Lecture 1.2:
Substitution Models

Popular phylogenetic methods

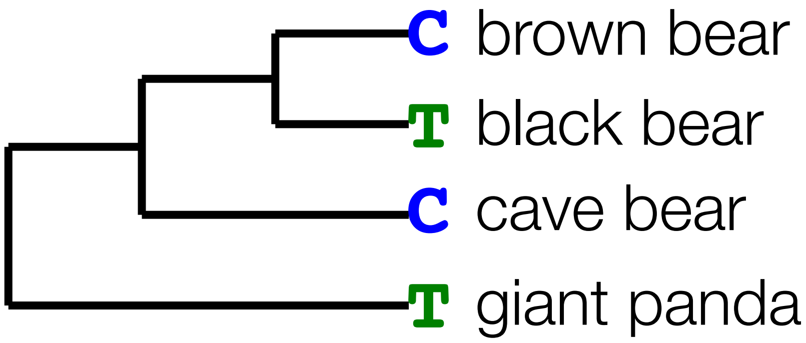
1. Maximum parsimony
2. Distance-based methods
3. Maximum likelihood
4. Bayesian inference

Model-based methods

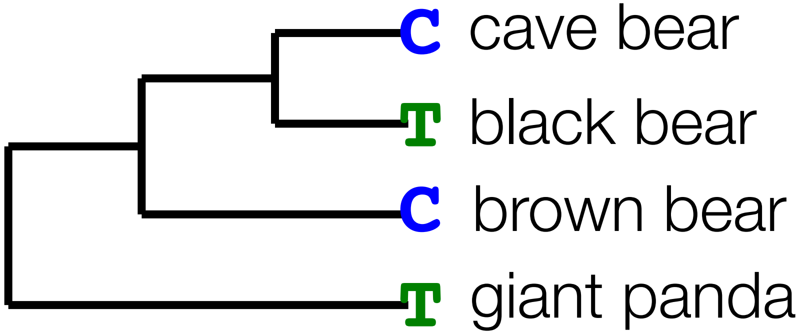
Maximum Parsimony

Maximum parsimony

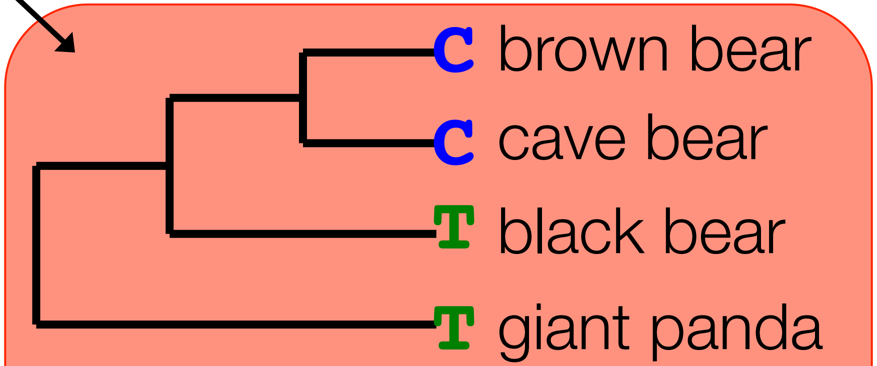
brown bear	CGTTAGTACACT
cave bear	CGATAGTTCACT
black bear	CGTTAGTTTACC
giant panda	CATTGGTTTACT



