MCB 371/372

quartets
positive selection
4/20/05

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Perl assignment #3

Write a script that takes all phylip formated aligned multiple sequence files present in a directory, and perfomes a bootstrap analyses using maximum parsimony.
I.e., the script should go through the same steps as we did in the exercises #4 tasks 1a and 1c

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 ☺

run phylip programs from perl

An example on how to solve the homework assignment is here, the cmd files are here, here, and here:

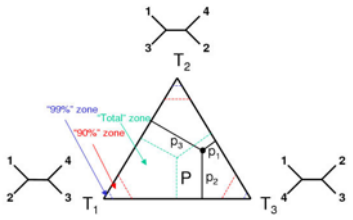
```
#!/usr/bin/perl -w
print "# This program runs seqboot, protpars and consense on all multiple \n
# sequence files with names *.phy\n";

while(defined($file=glob("*.phy"))){

        @parts=split(/\./,$file);
        $file=$parts[0];

        system ("cp $file.phy infile");
        system ("seqboot < seqboot.cmd");
        system ("mv outfile infile");
        system ("protpars < protpars.cmd");
        system ("rm outfile");
        system ("mv outtree intree");
        system ("consense < consense.cmd");
        system ("mv outtree $file.outtree");
        system ("mv outfile $file.outfile");
};

# cleanup:
system ("rm infile");
system ("rm intree");
exit;
```

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

ml mapping



From: Olga Zhaxybayeva and J Peter Gogarten *BMC Genomics* 2002, 3:4

ml mapping can be used to assess the amount of phylogenetic information in a dataset:

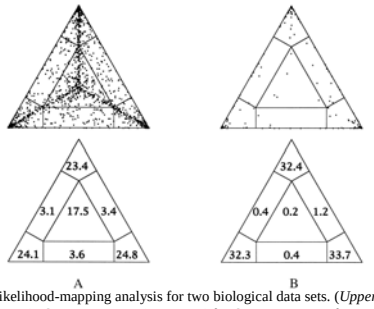


Figure 5. Likelihood-mapping analysis for two biological data sets. (*Upper*) The distribution patterns. (*Lower*) The occupancies (in percent) for the seven areas of attraction. (*A*) Cytochrome-*b* data from ref. 14. (*B*) Ribosomal DNA of major arthropod groups (15).

From: Korbinian Strimmer and Arndt von Haeseler Proc. Natl. Acad. Sci. USA Vol. 94, pp. 6815-6819, June 1997

ml mapping can asses the topology surrounding an individual branch :

E.g.: If we want to know if *Giardia lamblia* forms the deepest branch within the known eukaryotes, we can use ML mapping to address this problem.
To apply ml mapping we choose the "higher" eukaryotes as cluster a, another deep branching eukaryote (the one that competes against Giardia) as cluster b, Giardia as cluster c, and the outgroup as cluster d. For an example output see this sample ml-map.

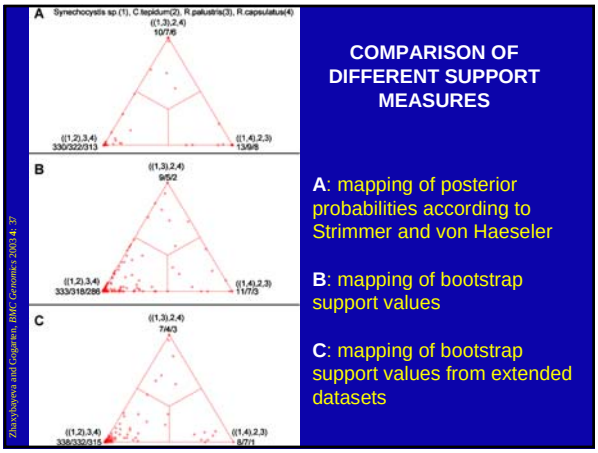
An analysis of the carbamoyl phosphate synthetase domains with respect to the root of the tree of life is here.

