Introduction to molecular dating methods

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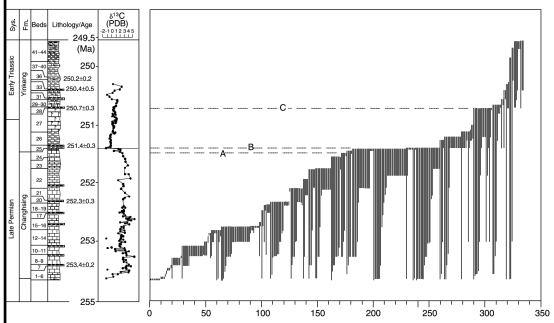
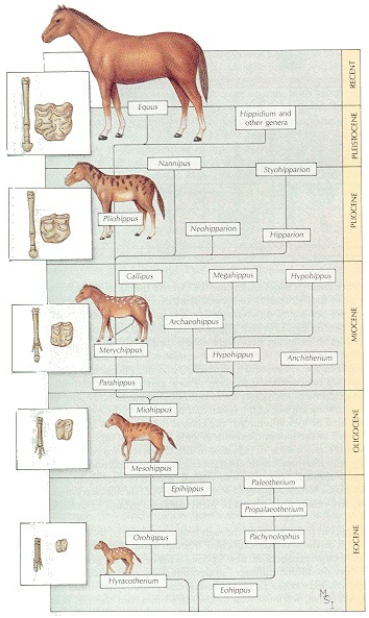
The fossil record is the direct evidence of past events and the time at which they occurred





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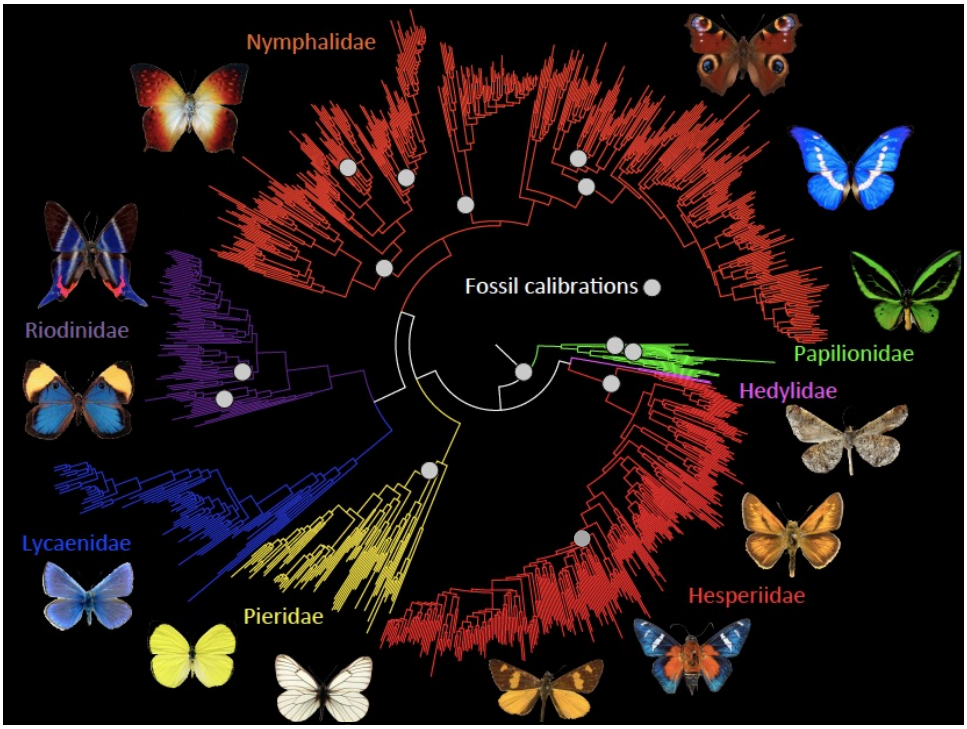
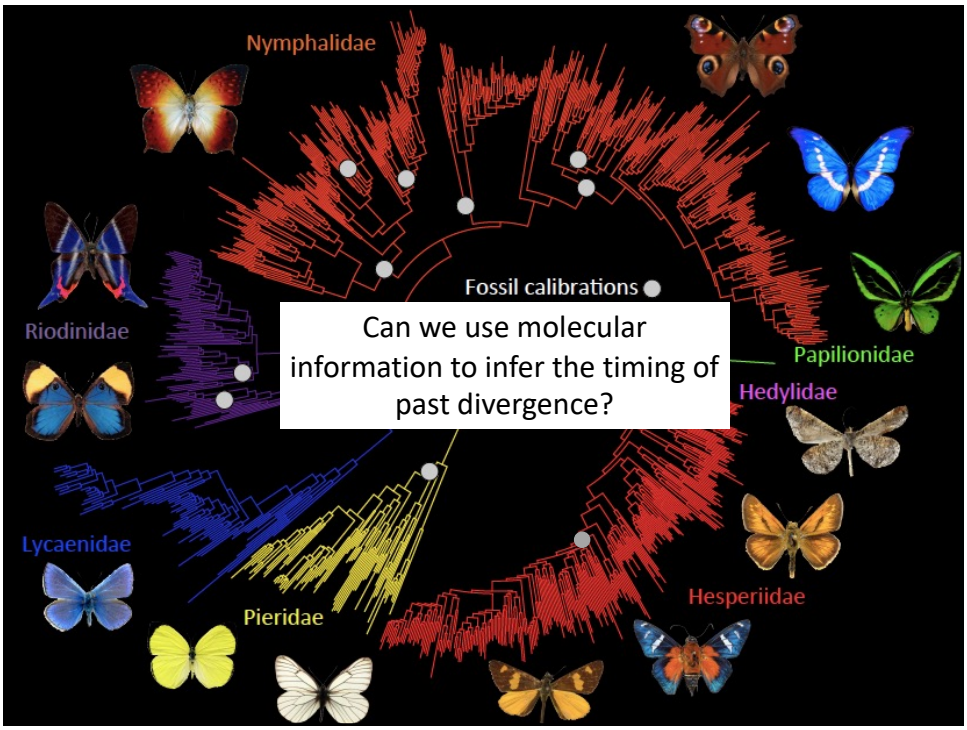

Nymphalidae
Fossil calibrations
Riodinidae
Papilionidae
Hedylidae
Lycaenidae
Hesperiidae
Pieridae

3


Nymphalidae
Fossil calibrations
Riodinidae
Can we use molecular information to infer the timing of past divergence?
Papilionidae
Hedylidae
Lycaenidae
Hesperiidae
Pieridae

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The Molecular Clock

Going back to ancient times

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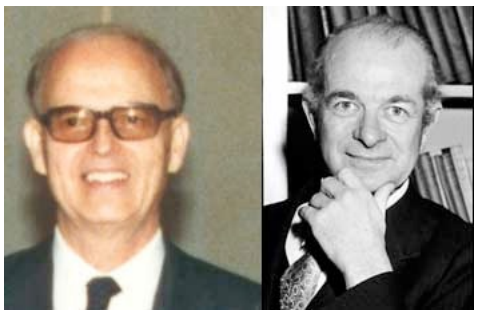
Is there a molecular clock?

