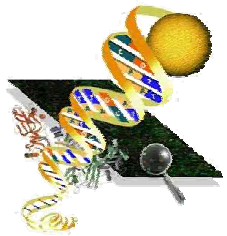
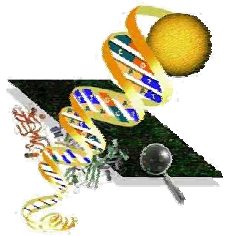
Multiple Sequence Alignments (MSA)



- Multiple sequence alignments
  - Definition
  - The need for MSA
  - The MSA problem
  - MSA methods
- Editing and formatting alignments
  - Software packages available

What is Multiple Sequence Alignment (MSA)?



- Multiple sequence alignment (MSA) can be seen as a generalization of Pairwise Sequence Alignment - instead of aligning two sequences, n sequences are aligned simultaneously, where n is > 2

- **Definition:**

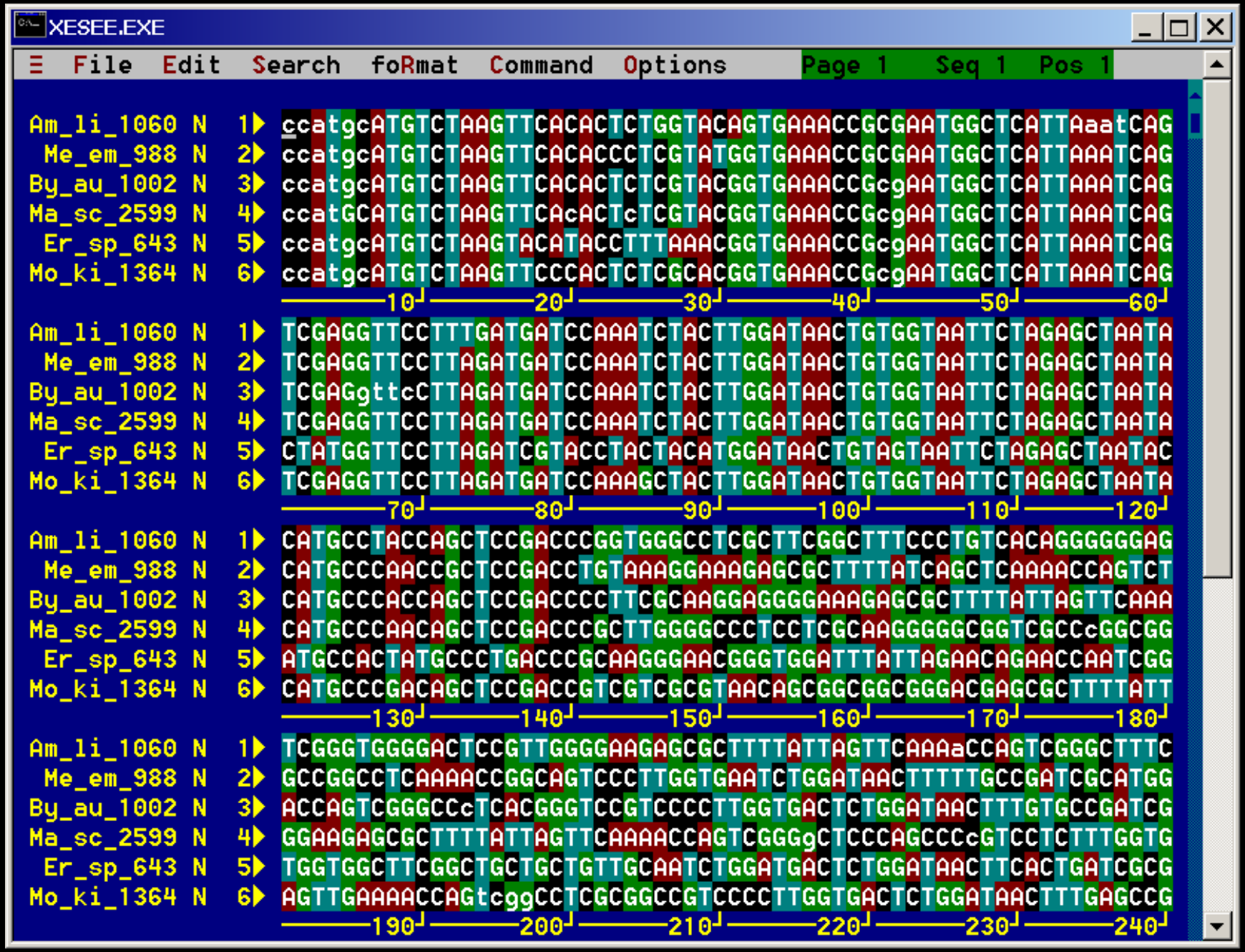
  A multiple sequence alignment is an alignment of n > 2 sequences obtained by inserting gaps ("-") into sequences such that the resulting sequences have all length L and can be arranged in a matrix of N rows and L columns where each column represents a homologous position

- **Note:**

  MSA applies both to nucleotide and amino acid sequences

  To construct a multiple alignment, one may have to introduce gaps in sequences at positions where there were no gaps in the corresponding pairwise alignment

