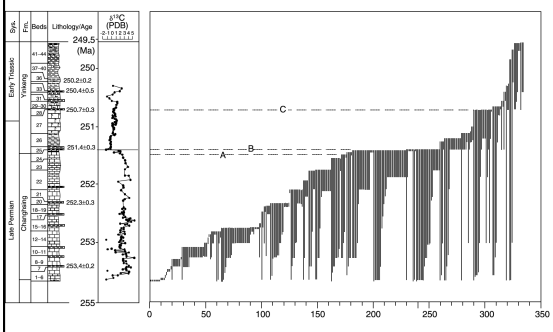
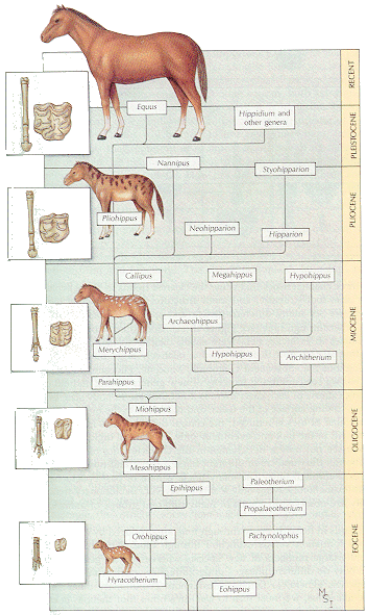
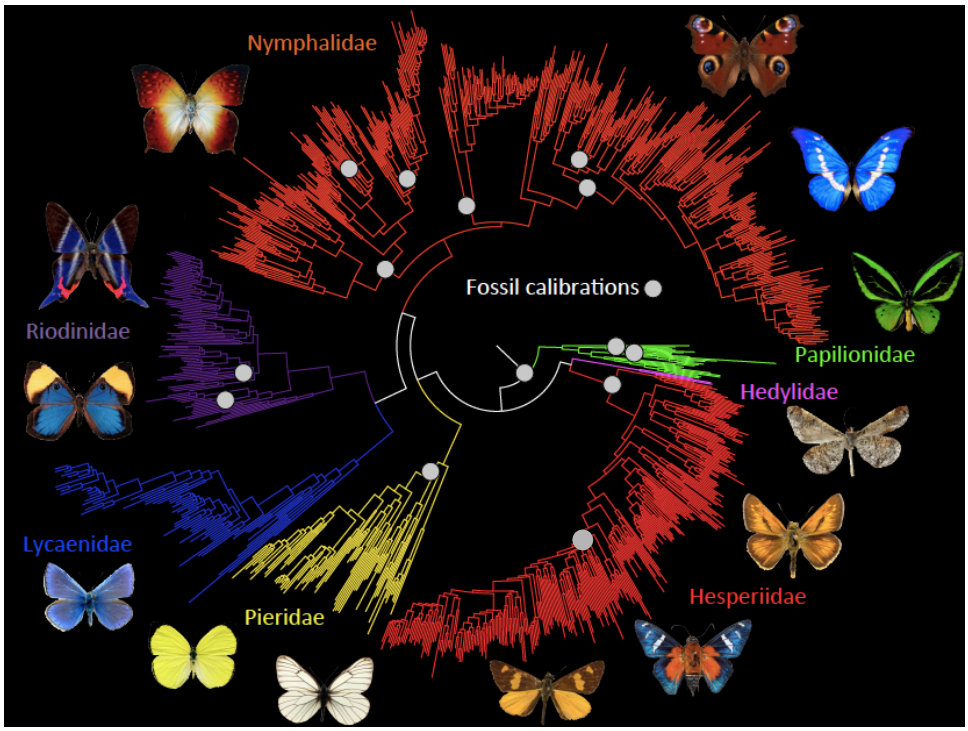
Introduction to molecular dating methods

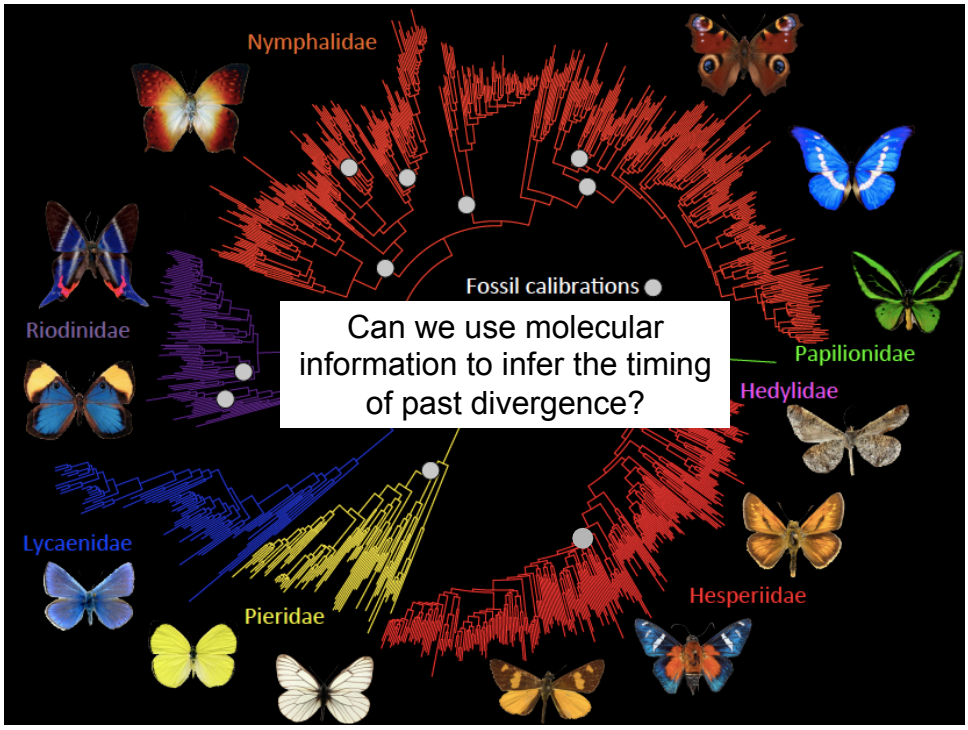
Many slides from Simon Ho, modified by Nicolas Chazot

