CMSC423: Bioinformatic Algorithms, Databases and Tools
Lecture 13

multiple alignment

motif finding

Recap

- Multiple alignment is expensive – $O(n^k)$ for k sequences of length n (use same DP as for pairwise but on a k-dimensional matrix)
- Approximation algorithm (star alignment) can find a solution in $O(n^2k^2)$ which is at most twice worse than the best alignment

Consensus sequence

- For every column j in the alignment, pick the amino-acid AA that minimizes $\sum_i d(AA, S_i[j])$ (usually becomes majority rule)
- Intuitively – this is the sequence of the ancestor of all the sequences in the multiple alignment
- We can define the multiple alignment problem as:
  - find the multiple alignment that minimizes $\sum_i D(CO, S_i)$
- Still NP – hard, but consensus sequence useful on it's own.

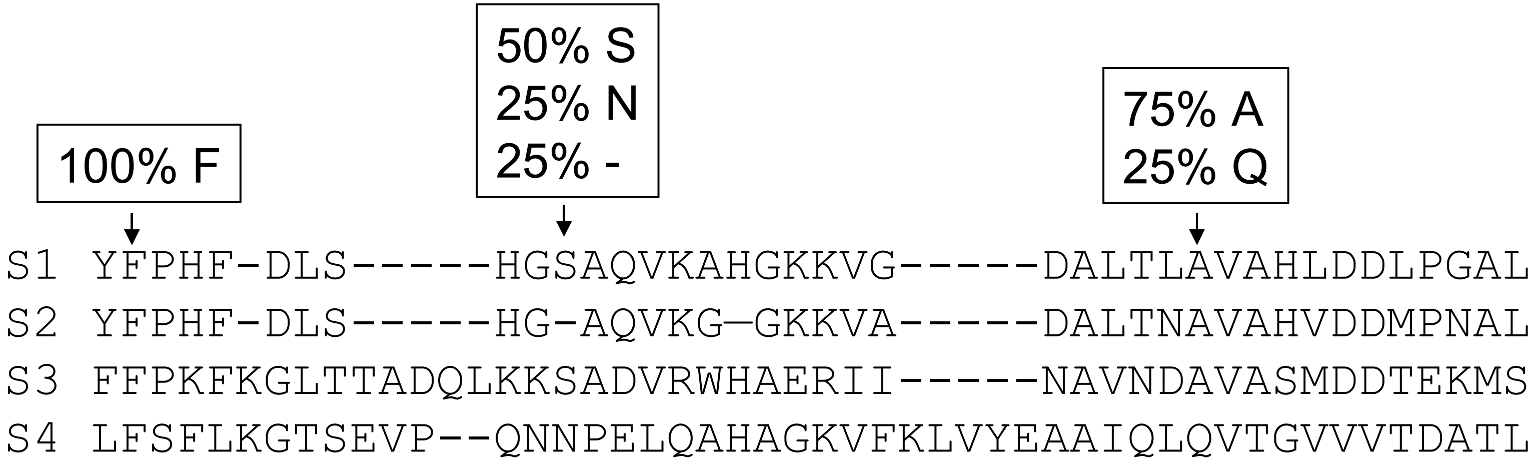
```
CO YFPHFKDLS-----HGSAQVKAHGKKVG-----DALTLAVAHVDDTPGAL
S1 YFPHF-DLS-----HGSAQVKAHGKKVG-----DALTLAVAHLDDLPGAL
S2 YFPHF-DLS-----HG-AQVKG—GKKVA-----DALTNAVAHVDDMPNAL
S3 FFPKFKGLTTADQLKKSADVRWHAERII-----NAVNDAVASMDDTEKMS
S4 LFSFLKGTSEVP--QNNPELQAHAGKVFKLVYEAAIQLQVTGVVVTDATL
```

Iterative alignment revisited

- Pick a sequence (e.g. SC) as a starting point
- Align S1 to it & build consensus for the alignment
- Take S2 and align it to the consensus (instead of SC)
- repeat...
- Problem: consensus (or any single sequence) ignores the other sequences being aligned.
- Solution: keep track of % of each amino-acid aligned in each column
- score of alignment to profile – combination of scores to each AA.

Profile alignment

- Solution: keep track of % of each amino-acid aligned in each column
- score of alignment to profile – combination of scores to each AA.