  → multiple alignments typically contain more gaps than any given pair of aligned sequences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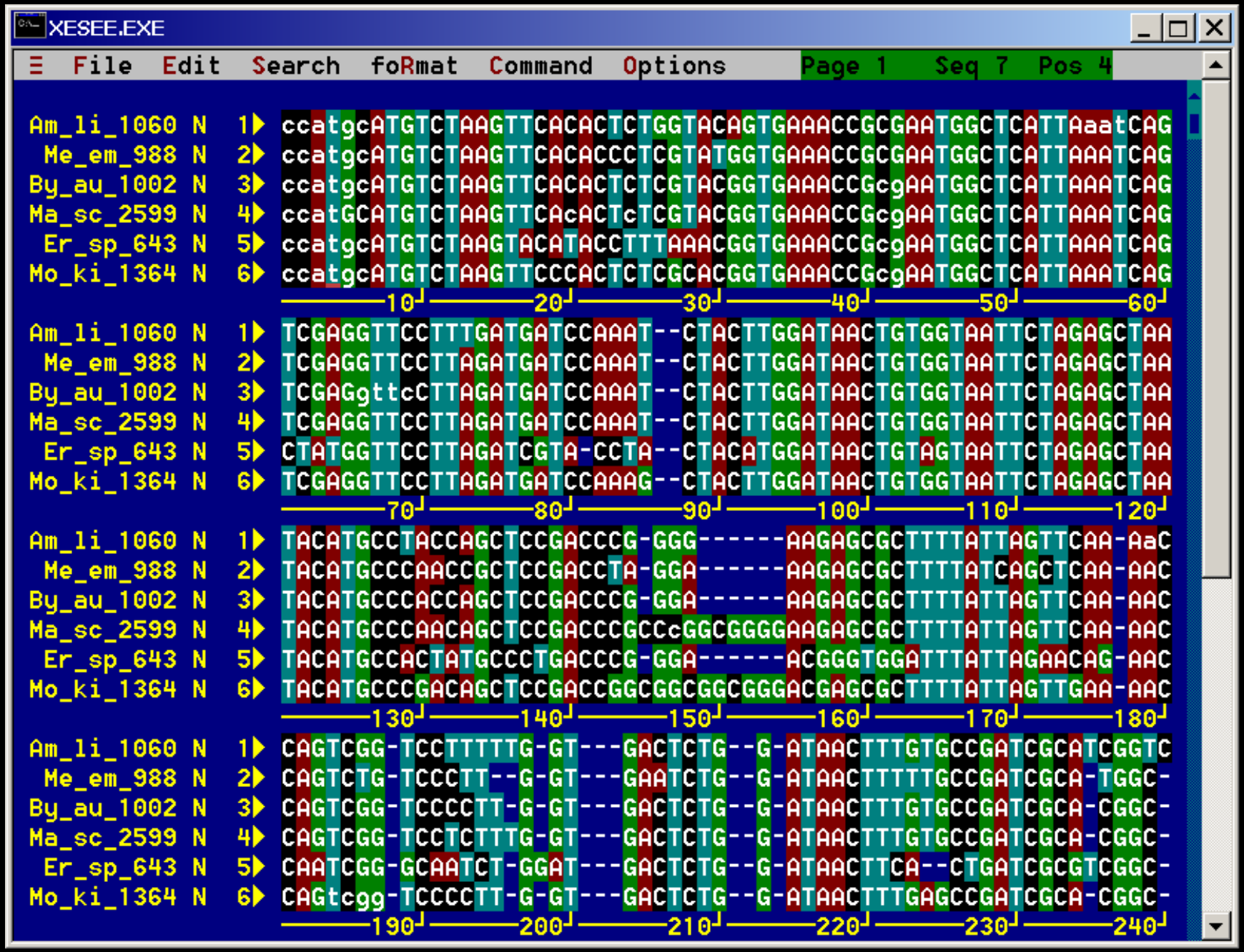
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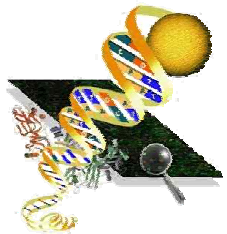
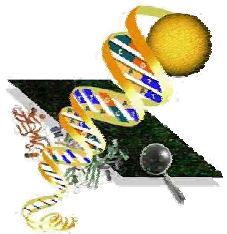
Why do we need MSA?



- Multiple sequence alignment can help to develop a sequence "finger print" which allows the identification of members of distantly related protein family (motifs)
- Formulate & test hypotheses about protein 3-D structure
- MSA can help us to reveal biological facts about proteins, e.g.:
  (e.g. how protein function has changed or evolutionary pressure acting on a gene)
- Crucial for genome sequencing:
  - Random fragments of a large molecule are sequenced and those that overlap are found by a multiple sequence alignment program.
  - There should be one correct alignment that corresponds to the genomic sequence rather than a range of possibilities
  - Sequence may be from one strand of DNA or the other, so complements of each sequence must also be compared
  - Sequence fragments will usually overlap, but by an unknown amount and in some cases, one sequence may be included within another
  - All of the overlapping pairs of sequence fragments must be assembled into large composite genome sequence
- To establish homology for phylogenetic analyses
- Identify primers and probes to search for homologous sequences in other organisms

The alignment problem



- How do we generate a multiple alignment? Given a pairwise alignment, just add the third, then the fourth, and so on, until all have been aligned. Does it work?

Example:

```
Taxon A   AGAC
Taxon B   --AC
```

```
Taxon A   AGAC
Taxon C   AG--
```

```
Taxon B   AC
Taxon C   AG
```

It is not self-evident how these sequences are to be aligned together. Here are some possibilities:

```
Taxon A   AGAC
Taxon B   --AC
Taxon C   AG--
```

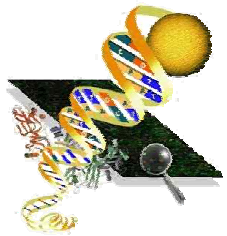
```
Taxon A   AGAC
Taxon C   AG--
Taxon B   --AC
```

```
Taxon B   AC--
Taxon C   AG--
Taxon A   AGAC
```

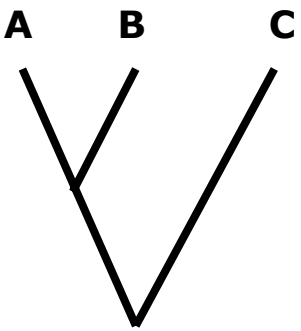
```
Taxon B   --AC
Taxon C   --AG
Taxon A   AGAC
```

- It depends not only on the various alignment parameters but also on the order in which sequences are added to the multiple alignment

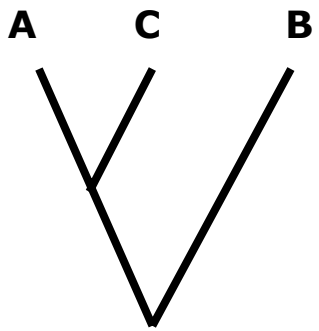
The alignment problem



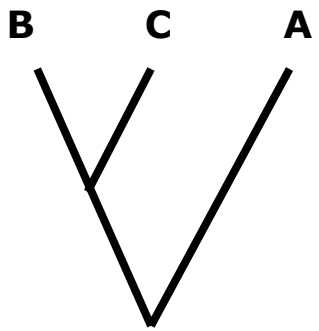
- What happens when a sequence alignment is wrong?