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






Nymphalidae
Fossil calibrations
Riodinidae
Papilionidae
Hedylidae
Lycaenidae
Hesperiidae
Pieridae

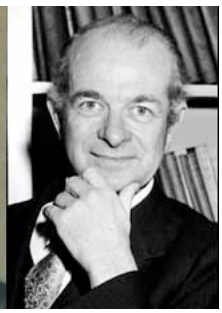

Nymphalidae
Fossil calibrations
Can we use molecular information to infer the timing of past divergence?
Riodinidae
Papilionidae
Hedylidae
Lycaenidae
Hesperiidae
Pieridae

The Molecular Clock

Going back to ancient times

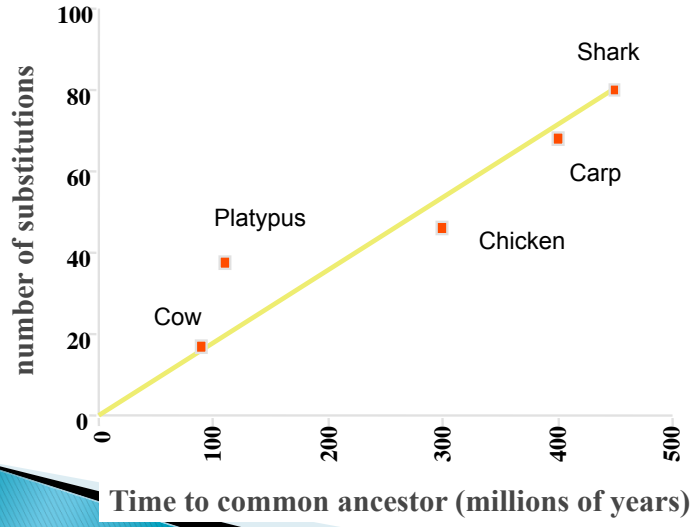
Is there a molecular clock?

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



The molecular clock for alpha-globin:

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



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

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

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

Assumptions of a perfect clock (cont.)

- 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

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

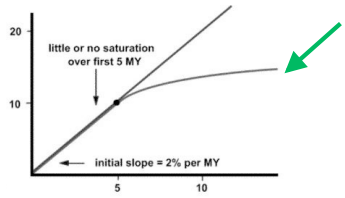
There is no universal molecular clock

- 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

Problems

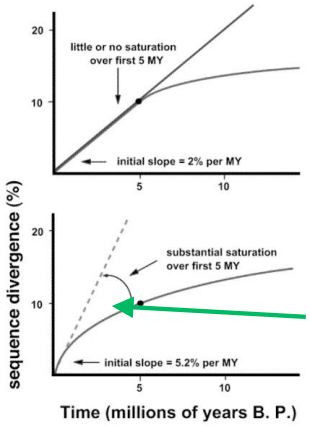
- Saturation

Saturation problems



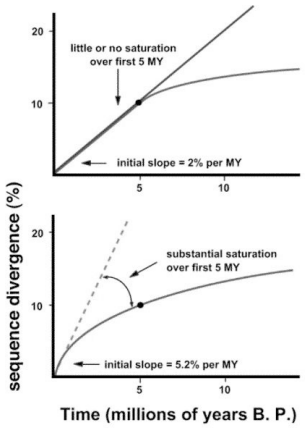
old divergences will be underestimated

Saturation problems

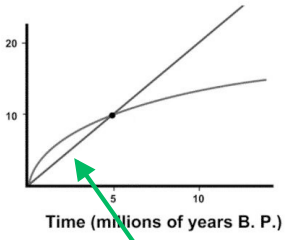


times of divergences are quickly underestimated

Saturation problems



And if we underestimate the rate of substitution...

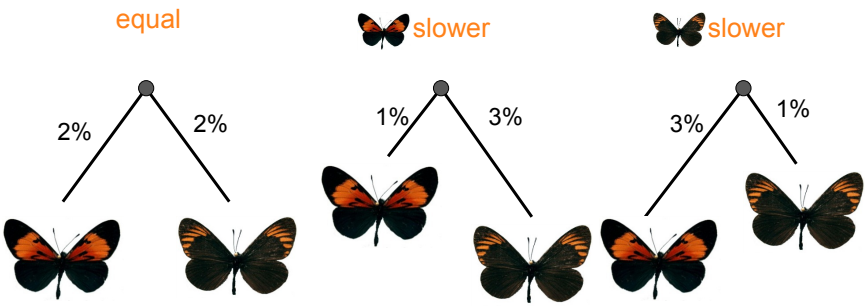


Both recent divergences will be overestimated and older divergences will be underestimated

