MCB 372

Phylogenetic reconstruction

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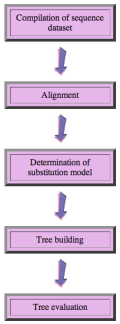
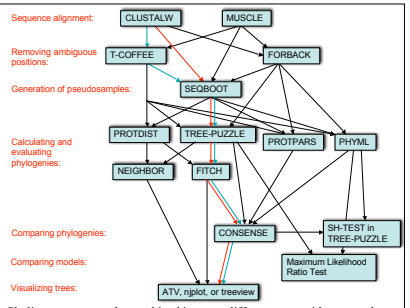
Steps of the phylogenetic analysis

Phylogenetic analysis is an inference of evolutionary relationships between organisms. Phylogenetics tries to answer the question "How did groups of organisms come into existence?"

Those relationships are usually represented by tree-like diagrams.

Note: the assumption of a tree-like process of evolution is controversial!





Phylip programs can be combined in many different ways with one another and with programs that use the same file formats.

From lab 6:

1) Protein parsimony analysis using Phylip

a) In phylip_temp execute the program seqboot by typing

```
> seqboot
```

Read the menu and enter the appropriate letters to generate 100 pseudo samples using bootstrap. If in doubt, read the manual.

Remember to move your output to a new name:

```
> mv outfile your_filename.boot.phy
```

b) do a protein parsimony analysis on the original dataset

```
> protpars
```

Read the menu and enter the appropriate letters to a heuristic search for the most parsimonious tree. Jumble the input order twice.

Again, remember to move your output to new names:

```
> mv outfile your_filename.protpars.outfile
> mv outtree your_filename.protpars.outtree
```

c) do a protein parsimony analysis on the pseudo samples (your_filename.boot.phy)

```
> protpars
```

Read the menu and enter the appropriate letters to a heuristic search for the most parsimonious tree. (Jumble the input order, but only once)

```
> mv outtree your_filename.boot.protpars.outtree
```

Calculate a consensus tree from the (100) trees in your_filename.boot.protpars.outtree

```
> consense
> mv outfile your_filename.boot.protpars.consense.outfile
> mv outtree your_filename.boot.protpars.consense.outtree
```

Is the topology of the consensus tree different from the most parsimonious tree(s)?

Perl assignment

Write a script that takes all phylip formated aligned multiple sequence files present in a directory, and performes a bootstrap analyses using maximum parsimony.

Files you might want to use are A.fa, B.fa, alpha.fa, beta.fa, and atp_all.phy. BUT you first have to convert them to phylip format AND you should replace some or all gaps with ?
(In the end you would be able to answer the question "does the resolution increase if a more related subgroup is analyzed independent from an outgroup?)

hints

Rather than typing commands at the menu, you can write the responses that you would need to give via the keyboard into a file (e.g. your_input.txt)

You could start and execute the program protpars by typing

protpars < your_input.txt

your input.txt might contain the following lines:

```
infile1.txt
r
t
10
y
r
r
```

in the script you could use the line

```
system ("protpars < your_input.txt");
```

The main problem are the owerwrite commands if the oufile and outtree files are already existing. You can either create these beforehand, or erase them by moving (mv) their contents somewhere else.

create *.phy files

the easiest (probably) is to run clustalw with the phylip option:
For example (here):

```
#!/usr/bin/perl -w
print "# This program aligns all multiple sequence files with names *.fa \n
# found in its directory using clustalw,  and saves them in phyip format.\n";
while(defined($file=glob("*.fa"))){
        @parts=split(/\./,$file);
        $file=$parts[0];
        system(" clustalw -infile=$file.fa -align -output=PHYLIP");
        };
# cleanup:
system ("rm *.dnd");
exit;
```

Alternatively, you could use a web version of readseq – this one worked great for me ☺

Alternative for entering the commands for the menu:

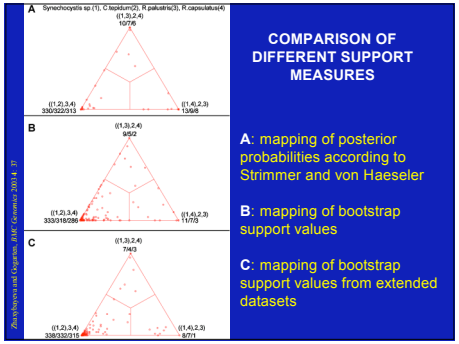
```
#!/usr/bin/perl -w
        system ("cp A.phy infile");
        system ("echo -e 'y\n9\n'|seqboot");
exit;
```

