MCB 371/372

positive selection

4/25/05

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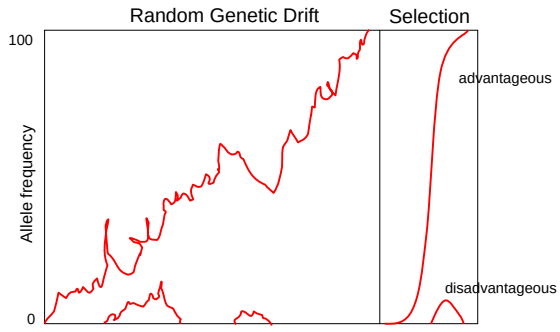


Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026_1+2.ppt

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 <u>possible</u> 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

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

Aside #1: Population genetics approach:

A selective sweep decreases the number of polymorphisms surrounding the gene that was driven into fixation due to positive selection. This provides an alternative to dN/dS ratios to detect genes under positive selection.

Aside #2: Number of non-synonymous substitutions

If a site or a gene repeatedly was driven into fixation due to positive selection, its substitution rate will be higher than the mutation rate. This diversifying selection is frequently observed for sites interating with immune system.

Positive selection

dN/dS > 1

- A new allele (mutant) confers some <u>increase</u> 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 <u>reproduce</u>

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

Negative selection

dN/dS < 1

- 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

Neutral mutations

dN/dS = 1

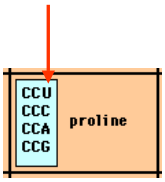
- Neither advantageous nor disadvantageous
- Invisible to selection (no selection)
- Frequency subject to 'drift' in the population
- **Random drift** – random changes in small populations

This seems to be true only for pseudogenes!

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

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

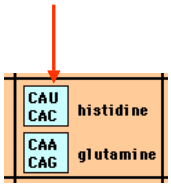
Genetic Code



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

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.

omega = dN/dS

According to the model:

omega < 1 purifying selection
omega = 0 neutral evolution
omega > 1 positive selection

Concern: If a gene is expressed, codon usage, nucleotide bias and other factors (protein toxicity) will generate some purifying selection even though the gene might not have a function that is selected for.
I.e., omega < 1 could be due to avoiding deleterious functions, rather than the loss of function.

Most proteins coding genes have omega between 0 and 1.

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.

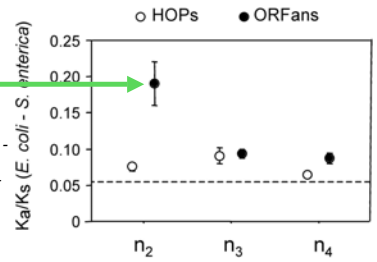
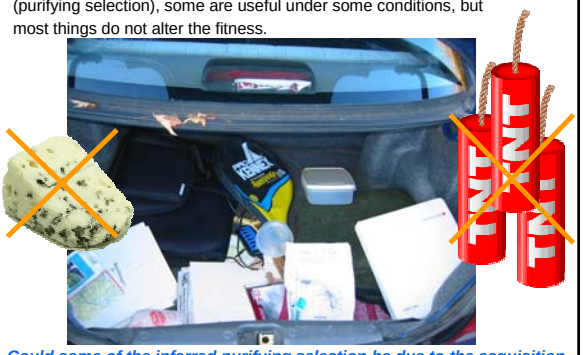


Fig. 3 from Vincent Daubin and Howard Ochman, *Genome Research 14:1036-1042, 2004*

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)?

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 to 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, usually this does not work well, because a single site on a single branch does not provide sufficient statistics ….

