

Phylogenetic Analysis by Maximum Likelihood (PAML)

Version 3.12, March 2002

[Ziheng Yang](#)

© Copyright 1993 –2001 by Ziheng Yang. The software package is provided "as is" without warranty of any kind. In no event shall the author be held responsible for any damage resulting from the use of this software, including but not limited to the frustration that you may experience in using the package. The program package, including source codes, example data sets, executables, and this documentation, is distributed free of charge for academic use only. Permission is granted to copy and use programs in the package provided no fee is charged for it and provided that this copyright notice is not removed.

Suggested citation:

Yang, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood *CABIOS* **13**:555-556
(<http://abacus.gene.ucl.ac.uk/software/paml.html>).

The author can be reached at
[Ziheng Yang](#)
[Department of Biology](#)
[University College London](#)
Gower Street, London WC1E 6BT
Email: z.yang@ucl.ac.uk
Phone: +44 (20) 7679 4379
Fax: +44 (20) 7679 7096

Table of Contents

0	Recent changes and bug fixes	3
1	Introduction	3
2	Getting started.....	4
	Windows 95/98NT/2000/XP and UNIX (including Mac OS X)	4
	MACs	6
3	Files in the Package	6
	Which files are needed?	8
4	Using Programs in the Package.....	8
	Sequence data format.....	8
	Tree file and representations of tree topology.....	11
	baseml	12
	basemlg	17
	codeml (codonml and aaml)	17
	Codon sequences (seqtype = 1)	18
	Amino acid sequences (seqtype = 2).....	21
	evolver	22
	yn00	24
	mcmctree.....	24
5	Models and Methods.....	25
	Nucleotide substitution models.....	25
	Codon substitution models	28
	Amino acid substitution models	28
	Models for combined analyses of heterogeneous data (multiple genes or codon positions)	29
	For nucleotides (baseml).....	29
	For codons and amino acids (codeml).....	31
	Global and local clocks, and dated sequences.....	31
	Reconstruction of ancestral sequences	32
	Analysing large data sets and iteration algorithms for parameter estimation.....	33
	Tree search algorithms.....	34
	Simulation.....	34
6	Technical Notes.....	36
	The rub file recording the progress of iteration	36
	How to specify initial values.....	36
	Fine-tuning the iteration algorithm	36
	Adjustable variables in the source codes	37
	PowerMAC memory allocation problem.....	37
	MS Windows version window auto-close	38
7	Acknowledgments.....	38
8	References	38

0 Recent changes and bug fixes

See the file `pamlHistory.txt` for recent changes and bug fixes.

The `examples/` folder in the package contain example data sets and results. Not all of them are described in this documentation. See the `readme` files in those folders.

1 Introduction

PAML is a package of programs for phylogenetic analyses of DNA or protein sequences using maximum likelihood (ML). The PAML web page (<http://abacus.gene.ucl.ac.uk/software/paml.html>) explains what the programs can and cannot do, how to download and compile the programs, and how to report bugs. Those will not be duplicated in this documentation. There is now also an FAQ page at <http://abacus.gene.ucl.ac.uk/software/pamlFAQs.html>.

The program `baseml` is for analyzing nucleotide sequences. The program `codeml` is formed by merging two old programs: `codonml`, which implements the codon substitution model of Goldman and Yang (1994) for protein-coding DNA sequences, and `aaml`, which implements models for amino acid sequences. These two are now distinguished by a variable named `seqtype` in the control file `codeml.ctl`, that is, 1 for codon sequences and 2 for amino acid sequences. In this document I use `codonml` and `aaml` to mean `codeml` with `seqtype = 1` and 2, respectively. The programs `baseml`, `codonml`, and `aaml` use similar algorithms to fit models, the difference being that the unit of evolution in the substitution model, referred to as a "site" in the sequence, is a nucleotide, a codon, or an amino acid for the three programs, respectively. Markov process models are used to describe substitutions between nucleotides, codons or amino acids, with substitution rates assumed to be either constant or variable among sites. A *discrete-gamma* model (Yang, 1994c) is used in `baseml`, `codonml` and `aaml` to accommodate rate variation among sites, by which rates for sites come from several (say, four or eight) categories used to approximate the continuous gamma distribution. When rates are variable at sites, the *auto-discrete-gamma* model (Yang, 1995) accounts for correlation of rates between adjacent sites.

The program `basemlg` implements the continuous gamma model of Yang (1993). It is slow and unfeasible for data of >6 or 7 species. The discrete-gamma model in `baseml` is recommended.

General assumptions of the models (programs) are

- Substitutions occur independently in different lineages;
- Substitutions occur independently among sites (except for the auto-discrete-gamma model which account for correlated substitution rates at neighboring sites);

- The process of substitution is described by a time-homogeneous Markov process. Further restrictions may be placed on the structure of the *rate matrix* of the process and lead to different substitution models;

The process of substitution is stationary. In other words, the frequencies of nucleotides (`baseml`), codons (`codonml`), or amino acids (`aaml`) have remained constant over the time period covered by the data.

The existence of a molecular clock (rate constancy among lineages) is not necessary but can be imposed. Variation (and dependence) of rates at sites is allowed by the discrete-gamma (or auto-discrete-gamma) models implemented in `baseml`, `codonml` and `aaml`.

The sequences must be aligned. If there are alignment gaps, they will either be removed from all sequences before analysis, with appropriate adjustment to the sequence length (if `cleandata = 1`), or treated as ambiguity characters (if `cleandata = 0`).

Other small programs in the package include `evolver` for simulating sequence data sets, `pamp` for parsimony-based analysis (Yang and Kumar 1996), and `yn00` for estimating synonymous and nonsynonymous substitution rates in pairwise comparisons using the method of Yang and Nielsen (2000).

This document is now mainly an explanation of the control variables in the control files for individual programs. Topics that seem too complicated to explain there are dealt with in a section in the Chapter "Models and Methods".

2 Getting started

Windows 95/98NT/2000/XP and UNIX (including Mac OS X)

In the good old days, we type commands on a command line. Nowadays most people know dragging and double-clicking or scrolling only. PAML programs do not have a graphics or menu based interface, and so you have to know some basic techniques of the good old days. Here is the basics for getting you started.

- (1) download and unpack the archive into a folder, say `/paml/` (or `~/paml/` on UNIX). You should remember the name of the folder.
- (2) Start a command box. You do this by choosing "Start - Programs" and look for a command called "MS-DOS prompt" or "Command Prompt". It is usually in the group "Accessories". On UNIX, you will be in a shell window as soon as you log on by telnet.
- (3) Change directory to the `paml` folder. For example you type one of the following.

```
cd paml
cd ~/paml
cd \paml
```

- (4) Run a program. Next you type a command, for example, codeml, and hit the “Enter” key.

```
codeml
```

This makes codeml to read the default control file codeml.ctl and do the analysis according to it. Now you can print out a copy of codeml.ctl, and open your favourite text editor to view the relevant sequence data and tree files.

Next you can prepare your own sequence data files and tree files. PAML data files are plain text files. If you use MS Word to prepare them, make sure you save them as “Text with line breaks” or “Text without line breaks”. Most likely only one of those two formats works.

A few commonly used DOS and UNIX commands

DOS/Windows	UNIX	Function
cd, chdir	cd, chdir, pwd	Sets and displays current directory (folder)
copy	cp, cat	Copies files
del	rm	Deletes files
dir	ls	Lists files
exit	exit	Exits from the command processor
find	fgrep	Searches for a string in files
help	man	Gets help
md	mkdir	Makes a new directory
more	more, less	Displays file contents by screenfuls
path	set PATH	Sets search path for commands
print	lpr	Prints files
rd, rmdir	rmdir, rm -r	Removes directories
ren	mv	Renames a file
time, date	date	Displays or sets time and date
type	cat	Displays the contents of a file
xcopy	cp	Copies files and subdirectories
ftp	ftp	Starts an ftp session
telnet	telnet	Starts a telnet session
	Ctrl-Z, followed by bg	Puts a foreground job into the background
	fg	Brings a job to the foreground
	nice, renice	Be nice to others by running your jobs at a lower priority

MACs

You double-click on the executable such as `codeml`. The compiled executables should ask you for the name of the control file. The default name is `codeml.ctl`. My experience of MACs was several years old and was not a very good one.

3 Files in the Package

The following files are included in the package:

Source codes:

- `baseml.c`: various models for nucleotide sequences
- `codeml.c`: models for codon (`seqtype = 1`) and amino acid (`seqtype = 2`) sequences
- `pamp.c`: parsimony analyses of nucleotide or amino acid sequences
- `mcmctree.c`: Markov chain Monte Carlo algorithm for Bayes estimation of phylogenies
- `evolver.c`: simulation of sequence data and comparison of trees
- `basemlg.c`: Nucleotide-based model with (continuous) gamma rates among sites
- `yn00.c`: Estimation of dN and dS by the method of Yang and Nielsen (2000)
- `treesub.c`: a few functions
- `treespace.c`: a few more functions
- `tools.c`: my toolkit
- `tools.h`: header file
- `eigen.c`: routines for calculating eigen values and vectors

Compiling commands

- Makefile: make file
- `paml.cc`: batch file for compiling PAML using the `cc` compiler
- `paml.gcc`: batch file for compiling PAML using the GNU `gcc` compiler
- `paml.acc`: batch file for compilation PAML using the SUN `acc` compiler

Control files:

- `baseml.ctl`: control file for running `baseml` and `basemlg`;
- `codeml.ctl`: control file for `codeml` (i.e., `codonml` and `aaml`)
- `pamp.ctl`: control file for `pamp`
- `yn00.ctl`: control file `yn00`
- `mcmctree.ctl`: control file for `mcmctree`

Data files for codeml (see the files for details):

- `grantham.dat`: amino acid distance matrix (Grantham 1974)
- `miyata.dat`: amino acid distance matrix (Miyata *et al.* 1980)
- `dayhoff.dat`: Empirical amino acid substitution matrix of Dayhoff *et al.* (1978)
- `jones.dat`: Empirical amino acid substitution matrix of Jones *et al.* (1992)

wag.dat: Empirical amino acid substitution matrix of Whelan and Goldman (in press)

mtREV24.dat: Empirical amino acid substitution matrix of Adachi and Hasegawa (1996b)

mtmam.dat: Empirical amino acid substitution matrix for mitochondrial proteins of mammals

Data files for `evolver` (see those small files for details):

MCbase.dat: data file for simulating nucleotide sequences

MCcodon.dat: data file for simulating codon sequences

MCaa.dat: data file for simulating amino acid sequences

Example tree files:

4s.trees: tree structure file for 4-sequence data

5s.trees: tree structure file for 5-sequence data

Documentations:

paml.readme: readme file

paml.html: paml web page, serving also as part of the manual (html file)

pamlDOC.pdf: this document

Example data sets:

Several example data sets are included. They were used in our papers to test new methods, and are included in the package for error-checking.

brown.nuc: the 895-bp mtDNA data of Brown *et al.* (1982), used in Yang *et al.* (1994) and Yang (1994c) to test models of variable rates among sites.

mtprim9.nuc: mitochondrial segment consisting 888 aligned sites from 9 primate species (Hayasaka *et al.* 1988), used by Yang (1994c) to test the discrete-gamma model and Yang (1995) to test the auto-discrete-gamma models.

abglobin.nuc: the concatenated alpha and beta globin genes, example data for `condonml`

exampleTipDate.phy (phylip format), exampleTipDate.trees: data set of 17 dingo viral strains sequenced at different dates from Andrew Rambaut's TipDate program. This is used for testing the TipDate models of Rambaut (2000). Run `baseml` by specifying `clock = 3`. The results are included in the file `exampleTipDate.rst`.

HIVenvSweden.paup (paup* format), HIVenvSweden.trees, HIVenvSweden.ctl: 13 HIV *env* genes used by Yang *et al.* (2000) in developing models of variable selective pressures among sites (the `Nssites` models). (Use command: `codeml sites HIVenvSweden.ctl`)

hummt25.nuc: 25 human D-loop sequences used in Yang and Kumar (1995). Run `baseml` by specifying `fix_alpha = 0`, or run `pamp`.

lysozymeSmall.nuc, lysozymeSmall.trees, lysozyme.ctl: primate lysozyme genes of Messier and Stewart 1997, used by Yang (1998) in developing tests of positive selection along lineages. This is the "small data set" analyzed in that paper. See the control file `lysozyme.ctl` for details for specifying the different models. Run the analysis by `codeml lysozyme.ctl`

`stewart.aa`, `stewart.trees`: lysozyme sequences of six mammals (Stewart *et al.* 1987), used by Yang *et al.* (1995) to test methods for reconstructing ancestral amino acid sequences.