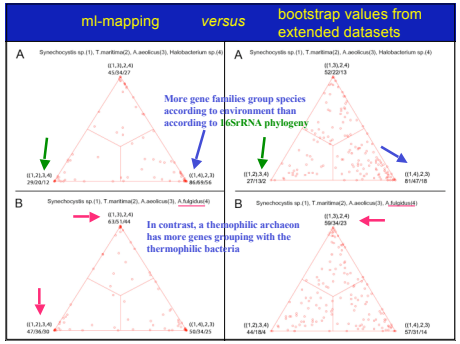
`echo` returns the string in ` `, i.e., y\n9\n.
The `-e` options allows the use of `\n`
The `|` symbol pipes the output from echo to seqboot

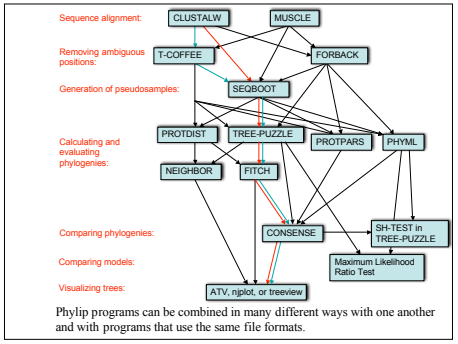
go through examples on bbcxsrv1

Assignments:

- Read through chapter 8
- Using the midterm script (informative.pl see script collection) as a starting point, write a program that reads in a multiple sequence alignment and returns the number of residues per alignment column (you could produce a tab delimited table the you can plot using Excel)
- Modify the program so that it returns the average number of different amino acids in a sliding window, whose size can be modified.







puzzle examples

archaea_euk.phy in puzzle_temp

usertrees (clock check outfile)

usertrees (determine confidence set - example if time)

Alternative Approaches to Estimate Posterior Probabilities

Bayesian Posterior Probability Mapping with MrBayes
(Huelsenbeck and Ronquist, 2001)

Problem:

Strimmer's formula $p_i = \frac{L_i}{L_1+L_2+L_3}$ only considers 3 trees (those that maximize the likelihood for the three topologies)

Solution:

Exploration of the tree space by sampling trees using a biased random walk (Implemented in MrBayes program)

Trees with higher likelihoods will be sampled more often

$p_i = \frac{N_i}{N_{total}}$,where N_i - number of sampled trees of topology i, i=1,2,3

N_{total} – total number of sampled trees (has to be large)

Illustration of a biased random walk

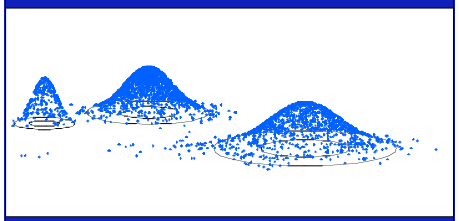


Figure generated using MCRobot program (Paul Lewis, 2001)

the gradualist point of view

Evolution occurs within populations where the fittest organisms have a selective advantage. Over time the advantages genes become fixed in a population and the population gradually changes.

Note: this is not in contradiction to the the theory of neutral evolution. (which says what ?)

Processes that MIGHT go beyond inheritance with variation and selection?

- Horizontal gene transfer and recombination
- Polyploidization (botany, vertebrate evolution) see here
- Fusion and cooperation of organisms (Kefir, lichen, also the eukaryotic cell)
- Targeted mutations (?), genetic memory (?) (see Foster's and Hall's reviews on directed/adaptive mutations; see here for a counterpoint)
- Random genetic drift
- Gratuitous complexity
- Selfish genes (who/what is the subject of evolution??)
- Parasitism, altruism, Morons

selection versus drift

see Kent Holsinger's java simulations at
http://darwin.eeb.uconn.edu/simulations/simulations.html
The law of the gutter.
compare drift versus select + drift
The larger the population the longer it takes for an allele to become fixed.
Note: Even though an allele conveys a strong selective advantage of 10%, the allele has a rather large chance to go extinct.
Note#2: Fixation is faster under selection than under drift.

BUT

s=0

Probability of fixation, P, is equal to frequency of allele in population.
Mutation rate (per gene/per unit of time) = u ;
freq. with which allele is generated in diploid population size N =u*2N
Probability of fixation for each allele = 1/(2N)

Substitution rate =
frequency with which new alleles are generated * Probability of fixation= u*2N *1/(2N) = u
Therefore:
If f s=0, the substitution rate is independent of population size, and equal to the mutation rate !!!! (NOTE: Mutation unequal Substitution!)
This is the reason that there is hope that the molecular clock might sometimes work.