A: AGT
B: AT
C: ATC



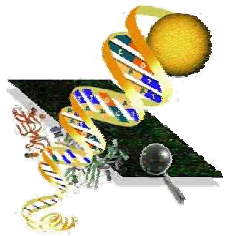
A: AGT
B: A -T
C: ATC



A: AGT
B: AT -
C: ATC

A: AGT -
B: A -T -
C: A -TC

From pairwise to multiple alignments

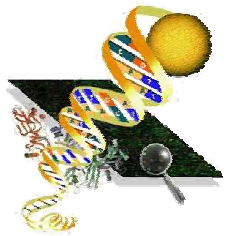


- In pairwise alignments, one has a two-dimensional matrix with the sequences on each axis. The number of operations required to locate the best "path" through the matrix is approximately proportional to the product of the lengths of the two sequences

- A possible general method would be to extend the pairwise alignment method into a simultaneous N-wise alignment, using a complete dynamical-programming algorithm in N dimensions. Algorithmically, this is not difficult to do

But what about execution time?

The big-O notation



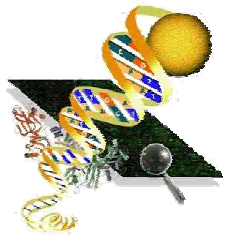
- One of the most important properties of an algorithm is how its execution time increases as the problem is made larger (e.g. more sequences to align).
  This is the so-called **algorithmic** (or computational) **complexity** of the algorithm

- There is a notation to describe the algorithmic complexity, called **the big-O notation**.
  If we have a problem size (number of input data points) n, then an algorithm takes **O(n)** time if the time increases linearly with n. If the algorithm needs time proportional to the square of n, then it is **$O(n^2)$**

- It is important to realize that an algorithm that is quick on small problems may be totally useless on large problems if it has a bad O() behavior. As a rule of thumb one can use the following characterizations, where n is the size of the problem, and c is a constant:

O(c)	utopian
O(log n)	excellent
O(n)	very good
$O(n^2)$	not so good
$O(n^3)$	pretty bad
$O(c^n)$	disaster

The big-O notation



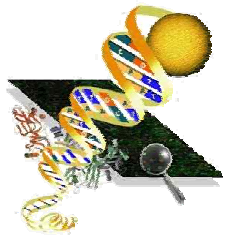
- To compute a N-wise alignment, the algorithmic complexity is something like **$O(c^{2n})$**, where c is a constant, and n is the number of sequences

- Example:

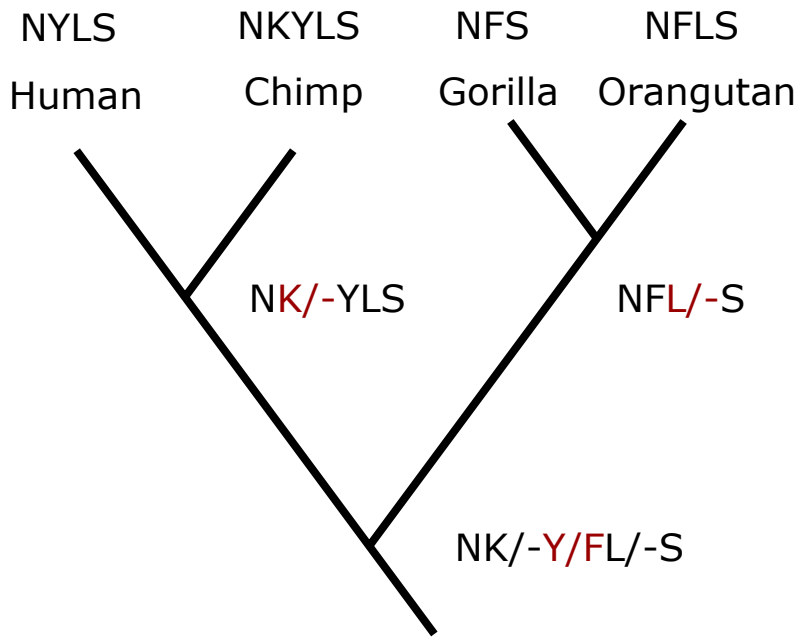
  A pairwise alignment of two sequences [$O(c^{2x2})$], takes 1 second, then four sequences [$O(c^{2x4})$], would take 10^4 seconds (2.8 hours), five sequences [$O(c^{2x5})$], 10^6 seconds (11.6 days), six sequences [$O(c^{2x6})$], 10^8 seconds (3.2 years), seven sequences [$O(c^{2x7})$], 10^{10} seconds (317 years), and so on

This is disastrous!

How to optimize alignment algorithms?

