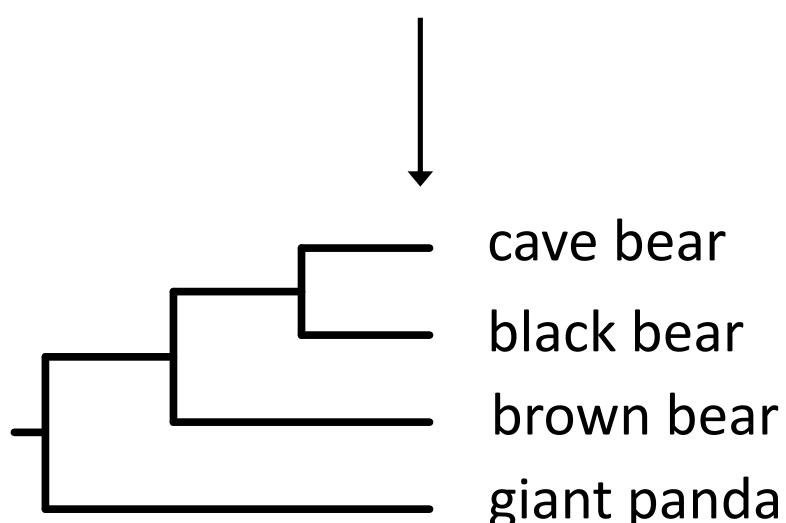
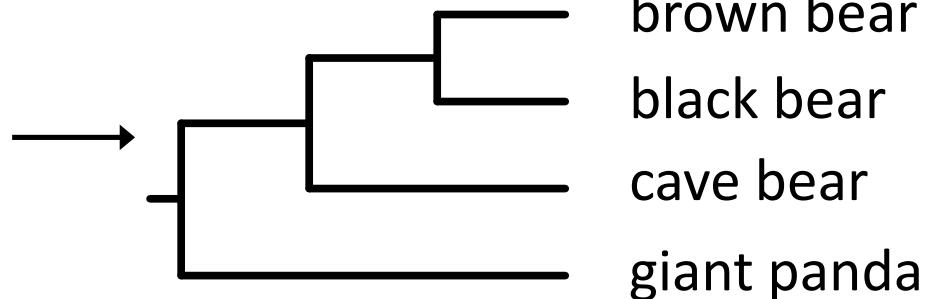

Lecture 1.4

Phylogenetic Methods

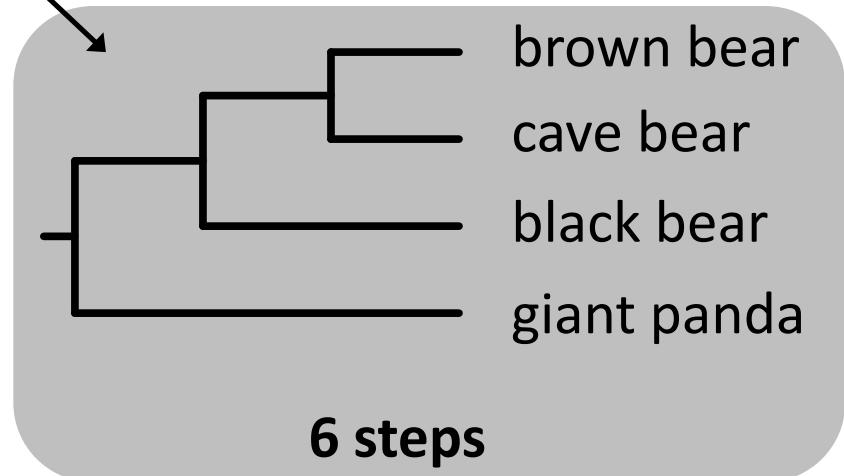
Maximum parsimony

brown bear	CGTTAGTACACT	
cave bear	CGATA GTTCACT	
black bear	CGTTAGTTTACC	
giant panda	CATTGGTTTACT	



7 steps

7 steps



6 steps

Popular phylogenetic methods

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

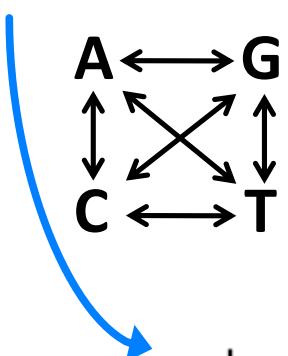
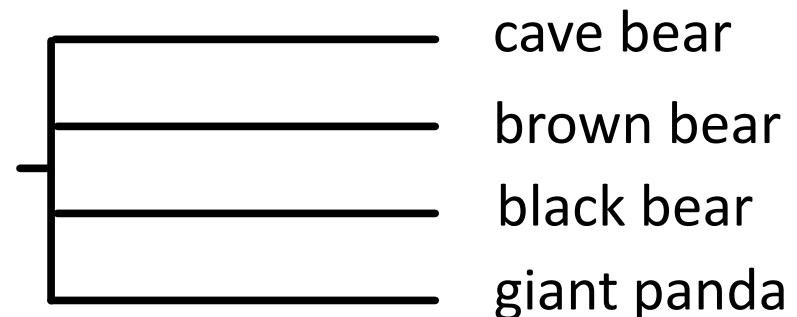
Model-based methods



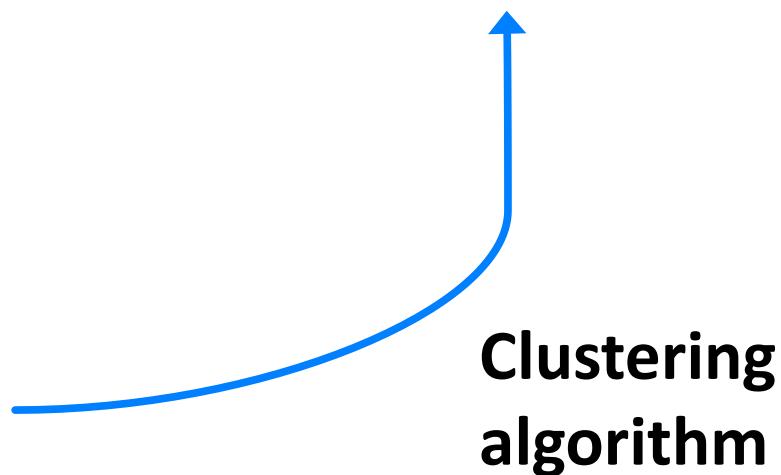
Distance-Based Methods

Neighbour joining

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

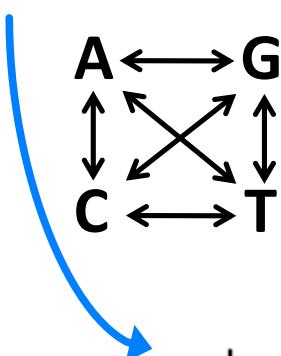
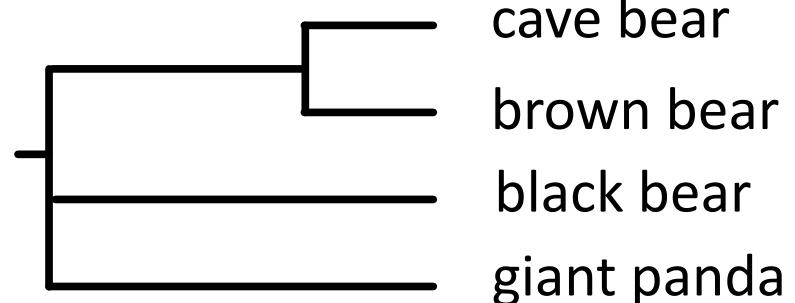


	brown bear	cave bear	black bear	giant panda
brown bear	-			
cave bear	.1	-		
black bear	.3	.3	-	
giant panda	.4	.5	.4	-



Neighbour joining

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

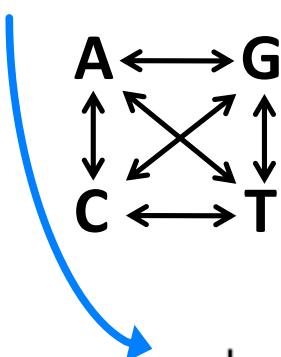
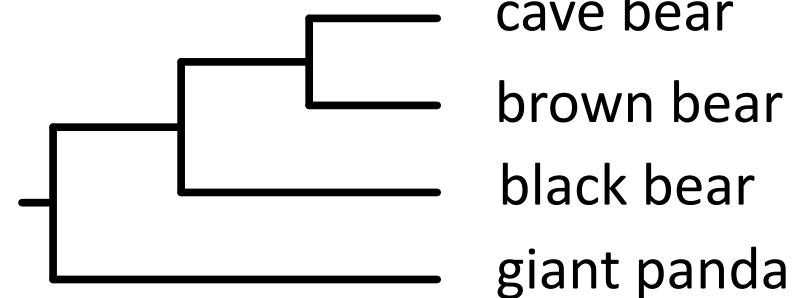


	brown bear	cave bear	black bear	giant panda
brown bear	-			
cave bear	.1	-		
black bear	.3	.3	-	
giant panda	.4	.5	.4	-

Clustering algorithm

Neighbour joining

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



	brown bear	cave bear	black bear	giant panda
brown bear	-			
cave bear	.1	-		
black bear	.3	.3	-	
giant panda	.4	.5	.4	-

Clustering
algorithm

Distance-based methods

- **Clustering algorithms**
 - Unweighted Pair Group Method with Arithmetic Mean (UPGMA)
 - Neighbour joining
- **Tree searching using optimality criteria**
 - Minimum evolution
 - Least-squares inference

Strengths and weaknesses

- **Strengths**
 - Very quick method
 - Deals with multiple substitutions and long-branch attraction
- **Weaknesses**
 - Does not use all information in alignment
 - Loss of information in pairwise comparisons
 - Unable to implement sophisticated evolutionary models

