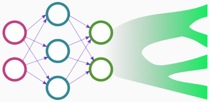
Deep end-to-end likelihood-free inference of phylogenetic trees

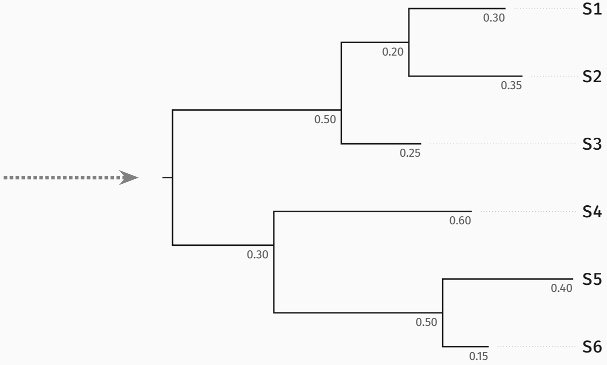


Luc Blassel, Nicolas Lartillot, Bastien Boussau, Laurent Jacob

MLCB - September 11th, 2025

Context - Phylogenetic inference

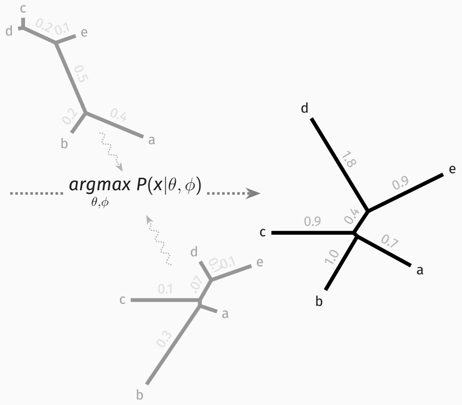
```
S1 TQPRLPTC
S2 TQPSVPKC
S3 TGVPVPAC
S4 TLGRSPSC
S5 TQPRAKTC
S6 TQPRIPAC
```



Goal: describe **evolutionary-history** of MSA

Context - Likelihood-based tree reconstruction

a	TQPRLPTC
b	TQPSVPKC
c	TGVPVPAC
d	TLGRSPSC
e	TQPRAKTC

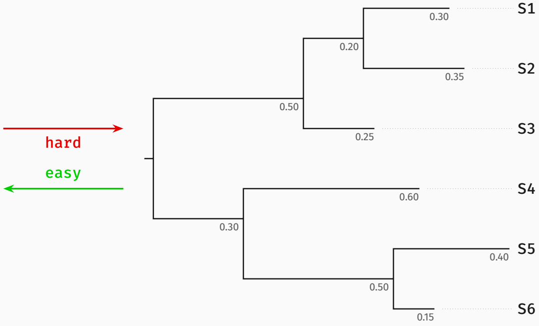


- **accurate** but **slow**
- $P(x|\theta,\phi)$ must be **computable**

x : MSA, $\theta = (\tau, \ell)$: Phylogenetic tree, ϕ : Evolution model Felsenstein 1993; Kleinman et al. 2010

Motivation - Likelihood-free inference

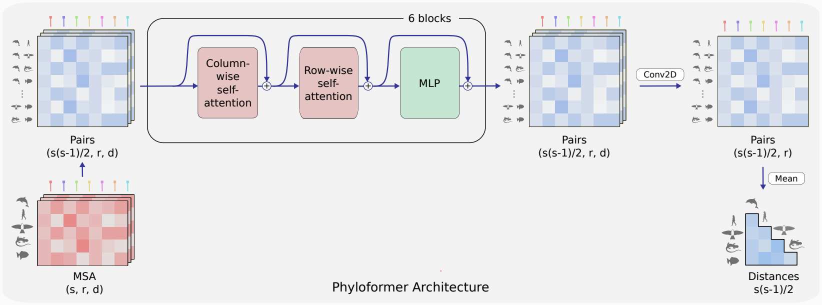
```
S1  TQPRLPTC
S2  TQPSVPKC
S3  TGVPVPAC
S4  TLGRSPSC
S5  TQPRAKTC
S6  TQPRIPAC
```



- We can simulate many[1] (tree, MSA) pairs
- Can we **learn** the mapping **from MSA to tree**?