- The idea of a molecular clock was initially suggested by Zuckerkandl and Pauling in 1962 and 1965



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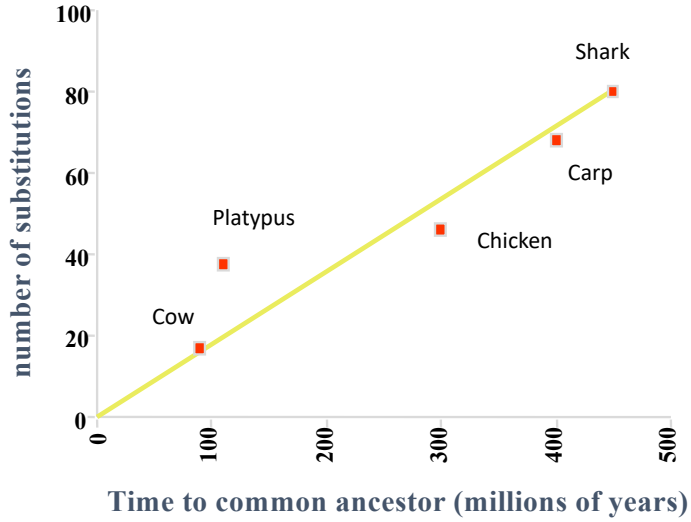
The molecular clock for alpha-globin:

Each point represents the number of substitutions separating each animal from humans



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Is there a molecular clock?

- The idea of a molecular clock was initially suggested by Zuckerkandl and Pauling in 1962 and 1965
- They noted that rates of amino acid replacements in animal haemoglobins were roughly proportional to time - as judged against the fossil record

=> implies the existence of a sort of molecular clock ticking faster or slower for different genes but at a more or less constant rate for a genes among different lineages

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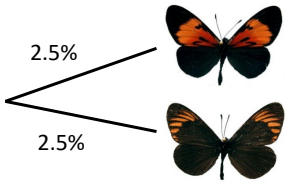
The molecular clock hypothesis

- Assumes an equal rate of molecular evolution over time



- A 5% difference between species means they have each diverged 2.5% since their common ancestor
- If a fossil or other evidence will let us calibrate this clock we can convert % difference to years

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Assumptions of a perfect clock

- Molecular change is a linear function of time with substitutions accumulating following a Poisson distribution - any variation will be stochastic [imagine 1 substitution / million yrs]
- Rate of change is equal across all sites and lineages
- The phylogeny can be estimated without error
- The number of substitutions along each lineage can be estimated without error
- Calibration dates for all times of divergence used to calculate the rate of the molecular clock are known without error
- A regression of time on number of substitutions can be conducted without error

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Dating with a molecular clock

- "Universal Molecular Clocks"
- Calibrations proposed for various taxa / genes
- eg. mtDNA molecular clock of animals
  - ~ 2% sequence divergence per million years for vertebrates
  - ~ 1% sequence divergence per million years for invertebrates

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There is no universal molecular clock

- The initial proposal saw the clock as a Poisson process with a constant rate
- Now known to be more complex - differences in rates occur for:
  - different sites in a molecule
  - different genes
  - different regions of genomes
  - different genomes in the same cell
  - different taxonomic groups for the same gene
- There is no universal molecular clock

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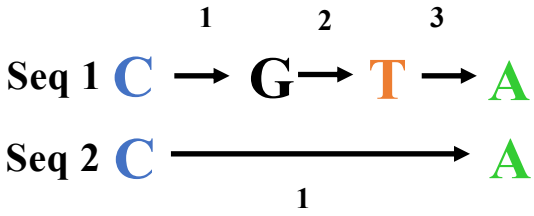
Challenges

- Saturation

```
Ancest GGCGCG
Seq 1  AGCGAG
Seq 2  GCGGAC
```

Number of changes



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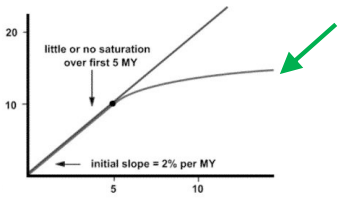
Saturation problems



old divergences will be underestimated

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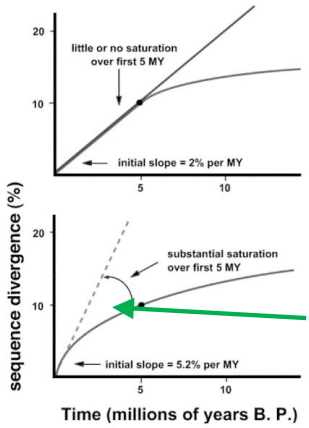
Saturation problems



times of divergences are quickly underestimated

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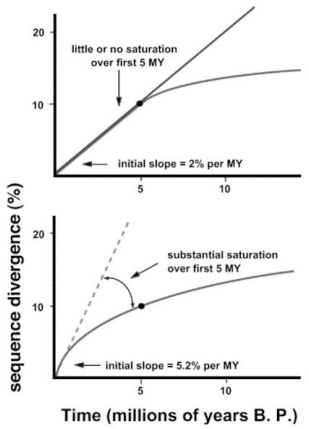
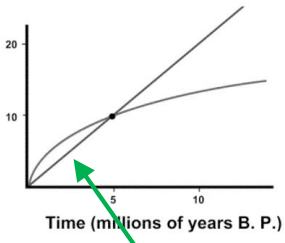
Saturation problems



And if we underestimate the rate of substitution…



Both recent divergences will be overestimated and older divergences will be underestimated

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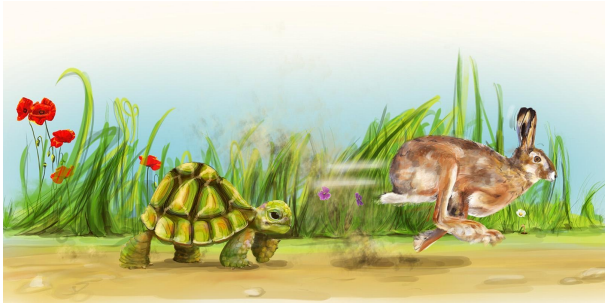
Challenges