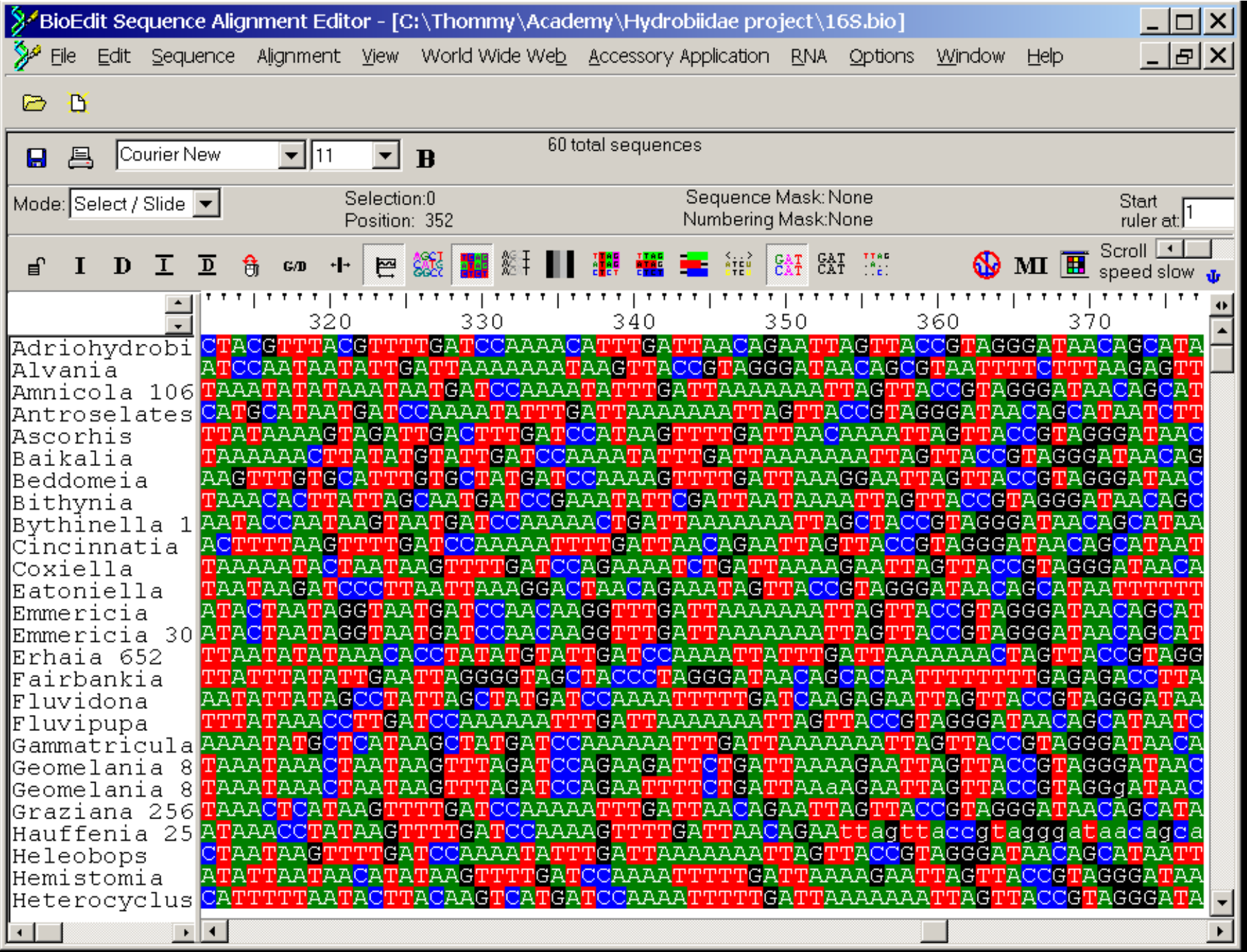


- Use structural information:
  - reading frame
  - protein structure
- Sequence elements are not truly independent but related by phylogeny



	Raw	Alignment
Human	N Y L S	N – Y L S
Chimp	N K Y L S	N K Y L S
Gorilla	N F S	N – F – S
Orangutan	N F L S	N – F L S

- Sequences often contain highly conserved regions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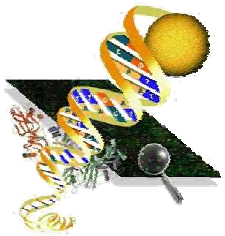
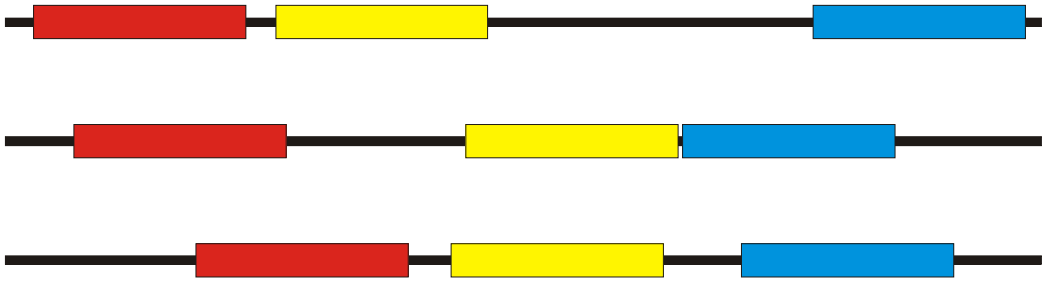
Antroselates
Ascorhis
Baikalia
Beddomeia
Bithynia
Bythinella 1
Cincinnatia
Coxiella
Eatoniella
Emmericia
Emmericia 30
Erhaia 652
Fairbankia
Fluvidona
Fluvipupa
Gammatricula
Geomelania 8
Geomelania 8
Graziana 256
Hauffenia 25
Heleobops
Hemistomia
Heterocyclus

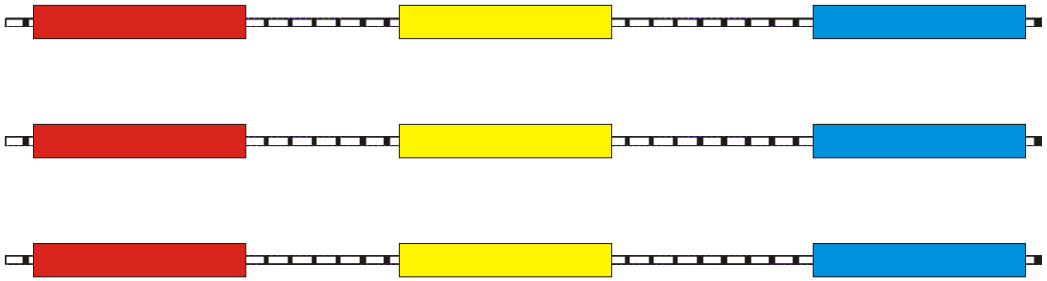
How to optimize alignment algorithms?



