Evolutionary Analysis

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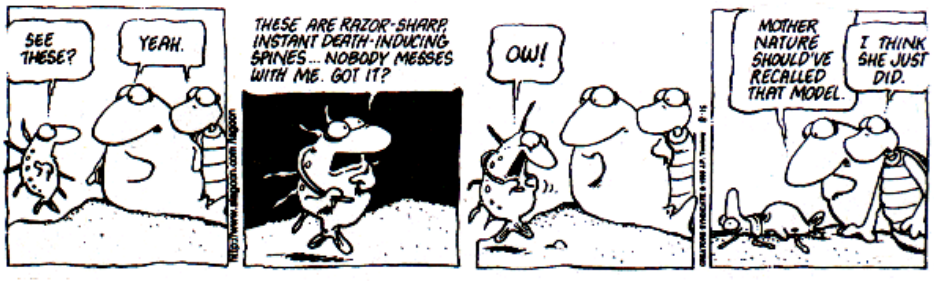


Why care about Evolutionary Analysis?

What do

- BLAST
- Protein motif searching
- Protein threading
- Multiple sequence alignment

Have in common?

Why care about Evolutionary Analysis?

Gene family identification

Gene discovery – inferring gene function, gene annotation

Origins of a genetic disease, characterization of polymorphisms

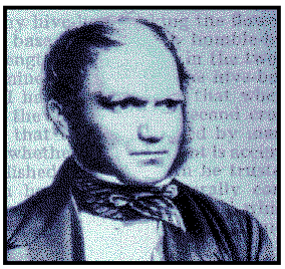
Why care about Evolutionary Analysis?

Koski LB, Golding GB

The closest BLAST hit is often not the nearest neighbor.
J Mol Evol. 2001 Jun;52(6):540-2.

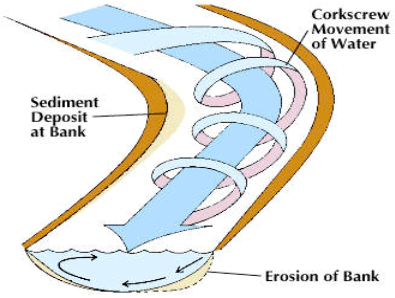
Evolutionary Analysis: Key Concepts

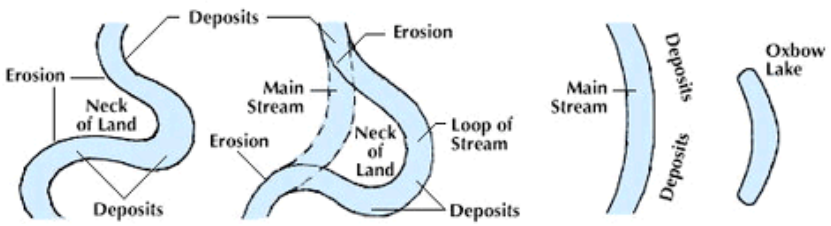
- Foundation of most bioinformatic analyses: Evolutionary theory
- Unique verses non-unique characters
- Sequence alignments are important!
- Fundamentals of phylogenetics and interpreting phylogenetic trees (with cautionary notes)
- Overview of some common phylogenetic methods
- Appreciate the need for new algorithms



18th and 19th centuries: The evolution of a theory

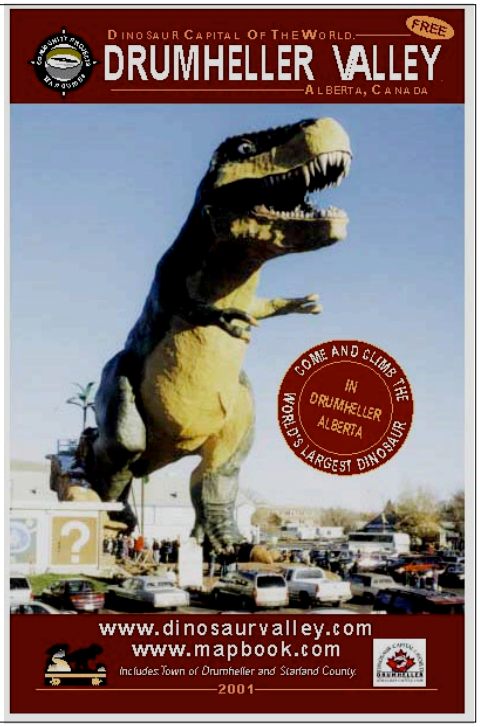
- Earth erosion, sediment deposition, strata – present earth conditions provide keys to the past

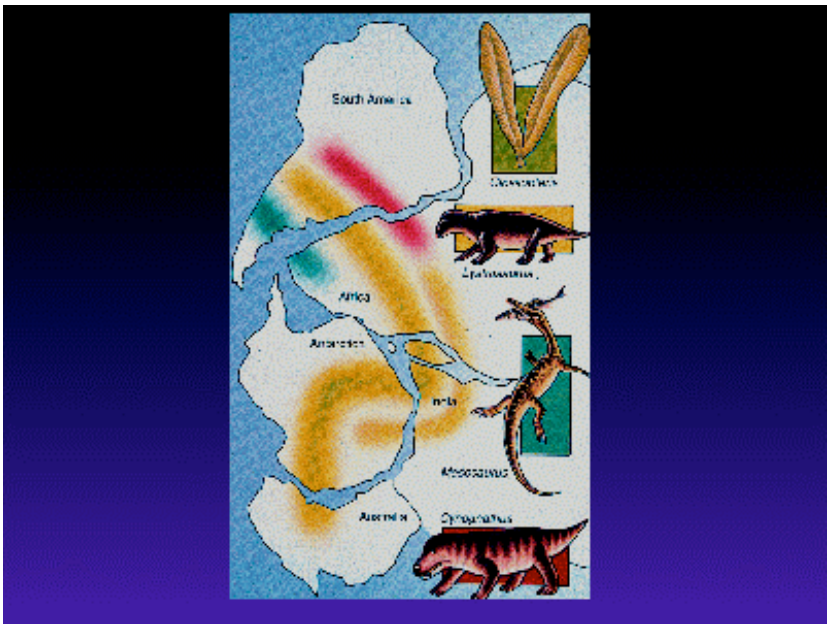




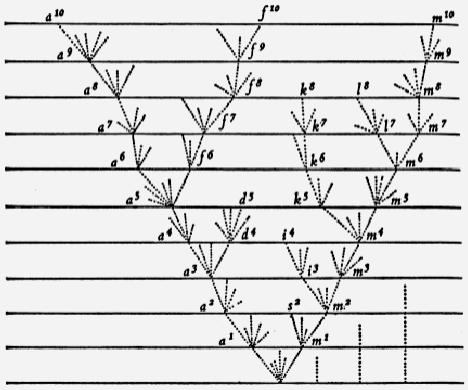
18th and 19th centuries: The evolution of a theory

- Discoveries of fossils accumulated
  - Remains of unknown but still living species that are elsewhere on the planet?
  - Cuvier (circa 1800): the deeper the strata, the less similar fossils were to existing species



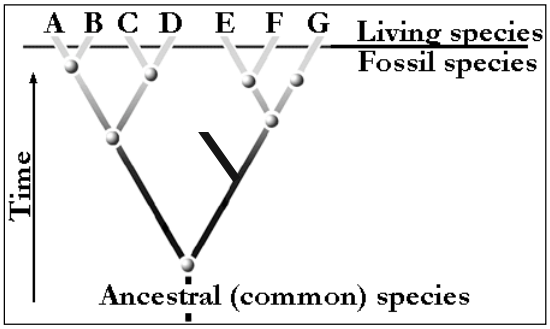


Darwin: "Origin of the species"



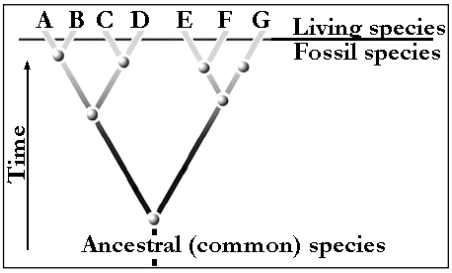
Part of Darwin's Theory

- The world is not constant, but changing
- All organisms are derived from common ancestors by a process of branching.



Part of Darwin's Theory

- This explained…
  - Fossil record
  - Similarities of organisms classified together (shared traits inherited from common ancestor)
  - Similar species in the same geographic region



  - Morphological character-based analysis

What is evolution?

- Come up with a definition in 10 words or less
- Bonus: 2 or 3 word definition!

