Seq-Gen

Sequence-Generator: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees.
Version 1.2.5

Andrew Rambaut and Nick C. Grassly

Supported by the Wellcome Trust (grants 50275)

Department of Zoology,
University of Oxford,
South Parks Road,
Oxford OX1 3PS, U.K.

New Features in version 1.2.5 - 25 Sep 2001

- New option to write the relative rate used for each for each site

Citation

If you use this program in a publication please cite the following reference:

Rambaut, A. and Grassly, N. C. (1997) Seq-Gen: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Comput. Appl. Biosci.* **1 3**: 235-238.

Introduction

Seq-Gen is a program that will simulate the evolution of nucleotide

sequences along a phylogeny, using common models of the substitution process. A range of models of molecular evolution are implemented including the general reversible model. Nucleotide frequencies and other parameters of the model may be given and site-specific rate heterogeneity may also be incorporated in a number of ways. Any number of trees may be read in and the program will produce any number of data sets for each tree. Thus large sets of replicate simulations can be easily created. It has been designed to be a general purpose simulator that incorporates most of the commonly used (and computationally tractable) models of DNA sequence evolution. The paper cited above contains details of the algorithm and a short discussion about the uses of Seq-Gen.

For the purposes of this manual, we assume that the user is familiar with the theory and practice of molecular evolution as well as the use of their computer system.

Requirements

Seq-Gen is a command-line controlled program written in ANSI C. It should be easily compiled and run on any UNIX system or workstation. The code will also compile on the Apple Macintosh using the Metrowerks Codewarrior compiler. A separate package is available that includes compiled executables, source and instructions for compiling and running the program on these machines. This paper describes the use of Seq-Gen on a UNIX machine. The application requires an amount of memory proportional to the size of each simulated sequence data set

Acknowledgements

A.R was supported by grant 50275 from the Wellcome Trust and N.C.G. by BBSRC. We would like to thank Ziheng Yang for allowing us to use some invaluable code from PAML.

The models of substitution

All three models of nucleotide substitution implemented in Seq-Gen are Markov models, and assume evolution is independent and indentical at each site and along each lineage. Almost all models used in the maximum likelihood reconstruction of phylogenies using nucleotide sequences are processes of this type (but see Yang, 1994).

The Hasegawa, Kishino and Yano (HKY) model (Hasegawa et al.,

1985) allows for a different rate of transitions and transversions as well as unequal frequencies of the four nucleotides (base frequencies). The parameters requires by this model are transition to transversion ratio (TS/TV) and the base frequencies. There are a number of simpler models that are specific cases of the HKY model. If the base frequencies are set equal (by not specifying base frequencies) then the model becomes equivalent to the Kimura 2-parameter (K2P) model (Kimura, 1980). If the TS TV rates are set to be equal (by not specifying a TS/TV ratio) as well, then it becomes equivalent to the Jukes-Cantor (JC69) model (Jukes and Cantor, 1969).

The F84 model (Felsenstein and Churchill, 1996), as implemented in DNAML in the PHYLIP package (Felsenstein, 1993), is very similar to HKY but differs slightly in how it treats transitions and transversions. This model requires the same parameters as HKY.

Finally, the general reversible process (REV) model (e.g. Yang, 1994) allows 6 different rate parameters and is the most general model that is still consistent with the requirement of being reversible. The 6 parameters are the relative rates for each type of substitution (i.e. A to C, A to G, A to T, C to G, C to T and G to T). As this is a time-reversible process, the rate parameter of one type of substitution (e.g., A to T) is assumed to be the same as the reverse (e.g., T to A).

Site-specific rate heterogeneity

Site-specific rate heterogeneity allows different sites to evolve at different rates. Two models of rate heterogeneity are implemented. The first is a codon-based model in which the user may specify a different rate for each codon position. This can be used simulate the protein-coding sequences for which the third codon position evolves faster than the first and second because a substitution at this position is considerably less likely to cause an amino-acid substitution. Likewise, the first codon position is expected to evolve slightly faster than the second.