7 steps



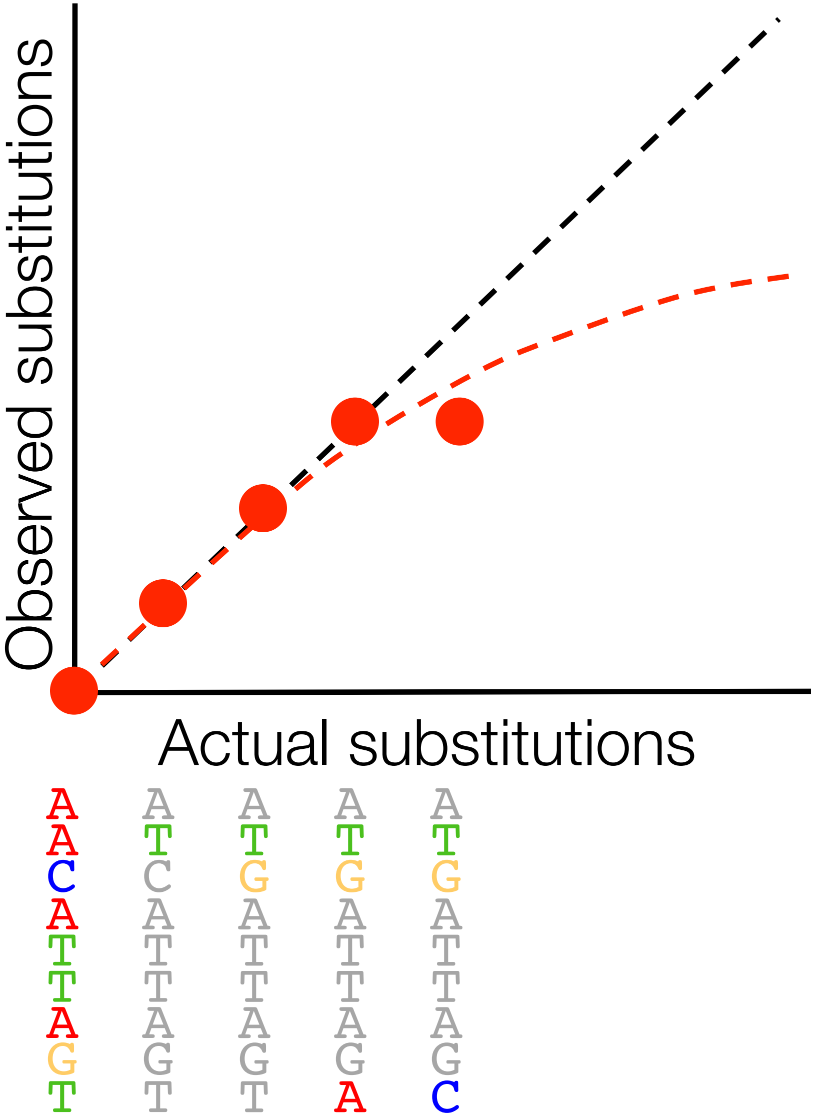
7 steps



6 steps

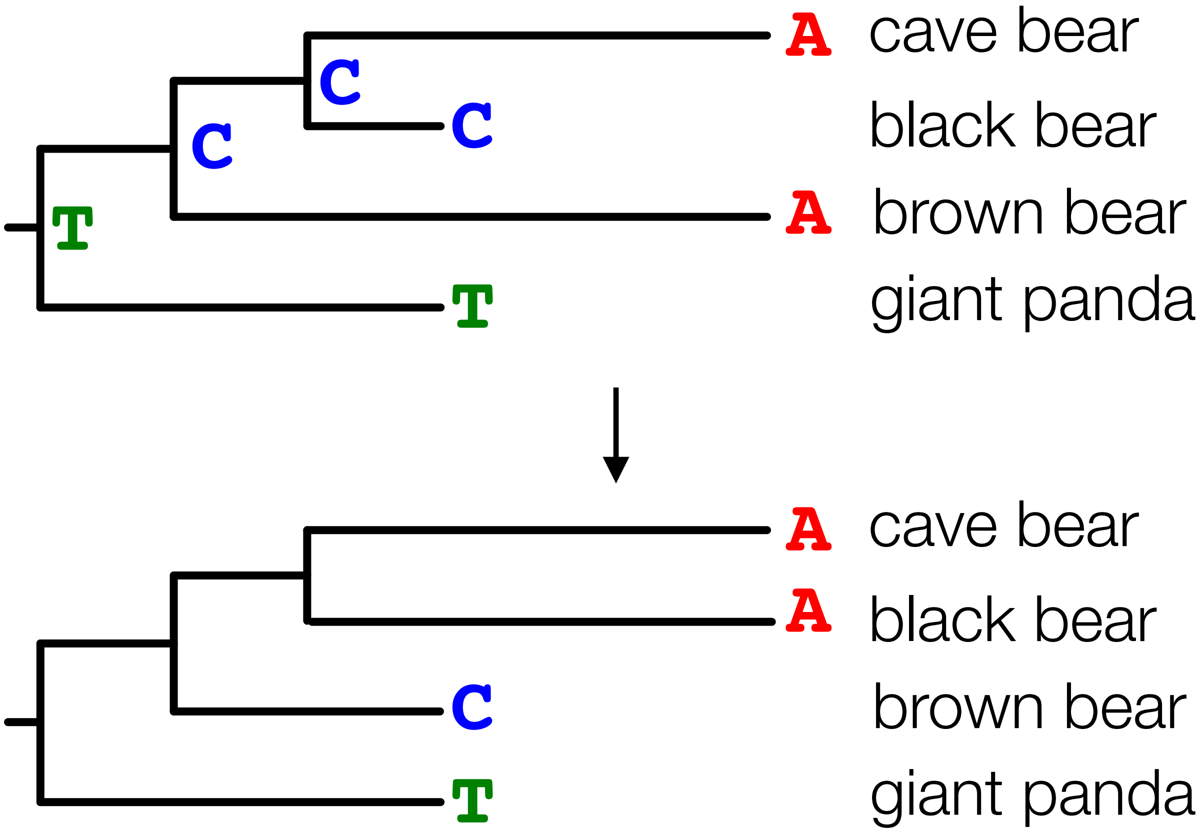
Maximum parsimony

- Identifies the tree topology that can explain the sequence data, using the smallest number of inferred substitution events
- Commonly used for morphological data
- Now rarely used for analysing genetic data
    - Cannot estimate evolutionary rates or timescales
    - Effects of multiple substitutions



- Maximum parsimony does not correct for multiple substitutions at the same site
- This leads to a problem known as ‘long-branch attraction’
    - Long branch = many substitutions
    - Similarities arise by chance
    - Long branches cluster together

Long-branch attraction



Weaknesses

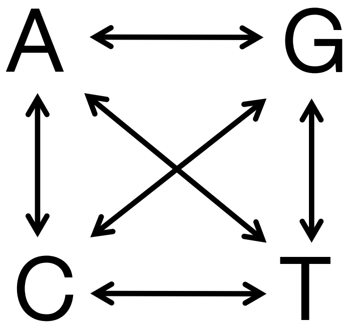
- Maximum parsimony does not correct for multiple substitutions at the same site
- This leads to a problem known as ‘long-branch attraction’
  - Long branches in the tree tend to group together

We can correct for multiple substitutions using **models** of the molecular evolutionary process

Evolutionary Models

Nucleotide substitution models

Rate Matrix



Base Frequencies

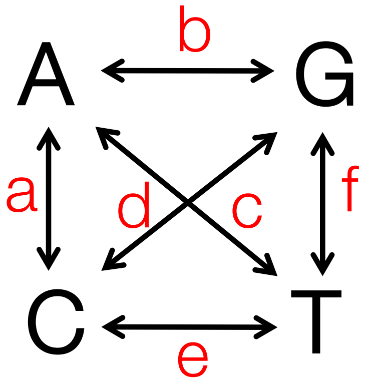
$\pi_A + \pi_C + \pi_G + \pi_T = 1$

Site Rates

+ I + G

Nucleotide substitution models

Rate Matrix



Base Frequencies

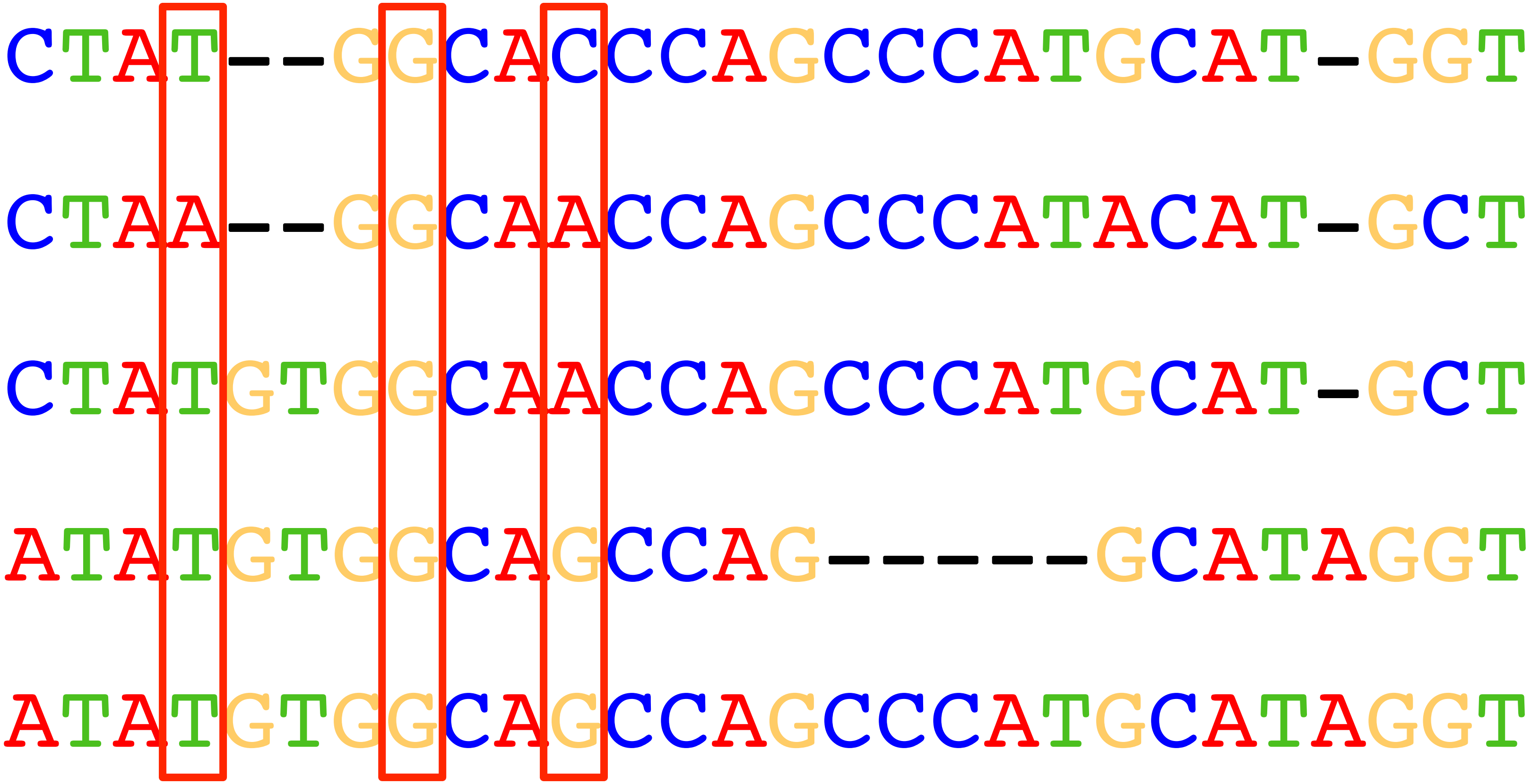
$$\pi_A + \pi_C + \pi_G + \pi_T = 1$$

Site Rates

+ I + G

JC	HKY	GTR	GTR+I+G
a=b=c=d=e=f	a=c=d=f, b=e	a, b, c, d, e, f	a, b, c, d, e, f
$\pi_A=\pi_C=\pi_G=\pi_T$	π_A, π_C, π_G, π_T	π_A, π_C, π_G, π_T	π_A, π_C, π_G, π_T
No I or G	No I or G	No I or G	I, G
0 free parameters	4 free parameters	8 free parameters	10 free parameters