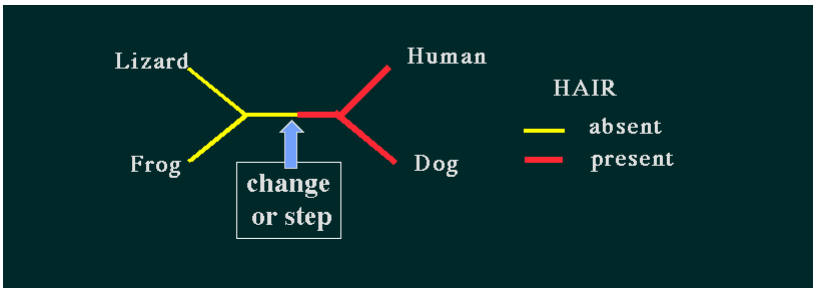
Think – Pair - Share

Characters

- Heritable changes in features (morphology, DNA sequence etc…)
- The more similar characters you have, the more related you are
- However….. characters can be unique and non-unique

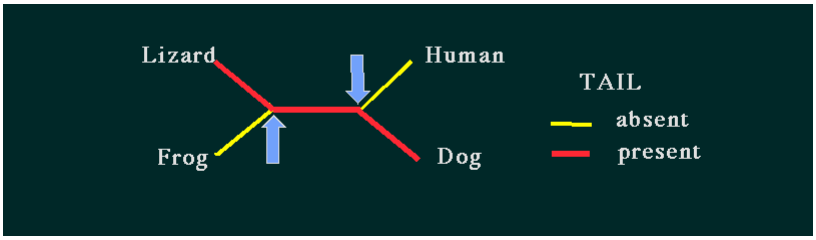
A Unique Character: Hair for Mammals

- Hair evolved only once and is "unreversed"
- Presence of hair → strong indication that organism is a mammal

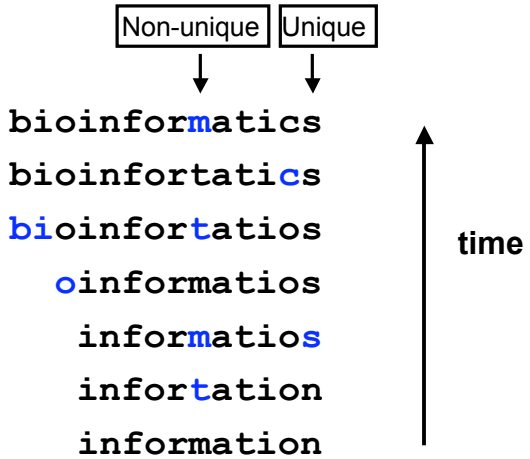


Homoplasy: The formation of tails

- Tails evolved independently in the ancestors of frogs and humans
- Presence of a tail → no useful conclusions



Unique and non-unique characters



Unique and non-unique characters

Example: Sequence analysis of functionally similar transporters

All share the same deleted sequence region, which is not found in any other transporter examined to date

→Unique character?

→Further investigate for possible functional significance, or use for classification

Unique and non-unique characters

Example: Sequence analysis of functionally similar transporters

All have isoleucine at the third position in the sequence, however some other transporters have isoleucine there too, while some other transporters have leucine at that position

→Non-unique.

→Changes from I → L → I are common (see BLOSUM OR PAM matrices). Not a high priority for further analysis of significance and not useful for classification.

Classification according to characters – more characters can be good

	Colour	Skin	Cost
Beef	red	no	$$$
Duck	red	yes	$$$
Pork	white	no	$$
Chicken	white	yes	$
Tofu	white	sometimes	$

Chicken most similar to Tofu?

Classification according to characters

	Colour	Skin	Cost	Legs
Beef	red	no	$$$	four
Duck	red	yes	$$$	two
Pork	white	no	$$	four
Chicken	white	yes	$	two
Tofu	white	sometimes	$	none

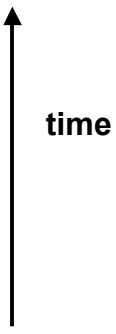
Classification according to characters – increasing the number of characters

	Colour	Skin	Cost	Legs	Feathers	Hair
Beef	red	no	$$$	four	no	yes
Duck	red	yes	$$$	two	yes	no
Pork	white	no	$$	four	no	yes
Chicken	white	yes	$	two	yes	no
Tofu	white	sometimes	$	none	no	no

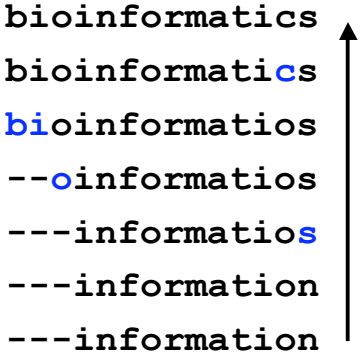
Chicken most similar to Duck?

Evolution and characters – the importance of comparing characters with common origins (homologous)

```
bioinformatics
bioinformatics
bioinformatios
oinformatios
informatios
information
information
```



Evolution and characters



- Gaps represent non-homologous positions in the sequence.
- They reflect the occurrence of insertions/deletions or other rearrangements during the evolutionary process.

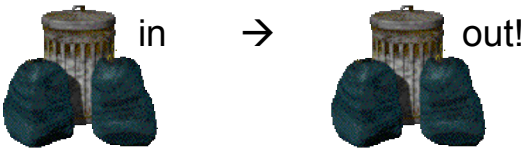
Multiple Sequence Alignment

```
VTISCTGSSSNIGAG-NHVKWYQQLPG
VTISCTGTSSNIGS--ITVNWYQQLPG
LRLSCSSSGFIFSS--YAMYWVRQAPG
LSLTCTVSGTSFDD--YYSTWVRQPPG
PEVTCVVVDVSHEDPQVKFNWYVDG--
ATLVCLISDFYPGA--VTVAWKADS--
AALGCLVKDYFPEP--VTVSWNSG---
VSLTCLVKGFYPSD--IAVEWESNG--
```

The sole purpose of multiple sequence alignments is to place *homologous positions* of *homologous sequences* into the *same column*.

Multiple sequence alignments and phylogenetic analysis

- *First step in any phylogenetic analysis*
- Phylogenetic analysis only as good as the alignment



Multiple Sequence Alignment - uses

Powerful tool

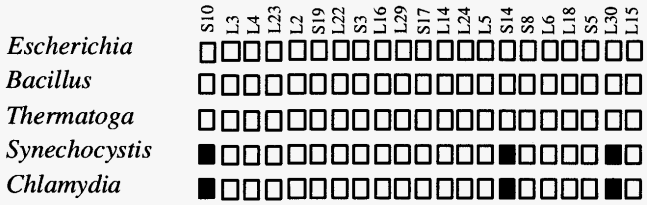
- Detect trends/patterns in homologous sequences (motifs, domains, indels)

  - Indels (insertions and deletions) of evolutionary interest, yet not incorporated into some phylogenetic tree algorithms

```
- ATTYNETCITRTQ -
- SITYNETCVTITQ -
- SVTY-----CIVR -
```

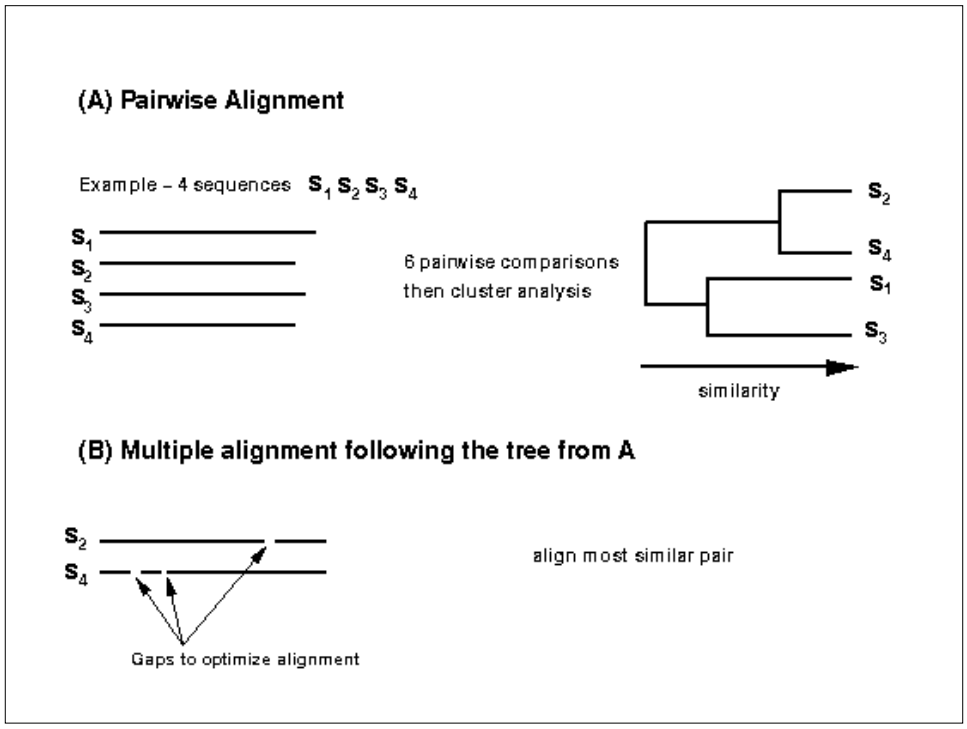
Multiple alignments – not just sequence…

