The impact of rate variation assumptions on phylogenetic accuracy

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Phylogenetic Tree Inference

Standard Assumptions

- Markov **substitution models** describe sequence evolution.
- Assumes **uniform evolutionary rates**.

T1	C	A	G	T	T	A	C	G
T2	T	A	G	A	T	A	C	T
T3	G	A	G	C	T	C	C	C
T4	A	A	G	G	T	T	C	A

Rate heterogeneity in phylogenetics

T1	C	A	G	T	T	A	C	G
T2	T	A	G	A	T	A	C	T
T3	G	A	G	C	T	C	C	C
T4	A	A	G	G	T	T	C	A

Biological Reality

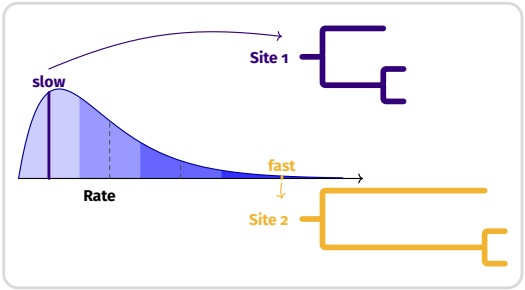
Different regions experience different selective pressures, leading to **among-site rate variation** (fast vs. slow sites).

- Yang (1994) proposed modeling these varying rates using a continuous **Gamma distribution** ($+\Gamma$).

Modified from Silvestro et al. (2024)

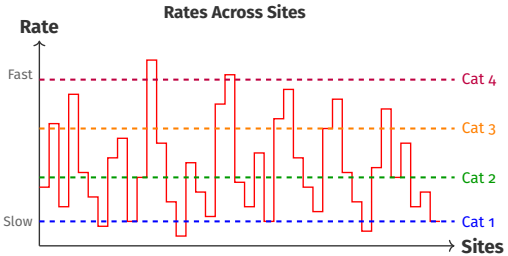
Among-site rate variation model

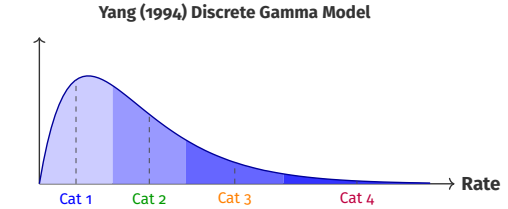
- Yang (1994) $\rightarrow$ **Discrete Gamma Model** ($+\Gamma$).
- To make this mathematically tractable, he suggested approximating the curve into **4 discrete rate categories** ($k = 4$).



Modified from Heckeberg et al. (2026)

Re-evaluating the $k = 4$ assumption





- Software extracts a single **Mean** or **Median** rate for each category to scale branch lengths.

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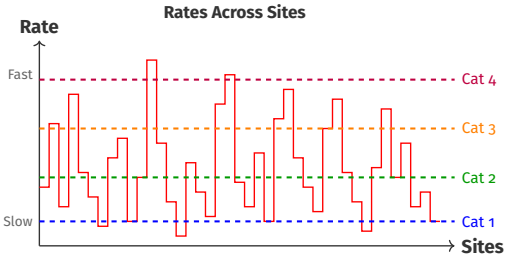
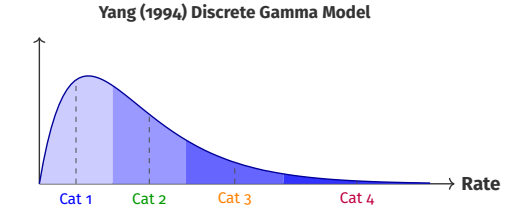
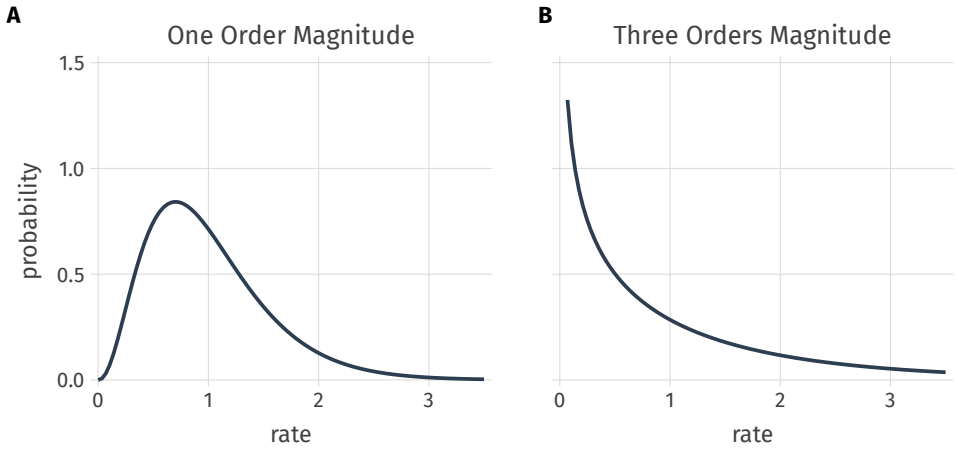