Sites model(s)

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:

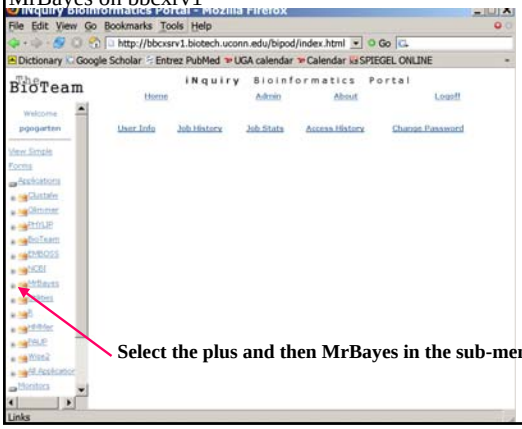
```
begin mrbayes;
set autoclose=yes;
lset nst=2 rates=gamma nucmodel=codon omegavar=Ny98;
mcmcp samplefreq=500 printfreq=500;
mcmc ngen=500000;
sump burnin=50;
sumt burnin=50;
end;
```

MrBayes on bbcxrv1

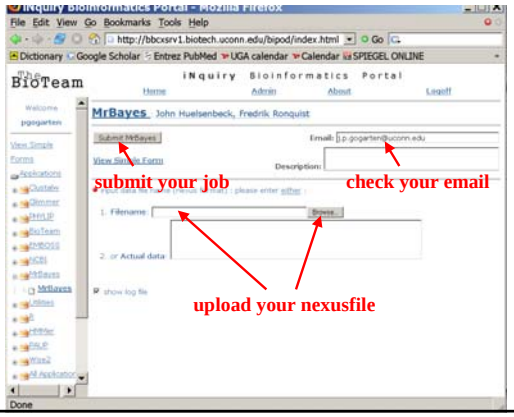
Create the nexus file on your computer.
It will help to have MrBayes installed locally, this way you can check that you don't have any typos in the MrBayes block.

Direct your browser to
http://bbcxsrv1.biotech.uconn.edu/bipod/index.html

MrBayes on bbcxrv1



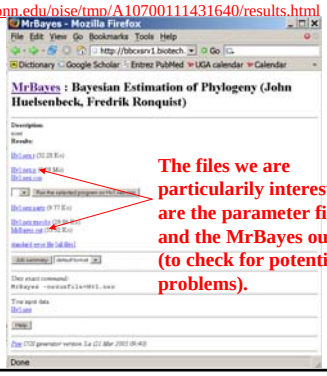
MrBayes on bbcxrv1



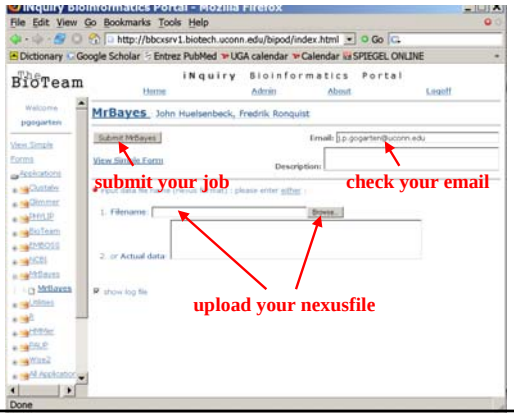
MrBayes on bbcxrv1

You will receive the results per email, and you will receive the link of a web page that lists all the output files. In this case:
http://bbcxsrv1.biotech.uconn.edu/pise/tmp/A10700111431640/results.html

You can save the files from your browser, or open the email attachments. .



