ModelTest Manual v0.1.10

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July 16, 2017

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1 Overview

ModelTest-NG is a tool to carry out statistical selection of best-fit models of nucleotide substitution or amino acid replacement. It implements five different model selection strategies: hierarchical and dynamical likelihood ratio tests (hLRT and dLRT), Akaike and Bayesian information criteria (AIC and BIC), and a decision theory method (DT). It also provides estimates of model selection uncertainty, parameter importances and model-averaged parameter estimates, including model-averaged tree topologies. *ModelTest-NG* gathers features of *jModelTest 2* [**?**] and *ProtTest 3* [Darriba *et al.*, 2011].

1.1 Download

The main project webpage is located at GitHub: https://github.com/ddarriba/modeltest.
New distributions of ModelTest-Light will be hosted in GitHub releases.

- https://github.com/ddarriba/modeltest/releases

Please use the jModelTest discussion group for any question:

- http://groups.google.com/group/jmodeltest.

1.2 Disclaimer

1.3 Last Updates

- 3 Mar 2016 Version 2.1.10 Revision 20160303
  - xxx

2 Getting Started

ModelTest-NG provides graphical and a command console interfaces.

2.1 Operating Systems

Sources can be compiled for every major Operating System, including Linux, Windows, and Mac OS X. For convenience, with each release you will find binaries for each of these systems. Nonetheless, it might happen that for certain distributions only some of theme are available, for example if the realease fixes a bug affecting one single OS.

2.2 Working with the repository

This tool is distributed under GPL v3 license. The source code is freely available at github repository. You can clone the repository at https://github.com/ddarriba/modeltest.

2.3 Example run

1. Execute the following command line:

```
$ ./modeltest-cmd -i example-data/dna/tiny.fas -h uigf -f ef
```

   This will test all 88 models (gamma models with 4 rate categories), and then perform the model selection using Akaike (AIC) and Bayesian (BIC) criteria.

   See Section 4 for information about supported arguments.

2. This will generate the following output:

   (a) Header:

```
                                _       _ _            _
                               | |     | | |          | |
 _ __ ___   ___   __| | ___| | |_ ___  ___| |_
| '_ ' _ \ / _ \ / _' |/ _ \ | __/ _ \/ __| __|
| | | | | | (_) | (_| |  __/ | ||  __/\__ \ |_
|_| |_| |_|\___/ \__,_|\___|_|\__\___||___/\__|
------------------------------------------------------------------------
modeltest 0.1.0
Copyright (C) 2017 Diego Darriba, David Posada, Alexandros Stamatakis
License GPLv3+: GNU GPL version 3 or later <http://gnu.org/licenses/gpl.html>.
This is free software: you are free to change and redistribute it.
There is NO WARRANTY, to the extent permitted by law.

Written by Diego Darriba.
------------------------------------------------------------------------
```

   (b) System and compilation details:

```
Physical cores: 2
Logical cores:  4
Memory:         3.57GB
Extensions:     AVX
```

   (c) Execution options:

```
------------------------------------------------------------------------
Input data:
  MSA:          example-data/dna/aP6.fas
  Tree:         Maximum parsimony
    file:       -
  #taxa:        6
  #sites:       631
  #patterns:    28

Output:
```

```
  Log:              test.log
  Starting tree:    test.tree
  Results:          test.out

Selection options:
  # dna schemes:       11
  # dna models:        88
  include model parameters:
    Uniform:          true
    p-inv (+I):       true
    gamma (+G):       true
    both (+I+G):      true
    fixed freqs:      true
    estimated freqs:  true
    #categories:      4
  asc bias:            none
  epsilon (opt):       0.01
  epsilon (par):       0.01

Additional options:
  verbosity:         very low
  threads:           1/2
  RNG seed:          12345
  subtree repeats:   enabled
--------------------------------------------------------------------------------
modeltest-ng was called as follows:
>> src/modeltest-cmd -i example-data/dna/aP6.fas -h uifg -f fe -o test
```

(d) Real time optimization results (progress):

```
Partition 1/1

 ----ID----  ----MODEL----  ---Time---  -Elapsed---  --------LnL--------  -Alpha-  -P-inv-
    1/88     JC             0h:00:00    0h:00:00      -1115.1193         -        -
    2/88     JC+I           0h:00:00    0h:00:00      -1103.3444         -   0.9082
    3/88     JC+G           0h:00:00    0h:00:00      -1106.6136    0.0200        -
    4/88     JC+I+G         0h:00:00    0h:00:00      -1103.6235    1.1674   0.8542
    5/88     F81            0h:00:00    0h:00:00      -1065.0339         -        -
    6/88     F81+I          0h:00:00    0h:00:00      -1053.6319         -   0.9032
    7/88     F81+G          0h:00:00    0h:00:00      -1056.6126    0.0200        -
    8/88     F81+I+G        0h:00:00    0h:00:00      -1053.8953    1.1494   0.8460

...

   85/88     GTR            0h:00:00    0h:00:01      -1063.2358         -        -
   86/88     GTR+I          0h:00:00    0h:00:01      -1051.9056         -   0.9001
   87/88     GTR+G          0h:00:00    0h:00:01      -1054.7872    0.0200        -
   88/88     GTR+I+G        0h:00:00    0h:00:01      -1052.1689    1.1396   0.8417
 ----ID----  ----MODEL----  ---Time---  -Elapsed---  --------LnL--------  -Alpha-  -P-inv-

Computation of likelihood scores completed. It took 0h:00:01
```