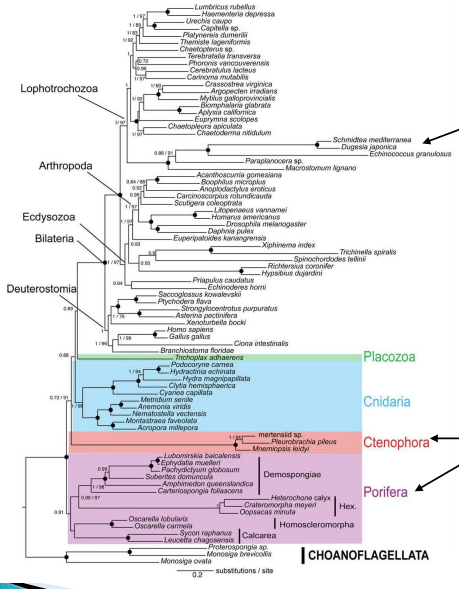
Problems

- Saturation
- Rate Heterogeneity – violation of homogeneity

No universal molecular clock



Molecular distance from  to  is the same in all cases



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

Teasing apart RATE and TIME

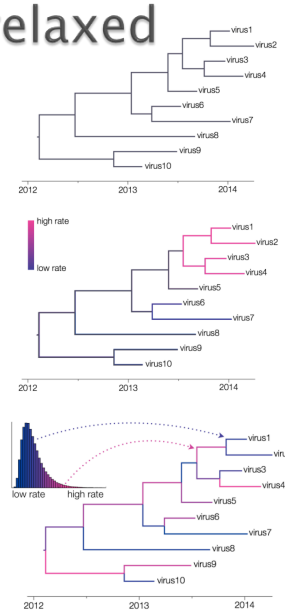
- Branch lengths are proportional to:

  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*

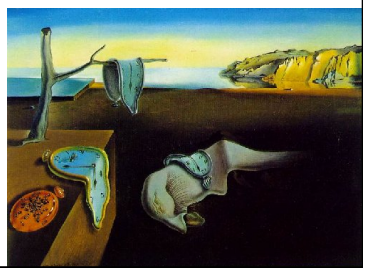
Molecular clocks can be relaxed

- 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)



What is a relaxed clock?

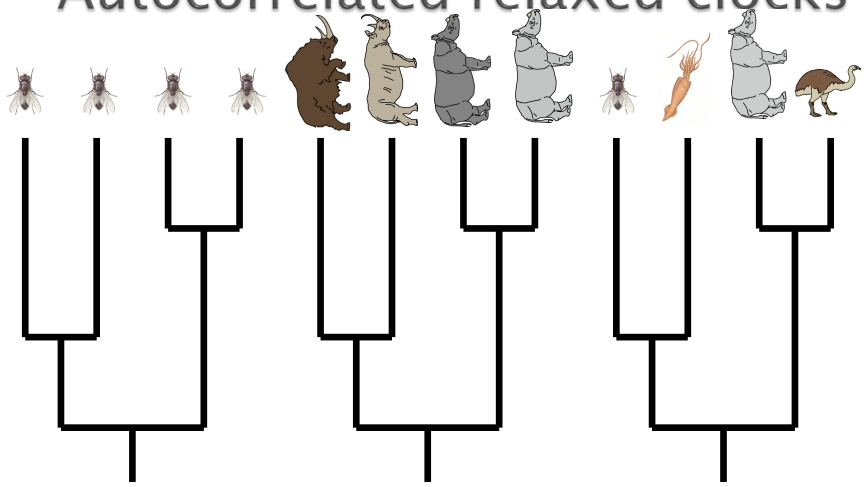
- Strict clock: rate identical in all branches
- Relaxed clock: rate allowed to vary among branches
  - Autocorrelated relaxed clock: rates in adjacent branches are related
  - Uncorrelated relaxed clock: rates identically and independently distributed among branches



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

Autocorrelated relaxed clocks



Very high autocorrelation

Moderate autocorrelation

Very low autocorrelation

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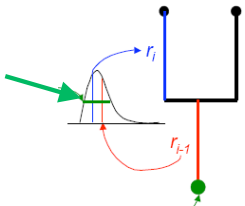
Modeling autocorrelation

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

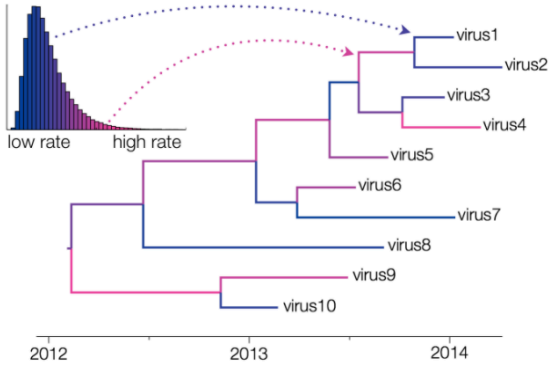
v controls the s.d. of the distribution



Further assumption needs to be made about rate at the root

Uncorrelated relaxed clocks

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



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

Problems

- Saturation
- Rate Heterogeneity – violation of homogeneity
- Calibration

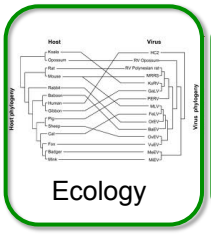
Calibration

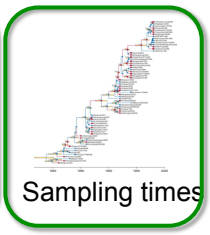
Separating rate and time

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


Fossil record


Biogeography


Ecology


Sampling times

Calibration: Fossil record

- Fossil record provides minimum estimates of divergence times


Fossil record

Identified as belonging to the family Aeshnidae and genus *Aeshna*

informative

Calibration: Fossil record

- Fossil record provides minimum estimates of divergence times

Fossil record

0 my

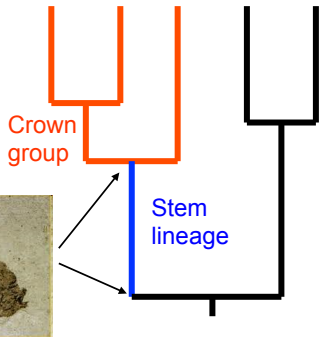
Identified as belonging to the family Phasianidae, to genus *Gallus*, to the species *Gallus gallus domesticus*

