nGICE Phylogenomics Course

Introduction to model-based methods

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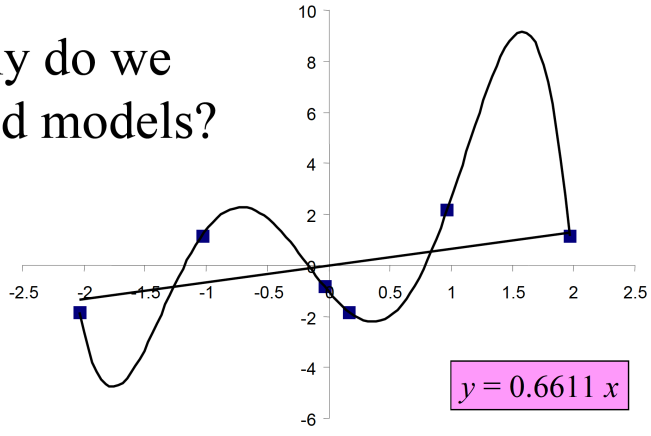
Slides by Jadranka Rota, Paul Lewis and Chris Simon (University of Connecticut, USA)

$y = -1.5972\,x^5 + 23.167\,x^4 - 126.18\,x^3 + 319.17\,x^2 - 369.22\,x + 155.67$

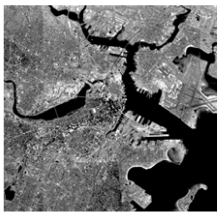
Why do we need models?



A very *practical* MBTA subway map

17

A very *realistic* MBTA subway map



18

- **Which is more useful?**

Models

- Models help us intelligently **interpolate between our observations** for purposes of predicting future observations
- **Adding parameters** to a model generally increases its fit to the data
- **Underparameterized** models lead to poor fit to observed data points
- **Overparameterized** models lead to poor prediction of future observations
- Criteria for choosing models include likelihood ratio tests, AIC, BIC, Bayes Factors, etc.
  - all provide a way to choose a model that is neither underparameterized nor overparameterized

Modelling nucleotide substitution

- **With thousands of genomes sequenced**
  - **Good understanding of how DNA sequences evolve**
  - **Different regions of the genome have their own substitution dynamics**
  - **Different lineages may have their own substitution dynamics**

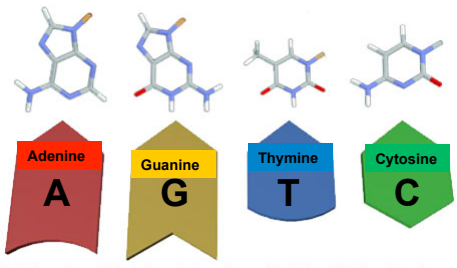
Main Challenge

- **DNA has only four characters**

Purines

Pyrimidines

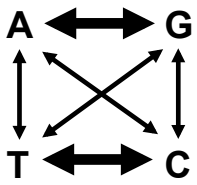
Figure B-3: The Four Nitrogenous Bases



Each base has a distinct shape that can be used to distinguish it form the others. 3D representations of the four bases are shown, with the corresponding chemical structures drawn above.

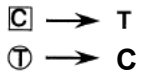
Substitution types

- Purines: A, G
- Pyrimidines: C, T
- Transversions
  - Pu → Pyr
  - Pyr → Pu
- Transitions – more common
  - Pu → Pu
  - Pyr → Pyr



Pur - Pyr mispairs lead to transitions

In next round of replication



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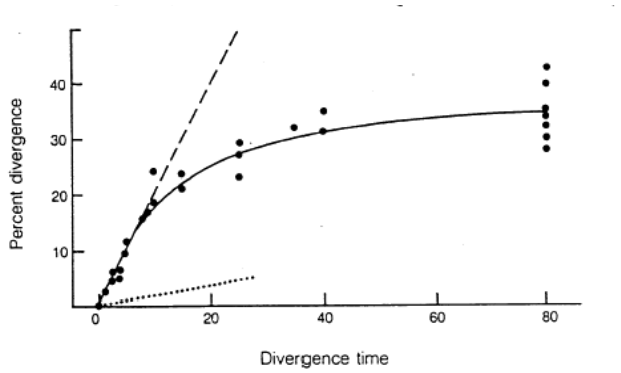
Saturation in sequence data:

- Saturation is due to multiple substitutions at the same site subsequent to lineage splitting
- Models of evolution attempt to infer the missing information through correcting for "multiple hits"
- Most data will contain some fast evolving sites which are potentially saturated
  - e.g. in protein-coding genes codon position 3
- In severe cases the data become essentially random and all information about relationships can be lost
- Probabilistic models of sequence evolution are used to calculate expected changes

Misleading DNA evolution

Multiple substitutions hide previous changes



Brown et al. 1979. PNAS 76:1967

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Difference between mutation and substitution

- **Substitutions** = mutational changes observed in populations
- **Mutations** = not all observed in populations, randomly distributed

  1) removed by proof reading enzymes

  2) cause death of cell, gamete, embryo

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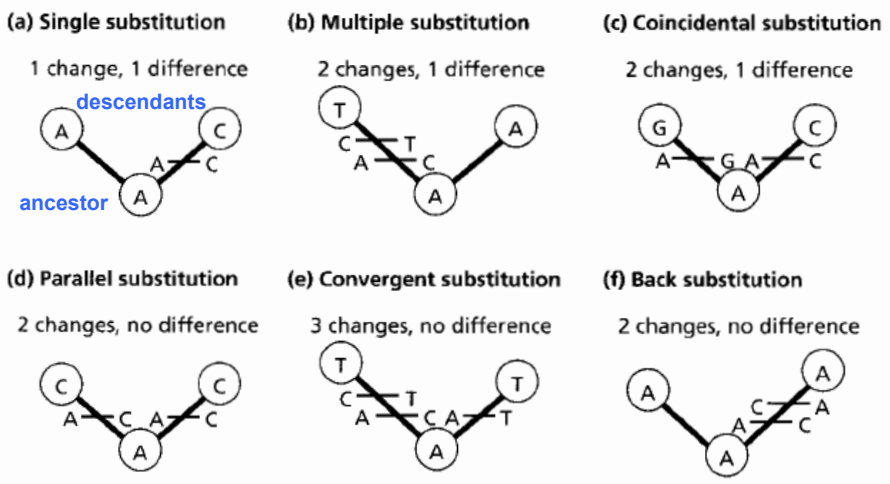
Types of Substitutions



Page, R. and E. Holmes. 1998. Molecular Evolution: A phylogenetic Approach. Blackwell.