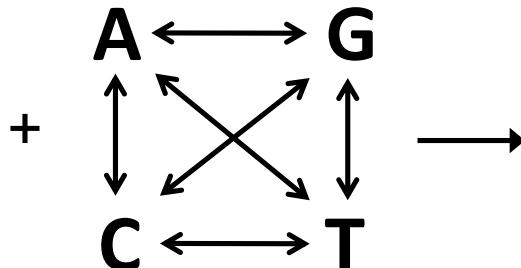
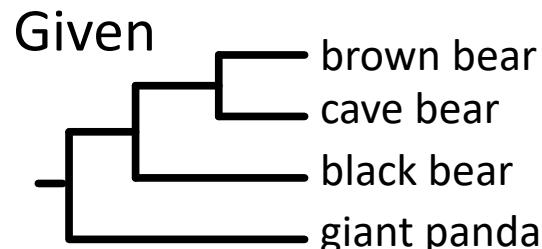
Maximum Likelihood

Maximum likelihood

Likelihood of hypothesis H =

$$P(D|H)$$

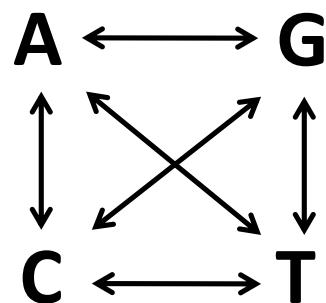
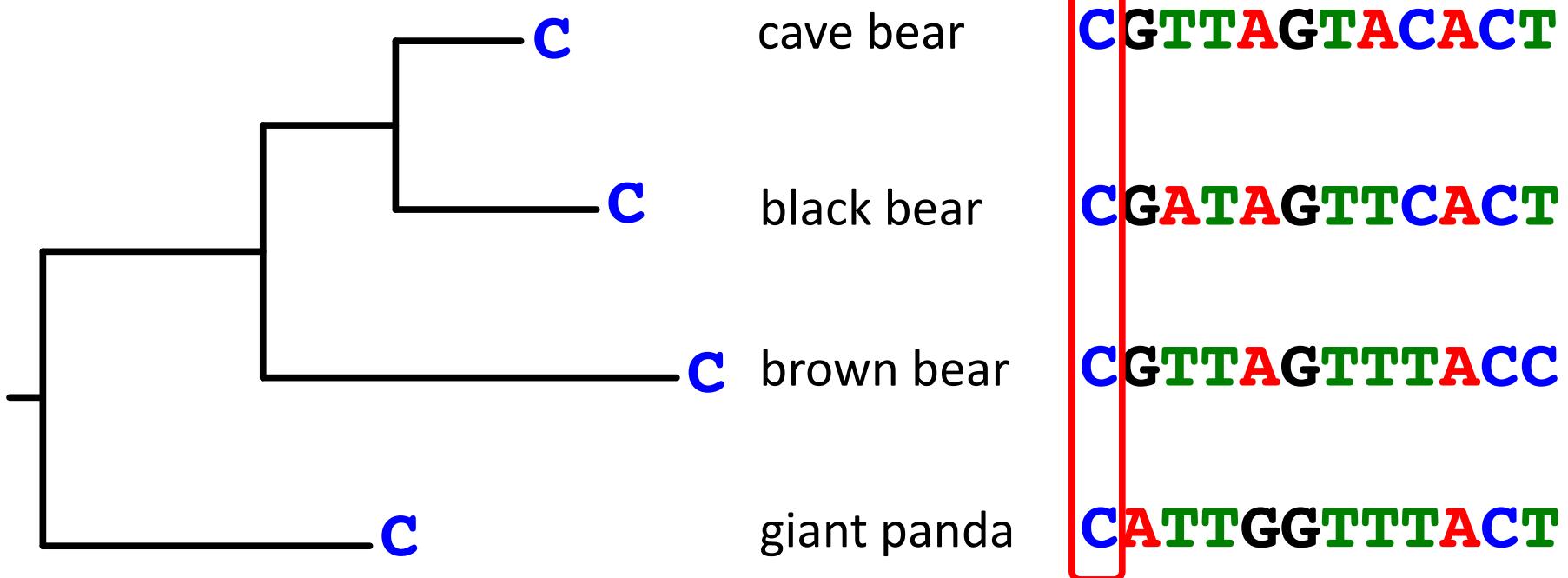
Probability of the data, given the hypothesis



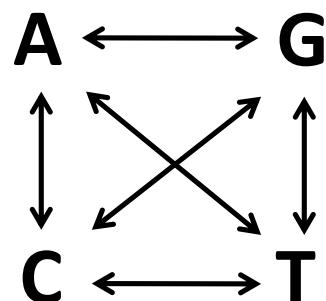
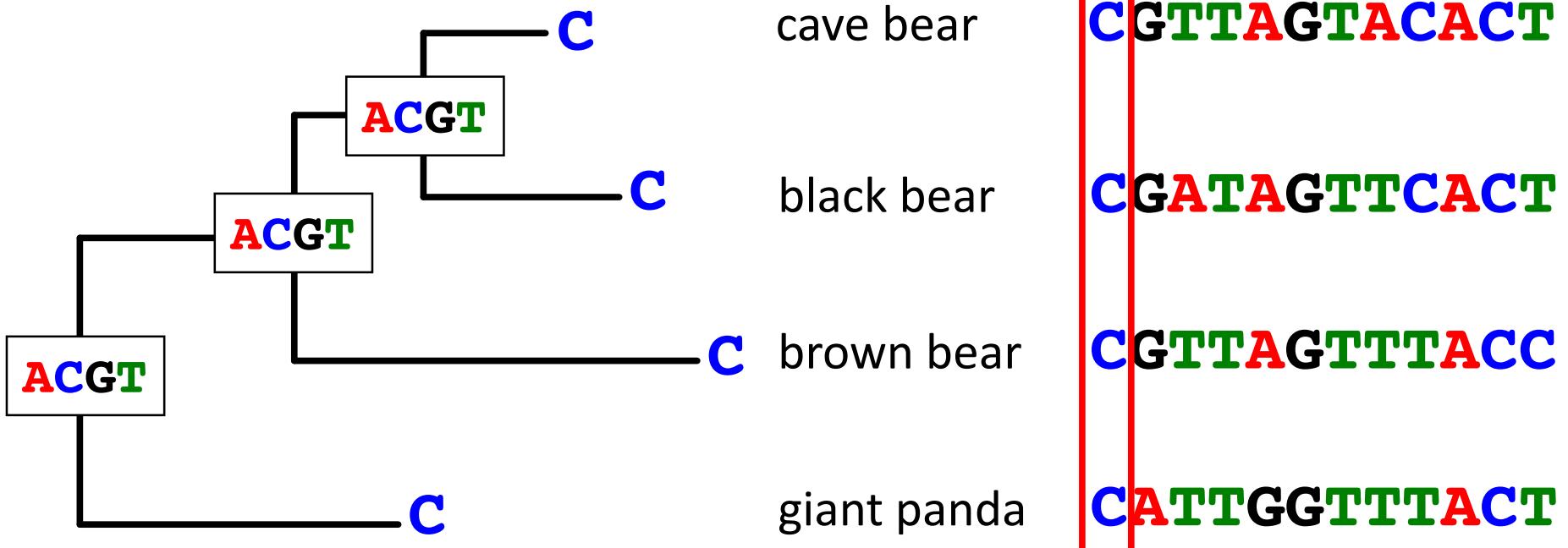
Probability of?

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

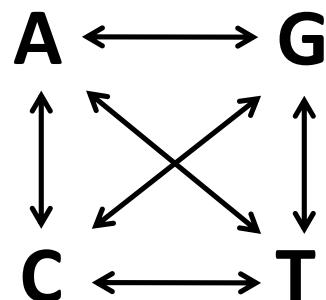
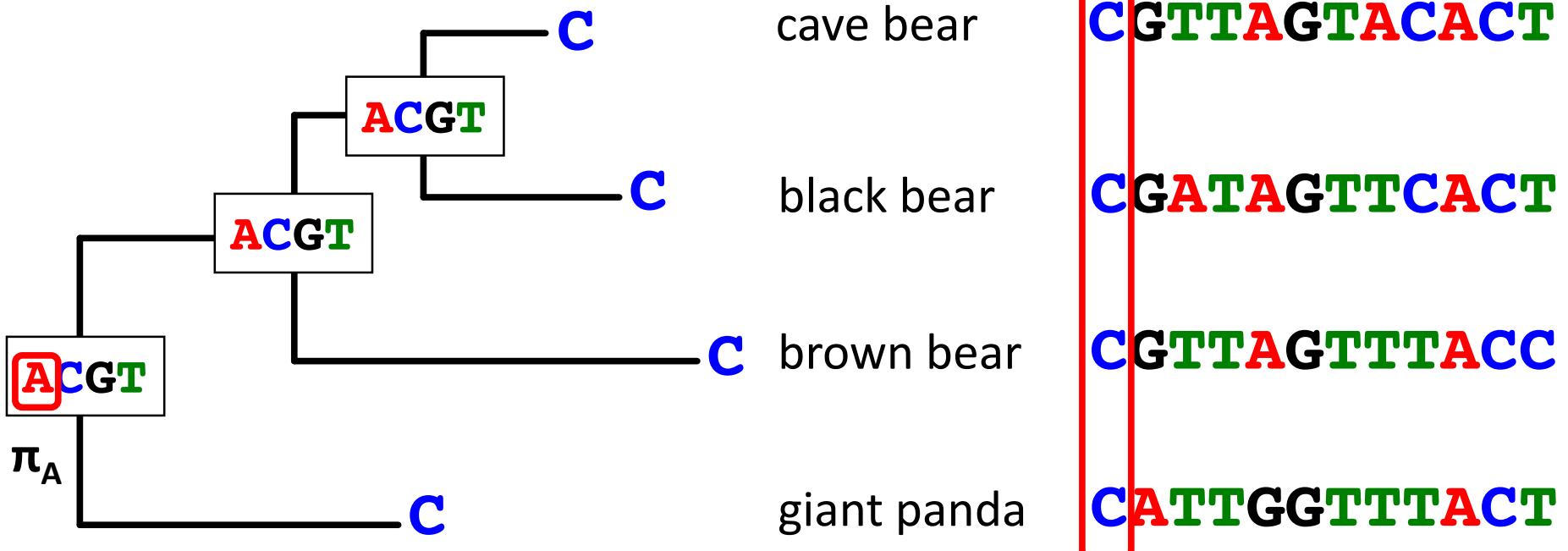
Maximum likelihood