(e) Selected Information Criteria (best model and all models sorted according to each criterion):

```
BIC          model                K          lnL          score          delta     weight
--------------------------------------------------------------------------------
      1      F81+I                4    -1053.6319     2191.0788         0.0000     0.8565
      2      HKY+I                5    -1053.1557     2196.5737         5.4949     0.0549
      3      F81+G                4    -1056.6126     2197.0401         5.9613     0.0435
      4      F81+I+G              5    -1053.8953     2198.0529         6.9741     0.0262
      5      TrN+I                6    -1052.6019     2201.9134        10.8346     0.0038
      6      TPM2uf+I             6    -1052.6600     2202.0296        10.9507     0.0036
      7      HKY+G                5    -1056.0996     2202.4615        11.3827     0.0029
      8      TPM3uf+I             6    -1052.9534     2202.6164        11.5376     0.0027
      9      TPM1uf+I             6    -1053.0742     2202.8579        11.7791     0.0024
     10      HKY+I+G              6    -1053.4340     2203.5777        12.4988     0.0017
--------------------------------------------------------------------------------
Best model according to BIC
--------------------------------------------------------------------------------
Model:              F81+I
lnL:                -1053.6319
Frequencies:        0.4253 0.1506 0.2010 0.2232
Subst. Rates:       1.0000 1.0000 1.0000 1.0000 1.0000 1.0000
```

```
Inv. sites prop:      0.9032
Gamma shape:          -
Score:                2191.0788
Weight:               0.8565
-------------------------------
Parameter importances
-------------------------------
P.Inv:                0.9244
Gamma:                0.0471
Gamma-Inv:            0.0282
Frequencies:          1.0000
-------------------------------
Model averaged estimates
-------------------------------
P.Inv:                0.9031
Alpha:                0.0200
Alpha-P.Inv:          1.1502
P.Inv-Alpha:          0.8459
Frequencies:          0.4253 0.1506 0.2010 0.2232

Commands:
  > phyml  -i example-data/dna/aP6.fas -m 000000 -f m -v e -a 0 -c 1 -o tlr
  > raxmlHPC-SSE3 -s example-data/dna/aP6.fas -c 1 -m GTRCATIX --JC69 -n EXEC_NAME -p
    PARSIMONY_SEED
  > paup -s example-data/dna/aP6.fas
  > iqtree -s example-data/dna/aP6.fas -m F81+I
```

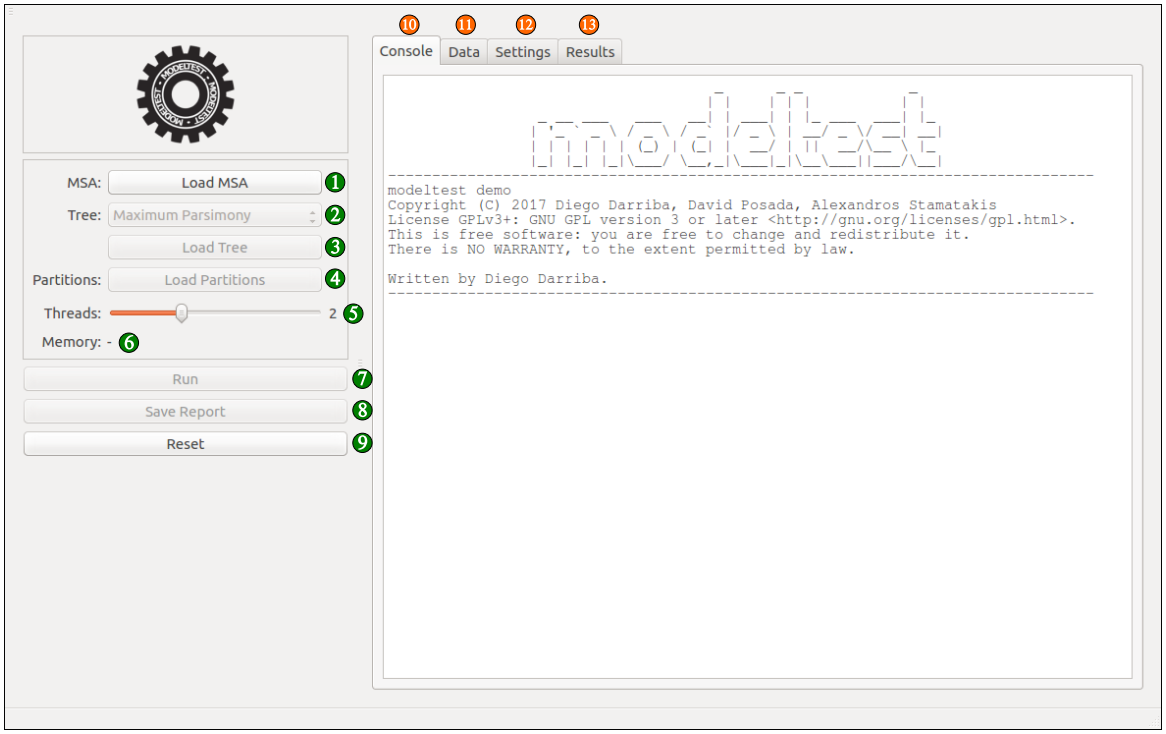
(f) Consensus tree of the optimized phylogenies using the criterion weights (only for **ML topologies**):

```
There are 2 different topologies
Topologies written to output.topos

topo_id    models_count    bic_support    aic_support    aicc_support
-------------------------------------------------------------------
      1              37        0.95897        0.66064         0.66964
      2              51        0.04103        0.33936         0.33036

extended majority-rule consensus: ((P4,(P6,P1)[1.00000])[0.95897],P5,(P2,P3)[1.00000]);
strict consensus: ((P6,P1)[1.00000],P4,P5,(P2,P3)[1.00000]);
```