Modelling nucleotide substitutions

- These dynamics can be modelled over a tree and they are incoporated into distance methods, maximum likelihood, and Bayesian inference
- Models incorporate information about the rates at which each nucleotide is replaced by each alternative nucleotide
  - For DNA this can be expressed as a 4 x 4 rate matrix (known as the Q matrix)
- Other model parameters may include:
  - Site by site rate variation (aka among-site rate variation - ASRV) - often modelled as a statistical distribution - for example a gamma distribution

Corrections for multiple substitutions:
First DNA substitution model

Jukes & Cantor (1969) assumptions:

1. A = T = G = C No nucleotide bias
2. Every base changes to every other base with equal probability (no TS/TV bias)
3. All sites change with the same probability (no ASRV - among-site rate variation)

Also: probability of substitution & base composition remains constant over time/across lineages

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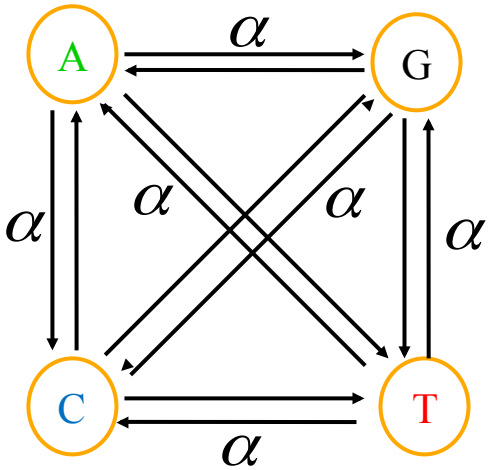
Jukes-Cantor model



t = time

- α = the rate of substitution (α changes from A to G every t)
- The rate of substitution for each nucleotide is 3α
- In t steps there will be 3αt changes

The Q matrix

From \ To	A	C	G	T
A	-3α	α	α	α
C	α	-3α	α	α
G	α	α	-3α	α
T	α	α	α	-3α

The Jukes-Cantor model: the simplest model

	A	C	G	T
A	-3α	α	α	α
C	α	-3α	α	α
G	α	α	-3α	α
T	α	α	α	-3α

JC model: one parameter model

1) It assumes that all bases are equally frequent (p=0.25)
2) It assumes that all sites can change and they do so at the same rate – α

The Jukes-Cantor model: the simplest model

	A	C	G	T
A	–	α	α	α
C	α	–	α	α
G	α	α	–	α
T	α	α	α	–

JC model: one parameter model

1) It assumes that all bases are equally frequent (p=0.25)
2) It assumes that all sites can change and they do so at the same rate – α

Improvements on Jukes-Cantor

- Allow base frequencies to be unequal
- Allow transitions to be more common than transversions, in fact, allow separate estimates of the probability of change of all six possible nucleotide substitutions
- Allow the probability of substitution to change along the molecule - ASRV

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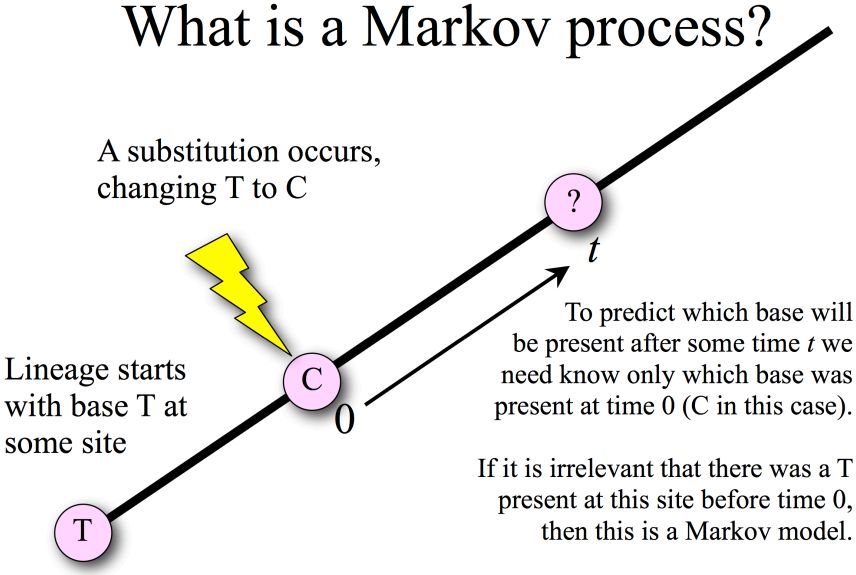
Parameters we are interested in

- The mean instantaneous substitution rate
  =the general mutation rate + rate of fixation in population
- The relative rates of substitution between each nucleotide
- The average frequencies of each base in the dataset
- Topology (part of the model) and branch lengths

Time-homogenous time-continuous stationary Markov models: Assumptions

- Rate of change from base *i* to base *j* is independent of the base that occupied a site prior to *i* (Markov property)

What is a Markov process?