[1] pretty much practically ∞

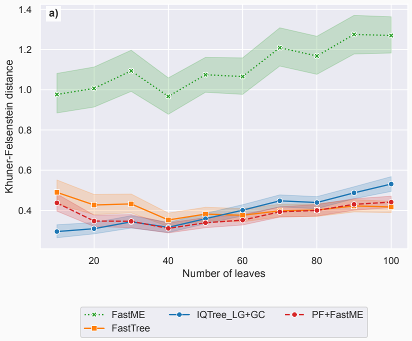
Related Work - Phyloformer, our first approach

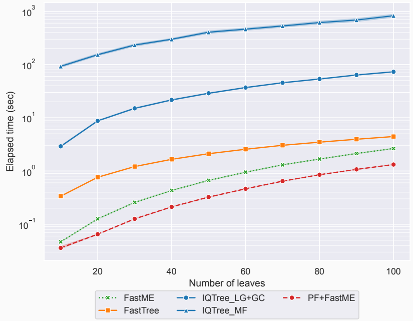


- Input an **MSA**, get a **Distance matrix**
- Feed Distance matrix to **FastME** to get **tree**

Nesterenko et al. 2025; Lefort et al. 2015

Related Work - Phyloformer is good!





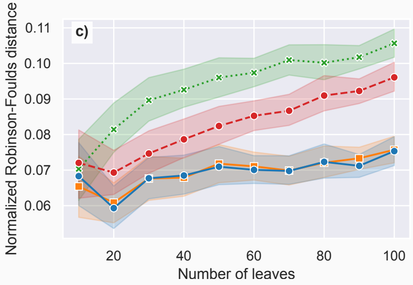
Tree inference accuracy (KF)

Runtime

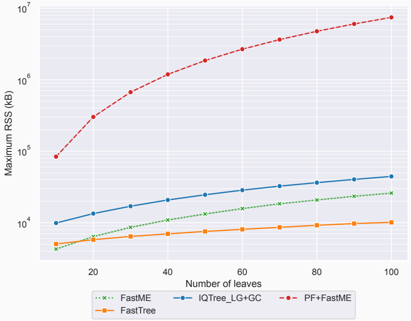
- Fairly **competitive** even on simple LG+GC model
- **Fast** because we use GPUs [1]

Nesterenko et al. 2025, [1]🙏 Jean-Zay

Related Work - But also sometimes less good...



Topological accuracy (RF)



Memory usage

- **Gap** between PF and **ML methods**
- PF is **by far** the most **memory intensive**

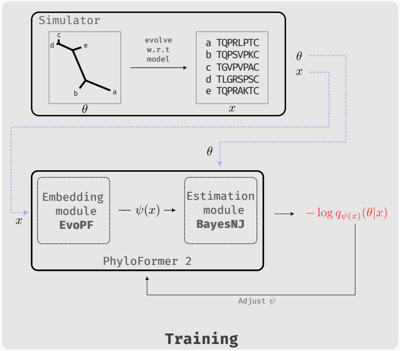
How to do phylogenetic inference end-to-end ?