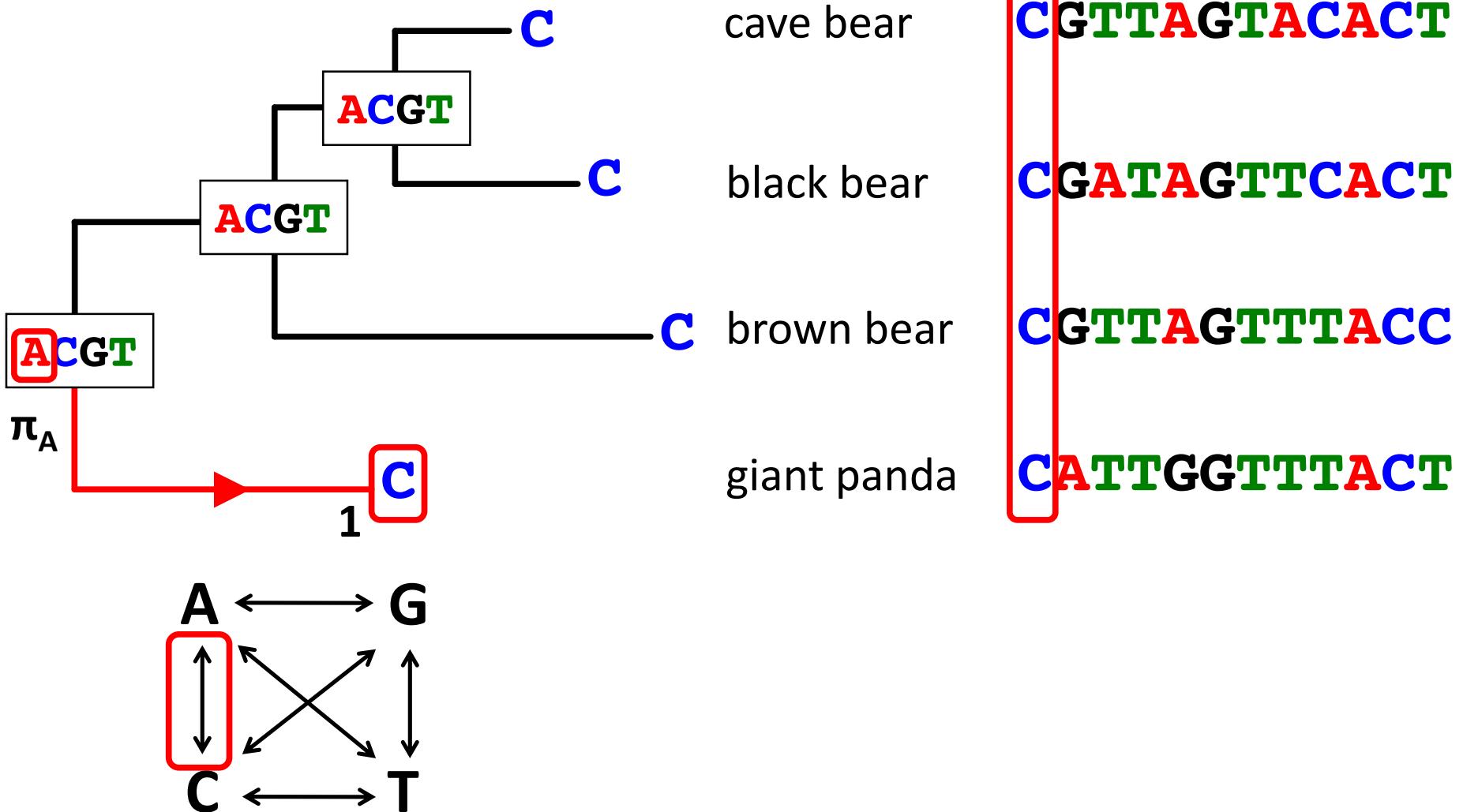
Maximum likelihood



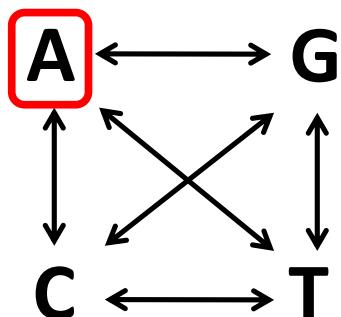
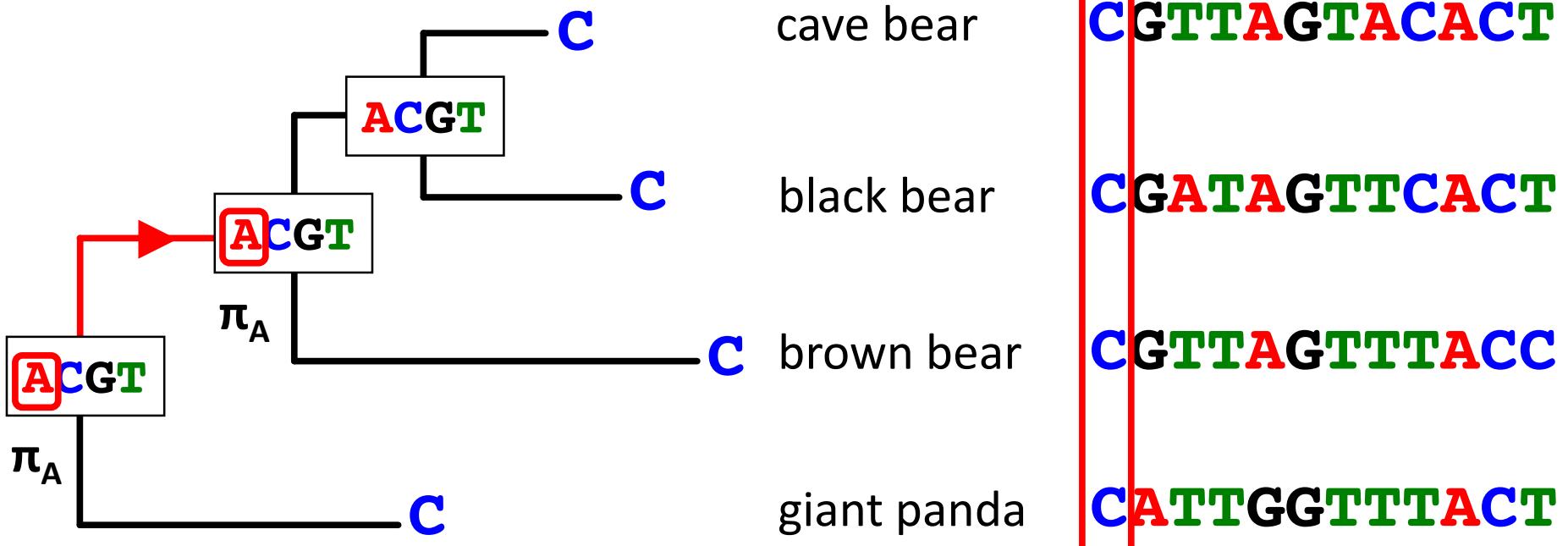
Maximum likelihood