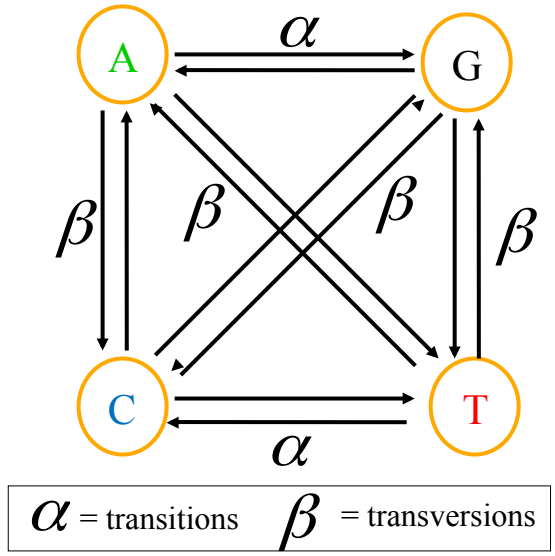
To predict which base will be present after some time *t* we need know only which base was present at time 0 (C in this case).

If it is irrelevant that there was a T present at this site before time 0, then this is a Markov model.

Time-homogenous time-continuous stationary Markov models

- Rate of change from base *i* to base *j* is independent of the base that occupied a site prior to *i* (Markov property)
- **Substitution rate does not change over time (homogeneity)**
- **Relative frequencies of A, G, C, and T are at equilibrium (stationarity)**
- **Rate of change from base *i* to base *j* is identical to the rate of change from base *j* to base *i* (time reversibility)**

Kimura (1980) model: K2P



The Kimura model has 2 parameters

	A	C	G	T
A	–	β	α	β
C	β	–	β	α
G	α	β	–	β
T	β	α	β	–

K2P model is more realistic, but still

1) **It assumes that all bases are equally frequent (p=0.25)**
2) **There are two substitution types (transitions – α and transversions - β**

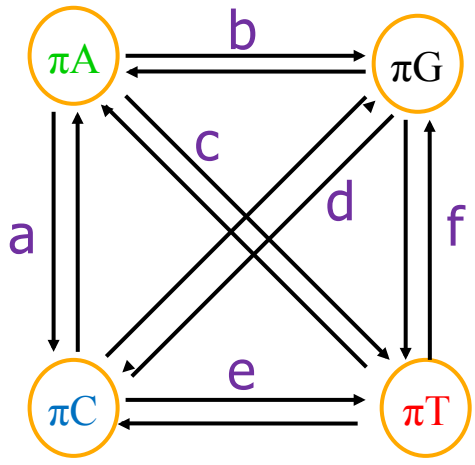
The Hasegawa-Kishino-Yano model

	A	C	G	T
A	–	$\pi_C\beta$	$\pi_G\alpha$	$\pi_T\beta$
C	$\pi_A\beta$	–	$\pi_G\beta$	$\pi_T\alpha$
G	$\pi_A\alpha$	$\pi_C\beta$	–	$\pi_T\beta$
T	$\pi_A\beta$	$\pi_C\alpha$	$\pi_G\beta$	–

HKY model:

1) **Base frequencies are allowed to vary: πA, πC, πG, πT**
2) **There are two substitution types (transitions – α and transversions - β**

The General Time-Reversible model



The General Time-Reversible model (GTR)

	A	C	G	T
A	–	$\pi_C a$	$\pi_G b$	$\pi_T c$
C	$\pi_A a$	–	$\pi_G d$	$\pi_T e$
G	$\pi_A b$	$\pi_C d$	–	$\pi_T f$
T	$\pi_A c$	$\pi_C e$	$\pi_G f$	–

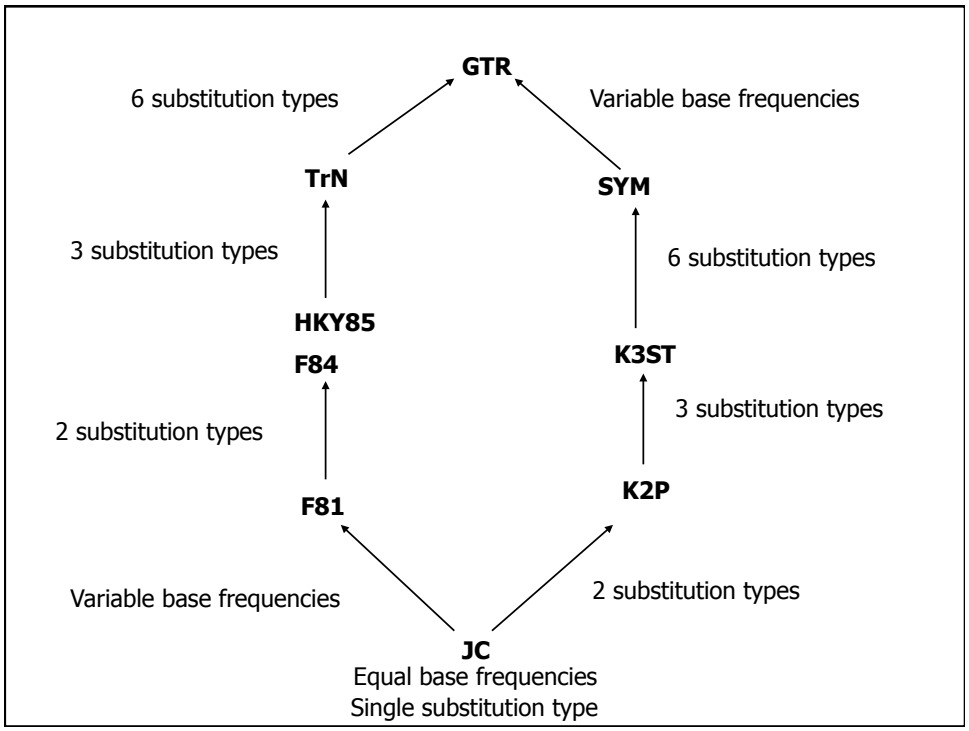
GTRmodel:

1) **Base frequencies are allowed to vary: πA, πC, πG, πT**
2) **There are six substitution types: a, b, c, d, e, f**

The most commonly used models

- **Almost all models used are special cases of one model:**
  - **The general time reversible model - GTR**

ACAGGTGAGGCTCAGCCAATTTGAGCTTTGTCGATAGGT



Models

- **Model parameters can be:**
  - **estimated from the data (using a likelihood function)**
  - **can be pre-set based upon assumptions about the data (for example that for all sequences all sites change at the same rate and all substitutions are equally likely - e.g. the Jukes-Cantor model)**
  - ***wherever possible avoid assumptions* which are violated by the data because they can lead to incorrect trees**

Modelling among-site rate variation (ASRV)

- All of the models so far assume that the rate of change is the same for every position in the alignment
- Biggest difference in substitution rate between variable and "invariable" sites
- Two classes of "invariable sites"
  - Highly restricted "not free to vary"
  - not observed to vary but in fact variable
    - due to convergence or reversal
    - % invariable sites can't be calculated by simple sequence comparison.

Slide by Chris Simon 2005

ASRV, Yang 1996, TREE 11(9):367-372

Why is modelling ASRV important?

- Protein-coding genes – 1st, 2nd, 3rd codon positions evolve differently from each other
- RNA molecules – stems and loops
- Introns vs. exons

RNA codon table

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	stop	stop	A
	Leu	Ser	stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Amino Acids

Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp:Aspartic acid
Cys:Cysteine
Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine
Leu: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
Thr: Threonine
Trp: Tryptophane
Tyr: Tyrosisne
Val: Valine

Typical pattern of variation among codon positions

E.g. in mtDNA in Collembola

56.7% of all variable sites are located in third positions

1st 27.9% 2nd 15.4% 3rd 56.7%

96.9% of all third positions are variable

1st 47.8% 2nd 26.3% 3rd 96.9%

Frati et al. 1997. J. Mol. Evol.

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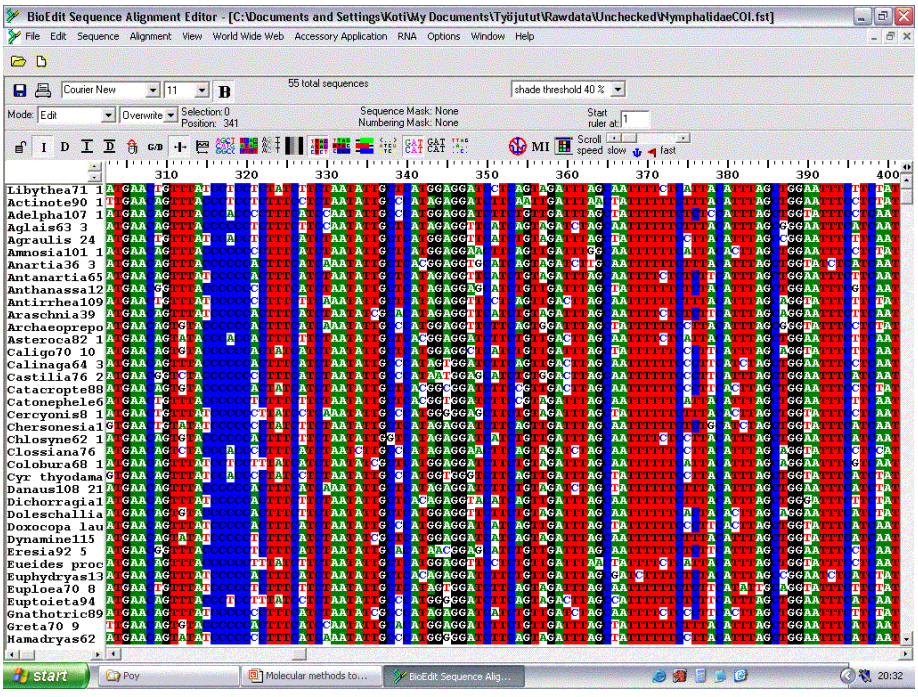

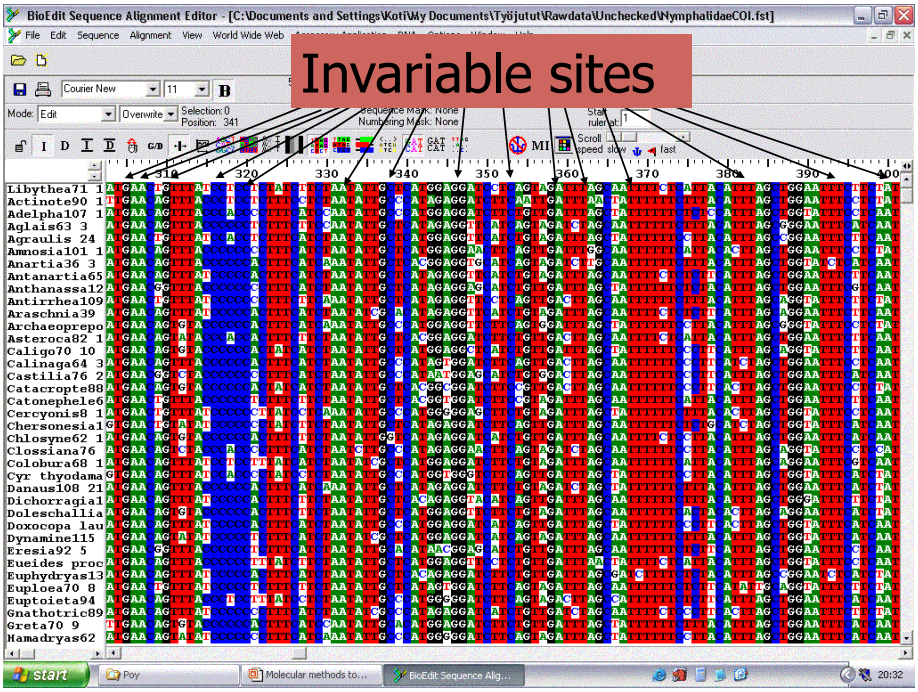

Modelling among-site rate variation (ASRV)