Minimum age for the birds…
BUT not informative

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)



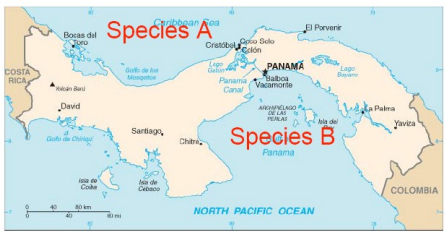
Calibration errors

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

Calibration: Biogeography

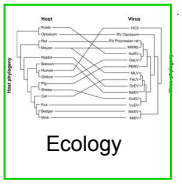
- Biogeographic events can provide maximum estimates of divergence times

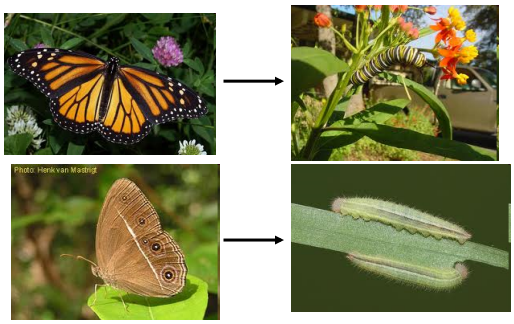

Biogeography



Calibration: Ecology

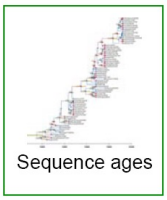
- Knowledge of tight ecological associations can be used to provide maximum estimates of divergence times

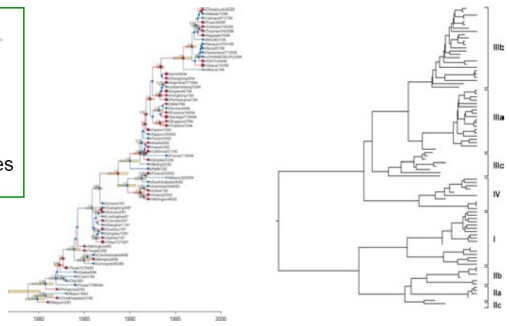




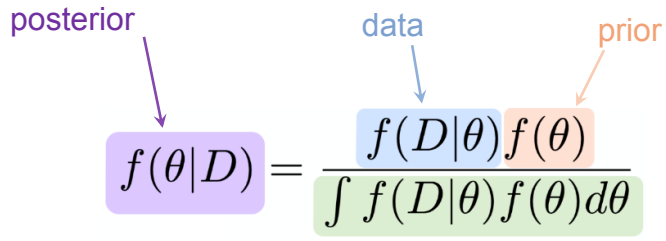
Calibration: Sequence ages

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





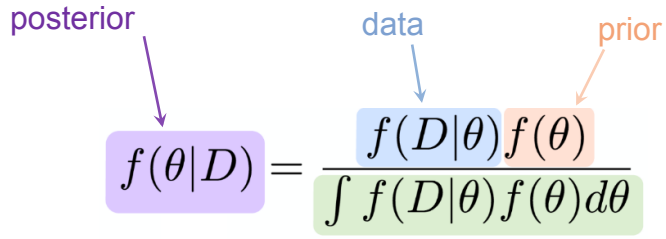
Calibration in Bayesian framework



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

prior: prior expectation we have for parameters of the model

Calibration in Bayesian framework



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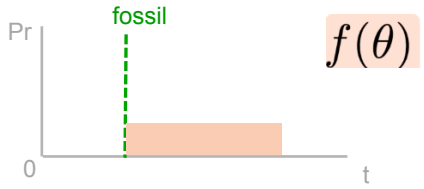
For example: age of nodes based on fossil information

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:



Calibration in Bayesian framework

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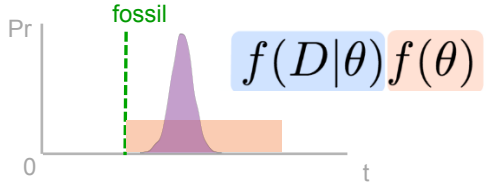
Prior probability distribution:

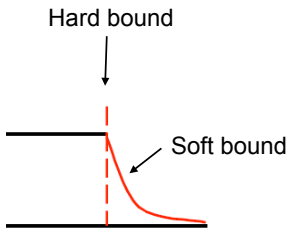


Calibration types

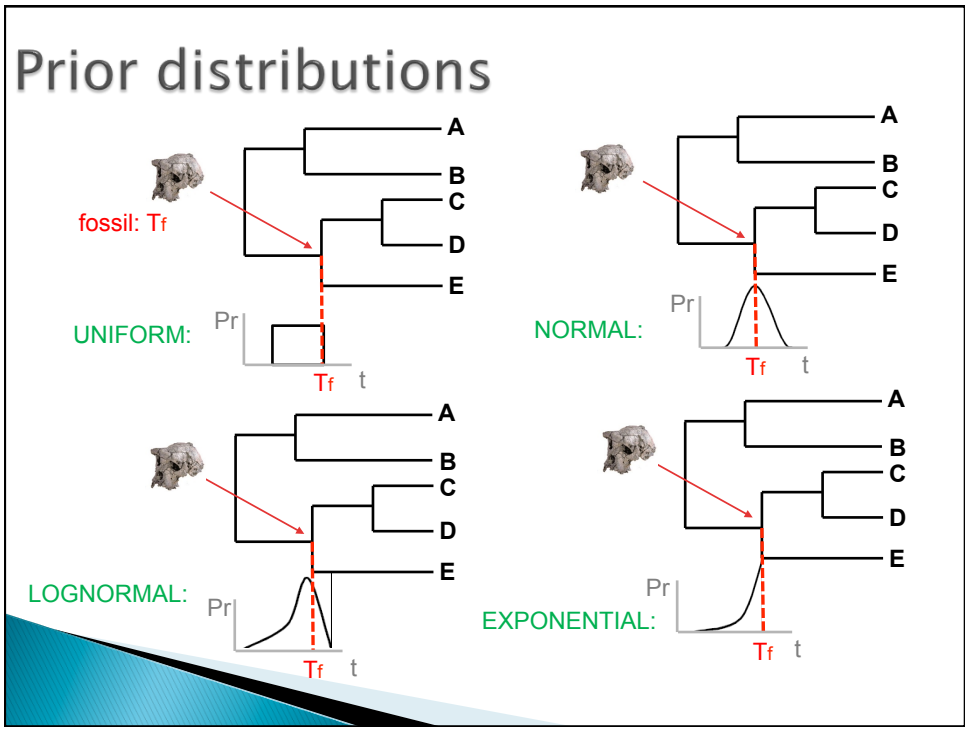
- Point calibrations
- Hard minimum/maximum bounds
- Soft minimum/maximum bounds
- Parametric prior distributions
  - Normal distribution
  - Lognormal distribution
  - Exponential distribution

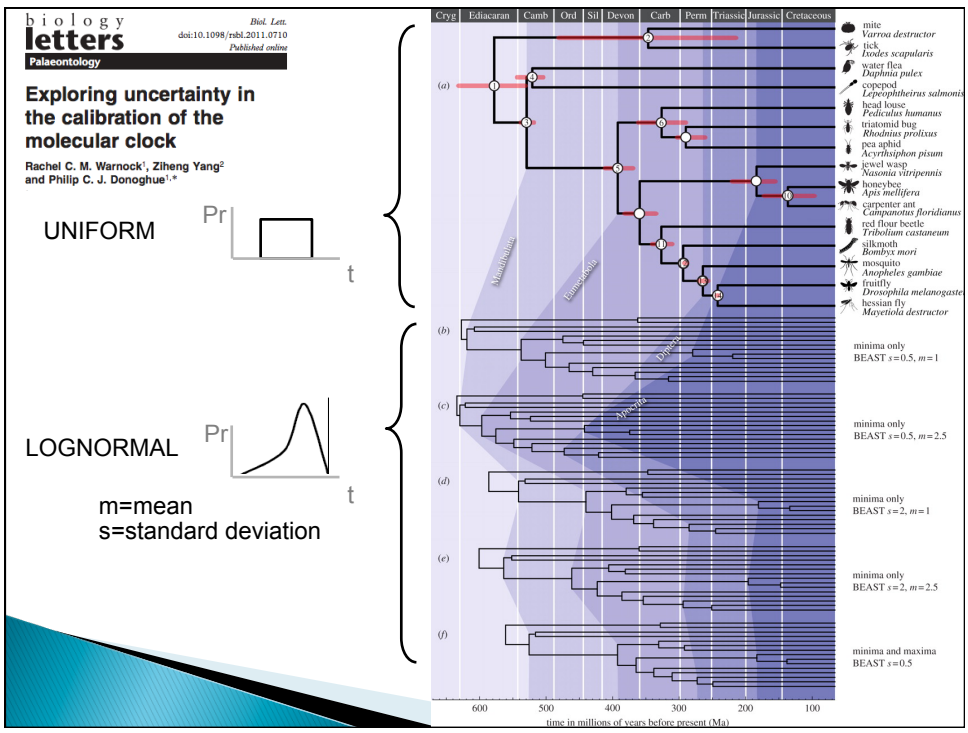
Hard/Soft Bounds

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

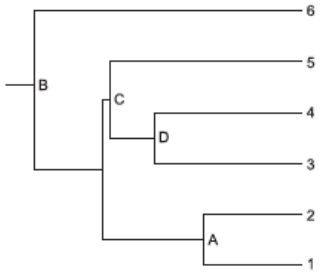


Prior distributions





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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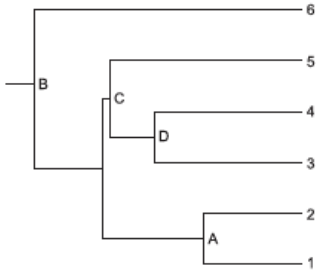
Multiple calibrations



- 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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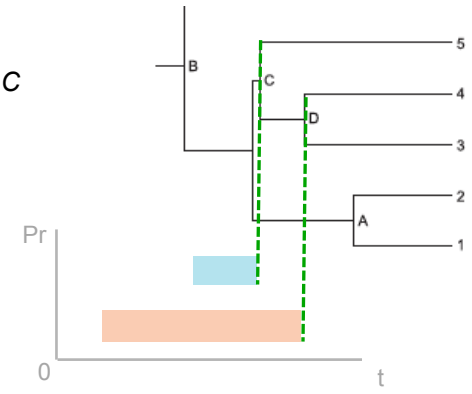
Multiple calibrations