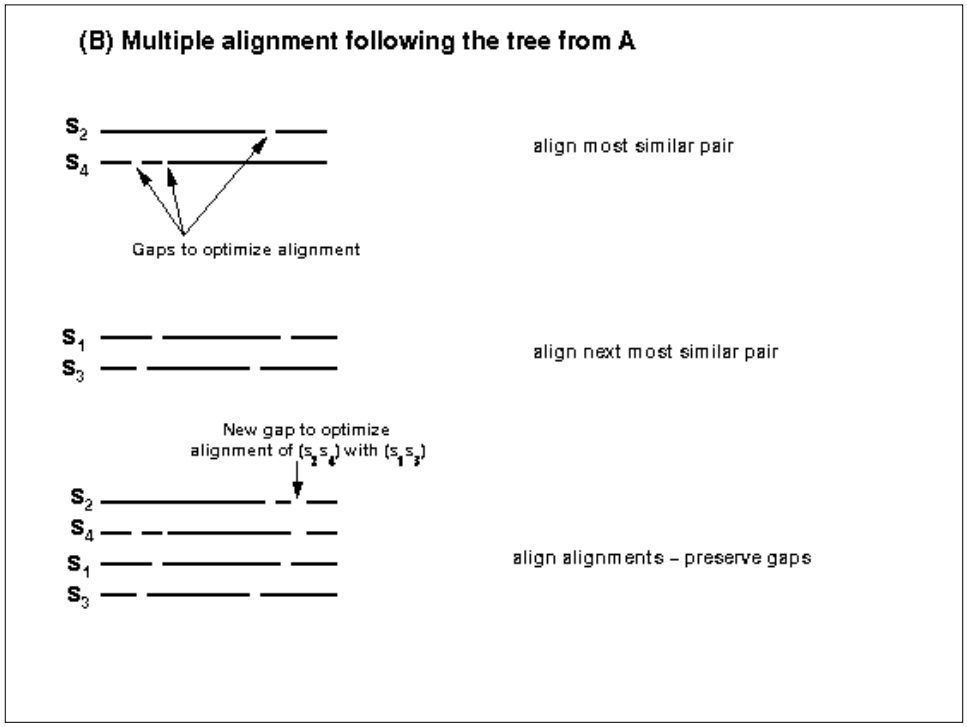
Unique shared-derived characters on the ribosomal super operon unite Cyanobacteria and Chlamydiae

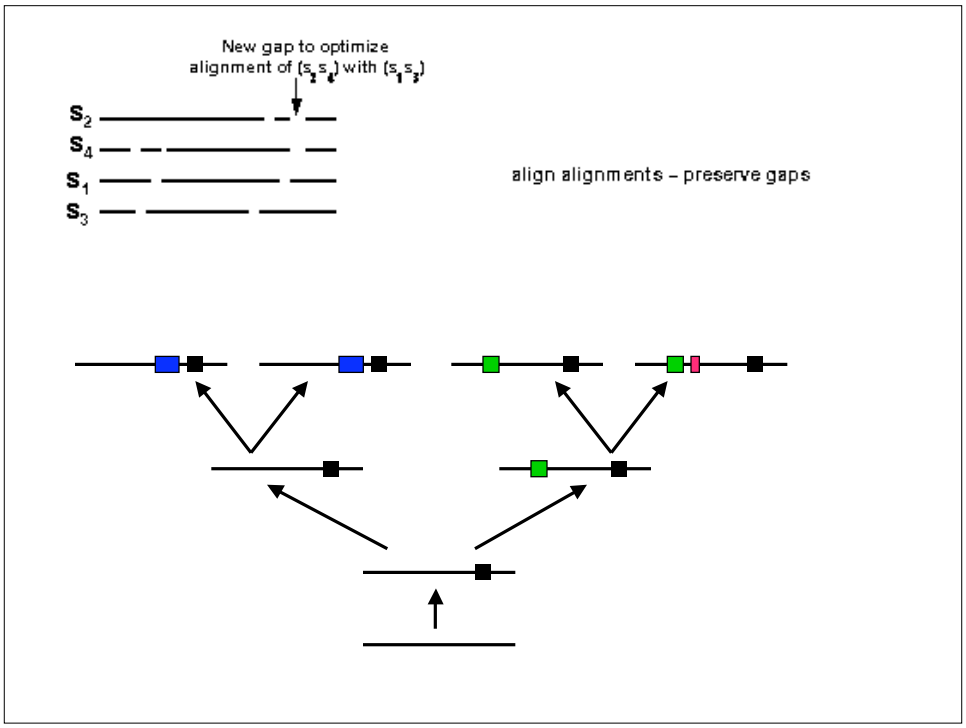


Clustal: Adding evolutionary theory to multiple sequence alignment

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680.


(A) Pairwise Alignment
Example – 4 sequences S1 S2 S3 S4
S1
S2
S3
S4
6 pairwise comparisons
then cluster analysis
S2
S4
S1
S3
similarity
(B) Multiple alignment following the tree from A
S2
S4
Gaps to optimize alignment
align most similar pair


(B) Multiple alignment following the tree from A
S2
S4
Gaps to optimize alignment
align most similar pair
S1
S3
align next most similar pair
New gap to optimize
alignment of (s2 s4) with (s1 s3)
S2
S4
S1
S3
align alignments – preserve gaps



Clustal: Incorporating Biology into Sequence Alignment Algorithms

- Matrices varied at different alignment stages according to the divergence of the sequences
- Gap penalties differ for hydrophilic regions to encourage new gaps in potential loop regions
- Gapped positions in early alignments - reduced gap penalties to encourage the opening up of new gaps at these positions
- (gaps not penalized as much at the end of proteins)

ClustalX

- Subset of sequences in alignment can be selected and realigned. Useful when trying to align very divergent sequences.
- A range of the sequence alignment can be selected for realignment. Guide tree built based only on the residue range selected.

Genedoc - for editing and flexible display of alignments

Statistics Report

1: # residues identical
2: # residues > zero score (similar residues)
3: # residues lined up with a gap

	human	rat	rabbit	turtle
human	1870	97%	96%	22%
	0	98%	96%	28%
	0	0%	2%	61%
rat	1830	1874	94%	22%
	1846	0	95%	28%
	18	0	2%	61%
rabbit	1818	1793	1863	22%
	1828	1815	0	28%
	45	53	0	61%
turtle	584	585	582	1660
	734	737	734	0

Standard multiple sequence alignment approach (first step for phylogenetic analysis)

- Be as sure as possible that the sequences included are homologous
- Know as much as possible about the gene/protein in question before trying to create an alignment (secondary structure etc..)
- Start with an automated alignment: preferably one that utilizes some evolutionary theory such as Clustal

- Ensure aligned residues/bases evolved from a common ancestor
- Note indels (insertions and deletions)
- Remove unreliably aligned regions for phylogenetic analysis

```
ILPITSPSKEGYESGKAPDEFSSGG
ILPEH--IKDDGELGAAPHSFSTAG
VLPLD-----S--AGRPADSFSAAG
VLPVDR-------DGQARDEYT-VG
VLPVDN-------KGEARDEYT-VG
LLPYDD-------QGRPQDDYSRAG
GIVSRSG---SNFDGEPKDSYGKVG
```

Delete?