- The most common additional parameters are:
  - A correction for the proportion of sites which are invariable (parameter *I*)
  - A correction for variable site rates at those sites which can change (parameter gamma, *G*)
- All models can be supplemented with these parameters (e.g. GTR+*I*+*G*, HKY+*I*+*G*)

Modelling ASRV in variable sites

- ASRV in variable sites commonly modelled with a gamma distribution
- Alpha – the shape parameter of this distribution

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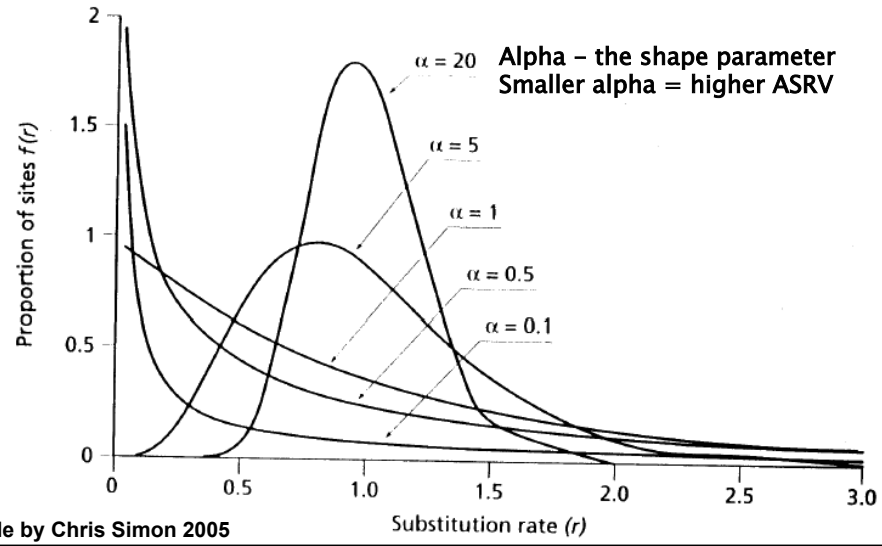
ASRV, Yang 1996, TREE 11(9):367-372

Gamma distribution:

Relative substitution rates for different α values



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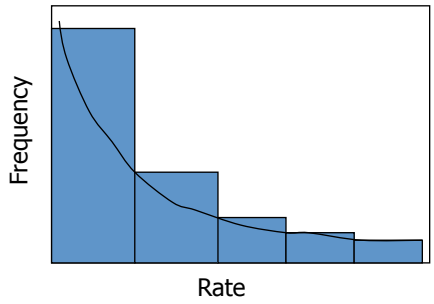
Gamma distribution computationally costly

- Computational difficulties in using continuous distribution
- Most programs use discrete categories



ASRV: Yang discrete model

- Continuous data divided into "n" discrete rate classes (generally 4)
- If $\alpha < 0.2$ Yang recommends more rate classes
- Less computer intensive than obtaining likelihoods by integrating over the continuous gamma distribution

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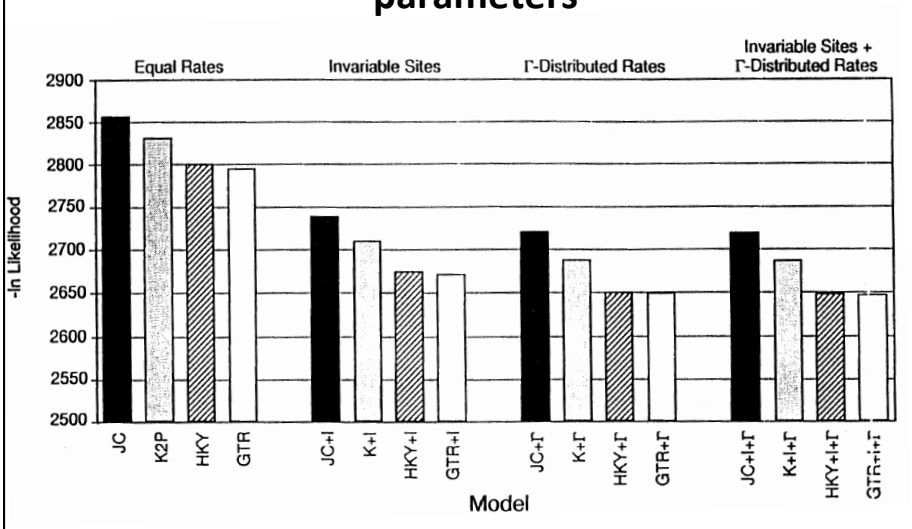
ASRV >> fit improvement than by other parameters