3 Graphical User Interface

3.1 Launching the Graphical User Interface

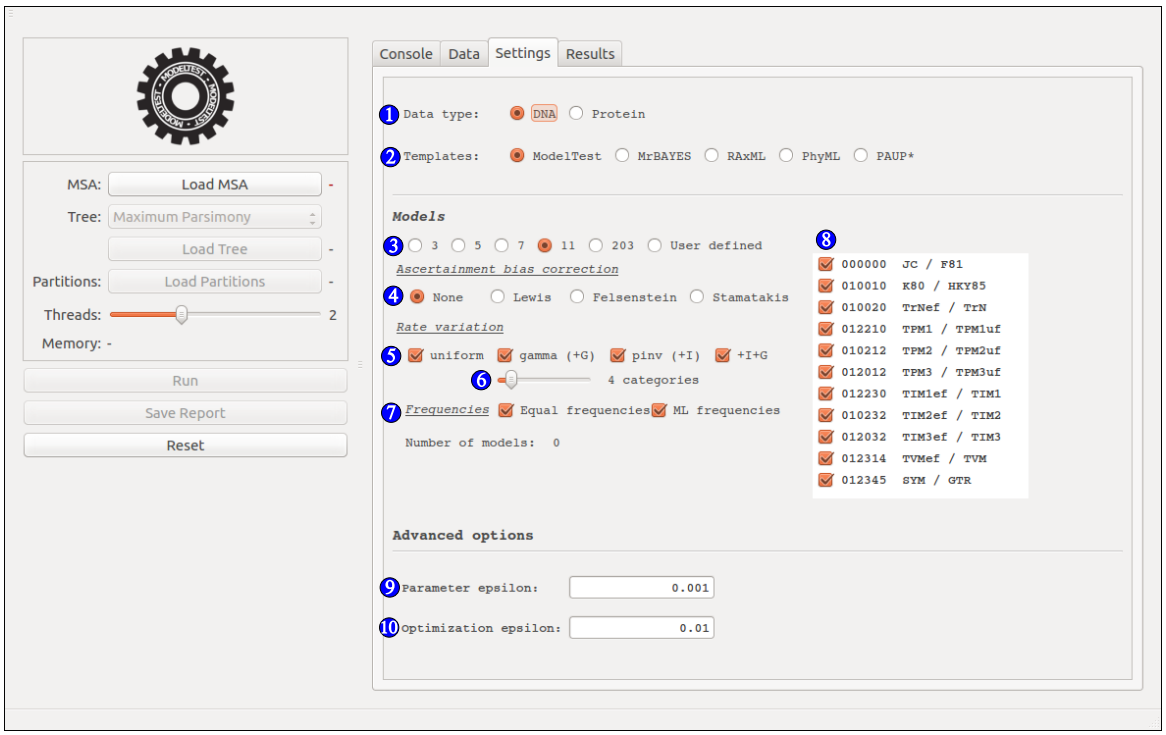
Running *modeltest-gui* with no arguments launches the graphical interface. The following window will show on the screen:



1	Load an MSA file in PHYLIP or FASTA format
2	Select the phylogenetic tree for each model
3	Load a fixed or starting tree in NEWICK format (optional)
4	Load a partitioning scheme file in RAxML format (optional)
5	Select the number of concurrent threads to use
6	Displays the estimated amount of memory needed as a function of the MSA size and the number of threads
7	Start model selection process
8	Save the results report in a file
9	Reset the interface
10	Pane containing the main output console
11	Pane containing data description
12	Pane containing the model selection configuration
13	Pane containing the model selection results

3.2 Custom settings

The settings tab (12) allows to change the model optimization settings. Although the default settings are the most commonly used, you might want to use different ones for your own purposes.



1 Data type (DNA or amino acids)
2 Use only models available in a particular phylogenetic inference tool
3 Use *a priori* defined subset of substitution schemes
4 Correct models for ascertainment bias
5 Include models of rate variation among sites
6 Select the number of discrete rate categories for Gamma model of rate variation
7 Include equal/model-defined or ML/empirical frequencies
8 Select individual candidate models
9 Tolerance for single parameter optimization
10 Global tolerance for model optimization

3.3 Example

If you want to start running a small example, press Ctrl+O in the main window. Select a MSA file from 'example-data/nucleic' or 'example-data/proteic' in the dialog, either in FASTA or PHYLIP format. Press Ctrl+T and select the corresponding tree file in the dialog, in NEWICK format. Press Ctrl+R and enjoy the execution.

4 Command Line Arguments

4.1 Overview

4.1.1 Main Arguments

-d	--datatype	**nt,aa**	Data type is 'nt' for nucleotide (default), 'aa' for amino-acid sequences.
-i	--input	*filename*	Input MSA file in FASTA or sequential PHYLIP format. Check section 5.1
-t	--topology	*topology_type*.	Check section 5.2
		ml	maximum likelihood
		mp	maximum parsimony (default)
		fixed-ml-jc	fixed maximum likelihood (JC)
		fixed-ml-gtr	fixed maximum likelihood (GTR)
		random	random generated tree
		user	fixed user defined (requires -u argument)
-u	--utree	*filename*	User-defined tree in NEWICK format. Check section 5.2
-q	--partitions	*filename*	Partitions filename in RAxML format. Check section 5.3
-o	--output	*filename*	Pipes the output into a file
-p	--processes	*number_of_threads*	Number of concurrent threads
-r	--rngseed	*seed*	Sets the seed for the random number generator