`abglobin.aa`: the concatenated alpha- and beta-globins, translated from `abglobin.nuc`

Which files are needed?

You may copy the executables to a directory containing your data files. Please note that the program `codeml` may need some of the data files in the package such as `grantham.dat`, `dayhoff.dat`, `jones.dat`, `wag.dat`, `mtREV24.dat`, or `mtmam.dat`. You should probably copy these files together. Other programs do not need such data files apart from the sequence and tree files you specify in the control file.

Note also that the programs produce result files. Some other files with names `rub`, `lnf`, `rst`, or `rates` may also be created. You should not use these names for your files.

4 Using Programs in the Package

Sequence data format

Have a look at the example data files in the package (`*.nuc`, `*.aa`, and `*.paup`). As long as you get your data file into one of the formats, PAML programs should be able to read it. PAML now has limited support for the NEXUS file format used by PAUP and MacClade. Only the sequence data or trees are read, and command blocks are all ignored. PAML does not deal with comment blocks in the sequence data block, so try to avoid them.

Below is an example of the PHYLIP format (Felsenstein, 1993). The first line contains the number of species and the sequence length (possibly followed by option characters). With `codonml` (`codeml` with `seqtype = 1`), the sequence length in the sequence file refers to the number of nucleotides rather than the number of codons. The only options allowed in the sequence file are I, S, C and G. The sequences may be in either *interleaved* format (option I, example data file `abglobin.nuc`), or *sequential* format (option S, example data file `brown.nuc`). The default option is S. (Option G is used for combined analysis of multiple gene data and is explained below.) The following is an example data set in the sequential format. It has 4 sequences each of 60 nucleotides.

```
4 60
sequence 1
AAGCTTCACCGGCGCAGTCATTCTCATAAT
CGCCCACGGACTTACATCCTCATTACTATT
sequence 2
AAGCTTCACCGGCGCAATTATCCTCATAAT
CGCCCACGGACTTACATCCTCATTATTATT
```



```

sequence 3
AAGCTTCACCGGCGCAGTTGTTCTTATAAT
TGCCACGGACTTACATCATCATTATTATT
sequence 4
AAGCTTCACCGGCGCAACCACCCTCATGAT
TGCCCATGGACTCACATCCTCCCTACTGTT

```

Species names. Do not use special symbols like , : # () in a species name as they may confuse the programs. The maximum number of characters in a species name (LSPNAME) is specified at the beginning of the main programs `baseml.c` and `codeml.c`. The default value is 30. In PHYLIP, exactly 10 characters are used for a species name. To make this discrepancy less a problem, PAML considers two consecutive spaces as the end of a species name, so that the species name does not have to have exactly 30 (or 10) characters. To make this rule work, you should not have two consecutive spaces *within* a species name. For example the above data set can have the following format too.

```

4 60
sequence 1 AAGCTTCACCGGCGCAGTCATTCTCATAAT
CGCCACGGACTTACATCCTCATTACTATT
sequence 2 AAGCTTCACCGGCGCAATTATCCTCATAAT
CGCCACGGACTTACATCCTCATTATTATT
sequence 3 AAGCTTCACC GGCGCAGTTG TTCTTATAAT
TGCCACGGACTTACATCATCATTATTATT
sequence 4 AAGCTTCACCGGCGCAACCACCCTCATGAT
TGCCCATGGACTCACATCCTCCCTACTGTT

```

Another thing you can do is to patch a few spaces after the species name in your PHYLIP data file, which will then be readable by both PHYLIP and PAML.

In a sequence, three special characters ".", "-", and "?" may be used: a dot means the same character as in the first sequence, a dash means an alignment gap, and a question mark means an undetermined site. Sites at which at least one sequence involves a "-" or "?" are excluded from all sequences before analysis, with the sequence length adjusted. For codon sequences, the whole codon is removed. Characters T, C, A, G, U, t, c, a, g, u are recognized as nucleotides (for `baseml`, `basemlg` and `codonml`), while the standard one-letter codes (A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V or their lowercase equivalents) are used for amino acids. Other alphabetic characters cause errors. Non-alphabetic symbols such as ><!"£\$%^0123456789 are simply ignored and can be freely used as landmarks. Lines do not have to be equally long and you can put the whole sequence on one line.

Notes may be placed at the end of the sequence file and will be ignored by the programs.

Option G: This option is for combined analyses of heterogeneous data sets such as data of multiple genes or data of the three codon positions. The sequences must be

The third format applies to protein-coding DNA sequences only (for `baseml`). You use option characters `GC` on the first line instead of `G` alone. The program will then treat the three codon positions differently in the nucleotide-based analysis. It is assumed that the sequence length is an exact multiple of three.

```
5 855 GC
human      GTG CTG TCT CCT ...
```

Tree file and representations of tree topology

A *tree structure file* is used when `runmode = 0` or `1`. The file name is specified in the appropriate control file. Two methods for representing a tree topology are used in PAML.

Parenthesis notation: The first is the familiar parenthesis representation, that is used in virtually every phylogenetic software. The species can be represented using either their names or their indexes corresponding to the order of their occurrences in the sequence data file. If species names are used, they have to match exactly those in the sequence data file (including spaces or strange characters). Branch lengths are allowed. The following is a possible tree structure file for a data set of four species (human, chimpanzee, gorilla, and orangutan, occurring in this order in the data file). The first tree is a star tree, while the next four trees are the same.

```
4 5 // 4 species, 5 trees
(1,2,3,4) // the star tree
((1,2),3,4) // species 1 and 2 are clustered together
((1,2),3,4) // Commas are needed with more than 9 species
(human,chimpanzee),gorilla,orangutan);
((human:.1,chimpanzee:.2):.05,gorilla:.3,orangutan:.5);
```

If the tree has branch lengths, some programs may ask you whether you want to use those branch lengths as fixed and estimate other parameters in the substitution model only. You will then have three options: ignore the branch lengths, use them as initial values, and fix them and estimate other parameters.

Whether you should use rooted or unrooted trees depends on the model, and in particular on whether a molecular clock is assumed. Without the clock (`clock = 0`), unrooted trees should be used, such as `((1,2),3,4)` or `(1,2,(3,4))`. With the clock assumption, the trees should be rooted and these two trees are different and both are different from `((((1,2),3),4)`. In PAML, a rooted tree has a bifurcation at the root, while an unrooted tree has a trifurcation or multifurcation at the root.

Branch labels: Sometimes (such as the local clock models specified by `clock = 2` in `baseml` and `codeml` and codon models with different ω s for branches), we need to label the branches. Branch labels are specified in the same way as branch lengths except that the symbol preceding the branch label is `#` or `$` rather than `:` which indicates a branch length. The branch labels are consecutive integers starting from 0, which is the default label and does not have to be specified. For example, the following tree

```
((Hsa_Human, Hla_gibbon) #1, ((Cgu/Can_colobus, Pne_langur), Mmu_rhesus),
(Ssc_squirrelM, Cja_marmoset));
```

is from the tree file `lysozyme.trees`, with branch labels for fitting models of different d_N/d_S ratios (ω) for branches. The internal branch ancestral to human and gibbon has the ratio ω_1 while all other branches have the background ratio ω_0 . This fits the model in table 1C for the small data set of lysozyme genes in Yang (1998). I have found it convenient to create the tree file with labels and read the tree in using Rod page's [TreeView](#) to check that the tree is right. However, TreeView recognizes labels for internal branches (nodes) only and do not allow the user to add labels. If you don't specify the labels in the tree structure, the program will ask for input from the keyboard. For large trees, this may be hard to use. Another program that you can use to create branch or node labels is Andrew Rambaut's [TreeEdit](#), available for the Mac.

If the model requires labeling branches but the tree does not has any branch labels, the program will ask for input from the keyboard. See the explanations of the variable `model` for the program `codonml`.

Representation of tree topology by branches: A second way of representing the tree topology used in PAML is by enumerating its branches. This is mainly used in the result files for outputting the estimated branch lengths. For example, the tree ((12)34) is specified by its 5 branches:

```
5 6, 6 1, 6 2, 5 3, 5 4
```

The nodes in a tree are marked with consecutive natural numbers, with 1, 2, ..., s representing the s known sequences, in the same order as in the data. A number larger than s means an interior node, at which the sequence is unknown. In case some sequences in the data are ancestral to some others, this method is convenient. To use this format in the tree structure file, give the number of branches, and then the branches as specified by the end nodes. For example, the tree in the following `5s.trees` file has 4 branches, with taxon 5 to be the common ancestor of taxa 1, 2, 3, and 4:

```
5 1
4      5 1      5 2      5 3      5 4
```

baseml

The default control file for `baseml` is `baseml.ct1`, and an example is shown below. Note that spaces are required on both sides of the equal sign, and blank lines or lines beginning with "*" are treated as comments. Options not used can be deleted from the control file.

```
seqfile = brown.nuc * sequence data file name
outfile = mlb * main result file
treefile = brown.trees * tree structure file name

noisy = 3 * 0,1,2,3: how much rubbish on the screen
verbose = 0 * 1: detailed output, 0: concise output
runmode = 0 * 0: user tree; 1: semi-automatic; 2: automatic
           * 3: StepwiseAddition; (4,5):PerturbationNNI

model = 4 * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85, 5:TN93, 6:REV, 7:UNREST
Mgene = 0 * 0:rates, 1:separate; 2:diff pi, 3:diff kapa, 4:all diff
```

```

fix_kappa = 0 * 0: estimate kappa; 1: fix kappa at value below
kappa = 2.5 * initial or fixed kappa

fix_alpha = 1 * 0: estimate alpha; 1: fix alpha at value below
alpha = 0. * initial or fixed alpha, 0:infinity (constant rate)
Malpha = 0 * 1: different alpha's for genes, 0: one alpha
ncatG = 5 * # of categories in the dG, AdG, or nparK models of rates

fix_rho = 1 * 0: estimate rho; 1: fix rho at value below
rho = 0. * initial or fixed rho, 0:no correlation
nparK = 0 * rate-class models. 1:rK, 2:rK&fK, 3:rK&MK(1/K), 4:rK&MK

clock = 0 * 0:no clock, 1:clock; 2:local clock; 3:TipDate
nhomo = 0 * 0 & 1: homogeneous, 2: kappa for branches, 3: N1, 4: N2
getSE = 0 * 0: don't want them, 1: want S.E.s of estimates
RateAncestor = 0 * (0,1,2): rates (alpha>0) or ancestral states

Small_Diff = 9e-6
* cleandata = 1 * remove sites with ambiguity data (1:yes, 0:no)?
* ndata = 1
method = 0 * 0: simultaneous; 1: one branch at a time

```

The control variables are described below.

seqfile, **outfile**, and **treefile** specifies the names of the sequence data file, main result file, and the tree structure file, respectively.

noisy controls how much output you want on the screen. If the model being fitted involves much computation, you can choose a large number for **noisy** to avoid loneliness. **verbose** controls how much output in the result file.

runmode = 0 means evaluation of the tree topologies specified in the tree structure file, and **runmode** = 1 or 2 means heuristic tree search by the star-decomposition algorithm. With **runmode** = 2, the algorithm starts from the star tree, while if **runmode** = 1, the program will read a multifurcating tree from the tree structure file and try to estimate the best bifurcating tree compatible with it. **runmode** = 3 means stepwise addition. **runmode** = 4 means NNI perturbation with the starting tree obtained by a parsimony algorithm, while **runmode** = 5 means NNI perturbation with the starting tree read from the tree structure file. The tree search options do not work well, and so use **runmode** = 0 as much as you can. For relatively small data set, the stepwise addition algorithm seems usable.

model specifies the model of nucleotide substitution. Models 0, 1, ..., 7 represent models JC69, K80, F81, F84, HKY85, TN93, REV, and UNREST, respectively. Check Yang (1994 JME 39:105-111) for notation. Two more models are implemented recently. **model** = 8 are special cases of the REV model, while **model** = 9 are special cases of the unrestricted model. The format is shown in the following examples and should be self-explanatory. Basically you include extra information on the same line that specifies model when **model** = 8 or 9. The number in the brackets [] are the number of free rate parameters. For example, this should be 5 for REV and 11 for UNREST. Following that number are equal number of parenthesis pairs (). The rate parameters in the output file will follow this order here. The pairs that are not mentioned here will have the rate 1. When **model** = 8, you specify TC or CT, but not both. When **model** = 9, TC and CT are different. See the following examples and Yang (1994a) for notation.

```

model = 8 [2 (CT) (AG)] /* TN93 */