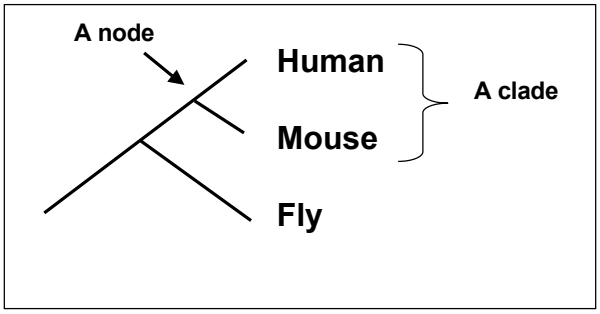
If aligning DNA sequence for phylogenetic analysis: may remove every third codon position

```
MMET  GLY  SER GLY
 MET  GLY  SER GLY
 MET  ARG  CYS ARG

AATG GGA AGT GGA
 ATG GGG AGC GGG
 ATG AGG TGC AGG
```

Another good reason to evolve

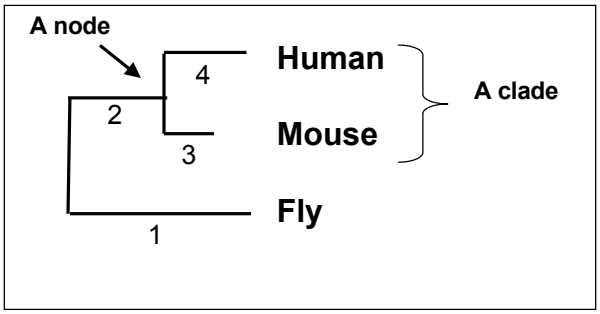
A phylogenetic tree



taxon -- Any named group of organisms – evolutionary theory not necessarily involved.

clade -- A monophyletic **taxon** (evolutionary theory utilized)

A phylogenetic tree with branch lengths



Branch length can be significant…

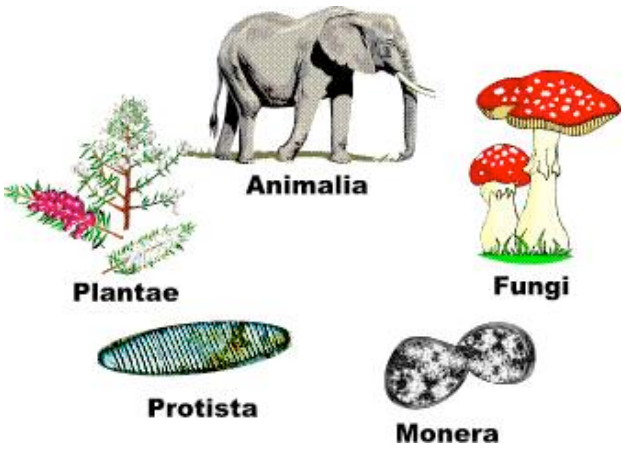
In this case it is and mouse is slightly more similar to fly than human is to fly

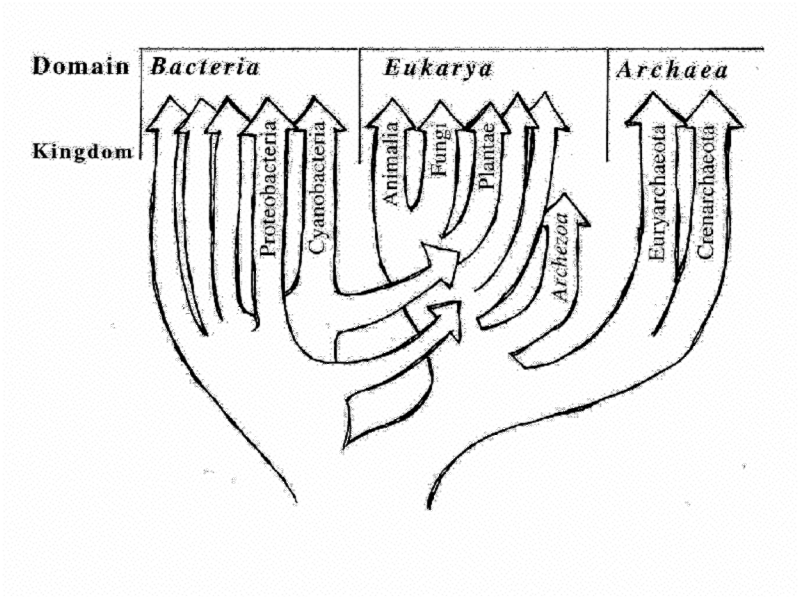
(sum of branches 1+2+3 is less than sum of 1+2+4)

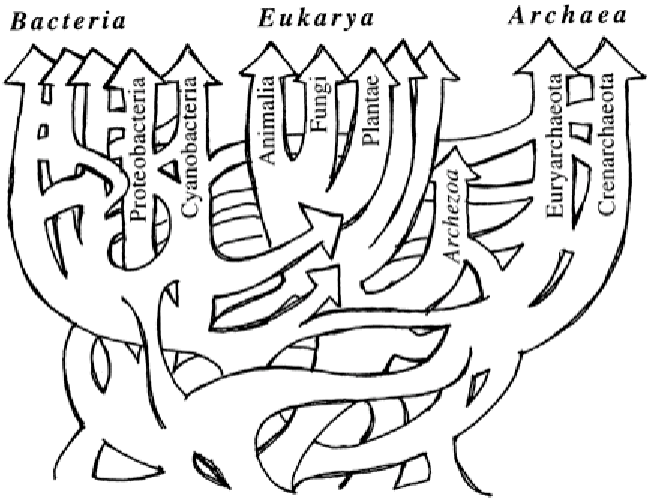
Phylogenetic analysis

- **Organismal relationships**
- **Gene/Protein relationships**

Organismal relationships

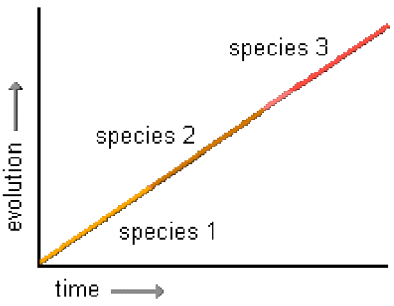


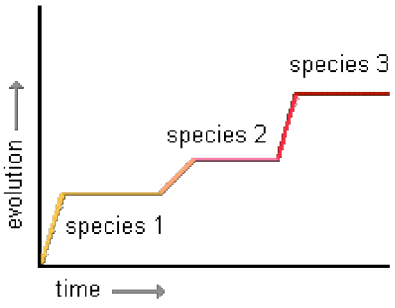




Improving our understanding of organismal relationships

Realization that rates of change are not constant





Improving our understanding of organismal relationships

Better appreciation for what sequences may be suitable for analysis of different degrees of divergence

For the tree of life:

rRNA genes

↓

Multiple genes

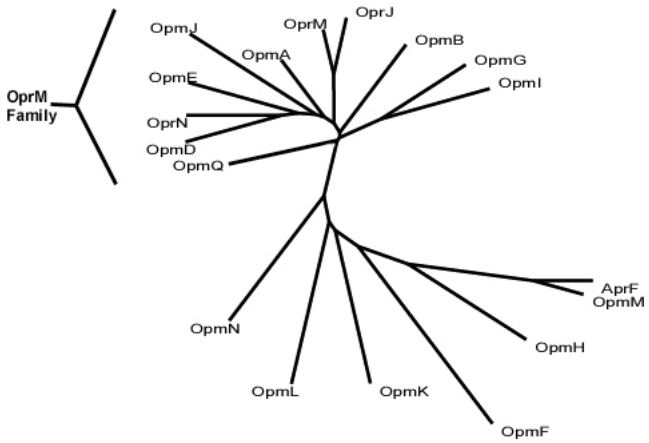
↓

"Whole genome" datasets of genes

↓

rRNA genes!

Gene/Protein Relationships



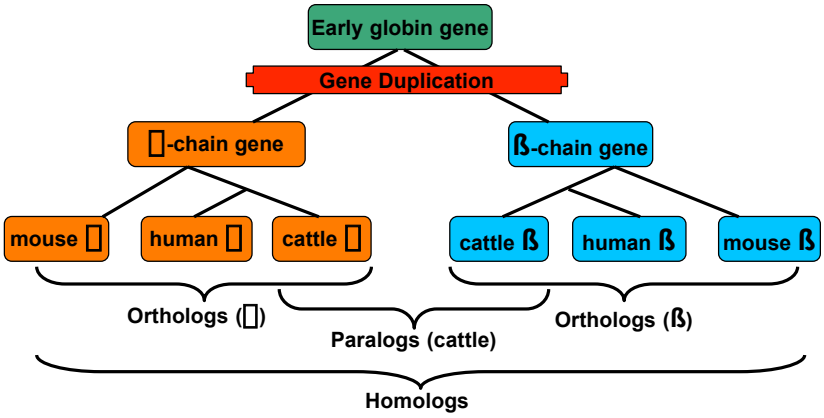
Homolog, ortholog, paralog??

Homologs

Have common origins but may or may not have common activity.

Homologous or not?: *Often* determined by arbitrary threshold level of similarity determined by alignment

Gene Orthology – Why care?



Orthologs – diverged after speciation – *tend to have similar function*

Paralogs – diverged after gene duplication – *some functional divergence occurs*

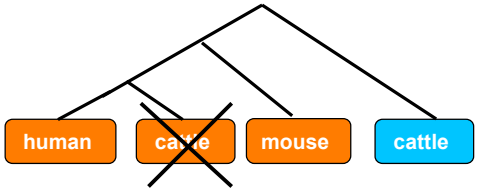
Therefore, for linking similar genes between species, or performing "annotation transfer", identify orthologs

Gene Orthology: How to detect?