100% F

50% S
25% N
25% -

75% A
25% Q

```
S1 YFPHF-DLS-----HGSAQVKAHGKKVG-----DALTLAVAHLDDLPGAL
S2 YFPHF-DLS-----HG-AQVKG—GKKVA-----DALTNAVAHVDDMPNAL
S3 FFPKFKGLTTADQLKKSADVRWHAERII-----NAVNDAVASMDDTEKMS
S4 LFSFLKGTSEVP--QNNPELQAHAGKVFKLVYEAAIQLQVTGVVVTDATL
```

- Score(prof1, prof2) = weighted average of all pairs of amino-acids

Practical algorithms

Iterative alignment

```
SC YFPHFDLSHGSAQVKAHGKKVGDALTLAVGHLDDLPGAL
```

- Take sequences si in order:
  - align s1 with sc - results in gaps being inserted in both sequences

```
SC YFPHFDLSHGSAQVKAHGKKVGDALTLAVGHLDDLPGAL
S1 YFPHFDLSHG-AQVKG--KKVADALTNAVAHVDDMPNAL
```

  - align s2 with sc - if gaps must be inserted – insert in previously aligned sequences

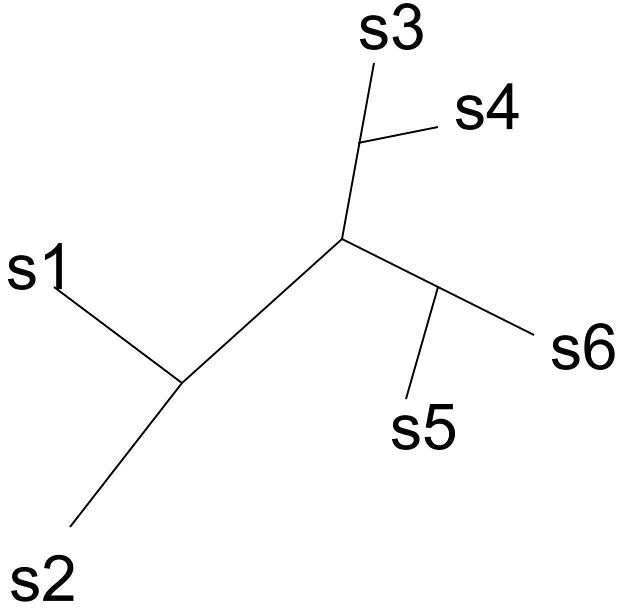
```
SC YFPHF-DLS-----HGSAQVKAHGKKVG-----DALTLAVAHLDDLPGAL
S1 YFPHF-DLS-----HG-AQVKG—GKKVA-----DALTNAVAHVDDMPNAL
S2 FFPKFKGLTTADQLKKSADVRWHAERII-----NAVNDAVASMDDTEKMS
```

  - and so on (note: if gaps coincide with previously introduced gaps no need to change previously aligned sequences)

```
SC YFPHF-DLS-----HGSAQVKAHGKKVG-----DALTLAVAHLDDLPGAL
S1 YFPHF-DLS-----HG-AQVKG—GKKVA-----DALTNAVAHVDDMPNAL
S2 FFPKFKGLTTADQLKKSADVRWHAERII-----NAVNDAVASMDDTEKMS
S3 LFSFLKGTSEVP--QNNPELQAHAGKVFKLVYEAAIQLQVTGVVVTDATL
```

CLUSTALW

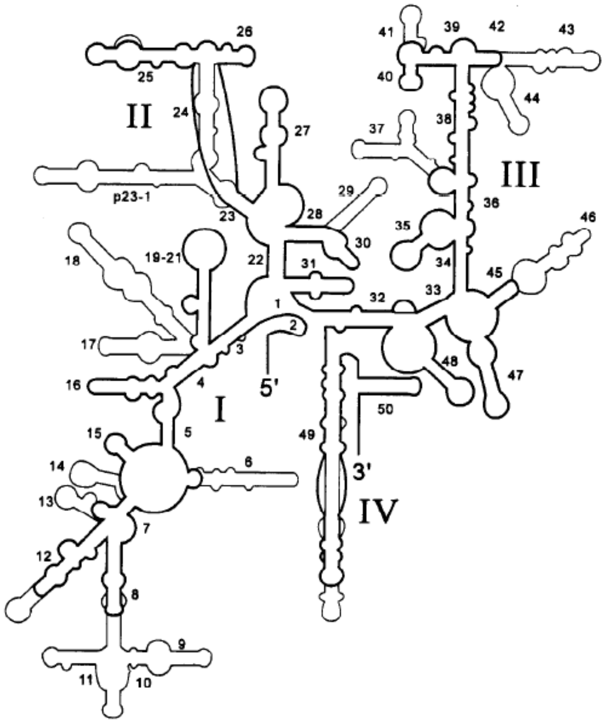
- Compute pairwise distances between strings
- Build phylogenetic tree
- Build iterative alignment by following tree edges