Frati, Simon, Sullivan, Swofford. 1997. JME 44:145-158

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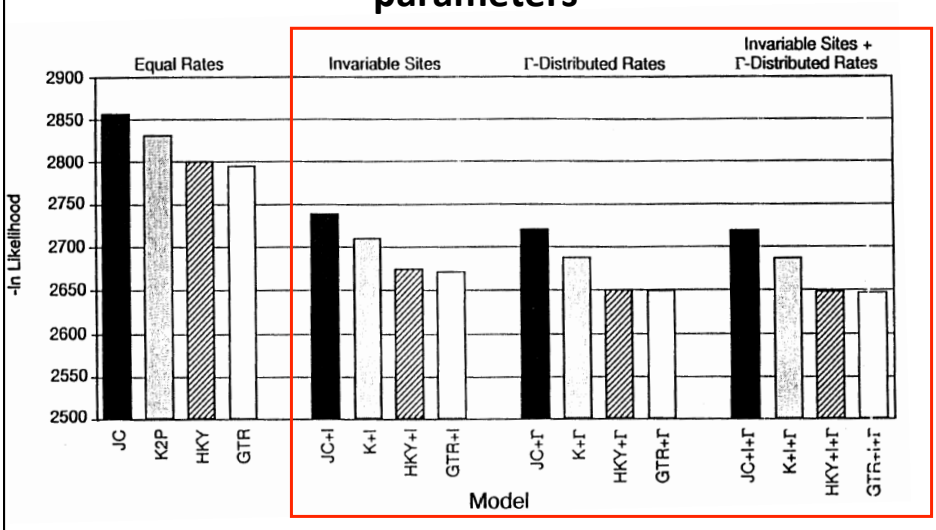
ASRV >> fit improvement than by other parameters



Frati, Simon, Sullivan, Swofford. 1997. JME 44:145-158

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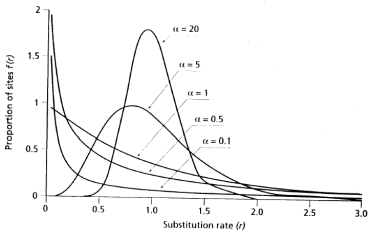
Difficulties in estimating ASRV

- The parameters *I* and *G* covary!
- (*I* + *G*) can be estimated, but the values of *I* and *G* are not easily teased apart
- Parameter *G* takes *I* into account, *I* not needed (in many/most? datasets)

Another method for modelling ASRV

- Gamma distribution is always unimodal
  - Not necessarily the case in our dataset!



- Flexible rate heterogeneity across sites model
  - Probability distribution free model so that you can find the distribution that fits your data (FreeRate Model)
  - Implemented in IQ-TREE

Kalyaanamoorthy et al. 2017 (Nature Methods) doi:10.1038/nmeth.4285

Parameters in models of DNA evolution

- **Numbers of parameters estimated:**
  - **Substitutions (up to 5; 1 fixed, 5 estimated)**
  - **Base composition (1 fixed, 3 estimated)**
  - **Among-site-rate variation**
    - **Gamma shape parameter = 1 parameter**
    - **Invariant sites = 1 parameter**
    - **Gamma + I = 2 parameters**
  - **Partitioned models – add up parameters of each partition**

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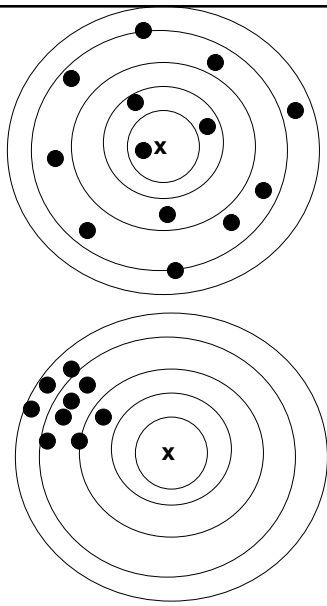
Models can be made more parameter rich to increase their realism

- **But the more parameters estimated, the more time needed, and the more sampling error accumulates**
  - **One might have a realistic model but large sampling errors**
  - **Realism comes at a cost in time and precision!**
  - **Fewer parameters may give an inaccurate estimate, but more parameters decrease the precision of the estimate**
  - **In general use the simplest model which fits the data**



Dave Swofford's Target Analogy

Trade-off between highly parameterized models & model error variance

- Many parameters, higher error variance but clustered around the true value (higher accuracy, lower precision)
- Few parameters, lower error variance but may not be centered around the mean (lower accuracy, higher precision)

