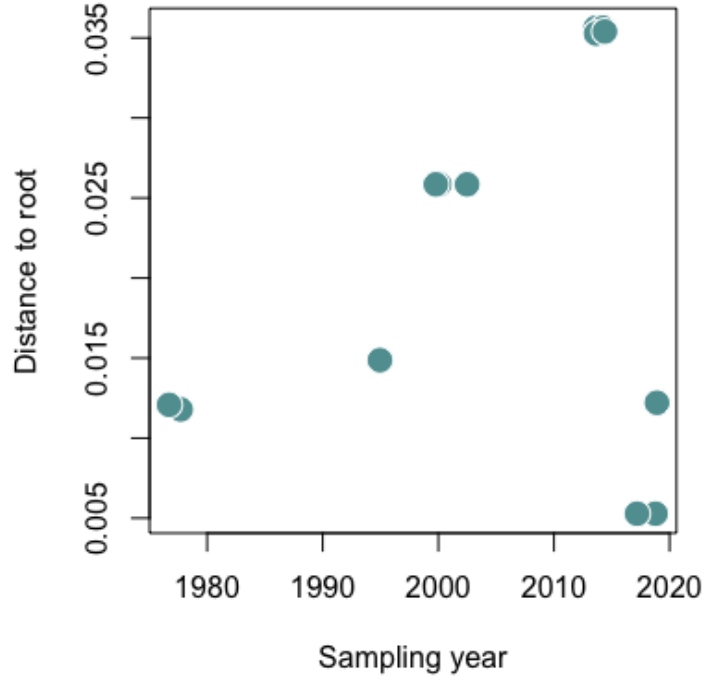
MH121168 1976
MH121166 1976
MH481611 2017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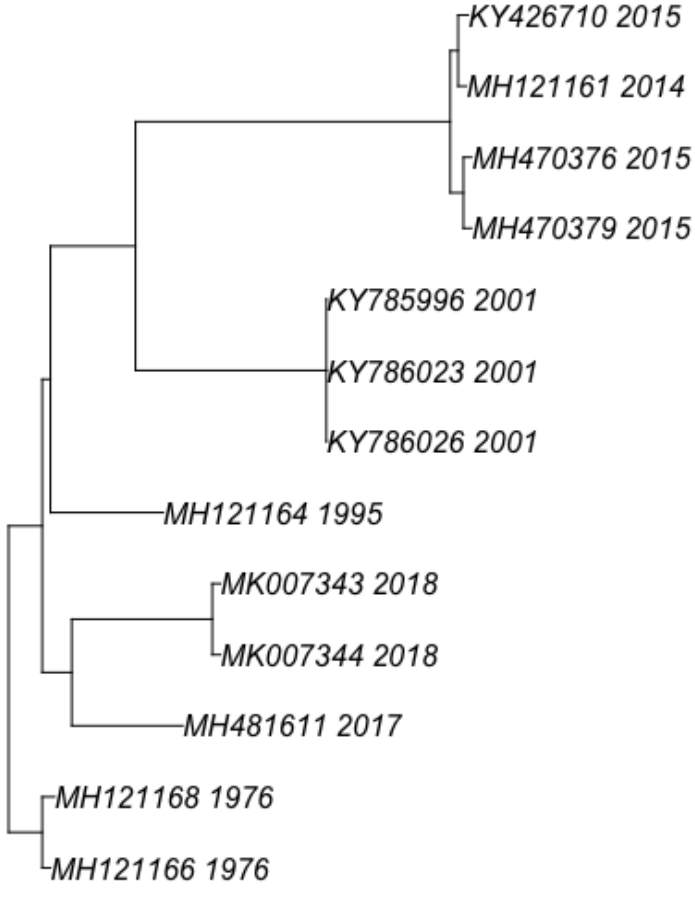
0.015
0.025
0.035
1980
1990
2000
2010
2020
Sampling year