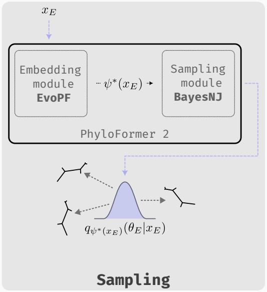
Methods - Neural Posterior Estimation (NPE)

- Given a **probabilistic model** $p(x|\theta)$ with some prior $p(\theta)$
- We want to **estimate the posterior**: $p(\theta|x)$
- We build $q_\psi(\theta|x)$ a **family** of distributions **parametrized** by ψ (our NN)
- We find $q_{\psi^*} = \underset{\psi}{\operatorname{argmin}}\, \mathbb{E}_{p(x)}[KL(q_\psi(\theta|x)||p(\theta|x)]$
- In practice we **maximize** $\mathbb{E}_{p(x,\theta)}[\log q_{\psi(x)}(\theta|x)]$ by **sampling** from $p(x,\theta)$

x : MSA, $\theta = (\tau, \ell)$: Phylogenetic tree, $\psi(x)$: NN applied to x

Methods - How do we do NPE?





- During **training** find $\psi^* = \underset{\psi}{\operatorname{argmin}} \; -\sum_i \log q_{\psi(x_i)}(\theta_i|x_i)$
- At **inference** time **sample** from: $q_{\psi^*(x_E)}(\theta_E|x_E)$ [1]

[1]WIP: so for now only point-estimation

Methods - The EvoPF module, intro

the EvoPF module is an **adaptation** of the **EvoFormer** module from **AlphaFold2**. The tasks are **transpositions** of each other:

given input MSA ($n \times r$)

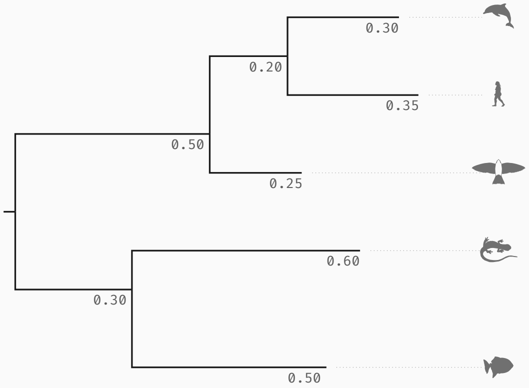
EvoFormer	represent $r \times r$ relationships between sites
EvoPF	represent $n \times n$ relationships between sequences

More expressive than MSA transformer
More lightweight than PF

Jumper et al. 2021; Rao et al. 2021

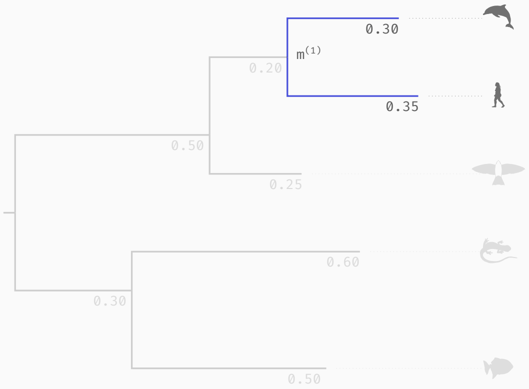
Methods - A tree is a series of merges

We want to describe the following tree:



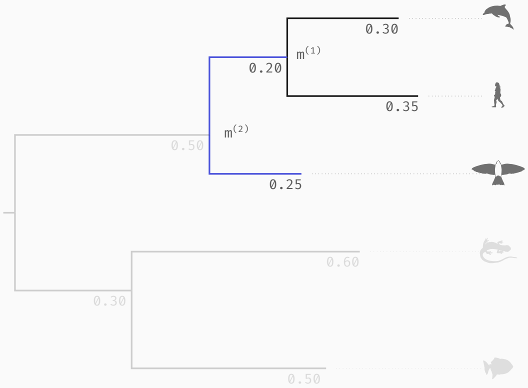
Methods - A tree is a series of merges

Iteratively merge shortest cherry:



Methods - A tree is a series of merges

Iteratively merge shortest cherry:



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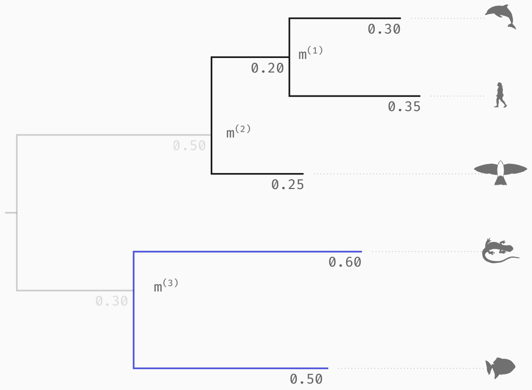

Methods - A tree is a series of merges

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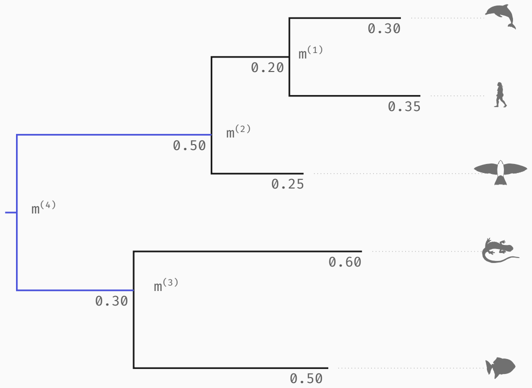


$$\tau = \{m^{(1)}, m^{(2)}, m^{(3)}, m^{(4)}\}$$

Methods - the BayesNJ module

- **Tree** is an **ordered set** of merges: $\theta : \{m^{(1)}, \dots, m^{(N-1)}\}$
- We **factorize** $q_{\psi(x)}(\theta|x)$ as the product of successive merge probabilities:

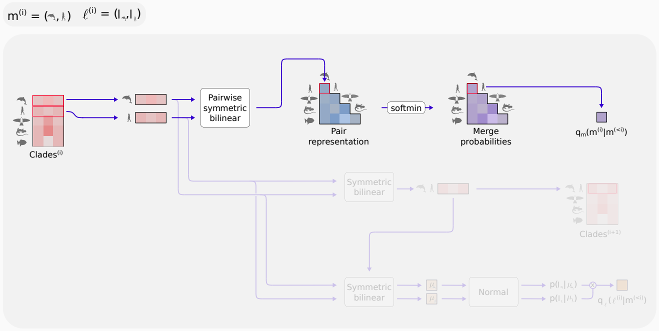
$$q_{\psi(x)}(\theta|x) = \prod_{k=1}^{N-1} q_m(m^{(k)}|m^{(<k)}) q_\ell(\ell^{(k)}|m^{(\leq k)})$$

- **Merge** probabilities have **2 components**:

topological: $q_m(m^{(k)}|m^{(<k)})$

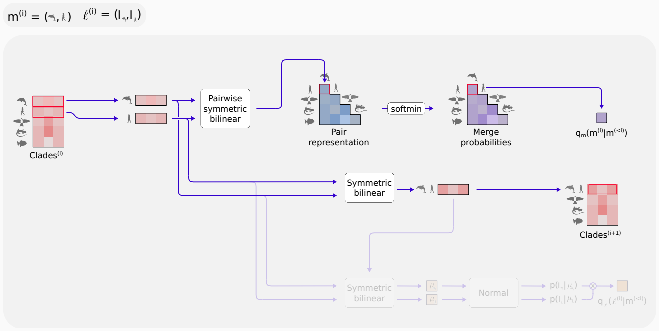
branch-length: $q_\ell(\ell^{(k)}|m^{(\leq k)})$