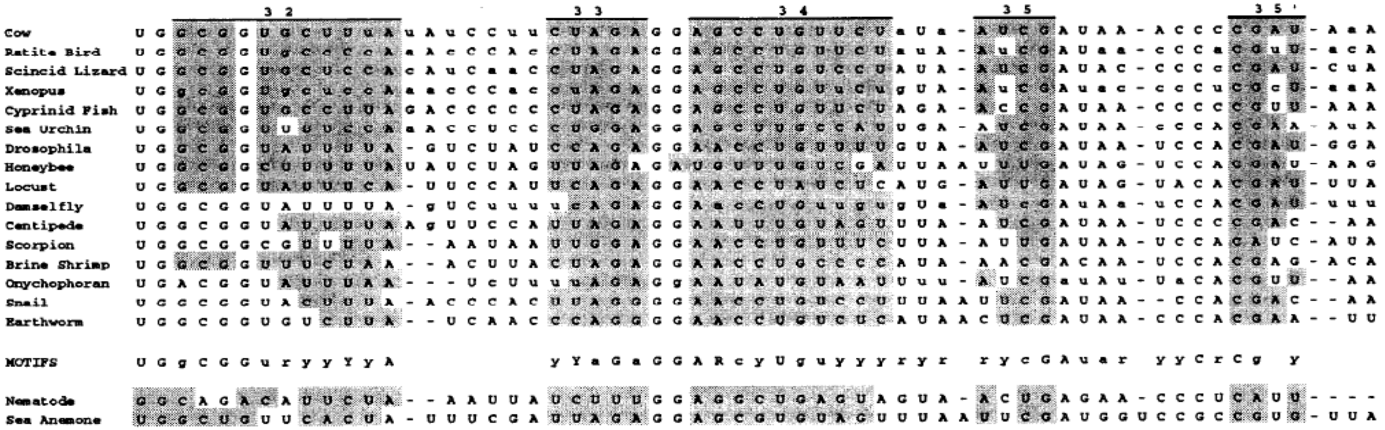


MUSCLE

- Just like ClustalW but different
- Build pairwise distances – uses fast heuristic (just count # of k-mers in common)
- Build phylogenetic tree
- Build multiple alignment based on tree
- Re-estimate distances based on tree
- Re-build tree
- Re-build multiple alignment
- etc. etc. etc.

Biological relevance of multiple alignments





```
                          3 2                         3 3                  3 4                      3 5                  3 5 '
Cow            U G G C G G U G C U U U A u A u C C u u C U A G A G G A G C C U G U U C U a U a - A U C G A U A A - A C C C C G A U - A a A
Ratite Bird    U G G C G G U g C c C c A a A c C C A c C U A G A G G A G C C U G U U C U a u A - A u C G A U a a - c C C a C G u U - a c A
Scincid Lizard U G G C G G U G C U C C A c A u C a a C C U A G A G G A G C C U G U C C U A U A - A U C G A U A C - C C C c C G A U - C u A
Xenopus        U G g C G G U g c u c c A a a c C C a c c u A G A G G A G C C U G U u C u g U A - A u C G A u a c - c c C u C G c U - a a A
Cyprinid Fish  U G G C G G U G C C U U A G A C C C C C C U A G A G G A G C C U G U U C U A G A - A C C G A U A A - C C C C C G U U - A A A
Sea Urchin     U G G C G G U U U U C C A a A C C U C C C U G G A G G A G C U U G C C A U U G A - A U C G A U A A - c C C A C G A A - A u A
Drosophila     U G G C G G U A U U U U A - G U C U A U C C A G A G G A A C C U G U U U U G U A - A U C G A U A A - U C C A C G A U - G G A
Honeybee       U G G C G G C U U U U U A U A U C U A G U U A G A G A U G U U U G U C G A U U A A U U U G A U A G - U C C A C G A U - A A G
Locust         U G G C G G U A U U U C A - U U C C A U U C A G A G G A A C C U A U C U C A U G - A U U G A U A G - U A C A C G A U - U U A
Damselfly      U G G C G G U A U U U U A - g U C u u u u c A G A G G A a c C U G u u g u g U a - A U c G A u A a - u C C A C G A U - u u u
Centipede      U G G C G G U A U U U U A A g U U C C A U U A G A G G A A U U U G U A G U U U A - A U C G A U A A - U C C C C G A C - - A A
Scorpion       U G G C G G C G U U U U A - - A A U A A U U G G A G G A A C C U G U U U C U U A - A U U G A U A A - U C C A C A U C - A U A
Brine Shrimp   U G G C G G U U U C U A A - - A C U U A C U A G A G G A A C C U G C C C C A U A - A A C G A C A A - U C C A C G A G - A C A
Onychophoran   U G A C G G U A U U U A A - - - U c U u u u A G A G g A A U A U G U A A U U u u - A U C G a u A u - U a C A C G U U - - A A
Snail          U G G C C G U A C U U U A - A C C C A C U U A G G G G A A C C U G U C C U U U A A U U C G A U A A - - C C A C G A C - - A A
Earthworm      U G G C G G U G U C U U A - - U C A A C C C A G G G G A A C C U G U C U C A U A A C U C G A U A A - C C C A C G A A - - U U

MOTIFS         U G g C G G u r y y Y y A               y Y a G a G G A R c y U g u y y y r y r   r y c G A u a r   y y C r C g   y

Nematode       G G C A G A C A U U C U A - - A A U U A U C U U U G G A G G C U G A G U A G U A - A C U G A G A A - C C C U C A U U - - - -
Sea Anemone    U G G C U G U U C A C U A - U U U C G A U U A G A G G A G C G U G U A G U U U A A U U C G A U G G U C C G C C G U G - U U A
```