4.1.2 Candidate Models

-a	--asc-bias	algorithm[:values]	Includes ascertainment bias correction. Check section 5.4 for more details **lewis**: Lewis (2001) **felsenstein**: Felsenstein (requires number of invariant sites) **stamatakis**: Leach et al. (2015) (requires invariant sites composition)
-f	--frequencies	[ef]	Sets the candidate models frequencies **e**: Estimated - maximum likelihood (DNA) / empirical (AA) **f**: Fixed - equal (DNA) / model defined (AA)
-h	--model-het	[uigf]	Sets the candidate models rate heterogeneity **u**: Uniform **i**: Proportion of invariant sites (+I) **g**: Discrite Gamma rate categories (+G) **f**: Both +I and +G (+I+G)
-m	--models	*list*	Sets the candidate model matrices separated by commas
		dna:	JC HKY TrN TPM1 TPM2 TPM3 TIM1 TIM2 TIM3 TVM GTR
		protein:	DAYHOFF LG DCMUT JTT MTREV WAG RTREV CPREV VT BLOSUM62 MTMAM MTART MTZOA PMB HIVB HIVW JTTDCMUT FLU STMTREV
-s	--schemes	*number_of_schemes*	Number of DNA substitution schemes. **3**: JC, HKY, GTR **5**: JC, HKY, TrN, TPM1, GTR **7**: JC, HKY, TrN, TPM1, TIM1, TVM, GTR **11**: All models defined in Table 1 **203**: All possible GTR submatrices
-T	--template	*tool*	Sets candidate models according to a specified tool
		raxml	RAxML (DNA 3 schemes / AA full search)
		phyml	PhyML (DNA full search / 14 AA matrices)
		mrbayes	MrBayes (DNA 3 schemes / 8 AA matrices)
		paup	PAUP* (DNA full search / AA full search)

4.1.3 Other options

	`--eps`	*epsilon_value*	Sets the m
	`--tol`	*tolerance_value*	Sets the p
	`--smooth-frequencies`	Forces frequencies smoothing	
`-H`	`--no-compress`	Disables pattern compression. *ModelTest-NG* ignores if there are missing states	
`-v`	`--verbose`	Run in verbose mode	
	`--help`	Display this help message and exit	
	`--version`	Output version information and exit	

5 Model Optimization Settings

5.1 Input data

The main and only required argument is the multiple sequence alignment file ($-i$ argument). *ModelTest-NG* supports PHYLIP and FASTA format. All sequences must be alignned and have thus have the same sequence length.

PHYLIP format starts with a header line containing 2 integer values corresponding to the number of sequences and the sequence length. The following lines are the individual taxa followed by the corresponding sequence. Taxon names and sequences must *not* contain whitespaces. If that is the case in your alignment, please remove or replace every white space with any arbitrary character, such for example an underscore.

Please note that at this moment *ModelTest-NG* does not support interleaved PHYLIP format.

```
TAXA_COUNT SEQ_LENGTH
TAXON_NAME_1 SEQUENCE_1
TAXON_NAME_2 SEQUENCE_2
TAXON_NAME_3 SEQUENCE_3
...
TAXON_NAME_N SEQUENCE_N
```

Example:

```
5 20
taxon1 acgctatcgcgatcgatagc
taxon2 aaactagggcgatcgatagg
taxon3 acactatcg---tcgatagg
taxon4 acgctatcg---ccgatagg
taxon5 acgctaacgcgaacgttatc
```

FASTA format does not contain any header, and it is formatted as a list of the sequences, each of them covering 2 lines: the taxon name, and the sequence.

```
>TAXON_NAME_1
SEQUENCE_1
>TAXON_NAME_2
SEQUENCE_2
>TAXON_NAME_3
SEQUENCE_3
...
>TAXON_NAME_N
SEQUENCE_N
```

The example below is analogous to the previous example in PHYLIP format:

```
>taxon1
acgctatcgcgatcgatagc
>taxon2
aaactagggcgatcgatagg
>taxon3
acactatcg---tcgatagg
>taxon4
acgctatcg---ccgatagg
>taxon5
acgctaacgcgaacgttatc
```

5.2 Topology type

By default, *ModelTest-NG* optimizes each single model using a fixed Maximum-Parsimony topology with Maximum-Likelihood optimized branch lengths. However, it allows other tree optimization techniques. The topology type can be selected with $-t$ argument and it accepts the following values:

- **ml:** Optimize topology and branch lengths for each model
- **fixed-ml-jc:** Build a ML topology with Jukes-Cantor model and fixes it for every other.
- **fixed-ml-gtr:** Build a ML topology with GTR model and fixes it for every other.
- **random:** Use a fixed randomly generated tree.
- **user:** Use fixed user-defined topology

In addition to that, you can set a custom tree topology using $-u$ argument, followed by a file containing the tree in NEWICK format. This argument is mandatory if the tree type was set to *u*ser, and optional for ML trees. In the latter case, the custom-defined tree is used as starting point for the ML optimization, while otherwise *ModelTest-NG* uses a MP tree.

A *r*andom tree topology can be interesting if one wants to measure how sensitive is the model selection process to the tree topology. If you want to test several different random trees, do not forget to use different RNG seeds ($-r$ argument).

5.3 Partitioning scheme

ModelTest-NG is able to select individual models of evolution for each partition defined on the data set ($-q$ argument). The partitioning scheme used may be defined in a file using RAxML-like format, where each partition is defined by one line in the file as follows:

```
DATA_TYPE, PARTITION_NAME = PARTITION_SITES
```

Where:

- **DATA_TYPE** can be *D*NA or *P*ROTEIN
- **PARTITION_NAME** is an arbitrary name for each partition
- **PARTITION_SITES** is the subset of sites that belong to the partition. They can be contiguous (e.g.,1-1000), or defined in several sections (e.g., $1-1000, 2500-3000$). Additionally, one can specify a stride. For example, a partition covering all first codon positions in the first 1,000 sites is defined as $1-1000$
  3, second codon position is $2-1000$
  3, and third $3-1000$
  3. Second and third codon positions together would be $2-1000$
  $3, 3-1000$
  3.

For example:

```
DNA, GENE1 = 1-800
DNA, GENE2 = 801-1700
DNA, GENE3_1 = 1701-2400\3
DNA, GENE3_2 = 1702-2400\3
DNA, GENE3_3 = 1703-2400\3
```

Partitions do not need to cover all sites in the MSA. Every site which does not belong to any partition is just ignored. Also, there must not be overlapping partitions (i.e., it is not allowed a site to belong to more than one partition).

5.4 Ascertainment Bias Correction

ModelTest-NG incorporates 3 algorithms for including ascertainment bias correction in the candidate models.