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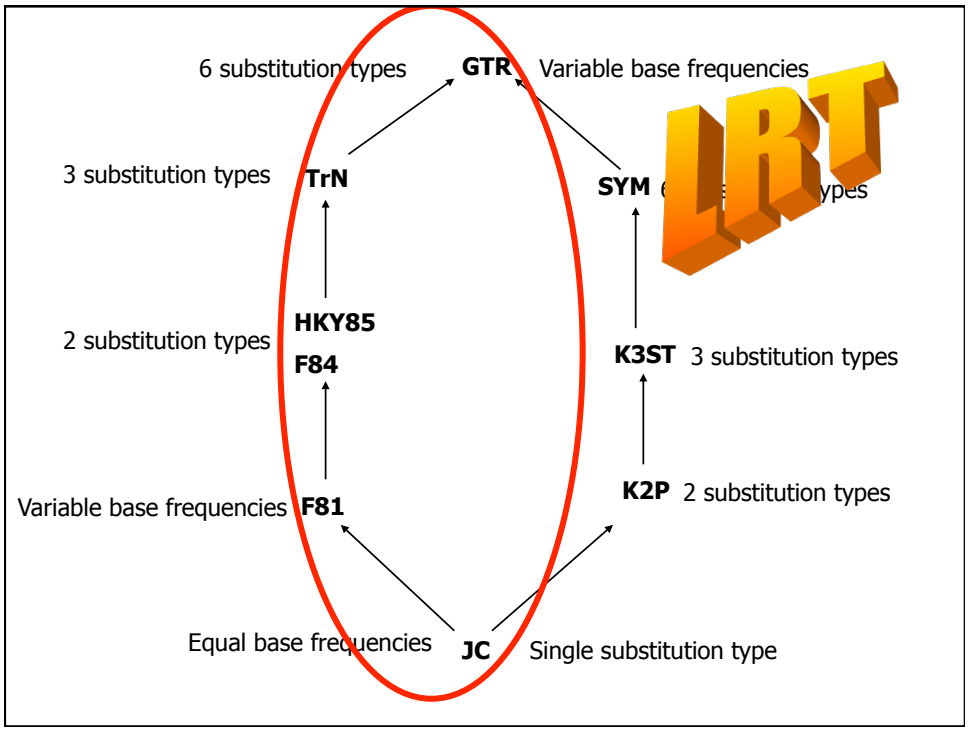
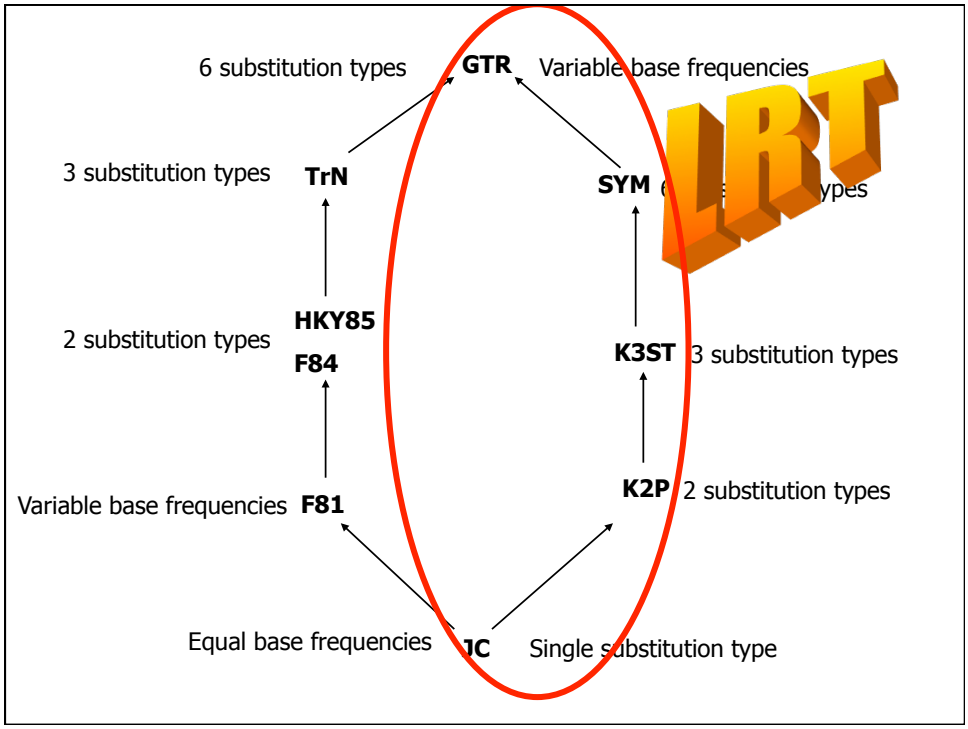
Choosing between models

- Tools to determine whether the model can estimate parameters from the data
- When models are nested
  - Likelihood ratio test (LRT)
- When models are not nested
  - Akaike Information Criterion (AIC)
  - Bayesian Information Criterion (BIC)