Motif finding

Motif finding

- Special case of multiple alignment – find short “motif” that occurs almost identically in multiple DNA sequences
- Local multiple alignment (the definition of multiple alignment sofar was global)
- Motif finding – special requirements
  - inexact alignment sought
  - but no gaps allowed
- Biological significance
  - gene promoters
  - transcription factor binding sites
  - other elements involved in gene regulation

Motif finding...example

TTAGAGGTTGACTA**TTCAACTT**TTGAGGAGGCCTAG *TAGAGC*

AGCCGACT**TGCAACTT**AGGCGTGGTCAGTGCCCTAA *TAGAGC*

GGCCTATTTGGGCCACTTAGACC**TTCAACTT**TTGCA *TAGAGC*

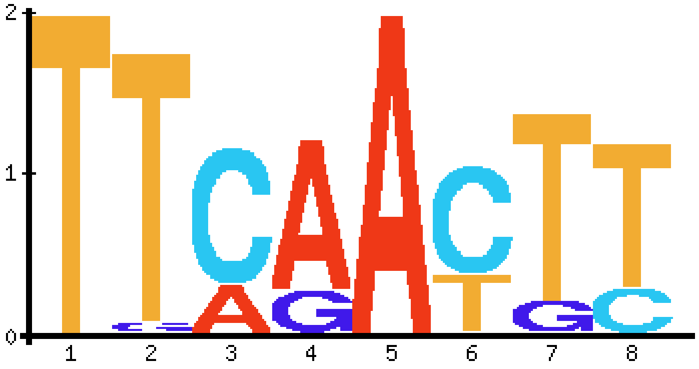
CCACAG**TTAGATGT**CCAAAAGACAAATATAGAGGGC *TAGAGC*

ACACGGACTGCG**TTCAATGC**TTACAGCAGATTGAGT *TAGAGC*

TTCAAAGACTTGACTATTG**TTCAACTT**TGAAGACTA *TAGAGC*

Promoter region → Gene

Motif "sequence logo"



From genetics.mgh.harvard.edu/sheenlab/

Finding motifs – Gibbs sampling

- Observations:
  - since no gaps – all motifs have equal length (assume known value - m)
  - exhaustive search of promoter region is impractical: all combinations of substrings of length m among k sequences of length L = $(L – m + 1)^k$
- Solution: random search

1. Pick random substring of length m from each of the strings
2. Construct multiple alignment (easy since no gaps) and compute profile
3. Pick random sequence s and remove from multiple alignment. Recompute profile.
4. Within removed sequence, search for best fit to profile and insert into alignment
5. Repeat until profile does not improve