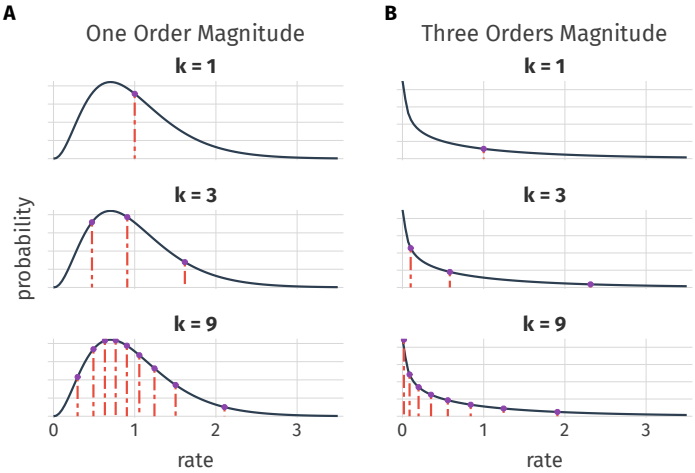
- Software extracts a single **Mean** or **Median** rate for each category to scale branch lengths.
- We now analyze massive phylogenomic datasets.

  $\rightarrow$ **Is $k = 4$ still valid for genome scale datasets?**

Continuous Gamma



Discrete Gamma



Choices?

To model Among-Site Rate Variation (ASRV) in practice, researchers must make two critical decisions:

1. What value of k to use?

- The historical default ($k = 4$).
- Higher resolution ($k = 8, 16, \ldots$).
- *Trade-off:* Precision vs. Computational Cost.

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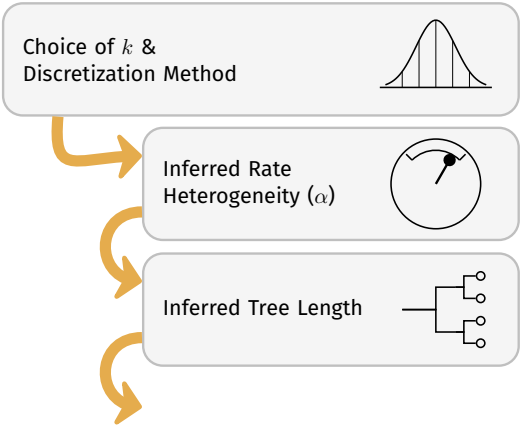
Different software packages use different "invisible" defaults!

Core Issue with ASRV

Using simulations we show that the choice of this “technical detail” can systematically distort two foundational parameters during tree inference: the **shape of rate distribution** (α) and thus the **tree length**.