The second model of rate heterogeneity assigns different rates to different sites according to a gamma distribution (Yang, 1993). The distribution is scaled such that the mean rate for all the sites is 1 but the user must supply a parameter which describes its shape. A low value for this parameter (<1.0) simulates a large degree of site-specific rate heterogeneity and as this value increases the simulated data becomes more rate-homogeneous. This can be performed as a continuous model, i.e. every site has a different

rate sampled from the gamma distribution of the given shape, or as a discrete model, i.e. each site falls into one of N rate categories approximating the gamma distribution. For a review of site-specific rate heterogeneity and its implications for phylogenetic analyses, see Yang (1996).

Seq-Gen also implements the invariable sites model. With this model, a specified proportion of sites are expected to be invariable across the whole tree. The expected number of substitutions then fall on the remaining variable sites.

The final way of introducing site-specific rate heterogeneity is to specify a number of partitions and give these partitions relative rates. See section 'Input File Format', below, for details about how to do this.

Compilation and Execution

Seq-Gen is written in ANSI C and should compile on most UNIX systems and workstations. It will compile using Metrowerks Codewarrior on the Apple Macintosh and a project file and compiled Power Macintosh executable are included in the Macintosh archive. In this manual I will describe the process of installation and compilation on a UNIX system. For details about compiling and running the Macintosh version see Appendix A. It could also be compiled on MSDOS machines this may be limiting in terms of memory (RAM) available. Some modifications would be necessary in order to compile and run it under Windows or with other Macintosh compilers.

Compilation on UNIX

A simple Makefile is included in the package. You should edit this and change the name of the compiler (by default this is **cc**) and add any flags for optimisation on your system (an example is given for SUN compilers). Once this is done, type:

make

The program will then compile and you will have an executable program: **seq-gen**.

Running Seq-Gen

To run Seq-Gen you type:

```
seq-gen [parameters] < [trees] > [sequences]
```

where [parameters] are the parameters for the program (described below), [trees] is the tree file and [sequences] is the name of the file that will contain the simulated sequences. The tree file must contain one or more trees in PHYLIP format (see below).

The sequences produced by Seq-Gen are written to the standard output (and can thus redirected to the output file using '> [filename]'). Other information and results are written to the standard error and thus will appear on the screen. This can also be saved to a file using '%> [filename]'.

Input file format

The tree format is the same as used by PHYLIP (also called the 'Newick' format). This is a nested set of bifurcations defined by brackets and commas. Here are two examples:

```
(((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);

((Taxon1:0.1,Taxon2:0.2):0.05,Taxon3:0.3,Taxon4:0.4);
```

The first is a rooted tree because it has a bifurcation at the highest level. The next tree is unrooted - it has a trifurcation at the top level. Each tree should finish with a semicolon. Any number of trees may be in the input file separated by a simi-colon and a new-line. Whilst PHYLIP only allows taxon names of up to 10 characters, Seq-Gen can read trees with taxon names of up to 64 characters. Unless the **-o** option is set (see above), the output file will conform to the PHYLIP format and the names will be truncated to 10 characters.

Optionally, the user can supply a sequence alignment as input, as well as the trees. This should be in relaxed PHYLIP format. The trees can then be placed in this file at the end, after a line stating how many trees there are. The file may look like this:

```
4 50
Taxon1      ATCTTTGTAGTCATCGCCGTATTAGCATTCTTAGATCTAA
Taxon2      ATCCTAGTAGTCGCTTGCGCACTAGCCTTCCGAAATCTAG
Taxon3      ACTTCTGTGTTTACTGAGCTACTAGCTTCCCTAAATCTAG
Taxon4      ATTCCTATATTCGCTAATTTCTTAGCTTTCCTGAATCTGG
1
(((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
```

Note that the labels in the alignment do not have to match those in the tree (the ones in the tree will be used for output) – there

doesn't even have to be the same number of taxa in the alignment as in the trees. The sequence length supplied by the alignment will be used to obtain the simulated sequence length (unless the –l option is set). The –k option also refers to one of the sequences to specify the ancestral sequence.