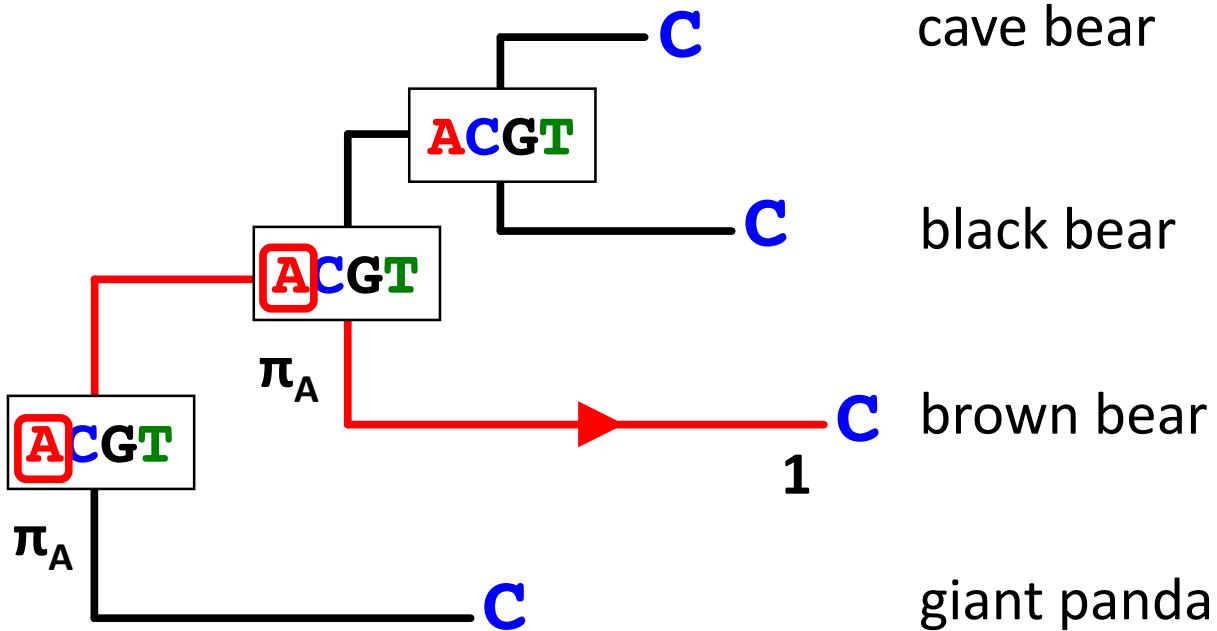
Maximum likelihood



Maximum likelihood

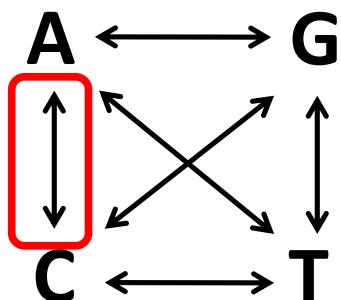


Maximum likelihood

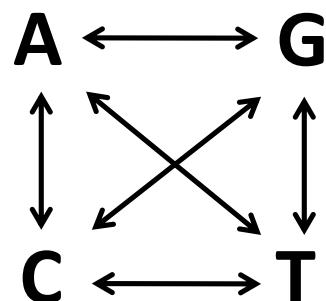
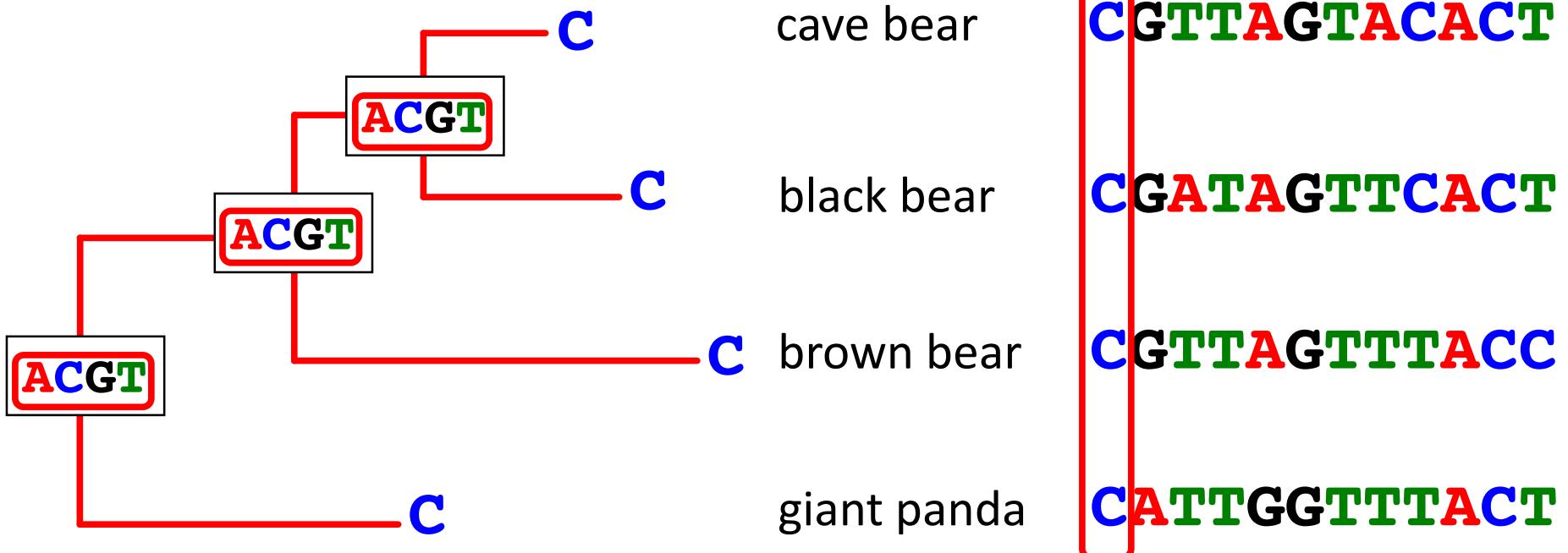