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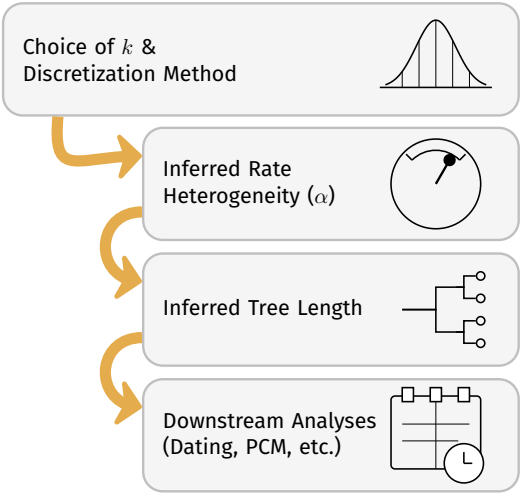

Simulation setup

10,000 sites, 8 Taxa

Generating Model
(Truth)
$k \in \{2, 4, 8\}$, Continuous

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Simulation setup

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Generating Model
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$k \in \{2, 4, 8\}$, Continuous

→

Tested across
3 Heterogeneity Levels:
$10\times, 100\times, 1000\times$
(Low → High)

→

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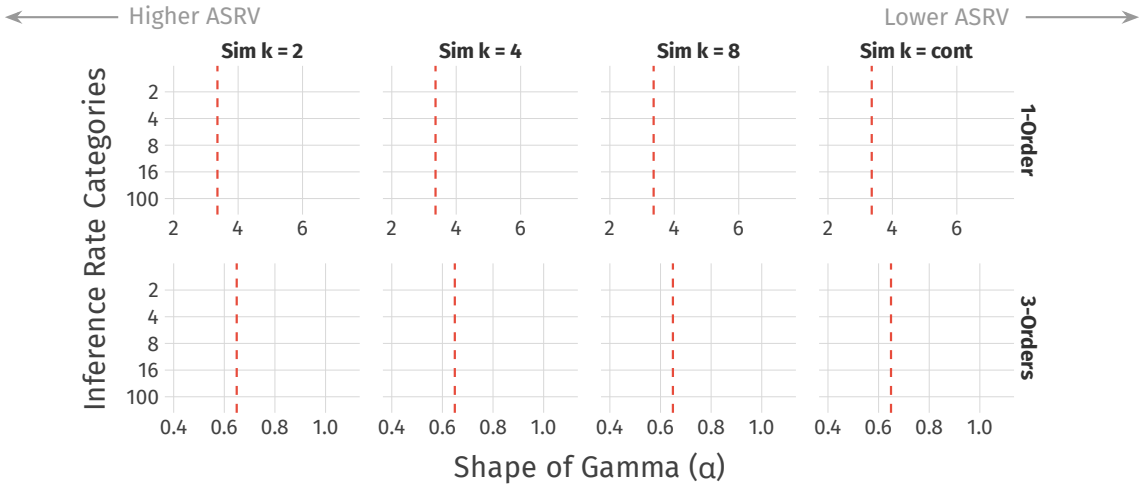
$k \in \{2, 4, 8, 16, 100\}$

Results: Impact of k mismatch on α



Khakurel et al. (in review)

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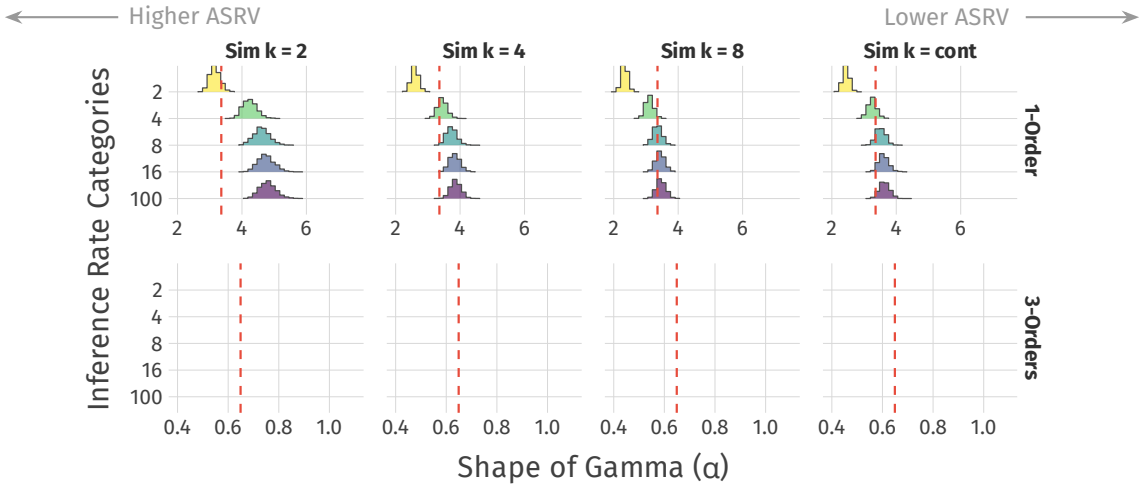