Rate variation across sites

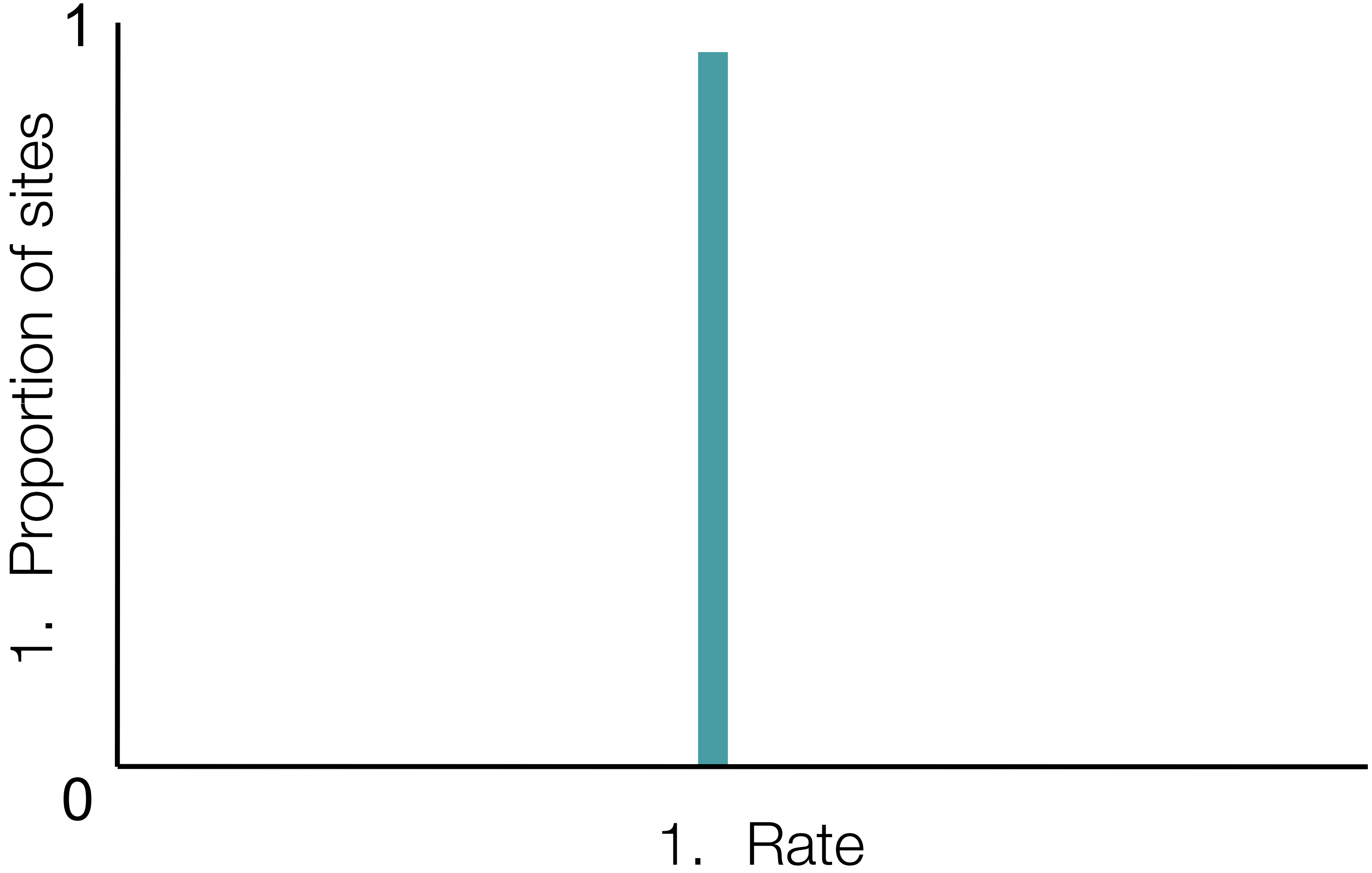


```
CTAT--GGCACCCAGCCCATGCAT-GGT
CTAA--GGCAACCAGCCCATACAT-GCT
CTATGTGGCAACCAGCCCATGCAT-GCT
ATATGTGGCAGCCAG-----GCATAGGT
ATATGTGGCAGCCAGCCCATGCATAGGT
```