- Most common high throughput computational method: Identify reciprocal best BLAST hits (EGO, COGs,…)

Example Problem:

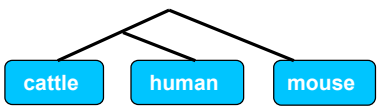
- If making comparisons between human and bovine, for example, the bovine gene dataset is still quite incomplete
- Therefore, current best hit may be a paralog now and the true ortholog not yet sequenced



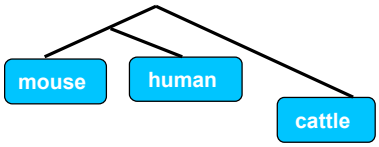
Can we improve orthology analysis for linking functionally similar genes?

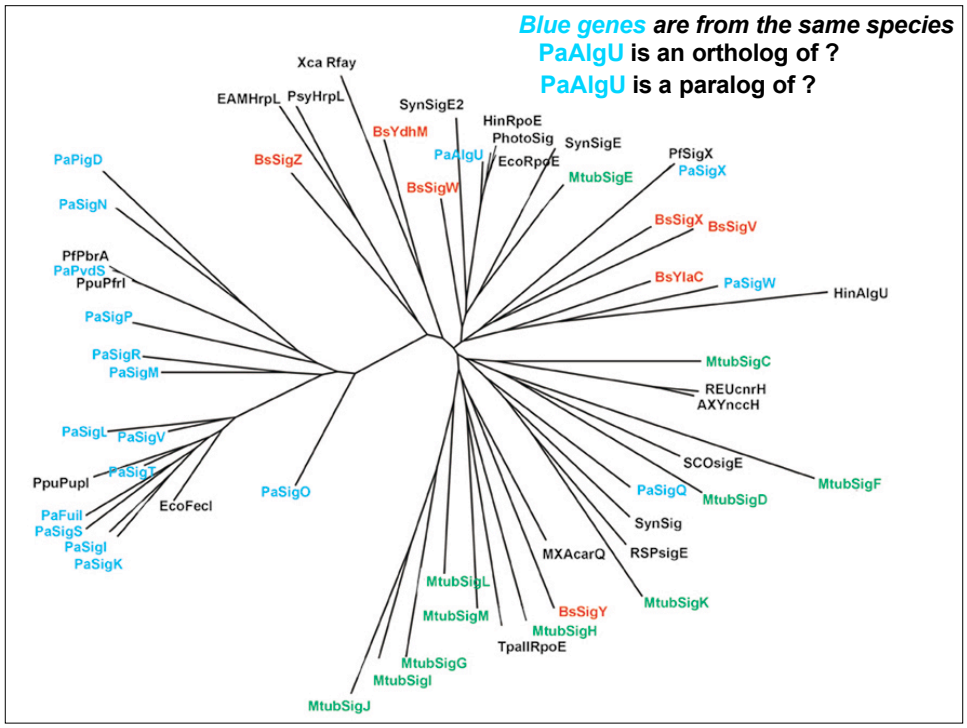
- One solution: Phylogenetic analysis of all putative human-bovine orthologs, using mouse as an outgroup
- Assumption:
  - Mouse and Human gene datasets are more complete, with more true orthologs identified

Expect (organismal phylogeny):



Reject:




Blue genes are from the same species
PaAlgU is an ortholog of ?
PaAlgU is a paralog of ?
Xca Rfay
EAMHrpL
PsyHrpL
SynSigE2
HinRpoE
PhotoSig
SynSigE
BsYdhM
PaAlgU
EcoRpoE
PfSigX
PaSigX
MtubSigE
PaPigD
BsSigZ
BsSigW
PaSigN
BsSigX
BsSigV
PfPbrA
PaPvdS
PpuPfrI
BsYlaC
PaSigW
HinAlgU
PaSigP
PaSigR
PaSigM
MtubSigC
REUcnrH
AXYnccH
PaSigL
PaSigV
SCOsigE
PpuPupI
PaSigT
MtubSigF
PaSigQ
MtubSigD
PaSigO
EcoFecI
PaFuiI
PaSigS
SynSig
PaSigI
MXAcarQ
RSPsigE
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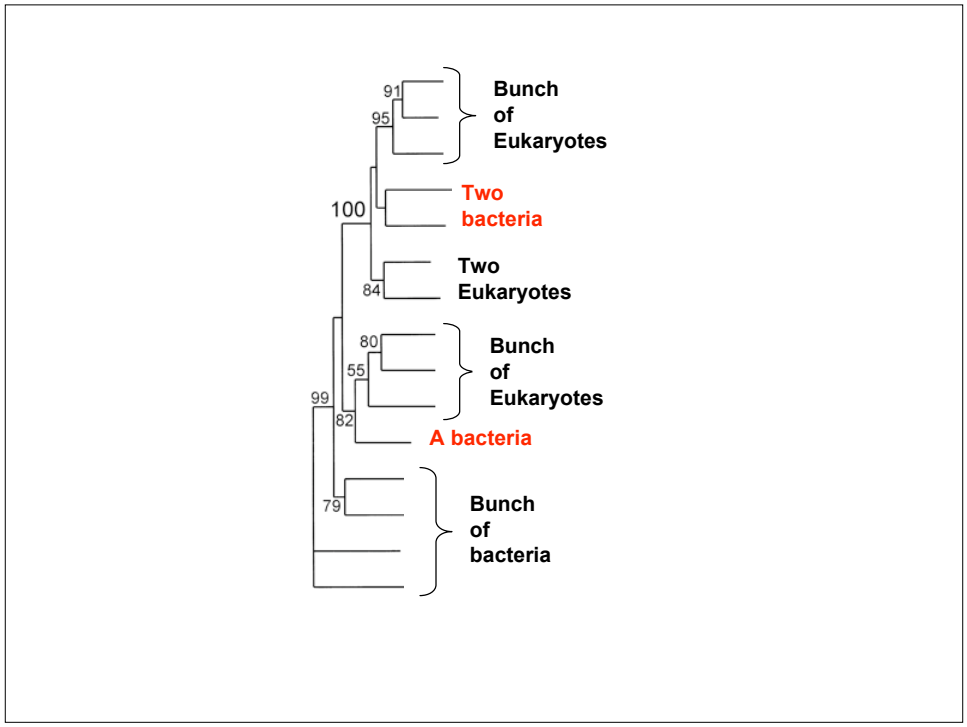
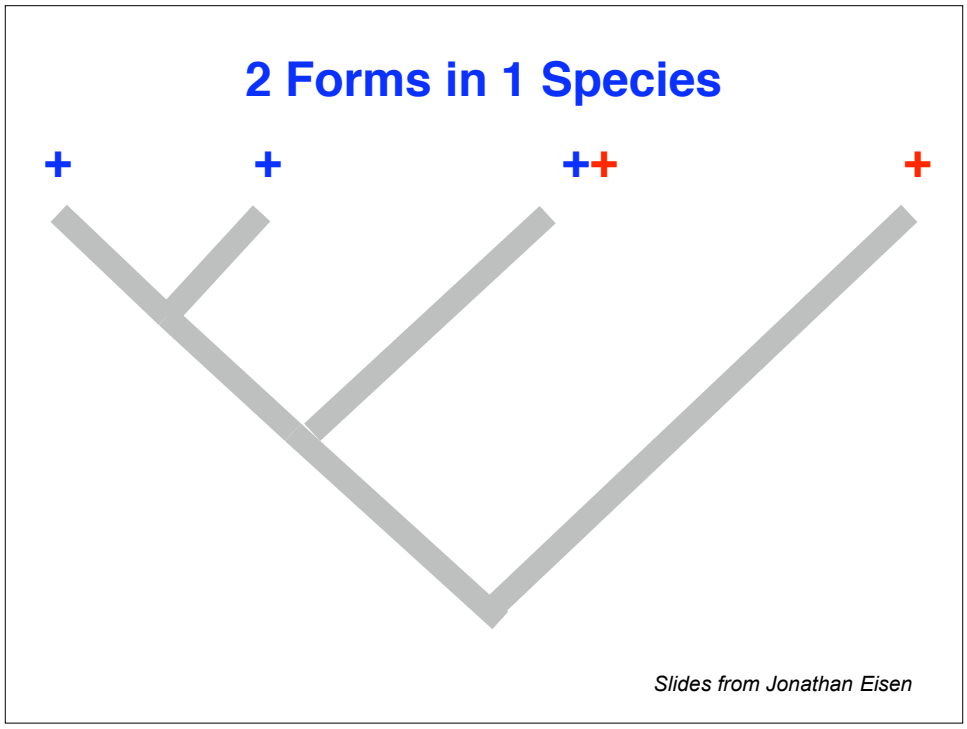
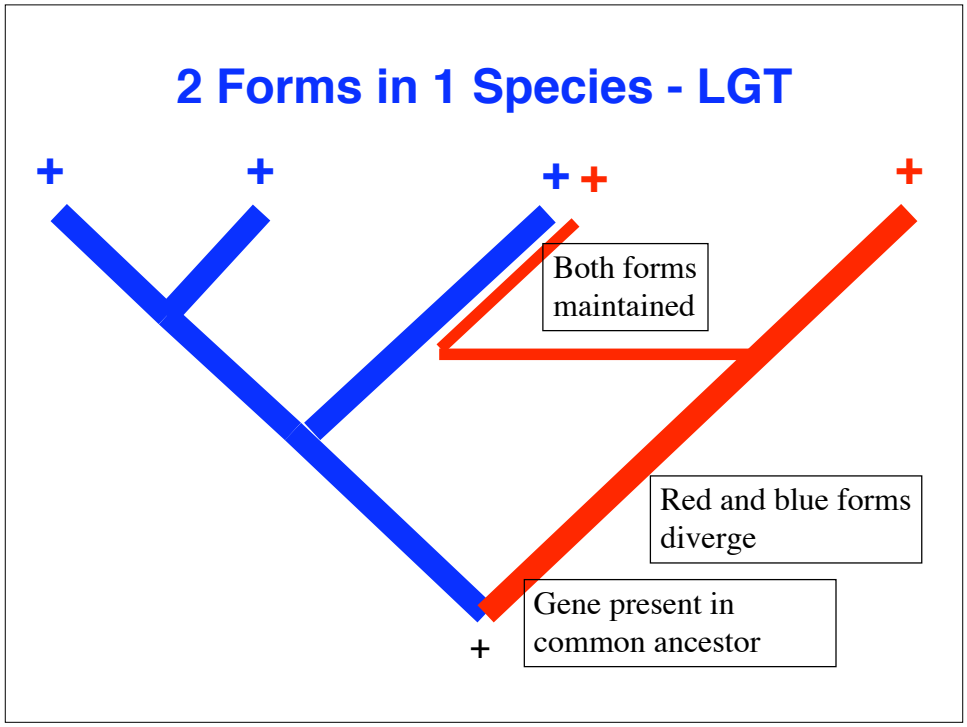
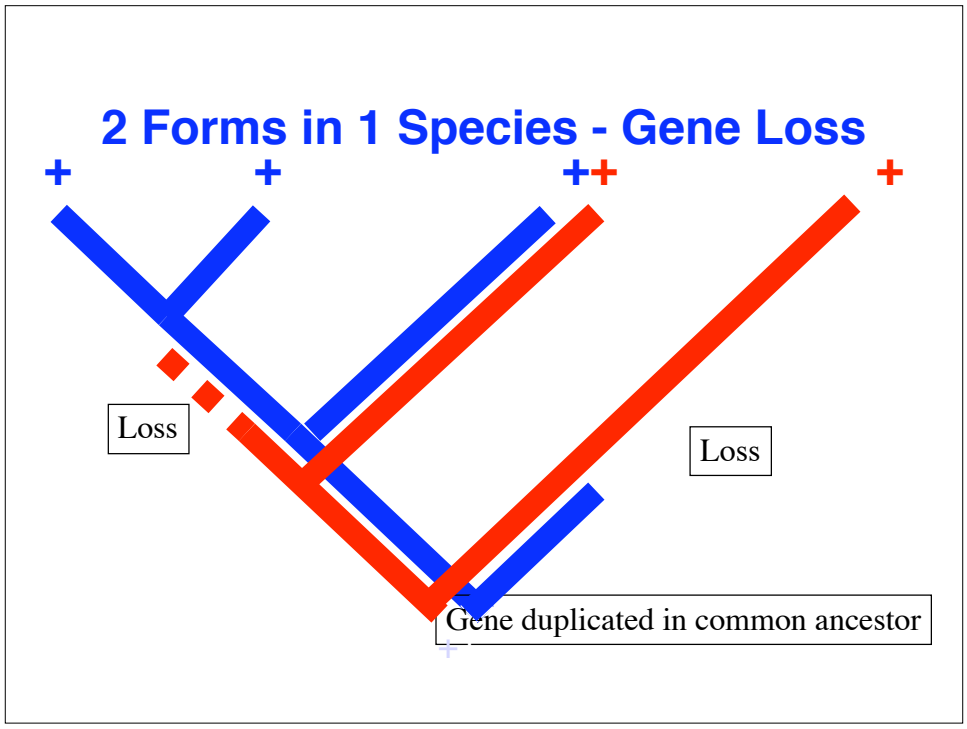
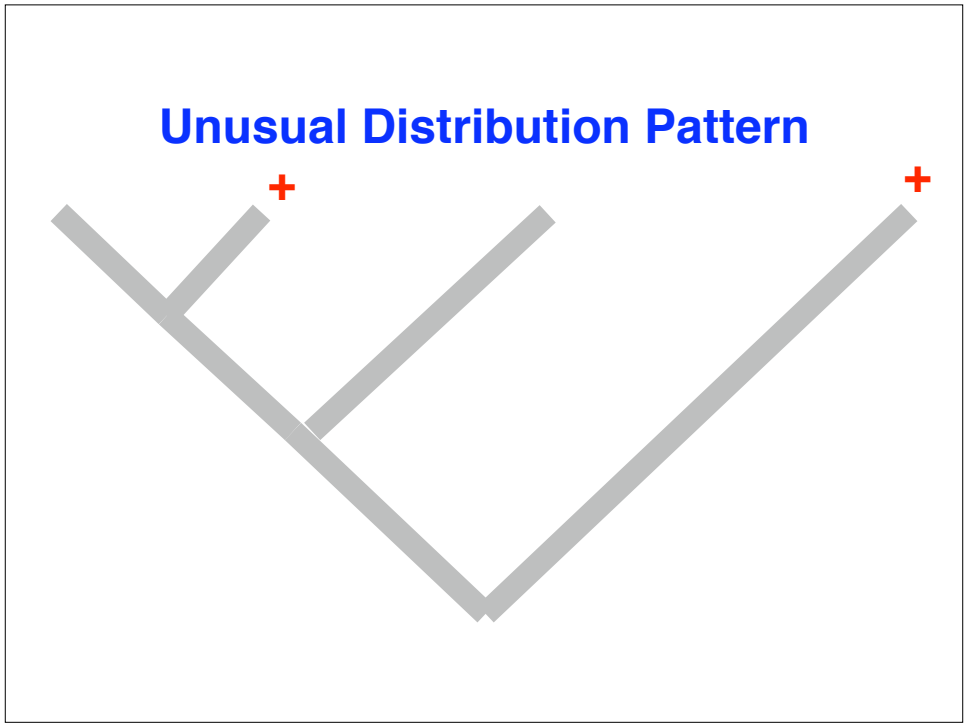
TpalIRpoE
MtubSigG
MtubSigI
MtubSigJ

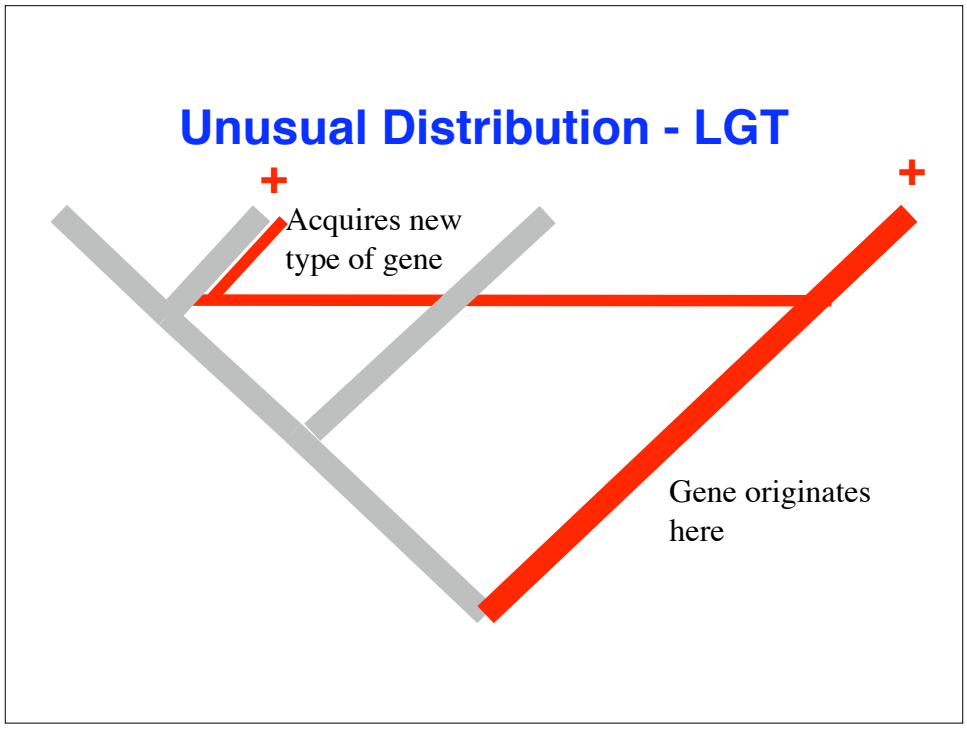

91
95
Bunch of Eukaryotes
100
Two bacteria
84
Two Eukaryotes
80
55
99
Bunch of Eukaryotes
82
A bacteria
79
Bunch of bacteria