6 substitution types
GTR
Variable base frequencies
3 substitution types
TrN
SYM
LRT
2 substitution types
HKY85
F84
K3ST
3 substitution types
K2P
2 substitution types
Variable base frequencies
F81
Equal base frequencies
JC
Single substitution type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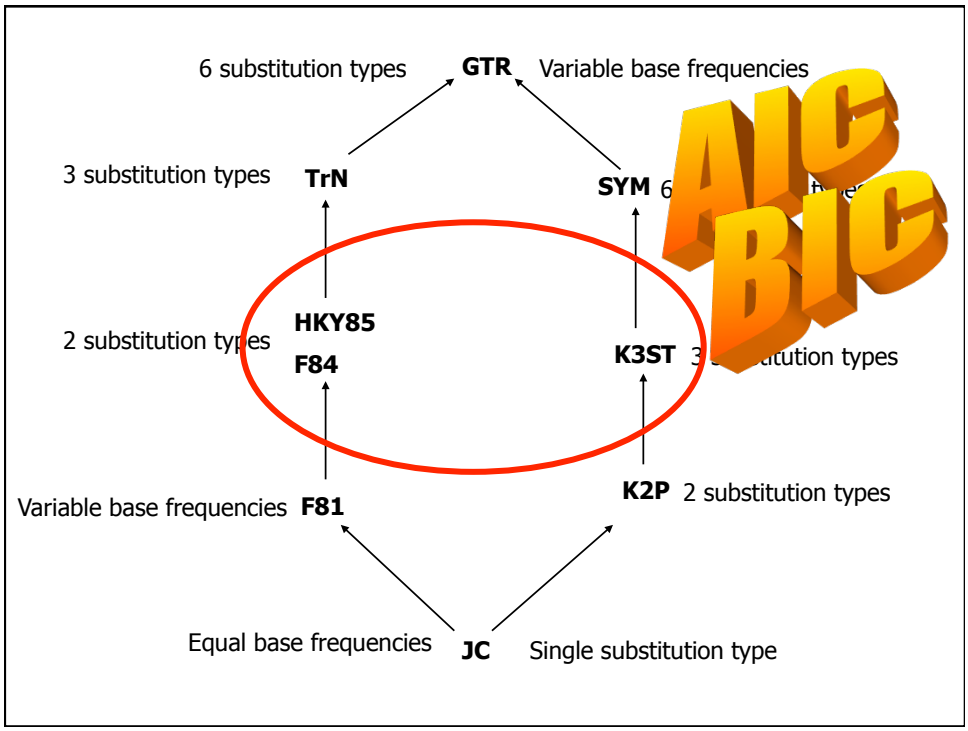
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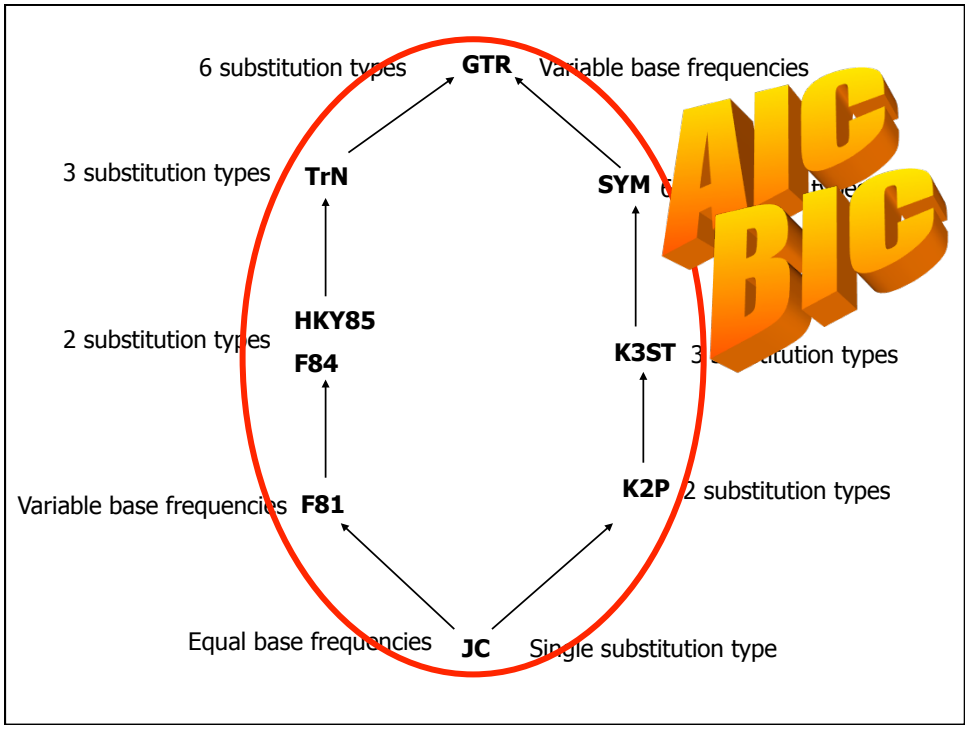
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F81
Equal base frequencies
JC
Single substitution type

Estimation of substitution model parameters

- Yang (1995) has shown that parameter estimates are reasonably stable across tree topologies provided trees are not "too wrong"
- Thus one can obtain a tree using a quick method and then estimate parameters on that tree
- These parameters can then be used to calculate the likelihood of a model for model comparison

Model-testing programs

- **Modeltest**
  - Posada & Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9): 817-818.
- **jModeltest**
  - Darriba et al. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8), 772.
- **PartitionFinder**
  - Lanfear et al. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. MBE 34(3), 772 − 773.
- **ModelFinder built into IQ-Tree**
  - S. Kalyaanamoorthy, B.Q. Minh, T.K.F. Wong, A. von Haeseler, and L.S. Jermiin (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates, Nature Methods, 14:587–589. https://doi.org/10.1038/nmeth.4285

Model testing easier nowadays

- Bayesian statistical framework
  - MrBayes has a model jumping feature
  - It samples over all possible models based on their probabilities
  - No longer necessary to test for which model is optimal
- Maximum Likelihood framework
  - IQ-Tree - ModelFinder implemented

Partitioned models (1/2)

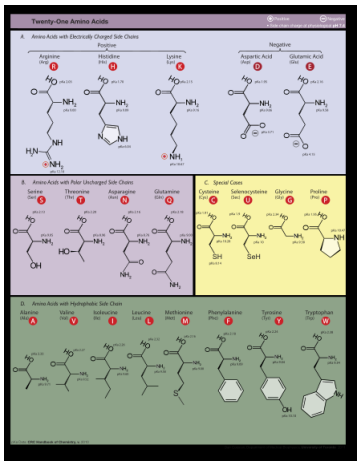
- Today's datasets tend to be large, including hundreds or thousands of genes
- Unrealistic to have the same model for the whole dataset (underparameterization)
- Modelling DNA substitution for separate sections of the data (partitions)
  - E.g. different genes, codon positions, introns/exons, etc.
- To avoid overparameterization, partitions with similar properties can be merged

Partitioned models (2/2)

- This approach allows us to accommodate heterogeneity across data subsets in overall rate and in substitution model parameters
- In some programs also possible to unlink topology and branch lengths so that each data subset evolves differently from each other
- Built into IQ-Tree

Models of amino acid substitution

- Empirical and mechanistic models
- Empirical models: based on empirical AA replacement with matrices from different taxa
  - 20 amino acids – 20x20 matrix too big for estimation
  - Examples: JTT, WAG, LG, MtREV (for mitochondria), Blosum62
- Mechanistic models:
  - e.g. codon models (61x61 matrix
  - Tend to outperform empirical models BUT
  - Computationally very intensive



Recommended reading

- Christoph Bleidorn (2017) Phylogenomics: An Introduction (DOI: 10.1007/978-3-319-54064-1)
- Hoff et al. 2016. Does the choice of nucleotide substitution models matter topologically? BMC Bioinformatics 17: 143. doi.org/10.1186/s12859-016-0985-x
- Kainer & Lanfear. 2015. The Effects of Partitioning on Phylogenetic Inference. Molecular Biology and Evolution, 32(6), 1611–1627. doi.org/10.1093/molbev/msv026