```

```

model = 8 [2 (TA AT TG CA CG) (AG)] /* TN93 */
model = 9 [1 (TC CT AG GA)] /* K80 */
model = 9 [0] /* JC69 */
model = 9 [11 (TA) (TG) (CT) (CA) (CG) (AT) (AC) (AG) (GT) (GC) (GA)]
/*UNREST*/

```

Mgene is used in combination with option G in the sequence data file, for combined analysis of data from multiple genes or the three codon positions. More details are given later in the Models and Methods section. Choose 0 if option G is not used in the data file.

fix_kappa specifies whether κ in K80, F84, or HKY85 is given at a fixed value or is to be estimated by iteration from the data. If `fix_kappa = 1`, the value of another variable, `kappa`, is the given value, and otherwise the value of `kappa` is used as the initial estimate for iteration. The variables `fix_kappa` and `kappa` have no effect with JC69 or F81 which does not involve such a parameter, or with TN93 and REV which have two and five rate parameters respectively, when all of them are estimated from the data.

fix_alpha and `alpha` work in a similar way, where `alpha` refers to the shape parameter α of the gamma distribution for variable substitution rates across sites (Yang 1994c). The model of a single rate for all sites is specified as `fix_alpha = 1` and `alpha = 0` (0 means infinity), while the (discrete-) gamma model is specified by a positive value for `alpha`, and `ncatG` is then the number of categories for the discrete-gamma model (`baseml`).

fix_rho and `rho` work in a similar way and concern independence or correlation of rates at adjacent sites, where ρ (`rho`) is the correlation parameter of the auto-discrete-gamma model (Yang 1995). The model of independent rates for sites is specified as `fix_rho = 1` and `rho = 0`; choosing `alpha = 0` further means a constant rate for all sites. The auto-discrete-gamma model is specified by positive values for both `alpha` and `rho`. The model of a constant rate for sites is a special case of the (discrete) gamma model with $\alpha = \infty$ (`alpha = 0`), and the model of independent rates for sites is a special case of the auto-discrete-gamma model with $\rho = 0$ (`rho = 0`).

nparK specifies nonparametric models for variable and Markov-dependent rates across sites: `nparK = 1` or `2` means several (`ncatG`) categories of independent rates for sites, while `nparK = 3` or `4` means the rates are Markov-dependent at adjacent sites; `nparK = 1` and `3` have the restriction that each rate category has equal probability while `nparK = 2` and `4` do not have this restriction (Yang, 1995). The variable `nparK` takes precedence over `alpha` or `rho`.

clock specifies models concerning rate constancy among lineages. `clock = 0` means no clock. `clock = 1` means the global clock. `clock = 2` implements local clock models (Yoder and Yang 2000), which assumes that branches in the phylogeny conform with the clock assumption and has the default rate ($r_0 = 1$) except for several pre-defined branches which have different rates. Rates for branches are specified using branch marks in the tree file. If you choose `noisy = 9`, the program will ask for a reference (calibration) node and date and calculate dates for other nodes. `clock = 3` implements

Rambaut (2000)'s TipDate models. Evolution conforms to a global clock but sequences in the data are determined at different dates. The dates are specified at the end of the sequence names, based on Andrew's format. This model has one extra parameter (the mutation rate) than the global clock model (`clock = 1`).

`nhomo` is for `baseml` only, and concerns the frequency parameters in the F81, F84, HKY85, TN93, or REV models. The option `nhomo = 1` fits a homogeneous model, but estimates the frequency parameters (π_T , π_C and π_A ; π_G is not a free parameter as the frequencies sum to 1) by maximum likelihood iteration. Normally (`nhomo = 0`) these are estimated by the averages of the observed frequencies. In both cases, you should count 3 free parameters for the base frequencies. The options `nhomo = 3` or `4`, in combination with F84 or HKY85, fit nonhomogeneous models of Yang and Roberts (1995). Substitutions are assumed to follow the pattern of F84 or HKY85, but with different frequency parameters assigned for different branches in the tree, to allow for unequal base frequencies in different sequences. The position of the root then makes a difference to the likelihood, and rooted trees are used. Because of the parameter richness, the model may only be used with small data sets when base frequencies are drastically different in different sequences. Choose `fix_kappa = 1`, which means one common κ is assumed for all branches. The option `nhomo = 4` assigns one set of frequency parameters for each node, which are the parameters for the rate matrix along the branch leading to the node or are the initial distribution if the node is the root of the tree. In the output, estimates of the frequency parameters are shown in the order of nodes $n + 1$, $n + 2$, ..., where n is the number of sequences.

`nhomo = 2` uses one transition/transversion rate ratio (κ) for each branch in the tree for the K80, F84, and HKY85 models (Yang 1994b; Yang and Yoder 1999).

`getSE` tells whether we want estimates of the standard errors of estimated parameters. These are crude estimates, calculated by the curvature method, *i.e.*, by inverting the matrix of second derivatives of the log-likelihood with respect to parameters. The second derivatives are calculated by the difference method, and are not always reliable. Even if this approximation is reliable, tests relying on the SE's should be taken with caution, as such tests rely on the normal approximation to the maximum likelihood estimates. The likelihood ratio test should always be preferred. The option is not available and choose `getSE = 0` when tree-search is performed.

`RateAncestor = 1` also works with `runmode = 0` only. For models of variable rates across sites, the program will calculate rates for sites along the sequence (output in the file `rates`) and performs marginal ancestral reconstruction (output in `rst`). For models of one rate for all sites, `RateAncestor = 1` does both marginal and joint ancestral sequence reconstruction. The program lists results site by site. You can also use the variable `verbose` to control the amount of output. If you choose `verbose = 0`, the program will list the best nucleotide at each node for the variable sites only and results for constant sites are suppressed. If `verbose = 1`, the program will list all sites for the best nucleotide at each node. If `verbose = 2`, the program also lists the full posterior probability distribution for each site at each ancestral node (for marginal reconstruction).

For nucleotide based (`baseml`) analysis of protein coding DNA sequences (option `GC` in the sequence data file), I have added the calculation of posterior probabilities of ancestral amino acids. In this analysis, branch lengths and other parameters are estimated under a nucleotide substitution model, but the reconstructed nucleotide triplets are examined to infer the most likely amino acid encoded by the triplet. Posterior probabilities for stop codons are small and reset to zero to scale the posterior probabilities for amino acids. To use this option, you need add the control variable `icode` in the control file `baseml.ctl`. This is not listed in the above. The variable `icode` can take a value out of 0, 1, ..., 10, corresponding to the 11 genetic codes included in `paml` (See the control file `codeml.ctl` for the definition of different genetic codes). A nucleotide substitution model that is very close to a codon-substitution model can be specified as follows. You add the option characters `GC` at the end of the first line in the data file and choose `model = 4` (HKY85) and `Mgene = 4`. The model then assumes different substitution rates, different base frequencies, and different transition/transversion rate ratio (κ) for the three codon positions. Ancestral reconstruction from such a nucleotide substitution should be very similar to codon-based reconstruction. (Thanks to Belinda Change for many useful suggestions.)

Small_Diff is a small value used in the difference approximation of derivatives.

cleandata = 1 means sites involving ambiguity characters or alignment gaps are removed from all sequences. This leads to faster calculation. `cleaddata = 0` (default) uses those sites.

method: This variable controls the iteration algorithm for estimating branch lengths under a model of no clock. `method = 0` implements the old algorithm in PAML, which updates all parameters including branch lengths simultaneously. `method = 1` specifies an algorithm newly implemented in PAML, which updates branch lengths one by one. `method = 1` does not work under the clock models (`clock = 1, 2, 3`).

ndata: specifies the number of separate data sets in the file. This variable is useful for simulation. You can use `evolver` to generate 200 replicate data sets, and then set `ndata = 200` to use `baseml` to analyze them.

Output: The output should be self-explanatory. Descriptive statistics are always listed. The observed site patterns and their frequencies are listed, together with the proportions of constant patterns. Nucleotide frequencies for each species (and for each gene in case of multiple gene data) are counted and listed. $\ln(L_{max})$ is the upper limit of the log likelihood and may be compared with the likelihood for the best (or true) tree under the substitution model to test the model's goodness of fit to data (Goldman, 1993a). You can ignore it if you don't know what it means.

With `getSE = 1`, the S.E.s are calculated as the square roots of the large sample variances and listed exactly below the parameter estimates. Zeros on this line mean errors, either caused by divergence of the algorithm or zero branch lengths. The S.Es of the common parameters measure the reliability of the estimates. For example, $(\kappa - 1)/SE(\kappa)$, when κ is estimated under K80, can be compared with a normal distribution to see whether there is real difference between K80 and JC69. The test can be more

reliably performed by comparing the log-likelihood values under the two models, using the likelihood ratio test. It has to be stressed that the S.E.'s of the estimated branch lengths should not be misinterpreted as an evaluation of the reliability of the estimated tree topology (e.g., Yang, 1994b, Goldman and Yang, 1994).

If the tree file has more than one tree, the programs `baseml` and `codeml` will calculate the bootstrap proportions using the RELL method (Kishino and Hasegawa 1989), as well as the method of Shimodaira and Hasegawa (1999) with a correction for multiple comparison. The bootstrap resampling accounts for possible data partitions (option G in the sequence data file). I did not bother to deal with ties, so if you include the same tree in the tree file more than once, you need to adjust the proportions for those trees yourself. The program `rell`, included in earlier versions, is now removed.

basemlg

`basemlg` uses the same control file `baseml.ctl`, as `baseml`. Tree-search or the assumption of a molecular clock are not allowed and so choose `runmode = 0` and `clock = 0`. Substitution models available for `basemlg` are JC69, F81, K80, F84 and HKY85, and a continuous gamma is always assumed for rates at sites. The variables `ncatG`, `given_rho`, `rho`, `nhomo` have no effect. The S.E.'s of parameter estimates are always printed out because they are calculated during the iteration, and so `getSE` has no effect.

Because of the intensive computation required by `basemlg`, the discrete-gamma model implemented in `baseml` is recommended for data analysis. If you choose to use `basemlg`, you should run `baseml` first, and then run `basemlg`. This allows `baseml` to collect initial values into a file named `in.basemlg`, for use by `basemlg`. Note that `basemlg` implements only a subset of models in `baseml`.

codeml (codonml and aaml)

Since the codon based analysis and the amino acid based analysis use different models, and some of the control variables have different meanings, it may be a good idea to use different control files for codon and amino acid sequences. The default control file for `codeml` is `codeml.ctl`, as shown below.

```
seqfile = stewart.aa * sequence data file name
outfile = mlc * main result file name
treefile = stewart.trees * tree structure file name

noisy = 9 * 0,1,2,3,9: how much rubbish on the screen
verbose = 0 * 1: detailed output, 0: concise output
runmode = 0 * 0: user tree; 1: semi-automatic; 2: automatic
          * 3: StepwiseAddition; (4,5):PerturbationNNI; -2: pairwise

seqtype = 2 * 1:codons; 2:AAs; 3:codons-->AAs
CodonFreq = 2 * 0:1/61 each, 1:F1X4, 2:F3X4, 3:codon table
aaDist = 0 * 0:equal, +:geometric; -:linear, 1-6:G1974,Miyata,c,p,v,a
aaRatefile = wag.dat * only used for aa seqs with model=empirical(_F)
               * dayhoff.dat, jones.dat, wag.dat, mtmam.dat, or your own

model = 2
          * models for codons:
```

```

* 0:one, 1:b, 2:2 or more dN/dS ratios for branches
* models for AAs or codon-translated AAs:
* 0:poisson, 1:proportional,2:Empirical,3:Empirical+F
* 6:FromCodon, 7:AAClasses, 8:REVaa_0, 9:REVaa(nr=189)

NSsites = 0 * 0:one w;1:neutral;2:selection; 3:discrete;4:freqs;
* 5:gamma;6:2gamma;7:beta;8:beta&w;9:beta&gamma;
* 10:beta&gamma+1; 11:beta&normal>1; 12:0&2normal>1;
* 13:3normal>0

icode = 0 * 0:universal code; 1:mammalian mt; 2-10:see below
Mgene = 0 * 0:rates, 1:separate;

fix_kappa = 0 * 1: kappa fixed, 0: kappa to be estimated
kappa = 2 * initial or fixed kappa
fix_omega = 0 * 1: omega or omega_1 fixed, 0: estimate
omega = .4 * initial or fixed omega, for codons or codon-based AAs

fix_alpha = 1 * 0: estimate gamma shape parameter; 1: fix it at alpha
alpha = 0. * initial or fixed alpha, 0:infinity (constant rate)
Malpha = 0 * different alphas for genes
ncatG = 3 * # of categories in dG of NSsites models

fix_rho = 1 * 0: estimate rho; 1: fix it at rho
rho = 0. * initial or fixed rho, 0:no correlation

clock = 0 * 0:no clock, 1:clock; 2:local clock; 3:TipDate
getSE = 0 * 0: don't want them, 1: want S.E.s of estimates
RateAncestor = 0 * (0,1,2): rates (alpha>0) or ancestral states (1 or 2)

Small_Diff = .5e-6
* cleandata = 0 * remove sites with ambiguity data (1:yes, 0:no)?
* ndata = 10
method = 0 * 0: simultaneous; 1: one branch at a time

```

The variables `seqfile`, `outfile`, `treefile`, `noisy`, `Mgene`, `fix_alpha`, `alpha`, `Malpha`, `fix_rho`, `rho`, `clock`, `getSE`, `RateAncestor`, `Small_Diff`, `cleandata`, `ndata`, and `method` are used in the same way as in `baseml.ctl` and are described in the previous section. The variable `seqtype` specifies the type of sequences in the data; `seqtype = 1` means codon sequences (the program is then `codonml`); `2` means amino acid sequences (the program is then `aaaml`); and `3` means codon sequences which are to be translated into proteins for analysis.