- 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

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

50

Multiple calibrations

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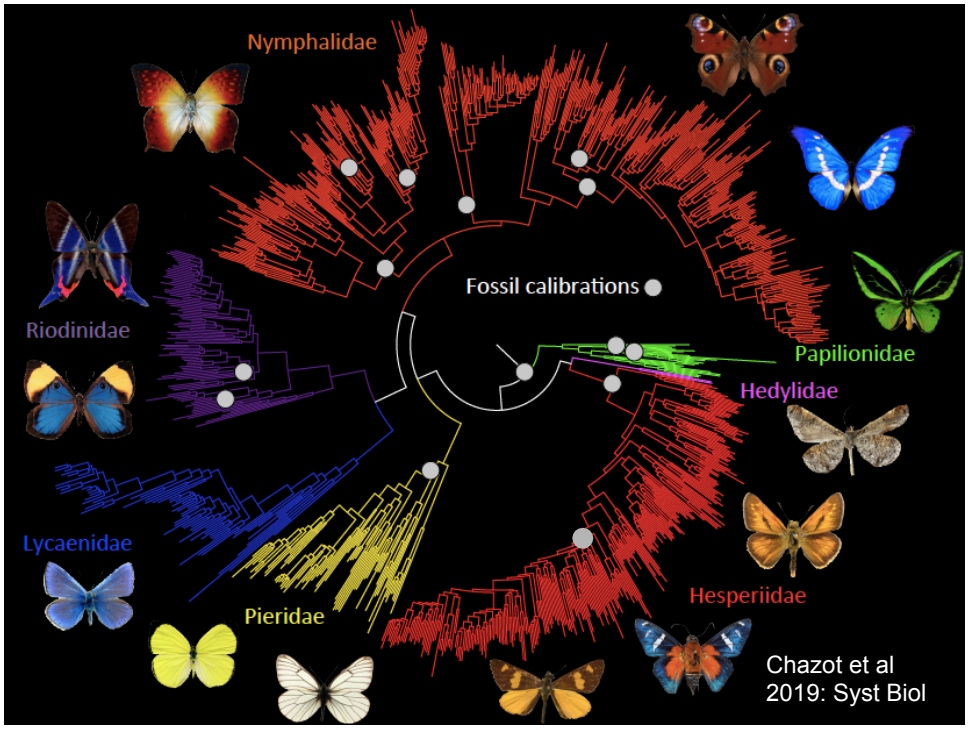
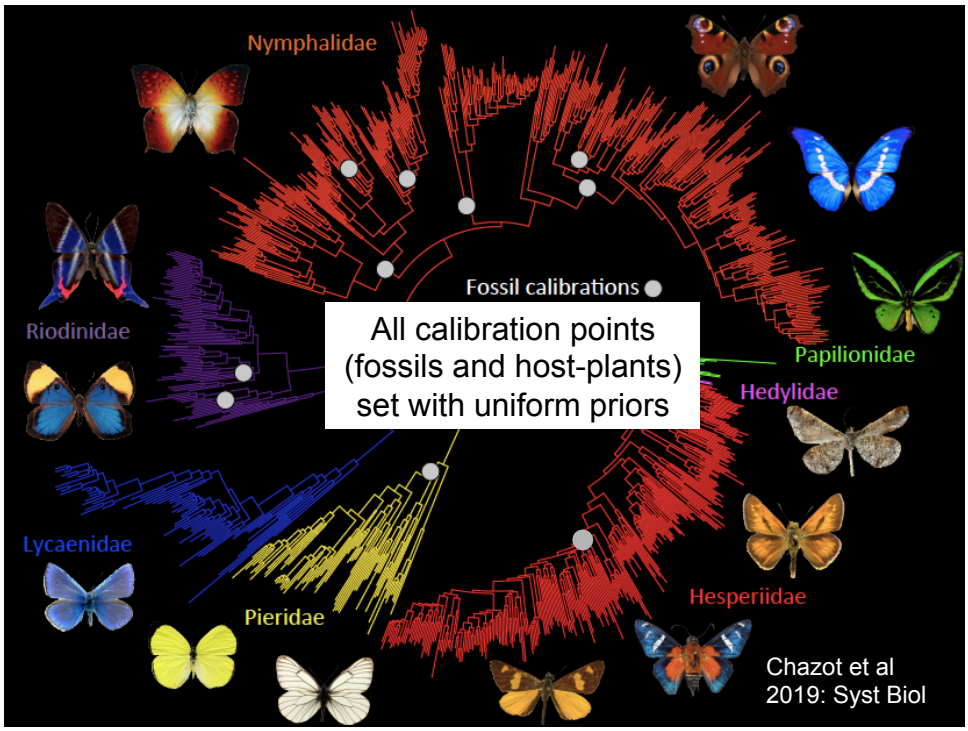
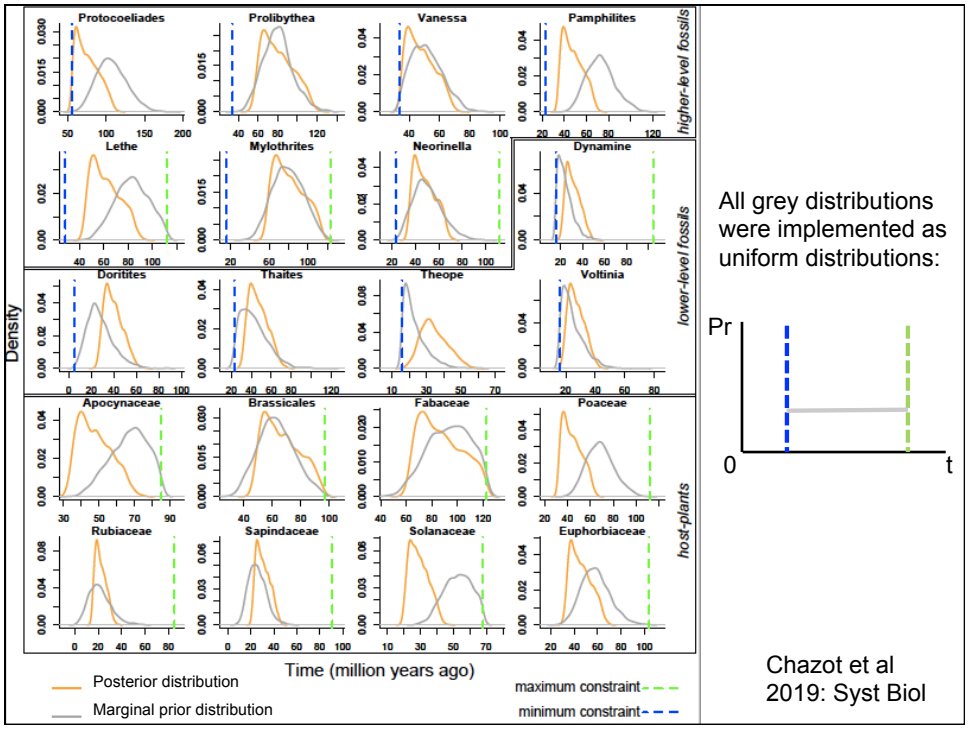
51