Let c be the sum of likelihoods (**not** log-likelihoods) of the 'dummy', or virtual invariant sites containing each of the states (eq. 1):, n is the number of sites, s is the number of states, ω is the number of invariant sites, and ω_i is the number of invariant sites for state i.

$$c = \sum_{i}^{s} L(s) \tag{1}$$

- Lewis (Lewis, 2001)

$$ln(L) = \sum_{i}^{n} ln(L_i) - n \cdot ln(1-c) \tag{2}$$

- Felsenstein (Felsenstein, xx)

$$ln(L) = \sum_{i}^{n} ln(L_i) + \omega \cdot ln(c) \tag{3}$$

- Stamatakis (Leaché et al. 2015)

$$ln(L) = \sum_{i}^{n} ln(L_i) + \sum_{j}^{s} \omega_j \cdot ln(L(j)) \tag{4}$$

You can set ascertainment bias correction in *ModelTest-NG* using the *-a* argument: -a algorithm[:values], where *a*lgorithm must be *l*ewis, *f*elsenstein or *s*tamatakis. Additionally, the weights of the dummy sites for Felsenstein's and Stamatakis' algorithms can be set using the *v*alue optional argument. For example:

- Lewis' algorithm (no weights required)

```
$ modeltest -i example-data/dna/aP6.fas -a lewis
```

- Felsenstein's algorithm (sum of dummy sites weights required, values=$w_a + ... + w_t$)

```
$ modeltest -i example-data/dna/aP6.fas -a felsenstein:20
```

- Stamatakis' algorithm (dummy sites weights required, values="w_a, w_c, w_g, w_t")

```
$ modeltest -i example-data/dna/aP6.fas -a stamatakis:10,5,7,15
```

The weights can also be set in the partitions file in a RAxML-like manner, because if the analysis involves several partitions, the dummy sites weights are likely unequal.

There are 2 important conditions for using ascertainment bias correction:

1. The input alignment must *n*ot contain invariant sites.
2. Models with a proportion of invariant sites (i.e., +I and +I+G must be excluded. If -h argument for selecting the rate variation is present and it includes 'g' or 'f', *ModelTest-NG* will complain and stop.

5.5 Frequencies

Nucleotide or amino acid stationary frequencies in a model of evolution can be either (i) defined *a-priori*, using fixed equal or empirical frequencies, or (ii) estimated from the data set at hand, computing the empirical frequencies or estimating ML ones. The latter involve $S-1$ additional degrees of freedom, where S is the number of states (4 for DNA, 20 for protein data).

For nucleotide substitution models, *ModelTest-NG* supports equal (no additional degrees of freedom) and ML frequencies (3 additional degrees of freedom).

For amino acid replacement models, *ModelTest-NG* supports model-defined (no additional degrees of freedom) and empirical frequencies (19 additional degrees of freedom).

With $-f$ argument you can choose whether you want to include models with fixed and/or estimated frequencies using one of both options below. By default, *ModelTest-NG* behaves as including the argument $-f$ ef.

Arg	Nucleotide	Amino acid
f	fixed equal	fixed model
e	ML estimated	empirical

5.6 Per-site rate heterogeneity

With $-h$ argument you can choose whether you want to include models with per-site rate heterogeneity using one or more options below. By default, *ModelTest-NG* behaves as including the argument $-h\ uigf$.

Arg	Rate heterogeneity model
u	No rate heterogeneity
i	proportion of invariant sites (+I)
g	discrete Gamma rates (+G)
f	both +I and +G together

5.7 Substitution schemes

5.8 Settings templates

In order to use the model of evolution selected by *ModelTest-NG* in other phylogenetic inference tool, you can select one of the settings templates such that you can make sure that the candidate models set contains only models available in specific tools:

- RAxML: JC/F81, K80/HKY and SYM/GTR models, with 4 gamma rate categories and a proportion of invariable sites.
- MrBayes: JC/F81, K80/HKY and SYM/GTR models, with 4 gamma rate categories and a proportion of invariable sites.

5.9 Custom optimization thoroughness

Thoroughness of the optimization process can be fine-tuned using 2 parameters: a local tolerance parameter controls the convergence criteria for optimizing individual parameters, and a global tolerance parameter decides whether to finish individual model optimization based on the log-likelihood score.

6 Common Use Cases

6.1 Basic Model Selection

Although *ModelTest-NG* has many options, most of the users would like to perform a model selection among the 11 substitution schemes, including models with unequal frequencies, gamma rate variation and/or a proportion of invariable sites. This is already the default option.

```
$ modeltest-cmd -i example-data/dna/aP6.fas
```

Note that, by default, *ModelTest-NG* uses a fast stepwise addition Maximum-Parsimony topology as the base tree for the models optimization.

6.2 Loading Checkpointing Files

ModelTest-NG saves a ".ckp" checkpointing files in the log directory. In case of an error occurs, the user can start again the process minimizing the loss of computation. If a checkpoint file exists for the input MSA, *ModelTest-NG* will ensure that the current arguments are the same (or compatible) with the saved search. If not, it will return an error, because that means that the stored models were evaluated under different conditions and the results would be inconsistent. You should then either restart the search with the previous arguments, or remove the ".ckp" file.

7 Theoretical Background

All phylogenetic methods make assumptions, whether explicit or implicit, about the process of DNA substitution [Felsenstein, 1988]. Consequently, all the methods of phylogenetic inference depend on their underlying substitution models. To have confidence in inferences it is necessary to have confidence in the models [Goldman, 1993]. Because of this, it makes sense to justify the use of a particular model. Statistical model selection is one way of doing this. For a review of model selection in phylogenetics see Sullivan and Joyce [2005] and Johnson and Omland [2003]. The strategies includes in *ModelTest-NG* include Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) and performance-based decision theory (DT).

