Bioinformatics CSM17 Week 7: Molecular Analysis

- Sequence comparison
- Molecular characters
- Homoplasy and convergence
- Multiple Sequence Alignment
- Cladograms from Molecular Data

Molecular data

A T G C A T G C **Sense Strand (Partner)**

| | | | | | | |

A **U** G C A **U** G C Messenger RNA

| | | | | | | |

T A C G T A C G Antisense (Template)

Sequence Comparison

Simple Alignment (see also Skelton & Smith [2002], Sect. 2.2 p29)

match score: 1 *mismatch score* 0

```
A A T C T A T A
A A G A T A       4 + 0 = 4 (best)

A A T C T A T A
  A A G A T A     1 + 0 = 1 (worst)

A A T C T A T A
    A A G A T A       3 + 0 = 3
```

Sequence Comparison

Simple Alignment with gap penalties

match score: 1 *mismatch score* 0 gap penalty -1

```
A A T C T A T A
A A G - A T - A      3 + 0 - 2 = 1     (worst)

A A T C T A T A
A A - G - A T A      5 + 0 – 2 = 3     (equal best)

A A T C T A T A
A A - - G A T A      5 + 0 – 2 = 3     (equal best)

A A T C T A T A
- A A G A T A -      1 + 0 – 2 = -1 (worst)
```

Sequence Comparison

Simple Alignment with origination and length penalties

match score: 1 *mismatch score* 0

origination penalty: -2 *length penalty* -1

```
A A T C T A T A
A A - G - A T A   5 + 0 - 4 - 2        = -1   (worst)

A A T C T A T A
A A - - G A T A   5 + 0 - 2 - 2        = 1    (best)
```

Origination penalty is applied for starting a series of gaps

Length penalty is also applied for each gap

Mutation (and copying errors)

Changes of nucleotide base sequences

- caused by
  - ionizing radiation, mutagenic chemicals, errors
- Mutations are usually harmful (damaging)
- may be
  - single base (changing one amino acid)
  - frameshift (more serious – indels in Open Reading Frames)

Transitions (most common)

- Purine to Purine

  A changed to G

  G changed to A

- Pyrimidine to Pyrimidine

  C changed to T

  T changed to C

Transversions (less common)