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


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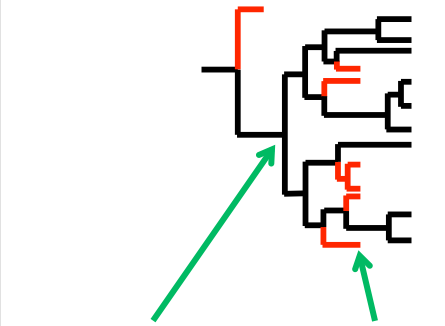
Chazot et al 2019: Syst Biol

Prior sensitivity



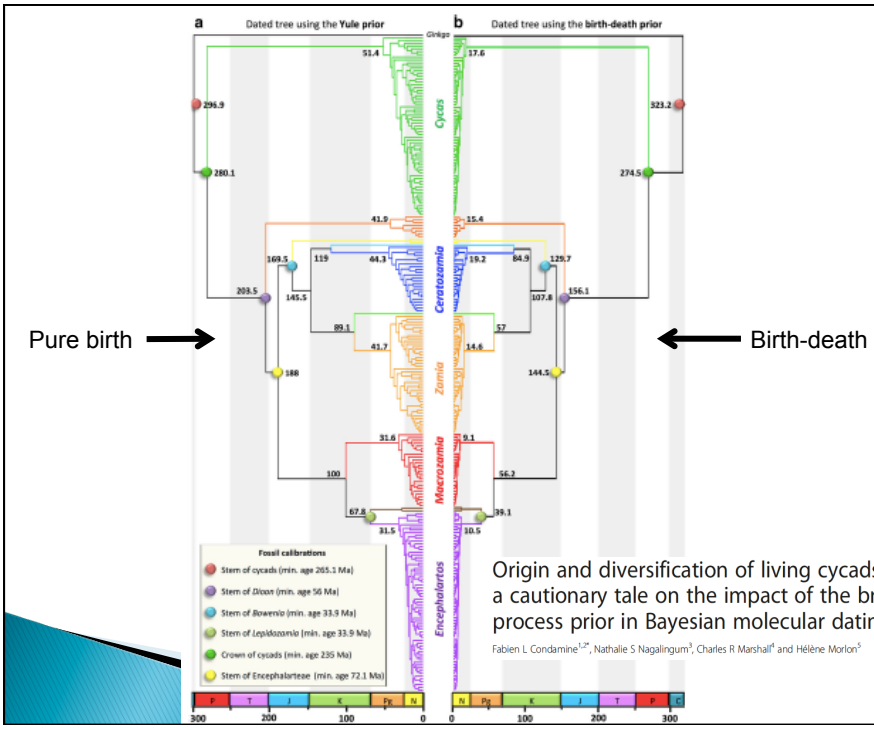
Bayesian methods include a prior on tree growth