- Saturation
- Rate Heterogeneity - violation of homogeneity



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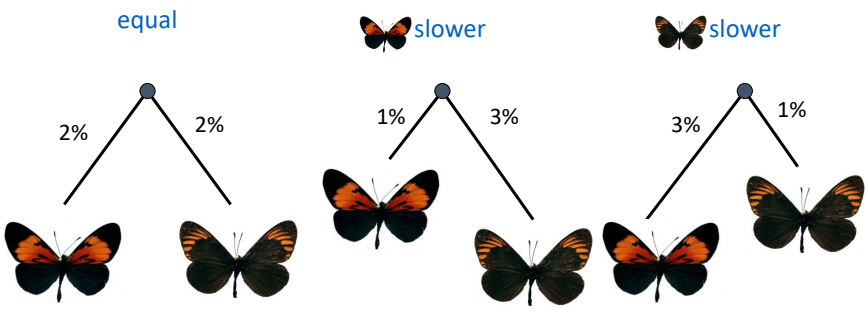
No universal molecular clock



Molecular distance from to is the same in all cases

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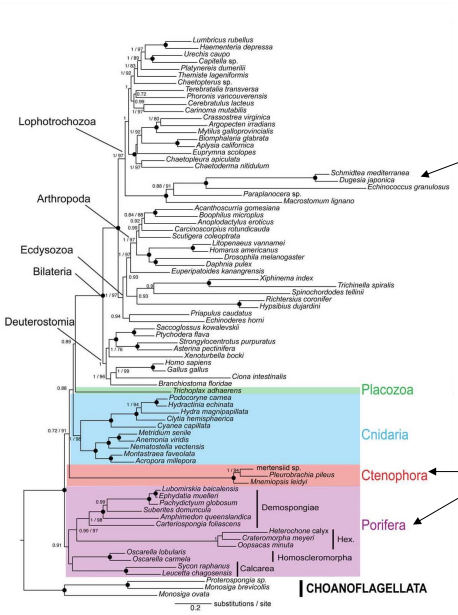

Long branches near the tip of a tree are probably "long" due to an increase in rate of change (not more time)

Long branches near the base of a tree are probably "long" due to time (not a faster rate of change)

Pick et al (2010) MBE 27:1983-1987

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Teasing apart RATE and TIME

- Branch lengths are proportional to:

  RATE * TIME

- If rates are constant then lengths are proportional to time
- If rates are not constant then *we have a hard time relating branch lengths to time*

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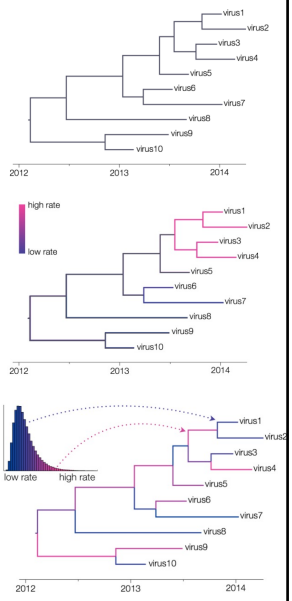
Molecular clocks can be relaxed

- Strict or ”global” clock
  - Many programs/methods/algorithms
- Local clocks
  - Maximum Likelihood (PAML, QDate)
  - Mean path length (Pathd8)
- Relaxed clocks
  - Non-parametric rate smoothing (r8s)
  - Penalized likelihood (r8s)
  - Bayesian, fixed tree (multidivtime, PhyBayes)
  - Bayesian, tree co-estimated (BEAST, MrBayes)



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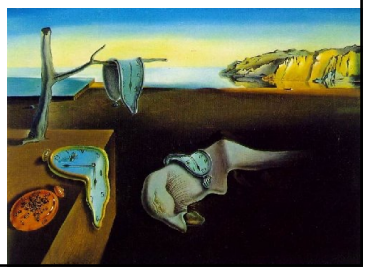
What is a relaxed clock?

- Relaxed clock: rate allowed to vary among branches
  - Autocorrelated relaxed clock: rates in adjacent branches are related
  - Uncorrelated relaxed clock: rates identically and independently distributed among branches



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Autocorrelated relaxed clocks

- Fixed topologies are input!
- Treat substitution rate as a heritable trait, so that it can 'evolve' through the tree
- Rate is assumed to be tied to:
  - Life history traits (e.g., generation time, population size, body size)
  - Cellular/biochemical environment
- Available in r8s, multidivtime, PhyBayes, BEAST, PAML

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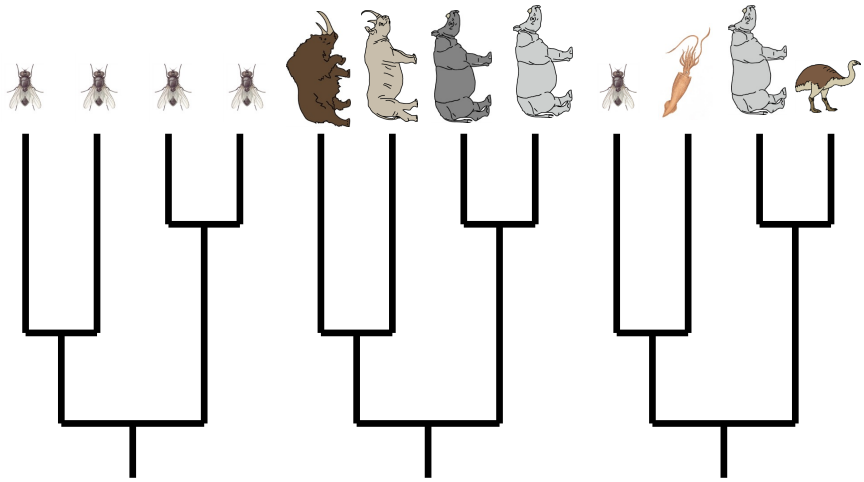
Autocorrelated relaxed clocks



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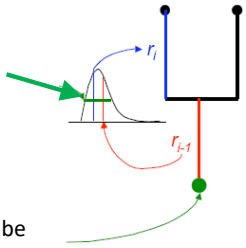
Modeling autocorrelation

- Model of autocorrelated rate change used to describe prior distribution of rates
- Lognormal
  - log(*ri*)~N(log(*ri*-1),*vt*)

v controls the s.d. of the distribution



Further assumption needs to be made about rate at the root

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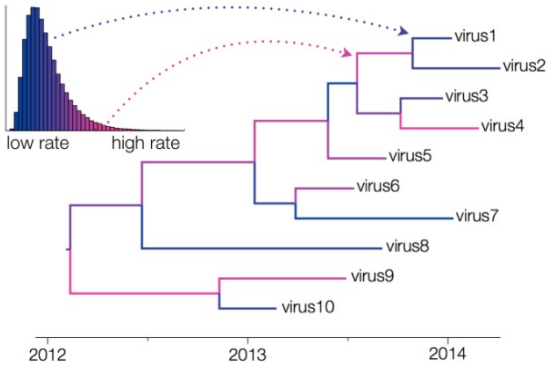
Uncorrelated relaxed clocks