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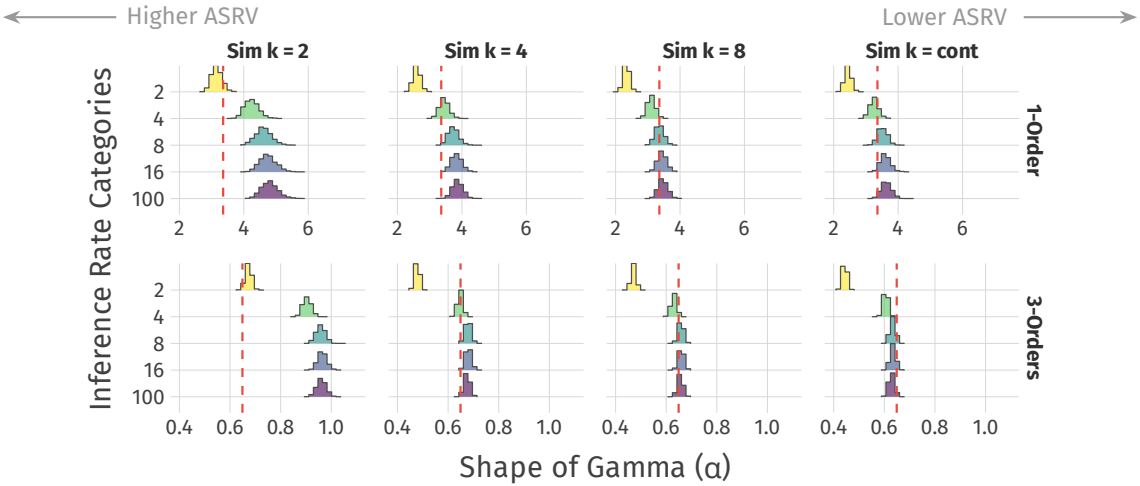

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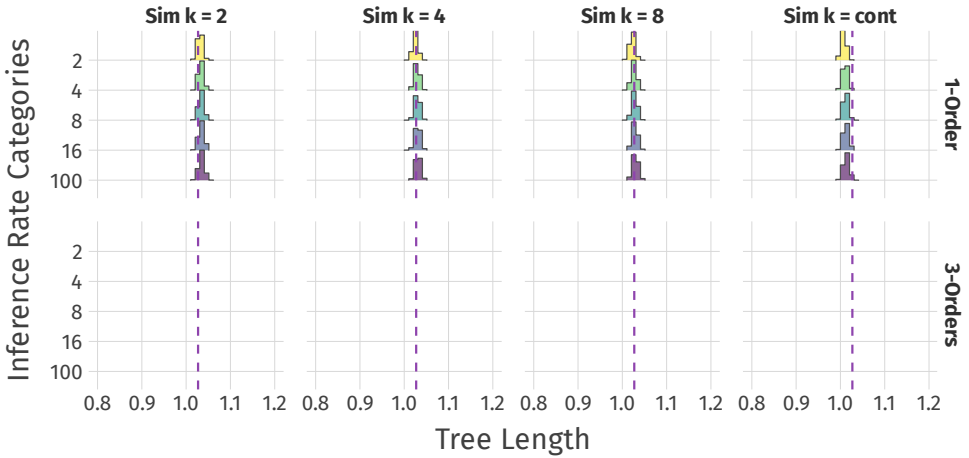
Results: Impact of k mismatch on the tree length