Medium Slow Fast

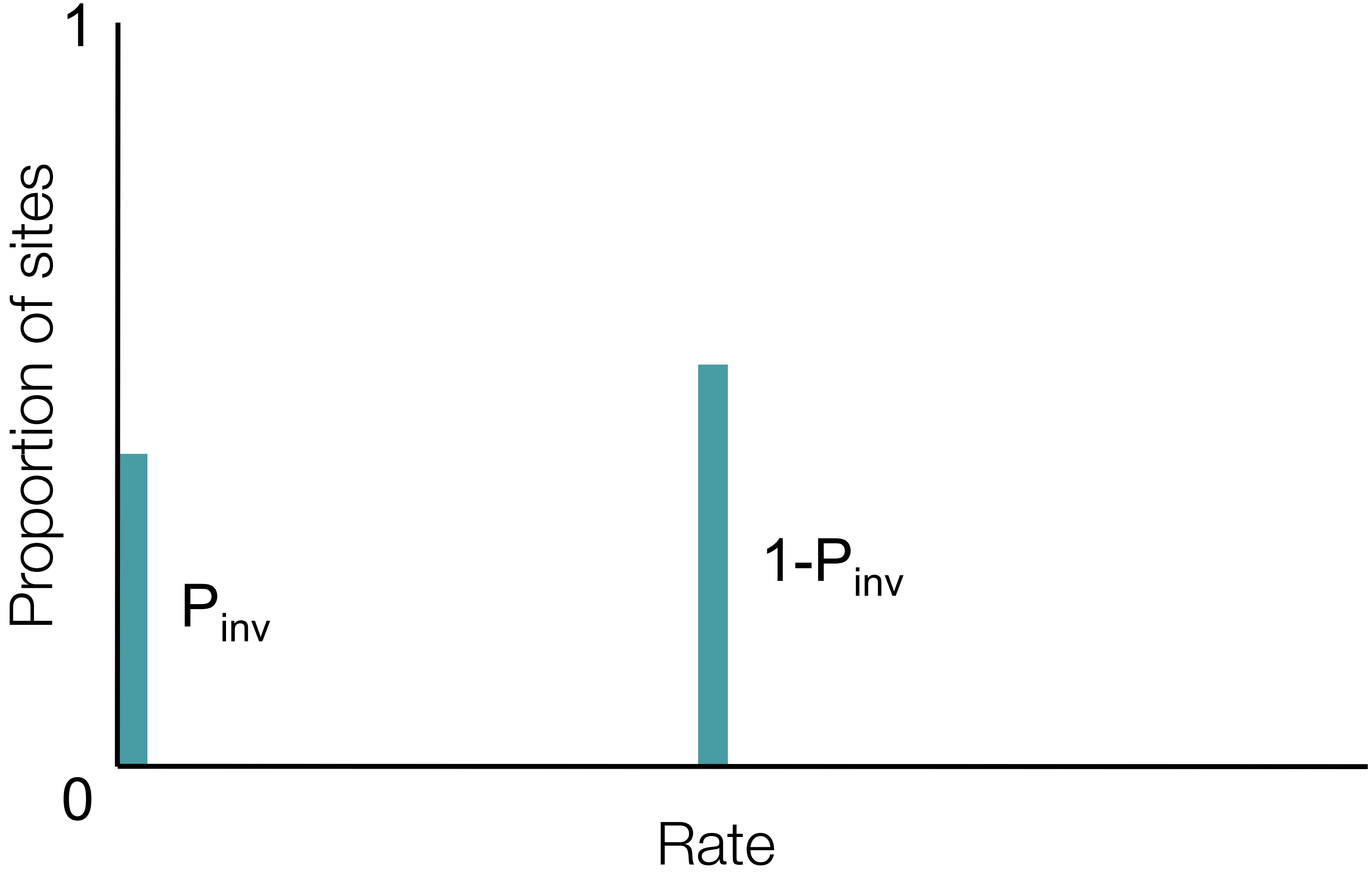
1. Rate variation among sites

1. Equal rates among sites (e.g., **JC, GTR, HKY** models)



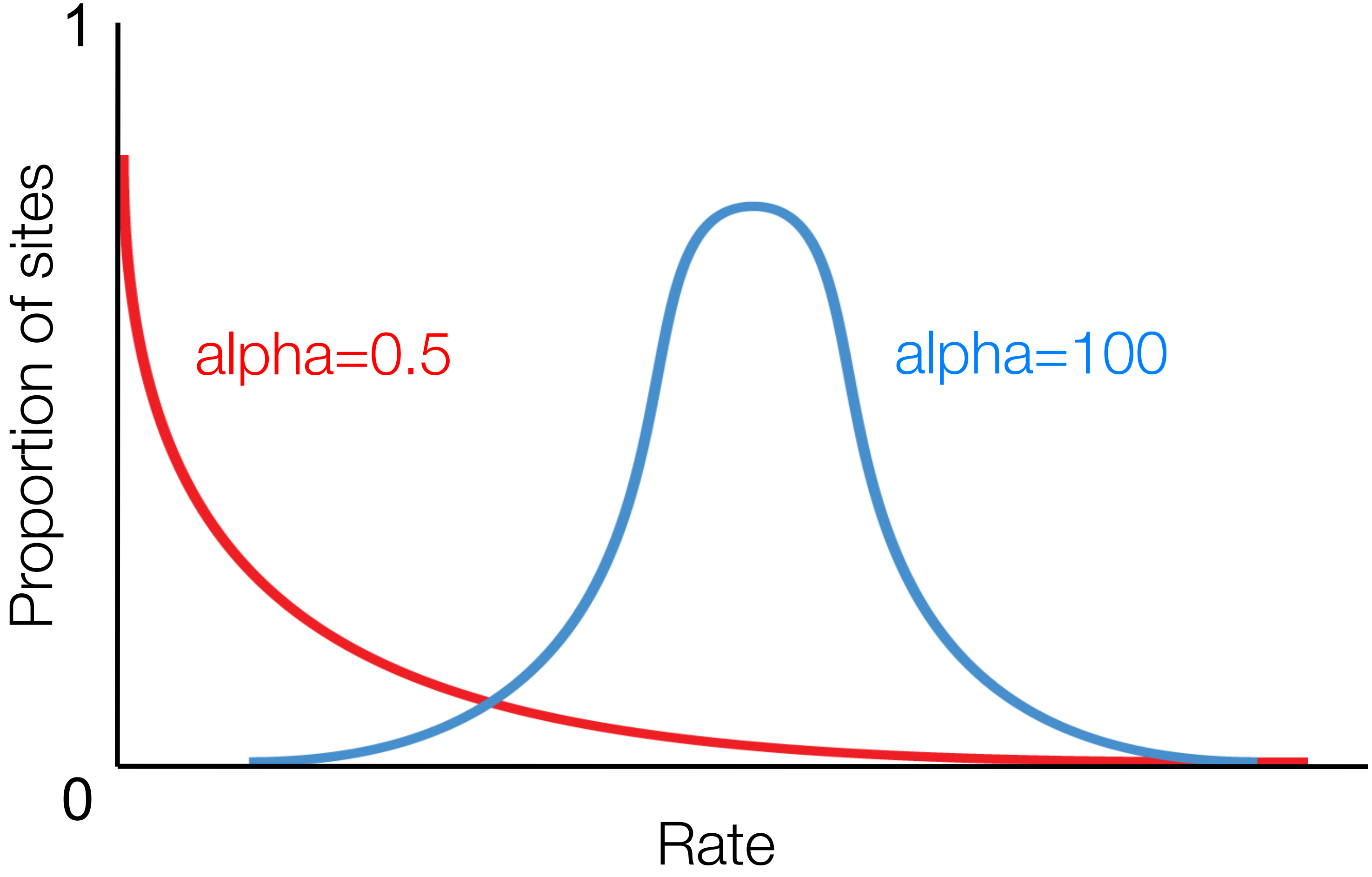
Rate variation among sites

- Proportion of invariable sites (e.g., JC+I, GTR+I, HKY+I models)



