Hierarchical clustering implementation

- **Single linkage (nearest neighbor):** **In this method the distance between two clusters is determined by the distance of the two closest objects (nearest neighbors) in the different clusters.**

- **Complete linkage (furthest neighbor):** In this method, the distances between clusters are determined by the greatest distance between any two objects in the different clusters (i.e., by the "furthest neighbors").

- **Group average linkage:** In this method, the distance between two clusters is calculated as the average distance between all pairs of objects in the two different clusters.

Single-Link Hierarchical Clustering

Iteration.

- Closest pair of clusters (i, j) is one with the smallest dist value.
- Replace row i by min of row i and row j.
- Infinity out row j and column j.
- Update dmin[i] and change dmin[i'] to i if previously dmin[i'] = j.

Closest pair

	dmin	dist
0	1	5.5
1	3	2.14
2	4	5.6
3	1	2.14
4	3	5.5

	0	1	2	3	4
gene0	-	5.5	7.3	8.9	5.8
1	5.5	-	6.1	2.14	5.6
2	7.3	6.1	-	7.8	5.6
3	8.9	2.14	7.8	-	5.5
4	5.8	5.6	5.6	5.5	-

Gene1 closest to gene3, dist=2.14

i=1, j=3

---

	dmin	dist
0	1	5.5
1	0	5.5
2	4	5.6
3	-	-
4	1	5.5

	0	1	2	3	4
0	-	5.5	7.3	-	5.8
node1	5.5	-	6.1	-	5.5
2	7.3	6.1	-	-	5.6
3	-	-	-	-	-
4	5.8	5.5	5.6	-	-

New min dist

Single-Link Clustering: Java Implementation

Single-link clustering.

- Read in the data.

```
public static void main(String[] args) {
   int M = StdIn.readInt();
   int N = StdIn.readInt();

   // read in N vectors of dimension M
   Vector[] vectors = new Vector[N];
   String[] names   = new String[N];
   for (int i = 0; i < N; i++) {
     names[i] = StdIn.readString();
     double[] d = new double[M];
     for (int j = 0; j < M; j++)
        d[j] = StdIn.readDouble();
     vectors[i] = new Vector(d);
   }
```

Single-Link Clustering: Java Implementation

Single-link clustering.

- Read in the data.
- Precompute `d[i][j]` = distance between cluster i and j.
- For each cluster i, maintain index `dmin[i]` of closest cluster.

```
double INFINITY = Double.POSITIVE_INFINITY;
double[][] d = new double[N][N];
int[] dmin = new int[N];
for (int i = 0; i < N; i++) {
   for (int j = 0; j < N; j++) {
      if (i == j) d[i][j] = INFINITY;
      else        d[i][j] = vectors[i].distanceTo(vectors[j]);
      if (d[i][j] < d[i][dmin[i]]) dmin[i] = j;
   }
}
```

Single-Link Clustering: Main Loop

```
for (int s = 0; s < N-1; s++) {
   // find closest pair of clusters (i1, i2)
   int i1 = 0;
   for (int i = 0; i < N; i++)
      if (d[i][dmin[i]] < d[i1][dmin[i1]]) i1 = i;
   int i2 = dmin[i1];

   // overwrite row i1 with minimum of entries in row i1 and i2
   for (int j = 0; j < N; j++)
      if (d[i2][j] < d[i1][j]) d[i1][j] = d[j][i1] = d[i2][j];
   d[i1][i1] = INFINITY;

   // infinity-out old row i2 and column i2
   for (int i = 0; i < N; i++)
      d[i2][i] = d[i][i2] = INFINITY;

   // update dmin and replace ones that previous pointed to
   // i2 to point to i1
   for (int j = 0; j < N; j++) {
      if (dmin[j] == i2) dmin[j] = i1;
      if (d[i1][j] < d[i1][dmin[i1]]) dmin[i1] = j;
   }
}
```

Store Centroids in Each Internal Node

Cluster analysis.
Centroids distance / similarity.