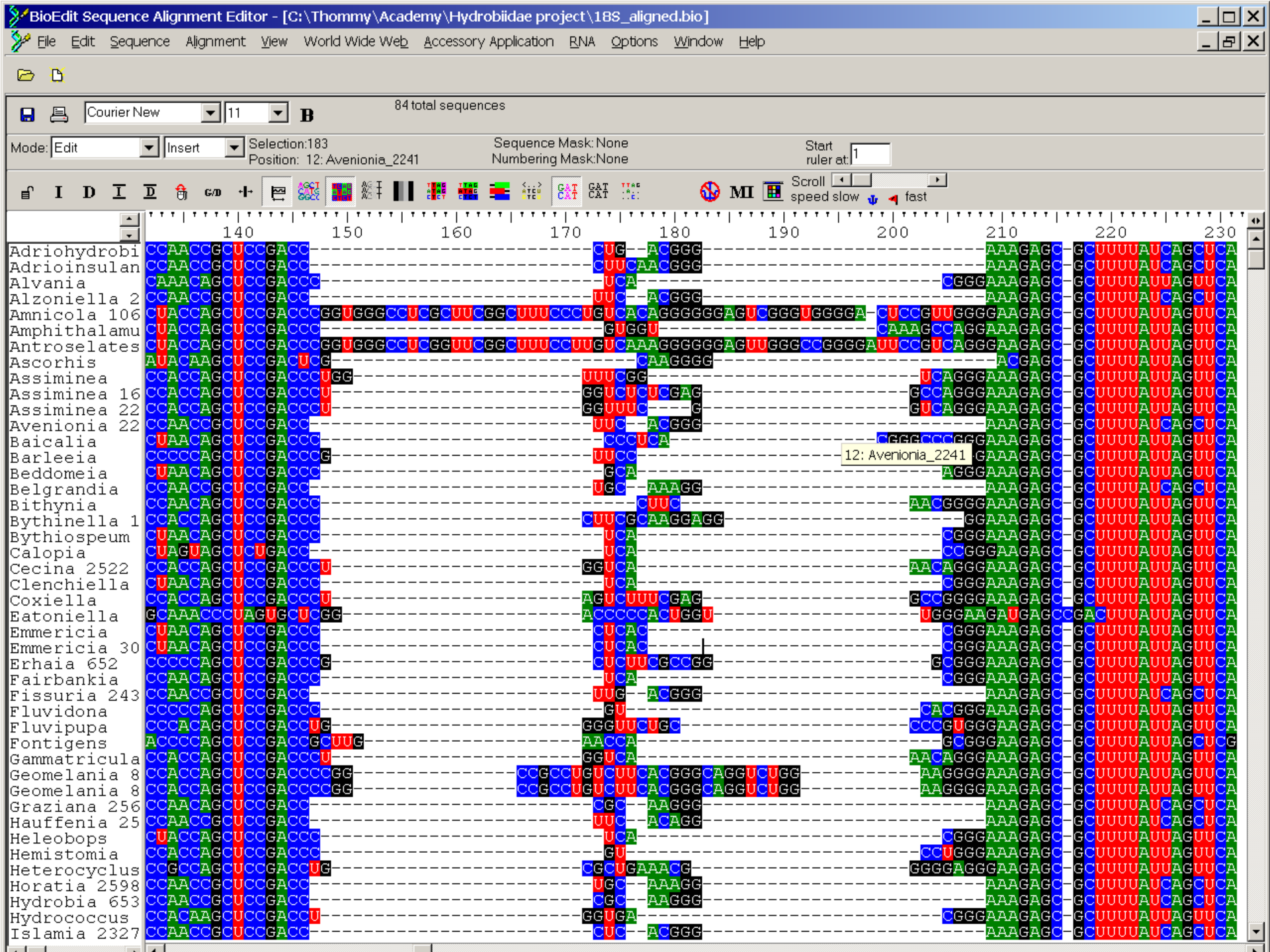
- Sequences often contain highly conserved regions



These regions can be used for an initial alignment



By analyzing a number of small, independent fragments, the algorithmic complexity can be drastically reduced!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Adrioinsulan
Alvania
Alzoniella 2
Amnicola 106
Amphithalamu
Antroselates
Ascorhis
Assiminea
Assiminea 16
Assiminea 22
Avenionia 22
Baicalia
Barleeia
Beddomeia
Belgrandia
Bithynia
Bythinella 1
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Calopia
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Fontigens
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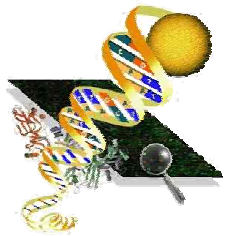
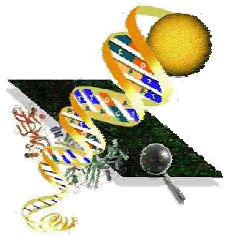
MSA methods



- Progressive global alignment of the sequences starting with an alignment of the most alike sequences and then building an alignment by adding more sequences
- Iterative methods that make an initial alignment of groups of sequences and then revise the alignment to achieve a better result
- Alignments based on locally conserved patterns found in the same order in the sequences

"Optimal" vs. "correct" alignment



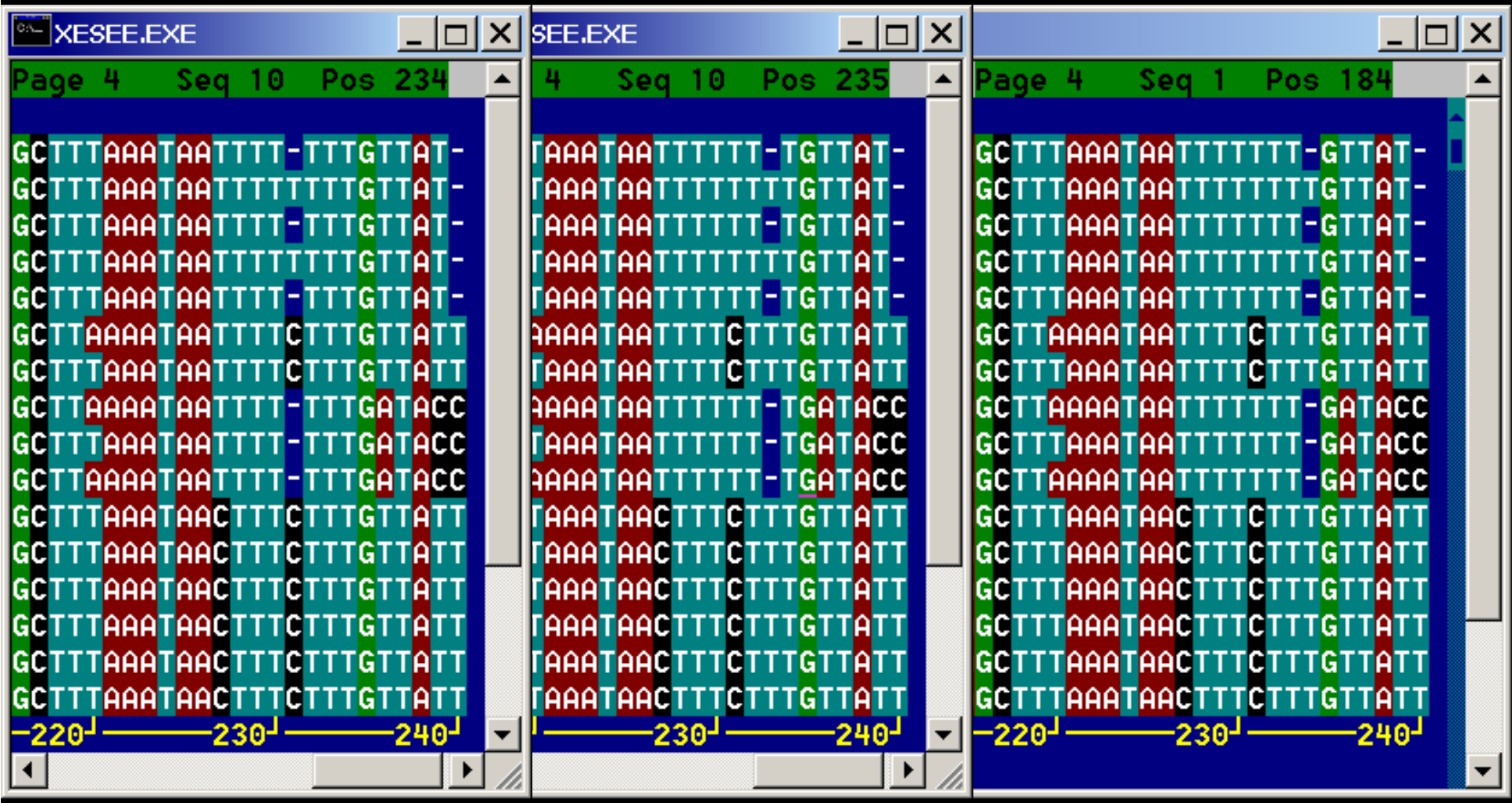
- For a given group of sequences, there is no single "correct" alignment, only an alignment that is "optimal" according to some set of calculations