Codon sequences (`seqtype = 1`)

CodonFreq specifies the equilibrium codon frequencies in codon substitution model. These frequencies can be assumed to be equal ($1/61$ each for the standard genetic code, `CodonFreq = 0`), calculated from the average nucleotide frequencies (`CodonFreq = 1`), from the average nucleotide frequencies at the three codon positions (`CodonFreq = 2`), or used as free parameters (`CodonFreq = 3`). The number of parameters involved in those models of codon frequencies is 0, 3, 9, and 60 (under the universal code), for `CodonFreq = 0, 1, 2, and 3` respectively.

aaDist specifies whether equal amino acid distances are assumed (= 0) or Grantham's matrix is used (= 1) (Yang *et al.* 1998).

runmode = -2 performs ML estimation of d_s and d_N in pairwise comparisons. The program will collect estimates of d_s and d_N into the files `2ML.dS` and `2ML.dN`. Since many users seem interested in looking at d_N/d_s ratios among lineages, examination of

the tree shapes indicated by branch lengths calculated from the two rates may be interesting although the analysis is *ad hoc*. If your species names have no more than 10 characters, you can use the output distance matrices as input to Phylip programs such as `neighbor` without change. Otherwise you need to edit the files to cut the names short.

The variable `model` concerns assumptions about the d_N/d_S rate ratios (or the ω parameters) among branches (Yang 1998; Yang and Nielsen 1998). `model = 0` means one d_N/d_S ratio for all lineages (branches), 1 means one ratio for each branch (the free-ratio model), and 2 means an arbitrary number of ratios (such as the 2-ratios or 3-ratios models). When `model = 2`, you may specify the ω ratios for branches using branch labels (see the section on tree structure file). This option seems rather easy to use. Otherwise, the program will ask the user to input a branch mark for the d_N/d_S ratio assumed for each branch. This should be an integral number between 0 to $k - 1$ if k different d_N/d_S ratios ($\omega_0 - \omega_{k-1}$) are assumed for the branches of the tree. This process may be frustrating if the tree is not very small. I run the program first to let it output the tree topology using the branch representation on the screen. I then prepare the branch labels in a file, say, `in`. Finally I run the program using redirection so that it will read the input from the prepared file

```
codeml < in
```

Redirection is not permitted on a MAC, but you can prepare the branch labels in a file and then copy and paste them into the session running the program. Furthermore, under this model, the variable `fix_omega` fixes the last d_N/d_S ratio (ω_{k-1}) at the value of `omega` specified in the file. This option can be used to test, for example, whether the ratio for a specific lineage is significantly different from one. It should, however, be noted that it is not proper to use the option `model = 1` to estimate d_N/d_S ratios for all branches to find out which ratios are greater than one, and then to use `model = 2` to test whether that difference is significant. This way the hypothesis is derived from the data and is tested using the same data. As a result, you tend to get significant results too often. Check the example data file `lysozymeSmall.nuc` and the control file `lysozymectl` and try to reproduce results published in Yang (1998).

`Nssites` specifies models that allow the d_N/d_S ratio (ω) to vary among sites (Nielsen and Yang 1998; Yang *et al.* 2000). `Nssites = m` corresponds to model `Mm` in Yang *et al.* (2000). The variable `ncatG` is used to specify the number of categories in the ω distribution under some models. The values of `ncatG` used to perform our analyses are 3 for M3 (discrete), 5 for M4 (freq), 10 for the continuous distributions (M5: gamma, M6: 2gamma, M7: beta, M8:beta&w, M9:beta&gamma, M10: beta&gamma+1, M11:beta&normal>1, and M12:0&2normal>1, M13:3normal>0). This means M8 will have 11 site classes (10 from the beta distribution plus 1 additional class). The posterior probabilities for site classes as well as the expected ω values for sites are listed in the file `rst`, which may be useful to pinpoint sites under positive selection, if they exist. To make it easy to run several `Nssites` models in one go, I compiled the executable `codemlsites`, which asks you how many and which models to run at the start of the program. The number of categories used will then match those used in

Yang *et al.* (2000). The HIV *env* data set used in Yang *et al.* (2000: table 12) is included in the package. Try

```
codeml sites HIVenvSweden.ctl
```

and duplicate our analysis of the 4 models, M0, 1, 2, 3, by

```
4 0 1 2 3
```

As noted in that paper, some of the models are hard to use, in particular, M12 and M13. Recommended models are 0 (one-ratio), 1 (neutral), 2 (selection), 3 (discrete), 7 (beta), and 8 (beta& ω). Some of the models like M2 and M8 are noted to be prone to the problem of multiple local optima. You are advised to run the program at least twice, once with a starting omega value <1 and a second time with a value > 1, and use the results corresponding to the highest likelihood.

The continuous neutral and selection models of Nielsen and Yang (1998) are not implemented in the program.

icode specifies the genetic code. About a dozen genetic code tables are implemented. These are 0 for the universal code; 1 for the mammalian mitochondrial code; 3 for mold mt., 4 for invertebrate mt.; 5 for ciliate nuclear code; 6 for echinoderm mt.; 7 for euplotid mt.; 8 for alternative yeast nuclear; 9 for ascidian mt.; and 10 for blepharisma nuclear. icode = 0 to 10 correspond to transl_table 1 to 11 in GenBank.

RateAncestor: For codon sequences, ancestral reconstruction is not implemented for the models of variable dN/dS ratios among sites. The output under codon-based models usually shows the encoded amino acid for each codon. The output under "Prob of best character at each node, listed by site" has two posterior probabilities for each node at each codon (amino acid) site. The first is for the best codon. The second, in parentheses, is for the most likely amino acid under the codon substitution model. This is a sum of posterior probabilities across synonymous codons. In theory it is possible although rare for the most likely amino acid not to match the most likely codon.

Output for codon sequences (`seqtype = 1`): The codon frequencies in each sequence are counted and listed in a genetic code table, together with their sums across species. Each table contains six or fewer species. For data of multiple genes (option G in the sequence file), codon frequencies in each gene (summed over species) are also listed. The nucleotide distributions at the three codon positions are also listed. The method of Nei and Gojobori (1986) is used to calculate the number of synonymous substitutions per synonymous site (d_s) and the number of nonsynonymous substitutions per nonsynonymous site (d_N) and their ratio (d_N/d_s). These are used to construct initial estimates of branch lengths for the likelihood analysis but are not MLEs themselves. Note that the estimates of these quantities for the a- and b-globin genes shown in Table 2 of Goldman and Yang (1994), calculated using the MEGA package (Kumar *et al.*, 1993), are not accurate.

Results of ancestral reconstructions (`RateAncestor = 1`) are collected in the file `rst`. Under models of variable d_N/d_S ratios among sites (`NSsites` models), the posterior probabilities for site classes as well as positively selected sites are listed in `rst`.

Amino acid sequences (`seqtype = 2`)

model specifies the model of amino acid substitution: 0 for the Poisson model assuming equal rates for any amino acid substitutions (Bishop and Friday, 1987); 1 for the proportional model in which the rate of change to an amino acid is proportional to the frequency of that amino acid. Model = 2 specifies a class of empirical models, and the empirical amino acid substitution rate matrix is given in the file specified by `aaRatefile`. Files included in the package are for the empirical models of Dayhoff *et al.* (1978) (`dayhoff.dat`), Jones *et al.* 1992 (`jones.dat`) (see Kishino *et al.*, 1990 for the construction), and Whelan and Goldman (`wag.dat`). The file `mtmam.dat` has a matrix for mitochondrial proteins estimated by maximum likelihood from a data set of 20 mammals. The mtREV24 model of the MOLPHY package is also provided (the file `mtREV24.dat`). These two are similar, and the difference is that the former is derived from proteins from mammals only while the latter came from more-diverse species including chicken, fish, frog, and lamprey. Due to differences in the implementation, you may see small differences in log-likelihood values and branch lengths between `aaml` and `protml` in the MOLPHY package. Such differences are normal and you should use the same program to compare different trees. Under the mtREV24 model, the two programs should give almost identical results.

If you want to specify your own substitution rate matrix, have a look at one of those files, which has notes about the file structure. Other options for amino acid substitution models should be ignored. To summarize, the variables `model`, `aaDist`, `CodonFreq`, `NSsites`, and `icode` are used for codon sequences (`seqtype = 1`), while `model`, `alpha`, and `aaRatefile` are used for amino acid sequences.

`model = 7` (AAClasses), which is implemented for both codon and amino acid sequences, allow you to have several types of amino acid substitutions and let the program estimate their different rates. The model was implemented in Yang *et al.* (1998). The number of substitution types and which pair of amino acid changes belong which type is specified in a file called `OmegaAA.dat`. You can use the model to fit different d_N/d_S (ω) ratios for “conserved” and “charged” amino acid substitutions. The folder `examples/mtCDNA` contain example files for this model; check the `readme` file in that folder.

runmode also works in the same way as in `baseml.ctl`. Specifying `runmode = -2` will forces the program to calculate the ML distances in pairwise comparisons. You can change the following variables in the control file `codeml.ctl`: `aaRatefile`, `model`, and `alpha`.

If you do pairwise ML comparison (`runmode = -2`) and the data contain ambiguity characters or alignment gaps, the program will remove all sites which have such characters from all sequences before the pairwise comparison if `cleandata = 1`. This is known as “complete deletion”. It will remove alignment gaps and ambiguity

characters in each pairwise comparison ("pairwise" deletion) if `cleandata = 0`. (In a likelihood analysis of multiple sequences on a phylogeny, alignment gaps are treated as ambiguity characters if `cleandata = 0`, and both alignment gaps and ambiguity characters are deleted if `cleandata = 1`. Note that removing alignment gaps and treating them as ambiguity characters both underestimate sequence divergences. Ambiguity characters in the data (`cleandata = 0`) make the likelihood calculation slower.