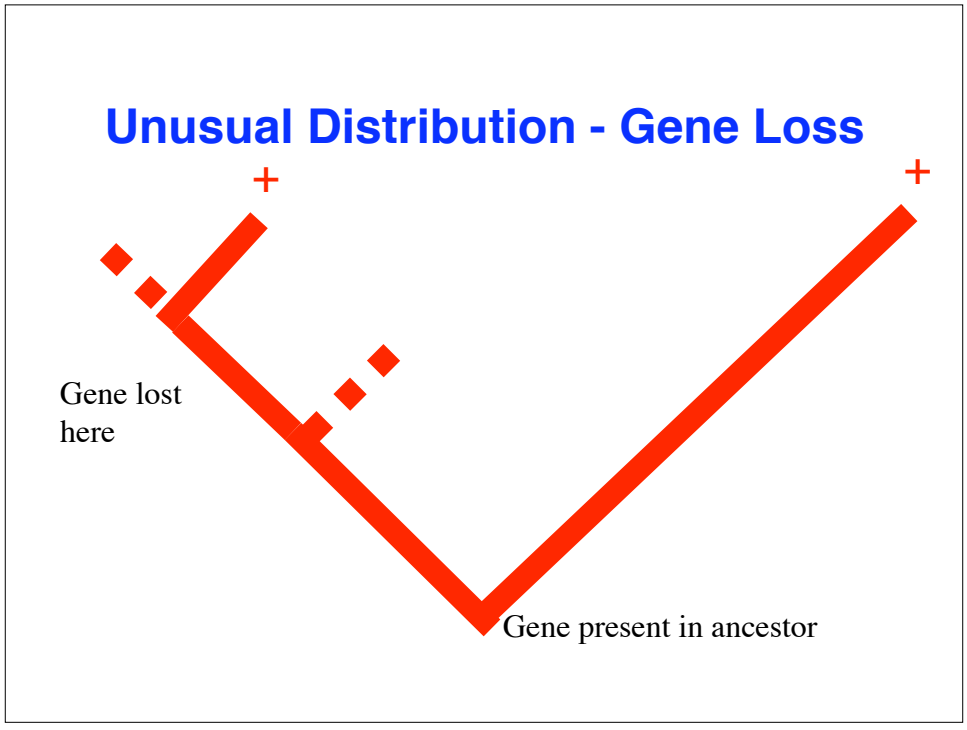

2 Forms in 1 Species
+
+
++
+
Slides from Jonathan Eisen

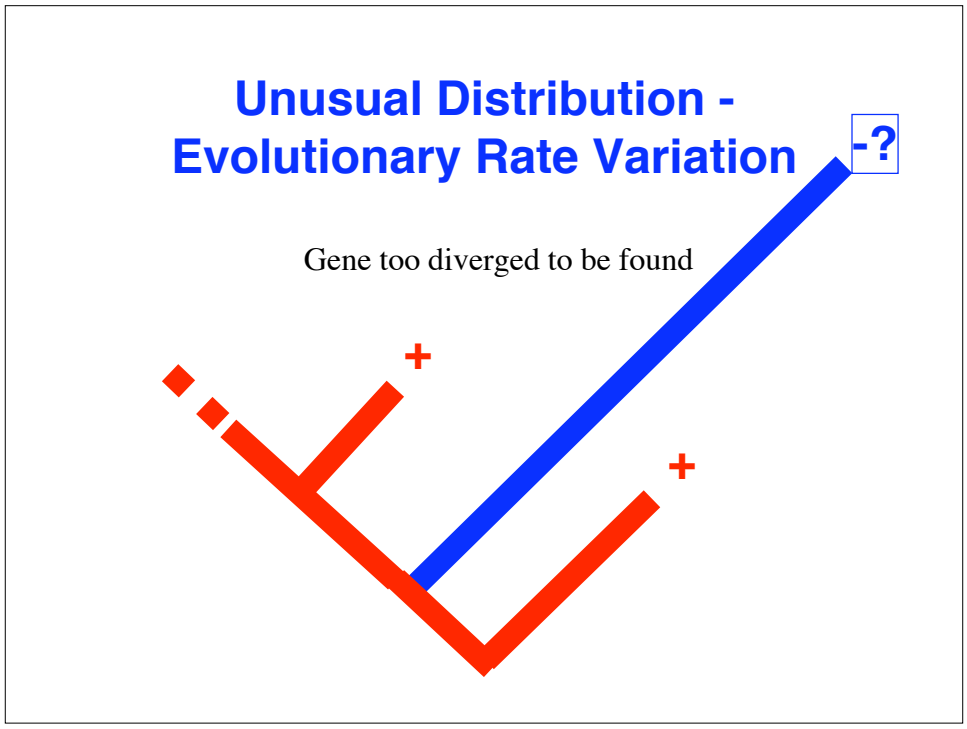

2 Forms in 1 Species - LGT
+
+
+ +
+
Both forms maintained
Red and blue forms diverge
Gene present in common ancestor
+

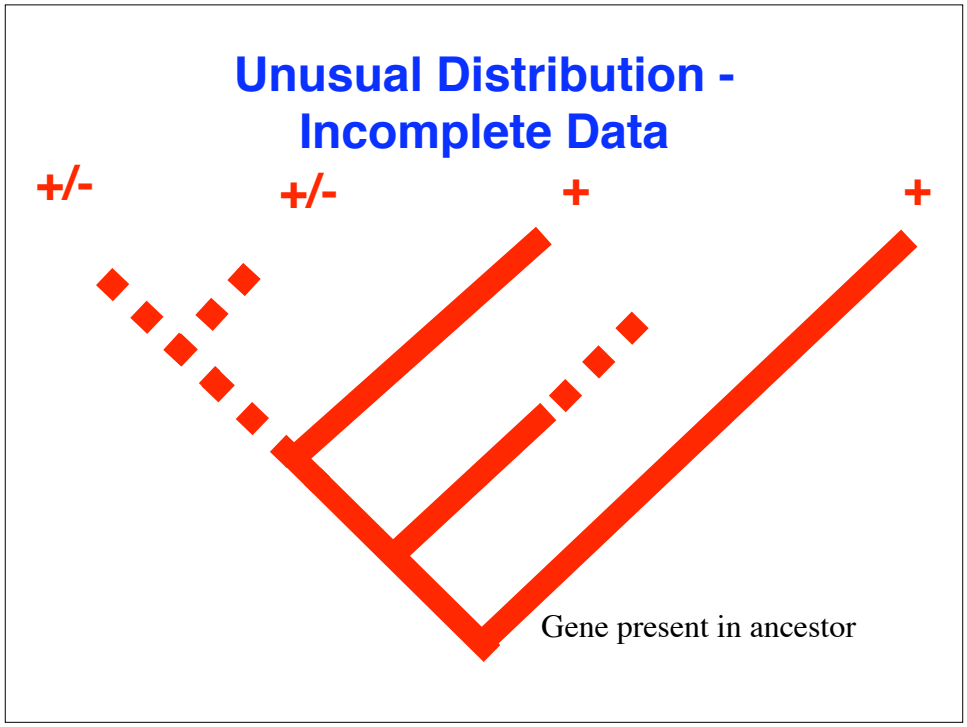

2 Forms in 1 Species - Gene Loss
+
+
++
+
Loss
Loss
Gene duplicated in common ancestor


Unusual Distribution Pattern
+
+


Unusual Distribution - LGT
+
+
Acquires new
type of gene
Gene originates
here


Unusual Distribution - Gene Loss
+
+
Gene lost
here
Gene present in ancestor


Unusual Distribution -
Evolutionary Rate Variation
-?
Gene too diverged to be found
+
+


Unusual Distribution -
Incomplete Data
+/-
+/-
+
+
Gene present in ancestor

Hope for the future

Better sampling of all the species in our world

Amazing but true!

More bacteria in our bodies than human cells!

More different types of bacterial genes in our body then there are human genes!

"So….. how do we construct a phylogenetic tree??"