"Good" root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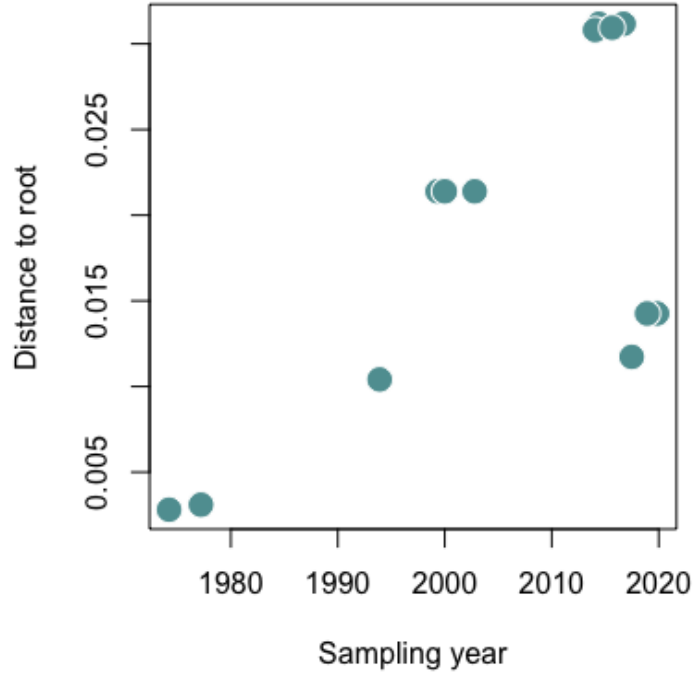
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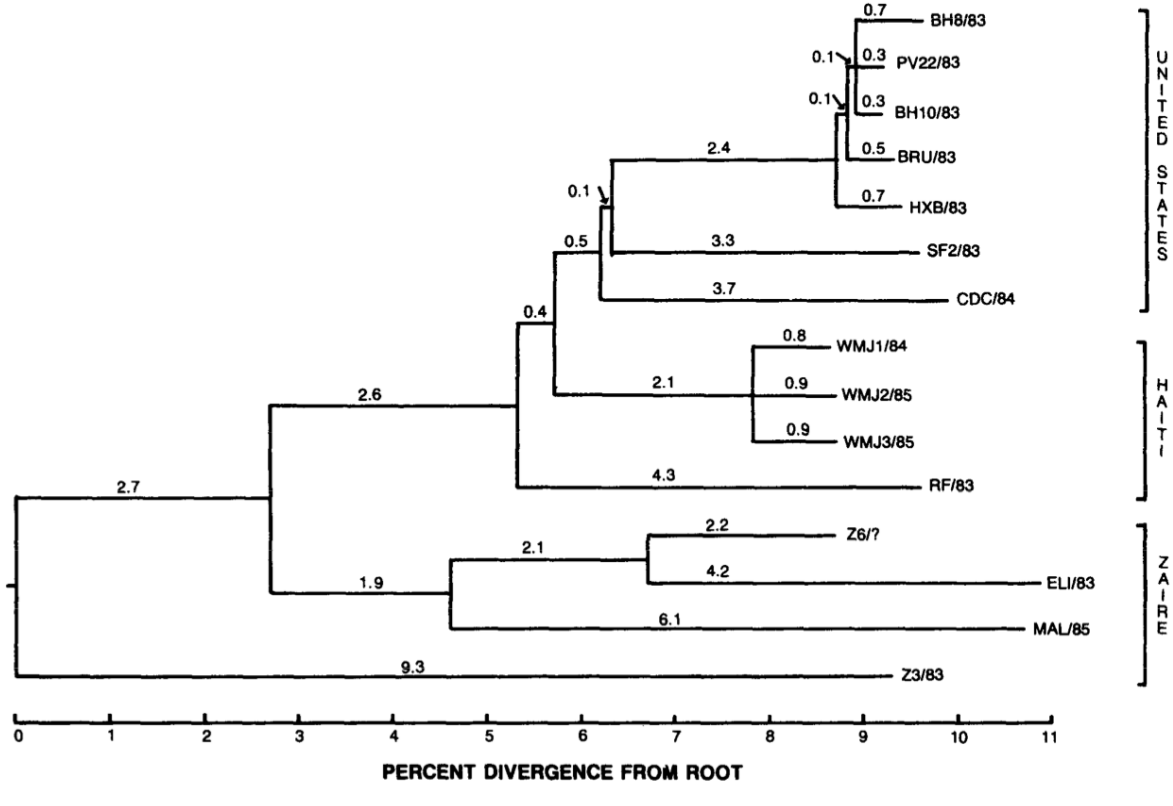
Origin of HIV-1

- This concept was first used to estimate the origin date of HIV-1.



Tree from Li, Tanimura and Sharp (1988) Mol Biol Evol 5: 313.

Origin of HIV-1

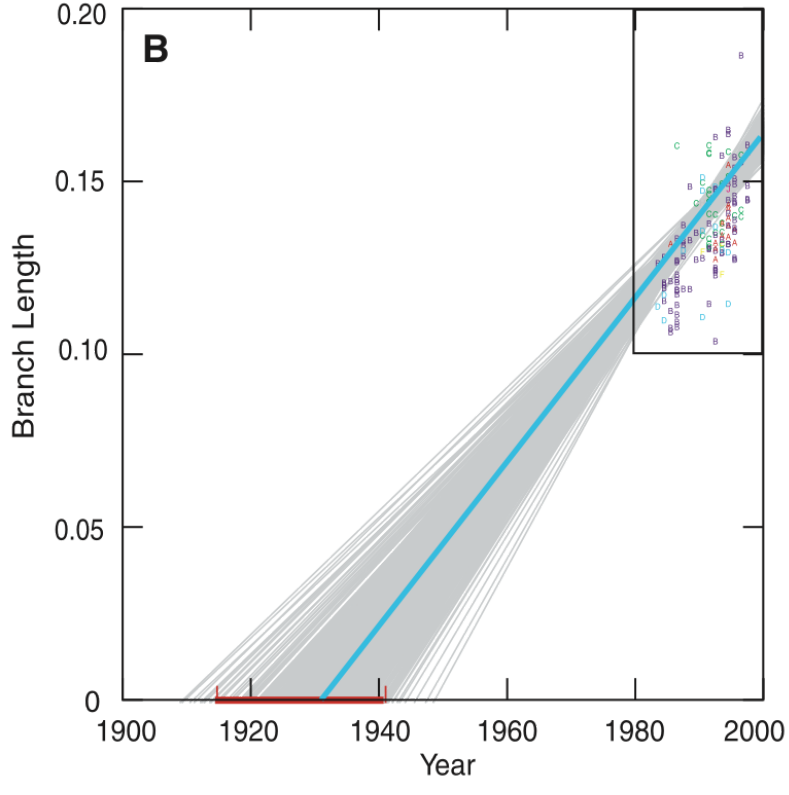


Figure from B Korber *et al.* (2000) Science 288.