Output for amino acid sequences (`seqtype = 2`): The output file is self-explanatory and very similar to the result files for the nucleotide- and codon-based analyses. The empirical models of amino acid substitution (specified by `dayhoff.dat`, `jones.dat`, `wag.dat`, `mtmam.dat`, or `mtREV24.dat`) do not involve any parameters in the substitution rate matrix. When `RateAncestor = 1`, results for ancestral reconstruction are in the file `rst`. Calculated substitution rates for sites under models of variable rates for sites are in `rates`.

evolver

The program `evolver` simulates nucleotide, codon, and amino acid sequences with user-specified tree topology and branch lengths. The user specifies the substitution model and parameters. The program generates multiple data sets in one file in either PAML (output `mc.paml`) or PAUP* (output `mc.paup`) format. If you choose the PAUP* format, the program will look for files with the following names: `paupstart` (which the program copies to the start of the data file), `paupblock` (which the program copies to the end of each simulated data set), and `paupend` (which the program incorporates at the end of the file). This makes it possible to use PAUP* to analyze all data sets in one run. Parameters for simulation are specified in three files: `MCbase.dat`, `MCcodon.dat`, and `MCAA.dat` for simulating nucleotide, codon, and amino acid sequences, respectively. Run the default options while watching out for screen output. Then have a look at the appropriate `.dat` files. As an example, the `MCbase.dat` file is reproduced below, with some notes. Note that the first block of the file has the inputs for `evolver`, while the rest is notes. The tree length is the expected number of substitutions per site along all branches in the phylogeny, calculated as the sum of the branch lengths. This variable was introduced when I was doing simulations to evaluate the effect of sequence divergence while keeping the shape of the tree fixed. `evolver` will scale the tree so that the branch lengths sum up to the specified tree length. If you use `-1` for the tree length, the program will use the branch lengths given in the tree. In the example, the sum of branch lengths is 1.12, and so using either 1.12 or `-1` for the tree length has the same effect. Also note that the base frequencies have to be in a fixed order; this is the same for the amino acid and codon frequencies in `MCAA.dat` and `MCcodon.dat`.

```
0
234567
4 200 2
-1
((1:.1, 2:.2):.12, 3:.3, 4:.4);
6
1 2 3 4 5
.5 4
```

```
0.25 0.25 0.25 0.25
T    C    A    G (fixed order)
```

```
=====
The rest of this data file are notes, ignored by the program evolver.
evolver simulates nucleotide sequences under the REV+Gamma model
and its simpler forms.
The variables in this file are defined below:
```

```
=====
<format,0=paml,1=paup>
<random number seed>
<# seqs> <# nucleotide sites> <# replicates>
<tree length, use -1 if tree has absolute branch lengths>
<tree with relative branch lengths>
<model: 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85, 5:TN93, 6:REV>
<kappa or rate parameters in model>
<alpha> <#categories for discrete gamma>
<base frequencies>
=====
```

The simulation options (5, 6, 7) of evolver can be run using a command line format. So here are all the possible ways of running evolver:

```
evolver
evolver 5 MyMCbaseFile
evolver 6 MyMCcodonFile
evolver 7 MyMCaaFile
```

This evolver program evolved from the old boring program `listtree` and still has the options for listing all trees for a specified small number of species, and for generating random trees from a model of cladogenesis, the birth-death process with species sampling (Yang and Rannala, 1997). It also has an option for calculating the partition distance between tree topologies.

Simulation algorithm used in evolver. Evolver simulates data sets by “evolving” sequences along the tree. First, a sequence is generated for the root using the equilibrium nucleotide, amino acid, or codon frequencies specified by the model and/or the data file (`MCbase.dat`, `MCcodon.dat`, and `MCAa.dat`, respectively). Then each site evolves along the branches of the tree according to the branch lengths, parameters in the substitution model etc. When the sites in the sequence evolve according to the same process, the transition probability matrix is calculated only once for all sites for each branch. For so called site-class models (such as the gamma, and the NSsites codon models), different sites might have different transition probability matrices. Given the character at the start of the branch, the character at the end of the branch is sampled from a multinomial distribution specified by the transition probabilities from the source character. Check any book on Monte Carlo simulation for procedures of sampling from a multinomial distribution, and see, e.g., Yang (1996c; 1997) for more details of simulations on phylogenies. Sequences at the ancestral nodes are generated during the simulation but not included in the output. If you want those ancestral sequences, you can search for the following line in the routine `Simulate()` in the file `evolver.c`, and change the value from 0 to 1.

```
int verbose=0;
```

Recompile the `evolver` program. The program will then output the ancestral sequences in a file named `ancestral.seq`.

yn00

The program `yn00` implements the method of Yang and Nielsen (2000) for estimating synonymous and nonsynonymous substitution rates between two sequences (d_s and d_n). The method of Nei and Gojobori (1986) is also included. The ad hoc method implemented in the program accounts for the transition/transversion rate bias and codon usage bias, and is an approximation to the ML method accounting for the transition/transversion rate ratio and assuming the F3x4 codon frequency model. We recommend that you use the ML method (`runmode= -2`, `CodonFreq= 2` in `codeml.ctl`) as much as possible even for pairwise sequence comparison.

```
seqfile = abglobin.nuc * sequence data file name
outfile = yn           * main result file
verbose = 0           * 1: detailed output (list sequences), 0: concise output

icode = 0 * 0:universal code; 1:mammalian mt; 2-10:see below
weighting = 0 * weighting pathways between codons (0/1)?
commonf3x4 = 0 * use one set of codon freqs for all pairs (0/1)?
```

The control file `yn00.ctl`, an example of which is shown above, specifies the sequence data file name (`seqfile`), output file name (`outfile`), and the genetic code (`icode`). Sites (codons) involving alignment gaps or ambiguity nucleotides in any sequence are removed from all sequences. The variable `weighting` decides whether equal weighting or unequal weighting will be used when counting differences between codons. The two approaches will be different for divergent sequences, and unequal weighting is much slower computationally. The transition/transversion rate ratio κ is estimated for all sequences in the data file and used in subsequent pairwise comparisons. I hope to add an option to allow κ to be estimated for each pair. `commonf3x4` specifies whether codon frequencies (based on the F3x4 model of `codonml`) should be estimated for each pair or for all sequences in the data. Besides the main result file, the program also generates three distance matrices: `2YN.dS` for synonymous rates, `2YN.dN` for nonsynonymous rates, `2YN.t` for the combined codon rate (t is measured as the number of nucleotide substitutions per codon). It should be possible to use those files directly with distance programs such as `NEIGHBOR` in Felsenstein's `PHYLIP` package.

mcmctree

The program `mcmctree` performs Bayesian estimation of phylogenies (Rannala and Yang, 1996; Yang and Rannala, 1997). The birth-death process with species sampling is used to specify the prior distribution of phylogenies ("labeled histories", which are rooted tree topologies with the interior nodes ordered according their associated speciation times), and the posterior probabilities of the labeled histories are compared to select the maximum posterior probability tree. The program implementing the method of Rannala and Yang (1996) is not distributed as the algorithm involves extensive computation. Instead, `mcmctree` implements a refined method (Yang and

Rannala 1997), which uses Markov chain Monte Carlo to select candidate labeled histories, and Monte Carlo integration to integrate over the distribution of the ancestral speciation times.

The default control file name is `mcmctree.ctl`, and a sample copy is shown below.

```
seqfile = mtprim9.nuc * sequence data file name
outfile = mcmctree.out * main result file name
treefile = 9s.trees * tree structure file name
LHfile = Lhs * LH file name. read (MCMC=0) or overwritten (MCMC=1)
MCMC = 0 * 0: read LHs from LHfile, 1: use MCMC to generate Lhs
beta = 0.15 * prob{change labeled history}, used only if MCMC=1
delta0 = 1 * small number for MCMC, used only if MCMC=1
delta1 = .2 * smaller number for comparing candidate LHs

model = 2 * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85

kappa = 2. * given kappa and omega in GY94.
alpha = 0 * given alpha, 0:infinity
ncatG = 8 * # of categories in the dG or AdG models of rates

hierarch = 0 * 1:hierarchical; 0:empirical Bayes analysis
birth = 6.7 * lineage birth rate
death = 2.5 * lineage death rate
sample = .06 * sampling proportion
mutate = .24 * mutation rate (# of mutations from root to present)
```

The variables `seqfile`, `outfile`, `treefile`, `model`, `kappa`, `alpha`, and `ncatG` are defined as in `baseml.ctl` for program `baseml`. The program first collects a set of candidate labeled histories. If `MCMC = 0`, the candidate labelled histories are read from the file `LHfile`, while if `MCMC = 1`, they are generated from the Markov chain Monte Carlo, with the Monte Carlo integration over ancestral speciation times evaluated at a low accuracy level (controlled by `delta0`). The program then calculates the (relative) posterior probabilities of these candidate labelled histories, with the Monte Carlo integration evaluated at a higher accuracy level (controlled by `delta1`). The Markov chain moves with probability `beta` to another labelled history of the same tree topology, or to a labelled history of a different tree topology through a nearest neighbor interchange. `hierarch` controls whether an empirical Bayes analysis or a hierarchical Bayes analysis is to be performed. `birth`, `death`, and `sample` are parameters of the prior distribution specified by the birth-death process with species sampling.

5 Models and Methods

This section provides some background information about the analysis that the programs in the `paml` package perform.

Nucleotide substitution models

Markov process models of nucleotide substitution implemented in PAML include JC69 (Jukes and Cantor, 1969), K80 (Kimura, 1980), F81 (Felsenstein, 1981), F84 (Felsenstein, DNAML program since 1984, PHYLIP Version 2.6), HKY85 (Hasegawa *et*

al., 1985), Tamura and Nei (1993), and REV (Yang, 1994a). The rate matrices of these models are given below

$$\text{JC69 : } Q = \begin{pmatrix} . & 1 & 1 & 1 \\ 1 & . & 1 & 1 \\ 1 & 1 & . & 1 \\ 1 & 1 & 1 & . \end{pmatrix}$$

$$\text{K80 : } Q = \begin{pmatrix} . & \kappa & 1 & 1 \\ \kappa & . & 1 & 1 \\ 1 & 1 & . & \kappa \\ 1 & 1 & \kappa & . \end{pmatrix}$$

$$\text{F81 : } Q = \begin{pmatrix} . & \pi_C & \pi_A & \pi_G \\ \pi_T & . & \pi_A & \pi_G \\ \pi_T & \pi_C & . & \pi_G \\ \pi_T & \pi_C & \pi_A & . \end{pmatrix}$$

$$\text{F84: } Q = \begin{pmatrix} . & (1 + \kappa / \pi_Y)\pi_C & \pi_A & \pi_G \\ (1 + \kappa / \pi_Y)\pi_T & . & \pi_A & \pi_G \\ \pi_T & \pi_C & . & (1 + \kappa / \pi_R)\pi_G \\ \pi_T & \pi_C & (1 + \kappa / \pi_R)\pi_A & . \end{pmatrix}$$

with $\pi_Y = \pi_T + \pi_C$ and $\pi_R = \pi_A + \pi_G$.

$$\text{HKY85: } Q = \begin{pmatrix} . & \kappa\pi_C & \pi_A & \pi_G \\ \kappa\pi_T & . & \pi_A & \pi_G \\ \pi_T & \pi_C & . & \kappa\pi_G \\ \pi_T & \pi_C & \kappa\pi_A & . \end{pmatrix}$$

$$\text{TN93: } Q = \begin{pmatrix} . & \kappa_1\pi_C & \pi_A & \pi_G \\ \kappa_1\pi_T & . & \pi_A & \pi_G \\ \pi_T & \pi_C & . & \kappa_2\pi_G \\ \pi_T & \pi_C & \kappa_2\pi_A & . \end{pmatrix}$$

$$\text{REV (GTR): } Q = \begin{pmatrix} . & a\pi_C & b\pi_A & c\pi_G \\ a\pi_T & . & d\pi_A & e\pi_G \\ b\pi_T & d\pi_C & . & \pi_G \\ c\pi_T & e\pi_C & \pi_A & . \end{pmatrix}$$

$$\text{UNREST } Q = \begin{pmatrix} . & q_{TC} & q_{TA} & q_{TG} \\ q_{CT} & . & q_{CA} & q_{CG} \\ q_{AT} & q_{AC} & . & q_{AG} \\ q_{GT} & q_{GC} & q_{GA} & . \end{pmatrix} = \begin{pmatrix} . & a & b & c \\ d & . & e & f \\ g & h & . & i \\ j & k & l & . \end{pmatrix}$$

The element q_{ij} ($i \neq j$) represents the rate of substitution from nucleotide i to j , with the diagonals q_{ii} specified by the mathematical requirement that each row of Q sums to zero. The nucleotides are ordered T, C, A, G. The transition probability matrix over

time t is then given as $P(t) = \{p_{ij}(t)\} = \exp(Qt)$, where $p_{ij}(t)$ is the probability that nucleotide i will become nucleotide j after time t . The sequence data does not permit separation of rate (Q) and time (t), and so Q specifies relative rates only. In the programs, Q is multiplied by a constant so that the average rate of substitution is 1 when the process is in equilibrium. This scaling means that time t , or the branch length in a tree, is measured by the expected number of nucleotide substitutions per site. Q thus represents the pattern of substitution, while the amount of evolution is reflected in time or the branch length. The frequency parameters $\pi_T, \pi_C, \pi_A, \pi_G$ (with the sum to be 1) give the equilibrium distribution of the process for the F81, F84, HKY85, TN93 and REV models; the equilibrium distribution under the JC69 and K80 models has equal frequencies (1/4) for the four nucleotides. Parameters a, b, c, d, e in REV, κ in F84 or HKY85, and κ_1 and κ_2 in TN93 may be termed rate ratio parameters. So the JC69, K80, F81, F84, HKY85, TN93 and REV models contain 0, 1, 0, 1, 1, 2, 5 rate ratio parameters respectively, and 0, 0, 3, 3, 3, 3, 3 frequency parameters respectively. Normally the frequency parameters are estimated using the averages of the observed frequencies, which should be very close to the true maximum likelihood estimates if the assumptions of homogeneity and stationarity are acceptable.