- Models available in *BEAST*
  - **Lognormal distribution**
    Most rates cluster around the mean
  - **Exponential distribution**
    Most rates are quite low



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Lognormal uncorrelated relaxed clock

- In the uncorrelated lognormal relaxed clock, two statistics can be obtained:
  1. **Coefficient of variation of rates**
     Measures the rate variation among branches
     A value of 0 indicates clocklike evolution
  2. **Covariance of rates**
     Measures autocorrelation of rates between adjacent branches

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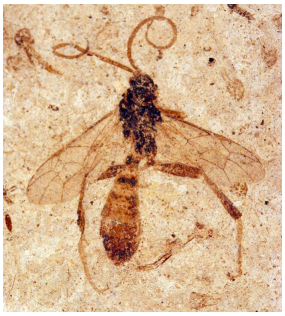
Challenges

- Saturation
- Rate Heterogeneity - violation of homogeneity
- Calibration



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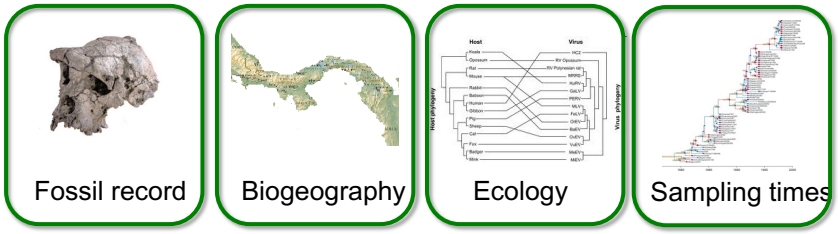
Separating rate and time

- Information about rate
  - Substitution rate obtained from an independent study
- Information about time – *prior information*:



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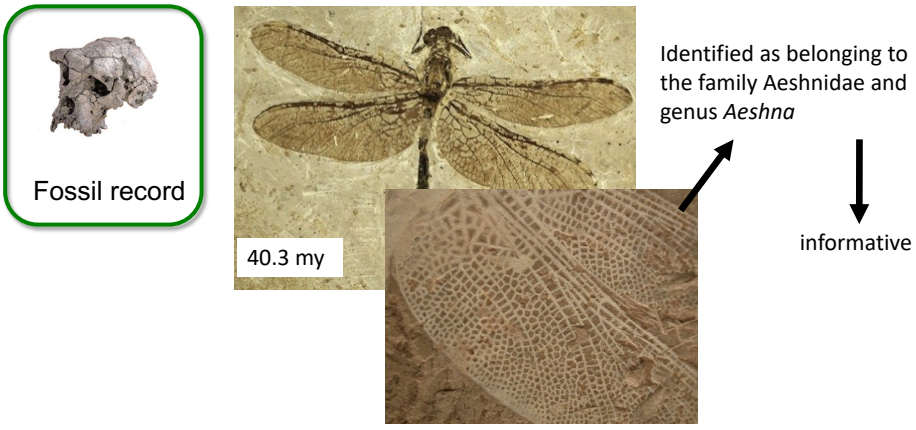
Calibration: Fossil record

- Fossil record provides minimum estimates of divergence times



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Calibration: Fossil record

- Fossil record provides minimum estimates of divergence times

Identified as belonging to the family Phasianidae, to genus *Gallus*, to the species *Gallus gallus domesticus*

Minimum age for the birds…
BUT not informative

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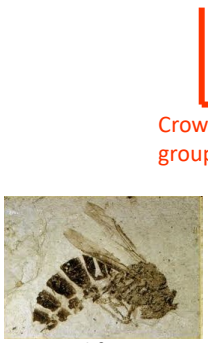
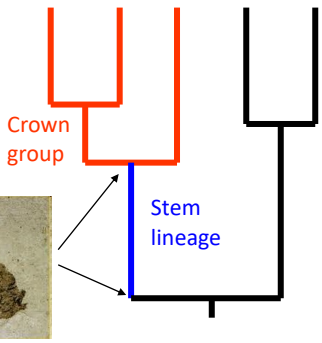
Problems with fossils

- Incompleteness of fossil record
- Identification
  - Species / Genus / Family?
- Position
  - Stem or crown?
- Which date?
  - Min / Mid / Max of Epoch?



Fossil from Eocene (55-33 Mya)



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Calibration errors

- Preservational bias
  - Hard parts
  - Environment, proximity to water bodies
  - Age
  - Sampling effort
- Taxonomic affinity
  - Fragmentary fossils
  - Extinct, stem lineages
- Stratigraphic and isotopic dating errors

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Calibration: Biogeography

- Biogeographic events can provide maximum estimates of divergence times

Biogeography

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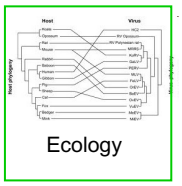
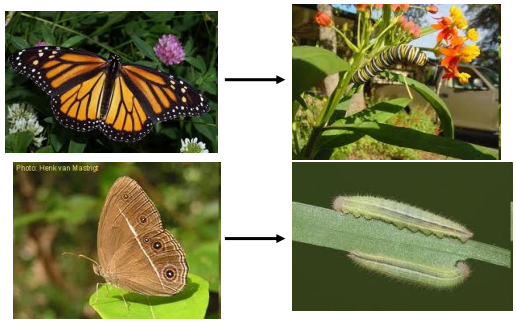
Calibration: Ecology

- Knowledge of tight ecological associations can be used to provide maximum estimates of divergence times





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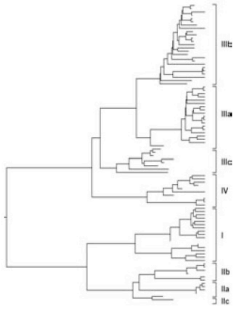
Calibration: Sequence ages

- Sequence ages provide sufficient age information for e.g. viruses



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Calibration in Bayesian framework

posterior

data

prior

$$f(\theta|D) = \frac{f(D|\theta)f(\theta)}{\int f(D|\theta)f(\theta)d\theta}$$

θ : model (substitution model(s), tree, etc)

prior: prior expectation we have for parameters of the model

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Calibration in Bayesian framework

posterior

data

prior

$$f(\theta|D) = \frac{f(D|\theta)f(\theta)}{\int f(D|\theta)f(\theta)d\theta}$$

θ : model (substitution model(s), tree, etc)

prior: prior expectation we have for parameters of the model

For example: age of nodes based on fossil information

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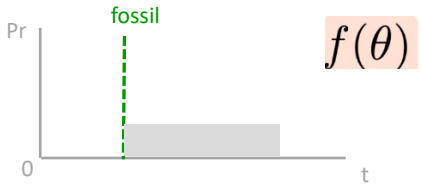
Calibration in Bayesian framework

posterior ↓ $f(\theta|D)$ = data ↓ $f(D|\theta)$ prior ↓ $f(\theta)$ / $\int f(D|\theta)f(\theta)d\theta$