Fixation time due to drift alone:
$t_{av}=4*N_e$ generations
(N_e=effective population size; For n discrete generations
$N_e = n/(1/N_1+1/N_2+.....1/N_n)$

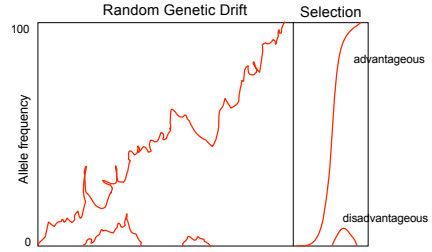
s>0

Time till fixation on average:
t_{av} = (2/s) ln (2N) generations
(also true for mutations with negative "s" ! discuss among yourselves)

E.g.: N=10^6,
s=0: average time to fixation: 4*10^6 generations
s=0.01: average time to fixation: 2900 generations

N=10^4,
s=0: average time to fixation: 40.000 generations
s=0.01: average time to fixation: 1.900 generations

=> substitution rate of mutation under positive selection is larger than the rate wite which neutral mutations are fixed.



Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

Positive selection

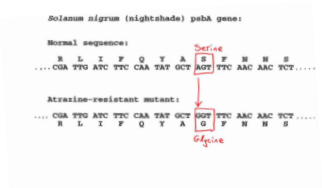
- A new allele (mutant) confers some increase in the **fitness** of the organism
- Selection acts to favour this allele
- Also called adaptive selection or Darwinian selection.

NOTE: **Fitness** = ability to survive and reproduce

Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

Advantageous allele

Herbicide resistance gene in nightshade plant



Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

Negative selection

- A new allele (mutant) confers some decrease in the fitness of the organism
- Selection acts to remove this allele
- Also called purifying selection

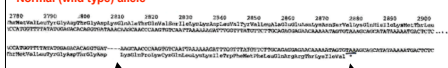
Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

Deleterious allele

Human breast cancer gene, BRCA2

5% of breast cancer cases are familial
Mutations in BRCA2 account for 20% of familial cases

Normal (wild type) allele



Mutant allele (Montreal 440 Family)

Stop codon

4 base pair deletion
Causes frameshift

Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

Neutral mutations

- Neither advantageous nor disadvantageous
- Invisible to selection (no selection)
- Frequency subject to 'drift' in the population
- **Random drift** – random changes in small populations

Types of Mutation-Substitution

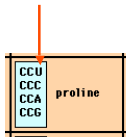
- Replacement of one nucleotide by another
- Synonymous (Doesn't change amino acid)
  - Rate sometimes indicated by Ks
  - Rate sometimes indicated by d_s
- Non-Synonymous (Changes Amino Acid)
  - Rate sometimes indicated by Ka
  - Rate sometimes indicated by d_n

(this and the following 4 slides are from mentor.lscf.ucsb.edu/course/ spring/eemb102/lecture/Lecture7.ppt)

Genetic Code – Note degeneracy of 1st vs 2nd vs 3rd position sites

UUU UUC	phenyl alanine	UCU UCC UCA UCG	serine	UAU UAC	tyrosine	UGU UGC	cysteine
UUA UUG	leucine			UAA UAG	stop	UGA	stop
						UGG	tryptophan
CUU CUC CUA CUG	leucine	CCU CCC CCA CCG	proline	CAU CAC	histidine	CGU CGC CGA CGG	arginine
				CAA CAG	glutamine		
AUU AUC AUA	isoleucine	ACU ACC ACA ACG	threonine	AAU AAC	asparagine	AGU AGC	serine
AUG	methionine			AAA AAG	lysine	AGA AGG	arginine
GUU GUC GUA GUG	valine	GCU GCC GCA GCG	alanine	GAU GAC	aspartic acid	GGU GGC GGA GGG	glycine
				GAA GAG	glutamic acid		

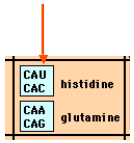
Genetic Code



Four-fold degenerate site – Any substitution is synonymous

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Genetic Code



Two-fold degenerate site – Some substitutions synonymous, some non-synonymous

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Measuring Selection on Genes

- Null hypothesis = neutral evolution
- Under neutral evolution, synonymous changes should accumulate at a rate equal to mutation rate
- Under neutral evolution, amino acid substitutions should also accumulate at a rate equal to the mutation rate

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Counting #s/#a

	Ser	Ser	Ser	Ser	Ser
Species1	TGA	TGC	TGT	TGT	TGT
	Ser	Ser	Ser	Ser	Ala
Species2	TGT	TGT	TGT	TGT	GGT

#s = 2 sites
#a = 1 site

#a/#s=0.5

To assess selection pressures one needs to calculate the rates (Ka, Ks), i.e. the occurring substitutions as a fraction of the possible syn. and nonsyn. substitutions.

Things get more complicated, if one wants to take transition transversion ratios and codon bias into account. See chapter 4 in Nei and Kumar, Molecular Evolution and Phylogenetics.