7.1 Models of nucleotide substitution

Models of evolution are sets of assumptions about the process of nucleotide substitution. They describe the different probabilities of change from one nucleotide to another along a phylogenetic tree, allowing us to choose among different phylogenetic hypotheses to explain the data at hand. Comprehensive reviews of model of evolution are offered elsewhere. *ModelTest-NG* implementes all 203 types of reversible substitution matrices, with when combined with unequal/equal base frequencies, gamma-distributed among-site rate variation and a proportion of invariable sites makes a total of 1624 models. Some of the models have received names (see Table 1):

Table 1: Named substitution models *ModelTest-NG* (a few of the 1624 possible). Any of these models can include invariable sites (+I), rate variation among sites (+G), or both (+I+G).

Model	Reference	Free param.	Base freq.	Substitution rates	Substitution code
JC	[Jukes and Cantor, 1969]	0	equal	AC=AG=AT=CG=CT=GT	000000
F81	[Felsenstein, 1981]	3	unequal	AC=AG=AT=CG=CT=GT	000000
K80	[Kimura, 1980]	1	equal	AC=AT=CG=GT;AG=GT	010010
HKY	[Hasegawa *et al.*, 1985]	4	unequal	AC=AT=CG=GT;AG=GT	010010
TrNef	[Tamura and Nei, 1993]	2	equal	AC=AT=CG=GT;AG;GT	010020
TrN	[Tamura and Nei, 1993]	5	unequal	AC=AT=CG=GT;AG;GT	010020
TPM1	=K81 [Kimura, 1981]	2	equal	AC=GT;AG=CT;AT=CG	012210
TPM1uf	[Kimura, 1981]	5	unequal	AC=GT;AG=CT;AT=CG	012210
TPM2		2	equal	AC=AT;CG=GT;AG=CT	010212
TPM2uf		5	unequal	AC=AT;CG=GT;AG=CT	010212
TPM3		2	equal	AC=AT;AG=GT;AG=CT	012012
TPM3uf		5	unequal	AC=CG;AT=GT;AG=CT	012012
TIM1	[Posada, 2003]	3	equal	AC=GT;AT=CG;AG;CT	012230
TIM1uf	[Posada, 2003]	6	unequal	AC=GT;AT=CG;AG;CT	012230
TIM2		3	equal	AC=AT;CG=GT;AG;CT	010232
TIM2uf		6	unequal	AC=AT;CG=GT;AG;CT	010232
TIM3		3	equal	AC=CG;AT=GT;AG;CT	012032
TIM3uf		6	unequal	AC=CG;AT=GT;AG;CT	012032
TVMef	[Posada, 2003]	4	equal	AC;CG;AT;GT;AG=CT	012314
TVM	[Posada, 2003]	7	unequal	AC;CG;AT;GT;AG=CT	012314
SYM	[Zharkikh, 1994]	5	equal	AC;CG;AT;GT;AG;CT	012345
GTR	=REV [Tavaré, 1986]	8	unequal	AC;CG;AT;GT;AG;CT	012345

7.2 Models of amino acid replacement

ModelTest-NG includes the empirical amino acid matrices described in the table below. If you expect a very long runtime according to the size of your data, it is recommended to select *a priori* a sensible set of candidate matrices instead of evaluating all the available ones (e.g., discarding those matrices estimated from different data).

Model	Description
Dayhoff	General matrix [Dayhoff and Schwartz, 1978]
JTT	General matrix [Jones *et al.*, 1992]
DCMut/JTT-DCMut	Revised Dayhoff and JTT matrices [Kosiol and Goldman, 2005]
WAG	General matrix [Whelan and Goldman, 2001]
VT	General matrix [Müller and Vingron, 2000]
cpREV	Chloroplast matrix [Adachi *et al.*, 2000]
rtREV	Retrovirus [Dimmic *et al.*, 2002]
stmtREV	Streptophyte mitochondrial land plants [Liu *et al.*, 2014]
mtArt	Mitochondrial Arthropoda [Abascal *et al.*, 2007]
mtMam	Mitochondrial Mammals [Yang and Nielsen, 1998]
mtREV	Mitochondrial Verterbrate [Adachi and Hasegawa, 1996]
mtZoa	Mitochondrial Metazoa (Animals) [Rota-Stabelli *et al.*, 2009]
HIVb/HIVw	HIV matrices [Nickle *et al.*, 2007]
LG	General matrix [Le and Gascuel, 2008]
Blosum62	BLOcks SUbstitution Matrix [Henikoff and Henikoff, 1992]
PMB	Revised Blosum matrix [Veerassamy *et al.*, 2003]
FLU	Influenza virus [Dang *et al.*, 2010]
LG4M	4-matrix mixture model with discrete Γ rates [Le *et al.*, 2012]
LG4X	4-matrix mixture model with free rates [Le *et al.*, 2012]

7.3 Information Criteria

7.3.1 Akaike Information Criterion

The Akaike information criterion (AIC, [Akaike, 1974] is an asymptotically unbiased estimator of the Kullback-Leibler information quantity [S. Kullback, 1951]. We can think of the AIC as the amount of information lost when we use a specific model to approximate the real process of molecular evolution. Therefore, the model with the smallest AIC is preferred. The AIC is computed as:

$$AIC = -2l + 2k$$

where l is the maximum log-likelihood value of the data under this model and k is the number of free parameters in the model, including branch lengths if they were estimated *de novo*. When sample size (n) is small compared to the number of parameters (say, $\frac{n}{K} < 40$) the use of a second order AIC, AICc [Hurvich and Tsai, 1989; Sugiura, 1978], is recommended:

$$AIC_c = AIC + \frac{(2k(k+1))}{(n-k-1)}$$

The AIC compares several candidate models simultaneously, it can be used to compare both nested and non-nested models, and model-selection uncertainty can be easily quantified using the AIC differences and Akaike weights (see Model uncertainty below). Burnham and Anderson [2003] provide an excellent introduction to the AIC and model selection in general.