For example: age of nodes based on fossil information

Prior probability distribution:



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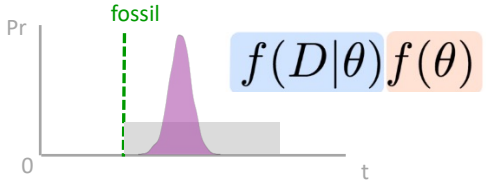
Calibration in Bayesian framework

posterior ↓ $f(\theta|D)$ = data ↓ $f(D|\theta)$ prior ↓ $f(\theta)$ / $\int f(D|\theta)f(\theta)d\theta$

For example: age of nodes based on fossil information

Prior probability distribution:



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Calibration types

- Point calibrations
- Hard minimum/maximum bounds
- Soft minimum/maximum bounds
- Parametric prior distributions
  - Normal distribution
  - Lognormal distribution
  - Exponential distribution

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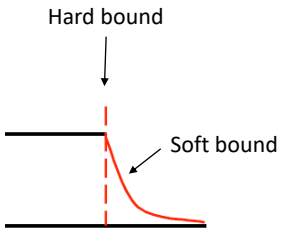
Hard/Soft Bounds

- Extension of hard bounds
- Soft:
  - Assign non-zero probability to values outside bound
  - Able to forgive calibration errors



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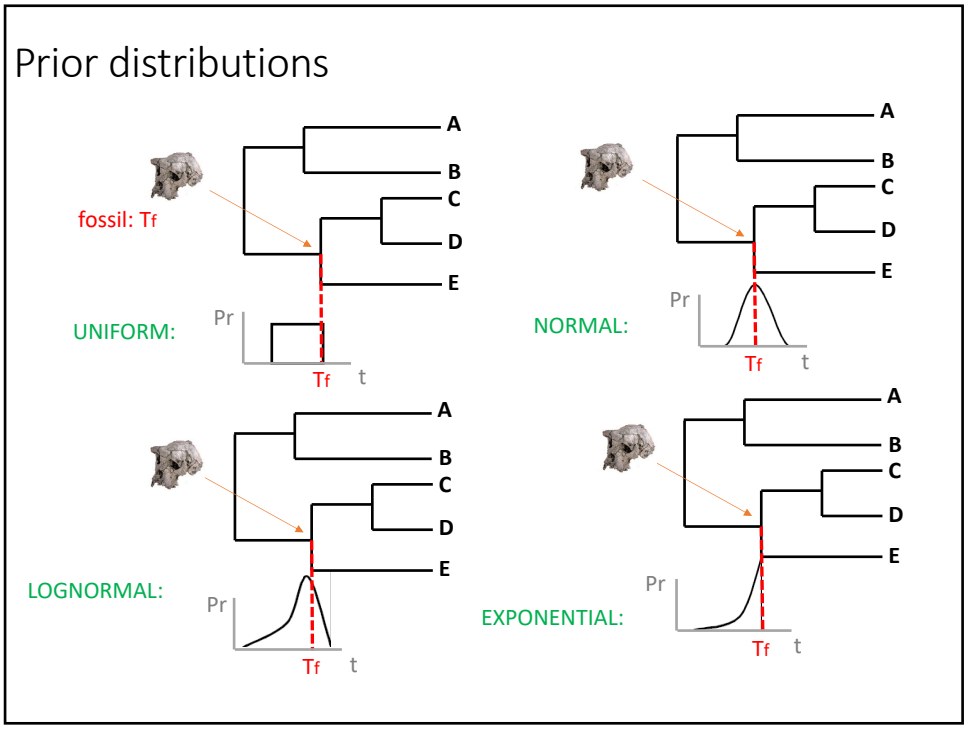
Prior distributions



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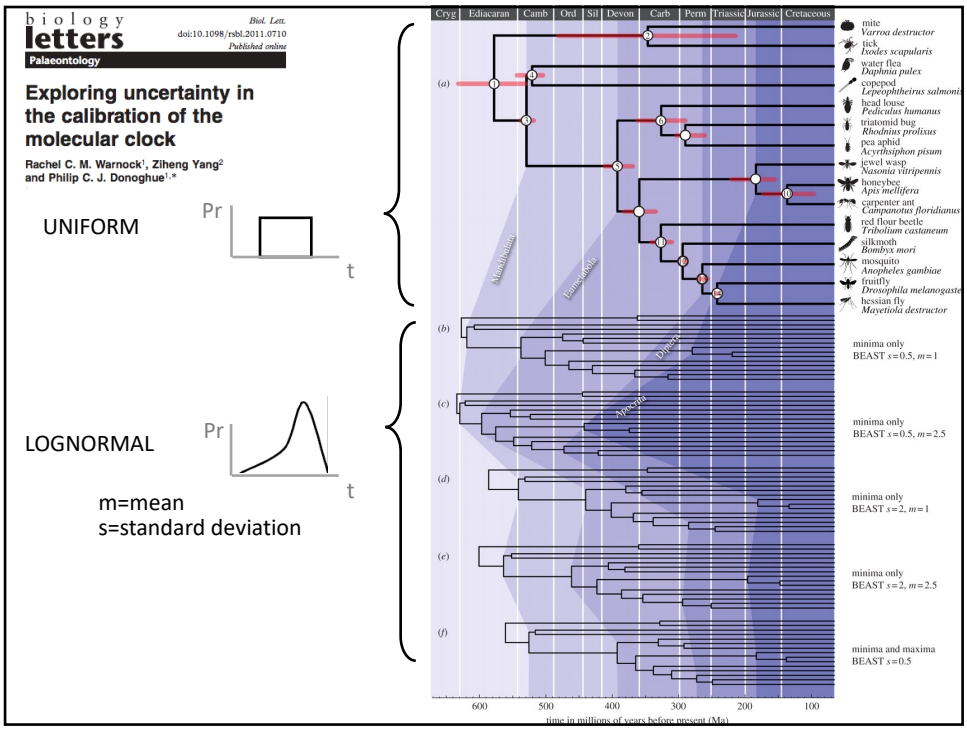

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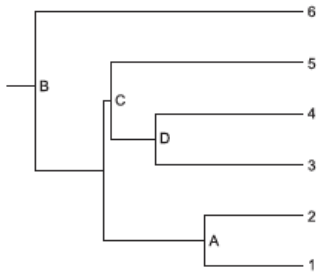
Multiple calibrations



- Molecular-clock estimates can be sensitive to the positions of the calibrations in the phylogenetic tree, especially when only a single or very few calibrations are available
- a small number of calibrations can lead to a biased estimate of the substitution rate if there is substantial among-lineage rate variation

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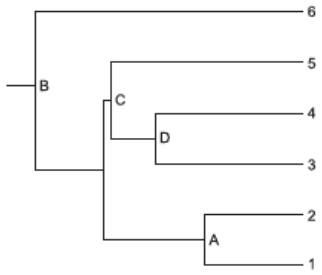
Multiple calibrations



- can improve the accuracy of date estimates in the presence of taxon undersampling
- substitution rate is primarily estimated from the branches between the calibrating nodes and the tips

=> deeper calibrations capture a larger proportion of the overall genetic variation.