cave bear
black bear
brown bear
giant panda

CGTTAGTACACT
CGATAGTTCACT
CGTTAGTTACC
CATTGGTTACT

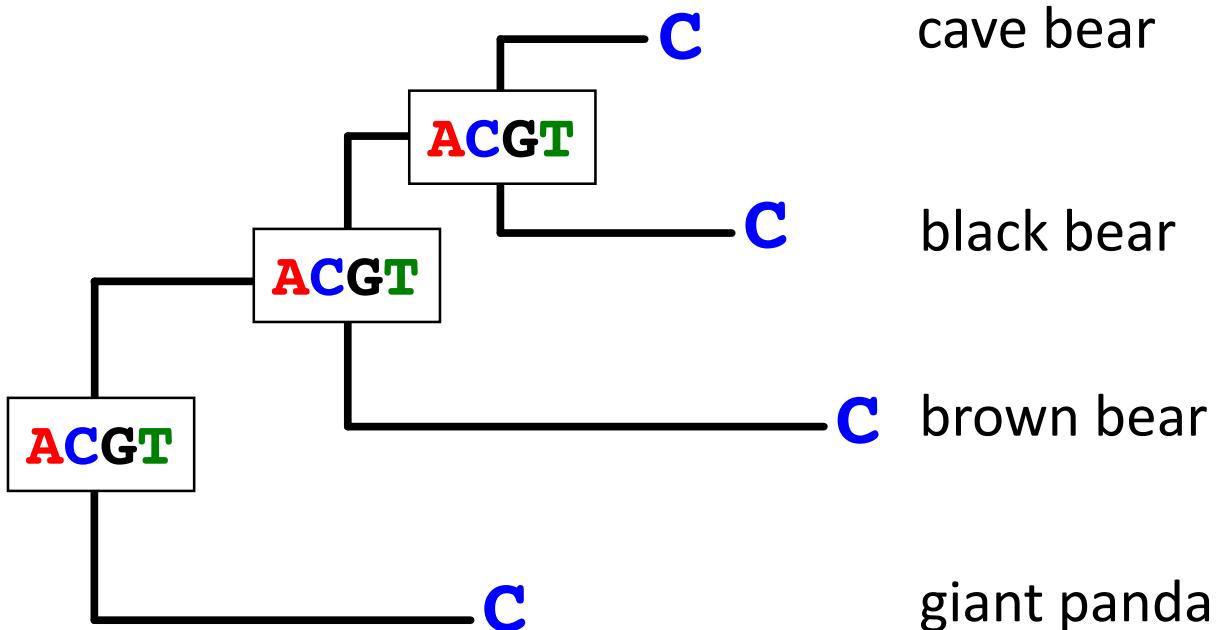


Maximum likelihood

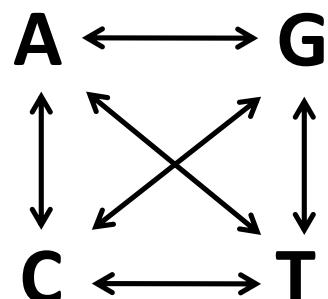


Likelihood is summed over all possibilities

Maximum likelihood



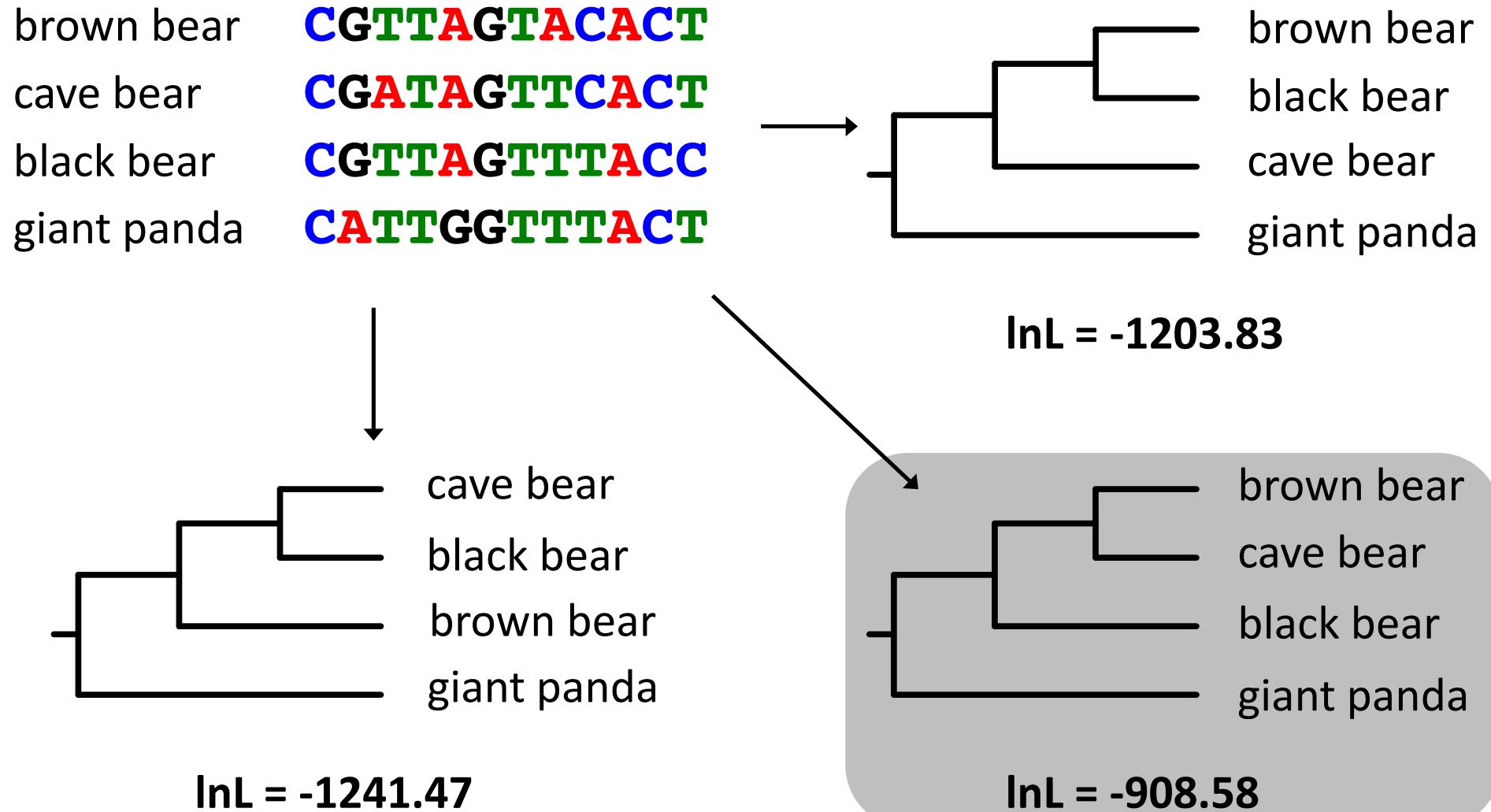
CGTTAGTACACT
CGATAGTTCACT
CGTTAGTTACC
CATGGGTTACT



Likelihood is multiplied across all sites

Very low probability of observing any particular alignment

Maximum likelihood



Likelihood optimisation

- Search through the space of possible trees and parameter values
- Calculate the likelihood for these
- Find best tree and model parameter values
- Multivariate optimisation

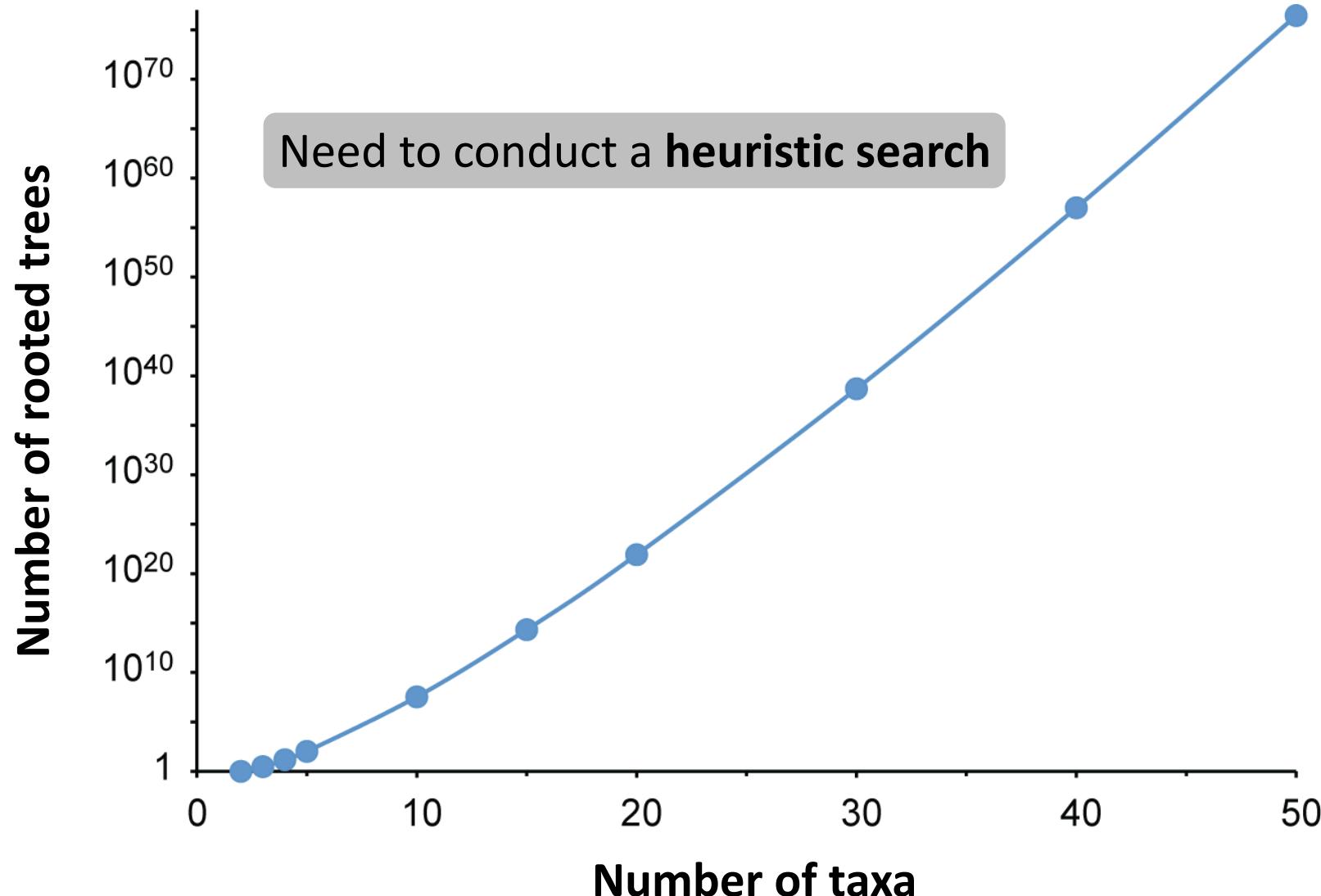
Finding the best tree

- For n taxa, the number of possible unrooted trees (B_n) is:

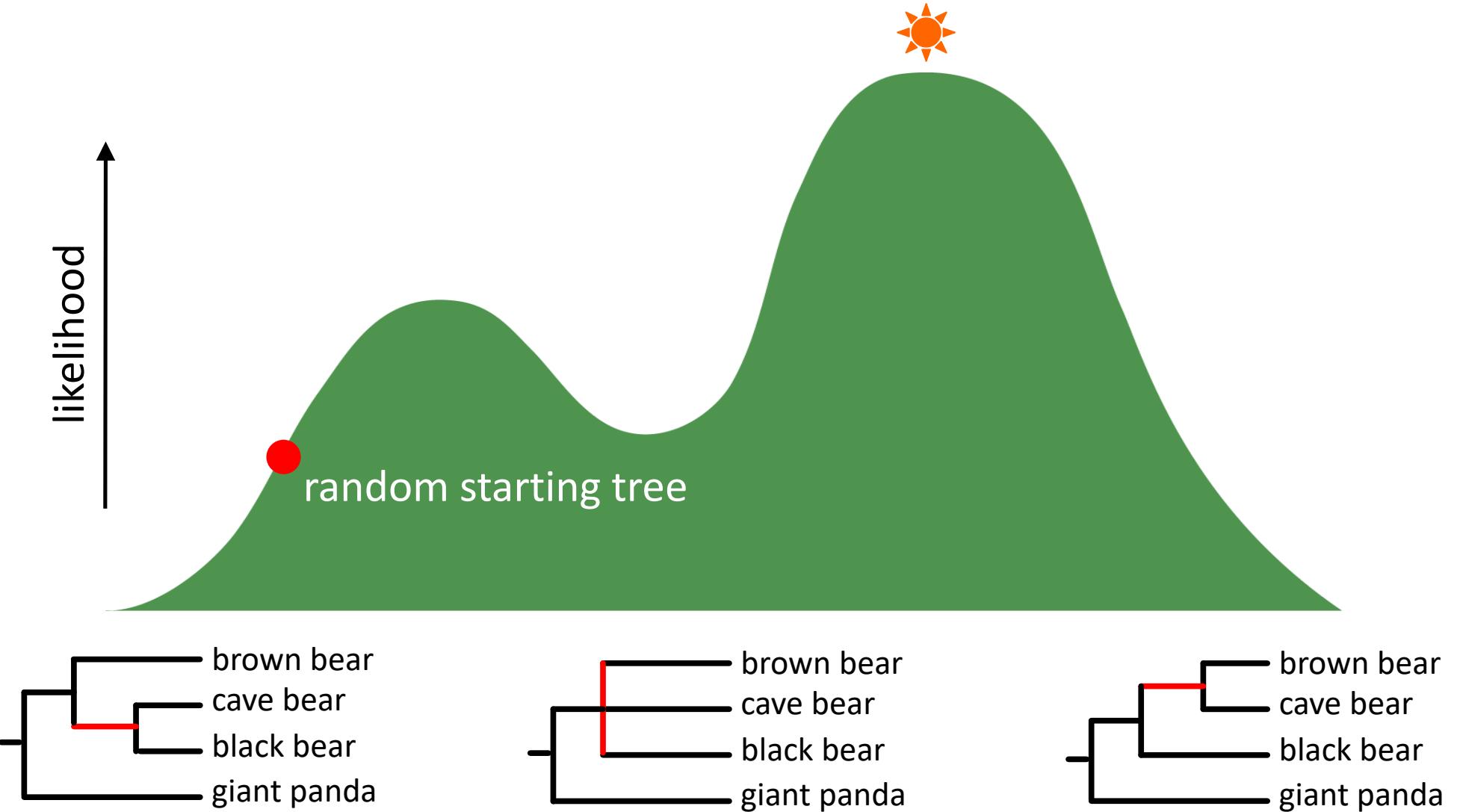
$$B_n = 1 \times 3 \times 5 \times \dots \times (2n - 5) = \prod_{i=3}^n (2i - 5)$$

- For example:
 - 4 taxa → 3 trees
 - 5 taxa → 15 trees
 - 10 taxa → 2,027,025 trees