- Purine to Pyrimidine

  A changed to C or T

  G changed to C or T

- Pyrimidine to Purine

  C changed to A or G

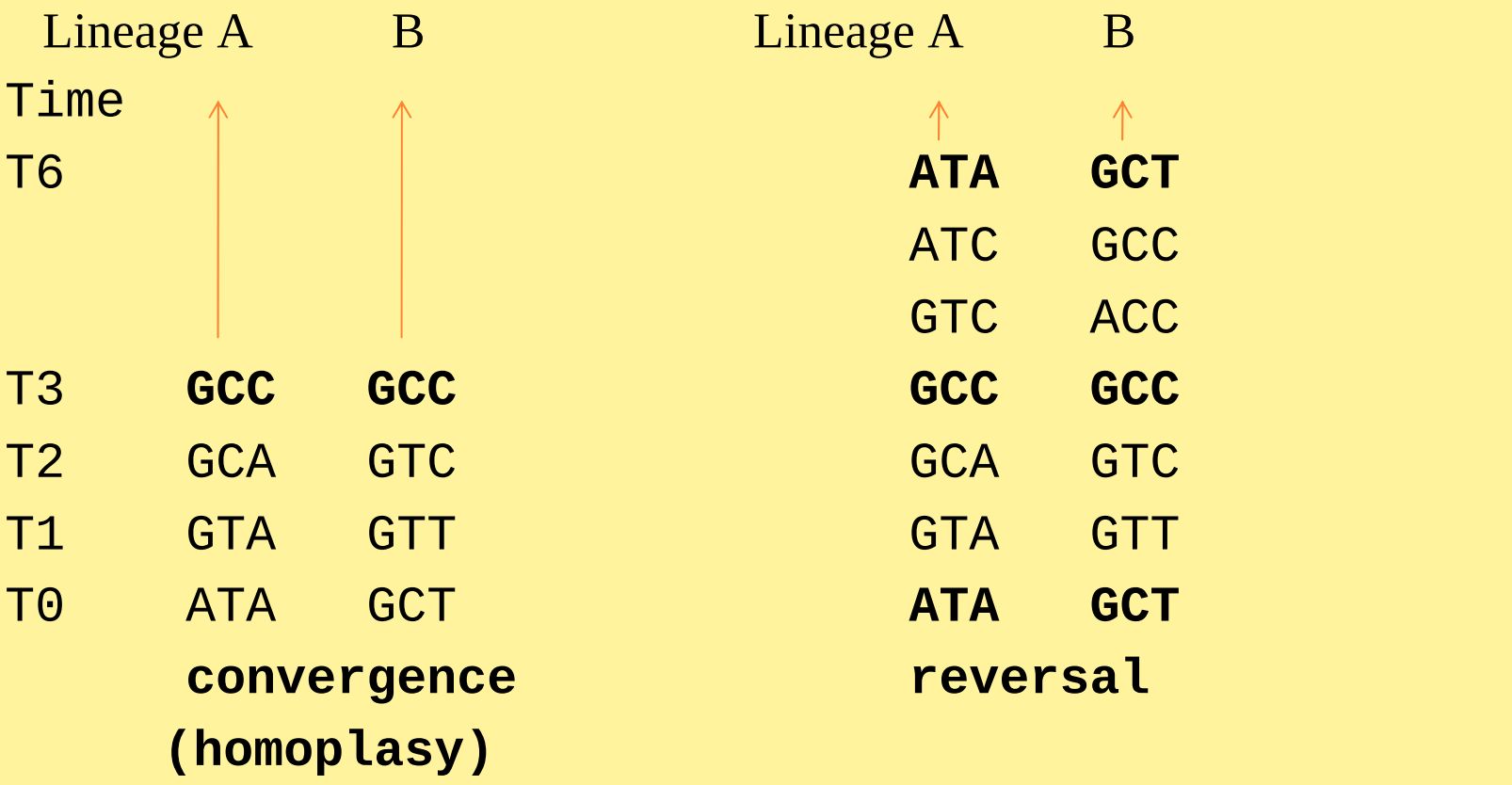
  T changed to A or G

Molecular Character Definitions

See also Skelton & Smith [2002], Sect. 2.3 p33)

- Uninformative Sites
  - invariant sites (all bases the same)
  - phylogenetically uninformative
- Informative Sites
  - cause some trees to be more parsimonious