Easy modification to `TreeNode` data structure.

- Store `Vector` in each node.
  - leaf nodes: directly corresponds to a gene
  - internal nodes: centroid = average of all leaf nodes beneath it
- Maintain count field in each `TreeNode`, which equals the number of leaf nodes beneath it.
- When setting z to be parent of x and y,
  - set z.count = x.count + y.count
  - set z.vector = $\alpha p + (1-\alpha)q$, where p = x.vector and q = y.vector, and α = x.count / z.count

Analysis and Micro-Optimizations

Running time. Proportional to MN^2 (N genes, M arrays)
Memory. Proportional to N^2.

Ex. [M = 50, N = 6,000] Takes 280MB, 48 sec on fast PC.

Some optimizations. ⟵ input size proportional to MN

- Use `float` instead of `double`
- Store only lower triangular part of distance matrix
- Use squares of distances instead of distances.

How much do you think would this help?

Sequence!

Some slides from Mona Singh, Serafim Batzoglou, Olga Troyanskaya

Bio-Sequences

Complete genomes of >1000 organisms
www.ncbi.nlm.nih.gov/Genomes/index.html

> 100 billion bases in Genbank (ncbi)

>509,000 proteins in SWISSPROT (hand curated); >9,300,000 proteins in TREMBL (computer annotated).

us.expasy.org/sprot

Next Gen Sequencers

Illumina/Solexa High Throughput Sequencing Machine

>20 billion bases per run

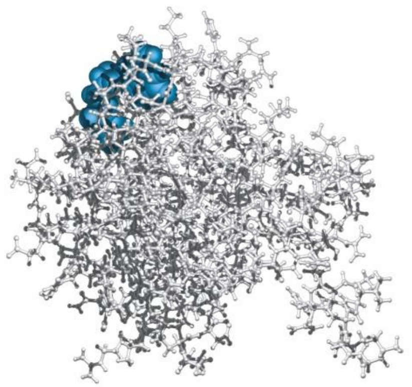
Illumina's Spring 2009 charge for sequencing your genome:

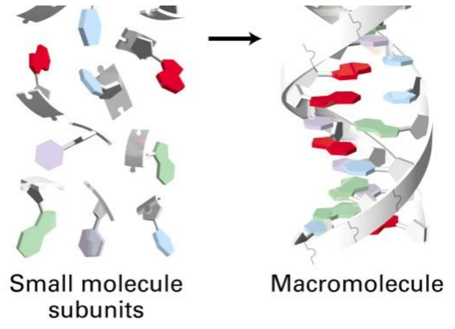
$48,000 - 30 fold coverage

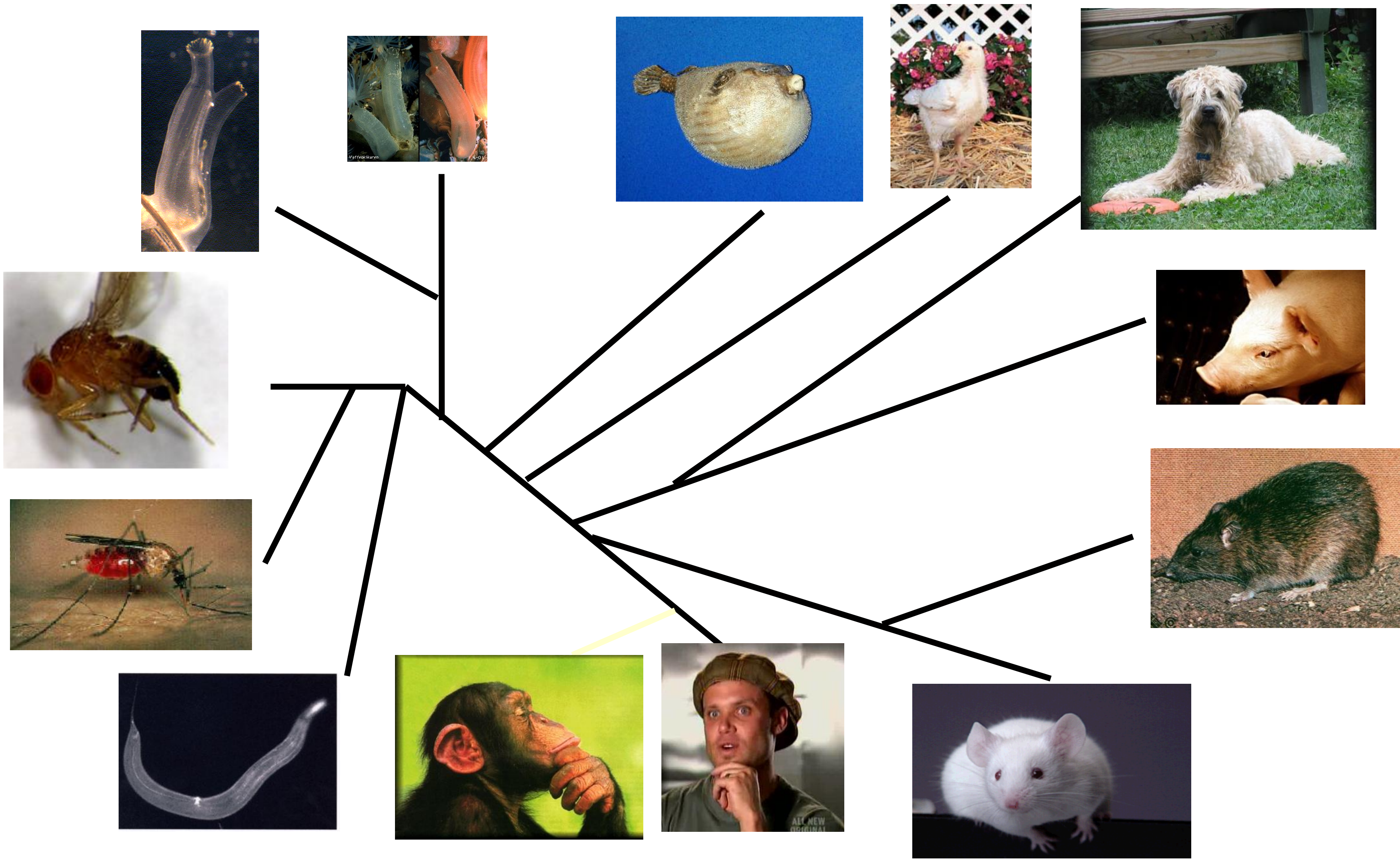
Biomolecules as Strings

Macromolecules are the chemical building blocks of cells

- Proteins
  - 20 amino acids
- Nucleic acids
  - 4 nucleotides {A, C, G, ,T}







Role of Evolution

Molecular structures and mechanisms are reused and changed during evolution

Often mechanisms that are conserved can be detected based on sequence similarity

Powerful tool for annotation

Ex: Protein Sequences

Horse vs Human Myoglobin (Global alignment of sequences)

```
GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASED
GLSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKASED

LKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHP
LKKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHP

GDFGADAQGAMTKALELFRNDIAAKYKELGFQG
GDFGADAQGAMNKALELFRKDMASNYKELGFQG
```

Same protein in two different organisms, can ID based on sequence similarity – 88% identical

Myoglobin - intracellular storage of oxygen

Global alignment: Issues with transferring annotations

Horse Myoglobin vs Human Hemoglobin Alpha

```
MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASEDL
MVLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKG----

KKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHPG
--HGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPA

DFGADAQGAMTKALELFRNDIAAKYKELGFQG
EFTPAVHASLDKFLASVSTVLTSKYR------
```

~25% identical; other “similar” amino acids
Myoglobin - intracellular storage of oxygen
Hemoglobin - transports oxygen

Basic Tool to Detect Sequence Similarity: Alignments

Given:

- a pair (or more) of sequences (DNA or protein)
- a method for scoring the similarity of a pair of characters (=bases or amino acids)

Determine: correspondences between characters in the sequences such that the similarity score is maximized

Pairwise global aligment

Given two sequences, a scoring scheme with a gap function, line up the sequences (with insertion of gaps) to maximize the score

E.g., match = 1
mismatch = -1
gap = -2

E.g., say your two sequences are

```
AACAGTTACC,  TAAGGTCA
```

```
AACAGTTACC
TA-AGGT-CA
```

Score = ?