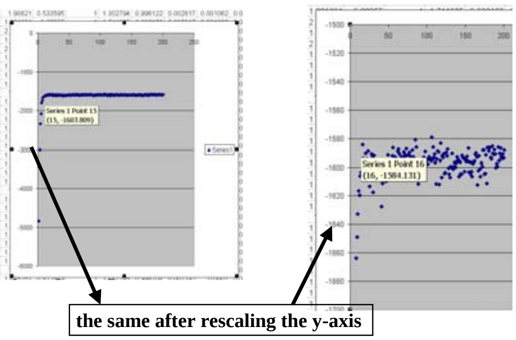
MrBayes on bbcxrv1



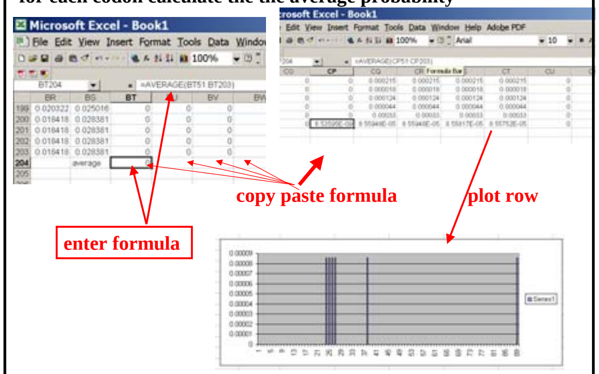
MrBayes analyzing the *.nex.p file

1. **The easiest is to load the file into excel. (if your alignment is too long, you need to load the data into separate speadsheets – see here execise 2 item 2 for more info)**
2. **plot LogL to determine which samples to ignore**
3. **for each codon calculate the the average probability (from the samples you do not ignore) that the codon belongs to the group of codons with omega>1.**
4. **plot this quantity using a bar graph.**

plot LogL to determine which samples to ignore



for each codon calculate the the average probability



MrBayes on bbcxrv1

If you do this for your own data,
•run the procedure first for only 50000 generations (takes about 30 minutes) to check that everthing works as expected,
•then run the program overnight for at least 500 000 generations.
•Especially, if you have a large dataset, do the latter twice and compare the results for consistency. (I prefer two runs over 500000 generations each over one run over a million generations.

PAML – codeml – sites model

the paml package contains several distinct programs for nucleotides (baseml) protein coding sequences and amino acid sequences (codeml) and to simulate sequences evolution.
The input file needs to be in phylip format.
By default it assumes a sequential format (e.g. here).
If the sequences are interleaved, you need to add an "I" to the first line, as in these example headers:

```
        6    467             I
gi|1613157 ---------- MSDNDTIVAQ ATPPGRGGVG ILRISGFKAR EVAETVLGKL
gi|2212798 ---------- MSTTDTIVAQ ATPPGRGGVG ILRVSGRAAS EVAHAVLGKL
gi|1564003 MALIQSCSGN TMTTDTIVAQ ATAPGRGGVG IIRVSGPLAA HVAQTVTGRT
gi|1560076 ---------M QAATETIVAI ATAQGRGGVG IVRVSGPLAG QMAVAVSGRQ
gi|2123365 -----MN--- -ALPSTIVAI ATAAGTGGIG IVRLSGPQSV QIAAALGIAG
gi|1583936 -----MSQRS TKMGDTIAAI ATASGAAGIG IIRLSGSLIK TIATGLGMTT

           PKPRYADYLP FKDADGSVLD QGIALWFPGP NSFTGEDVLE LQGHGGPVIL
           PKPRYADYLP FKDVDGSTLD QGIALYFPGP NSFTGEDVLE LQGHGGPVIL
           LRPRYAEYLP FTDEDGQQLD QGIALFFPNP HSFTGEDVLE LQGHGGPVVM
           LKARHAHYGP FLDAGGQVID EGLSLYFPGP NSFTGEDVLE LQGHGGPVVL
           LQSRHARYAR FRDAQGEVID DGIAVWFPAP HSFTGEEVVE LQGHGSPVLL
           LRPRYAHYTR FLDVQDEVID DGLALWFPAP HSFTGEDVLE LQGHGSPLLL
```

```
 5   855   I

human
goat-cow
rabbit
rat
marsupial
1
GTG CTG TCT CCT GCC GAC AAG ACC AAC GTC AAG GCC GCC TGG GGC AAG GTT GGC GCG CAC
... ... ... G.C ... ... ... T.. ..T ... ... ... ... ... ... ... ... ... .GC A..
... ... ... ..C ..T ... ... ... ... A.. ... A.T ... ... .AA ... A.C ... AGC ...
... ..C ... G.A .AT ... ..A ... ... A.. ... AA. TG. ... ..G ... A.. ..T .GC ..T
... ..C ..G GA. ..T ... ... ..T C.. ..G ..A ... AT. ... ..T ... ..G ..A .GC ...
61

GCT GGC GAG TAT GGT GCG GAG GCC CTG GAG AGG ATG TTC CTG TCC TTC CCC ACC ACC AAG
... ..A .CT ... ..C ..A ... ..T ... ... ... ... ... ... AG. ... ... ... ... ...
.G. ... ... ... ..C ..C ... ... G.. ... ... ... ... T.. GG. ... ... ... ... ...
.G. ..T ..A ... ..C .A. ... ... ..A C.. ... ... ... GCT G.. ... ... ... ... ...
..C ..T .CC ..C .CA ..T ..A ..T ..T .CC ..A .CC ... ..C ... ... ... ..T ... ..A
```