Data partitions with different trees

The user can input different trees for different partitions of the dataset. A partition is a set of contiguous sites that has evolved under a single tree. Using multiple partitions with different trees, a recombinant history for the sequences can be simulated. Assuming a 1000 bp sequence length and 2 partitions consisting of 400bp and 600bp respectively, the following input treefile could be used:

```
[400](((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
[600]((Taxon1:0.1,Taxon3:0.2):0.05,Taxon2:0.3,Taxon4:0.4);
```

Note the partition lengths in square brackets before each tree. These must sum to the specified total sequence length (given by the **-l** option). Multiple sets of partition trees may be input with different trees, numbers of partitions and partition lengths. Seq-Gen will work out the number of partitions for each replicate by the partition lengths (the maximum number of partitions must be given by the **-p** option.

For example:

```
[400](((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
[600]((Taxon1:0.1,Taxon3:0.2):0.05,Taxon2:0.3,Taxon4:0.4);
[300](((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
[400]((Taxon1:0.1,Taxon3:0.2):0.05,Taxon2:0.3,Taxon4:0.4);
[300]((Taxon1:0.1,Taxon2:0.2):0.05,Taxon3:0.3,Taxon4:0.4);
```

will generate 2 datasets, the first consisting of 2 partitions (400bp and 600bp) and the second consisting of 3 partitions (300bp, 400bp and 300bp).

Data partitions with different rates

The user can also input the same tree for all partitions of the dataset and then specify a relative rate of evolution for each. This allows partition rate heterogeneity. The relative rates should have a mean of 1.0 (although, if they don't the program will scale them so that they do).

For example:

[300,

```
0.5](((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
[400,
1.75](((Taxon1:0.2,Taxon3:0.2):0.1,Taxon2:0.3):0.1,Taxon4:0.4);
[300,
0.75](((Taxon1:0.2,Taxon2:0.2):0.1,Taxon3:0.3):0.1,Taxon4:0.4);
```

will generate 3 partitions (300bp, 400bp and 300bp) with relative rates of 0.5, 1.75 and 0.75 along the same tree.

Output File Format

The default format for the output files was chosen for its simplicity and for the wide range of programs that use it. All of the programs in the PHYLIP package that accept DNA sequences can analyse multiple data sets in the format produced by Seq-Gen. Seq-Gen can now also generate NEXUS format output for use with the PAUP program (Swofford, 1993). A PAUP command block (or any other text) can be inserted between each simulated dataset to automate the analysis process (see the **-x** command, below).

Parameters to control Seq-Gen

Options and parameters to Seq-Gen are supplied on the command line. The general format is a minus sign followed by a code letter. If required, the values of any parameters come after the code, separated from both code and each other with either a comma or a space. Some options act like switches and require no parameters. The case of the options is ignored.

Model

This option sets the model of nucleotide substitution with a choice of either *F84*, *HKY* (also known as *HKY85*) or *REV* (markov general reversable model). The first two models are quite similar but not identical. They both require a transition transversion ratio and relative base frequencies as parameters. Other models such as *K2P*, *F81* and *JC69* are special cases of *HKY* and can be obtained by setting the nucleotide frequencies equal (for *K2P*) or the transition transversion ratio to 1.0 (for *F81*) or both (for *JC69*). The usage is:

```
-m <MODEL>
```

Where <MODEL> is a three letter code: HKY ,F84 or REV. If no model is specified, the default is F84 which is computationally

simpler.

Length of Sequences

This option allows the user to set the length in nucleotides that each simulated sequence should be.

```
-l <SEQUENCE_LENGTH>
```

Where <SEQUENCE_LENGTH> is an integer number greater than zero. If an alignment is supplied as input and this option is not set, then Seq-Gen will use the length of the sequences in the alignment.

Number of Replicate Datasets

This option specifies how many separate datasets should be simulated for each tree in the tree file.

```
-n <NUMBER_OF_DATASETS>
```

Where <NUMBER_OF_DATASETS> is an integer number that corresponds to the number of datasets to be simulated.