Naïve way to find optimal alignments

1. Enumerate all possible alignments
2. Score all possible alignments
3. Take best scoring alignment
4. Problem: There are too many possible alignments between 2 sequences !!
5. Solution: dynamic programming

- RECALL: homework assignment from last term!

Pairwise Alignment

Needleman & Wunsch, *Journal of Molecular Biology*, 1970

Dynamic programming (DP): general technique to solve an instance of a problem by taking advantage of computed solutions for smaller subparts of the problem

Here, determine alignment of two sequences by determining alignment of all suffixes of the sequences

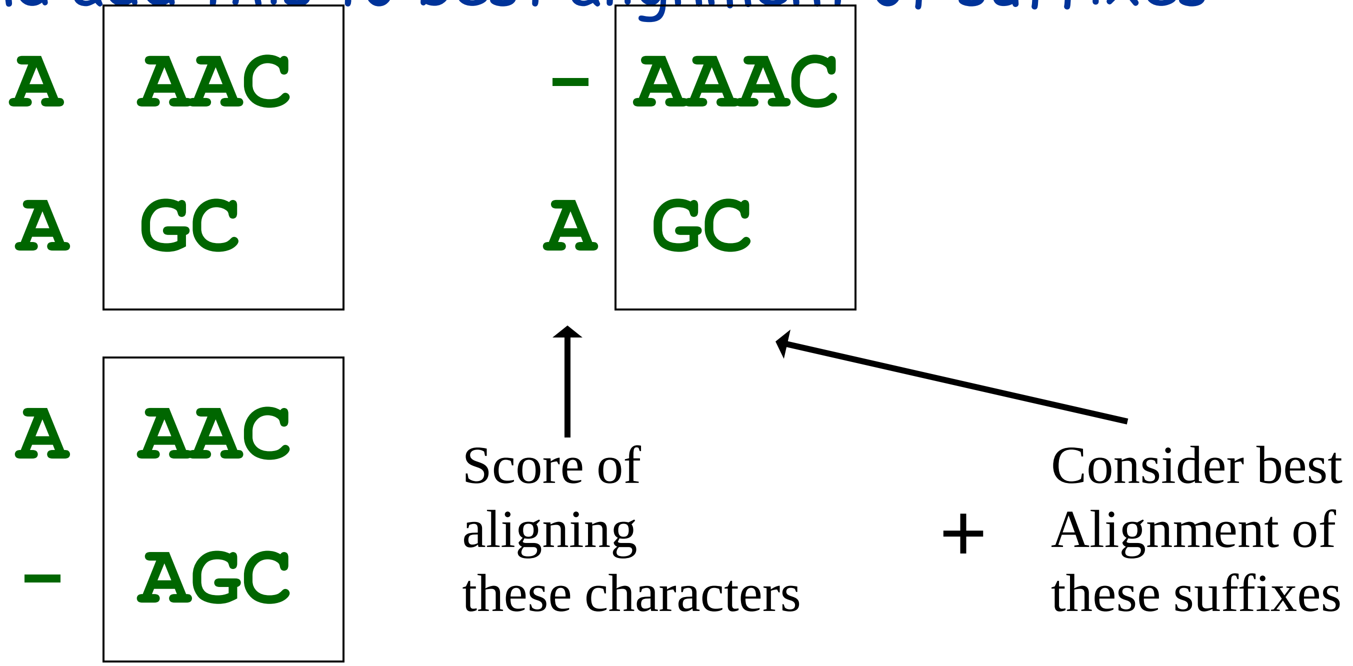
- (suffixes are subparts we'll save solutions for…)