Parameter κ in the K80 and HKY85 models is equivalent to α/β in the notation of Kimura (1980) and Hasegawa *et al.* (1985). The present notation is more convenient in a maximum likelihood analysis as the ratio is assumed to be constant for different branches of the tree. F84 is the model implemented in J. Felsenstein's DNAML program. The rate matrix for this model was given by Hasegawa and Kishino (1989), Kishino and Hasegawa (1989), Yang (1994b, 1994c) and Tateno *et al.* (1994). Thorne *et al.* (1992) described the transition probability matrix, and Yang (1994c) and Tateno *et al.* (1994) derived formulae for estimating sequence distances under the model. REV is the general time-reversible process model (also known as GTR; Yang, 1994a; see also Tavare, 1986; Zharkikh, 1994). It is used in `baseml` only. It seems sufficiently general to enable accurate estimation of the substitution pattern from real data. See Gillespie (1986), Tavare (1986), Rodriguez *et al.* (1990), Yang (1994a), and Zharkikh (1994) for reviews of substitution models.

Unfortunately there are a few different definitions of the “transition/transversion rate ratio”. The worst is the ratio of the observed numbers of transitional and transversional differences between two sequences, without correcting for multiple hits, also known as P/Q in Kimura's (1980) notation (see, e.g., Wakeley 1994). The measure used in `baseml` is κ as specified in the above formulas for K80 or HKY95. In Kimura's (1980) notation, $\kappa = \alpha/\beta$. A third measure (R) is the ratio averaged over base frequencies; this is the ratio of the expected number of transitions to the expected number of transversions if one observes the substitution process over time. In Kimura's (2000) notation, $R = \alpha/(2\beta)$. PHYLIP and PAUP* use R and name it the “transition/transversion rate ratio”, while referred to κ as the “transition/transversion rate parameter”. For a general substitution model $Q = \{q_{ij}\}$, κ and R are related by the formula

$$R = (\pi_T q_{TC} + \pi_C q_{CT} + \pi_A q_{AG} + \pi_G q_{GA}) / (\pi_T q_{TA} + \pi_T q_{TG} + \pi_C q_{CA} + \pi_C q_{CG} + \pi_A q_{AT} + \pi_A q_{AC} + \pi_G q_{GT} + \pi_G q_{GC}).$$

Special examples are listed in the following table.

Model	Average transition/transversion rate ratio (R)
JC69	$1/2$
K80	$\kappa/2$
F81	$(\pi_T\pi_C + \pi_A\pi_G)/(\pi_Y\pi_R)$
F84	$[\pi_T\pi_C(1 + \kappa/\pi_Y) + \pi_A\pi_G(1 + \kappa/\pi_R)] / (\pi_Y\pi_R)$
HKY85	$(\pi_T\pi_C + \pi_A\pi_G)\kappa/(\pi_Y\pi_R)$
TN93	$(\pi_T\pi_C\kappa_1 + \pi_A\pi_G\kappa_2)/(\pi_Y\pi_R)$
REV (GTR)	$(\pi_T\pi_{Ca} + \pi_A\pi_G)/(\pi_T\pi_{Ab} + \pi_T\pi_{Gc} + \pi_C\pi_{Ad} + \pi_C\pi_{Ge})$

The case of no transition-transversion bias is represented by $\kappa = 1$ and $R = 1/2$ under K80; $\kappa = 1$ and $R = (\pi_T\pi_C + \pi_A\pi_G)/(\pi_Y\pi_R)$ under HKY85; and $\kappa = 0$ and $R = (\pi_T\pi_C + \pi_A\pi_G)/(\pi_Y\pi_R)$ under F84.

Codon substitution models

The model of Goldman and Yang (1994) specifies the probability of substitution between the sense codons, by using the matrix of amino acid distances of Grantham (1974). The model does not seem to fit real data well, however, and the user is advised to use the following simpler version, which is equivalent to use equal distances for any pair of amino acids. The substitution rate from codon i to codon j is given as

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

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 model forms the basis for more sophisticated models implemented in `codeml`, such as those that allow the ω ratio to vary among branches in the phylogeny (Yang 1998; Yang and Nielsen 1998) implemented through the variable `model`, and those that allow the ω ratio to vary among sites (among codons or amino acids in the protein), implemented through the variable `Nssites`.

Amino acid substitution models

“Empirical” models based on the Dayhoff substitution matrix (model = 2) or its updated version of Jones *et al.* (1992) are constructed using the same strategy. The transition probability matrix over a very short time period such as one PAM, i.e.,

P(0.01), is used to approximate the matrix of instantaneous rates (Q). The empirical matrices of Dayhoff *et al.* (1978) and Jones *et al.* (1992) were made to satisfy the reversibility condition, that is,

$$\pi_i q_{ij} = \pi_j q_{ji}$$

for any i and j , so that my implementations may be slightly different from that of Kishino *et al.* (1990). These models assume a fixed pattern of amino acid substitution. The package also include an empirical model for globular proteins, the WAG model of Whelan and Goldman (in press) which is given by the file `wag.dat`, and two similar empirical models for mitochondrial proteins. The first of these is given by the file `mtREV24.dat` and is the mtREV24 model of Adachi and Hasegawa (1996a, b) estimated from a diverse range of species including mammals, chicken, frog, fish, and lamprey. The matrix was estimated by maximum likelihood from real data. The second is given by the file `mtmam.dat` and is estimated from 20 mammalian species using maximum likelihood under the REV model with variable rates among sites (Yang *et al.* 1998). You can check those files for more details, or if you want to supply your own empirical matrix.

"Mechanistic" models of amino acid substitution requires consideration of both the mutational distance between the amino acids as determined by the locations of their encoding codons in the genetic code table, and the effects that the potential change may have on the structure and function of the protein, which may be related to the physical, chemical and structural differences between amino acids. It seems natural that such a model should be formulated at the level of codons. The program `aaml` implements a few such models, specified by the variable `aaDist`.

Models of variable substitution rates across site (see Yang 1996b for review) are implemented for both nucleotide (`baseml`) and amino acid (`aaml`) sequences. Although the option variables such as `fix_alpha` and `alpha` are also available for codon models (`codonml`), the gamma model for codons is unrealistic as it applies the same gamma rate to both synonymous and nonsynonymous substitutions, with their rate ratio held constant among sites. You are recommended to use the `Nssites` models instead, which assume homogeneous synonymous rates but variable nonsynonymous rates.

Models for combined analyses of heterogeneous data (multiple genes or codon positions)

For nucleotides (`baseml`)

Several models are described by Yang (1996a) and implemented in programs `baseml` and `codeml` (`codonml` and `aaml`) for analyzing heterogeneous data sets (such as those of multiple genes or different codon positions). The implementation and description below refer to the case of multiple genes, but in the case of nucleotide-based models (`baseml`), the method can be used to analysed data of different codon positions. These models account for different aspects of heterogeneity among the

different data sets and are useful for testing hypotheses concerning the similarities and differences in the evolutionary process of different data sets.

The simplest model which assumes complete homogeneity among genes can be fitted by concatenating different genes into one sequence without using the option G (and by specifying $M_{\text{gene}} = 0$ in the control file). The most general model is equivalent to a separate analysis. This can be done by fitting the same model to each data set (each gene), but can also be done by specifying $M_{\text{gene}} = 1$ with the option G in the combined data file. The sum of the log-likelihood values over different genes is then the log likelihood of the most general model considered here. Models accounting for some aspects of the heterogeneity of multiple genes are fitted by specifying M_{gene} in combination with the option G in the sequence data file. $M_{\text{gene}} = 0$ means a model that assumes different substitution rates but the same pattern of nucleotide substitution for different genes. $M_{\text{gene}} = 2$ means different frequency parameters for different genes but the same rate ratio parameters (κ in the K80, F84, HKY85 models or the rate parameters in the TN93 and REV models). $M_{\text{gene}} = 3$ means different rate ratio parameters and the same frequency parameters. $M_{\text{gene}} = 4$ means both different rate ratio parameters and different frequency parameters for different genes. Parameters and assumptions made in these models are summarized in the following table, with the HKY85 model used as an example. When substitution rates are assumed to vary from site to site, the control variable M_{alpha} specifies whether one gamma distribution will be applied across all sites ($M_{\text{alpha}} = 0$) or a different gamma distribution is used for each gene (or codon position).

Sequence file	Control file	Parameters across genes
No G	$M_{\text{gene}} = 0$	everything equal
Option G	$M_{\text{gene}} = 0$	the same κ and π , but different α s (proportional branch lengths)
Option G	$M_{\text{gene}} = 2$	the same κ , but different π s and α s
Option G	$M_{\text{gene}} = 3$	the same π , but different κ s and α s
Option G	$M_{\text{gene}} = 4$	different κ , π s, and α s
Option G	$M_{\text{gene}} = 1$	different κ , π s, and different (unproportional) branch lengths

The different α s for different genes mean that branch lengths estimated for different genes are proportional. Parameters π represent the equilibrium nucleotide frequencies, which are estimated using the observed frequencies ($n_{\text{homo}} = 0$). The transition/transversion rate ratio κ in HKY85 can be replaced by the two or five rate ratio parameters under the TN93 or REV models, respectively. The likelihood ratio test can be used to compare these models, using an approach called the analysis of deviance, which is very similar to the more familiar analysis of variance.

For codons and amino acids (codeml)

For codons (see Yang 1996 JME; Yang and Swanson 2002)

Sequence file	Control file	Parameters across genes
No G	Mgene = 0	everything equal
Option G	Mgene = 0	the same (κ , ω) and π , but different ϵ s (proportional branch lengths)
Option G	Mgene = 2	the same (κ , ω), but different π s and ϵ s
Option G	Mgene = 3	the same π , but different (κ , ω) and ϵ s
Option G	Mgene = 4	different (κ , ω), π s, and ϵ s
Option G	Mgene = 1	separate analysis

For amino acids (see Yang 1996 JME for nucleotides)

Sequence file	Control file	Parameters across genes
No G	Mgene = 0	everything equal
Option G	Mgene = 0	the same π , but different ϵ s (proportional branch lengths)
Option G	Mgene = 2	different π s and ϵ s
Option G	Mgene = 1	separate analysis

Global and local clocks, and dated sequences

PAML (baseml and codeml) implements three ML models regarding rate constancy among lineages. `clock = 0` means no clock and each branch has an independent rate. For a binary tree with n species (sequences), this model has $(2n - 3)$ parameters (branch lengths). `clock = 1` means the global clock, and all branches have the same rate. This model has $(n - 1)$ parameters corresponding to the $(n - 1)$ internal nodes in the binary tree. So a test of the molecular clock assumption, which compares those two models, should have d.f. = $n - 2$.

Between those two extremes are the local clock models, specified by `clock = 2` (Yoder and Yang 2000), which assume that branches in the phylogeny conform with the clock assumption and has the default rate ($r_0 = 1$) except for several pre-defined branches, which have different rates. Rates for branches are specified using branch labels in the tree file, and, if they are not, can be inputted from the keyboard. For example, the tree `((1,2) #1, 3), 4)` specifies rate r_1 for the branch ancestral to species 1 and 2 while all other branches have the default rate r_0 , which does not have to be specified. The user need to specify which branch has which rate, and the program estimates the unknown rates (such as r_1 in the above example; $r_0 = 1$ is the default rate). You need to be careful when specifying rates for branches to make sure that all rates can be estimated by the model; if you specify too many rate parameters, especially for branches around the root, it may not be possible to estimate all of them and you will have a problem with identifiability. The number of parameters for a binary tree in the local clock

model is $(n - 1)$ plus the number of extra rate parameters for branches. In the above tree of 4 species, you have only one extra rate parameter r_1 , and so the local clock model has $(n - 1) + 1 = n = 4$ parameters. The no-clock model has 5 parameters while the global clock has 3 parameters for that tree.