  This is partly due to:

  - the complexity of the problem,
  - limitations of the scoring systems used,
  - our limited understanding of life and evolution

- Determining what alignment is best for a given set of sequences is really up to the judgment of the investigator

- Success of the alignment will depend on the similarity of the sequences. If sequence variation is great it will be very difficult to find an optimal alignment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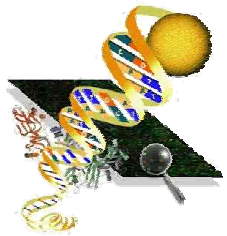
240

MSA and gaps



- **Gaps can occur:**

Before the first character of a string

```
CTGCGGG---GGTAAT
   ||||     || ||
--GCGG-AGAGG-AA-
```

Inside a string

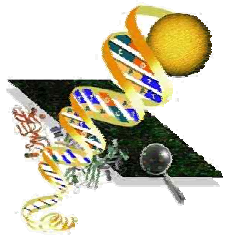
```
CTGCGGG---GGTAAT
   ||||     || ||
--GCGG-AGAGG-AA-
```

After the last character of a string

```
CTGCGGG---GGTAAT
   ||||     || ||
--GCGG-AGAGG-AA-
```

- **Note:** In protein-coding nucleotide sequences most gaps have a length of 3N

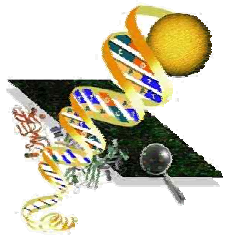
MSA and gaps



Gap Penalties

- In the MSA scoring scheme, a penalty is subtracted for each gap introduced into an alignment because the gap increases uncertainty into an alignment
- The gap penalty is used to help decide whether or not to accept a gap or insertion in an alignment
- Biologically, it should in general be easier for a sequence to accept a different residue in a position, rather than having parts of the sequence chopped away or inserted. Gaps/insertions should therefore be more rare than point mutations (substitutions)
- In general, the lower the gapping penalties, the more gaps and more identities are detected but this should be considered in relation to biological significance
- Most MSA programs allow for an adjustment of gap penalties

MSA with ClustalW



- Works by progressive alignment: it aligns a pair of sequences then aligns the next one onto the first pair
- Most closely related sequences are aligned first, and then additional sequences and groups of sequences are added, guided by the initial alignments

  Uses alignment scores to produce a phylogenetic tree
- Aligns the sequences sequentially, guided by the phylogenetic relationships indicated by the tree
- Gap penalties can be adjusted based on specific amino acid residues, regions of hydrophobicity, proximity to other gaps, or secondary structure
- Is available with a great web interface: http://www.ebi.ac.uk/clustalw/
- Also available as **ClustalX** (stand-alone MS-Windows software)

File Edit View Go Window Help

EMBL-EBI European Bioinformatics Institute

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SEQUENCE ANALYSIS

- Help Index
- General Help
- Formats
- Gaps
- Matrix
- References
- ClustalW Help

ClustalW Submission Form

Clustal W is a general purpose multiple sequence alignment program for DNA or proteins.It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can de seen via viewing Cladograms or Phylograms.

YOUR EMAIL	ALIGNMENT TITLE	CPU MODE	ALIGNMENT	OUTPUT FORMAT
	-NONE-	clustalw_mp	full	aln w/numbers
OUTPUT ORDER	**COLOR ALIGNMENT**	**KTUP (WORD SIZE)**	**WINDOW LENGTH**	**SCORE TYPE**
aligned	no	def	def	percent
TOPDIAG	**PAIRGAP**	**PHYLOGENETIC TREE**	**MATRIX**	**GAP OPEN**
def	def	TREE TYPE: none CORRECT DIST.: off IGNORE GAPS: off	def	def
END GAPS	**GAP EXTENSION**	**GAP DISTANCES**	**TREE TYPE:**	**TREE GRAPH DISTANCES:**
def	def	def	cladogram	hide