Dynamic Programming Idea

Say aligning **AAAC** with **AGC**

Consider what happens in the first column

Three possible options; each corresponds to different alignment of first column, choose each one and add this to best alignment of suffixes


A AAC
A GC
A AAC
– AGC
– AAAC
A GC
Score of aligning these characters
+
Consider best Alignment of these suffixes

Dynamic Programming Idea

```
-  AAAC

A  GC
```

```
A  AAC

A  GC
```

```
A  AAC

-  AGC
```

If we knew answers to these *three* subproblems, then we'd know the best alignment score between *AAAC* and *AGC*

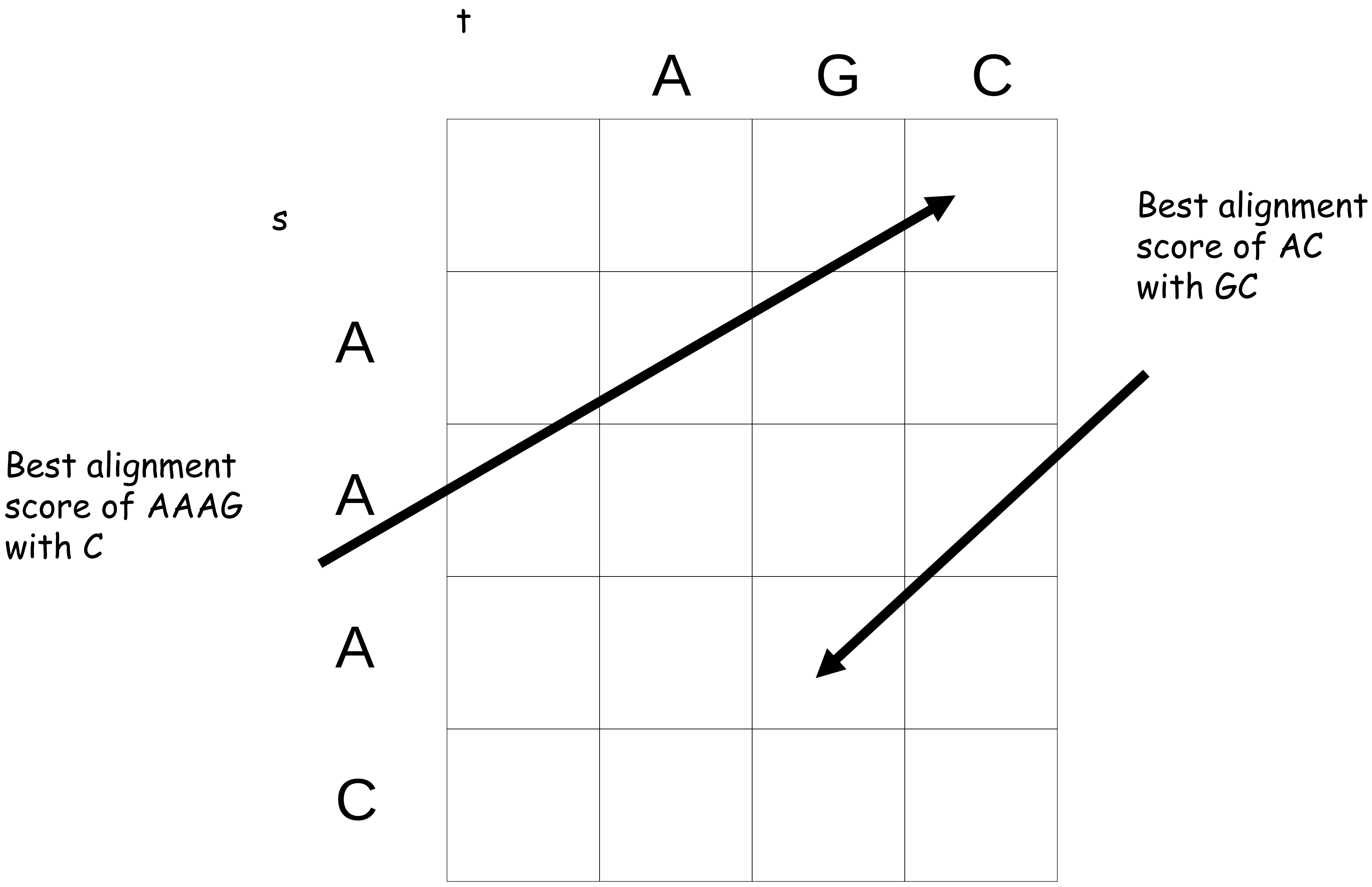
Consider minimum of these three cases

Dynamic Programming Idea

Given an *m*-character sequence *s*, and an *n*-character sequence *t* construct an $(m+1) \times (n+1)$ matrix *sim* where we'll store answers to subproblems

sim[*i*, *j*] = score of the best alignment of the suffix *i…m* of *s* with the suffix *j…n* of *t*.