7.3.2 Bayesian Information Criterion

An alternative to the use of the AIC is the Bayesian Information Criterion (BIC) [Schwarz, 1978]:

$$BIC = -2l + klog(n)$$

Given equal priors for all competing models, choosing the model with the smallest BIC is equivalent to selecting the model with the maximum posterior probability. Alternatively, Bayes factors for models of molecular evolution can be calculated using reversible jump MCMC [Huelsenbeck *et al.*, 2004]. We can easily use the BIC instead of the AIC to calculate BIC differences or BIC weights.

7.3.3 Performance Based Selection

Minin *et al.* [2003] developed a novel approach that selects models on the basis of their phylogenetic performance, measured as the expected error on branch lengths estimates weighted by their BIC. Under this decision theoretic framework (DT) the best model is the one with that minimizes the risk function:

$$C_i \approx \sum_{j=1}^{n} ||\hat{B}_i - \hat{B}_j|| \frac{e^{\frac{-BIC_j}{2}}}{\sum_{j=1}^{R}(e^{\frac{-BIC_i}{2}})}$$

where

$$||\hat{B}_i - \hat{B}_j||^2 = \sum_{l=1}^{2t-3} (\hat{B}_{il} - \hat{B}_{jl})^2$$

and where t is the number of taxa. Indeed, simulations suggested that models selected with this criterion result in slightly more accurate branch length estimates than those obtained under models selected by the hLRTs [Abdo *et al.*, 2005; Minin *et al.*, 2003].

7.4 Model Uncertainty

The AIC, Bayesian and DT methods can rank the models, allowing us to assess how confident we are in the model selected. For these measures we could present their differences (Δ). For example, for the i^{th} model, the AIC (BIC, DT) difference is:

$$\Delta_i = AIC_i - min(AIC)$$

where $min(AIC)$ is the smallest AIC value among all candidate models. The AIC differences are easy to interpret and allow a quick comparison and ranking of candidate models. As a rough rule of thumb, models having Δ_i within 1-2 of the best model have substantial support and should receive consideration. Models having Δ_i within 3-7 of the best model have considerably less support, while models with $\Delta_i > 10$ have essentially no support. Very conveniently, we can use these differences to obtain the relative AIC (BIC) weight (w_i) of each model:

$$\omega_i = \frac{e^{\frac{-\Delta_i}{2}}}{\sum_{r=1}^{R}(e^{\frac{-\Delta_r}{2}})}$$

which can be interpreted, from a Bayesian perspective, as the probability that a model is the best approximation to the truth given the data. The weights for every model add to 1, so we can establish an approximate 95% confidence set of models for the best models by summing the weights from largest to smallest from largest to smallest until the sum is 0.95 [Burnham and Anderson, 1998, 2003].

7.5 Model Averaging

Often there is some uncertainty in selecting the best candidate model. In such cases, or just one when does not want to rely on a single model, inferences can be drawn from all models (or an optimal subset) simultaneously. This is known as model averaging or multimodel inference. See Posada and Buckley [2004] and references therein for an explanation of application of these techniques in the context of phylogenetics.

Within the AIC or Bayesian frameworks, it is straightforward to obtain a model-averaged estimate of any parameter [Burnham and Anderson, 2003; Hoeting *et al.*, 1999; Madigan and Raftery, 1994; Posada, 2003; Raftery, 1996; Wasserman, 2000]. For example, a model-averaged estimate of the substitution rate between adenine and cytosine using the Akaike weights for R candidate models would be:

$$\widehat{\phi_{A-C}} = \frac{\sum_{r=1}^{R} \omega_i I_{\phi_{A-C}}(M_i)\phi_{A-C_i}}{\omega_+(\phi_{A-C})}$$

where

$$\omega_+(\phi_{A-C}) = \sum_{i=1}^{R} \omega_i I_{\phi_{A-C}}(M_i)$$

and

$$I_{\phi_{A-C}}(M_i) = \begin{cases} 1 & \phi_{A-C} \text{ is in model } M_i \\ 0 & \text{otherwise} \end{cases}$$

Note that need to be careful when interpreting the relative importance of parameters. When the number of candidate models is less than the number of possible combinations of parameters, the presence-absence of some pairs of parameters can be correlated, and so their relative importances.

7.6 Model Averaged Phylogeny

Indeed, the averaged parameter could be the topology itself, so we could construct a model-averaged estimate of phylogeny. For example, one could estimate a ML tree for all models (or a best subset) and with those one could build a weighted consensus tree using the corresponding Akaike weights. See Posada and Buckley [2004] for a practical example.

7.7 Parameter Importance

It is possible to estimate the relative importance of any parameter by summing the weights across all models that include the parameters we are interested in. For example, the relative importance of the substitution rate between adenine and cytosine across all candidate models is simply the denominator above, $\omega_+(\phi_{A-C})$

References

Abascal, F., Posada, D., and Zardoya, R. (2007). Mtart: a new model of amino acid replacement for arthropoda. *Molecular biology and evolution*, **24**(1), 1–5.

Abdo, Z., Minin, V., Joyce, P., and Sullivan, J. (2005). Accounting for uncertainty in the tree topology has little effect on the decision-theoretic approach to model selection in phylogeny estimation. *Molecular Biology and Evolution*, **22**, 691–703.

Adachi, J. and Hasegawa, M. (1996). Model of amino acid substitution in proteins encoded by mitochondrial dna. *Journal of molecular evolution*, **42**(4), 459–468.

Adachi, J., Waddell, P. J., Martin, W., and Hasegawa, M. (2000). Plastid genome phylogeny and a model of amino acid substitution for proteins encoded by chloroplast dna. *Journal of Molecular Evolution*, **50**(4), 348–358.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723.

Burnham, K. and Anderson, D. (1998). Model selection and inference: a practical information-theoretic approach. *Springer-Verlag, New York, NY*.

Burnham, K. and Anderson, D. (2003). Model selection and multimodel inference: a practical information-theoretic approach. *Springer-Verlag, New York, NY*.