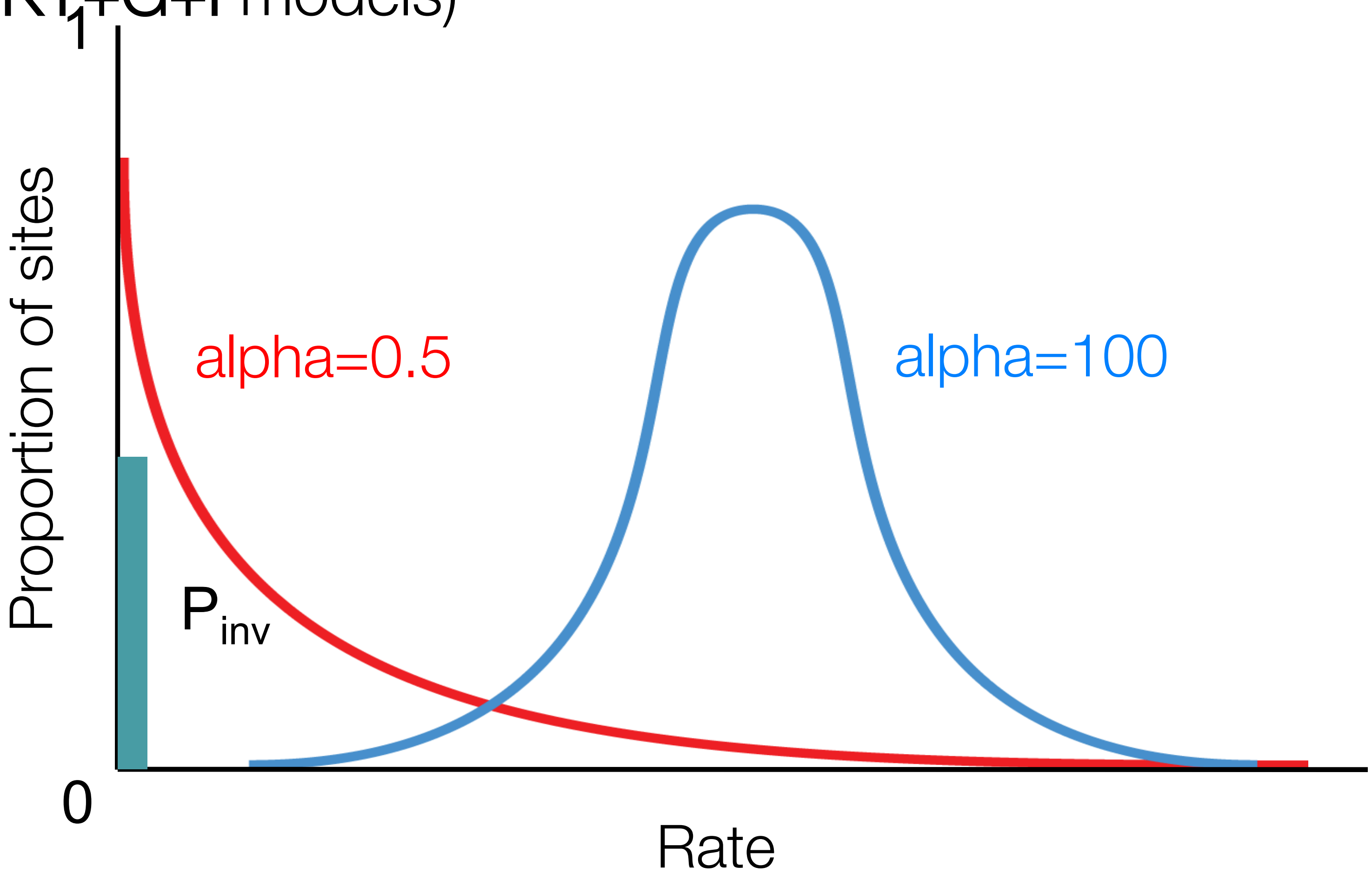
Rate variation among sites

- Gamma-distributed rate variation among sites (e.g., JC+G, GTR+G, HKY+G models)



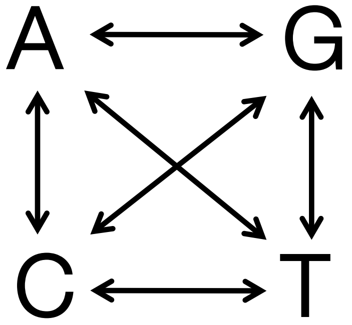
Rate variation among sites

- Gamma-distributed rate variation among sites and a proportion of invariable sites (e.g., JC+G+I, GTR+G+I, HKY+G+I models)



Nucleotide substitution models

Rate Matrix



Base Frequencies

$\pi_A + \pi_C + \pi_G + \pi_T = 1$

Site Rates

+ I + G

#Models

203 x 15 x 4 = 12,180

In phylogenetics, we typically consider a small subset of these

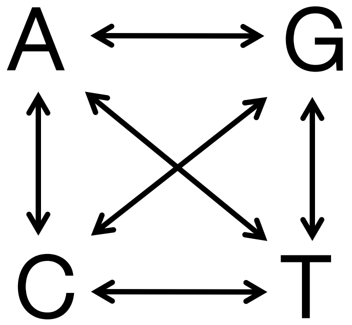
Amino acid substitution matrices

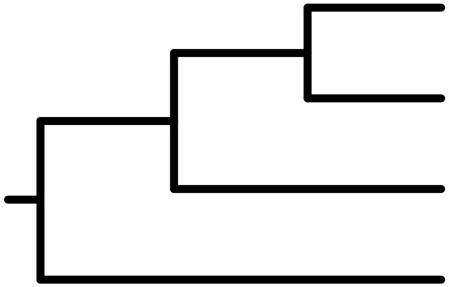
- 20x20 matrix of substitution probabilities
- Too many parameters to estimate
  - GTR model for DNA: 6 parameters
  - GTR model for proteins: 190 parameters
- Estimate substitution probabilities using a large data set
- Standard matrices:
  - PAM, BLOSUM, etc.

Fundamental assumptions

- Stationary
- Reversible
- Homogeneous
- Independent across sites

$\pi_A \quad \pi_C \quad \pi_G \quad \pi_T$





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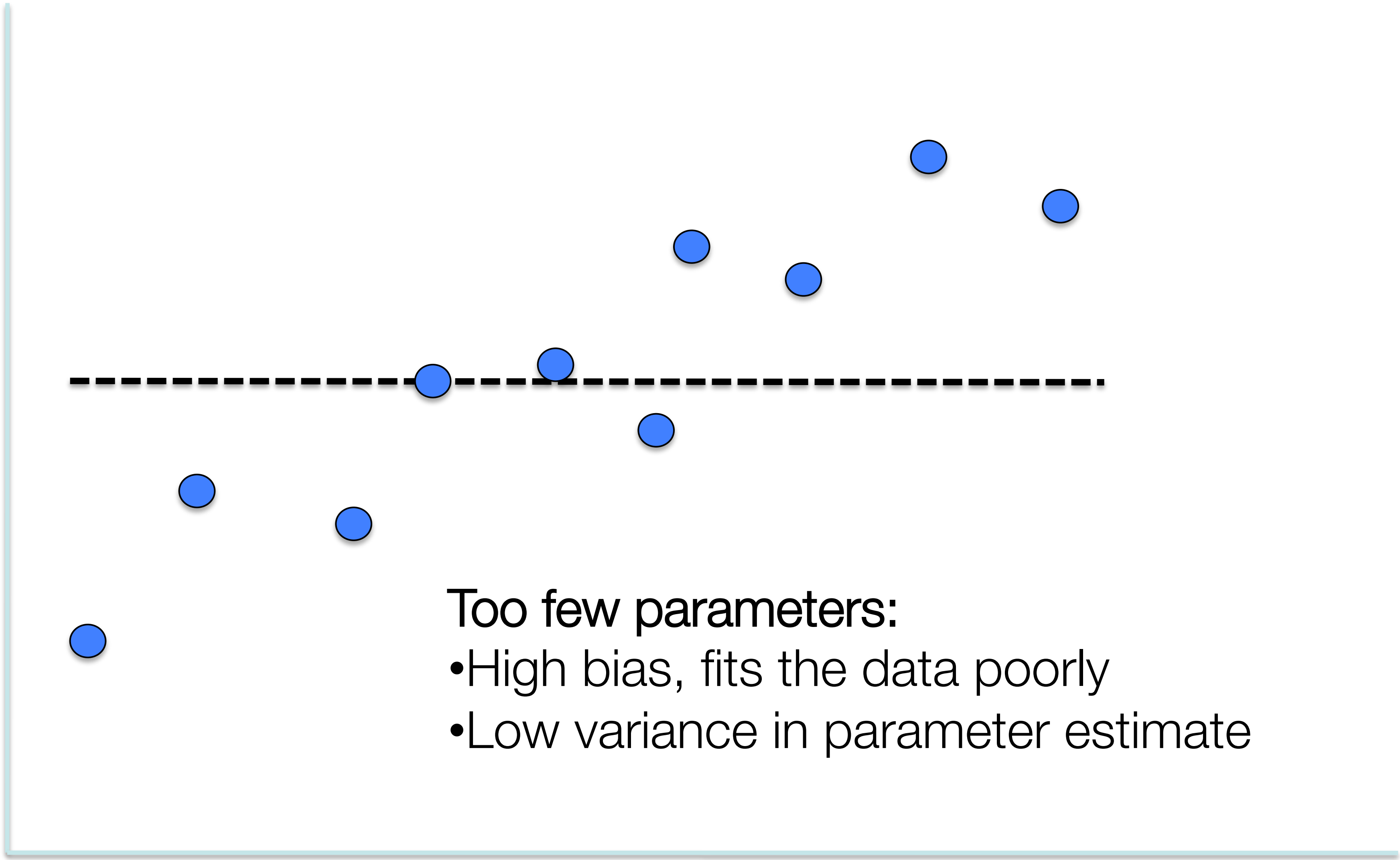
Model Selection