Enter or Paste a set of Sequences in any supported format : Help

Operational options

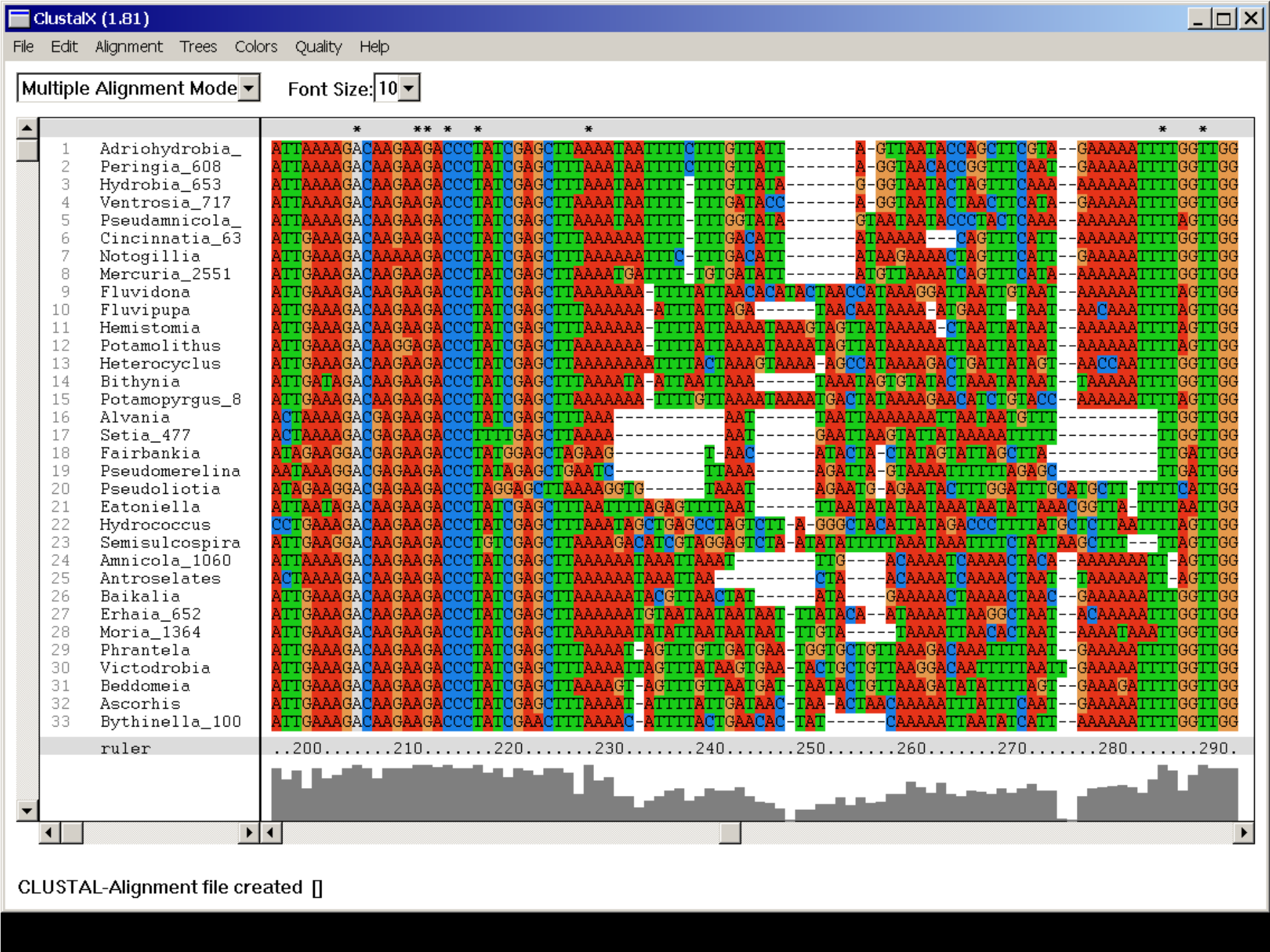
Output options

Input options, matrix choice, gap opening penalty

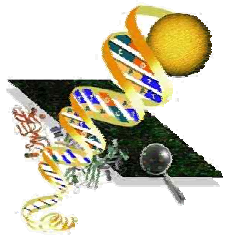
Gap information, output tree type

File input in GCG, FASTA, EMBL, GenBank, Phylip, or several other formats

Document: Done

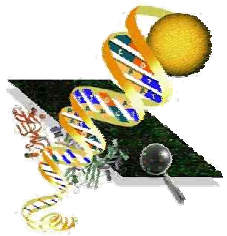

ClustalX (1.81)
File Edit Alignment Trees Colors Quality Help
Multiple Alignment Mode
Font Size: 10
1 Adriohydrobia_
2 Peringia_608
3 Hydrobia_653
4 Ventrosia_717
5 Pseudamnicola_
6 Cincinnatia_63
7 Notogillia
8 Mercuria_2551
9 Fluvidona
10 Fluvipupa
11 Hemistomia
12 Potamolithus
13 Heterocyclus
14 Bithynia
15 Potamopyrgus_8
16 Alvania
17 Setia_477
18 Fairbankia
19 Pseudomerelina
20 Pseudoliotia
21 Eatoniella
22 Hydrococcus
23 Semisulcospira
24 Amnicola_1060
25 Antroselates
26 Baikalia
27 Erhaia_652
28 Moria_1364
29 Phrantela
30 Victodrobia
31 Beddomeia
32 Ascorhis
33 Bythinella_100
ruler
..200.....210.....220.....230.....240.....250.....260.....270.....280.....290.
CLUSTAL-Alignment file created []

MSA with PILEUP



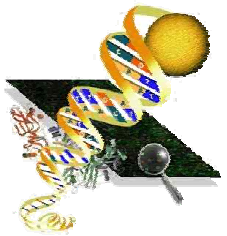
- PILEUP is the MSA program that is part of the Genetics Computer Group (GCG) sequence analysis package
- Sequences are aligned pairwise using dynamic programming algorithm
- The scores are used to produce a phylogenetic tree, which is then used to guide the alignment of the most closely related sequences and groups of sequences
- Resulting alignment is a global alignment produced by the Needleman-Wunsch algorithm

Iterative MSA methods



- Attempt to correct initial alignment problems by repeatedly aligning subgroups of the sequences and then by aligning these subgroups into a global alignment of all the sequences
- MultAlin – recalculates pair-wise scores during the production of the progressive alignment and uses these scores to recalculate the tree
- PRRP – initial alignment is made to predict a tree, the tree is used to produce weights where the sequences are analyzed for the presence of aligned regions that include gaps
- SAGA – based on genetic algorithm that is a machine-learning algorithm that attempts to produce alignments by the simulations of evolutionary changes in sequences