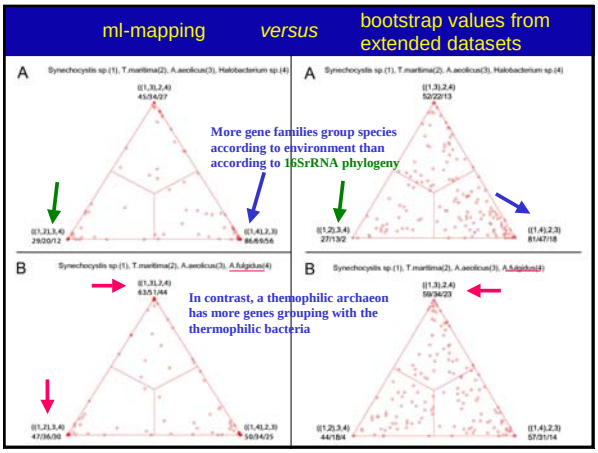
ml mapping can asses the not necessarily treelike histories of genome

Application of ML mapping to comparative Genome analyses

see here for a comparison of different probability measures.
Fig. 3: outline of approach
Fig. 4: Example and comparison of different measures

see here for an approach that solves the problem of poor taxon sampling that is usually considered inherent with quartet analyses.
Fig. 2: The principle of "analyzing extended datasets to obtain embedded quartets"
Example next slides:





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:
$\mathbf{t}_{av}=4*N_e$ generations
(N_e=effective population size; For n discrete generations
$N_e= n/(1/N_1+1/N_2+.....1/N_n)$

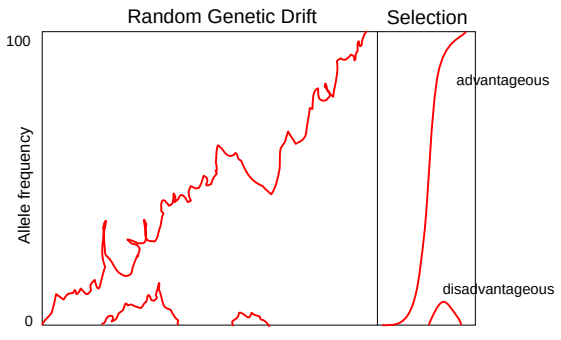
s>0

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

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

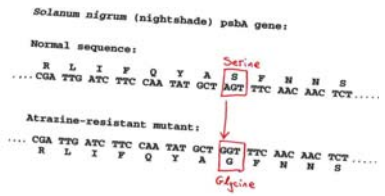
- A new allele (mutant) confers some increase in the **fitness** of the organism
- Selection acts to favour this allele
- Also called adaptive selection or Darwinian selection.

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

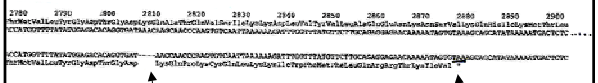
Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

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)

4 base pair deletion
Causes frameshift

Stop codon

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

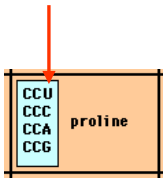
- Replacement of one nucleotide by another
- Synonymous (Doesn't change amino acid)
  - Rate sometimes indicated by Ks
  - Rate sometimes indicated by d_s
- Non-Synonymous (Changes Amino Acid)
  - Rate sometimes indicated by Ka
  - Rate sometimes indicated by d_n

(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	

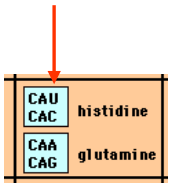
Genetic Code



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

reading assignment

Next week we will use the PAML software
In preparation please read the documentation
pages 38-43.

dambe

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

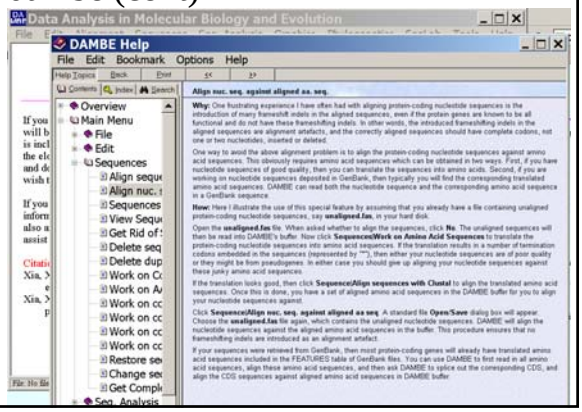
One is DAMBE (only for windows). This is a handy program for a lot of things, including reading a lot of different formats, calculating phylogenies, it even runs codeml (from PAML) for you.

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;
```