The option `clock = 3` implements Andrew Rambaut's TipDate models (Rambaut 2000; see also the TipDate program web page at

<http://evolve.zps.ox.ac.uk/software/TipDate/main.html>). For viral sequences determined in different years, a global molecular clock can be fitted to the data with the dates of sequence determination used in the likelihood calculation. I have used Andrew's format, which specifies the dates at the end of the sequence names; see `exampleTipDate.phy`, which is the example file in Rambaut's package. This model has one extra parameter (the mutation rate) than the global clock model (`clock = 1`). Thanks to Andrew for help with the implementation.

Reconstruction of ancestral sequences

Nucleotides or amino acids of extinct ancestors can be reconstructed using information of the present-day sequences. Parsimony reconstructs ancestral character states by the criterion that the number of changes along the tree at the site is minimized. Algorithms based on this criterion were developed by Fitch (1971) and Hartigan (1973), and are implemented in the program `pamp`. The likelihood approach uses branch lengths and the substitution pattern for ancestral reconstruction. It was developed by Yang *et al.* (1995) and is implemented in `baseml` for nucleotide sequences and in `aaml` (`codeml.c` with `seqtype = 2`) for amino acid sequences. Results are collected in the file `rst`.

Marginal reconstruction: This approach compares the probabilities of different character assignments to an interior node at a site and select the character that has the highest posterior probability (eq. 4 in Yang *et al.* 1995). The algorithm implemented in `paml` works under both the model of a constant rate for all sites and the gamma model of rates at sites. If `verbose = 1`, the output will include the full probability distribution at each node at each site.

Joint reconstruction: This approach considers the assignment of a set of characters to all interior nodes at a site as a reconstruction and select the reconstruction that has the highest posterior probability (eq. 2 in Yang *et al.* 1995). The implementation in `paml` now is based on the algorithm of Pupko *et al.* (2000), which gives the best reconstruction at each site and its posterior probability. The algorithm works under the model of a constant rate for sites only and does not work for the gamma model. (It works under models for multiple genes or data partitions as well. My old algorithm looks at alternatives (sub-optimal reconstructions) although it is inefficient and may miss important reconstructions. I have taken that algorithm out, as well as the old option (`RateAncestor = 2`) of allowing the user to specify the reconstruction to be evaluated. If you need those options, let me know.

The marginal and joint approaches use slightly different criteria, and none is better than the other. They are expected to produce very similar results; that is, the most

probable joint reconstruction for a site should almost always consist of characters that are also the best in the marginal reconstruction. Differences may arise when the competing reconstructions have similar probabilities. Since the marginal reconstruction works with models of variable rates among sites, it is recommended for data analysis.

Analysing large data sets and iteration algorithms for parameter estimation

The maximum likelihood method estimates parameters by maximizing the likelihood function. This is multi-dimensional optimisation problem that has to be solved numerically (except for the simplest possible case; see Yang 2000). PAML implements two iteration algorithms. The first one (`method = 0`) is a general-purpose minimization algorithm that deals with upper and lower bounds for parameters but not general equality or inequality constraints. The algorithm requires first derivatives, which are calculated using the difference approximation, and accumulates information about the curvature (second derivatives) during the iteration using the BFGS updating scheme. At each iteration step, it calculates a search direction, and does a one-dimensional search along that direction to determine how far to go. At the new point, the process is repeated, until there is no improvement in the log-likelihood value, and changes to the parameters are very small. The algorithm updates all parameters including branch lengths simultaneously.

Another algorithm (`method = 1`) works if an independent rate is assumed for each branch (`clock = 0`) (Yang submitted). This algorithm cycles through two phases. Phase I estimates branch lengths with substitution parameters (such as the transition/transversion rate ratio κ and the gamma shape parameter α) fixed. Phase II estimates substitution parameters using the BFGS algorithm, mentioned above, with branch lengths fixed. The procedure is repeated until the algorithm converges. In phase I of the algorithm, branch lengths are optimized one at a time. The advantage of the algorithm is that when the likelihood is calculated for different values of one single branch length, as is required when that branch length only is optimised, much of likelihood calculations on the phylogeny is the same and can be avoided by storing intermediate results in the computer memory. A cycle is completed after all branch lengths are optimized. As estimates of branch lengths are correlated, several cycles are needed to achieve convergence of all branch lengths in the tree, that is, to complete phase I of the algorithm.

If branch lengths are the only parameters to be estimated, that is, if substitution parameters are fixed, the second algorithm (`method = 1`) is much more efficient. Thus to perform heuristic tree search using stepwise addition, for example, you are advised to fix substitution parameters (such as κ and α). The second algorithm is also more efficient if the data contain many sequences so that the tree has many branch lengths.

Tip: To get good initial values for large data sets of protein coding DNA sequences, you can use `baseml`. Add the options characters “GC” at the end of the first line in the sequence data file. Then run the data with `baseml`. In the result file generated by

baseml (say mlb), look for “branch lengths for codon models” and copy the tree with branch lengths into the tree file. Then run codeml and choose “1: initial values” when asked about what to do with the branch lengths in the tree.

Tree search algorithms

One heuristic tree search algorithm implemented in baseml, codonml and aaml is a divisive algorithm, called "star-decomposition" by Adachi and Hasegawa (1996a). The algorithm starts from either the star tree (`runmode = 2`) or a multifurcating tree read from the tree structure file (`runmode = 1`). The algorithm joins two taxa to achieve the greatest increase in log-likelihood over the star-like tree. This will reduce the number of OTUs by one. The process is repeated to reduce the number of OTUs by one at each stage, until no multifurcation exists in the tree. This algorithm works either with or without the clock assumption. The stepwise addition algorithm is implemented with the option `runmode = 3`. Options `runmode = 4` or `5` are used for nearest neighbor interchanges, with the initial tree determined with stepwise addition under the parsimony criterion (`runmode = 4`) or read from the tree structure file (`runmode = 5`). The results are self-explanatory.

Besides the fact that ML calculations are slow, my implementations of these algorithms are crude. If the data set is small (say, with <20 or 30 species), the stepwise addition algorithm (`runmode = 3`) appears usable. Choose `clock = 0`, and `method = 1` to use the algorithm that updates one branch at a time, and fix substitution parameters in the model (such as κ and α) so that only branch lengths are optimized. Parameters κ and α can be fixed in the tree search using `fix_kappa` and `fix_alpha` in the control files. Other parameters (such as substitution rates for genes or codon positions or site partitions) cannot be fixed this way; they can instead be specified in the file of initial values (`in.baseml` or `in.codeml`). Suppose you use a candidate tree to estimate branch lengths and substitution parameters with `runmode = 0`. You can then move the substitution parameters (but not the branch lengths) into the file of initial values. You then change the following variables for tree search: `runmode = 3`, `method = 1`. The program will use the substitution parameters as fixed in the tree search, and optimizes branch lengths only. It is important that the substitution parameters are in the right order in the file; so copy-and-paste from paml output is probably the safest. It is also important that you do not change the parameter specifications in the control file; the control file should indicate that you want to estimate the substitution parameters, but when the program detects the file of initial values, fixed parameter values are used instead.

Simulation

Computer simulation is a widely used approach to evaluating estimation procedures. In molecular phylogenetics, there are two major methods for simulating sequence data. The first approach samples data at different sites (nucleotide, amino acid, or codon sites) from the multinomial distribution. Under most models of sequence

evolution, data at different sites are independently and identically distributed. This approach thus calculates the probability of observing each site pattern, and then sample from sites according to those site pattern probabilities. The number of categories in the multinomial distribution, that is, the number of distinct site patterns, is the number of character states raised to the power of the number of sequences. To simulate nucleotide sequences on a tree of 5 species, the multinomial will have $4^5 = 1024$ categories, and to simulate a pair of codon sequences under the universal code (with 61 sense codons), the multinomial will have $61^2 = 3721$ categories. This approach is faster for simulating data sets on small trees but impractical on large trees as the number of categories may be too large.

A second approach is to generate an ancestral sequence for the root of the tree, and then “evolve” the sequence along the tree according to the specified substitution model and using the specified branch lengths and substitution parameters. The `evolver` program implements this approach. The ancestral sequence is generated according to the equilibrium distribution of the characters, that is, by sampling characters repeatedly according to the equilibrium distribution. The program then evolves the sequence along branches of the tree, according to the transition probabilities calculated for each branch. For site-heterogeneous models, the substitution pattern may be different from site to site and the different sites may have different transition probabilities. See, for example, Huelsenbeck (1995) and Yang (1996c), for more details.

Tips:

1. For analyzing multiple simulated data sets, it is advisable that you copy the tree topology from the `Mcbase.dat` or `Mcaa.dat` file into the tree file for `baseml` or `codeml`. Then when you run `baseml` or `codeml`, the program will ask you what to do about the branch lengths in the tree topology and you choose “using them as initial values”. This should speed up the iteration since the true parameter values should be good initial values.
2. A good test of the simulation as well as the analysis program is to use a small tree to simulate a large data set of very long sequences (say 1 million nucleotides or amino acids) and then use `baseml` or `codeml` to analyse the data to see whether you get estimates very close to the true values. As ML is consistent, it should return the correct values with infinitely long sequences.
3. Programs `baseml` and `codeml` output one line of results for each data set in a file named `rst1`. The output typically includes the log likelihood, the estimated substitution parameters but not branch lengths. If you can modify the source codes, you can go into `baseml.c` or `codeml.c` and search for `frst1`, and add or remove output. However, this may require familiarity with the program, especially about how the variables are arranged during the iteration.

6 Technical Notes

This section contains some technical notes for running PAML programs. Also see the FAQs.

The rub file recording the progress of iteration

If you use a large value for the variable `noisy` (say >2), the programs `baseml` and `codeml` will log output to the screen, indicating the progress of the iteration process, i.e., the minimization of the negative log-likelihood. They will also print in the `rub` file, the size (norm) of the gradient or search direction (h), the negative log likelihood, and the current values of parameters for each round of iteration. A healthy iteration is indicated by the decrease of both h and the negative log likelihood, and h is particularly sensitive. If you run a complicated model hard to converge or analyzing a large data set with hundreds or thousands of sequences, you may switch on the output. You can check this file to see whether the algorithm has converged. A typical symptom of failure of the algorithm is that estimates of parameters are at the preset boundaries, with values like 2.00000, 5.00000. When `method = 1`, the output in the `rub` file lists the log likelihood and parameter estimates only.

How to specify initial values

You may change values of parameters in the control file such as `kappa`, `alpha`, `omega`, etc. to start the iteration from different initial values. Initial values for the second and later trees are determined by the program, and so you do not have much control in this way.

You can collect initial values into a file called `in.baseml` if you are running `baseml` or `in.codeml` if you are running `codeml`. When this file exists, the program will read initial values from it. This may be useful if the iteration is somehow aborted, and then you can collect current values of parameters from the file `rub` into this file of initial values, so that the new iteration can have a better start and may converge faster. The file of initial values may also be useful if you experience problems with convergence. If you have already obtained parameter estimates before and do not want the program to re-estimate them and only want to do some analysis base on those estimates such as reconstructing ancestral sequences, insert `-1` before the initial values.

The `rub` file records the iteration process and has one line for each round of iteration. Each line lists the current parameter values after the symbol `x`; you can copy those numbers into the file of initial values, and if you like, change one or a few of the parameter values too.

Fine-tuning the iteration algorithm

The iteration algorithm uses the difference approximation to calculate derivatives. This method changes the variable (x) slightly, say by a small number e , and see how

the function value changes. One such formula is $df/dx = [f(x + e) - f(x)]/e$. The small number e should be small to allow accurate approximation but should not be too small to avoid rounding errors. You can change this value by adding a line in the control files `baseml.ctl` or `codeml.ctl`

```
Small_Diff = 1e-6
```

The iteration is rather sensitive to the value of this variable, and reasonable values are between $1e-5$ and $1e-7$. This variable also affects the calculation of the SE's for parameters, which are much more difficult to approximate than the first derivatives. If the calculated SE's are sensitive to slight change in this variable, they are not reliable.