Number of Data Partitions

This option specifies how many partitions of each data set should be simulated. Each partition must have its own tree and a number specifying how many sites are in the partition. See the input format description in Appendix A for details. Multiple sets of trees are being inputted with varying numbers of partitions, then this should specify the maximum number of partitions that will be required.

```
-p <NUMBER_OF_PARTITIONS>
```

Where <NUMBER_OF_PARTITIONS> is an integer number that corresponds to the number of partitions for each dataset.

Scale branch lengths

This option allows the user to set a value with which to scale the branch lengths in order to make them equal the expected number of substitutions per site for each branch. Basically Seq-Gen multiplies each branch length by this value.

```
-s <SCALE>
```

Where <SCALE> is a decimal number greater than zero. For example

if you give an value of 0.5 then each branch length would be halved before using it to simulate the sequences.

Scale tree length

This option allows the user to set a value which is the desired length of each tree in units of subsitutions per site. The term 'tree length' here is the distance from the root to any one of the tips in units of mean number of subsitutions per site. This option can only be used when the input trees are rooted and ultrametric (no difference in rate amongst the lineages). This has the effect of making all the trees in the input file of the same length before simulating data.

```
-d <SCALE>
```

Where <SCALE> is a decimal number greater than zero. The option multiplies each branch length by a value equal to SCALE divided by the actual length of the tree.

Codon-Specific Rate Heterogeneity

Using this option the user may specify the relative rates for each codon position. This allows codon-specific rate heterogeneity to be simulated. The default is no site-specific rate heterogeneity.

```
-c <CODON_POSITION_RATES>
```

Where <CODON_POSITION_RATES> is three decimal numbers that specify the relative rates of substitution at each codon position, separated by commas or spaces.

Gamma Rate Heterogeneity

Using this option the user may specify a shape for the gamma rate heterogeneity called alpha. The default is no site-specific rate heterogeneity.

```
-a <ALPHA>
```

Where <ALPHA> is a real number >0 that specifies the shape of the gamma distribution to use with gamma rate heterogeneity. If this is used with the **-g** option, below, then a discrete model is used, otherwise a continuous one.

Discrete Gamma Rate Heterogeneity

Using this option the user may specify the number of categories for the discrete gamma rate heterogeneity model. The default is no site-specific rate heterogeneity (or the continuous model if only the **-a** option is specified).

```
-g <NUM_CATEGORIES>
```

Where <NUM_CATEGORIES> is an integer number between 2 and 32 that specifies the number of categories to use with the discrete gamma rate heterogeneity model.

Proportion of Invariable Sites

Using this option the user may specify the proportion of sites that should be invariable. These sites will be chosen randomly with this expected frequency. The default is no invariable sites. Invariable sites are sites that cannot change as opposed to sites which don't exhibit any changes due to chance (and perhaps a low rate).

```
-i <PROPORTION_INVARIABLE>
```

Where <PROPORTION_INVARIABLE> is an real number >= 0.0 and < 1.0 that specifies the proportion of invariable sites.

Relative Nucleotide Frequencies

This option is used to specify the relative frequencies of the four nucleotides. By default, Seq-Gen will assume these to be equal. If the given values don't sum to 1.0 then they will be scaled so that they do.

```
-f <NUCLEOTIDE_FREQUENCIES>
```

Where <NUCLEOTIDE_FREQUENCIES> are four decimal numbers for the frequencies of A, C, G and T respectively, separated by spaces or commas.

Transition Transversion Ratio

This option allows the user to set a value for the transition transversion ratio (TS/TV). This is only valid when either the HKY or F84 model has been selected.

```
-t <TRANSITION_TRANSVERSION_RATIO>
```

Where <TRANSITION_TRANSVERSION_RATIO> is a decimal number greater than zero.

General Reversable Rate Matrix

This option allows the user to set 6 values for the general reversable model's rate matrix. This is only valid when either the REV model has been selected.

```
-r <RATE_MATRIX_VALUES>
```

Where <RATE_MATRIX_VALUES> are size decimal numbers for the rates of transition from A to C, A to G, A to T, C to G, C to T and G to T respectively, separated by spaces or commas. The matrix is symmetrical so the reverse transitions equal the ones set (e.g. C to A equals A to C) and therefore only six values need be set. These values will be scaled such that the last value (G to T) is 1.0 and the others are set relative to this.