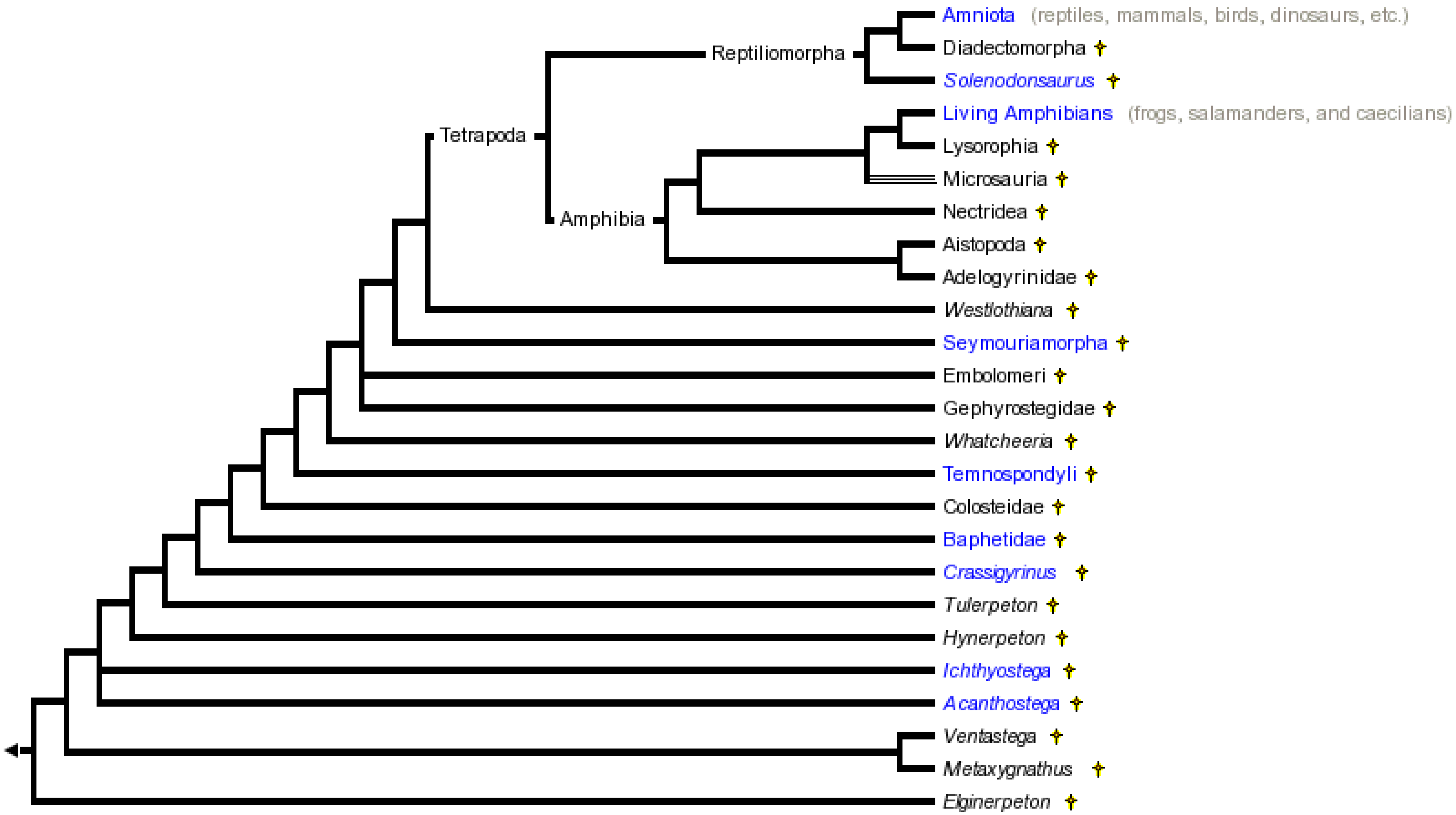
Gibbs sampling...cont

- How do you find best match to profile?
- What is overall running time of algorithm?