If you compile the source codes, you can also change the lower and upper bounds for parameters. I have not put these variables into the control files (See below).

Adjustable variables in the source codes

This section is relevant only if you compile the source codes yourself. The maximum values of certain variables are listed as constants in uppercase at the beginning of the main programs (`baseml.c`, `basemlg.c`, `codeml.c`). These values can be raised without increasing the memory requirement by too much.

NS: maximum number of sequences (species)
LSPNAME: maximum number of characters in a species name
NGENE: maximum number of "genes" in data of multiple genes (option G)
NCATG: maximum number of rate categories in the (auto-) discrete-gamma model (`baseml.c`, `codeml.c`)

You can change the value of LSPNAME. Other variables that may be changed include the bounds for parameters, specified at the beginning of the function `testx` or `SetxBound` in the main programs (`baseml.c` and `codeml.c`). For example, these variables are defined in the function `SetxBound` in `codeml.c`:

```
double tb[]={.0001,9}, rgeneb[]={0.1,99}, rateb[]={1e-4,999};  
double alphab[]={0.005,99}, rhob[]={0.01,0.99}, omegab[]={.001,99};
```

The pairs of variables specify lower and upper bounds for variables (`tb` for branch lengths, `rgeneb` for relative rates of genes used in multiple gene analysis, `alphab` for the gamma shape parameter, `rhob` for the correlation parameter in the auto-discrete-gamma model, and `omegab` for the d_N/d_S ratio in codon based analysis).

PowerMAC memory allocation problem

When your data set is large, you may see a message like "oom", which stands for "out of memory". If you think your data set should be manageable by the program/computer, you can change the memory that is allowed by the operating system for the program to use. If you select the file name and choose "File-Get information", you should see a pop up window. You can increase numbers in this window.

MS Windows version window auto-close

Run the Windows version from a DOS/Windows command box by typing the program names such as `baseml`. Do not run the programs by double clicking on the file names from Windows 95/98/2000/NT Explorer. Otherwise, the window will close automatically when the programs finish or abort and you won't have the chance to see any error messages.

7 Acknowledgments

I thank Nick Goldman, Adrian Friday, and Sudhir Kumar for many useful comments on different versions of the program package. I thank Tianlin Wang for the eigen routine used in the package. I also thank a number of users for reporting bugs and/or suggesting changes, especially Liz Bailes, Thomas Buckley, Belinda Chang, Adrian Friday, Nicolas Galtier, Nick Goldman, John Heulsenbeck, Sudhir Kumar, Robert D. Reed, Fransisco Rodriguez-Trelles, John Heulsenbeck, John Mercer, and Xuhua Xia.

8 References

- Adachi, J., and M. Hasegawa. 1996a. *MOLPHY Version 2.3: Programs for molecular phylogenetics based on maximum likelihood*. Computer science monographs, 28:1-150. Institute of Statistical Mathematics, Tokyo.
- Adachi, J., and M. Hasegawa. 1996b. Model of amino acid substitution in proteins encoded by mitochondrial DNA. *Journal of Molecular Evolution* **42**:459-468.
- Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates, tempo and mode of evolution. *Journal of Molecular Evolution* **18**:225-239.
- Dayhoff, M. O., R. M. Schwartz, and B. C. Orcutt. 1978. A model of evolutionary change in proteins. In *Atlas of Protein Sequence and Structure*, Vol 5, Suppl. 3 (ed M. O. Dayhoff), National Biomedical Research Foundation, Washington D.C., pp. 345-352.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* **17**:368-376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783-791.
- Felsenstein, J. 1993. *Phylogenetic Inference Package (PHYLIP), Version 3.5*. University of Washington, Seattle.
- Goldman, N. 1993a. Statistical tests of models of DNA substitution. *Journal of Molecular Evolution* **36**:182-198.
- Goldman, N. 1993b. Simple diagnostic statistical tests of models for DNA substitution. *Journal of Molecular Evolution* **37**:650-661.
- Goldman, N., and Z. Yang. 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Molecular Biology and Evolution* **11**:725-736.
- Grantham, R. 1974. Amino acid difference formula to help explain protein evolution. *Science* **185**:862-864.
- Hartigan, J. A. 1973. Minimum evolution fits to a given tree. *Biometrics* **29**: 53-65.

- Hasegawa, M., and H. Kishino. 1989. Confidence limits on the maximum likelihood estimation of the hominoid tree from mitochondrial DNA sequences. *Evolution* **43**:672-677.
- Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**:160-174.
- Hayasaka, K., T. Gojobori, and S. Horai. 1988. Molecular phylogeny and evolution of primate mitochondrial DNA. *Molecular Biology and Evolution* **5**:626-644.
- Huelsenbeck, J. P. 1995. The performance of phylogenetic methods in simulation. *Systematic Biology* **44**:17-48.
- Jones, D.T., W. R. Taylor, and J. M. Thornton. 1992. The rapid generation of mutation data matrices from protein sequences. *Computer Application in Biosciences* **8**:275-282.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**:111-120.
- Kishino, H., and M. Hasegawa. 1989. Evaluation of maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* **29**:170-179.
- Kishino, H., T. Miyata, and M. Hasegawa. 1990. Maximum likelihood inference of protein phylogeny and the origin of chloroplasts. *Journal of Molecular Evolution* **31**:151-160.
- Kumar, S., K. Tamura, and M. Nei. 1993. *MEGA: Molecular Evolutionary Genetics Analysis*. The Pennsylvania State University, University Park, PA 16802.
- Messier W. and C.-B. Stewart. 1997. Episodic adaptive evolution of primate lysozymes. *Nature* **385**:151-154.
- Nei, M., and T. Gojobori. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution* **3**:418-426.
- Nielsen, R., and Z. Yang. 1998. Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. *Genetics* **148**:929-936.
- Pupko, T., I. Pe, et al. 2000. A fast algorithm for joint reconstruction of ancestral amino acid sequences. *Molecular Biology and Evolution* **17**: 890-896.
- Rambaut, A. (2000) Estimating the rate of molecular evolution: incorporating non-contemporaneous sequences into maximum likelihood phylogenetics. *Bioinformatics* **16**:395-399.
- Rannala, B. and Z. Yang. 1996. Probability distributions of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43**:304-311.
- Rodriguez, F., J. F. Oliver, A. Marin, and J. R. Medina. 1990. The general stochastic model of nucleotide substitutions. *Journal of Theoretical Biology* **142**:485-501.
- Stewart, C.-B., J. W. Schilling, and A. C. Wilson. 1987. Adaptive evolution in the stomach lysozymes of foregut fermenters. *Nature* **330**:401-404.
- Swanson, W. J., Z. Yang, M. F. Wolfner and C. F. Aquadro. 2001. Positive Darwinian selection in the evolution of mammalian female reproductive proteins. *Proceedings of the National Academy of Sciences of U.S.A.* **98**:2509-2514.
- Swofford, D. L. 1993. *Phylogenetic Analysis Using Parsimony (PAUP), Version 3.2*. University of Illinois, Champaign.
- Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogeny Inference. Pp. 411-501 in D. M. Hillis, C. Moritz, and B. K. Mable eds. *Molecular Systematics*, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.
- Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**:512-526.
- Tavare, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. In *Lectures in Mathematics in the Life Sciences*, Vol. 17, pp. 57-86.

- Thorne, J. L., H. Kishino, and J. Felsenstein. 1991. An evolutionary model for maximum likelihood alignment of DNA sequences. *Journal of Molecular Evolution* **33**:114-124. (Erratum: *Journal of Molecular Evolution* **34**:91 [1992].)
- Thorne, J. L., H. Kishino, and J. Felsenstein. 1992. Inching toward reliability: An improved likelihood model of sequence evolution. *Journal of Molecular Evolution* **34**:3-16.
- Wakeley, J. 1993. Substitution rate variation among sites in hypervariable region 1 of human mitochondrial DNA. *Journal of Molecular Evolution* **37**:613-623.
- Wakeley, J. 1996. The excess of transitions among nucleotide substitutions: new methods of estimating transition bias underscore its significance. *Trends in Ecology and Evolution* **11**:158-163.
- Whelan, S. and N. Goldman, in press. A general empirical model of protein evolution derived from multiple protein families using a maximum likelihood approach. *Molecular Biology and Evolution*
- Yang, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* **10**:1396-1401.
- Yang, Z. 1994a. Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution* **39**:105-111.
- Yang, Z. 1994b. Statistical properties of the maximum likelihood method of phylogenetic estimation and comparison with distance matrix methods. *Systematic Biology* **43**:329-342.
- Yang, Z. 1994c. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution* **39**:306-314.
- Yang, Z. 1995. A space-time process model for the evolution of DNA sequences. *Genetics* **139**:993-1005.
- Yang, Z. 1996a. Maximum likelihood models for combined analyses of multiple sequence data. *Journal of Molecular Evolution* **42**:587-596.
- Yang, Z. 1996b. Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* **11**:367-372.
- Yang, Z. 1996c. Phylogenetic analysis using parsimony and likelihood methods. *Journal of Molecular Evolution* **42**:294-307.
- Yang, Z. 1997. How often do wrong models produce better phylogenies? *Molecular Biology and Evolution* **14**:105-108.
- Yang, Z. 1998. Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution. *Molecular Biology and Evolution* **15**:568-573
- Yang, Z. 2000. Complexity of the simplest phylogenetic estimation problem. *Proceedings of the Royal Society B: Biological Sciences* **267**:109-116.
- Yang, Z. 2000. Maximum likelihood estimation on large phylogenies and analysis of adaptive evolution in human influenza virus A. *Journal of Molecular Evolution* **51**: 423-432.
- Yang, Z. 2001. Adaptive molecular evolution, Chapter 12 (pp. 327-350) in *Handbook of statistical genetics*, eds. D. Balding, M. Bishop, and C. Cannings. Wiley, New York.
- Yang, Z., and B. Bielawski. 2000. Statistical methods for detecting molecular adaptation. *TREE* **15**:496-503.
- Yang, Z., and S. Kumar. 1996. New parsimony-based methods for estimating the pattern of nucleotide substitution and the variation of substitution rates among sites and comparison with likelihood methods. *Molecular Biology and Evolution* **13**:650-659.
- Yang, Z., and R. Nielsen. 1998. Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *Journal of Molecular Evolution* **46**:409-418.
- Yang, Z., and B. Rannala. 1997. Bayesian phylogenetic inference using DNA sequences: Markov chain Monte Carlo methods. *Molecular Biology and Evolution* **14**:717-724.
- Yang, Z., and D. Roberts. 1995. On the use of nucleic acid sequences to infer early branchings in the tree of life. *Molecular Biology and Evolution* **12**:451-458.

- Yang, Z., and W. J. Swanson. 2002. Codon-substitution models to detect adaptive evolution that account for heterogeneous selective pressures among site classes. *Mol. Biol. Evol.* **19**:49-57.
- Yang, Z., and T. Wang. 1995. Mixed model analysis of DNA sequence evolution. *Biometrics* **51**:552-561.
- Yang, Z., and A. D. Yoder. 1999. Estimation of the transition/transversion rate bias and species sampling. *Journal of Molecular Evolution* **48**:274-283.
- Yang, Z., N. Goldman, and A. E. Friday. 1994. Comparison of models for nucleotide substitution used in maximum likelihood phylogenetic estimation. *Molecular Biology and Evolution* **11**:316-324.
- Yang, Z., N. Goldman, and A. E. Friday. 1995. Maximum likelihood trees from DNA sequences: a peculiar statistical estimation problem. *Systematic Biology* **44**:384-399.
- Yang, Z., S. Kumar, and M. Nei. 1995. A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics* **141**:1641-1650.
- Yang, Z. and R. Nielsen. 2000. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Molecular Biology and Evolution* **17**: 32-43.
- Yang, Z., N. Nielsen, and M. Hasegawa. 1998. Models of amino acid substitution and applications to mitochondrial protein evolution. *Molecular Biology and Evolution* **15**:1600-1611.
- Yang, Z., N. Nielsen, N. Goldman, and A.-M. Pedersen. 2000. Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics* **155**:431-449.
- Yoder, A. D., and Z. Yang. 2000. Estimation of primate speciation dates using local molecular clocks. *Molecular Biology and Evolution* **17**: 1081-1090.
- Zharkikh, A. 1994. Estimation of evolutionary distances between nucleotide sequences. *Journal of Molecular Evolution* **39**:315-329.