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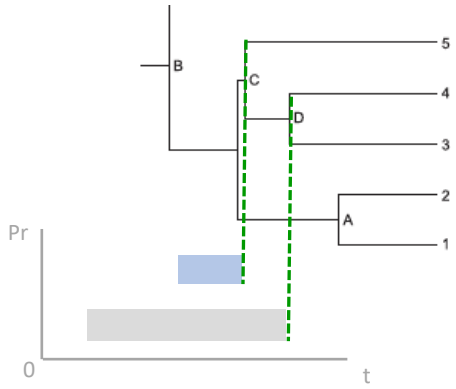
Multiple calibrations

- Be careful: priors interact with each others
- For example, node orders

D cannot be older than C



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Multiple calibrations

- Be careful: priors interact with each others
- For example, node orders
- Marginal priors resulting from prior interactions can differ from the initial user prior
  - This can be visualized by removing the data and running the same analysis

$$f(\theta|D) = \frac{f(D|\theta)f(\theta)}{\int f(D|\theta)f(\theta)d\theta}$$

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Multiple calibrations

- Be careful: priors interact with each others
- For example, node orders
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$$f(\theta|D) = \frac{f(\theta)}{\int f(D|\theta)f(\theta)d\theta}$$

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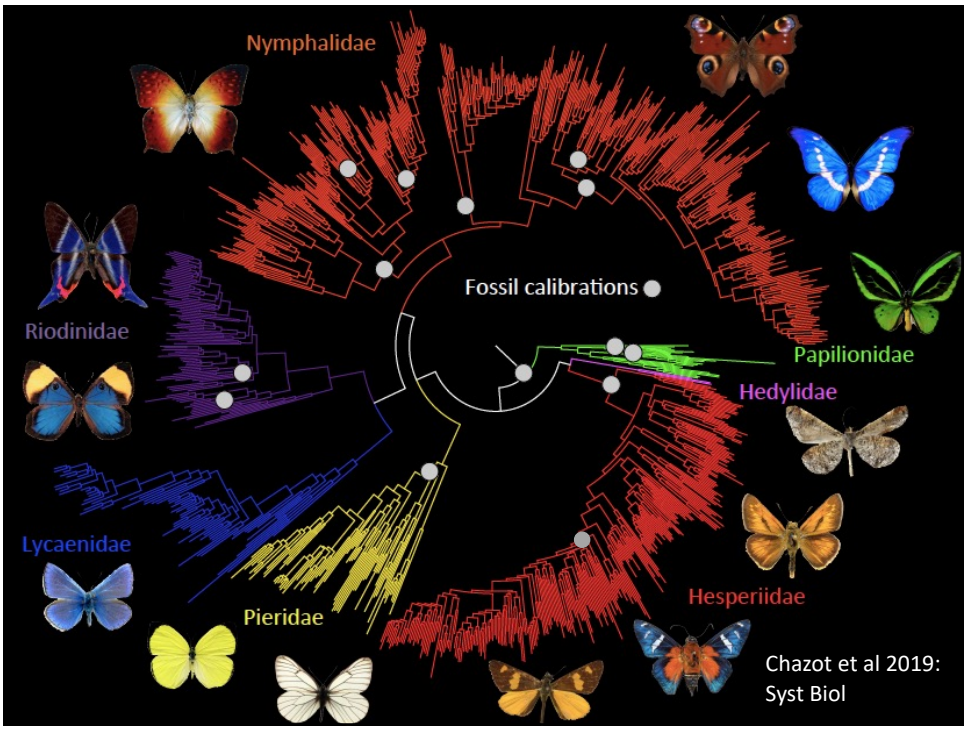
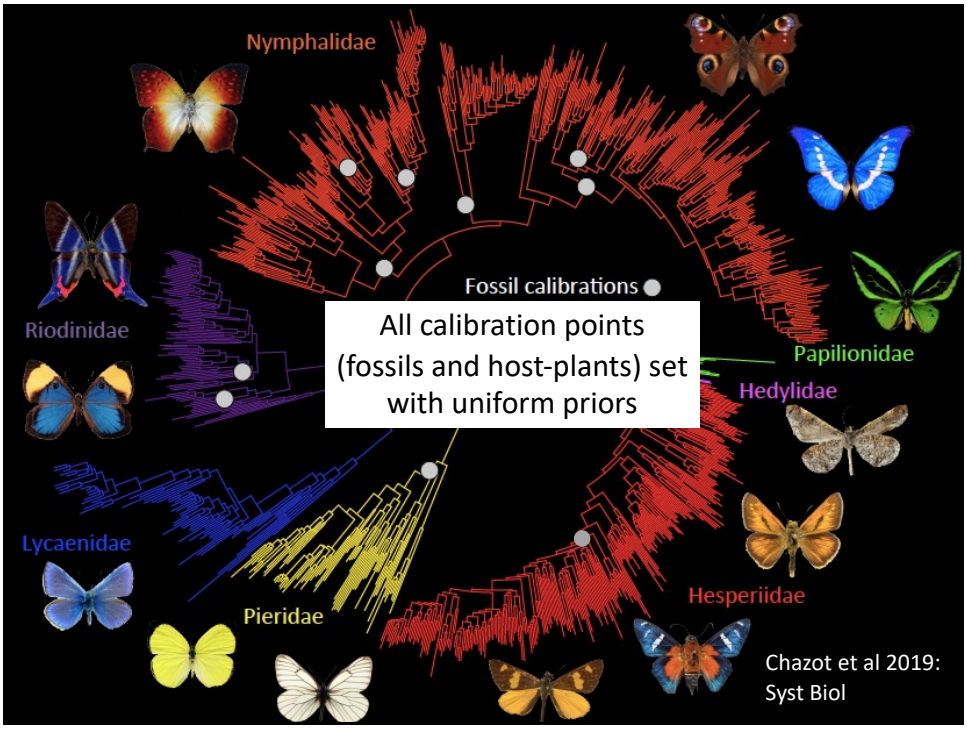
49



50


Nymphalidae
Fossil calibrations
All calibration points (fossils and host-plants) set with uniform priors
Riodinidae
Papilionidae
Hedylidae
Lycaenidae
Pieridae
Hesperiidae
Chazot et al 2019: Syst Biol