Methods - BayesNJ, evaluating topological probabilities



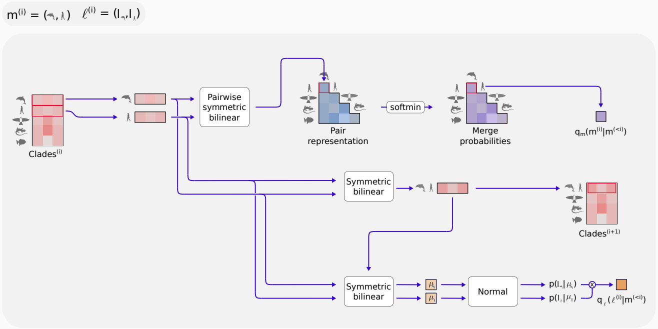
Compute **merge probability**

Methods - BayesNJ, evaluating topological probabilities



Update clade **representation** for next merge

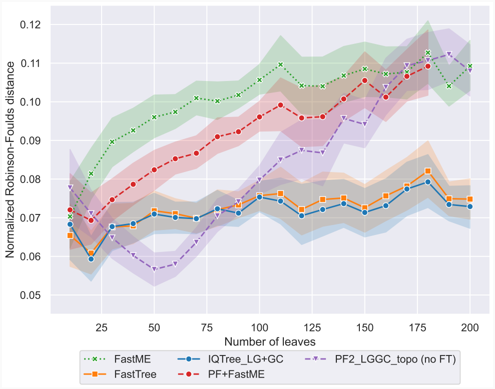
Methods - BayesNJ, evaluating topological probabilities



Compute **branch-length** probabilities

Does it work ?

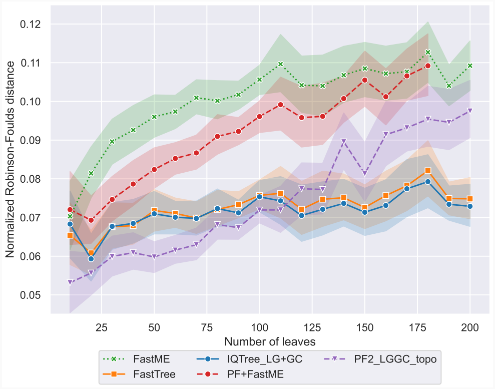
Results - Training topology only



- **overfitting** on tree-size is an **issue**

Same train set as PF1 paper: $\approx 170k$ 50 seq LG+GC MSAs on rescaled BD trees

Results - Training topology only



- **overfitting** on tree-size is an **issue**
- **Fine tuning** helps

Same train set as PF1 paper: $\approx 170k$ 50 seq LG+GC MSAs on rescaled BD trees

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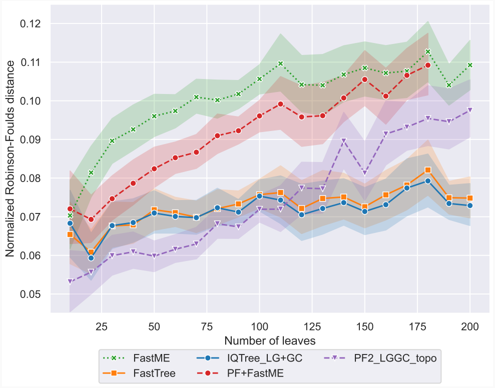
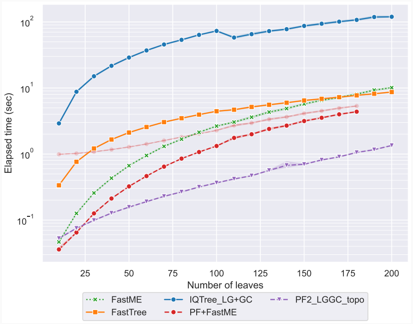


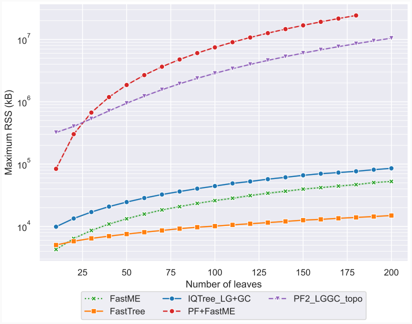
- **overfitting** on tree-size is an **issue**
- **Fine tuning** helps
- We **beat ML** methods in certain cases
- Marked **improvement** w.r.t **Phyloformer**

Same train set as PF1 paper: $\approx 170k$ 50 seq LG+GC MSAs on rescaled BD trees

Results - Scalability



Execution time



Memory usage[1]

[1] With $2\times$ bigger sequence, and $4\times$ bigger pair embeddings...