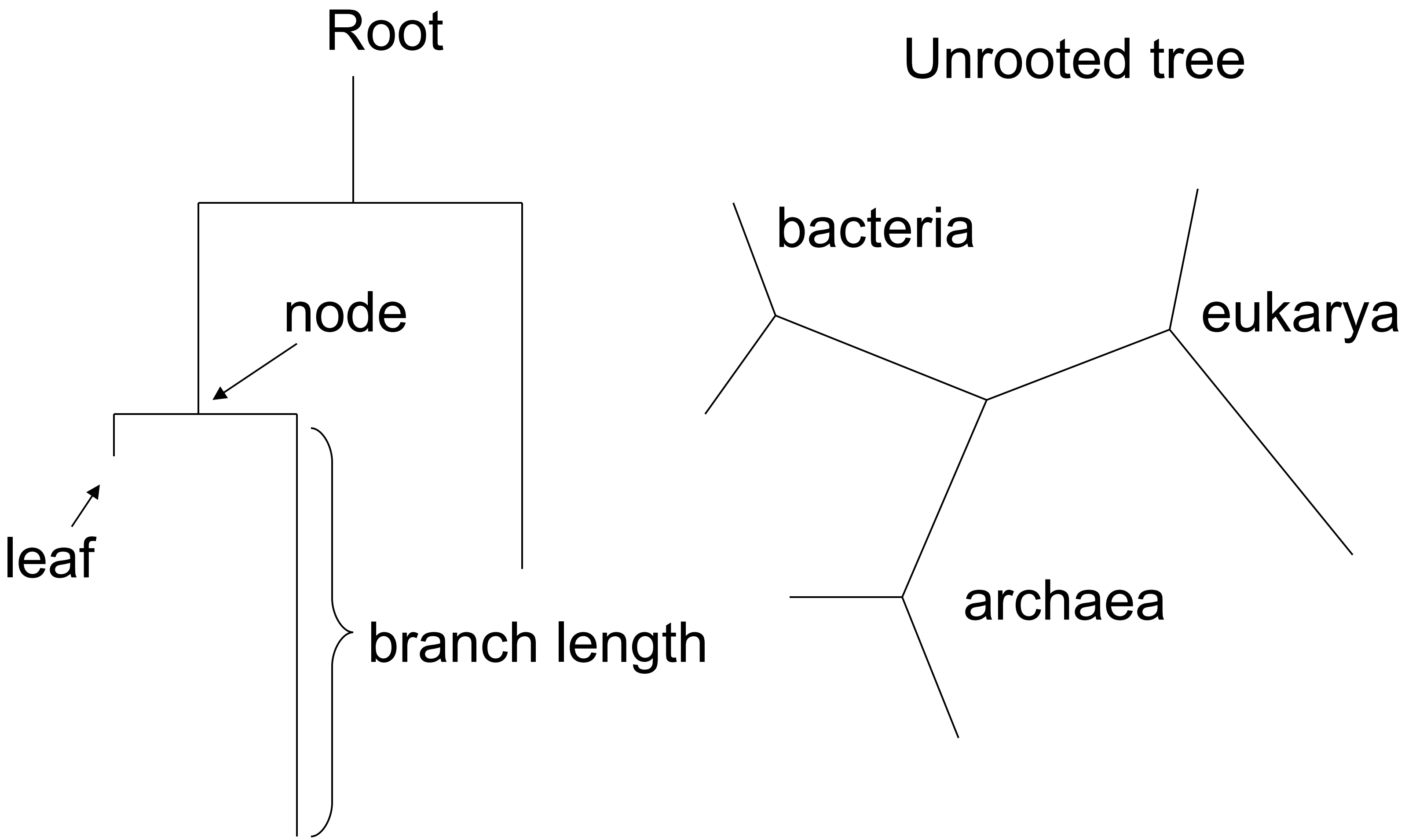
Phylogenetic trees

Phylogenetic trees – how evolution works

- http://www.tolweb.org/tree/ - the tree of life



Anatomy of a tree

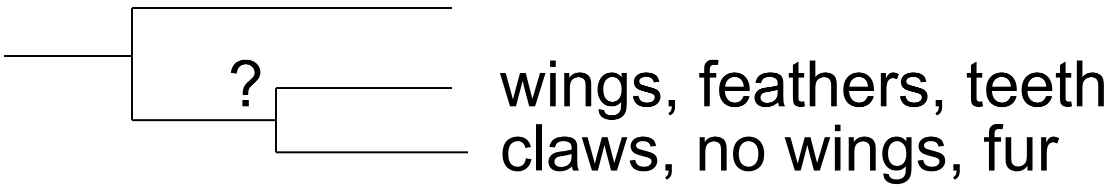


Phylogenetic trees are usually binary (though they don't have to)

Phylogeny questions

- Given several organisms & a set of features (usually sequence, but also morphological: wing shape/color...)

- A. Given a phylogenetic tree – figure out what the ancestors looked like (what are the features of internal nodes)



?

wings, feathers, teeth

claws, no wings, fur

- B. Find the phylogenetic tree that best describes the common evolutionary heritage of the organisms