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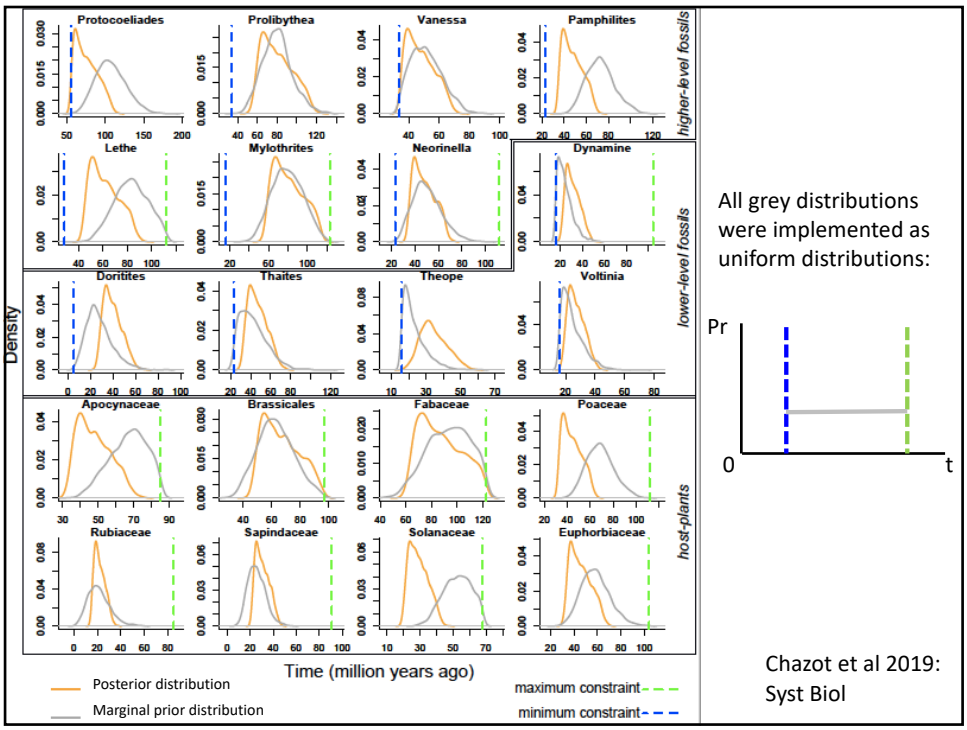
Protocoeliades
Prolibythea
Vanessa
Pamphilites
higher-level fossils
Lethe
Mylothrites
Neorinella
Dynamine
Doritites
Thaites
Theope
Voltinia
lower-level fossils
Density
Apocynaceae
Brassicales
Fabaceae
Poaceae
Rubiaceae
Sapindaceae
Solanaceae
Euphorbiaceae
host-plants
Time (million years ago)
Posterior distribution
Marginal prior distribution
maximum constraint
minimum constraint
All grey distributions were implemented as uniform distributions:
Pr
0
t
Chazot et al 2019: Syst Biol

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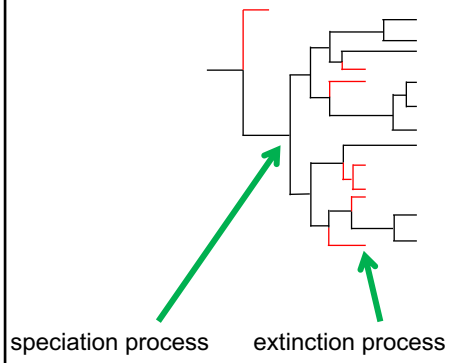
Prior sensitivity