PAML – codeml – sites model (cont.)

the program is invoked by typing codeml followed by the name of a control file that tells the program what to do.

paml can be used to find the maximumlikelihood tree, however, the program is rather slow. Phyml is a better choice to find the tree, which then can be used as a user tree.

An example for a codeml.ctl file is codeml.hv1.sites.ctl
This file directs codeml to run three different models:
one with an omega fixed at 1, a second where each site can be either have an omega between 0 and 1, or an omega of 1, and third a model that uses three omegas as discribed before for MrBayes.
The output is written into a file called Hv1.sites.codeml_out (as directed by the control file).

Point out log likelihoods and estimated parameter line (kappa and omegas)

Additional useful information is in the rst file generated by the codeml

Discuss overall result.

PAML – codeml – branch model

For the same dataset to estimate the dN/dS ratios for individual branches, you could use this file codeml.hv1.branches.ctl as control file.

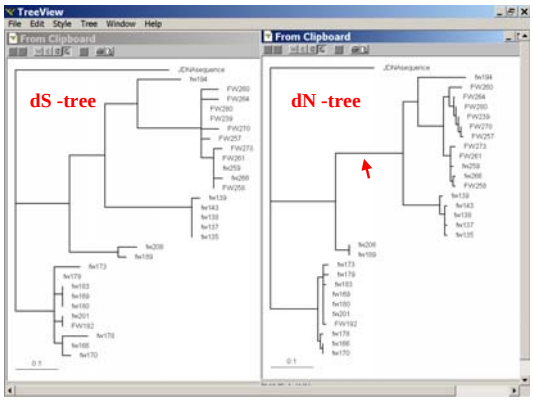
The output is written, as directed by the control file, into a file called Hv1.branch.codeml_out

A good way to check for episodes with plenty of non-synonymous substitutions is to compare the dn and ds trees.

Also, it might be a good idea to repeat the analyses on parts of the sequence (using the same tree). In this case the sequences encode a family of spider toxins that include the mature toxin, a propeptide and a signal sequence (see here for more information).

Bottom line: one needs plenty of sequences to detect positive selection.

PAML – codeml – branch model



where to get help

read the manuals and help files
check out the discussion boards at http://www.rannala.org/phpBB2/

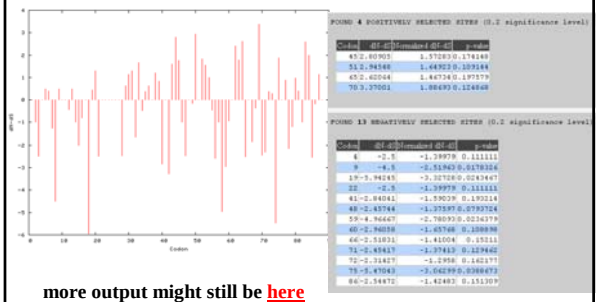
else

there is a new program on the block called hy-phy
(=hypothesis testing using phylogenetics).

The easiest is probably to run the analyses on the authors datamonkey.

hy-phy

Results of an anaylsis using the SLAC approach



more output might still be here