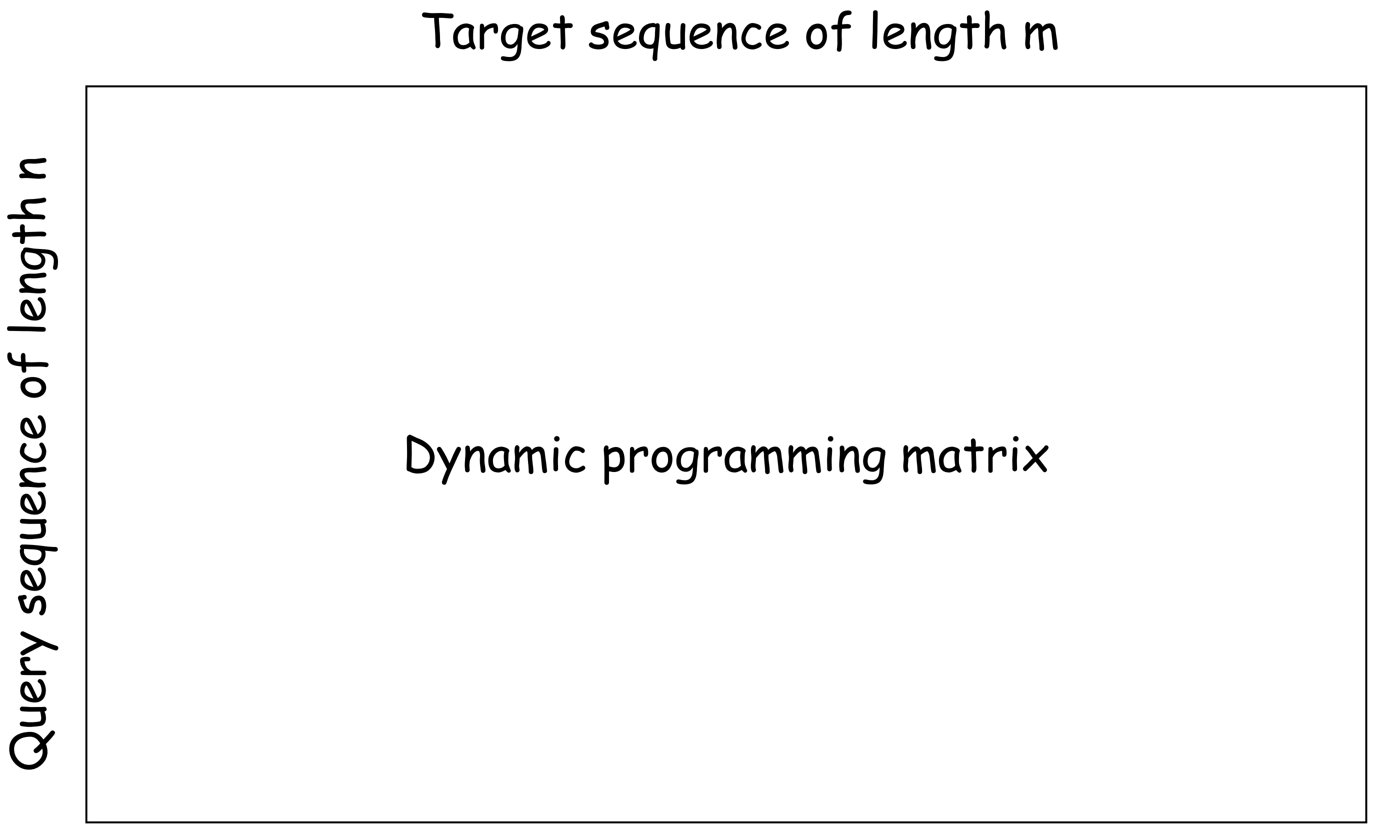
Aligning AAAC with AGC


t
A
G
C
s
A
A
A
C
Best alignment score of AC with GC
Best alignment score of AAAG with C