Bayesian methods include a prior on tree growth

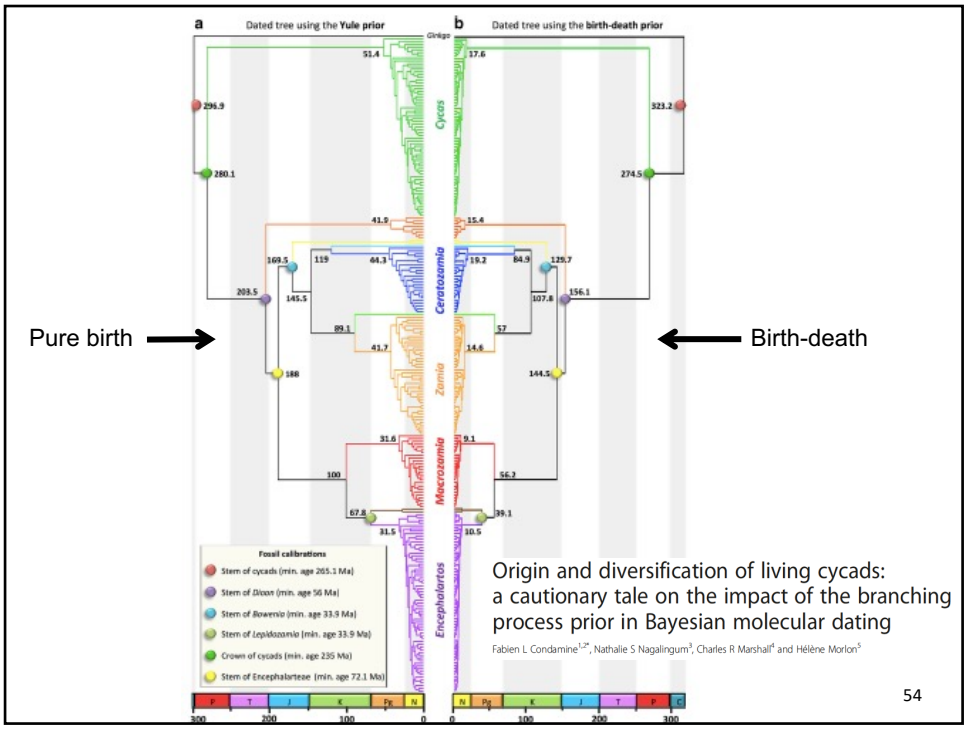
Branching process prior

Pure birth

Birth-death

53

53

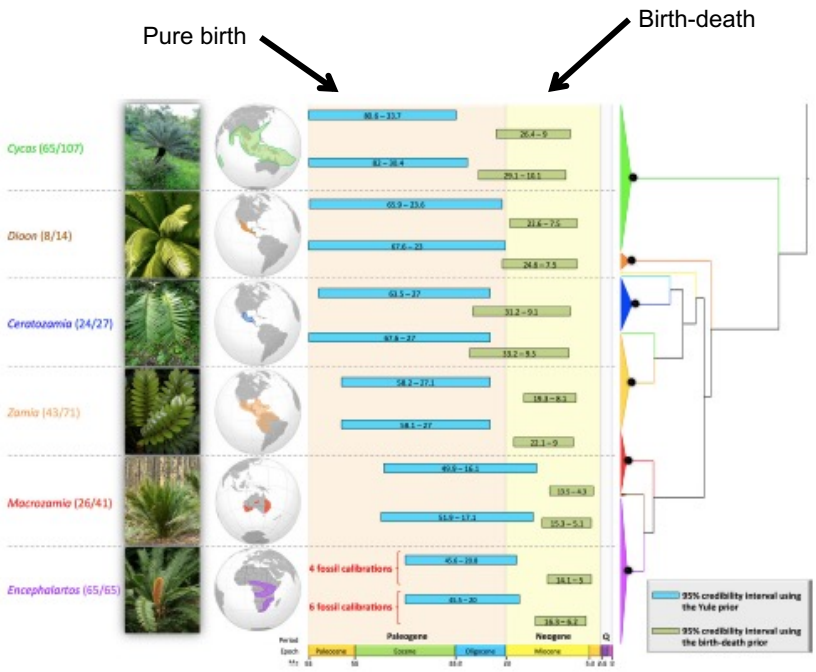


Pure birth

Birth-death

54

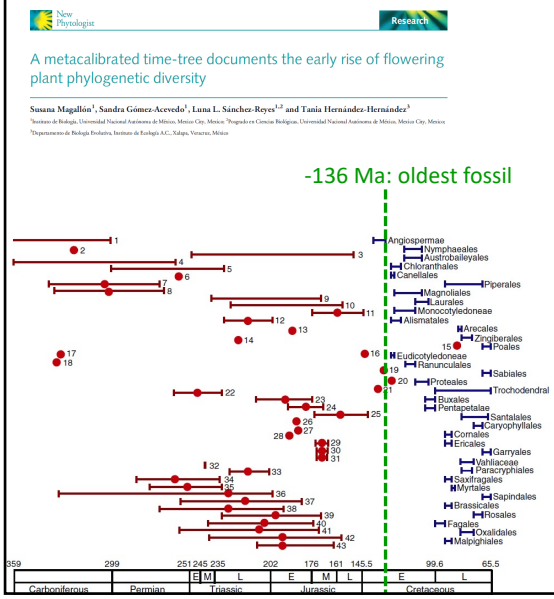
54



55

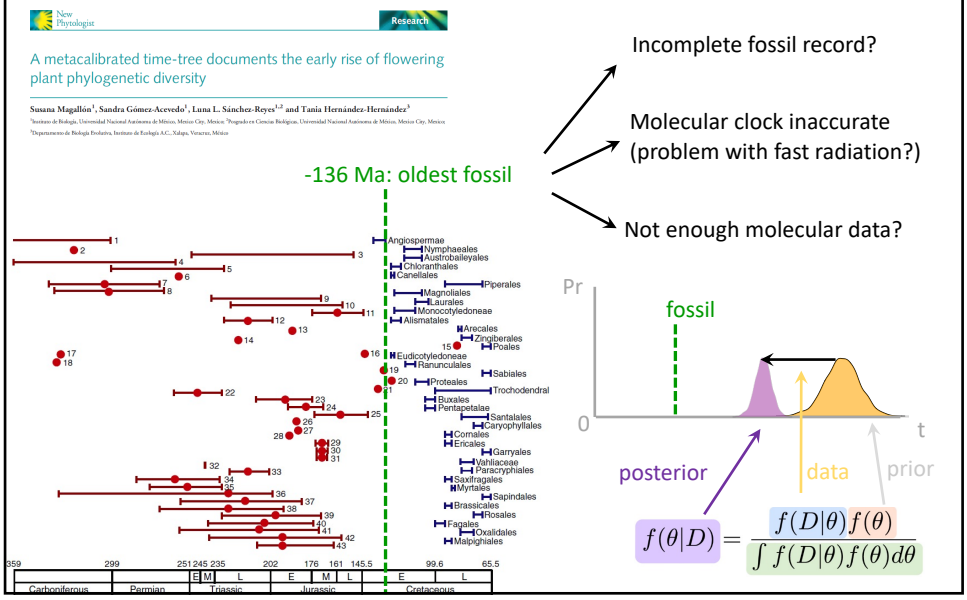
55

Timing the origin of Angiosperms



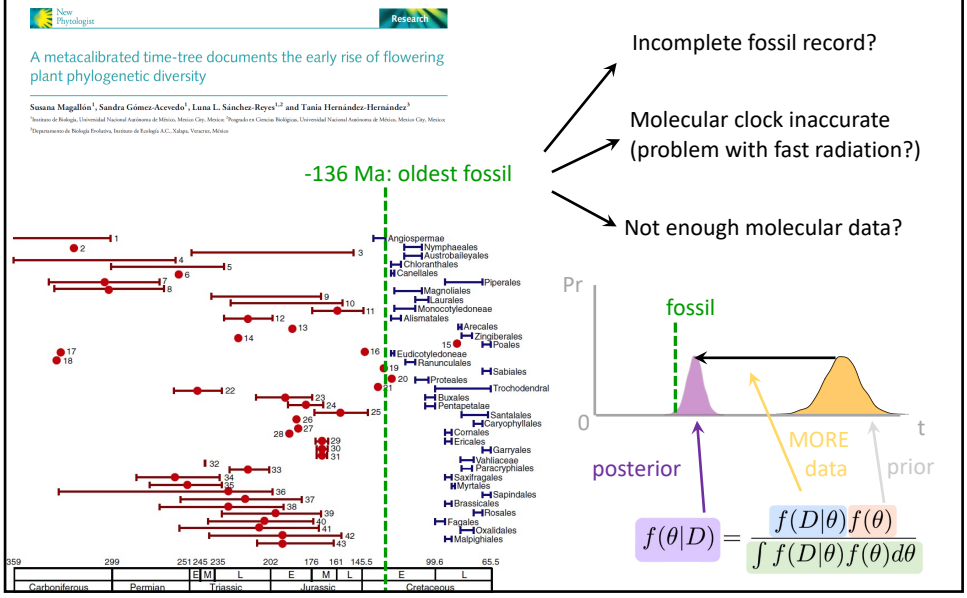
56

Timing the origin of Angiosperms



57

Timing the origin of Angiosperms



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Timing the origin of Angiosperms

Eocene lantern fruits from Gondwanan Patagonia and the early origins of Solanaceae

Peter Wilf[1,*], Mónica R. Carvalho[2], María A. Gandolfo[2], N. Rubén Cúneo[3]
+ See all authors and affiliations

Science 06 Jan 2017:
Vol. 355, Issue 6320, pp. 71-75
DOI: 10.1126/science.aag2737

Physalis infinemundi

Physalis

tomatillo group - 9 to 11 My

Nightshades - 35 to 51 My

59

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PRE Peer Reviewed ← see details

Physalis infinemundi

Physalis

tomatillo group - 9 to 11 My

Nightshades - 35 to 51 My

-52 Ma !!!

Wrong time estimate for the fossil?

Previous time-calibrations of Nightshade (and Angiosperms?) failed?

Over-estimation of the phylogenetic position?

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