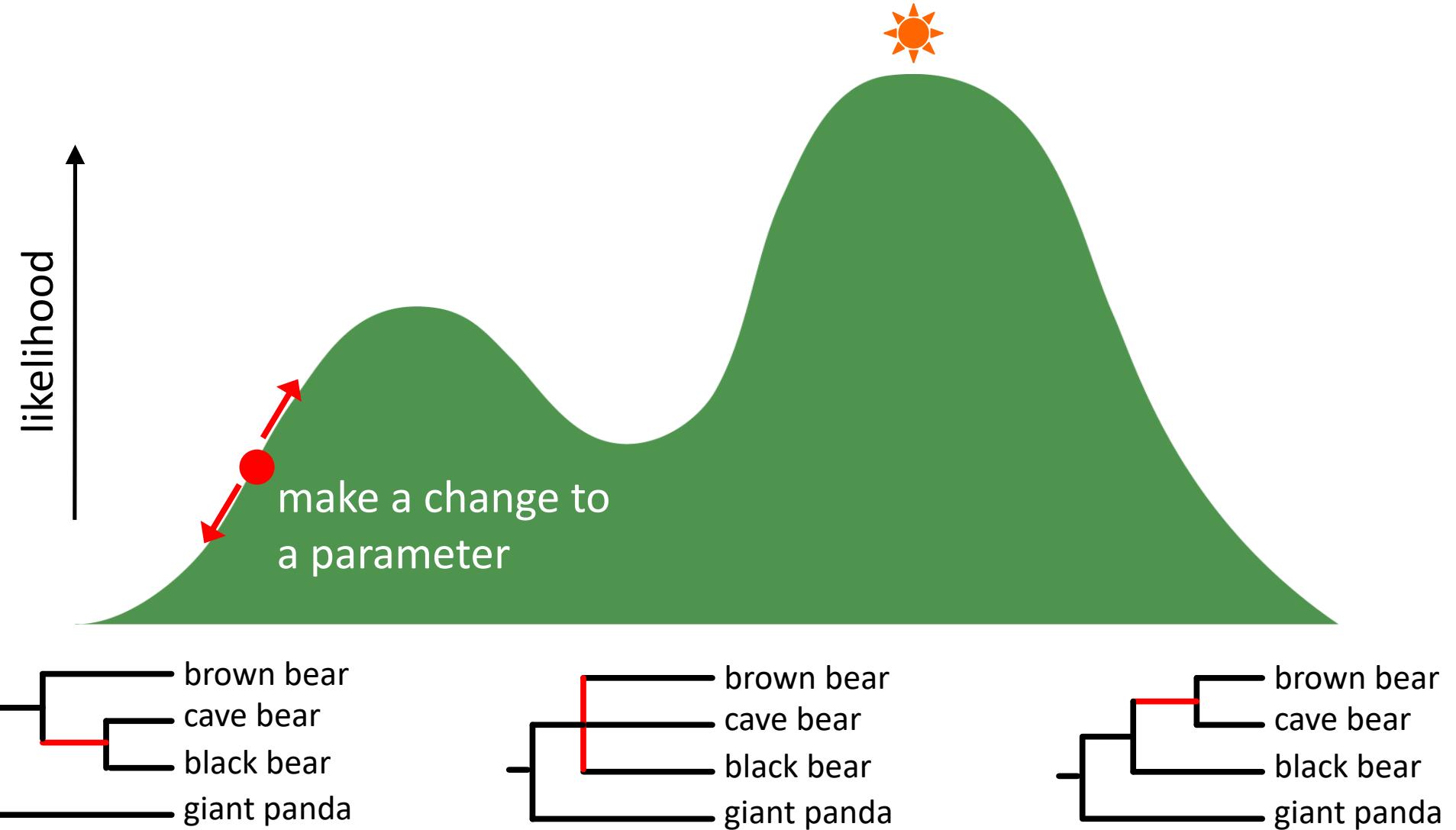
Finding the best tree



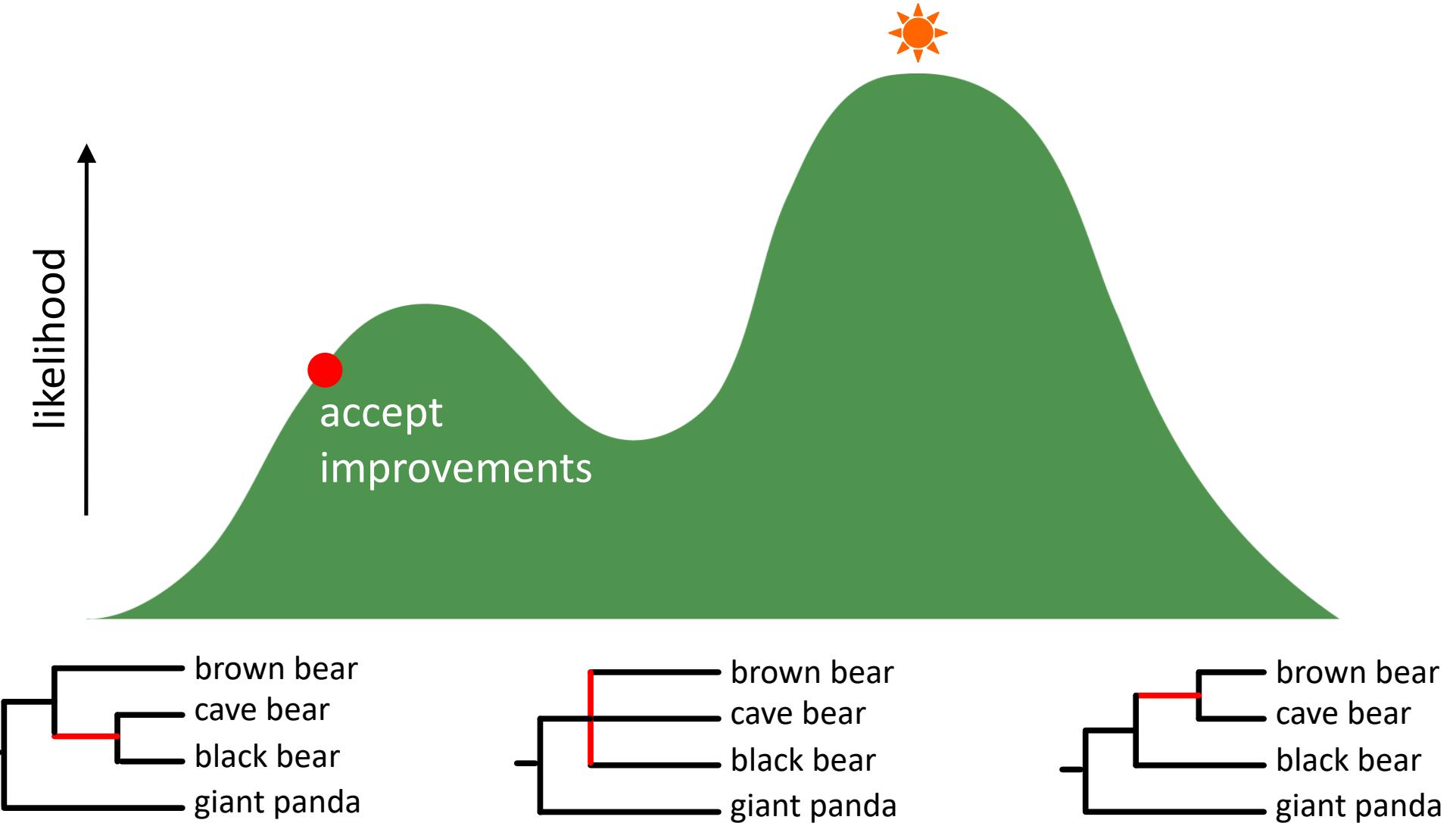
Heuristic search



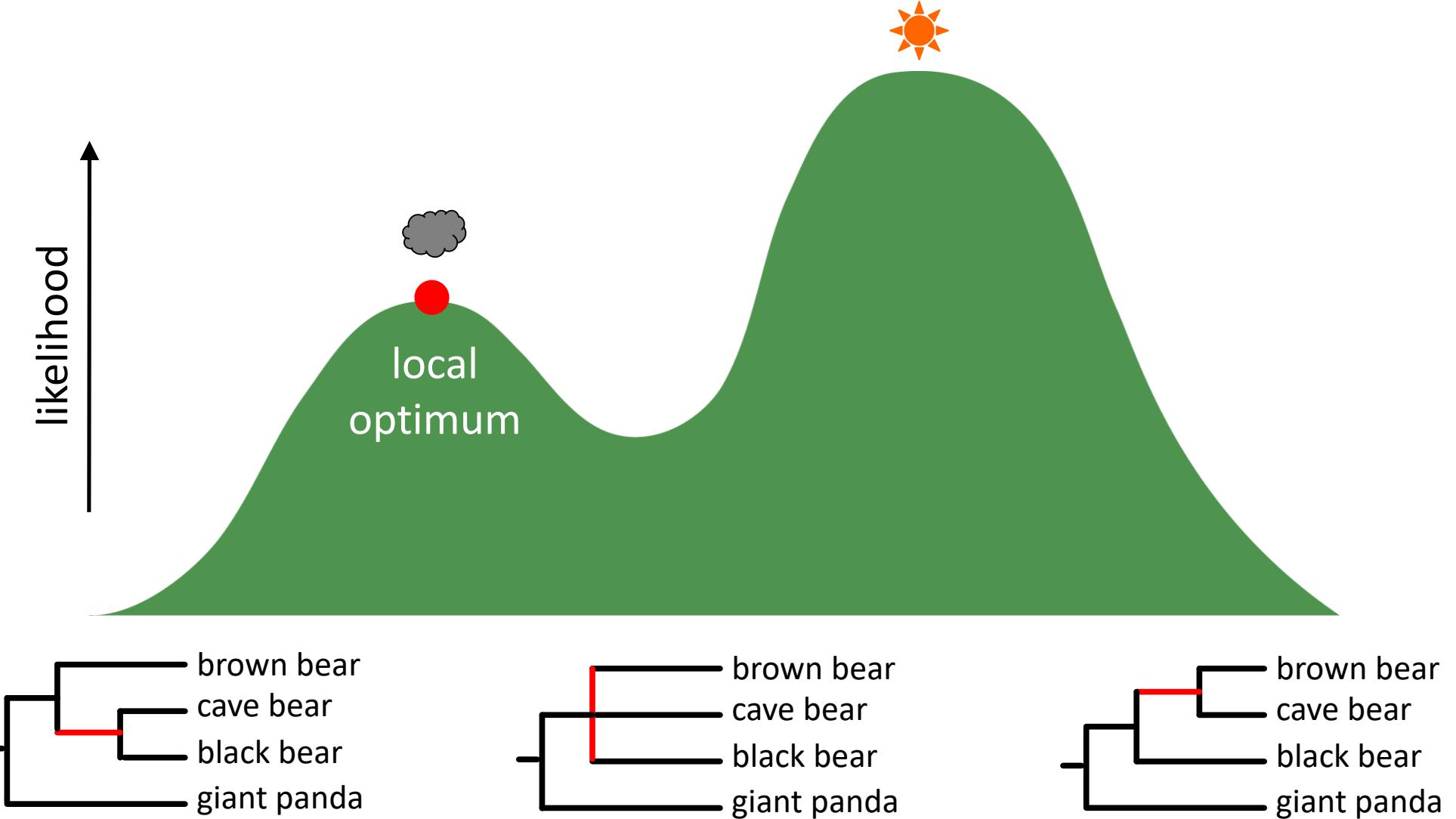
Heuristic search



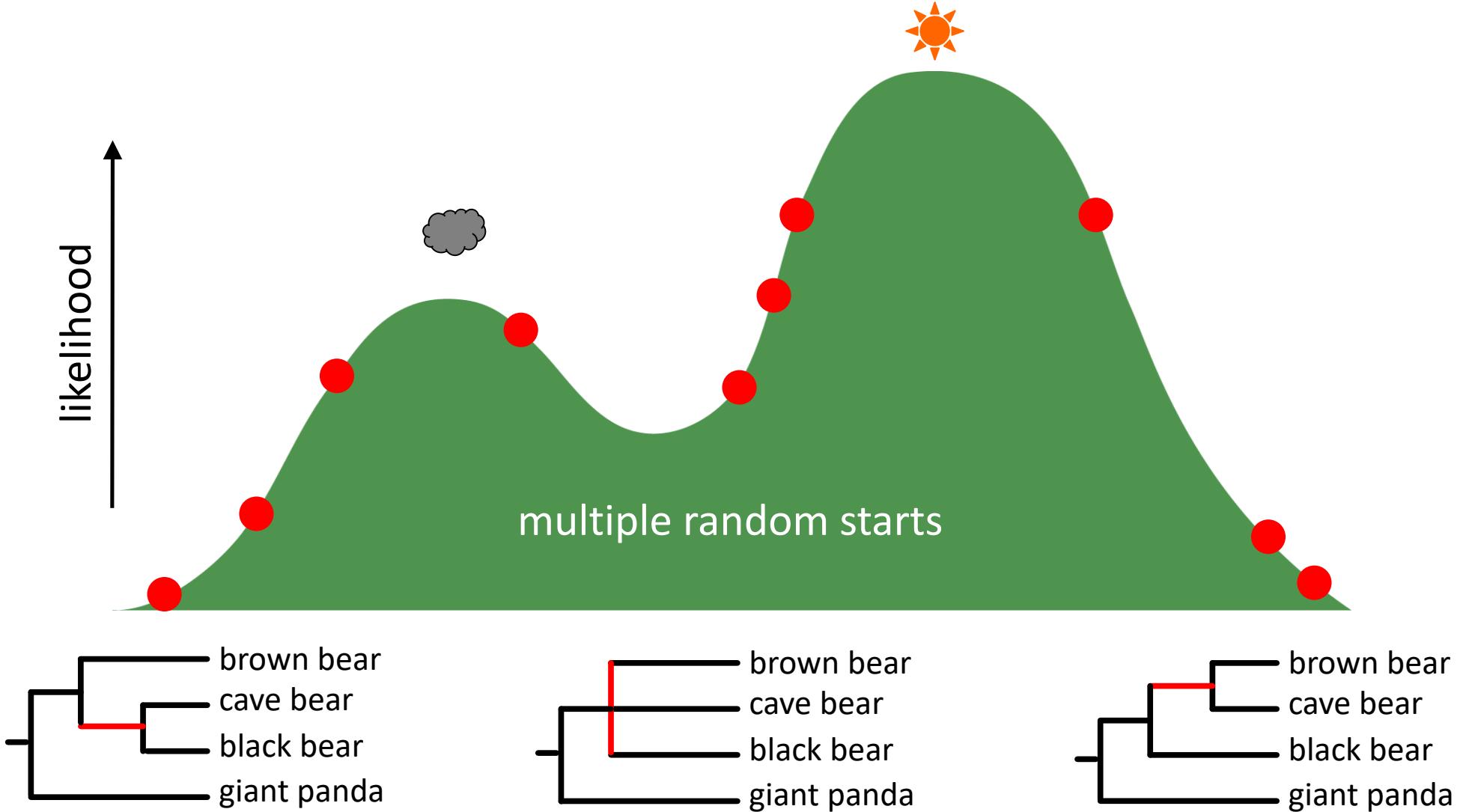
Heuristic search



Heuristic search

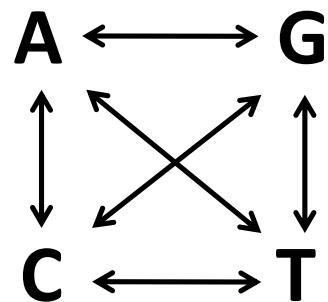


Heuristic search

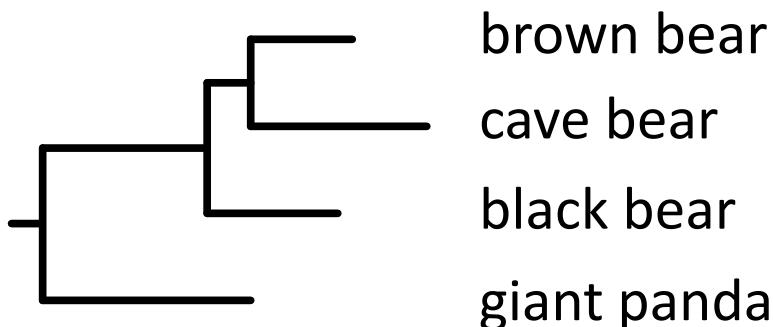


Maximum-likelihood estimates

A single set of maximum-likelihood estimates of model parameters



A single maximum-likelihood tree



Strengths and weaknesses

- **Strengths**
 - Rigorous statistical method
 - Deals with multiple substitutions and long-branch attraction
 - Highly robust to violations of assumptions
- **Weaknesses**
 - Not feasible to implement very parameter-rich models
 - Searching tree space can be difficult

Software

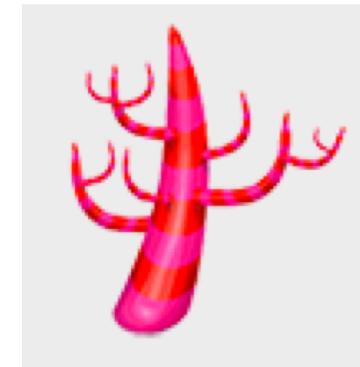
RAxML



PhyML



MEGA



PAML



IQ-TREE

RAxML

- Randomized Axelerated Maximum Likelihood
- Compile to suit your processor architecture
- Can run sequentially or in parallel
- Rapid bootstrapping (*Stamatakis et al. 2008*)



Bootstrapping

Nonparametric bootstrap

- Uncertainty in the estimate of the tree is inferred indirectly using **bootstrapping analysis**
- “pull oneself up by one's bootstraps”
 - Bootstrapping analysis can be used in various phylogenetic methods:
 - Maximum parsimony
 - Distance-based methods
 - Maximum likelihood