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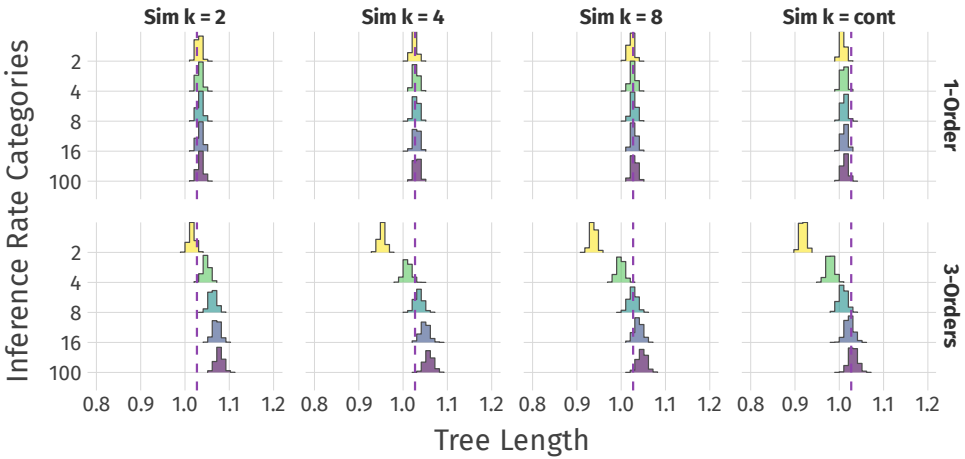

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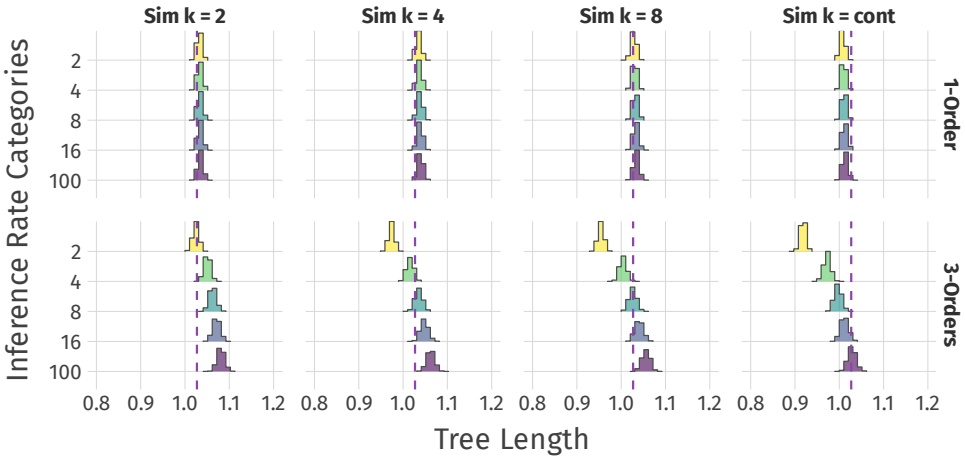
Median method: tree length