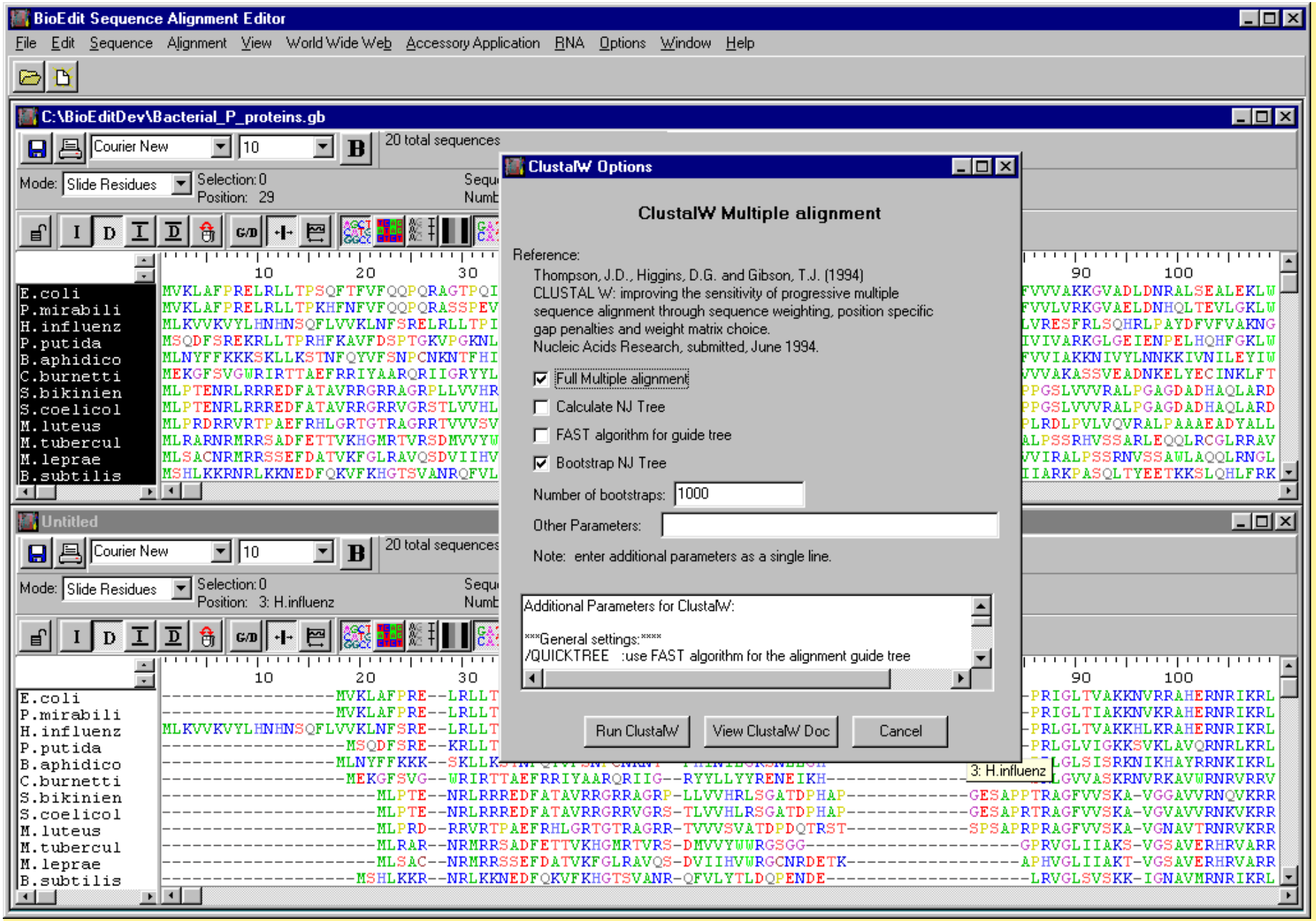
Homoplasy and convergence



Adapted from Skelton & Smith (2002)

Multiple Sequence Alignment

- … to enable production of cladogram
- Clustal W
- Using BioEdit (for Windows)
- Or MacClade (Mac OS X)
- Save alignment …


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View World Wide Web Accessory Application RNA Options Window Help
C:\BioEditDev\Bacterial_P_proteins.gb
Courier New
10
20 total sequences
Mode: Slide Residues
Selection: 0
Position: 29
E.coli
P.mirabili
H.influenz
P.putida
B.aphidico
C.burnetti
S.bikinien
S.coelicol
M.luteus
M.tubercul
M.leprae
B.subtilis
ClustalW Options
ClustalW Multiple alignment
Reference:
Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994)
CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice.
Nucleic Acids Research, submitted, June 1994.
Full Multiple alignment
Calculate NJ Tree
FAST algorithm for guide tree
Bootstrap NJ Tree
Number of bootstraps: 1000
Other Parameters:
Note: enter additional parameters as a single line.
Additional Parameters for ClustalW:
General settings:*
/QUICKTREE :use FAST algorithm for the alignment guide tree
Run ClustalW
View ClustalW Doc
Cancel
Untitled
Mode: Slide Residues
Selection: 0
Position: 3: H.influenz
3: H.influenz

Cladograms from Molecular Data

- Using PAUP (Phylogenetic Analysis Using Parsimony)
- … import alignment file
- Generate cladogram
- View Cladogram with TreeView

Useful Websites

- NCBI Genbank
  www.ncbi.nlm.nih.gov/Genbank/index.html
- PAUP
  http://paup.csit.fsu.edu/
- European Molecular Biology Laboratory
  www.embl.org
- BioEdit
  www.mbio.ncsu.edu/BioEdit/bioedit.html

References & Bibliography

- Skelton, P. & Smith, A (2002). *Cladistics – a practical primer on CD-ROM*. Cambridge University Press, UK. ISBN 0-521-52341 (hardback + CD-ROM)
- Kitching, I. J. *et al*. (1998) *Cladistics - the theory and practice of parsimony analysis*. Systematics Association Publication No. 11. Oxford University Press, UK. ISBN 0-19-850138 (paperback)
- Gibas, C. & Jambeck, P. (2001). *Developing bioinformatics computer skills*. O'Reilly, USA. Chapter 8, p191-214 ISBN 1-56592-664-1 (paperback)
- Page, R.D.M. & Holmes, E.C. (1998). Molecular *Evolution – A Phylogenetic Approach*, Blackwell Publishing, Malden, MA, USA. ISBN 978-0-86542-889-8 (softback)