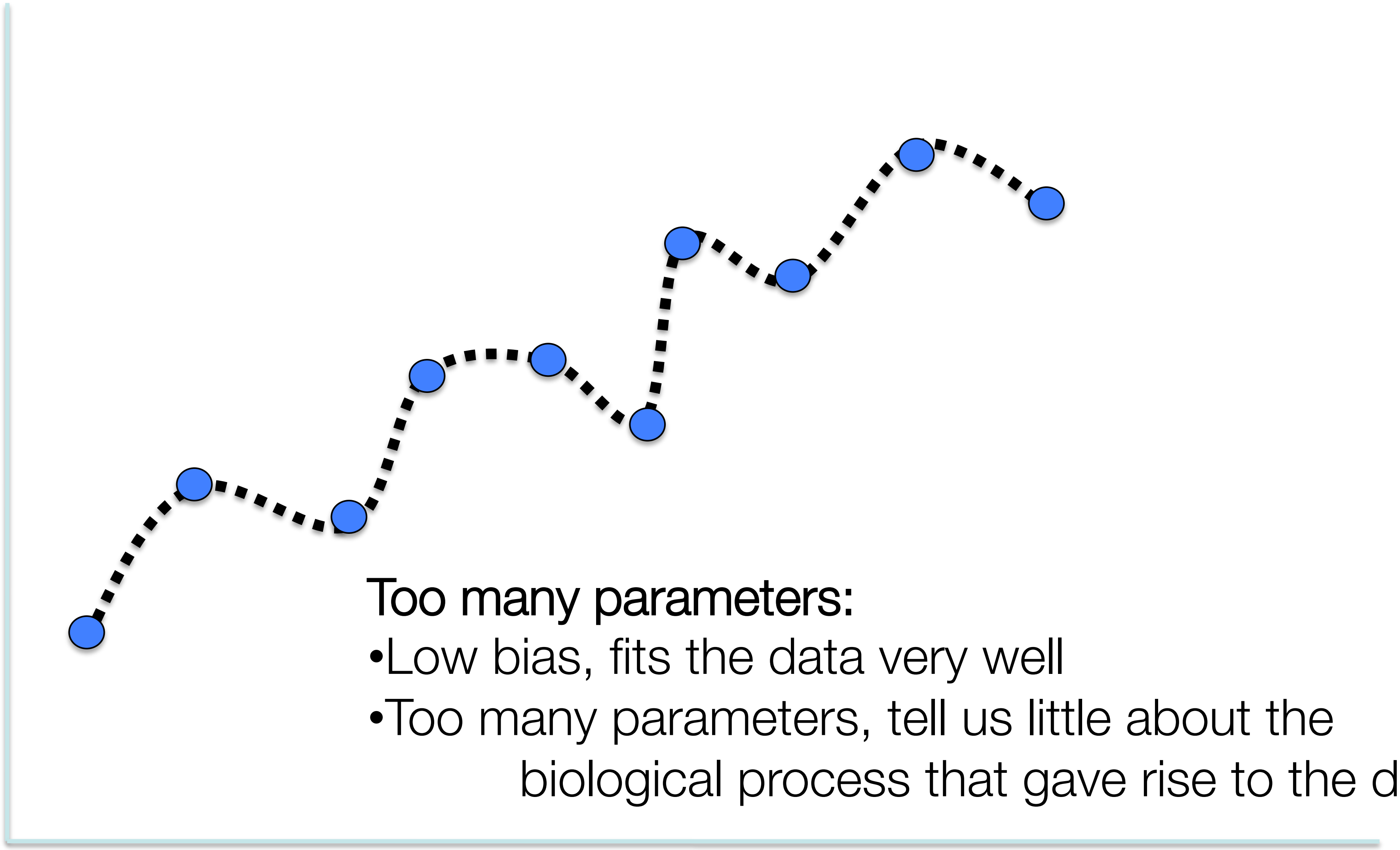
Model selection



Too few parameters:

- High bias, fits the data poorly
- Low variance in parameter estimate

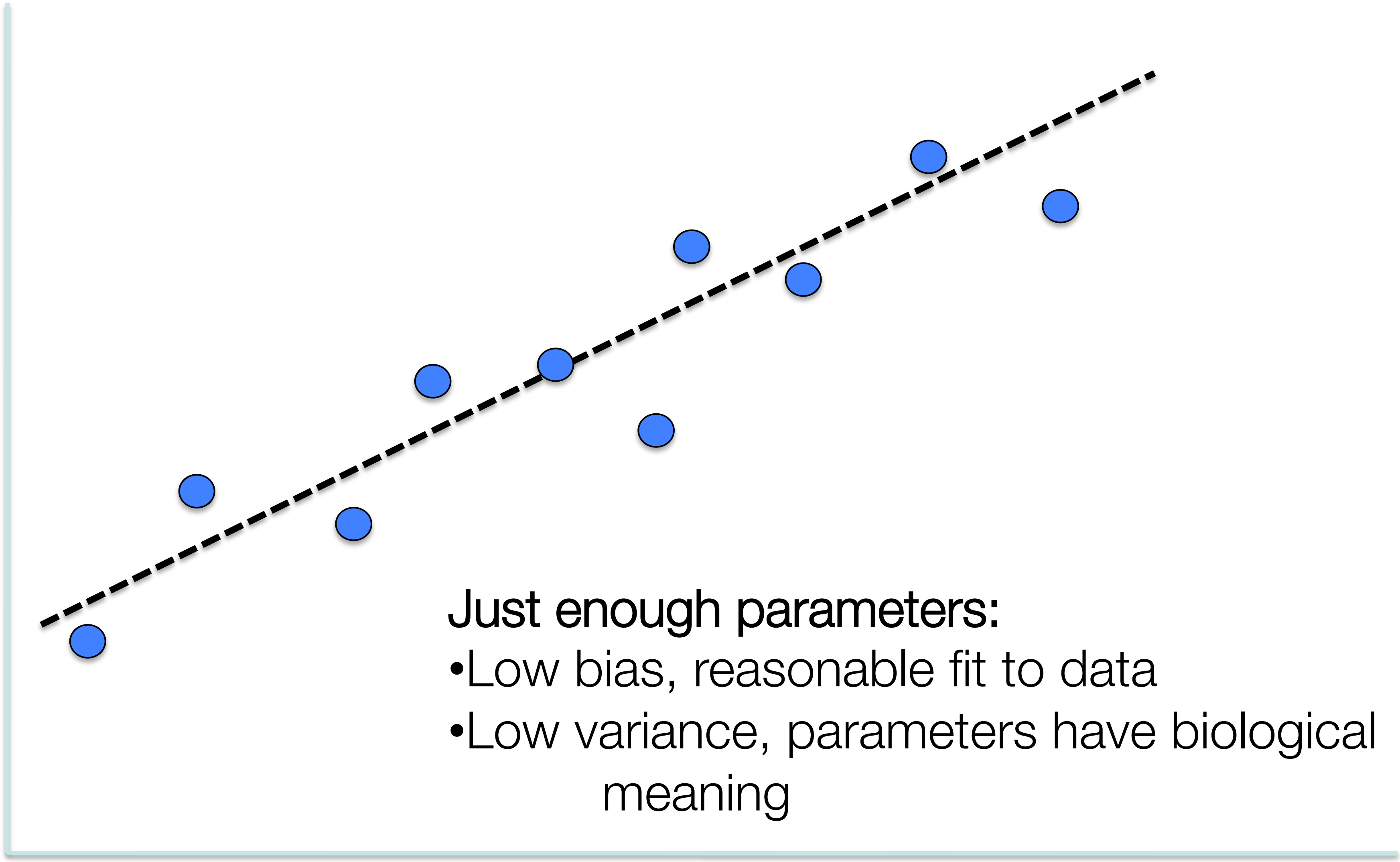
Model selection



Too many parameters:

- Low bias, fits the data very well
- Too many parameters, tell us little about the biological process that gave rise to the data

Model selection

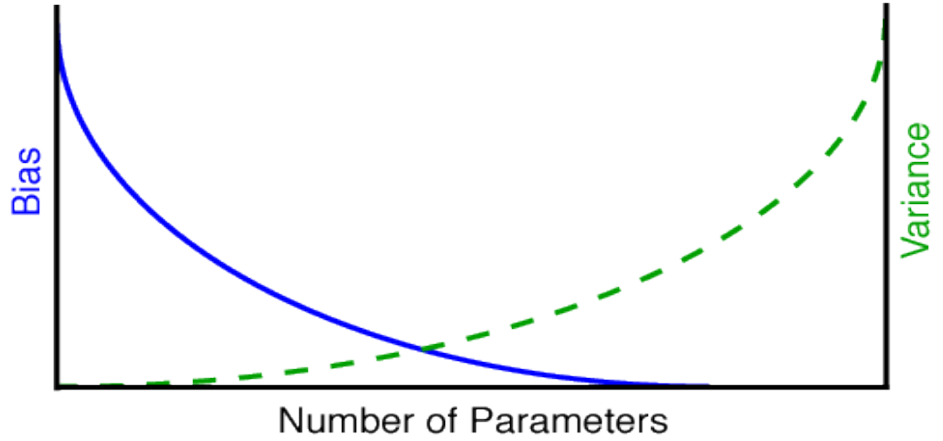


Just enough parameters:

- Low bias, reasonable fit to data
- Low variance, parameters have biological meaning

Model selection

- Adding more parameters *always* improves the fit of the model to the observed data
- More parameters → higher R^2 and better likelihood
- But it doesn’t necessarily improve the model!
- Goal is to find the best balance between bias and variance



Model selection

- Adding a parameter to the model:
  - Is the improvement in likelihood worth the cost of adding a parameter?
- Model selection methods
  - Likelihood-ratio test (LRT)
    Used to compare nested models
  - Akaike information criterion (AIC)
    AIC = -2ln(likelihood) + k
  - Bayesian information criterion (BIC)

Likelihood-ratio test

- **Likelihood ratio = 2(lnL_1 – lnL_0)**

  L_0 is the likelihood of the null model
  L_1 is the likelihood of the alternative model

- Used to compare nested models, such as:
  - HKY vs GTR substitution model
  - Strict clock vs unconstrained model

GTR

a, b, c, d, e, f

π_A, π_C, π_G, π_T

HKY

a=c=d=f, b=e

π_A, π_C, π_G, π_T

Likelihood-ratio test

- Test statistic is χ^2-distributed
  (d.f. = diff. in number of parameters)
- When multiple models are compared hierarchically, outcome can depend on order of tests
- χ^2 approximation
- Might be inappropriate when null model involves fixing a parameter at boundary of possible values
- Performs poorly when competing models are not nested

Akaike information criterion

- **AIC = -2ln*L* + 2*p***

  L is the likelihood under the model
  p is the number of parameters in the model

- Balances likelihood against number of parameters
- Prefer models with smaller AIC values
- Can be used to compare non-nested models, such as:
  - HKY+I vs GTR+G substitution model

Bayesian information criterion

- **BIC = -2ln*L* + *p*ln(*n*)**

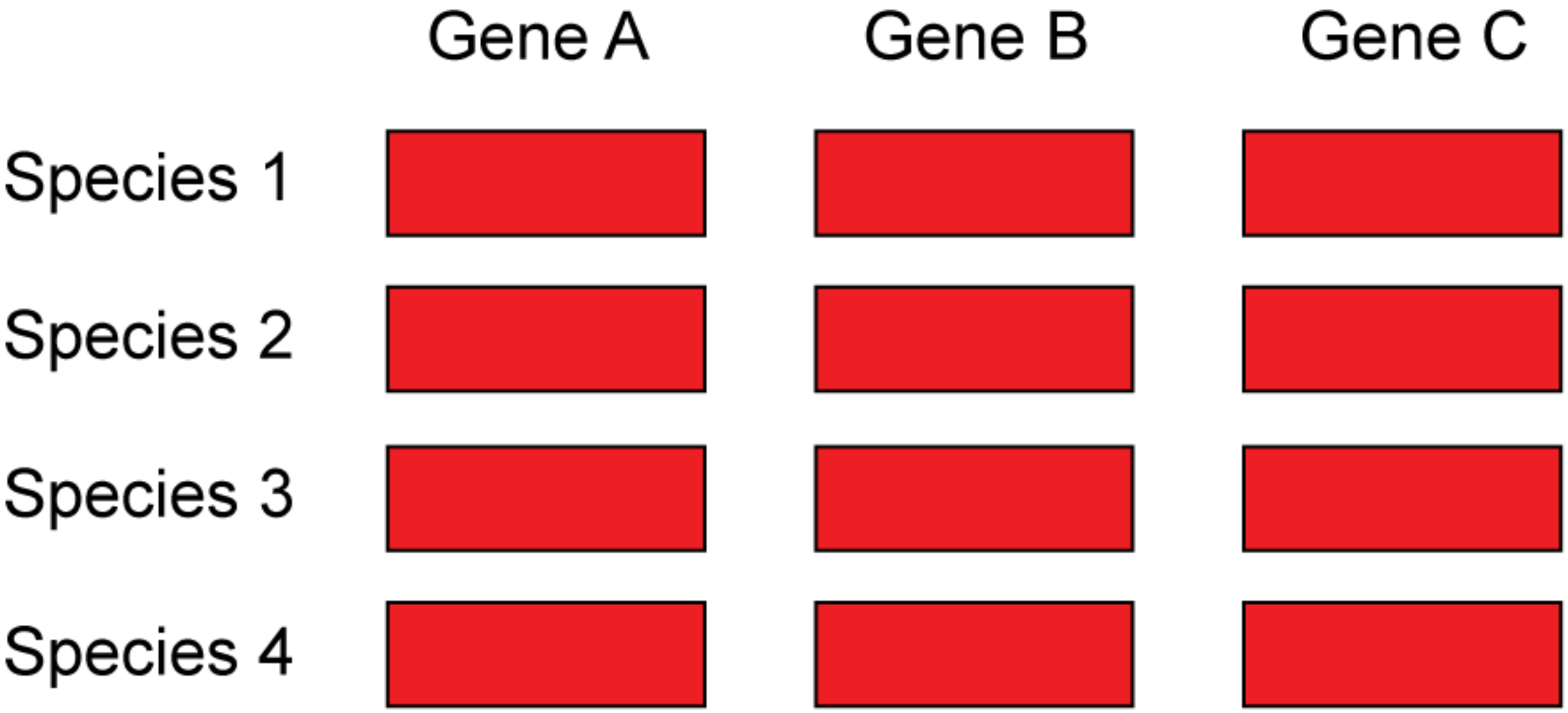
  L is the likelihood under the model
  p is the number of parameters in the model
  n is the sample size (sequence length)

- Stronger penalty on number of parameters
- Prefer models with smaller BIC values