Perspectives - Short term challenges

This is very much still a **work in progress...**

- Training on **more complex** data *(e.g. indels)* **increases** length-**overfitting**
- Learning **topology** and **branch-lengths** is also **challenging**
- How can we move **away** from **point-estimation** ?
- We might need to **adjust** our **priors** to compare with MCMC tools

Conclusion

Takeaways

- **Topologically** we manage to **beat** `IQTree`[1] on LG
- While being **more scalable** than PF1
- Still needs some **work** for a fully **end-to-end** phylogenetic **inference** tool

What next ?

- Can we do **better** where computing $p(\theta|x)$ is **difficult** or **intractable**? *(e.g. Potts, epistatis, Selection, ...)*
- **Confident** this can **work** given our experience with **PF**

Prillo et al. 2023; Duchemin et al. 2023; Latrille et al. 2021

[1] Yay!

Thanks to:

- Luca Nesterenko
- Laurent Jacob
- Bastien Boussau
- Nicolas Lartillot
- Philippe Veber
- Vincent Garot
- Amélie Leroy
- Anybody that listened to me!

Special thanks to Jean-Zay for all the GPUs!

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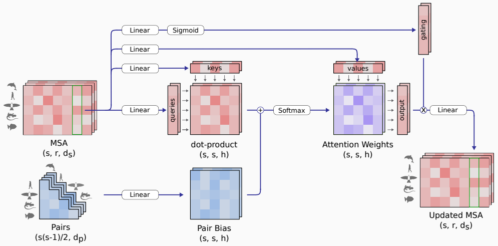
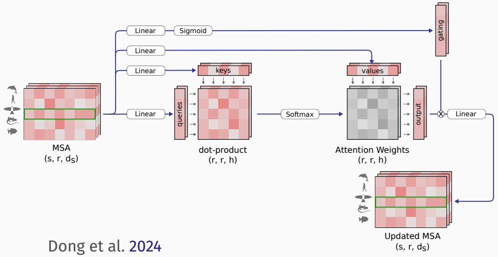
Supp. Methods - EvoPF, the MSA stack