Ancestral Sequence

This option allows the user to use a supplied sequence as the ancestral sequence at the root (otherwise a random sequence is used).

```
-k <ANCESTRAL_SEQUENCE_NUMBER>
```

Where <ANCESTRAL_SEQUENCE_NUMBER> is an integer number greater than zero which refers to one of the sequences supplied as input (see Appendix A).

Random Number Seed

This option allows the user to specify a seed for the random number generator. Using the same seed (with the same input) will result in identical simulated datasets. This is useful because you can then delete the (often large) simulated sequence files to save disk space. To recreate a set of simulations, you must use exactly the same model options.

```
-z <RANDOM_NUMBER_SEED>
```

Where <RANDOM_NUMBER_SEED> is an integer number.

Output file format

This option selects the format of the output file. The default is PHYLIP format.

```
-op
```

PHYLIP format.

-or

Relaxed PHYLIP format: PHYLIP format expects exactly 10 characters for the name (padded with spaces if the name is actually less than 10). With this option the output file can have up to 64 characters in the name, followed by a single space before the sequence. The longer taxon names are read from the tree. Some programs can read this and it keeps long taxon names.

-on

NEXUS format: This creates a NEXUS file which will load into PAUP. It generates one DATA block per dataset. It also includes the simulation settings as comments which will be ignored by PAUP.

Insert Text File into Output

This option allows the user to specify text file which will be inserted into the output file after every dataset. This allows the user to insert a PAUP command block or a tree (or anything else) into the file to automate the analysis.

-x <TEXT_FILE_NAME>

Where <TEXT_FILE_NAME> is the name of a file. For Macintosh users this file must be in the same folder as the Seq-Gen program (I find it convenient to copy the Seq-Gen program and move it into the folder in which my datafile are and then delete it afterwards). For UNIX users, this can be specified with a path or should be in the current directory (the one you were in when you run Seq-Gen). This option plus NEXUS format (**-on** option) replaces the previously included separate program, Phy2Nex.

Write Ancestral Sequences

This option allows the user to obtain the sequences for each of the internal nodes in the tree. The sequences are written out along with the sequences for the tips of the tree in relaxed PHYLIP format (see above).

-wa

Write Site Rates

This option allows the user to obtain the relative rate of substitution for each site as used in each simulation. This will go to stderr (or the screen) and will be produced for each replicate simulation.

```
-wr
```

Minimum Information

This option prevents any output except the final trees and any error messages.

```
-q
```

Help

This option prints a help message describing the options and then quits.

```
-h
```

An example of performing simulations using Seq-Gen

An example phylogeny is included with this package (called “example.tree”). This is an unrooted tree in PHYLIP format (see Appendix A). To use this tree to simulate 3 sets of sequences 50 nucleotide long using the HKY model and a transition-transversion ratio of 3.0, type the following:

```
seq-gen -mHKY -t3.0 -l40 -n3 < example.tree > example.dat
```

This produces a PHYLIP format data file called ‘example.dat’ which looks something like this:

```
 4 50
Taxon1     ATCTTTGTAGTCATCGCCGTATTAGCATTCTTAGATCTAA
Taxon2     ATCCTAGTAGTCGCTTGCGCACTAGCCTTCCGAAATCTAG
Taxon3     ACTTCTGTGTTTACTGAGCTACTAGCTTCCCTAAATCTAG
Taxon4     ATTCCTATATTCGCTAATTTCTTAGCTTTCCTGAATCTGG
 4 50
Taxon1     AGAACACAAGTCCCAAATAACCGAACTGGAGCGGGCAGTT
Taxon2     AAGACACAGGCCTAAACTGAGCGTACTGGAACGAGTCGTT
Taxon3     AAGACACAGGTCTCACTTGACTGCGTTGAAACGGTCCGTT
Taxon4     AAGACCCAGACTGTAACTTGTCGGACTGGAATGGACCGTT
 4 50
Taxon1     CAGCTGAGGCATTACGAAGCGCCCGGCCGGCCGGACGATT
Taxon2     TAACTGAGACAGTACGAAACGCGCAATGGGCCCCAAAACC
Taxon3     CGGTTAGGACATGACGAATCGCCCAGCGGGCCTCCGGACC
```

Taxon4 CAACTGGAATGTTACCAGCTGCCTAGGGTGCTCCGAGGAC

References

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol., 17, 368-376.