Editing and formatting alignments



- **Sequence editors are used for:**

  - manual alignment/editing of sequences

  - visualization of data

  - data management

  - import/export of data

  - graphical enhancement of data for presentations

- **Examples:**

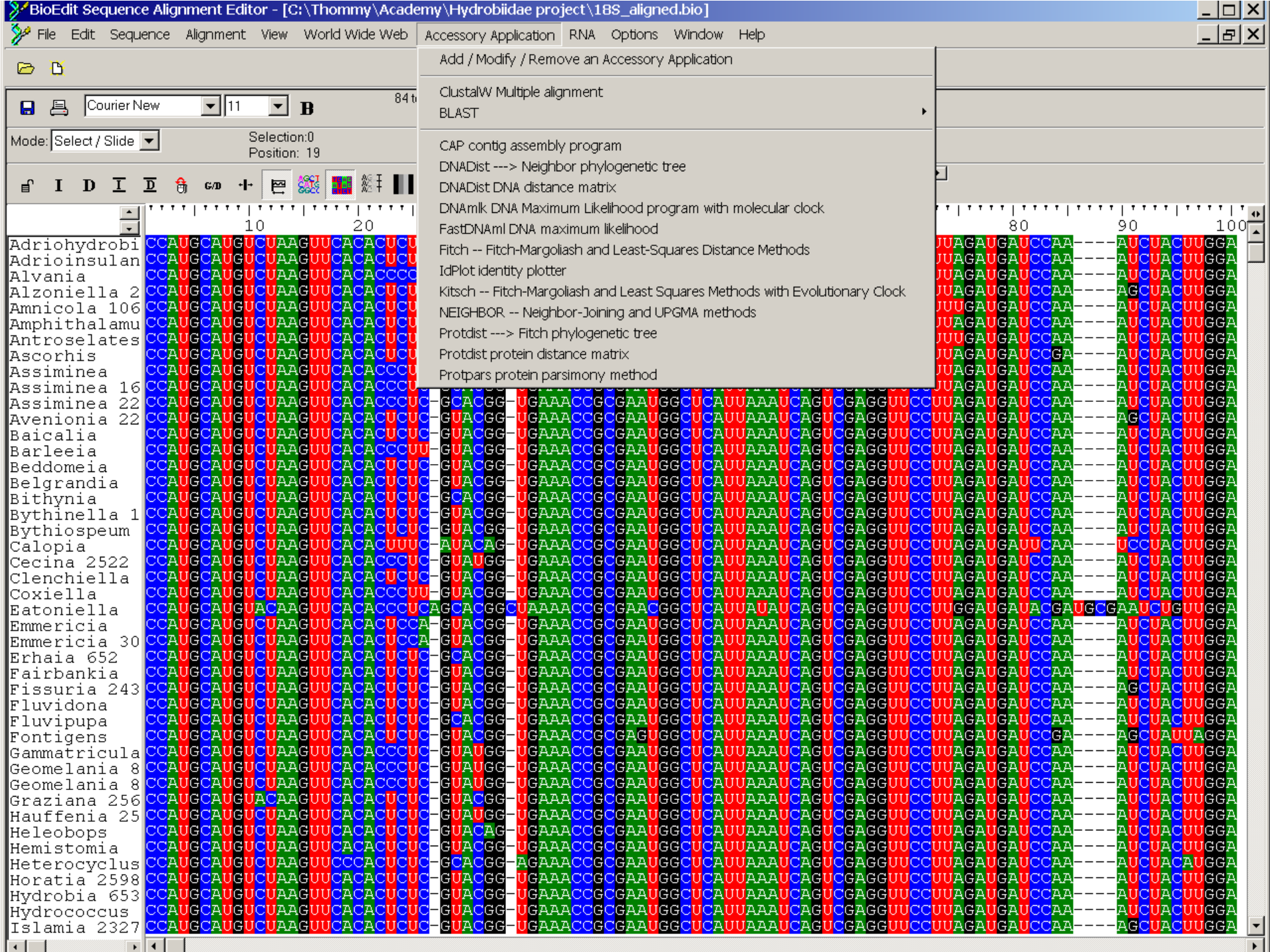
  - **CINEMA** (Color Interactive Editor for Multiple Alignments) web applet http://www.biochem.ucl.ac.uk/bsm/dbbrowser/CINEMA2.02/kit.html

  - **GDE** (Genetic Data Environment) - UNIX based http://bimas.dcrt.nih.gov/gde_sw.html

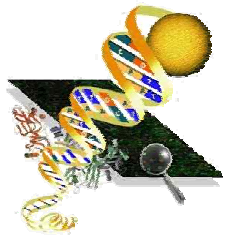
  - **GeneDoc** - MS Windows http://www.psc.edu/biomed/genedoc/

  - **MACAW** - local multiple sequence alignment program and sequence editing tool available by anonymous FTP from ncbi.nih.gov/pub/schuler/macaw

  - **BioEdit** - sequence alignment editor for MS Windows with web access and accessory applications (BLAST, local BLAST, ClustalW, Phylip and more)


BioEdit Sequence Alignment Editor - [C:\Thommy\Academy\Hydrobiidae project\18S_aligned.bio]
File
Edit
Sequence
Alignment
View
World Wide Web
Accessory Application
RNA
Options
Window
Help
Add / Modify / Remove an Accessory Application
ClustalW Multiple alignment
BLAST
CAP contig assembly program
DNADist ---> Neighbor phylogenetic tree
DNADist DNA distance matrix
DNAmlk DNA Maximum Likelihood program with molecular clock
FastDNAml DNA maximum likelihood
Fitch -- Fitch-Margoliash and Least-Squares Distance Methods
IdPlot identity plotter
Kitsch -- Fitch-Margoliash and Least Squares Methods with Evolutionary Clock
NEIGHBOR -- Neighbor-Joining and UPGMA methods
Protdist ---> Fitch phylogenetic tree
Protdist protein distance matrix
Protpars protein parsimony method
Courier New
11
Mode: Select / Slide
Selection:0
Position: 19
10
20
80
90
100
Adriohydrobi
Adrioinsulan
Alvania
Alzoniella 2
Amnicola 106
Amphithalamu
Antroselates
Ascorhis
Assiminea
Assiminea 16
Assiminea 22
Avenionia 22
Baicalia
Barleeia
Beddomeia
Belgrandia
Bithynia
Bythinella 1
Bythiospeum
Calopia
Cecina 2522
Clenchiella
Coxiella
Eatoniella
Emmericia
Emmericia 30
Erhaia 652
Fairbankia
Fissuria 243
Fluvidona
Fluvipupa
Fontigens
Gammatricula
Geomelania 8
Geomelania 8
Graziana 256
Hauffenia 25
Heleobops
Hemistomia
Heterocyclus
Horatia 2598
Hydrobia 653
Hydrococcus
Islamia 2327

Summary MSA



- **Definition:**

  A multiple sequence alignment is an alignment of n > 2 sequences obtained by inserting gaps ("-") into sequences such that the resulting sequences have all length L and can be arranged in a matrix of N rows and L columns where each column represents a homologous position

- **Why do we need MSA?**

  - Formulate & test hypotheses about protein 3-D structure
  - MSA can help us to reveal biological facts about proteins
  - Crucial for genome sequencing
  - To establish homology for phylogenetic analyses
  - Identify primers and probes to search for homologous sequences in other organisms

- **The MSA problem**

  - Most pairwise alignment algorithms are too complex to be used for n-wise alignments
  - Alignment algorithms need to be optimized
    * use structural information
    * use phylogenetic information
    * use conserved regions

- **MSA methods**

  - Progressive global alignment (starts with the most alike sequences)
    * e.g., ClustalW, ClustalX, Pileup
  - Iterative methods (initial alignment of groups of sequences that are revised)
    * MultAlin, PRRP, SAGA
  - Alignments based on locally conserved patterns

- **Sequence editors**

  - CINEMA GDE, GeneDoc, MACAW, BioEdit