Data Partitioning

Data partitioning

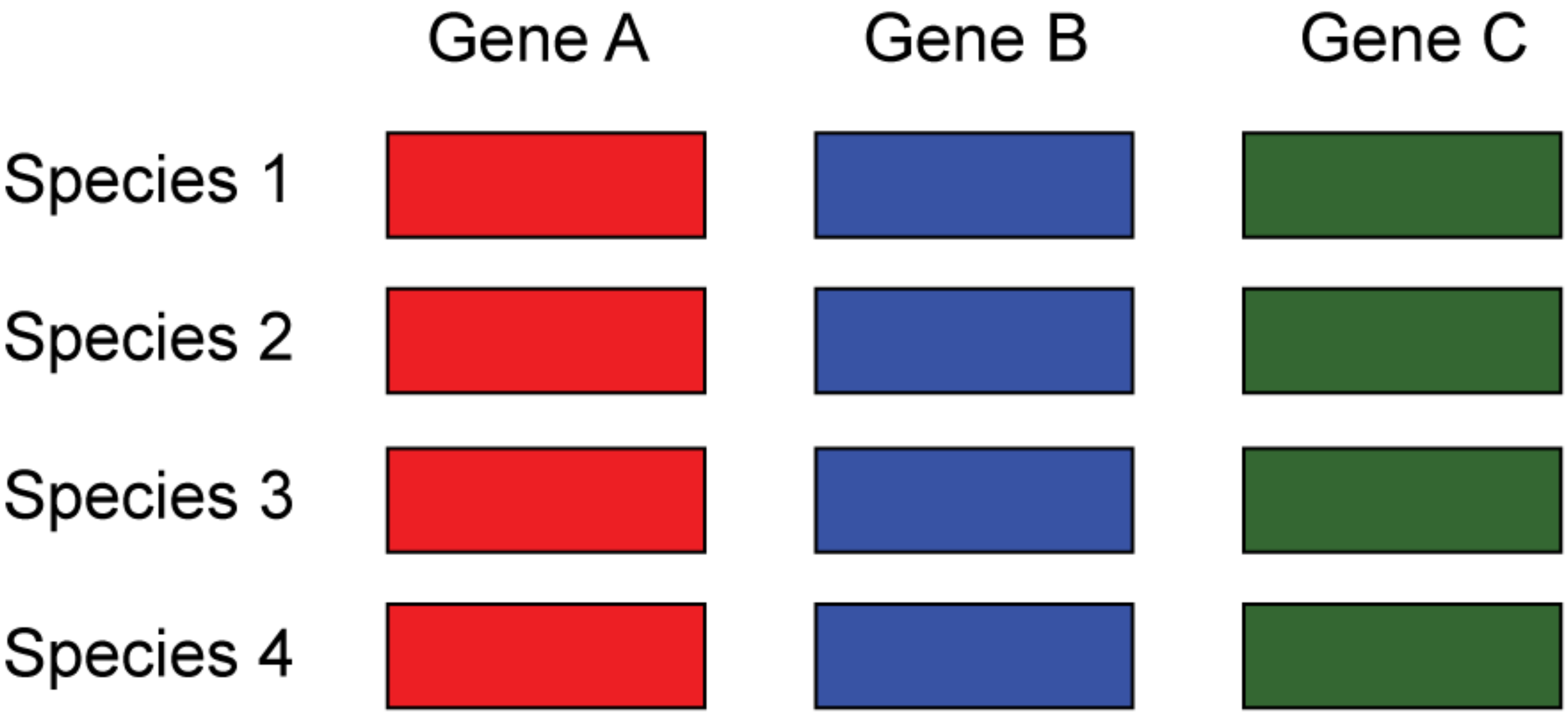
- Single substitution model across 3 genes



	Gene A	Gene B	Gene C
Species 1	🟥	🟥	🟥
Species 2	🟥	🟥	🟥
Species 3	🟥	🟥	🟥
Species 4	🟥	🟥	🟥

Data partitioning

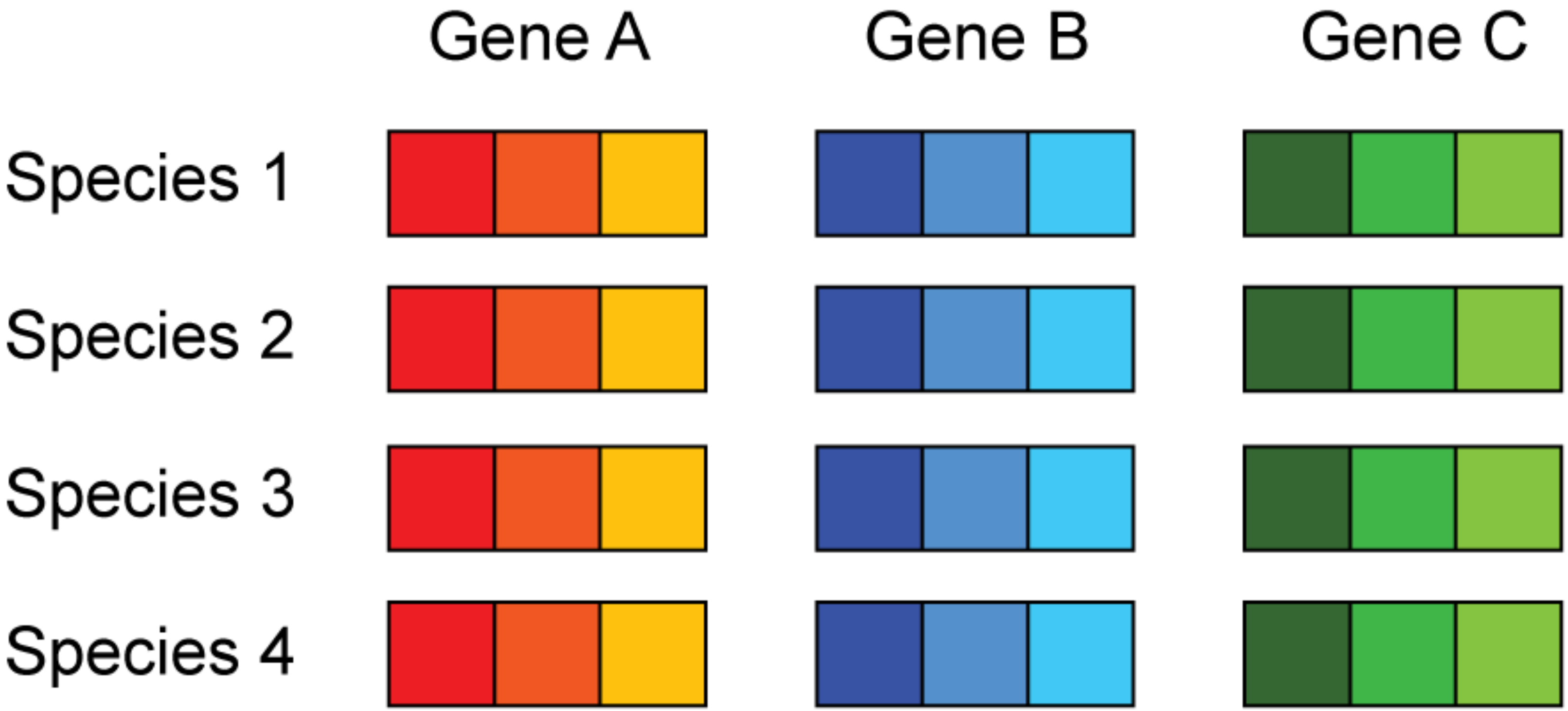
- Separate substitution model for each gene



	Gene A	Gene B	Gene C
Species 1	🟥	🟦	🟩
Species 2	🟥	🟦	🟩
Species 3	🟥	🟦	🟩
Species 4	🟥	🟦	🟩

Data partitioning

- Separate substitution model for each gene and codon position



Gene A

Gene B

Gene C

Species 1

Species 2

Species 3

Species 4