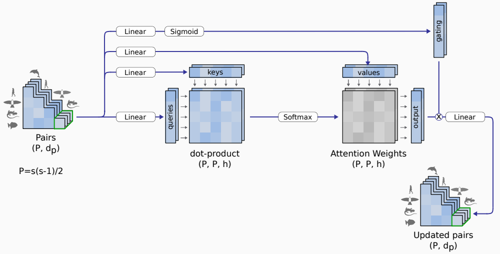
Column-wise attention with pair-bias



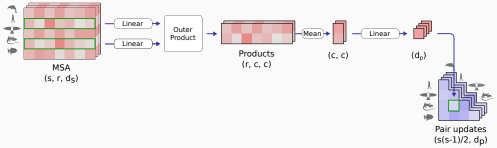
Row-wise attention

Dong et al. 2024

Sup. Methods - EvoPF, the pair stack



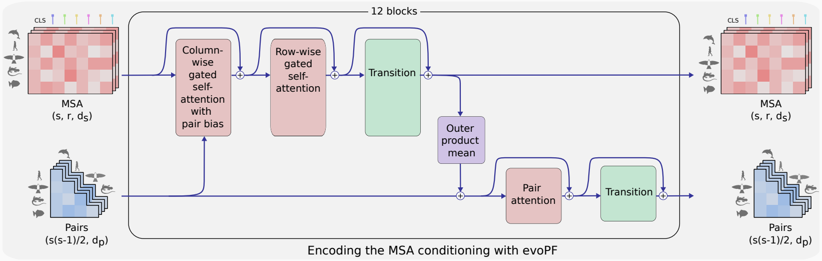
Pair attention



Outer product mean

Dong et al. 2024

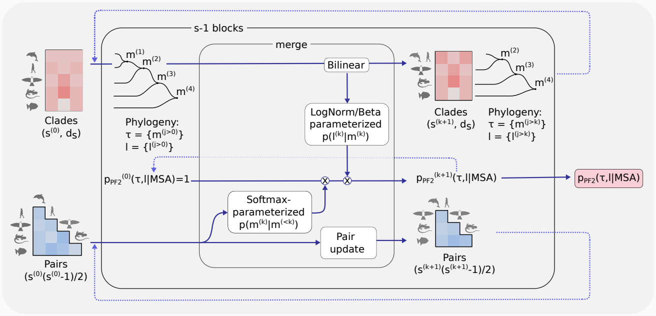
Sup. Methods - The EvoPF module, details



- Input an **MSA** and get:
  sequence embedding $\{s_i\}$
  sequence-pair embeddings $\{z_{ij}\}$
- **Both** embedding-types used to **update each-other**

Figure inspired by Jumper et al. 2021

Sup. Methods - BayesNJ evaluation mode


s-1 blocks
merge
$m^{(1)}$
$m^{(2)}$
$m^{(3)}$
$m^{(4)}$
Clades $(s^{(0)}, d_S)$
Phylogeny: $\tau = \{m^{(j>0)}\}$ $l = \{l^{(j>0)}\}$
Bilinear
LogNorm/Beta parameterized $p(l^{(k)}|m^{(k)})$
Clades $(s^{(k+1)}, d_S)$
Phylogeny: $\tau = \{m^{(j>k)}\}$ $l = \{l^{(j>k)}\}$
$p_{PF2}^{(0)}(\tau,l|MSA)=1$
$p_{PF2}^{(k+1)}(\tau,l|MSA)$
$p_{PF2}(\tau,l|MSA)$
Softmax-parameterized $p(m^{(k)}|m^{(<k)})$
Pair update
Pairs $(s^{(0)}(s^{(0)}-1)/2)$
Pairs $(s^{(k+1)}(s^{(k+1)}-1)/2)$

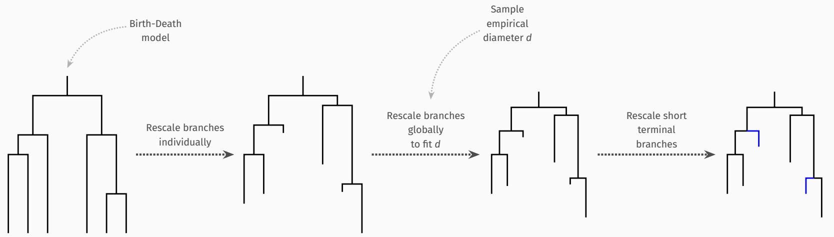
Sup. Methods - Ensuring the merge order is unique

Ensuring a **unique order** on merges ensures that we **define a distribution**. It also keeps **training** and **sampling** comparable [1]

- On a given tree τ always **merge** the **shortest** available **cherry**
- When **sampling**, add **constraints**:
  1. Start with a $N \times N$ constraints matrix $M_{ij} = 0$
  2. At iteration k sample merge $m^{(k)} = (i, j)$ and cherry length $s^{(k)} = M_{ij} + X$
  3. **Update constraints** for cherries **available** when sampling $m^{(k)}$: $M'_{ij} = max(M_{ij}, s^{(k)}) \quad M'_{ui} = 0$
- During evaluation compute $p_{PF2}(s^{(k)} - M_{ij} | m^{(\leq k)})$

[1] Which is not the same if we use the NJ merge order

Sup. Methods - Tree simulation


Birth-Death
model
Rescale branches
individually
Sample
empirical
diameter *d*
Rescale branches
globally
to fit *d*
Rescale short
terminal
branches