Branching process prior

Pure birth

Birth-death

55

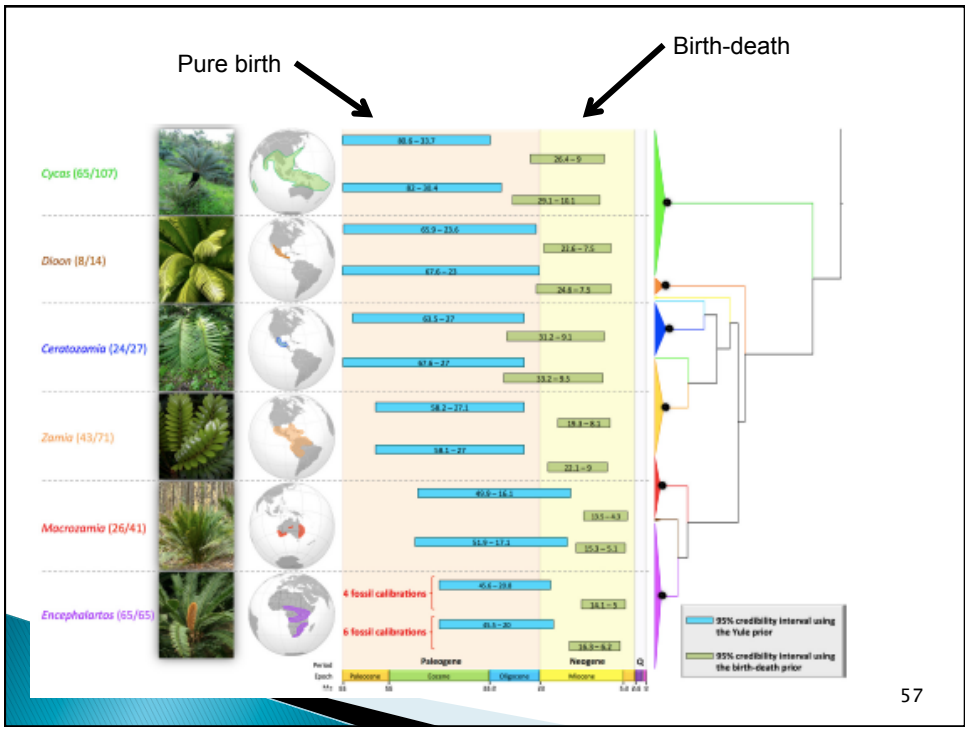


Pure birth → ← Birth-death

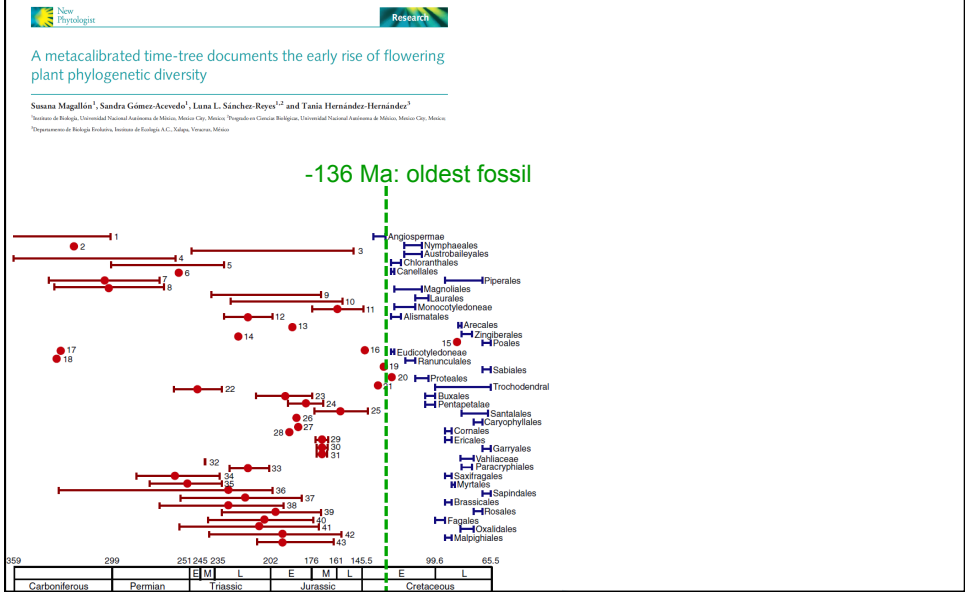
Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating

Fabien L Condamine[1,2*], Nathalie S Nagalingum[3], Charles R Marshall[4] and Hélène Morlon[5]

56



Timing the origin of Angiosperms



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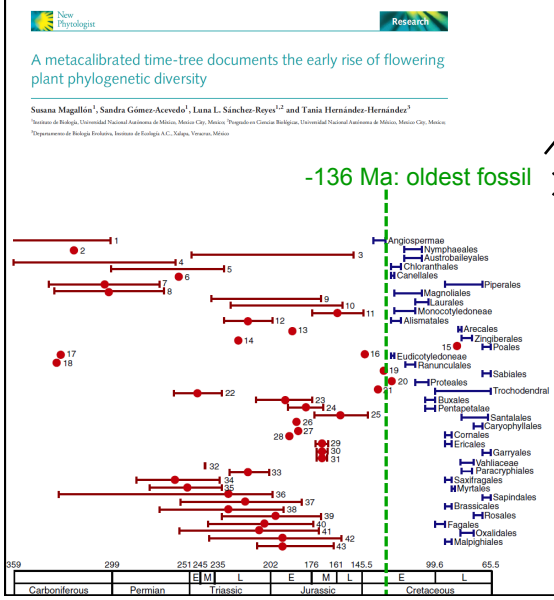
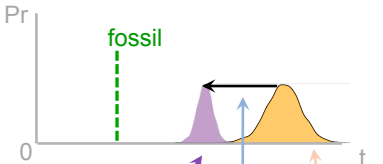


Incomplete fossil record?

Molecular clock inaccurate (problem with fast radiation?)

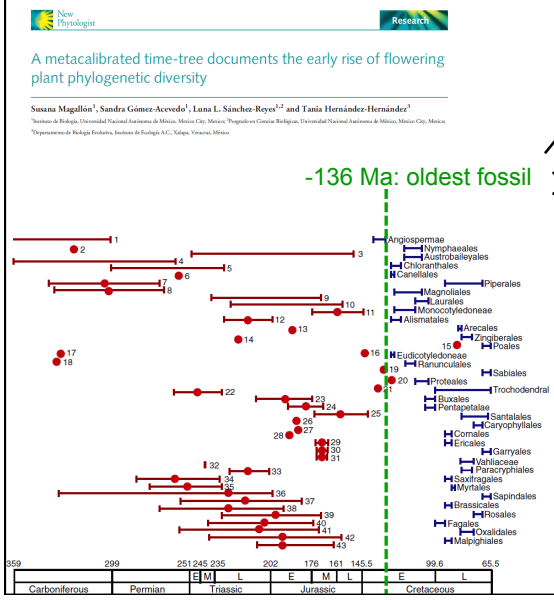
Not enough molecular data?



posterior data prior

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

Timing the origin of Angiosperms



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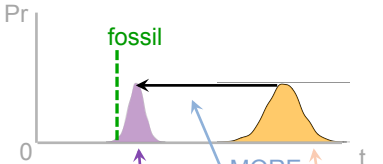

posterior MORE data prior

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

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

Peer Reviewed
← see details

Physalis infinemundi

Physalis

tomatillo group - 9 to 11 My

Nightshades - 35 to 51 My

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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?