Modified from: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

dambe

Two programs worked well for me to align nucleotide sequences based on the amino acid alignment,

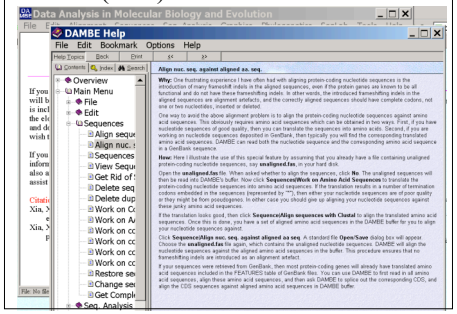
One is DAMBE (only for windows). This is a handy program for a lot of things, including reading a lot of different formats, calculating phylogenies, it even runs codeml (from PAML) for you.

The procedure is not straight forward, but is well described on the help pages. After installing DAMBE go to HELP -> general HELP -> sequences -> align nucleotide sequences based on …->

If you follow the instructions to the letter, it works fine.

DAMBE also calculates Ka and Ks distances from codon based aligned sequences.

dambe (cont)



aa based nucleotide alignments (cont)

An alternative is the tranalign program that is part of the emboss package. On bbcxsrv1 you can invoke the program by typing tranalign.

Instructions and program description are here .

If you want to use your own dataset in the lab on Monday, generate a codon based alignment with either *dambe* or *tranalign* and save it as a nexus file **and** as a phylip formated multiple sequence file (using either clustalw, PAUP (export or tonexus), dambe, or **readseq** on the web)

PAML (codeml) the basic model

$$q_{ij} = \begin{cases} 0, & \text{if the two codons differ at more than one position,} \\ \pi_j, & \text{for synonymous transversion,} \\ \kappa\pi_j, & \text{for synonymous transition,} \\ \omega\pi_j, & \text{for nonsynonymous transversion,} \\ \omega\kappa\pi_j, & \text{for nonsynonymous transition,} \end{cases}$$

The equilibrium frequency of codon j (π_j) can be considered a free parameter, but can also be calculated from the nucleotide frequencies at the three codon positions (control variable `CodonFreq`). Under this model, the relationship holds that $\omega = d_N/d_S$, the ratio of nonsynonymous/synonymous substitution rates. This basic model is fitted by specifying model = 0 NSsites = 0, in the control file codeml.ctl. It forms the basis for more sophisticated models implemented in codeml.

sites versus branches

You can determine omega for the whole dataset; however, usually not all sites in a sequence are under selection all the time.

PAML (and other programs) allow to either determine omega for each site over the whole tree, *Branch Models* **,**
or determine omega for each branch for the whole sequence, *Site Models* **.**

It would be great to do both, i.e., conclude codon 176 in the vacuolar ATPases was under positive selection during the evolution of modern humans – alas, a single site does not provide any statistics ….

Sites model(s)

work great have been shown to work great in few instances.
The most celebrated case is the influenza virus HA gene.

A talk by Walter Fitch (slides and sound) on the evolution of this molecule is here .
This article by Yang et al. 2000 gives more background on ml aproaches to measure omega. The dataset used by Yang et al is here: flu_data.paup .

sites model in MrBayes

The MrBayes block in a nexus file might look something like this:

```
begin mrbayes;
set autoclose=yes;
lset nst=2 rates=gamma nucmodel=codon omegavar=Ny98;
mcmcp samplefreq=500 printfreq=500;
mcmc ngen=500000;
sump burnin=50;
sumt burnin=50;
end;
```

Vincent Daubin and Howard Ochman: Bacterial Genomes as New Gene Homes: The Genealogy of ORFans in *E. coli*. *Genome Research 14:1036-1042, 2004*

The ratio of non-synonymous to synonymous substitutions for genes found only in the E.coli - Salmonella clade is lower than 1, but larger than for more widely distributed genes.

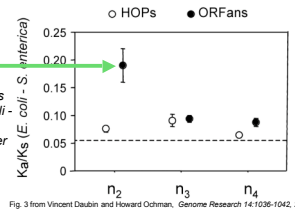
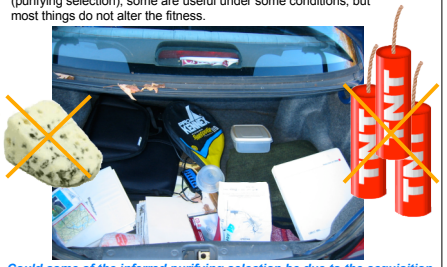


Fig. 3 from Vincent Daubin and Howard Ochman, *Genome Research 14:1036-1042, 2004*

Trunk-of-my-car analogy: Hardly anything in there is the is the result of providing a selective advantage. Some items are removed quickly (purifying selection), some are useful under some conditions, but most things do not alter the fitness.



Could some of the inferred purifying selection be due to the acquisition of novel detrimental characteristics (e.g., protein toxicity)?