Felsenstein, J. (1993) Phylogeny Inference Package (PHYLIP), Version 3.5. Department of Genetics, University of Washington, Seattle.

Felsenstein, J. and Churchill, G. (1996) A hidden markov model approach to variation among sites in rate of evolution. Molecular Biology and Evolution, 13, 93-104.

Hasegawa, M., Kishino, H. and Yano, T. (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol., 22, 160-174.

Jukes, T. H. and Cantor, C. R. (1969) Evolution of protein molecules. In Munro, H. N. (ed.) Mammalian Protein Metabolism, Academic Press, New York, pp. 21-123.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16, 111-120.

Swofford, D. L. (1993) Phylogenetic analysis using parsimony (PAUP), Version 3.1.1. Illinois Natural History Survey, Champaign.

Thorne, J. L., Kishino, H. and Felsenstein, J. (1992) Inching toward reality: An improved likelihood model of sequence evolution. J. Mol. Evol., 34, 3-16.

Yang, Z. (1993) Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. Mol. Biol. Evol., 10, 1396-1401.

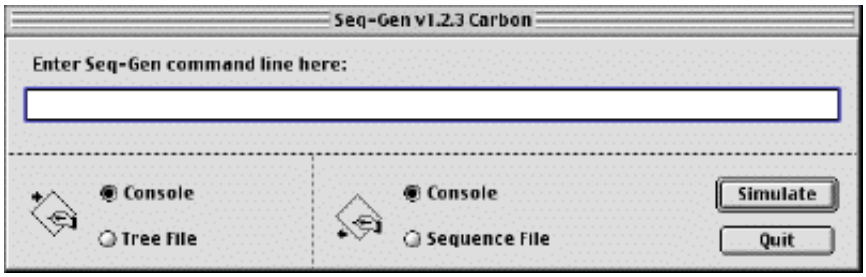
Yang, Z. (1994) Estimating the pattern of nucleotide substitution. J. Mol. Evol., 39, 105-111.

Yang, Z. (1996) Among-site rate variation and its impact on phylogenetic analyses. Tr. Ecol. Evol., 11, 367-372.

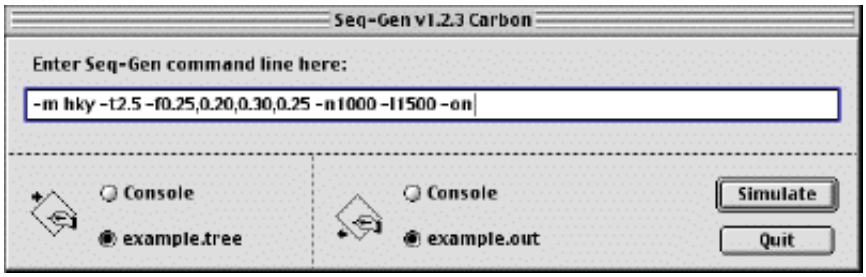
Appendix A - Running the Power Macintosh version.

The Apple Macintosh version of Seq-Gen is compiled with Metrowerks Codewarrior. Only two extra lines are code are required to allow Seq-Gen to compile on the Mac. These are included in the source code but will only be added to the program if compiled under the Codewarrior compiler. The effect of these lines is to bring up a dialog box when the program is run that allows the user to redirect a file to input and redirect the output to a file. These are the equivalent of the UNIX < input_file and > output_file redirection. Also command-line options can be entered in a text box. If you are not familiar with UNIX systems just follow the instructions below.