Most common methods

- Parsimony
- Neighbor-joining
- Maximum Likelihood

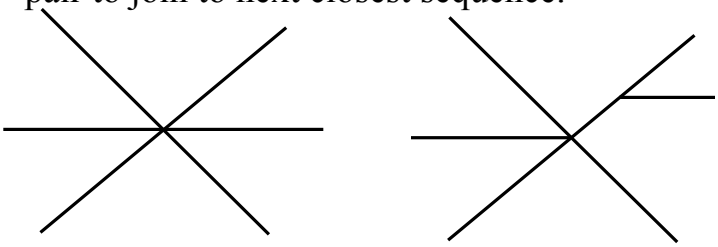
Parsimony

- "Shortest-way-from-A-to-B" method
- The tree implying the least number of changes in character states (most parsimonious) is the best.

- Note:
  - May get more than one tree
  - No branch lengths
  - Uses all character data

Neighbor-joining (and other distance matrix methods)

- "speedy-and-popular" method
- distance matrix constructed
- distance estimates the total branch length between a given two species/genes/proteins
- Neighbor-joining approach: Pairing those sequences that are the most alike and using that pair to join to next closest sequence.



Practical comparison of common distance matrix methods: some PHYLIP and PAUP programs as an example

- Neighbor-joining: fast – not so good for highly divergent sequences
- Fitch: Better but slower and result not that different (seeks to maximize fit of pairwise distances)
- Kitsch: Assumes equal rate of evolution – can greatly bias results so do not use!
- Minimum Evolution (PAUP): Similar to Fitch but fixes location of internal verses external nodes when maximizing fits

- Note: gap info not incorporated into analysis

Maximum Likelihood

- "Inside-out" approach
- produces trees and then sees if the data could generate that tree.
- gives an estimation of the likelihood of a particular tree, given a certain model of nucleotide substitution.
- Notes:
  - All sequence info (including gaps) is used
  - Based on a specific model of evolution – gives probability
  - Verrrrrrrrrrry slow (unless topology of tree is known)

How reliable is a result?

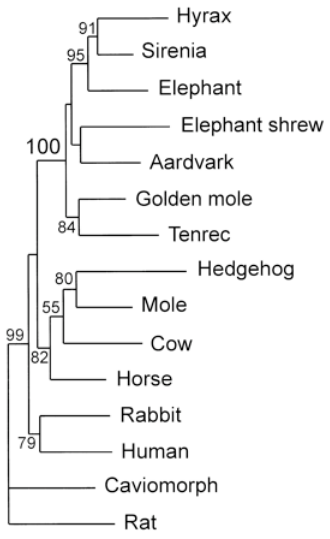
- **Non-parametric bootstrapping**
  - analysis of a sample of (eg. 100 or 1000) randomly perturbed data sets.
  - perturbation: random resampling with replacement, (some characters are represented more than once, some appear once, and some are deleted)
  - perturbed data analysed like real data
  - number of times that each grouping of species/genes/proteins appears in the resulting profile of cladograms is taken as an index of relative support for that grouping

Bootstrapping

The number of times a particular branch is formed in the tree (out of the X times the analysis is done) can be used to estimate its probability, which can be indicated on a consensus tree

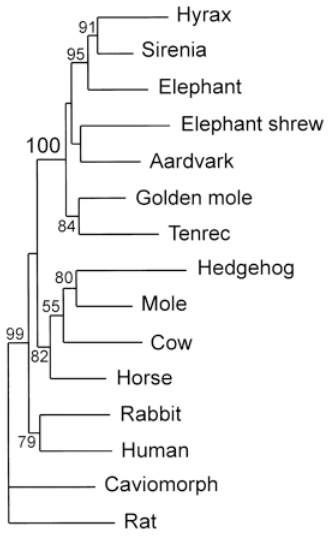
High bootstrap values don't mean that your tree is the true tree!

Alignment and evolutionary assumptions are key



Parametric Bootstrapping

Data are simulated according to the hypothesis being tested.



Phylogenetics – More info

Li, Wen-Hsiung. 1997. Molecular evolution Sunderland, Mass. Sinauer Associates.

- a good starting book, clearly describing the basis of molecular evolution theory. It is a 1997 book, so is starting to get a bit out of date.

Nei, Masatoshi & Kumar, Sudhir. 2000. Molecular evolution and phylogenetics Oxford ; New York. Oxford University Press.

- a relatively new book, by two very well respected researchers in the field. A bit more in-depth than the previous book, but very useful.

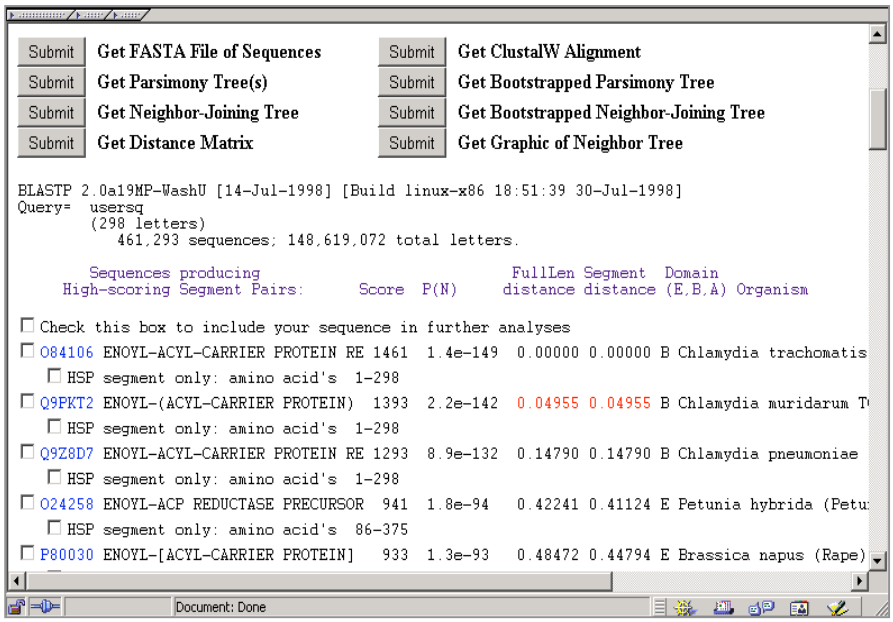
Phylogenetic Tree Construction: Examples of Common Software

PHYLIP
http://evolution.genetics.washington.edu/phylip.html
PAUP
http://paup.csit.fsu.edu/
MEGA 2.1
www.megasoftware.net/

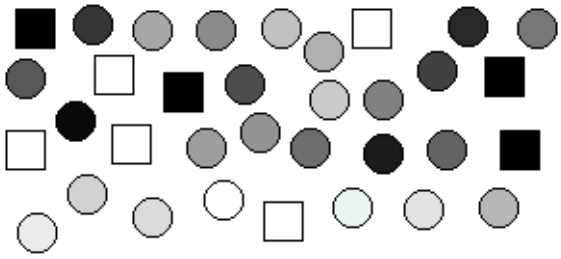
TREEVIEW
http://taxonomy.zoology.gla.ac.uk/rod/treeview.html

Extensive list of software
http://evolution.genetics.washington.edu/phylip/software.html

PhyloBLAST – a tool for analysis



Challenges



How do we classify?

Computational Challenges

- Need to incorporate more evolutionary theory into the multiple sequence alignment and phylogenetic algorithms used in phylogenetic analysis
- Phylogenetic analyses are computationally intensive – great way to benchmark your CPU speed!

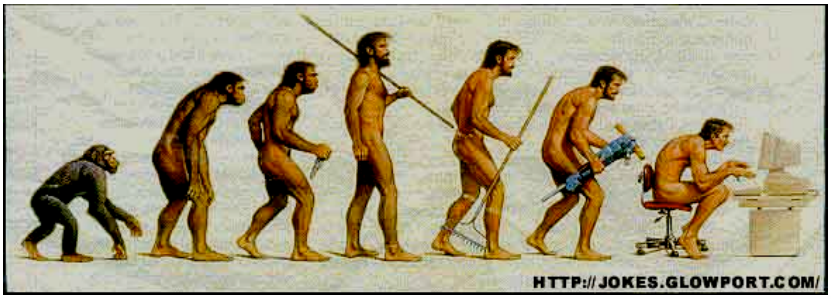
More Challenges

- *Increasing the sampling of our genetic world*
- More accurately differentiating orthologs, paralogs, and horizontally acquired genes
- How frequent is gene loss, gene duplication, and horizontal gene transfer in genome evolution?
- To what degree can we predict protein/gene function using phylogenetic analysis?

Evolution

"To study history one must know in advance that one is attempting something fundamentally impossible, yet necessary and highly important."

Father Jacobus (Hesse's Magister Ludi)



Evolutionary theory is evolving

"I've only just bought this bronze stuff and you're telling me I ought to upgrade to iron?"