Bootstrapping

brown bear
cave bear
black bear
giant panda

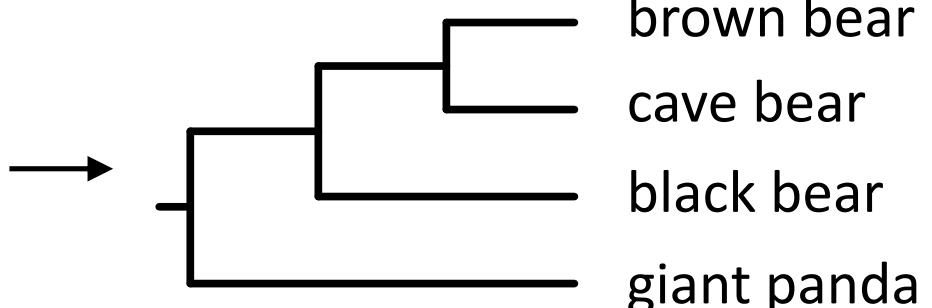
CGTTAGTACACT
CGATA GTTCACT
CGTTAGTTTACCC
CATTGGTTACT

Repeat 1,000 times

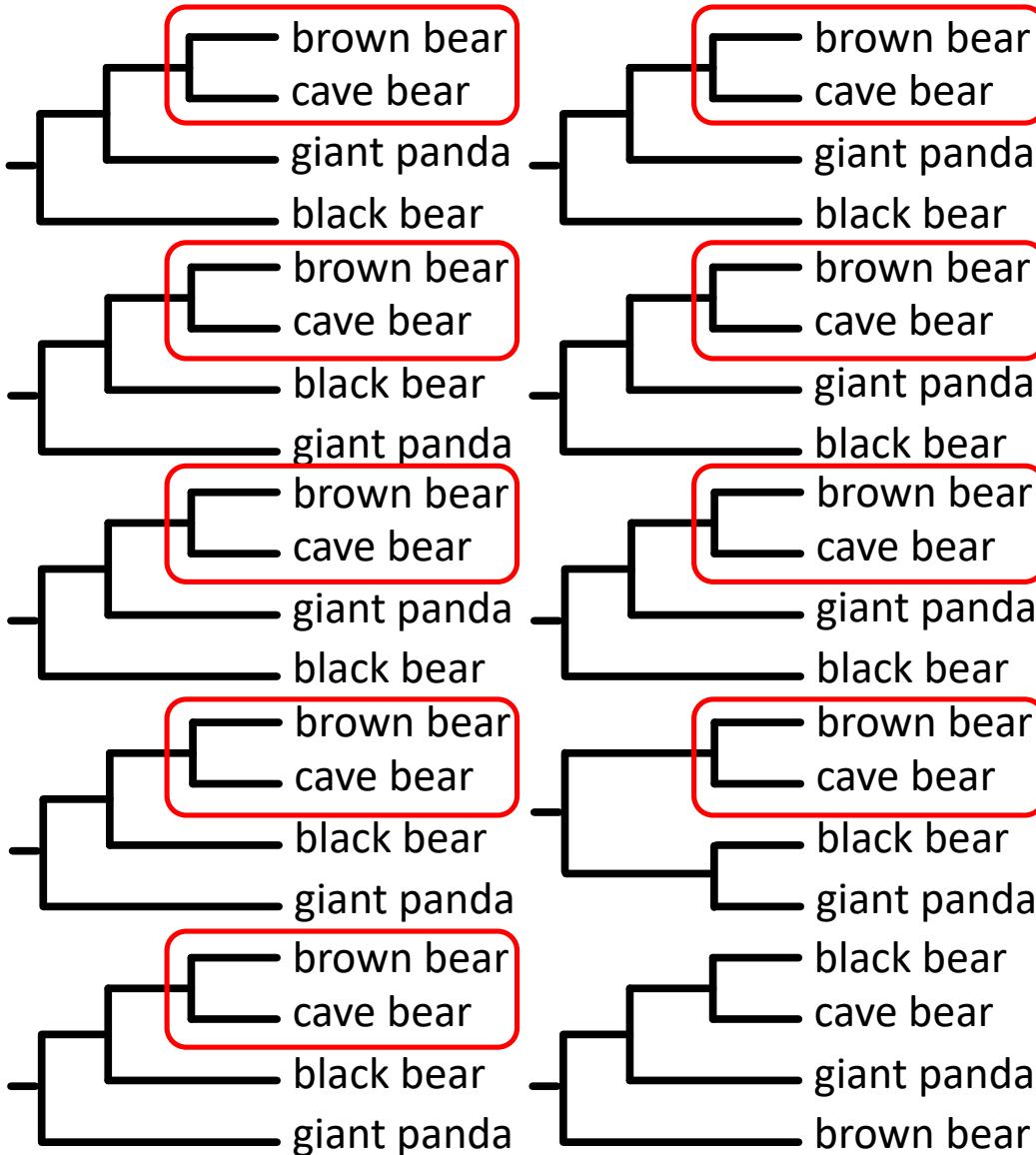
Pseudoreplication

brown bear
cave bear
black bear
giant panda

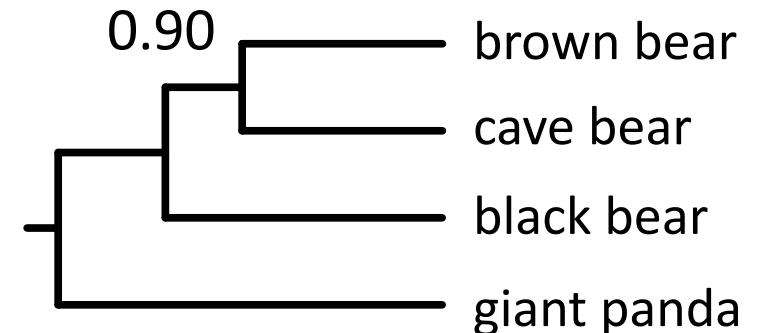
ATTACTGTCCCT
ATTACTGTCCCCA
ATCACTGTTCCCT
GTTGCTATTCCCT



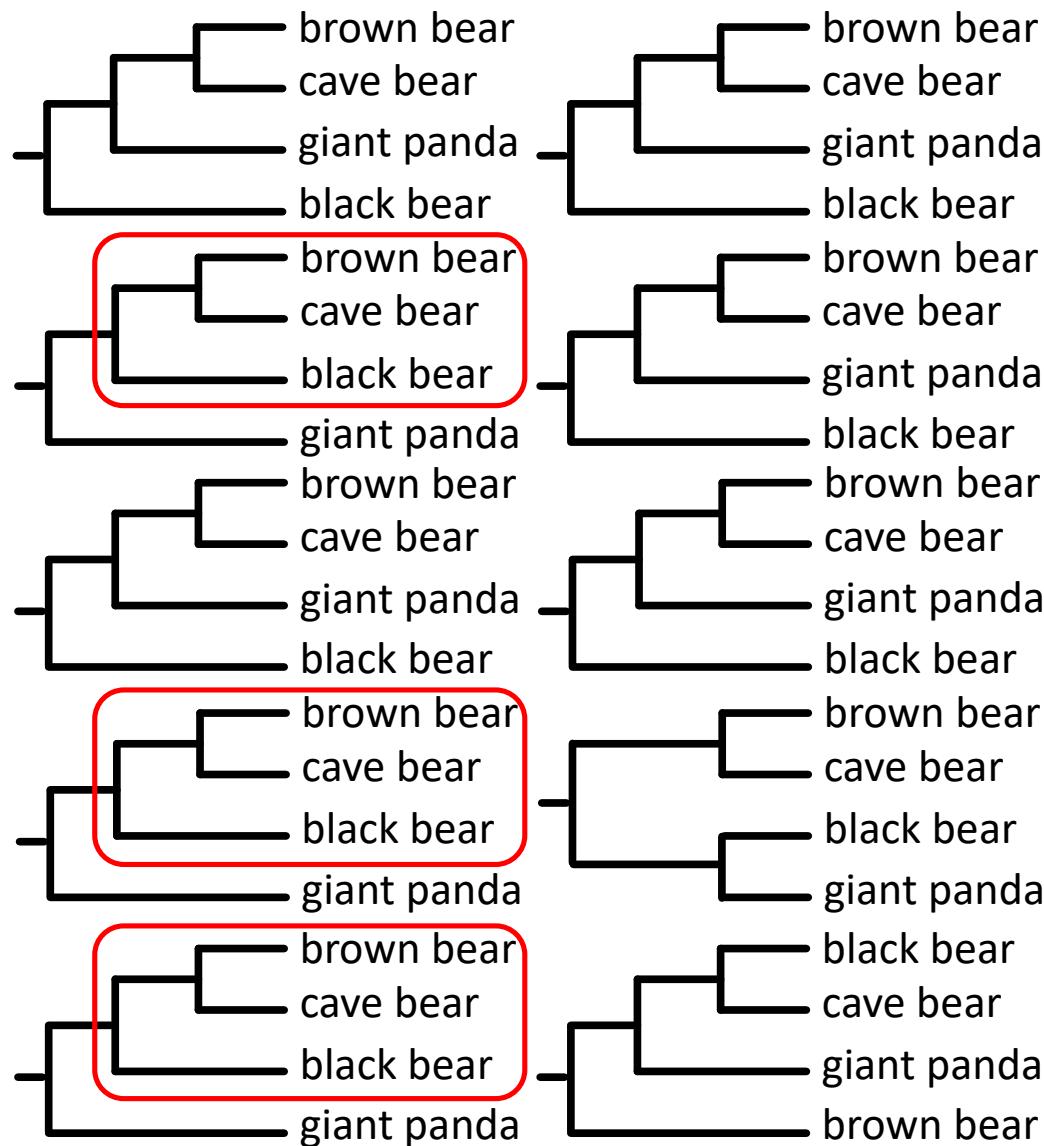
Bootstrapping



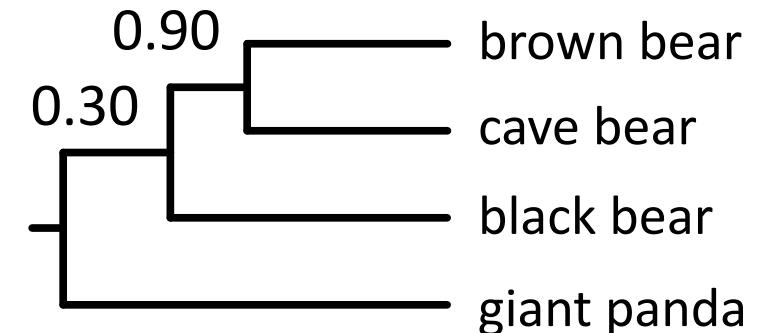
ML tree



Bootstrapping



ML tree



Interpreting bootstrap values

- **Felsenstein (1985)**
bootstrapping provides a confidence interval that contains the *phylogeny that would be estimated from repeated sampling of many characters from the underlying set of all characters*
- Bootstrap values are **measures of repeatability**
 - High when the data set is large
 - Not meaningful when analysing genome-scale data

Methods in practice

- **Maximum parsimony**
 - Commonly used to analyse morphological data
 - Rarely used to analyse molecular data
- **Distance-based methods**
 - Popular in some fields of research
 - Used to analyse very large data sets with many taxa
- **Maximum likelihood**
 - Widely used, but has been losing ground to Bayesian methods

Useful references

- **Molecular phylogenetics: principles and practice**
Yang & Rannala (2012) *Nature Reviews Genetics* 13: 303–314.