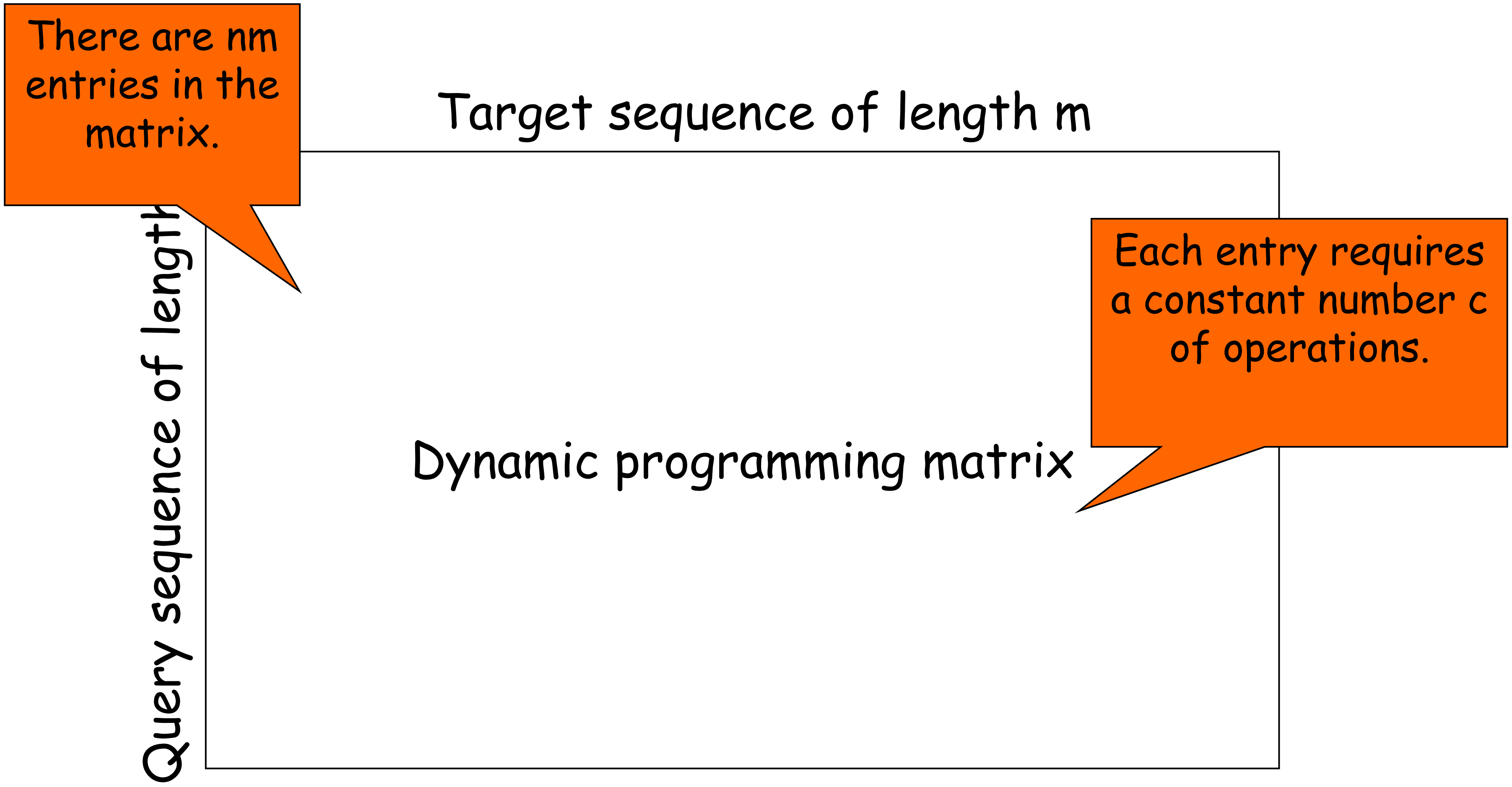
Dynamic Programming Rule

$$\begin{tikzcd}[column sep=huge, row sep=huge]
\text{sim}[i, j] \arrow[r, "\overset{\text{(gap cost)}}{+\ g}"] \arrow[d, "\underset{\text{(gap cost)}}{+\ g}"'] \arrow[dr, "\underset{\text{(similarity score between s[i] and t[j])}}{+\ sc(s[i],t[j])}"] & \text{sim}[i, j+1] \\
\text{sim}[i+1, j] & \text{sim}[i+1, j+1]
\end{tikzcd}$$

How long does DP take?


Target sequence of length m
Query sequence of length n
Dynamic programming matrix

How long does DP take?


There are nm entries in the matrix.
Target sequence of length m
Query sequence of length
Dynamic programming matrix
Each entry requires a constant number c of operations.

The total number of required operations is approximate nmc.
We say that the algorithm is “order nm” or “O(nm).”

Local Alignment

Just described *global alignment*, where we are looking for best match between sequences from one end to the other.

Often (and more commonly), we will want a *local alignment*, the best match between subsequences of *s* and *t*.

Local Alignment DP Algorithm

Original formulation: Smith & Waterman, *Journal of Molecular Biology*, 1981

Interpretation of array values is different from global sequence alignment

sim [*i*, *j*] = score of the best alignment of a prefix of *the i..m* suffix *of s* and a prefix of the *j…n* suffix of *t*

Algorithm is simple modification of DP just described - whenever score goes below 0, start from scratch !

I.e., consider four cases and take max

Database search