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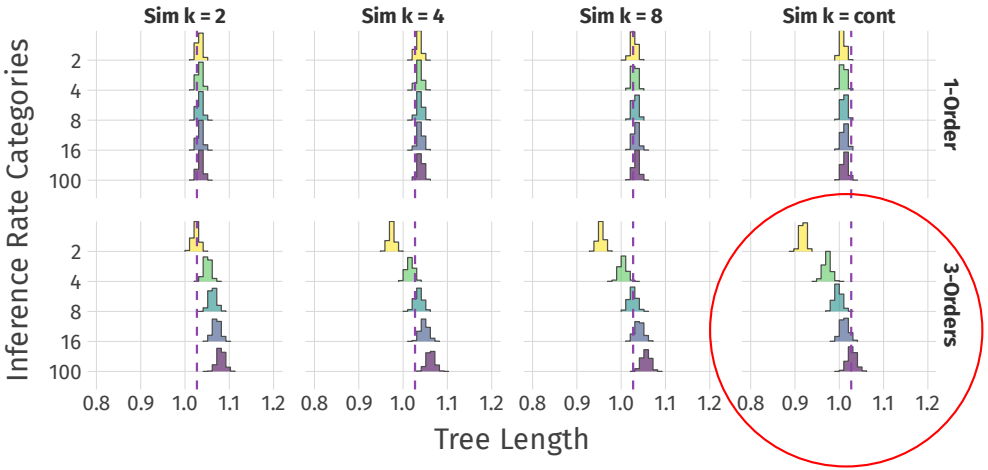

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Living in the Danger Zone?

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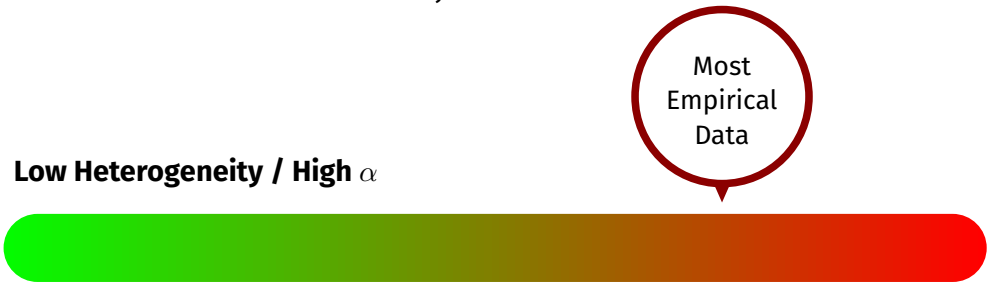
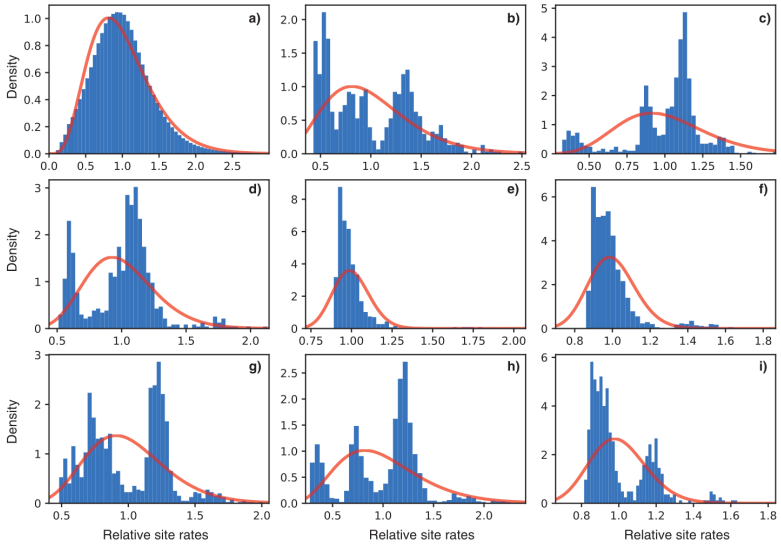


based on Buckley et al. (2001)



* rates > 3 (N = 53,061) are not shown in panel a)

Silvestro et al. (2024)

Recommendations

Warning

- **Avoid defaults** without testing.

  Different software use different discretization method and default k.

- **Caution** with high heterogeneity data:

  Strong rate variation + Wrong k = Biased Tree Lengths.

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Best Practices

- Report discretization method & k explicitly.
- Test sensitivity across multiple k values.
- Select high α (low rate variation) data subsets for dating.

Thank you!

Questions?

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