When Seq-Gen is run on the Mac, the following dialog box will appear:



To specify this input file, click on the left hand radio button labelled **Tree File**. A standard file selection box will appear. To specify an output file, click the right hand **Sequence File** button. Note that only the sequences will be written to the output file - all other information will appear on the screen (and can be saved to a file once the program has finished running). You can now type you options into the text box and click on the **Simulate** button.



Once Seq-Gen starts to run, an output window (console) will appear and in this all the programs output will appear. While running, the Mac will not respond to any input. Once finished the user can save the contents of the console window (or copy it to the clipboard) and quit using the standard **File** menus.

Appendix B - Version History.

New Features in version 1.2.4 - 6 Jul 2001

- Can now specify a relative rate for each partition. The partitions are specified in the tree files but all the partitions can be given the same tree but different rates.

Bug fixed which resulted in missing 'Begin Data' in NEXUS files when creating a single set of sequences.

New Features in version 1.2.3 - 6 Apr 2001

- Added feature write ancestral sequences (-w option).
- Improved the interface of the Macintosh version. Can now drag a tree onto the application - this tree will then be selected as input in the opening dialog box. This box has been made wider to allow longer command-lines.
- New Macintosh Carbon version that will run on MacOS X and MacOS 9.0 this can be found in the Macintosh package along with a version that will run on pre-MacOS 9 computers.

Bug fixed in Macintosh which would result in some of the end of a long command line being ignored.

Bug fixed in Version 1.22 - 4 Feb 2001

Fixed a bug which prevented unrooted trees from loading (complained about polytomies in the tree).

Bug fixed in version 1.21

Fixed a bug which prevented single partitions being simulated (i.e. most people's simulations). Updated make file in UNIX version.

New Features in version 1.2

- Invariable sites model. You can now specify a proportion of invariable sites using the **-i** option, outlined below.
- The default model is now HKY (instead of F84). I think it is now more widely used and the computational difference between them has become small.
- If you don't specify a TS/TV under either the HKY or F84 models, then the TS/TV is chosen to make the instantaneous rate of TSs and TVs equal. This has the effect of collapsing both models to K2P or F81 (depending on whether the base frequencies are equal or note). For the F84 this TS/TV will be 0.5 but for HKY this will be dependent on the base frequencies (for equal base frequencies this will be 0.5). TS/TV used to always default to an arbitrary value of 2.0.

- Output in NEXUS format. You can choose the format of the output using the o option followed by a code specifying PHYLIP, relaxed PHYLIP or NEXUS. When creating NEXUS format files, the name of a file containing a PAUP command block (or any other text) can be specified and this will be inserted into the output after every simulated dataset. This is done with the **-x** command.
- Simulate different partitions of the data under different trees. This allows the simulation of a recombinant history. The trees for each partition are given with the length of the partition in square brackets before it. The **-p** option specifies the number of partitions (the **-p** option used to specify the output format).
- Will now detect and disallow trees containing polytomies. Polytomies can be simulated by Seq-Gen if they are resolved arbitrarily with zero internal branches. This can be done automatically by TreeEdit, available at http://evolve.zoo.ox.ac.uk/software/TreeEdit/.
- Version 1.2 no longer has a Mac 68K executable. It should still compile for these machines but I don't have the time to support it.
- The default seed to the random number generator now has more resolution. Previously Seq-Gen used the system time in seconds. This means that if two short runs of Seq-Gen we executed in less than a second, the same random number seed could be used resulting in two identical datasets. It now adds a higher resolution clock to the seed. It will also print out the seed used so that you can check for this problem. You can also specify your own seed using the **-z** option. If you write down the seed outputted, you can use this to recreate an exact set of simulations. This is useful because you can then delete the (often large) simulated sequence files to save disk space. To recreate a set of simulations, you must use exactly the same model options.

Bug fixed in version 1.1

WARNING: a very important bug has been fixed in this release. Many apologies to anyone to whom this is relevent. All versions of Seq-Gen prior to this have not reassigned the gamma rate categories for each site between replicate simulations. This means that the same site will have the same rate (in both the discrete and continuous model) between replicates. This will reduce the amount of variability in a set of simulations.