Given a sequence of interest, can you find other similar sequences (to get a hint about structure/function)?

- E.g, NCBI BLAST site
  - Input sequence, gives back all significant sequence matches
  - Performs local alignments

Heuristic Methods for Sequence Database Searching

Quadratic algorithm too slow for large databases with high query traffic heuristic methods do fast approximation to dynamic programming

- FASTA [Pearson & Lipman (1988) PNAS 85, p2444]
  - http://www2.ebi.ac.uk/fasta3
- BLAST [Altschul *et al.* (1990) JMB 215, p403]
  - http://www.ncbi.nlm.nih.gov/BLAST

Speeding up searches

Give up optimality, use heuristics

For a query sequence, require its matches to share a k-mer exactly (e.g., k=11)

Fundamental innovation: use hashing (or other search data structures) to find (quickly) places in database where each k-mer in the query sequence occurs

BLAST algorithm

- Remove low-complexity regions.
- Make a list of all words of length 3 amino acids or 11 nucleotides.
- Augment the list to include similar words.
- Scan the database for occurrences of the words
- Connect nearby occurrences.
- Extend the matches.
- Prune the list of matches using a score threshold.
- Evaluate the significance of each remaining match.
    - Very important !
- Perform Smith-Waterman to get an alignment.

BLAST Notes

May fail to find all high-scoring segment pairs
-Heuristic approach

Empirically, more than an order of magnitude faster than Smith-Waterman

Large impact:

- NCBI's BLAST server handles thousands of queries a day
- most used (and cited) bioinformatics program