Dang, C. C., Le, Q. S., Gascuel, O., and Le, V. S. (2010). Flu, an amino acid substitution model for influenza proteins. *BMC evolutionary biology*, **10**(1), 99.

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2011). Prottest 3: fast selection of best-fit models of protein evolution. *Bioinformatics*, **27**(8), 1164–1165.

Dayhoff, M. O. and Schwartz, R. M. (1978). A model of evolutionary change in proteins. In *In Atlas of protein sequence and structure*. Citeseer.

Dimmic, M. W., Rest, J. S., Mindell, D. P., and Goldstein, R. A. (2002). rtrev: an amino acid substitution matrix for inference of retrovirus and reverse transcriptase phylogeny. *Journal of molecular evolution*, **55**(1), 65–73.

Felsenstein, J. (1981). Evolutionary trees from dna sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, **17**, 368–376.

Felsenstein, J. (1988). Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics*, **22**, 521–565.

Goldman, N. (1993). Statistical tests of models of dna substitution. *Journal of Molecular Evolution*, **36**, 182–198.

Hasegawa, M., Kishino, K., and Yano, T. (1985). Dating the human-ape splitting by a molecular clock of mitochondrial dna. *Journal of Molecular Evolution*, **22**, 160–174.

Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. *Proceedings of the National Academy of Sciences*, **89**(22), 10915–10919.

Hoeting, J., Madigan, D., and Raftery, A. (1999). Bayesian model averaging: A tutorial. *Statistical Science*, **14**, 382–417.

Huelsenbeck, J., Larget, B., and Alfaro, M. (2004). Bayesian phylogenetic model selection using reversible jump markov chain monte carlo. *Molecular Biology and Evolution*, **21**, 1123–1133.

Hurvich, C. and Tsai, C. (1989). Regression and time series model selection in small samples. *Biometrika*, **76**, 297–307.

Johnson, J. and Omland, K. (2003). Model selection in ecology and evolution. *Trends in Ecology and Evolution*, **19**, 101–108.

Jones, D. T., Taylor, W. R., and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Computer applications in the biosciences: CABIOS*, **8**(3), 275–282.

Jukes, T. and Cantor, C. (1969). Evolution of protein molecules. *Academic Press, New York, NY*, pages 21–132.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Kimura, M. (1981). Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences, U.S.A*, **78**, 454–458.

Kosiol, C. and Goldman, N. (2005). Different versions of the dayhoff rate matrix. *Molecular biology and evolution*, **22**(2), 193–199.

Le, S. Q. and Gascuel, O. (2008). An improved general amino acid replacement matrix. *Molecular biology and evolution*, **25**(7), 1307–1320.

Le, S. Q., Dang, C. C., and Gascuel, O. (2012). Modeling protein evolution with several amino acid replacement matrices depending on site rates. *Molecular biology and evolution*, page mss112.

Liu, Y., Cox, C. J., Wang, W., and Goffinet, B. (2014). Mitochondrial phylogenomics of early land plants: mitigating the effects of saturation, compositional heterogeneity, and codon-usage bias. *Systematic biology*, **63**(6), 862–878.

Madigan, D. and Raftery, A. (1994). Model selection and accounting for model uncertainty in graphical models using occam's window. *Journal of the American Statistical Association*, **59**, 1335–1346.

Minin, V., Abdo, Z., and P. Joyce, J. S. (2003). Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology*, **52**, 674–683.

Müller, T. and Vingron, M. (2000). Modeling amino acid replacement. *Journal of Computational Biology*, **7**(6), 761–776.

Nickle, D. C., Heath, L., Jensen, M. A., Gilbert, P. B., Mullins, J. I., and Pond, S. K. (2007). Hiv-specific probabilistic models of protein evolution. *PLoS One*, **2**(6), e503.

Posada, D. (2003). Using modeltest and paup to select a model of nucleotide substitution. pages 6.5.1–6.5.14.

Posada, D. and Buckley, T. (2004). Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology*, **53**, 793–808.

Raftery, A. (1996). Hypothesis testing and model selection. *Markov chain Monte Carlo in practice. Chapman and Hall, London*, pages 163–187.

Rota-Stabelli, O., Yang, Z., and Telford, M. J. (2009). Mtzoa: a general mitochondrial amino acid substitutions model for animal evolutionary studies. *Molecular phylogenetics and evolution*, **52**(1), 268–272.

S. Kullback, R. L. (1951). On information and sufficiency. *Annals of Mathematical Statistics*, **22**, 79–86.

Schwarz, G. (1978). Estimating the dimension of a model. *The Annals of Statistics*, **6**, 461–464.

Sugiura, N. (1978). Further analysis of the data by akaike's information criterion and the finite corrections. *Communications in StatisticsTheory and Methods*, **A7**, 13–26.

Sullivan, J. and Joyce, P. (2005). Model selection in phylogenetics. *Annual Review of Ecology, Evolution and Systematics*, **36**, 445–466.

Tamura, K. and Nei, M. (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.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of dna sequences. *Some mathematical questions in biology - DNA sequence analysis. Amer. Math. Soc., Providence, RI*, pages 57–86.

Veerassamy, S., Smith, A., and Tillier, E. R. (2003). A transition probability model for amino acid substitutions from blocks. *Journal of Computational Biology*, **10**(6), 997–1010.

Wasserman, L. (2000). Bayesian model selection and model averaging. *Journal of Mathematical Psychology 44:92-107*, **44**, 92–107.

Whelan, S. and Goldman, N. (2001). A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. *Molecular biology and evolution*, **18**(5), 691–699.

Yang, Z. and Nielsen, R. (1998). Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *Journal of molecular evolution*, **46**(4), 409–418.

Zharkikh, A. (1994). Estimation of evolutionary distances between nucleotide sequences. *Journal of Molecular Evolution*, **39**, 315–329.