Molecular Phylogenetics Course

Introduction to model-based methods

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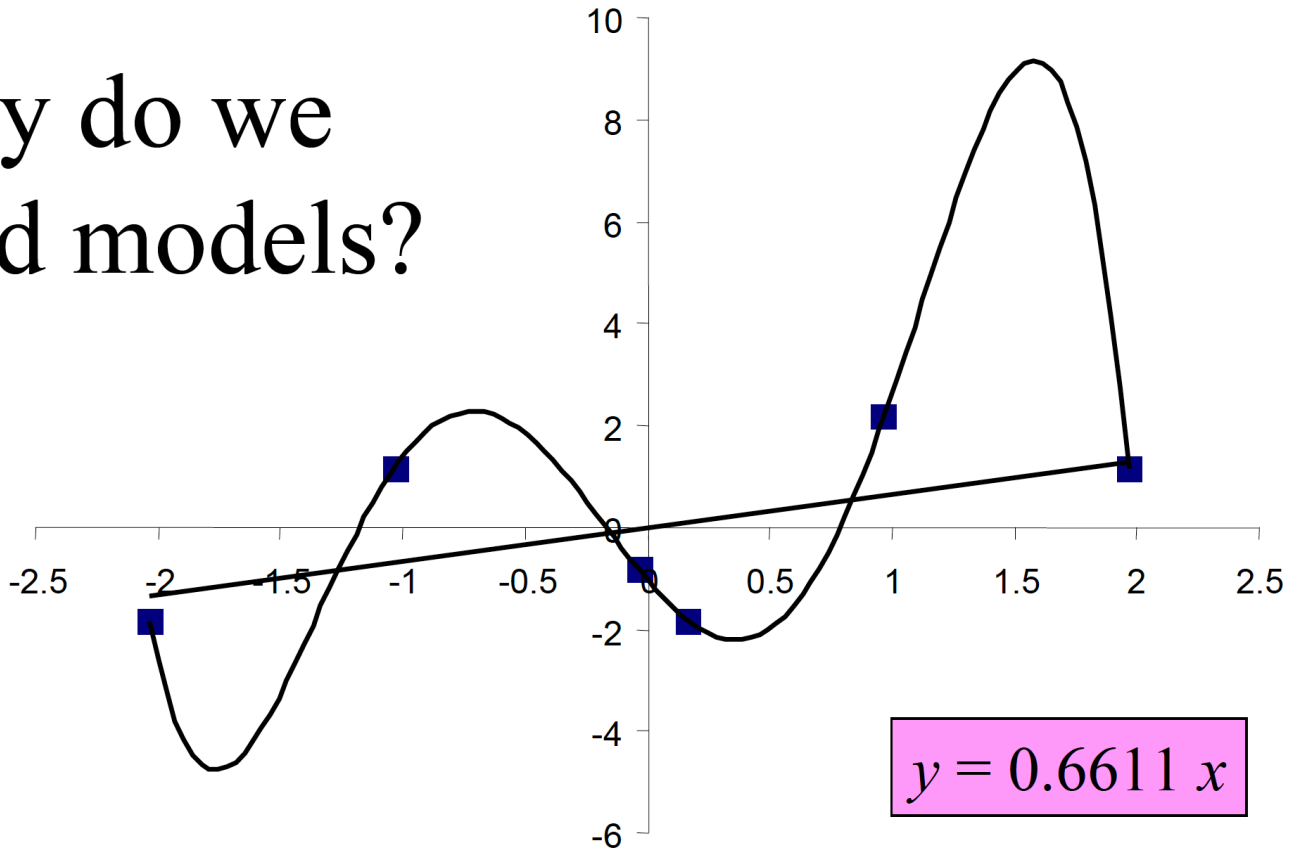
Some slides by Paul Lewis and Chris Simon (University of Connecticut, USA)

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

Why do we need models?

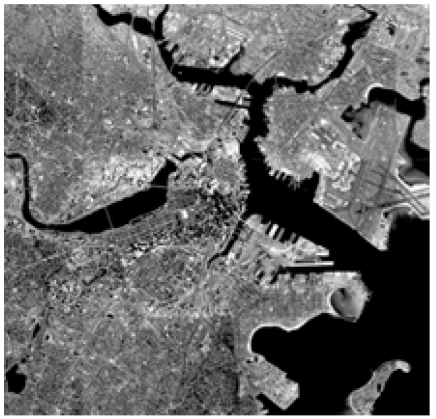


$$y = 0.6611\,x$$

A very *practical* MBTA subway map

17

A very *realistic* MBTA subway map



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- **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 evolution of DNA sequences

- **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**

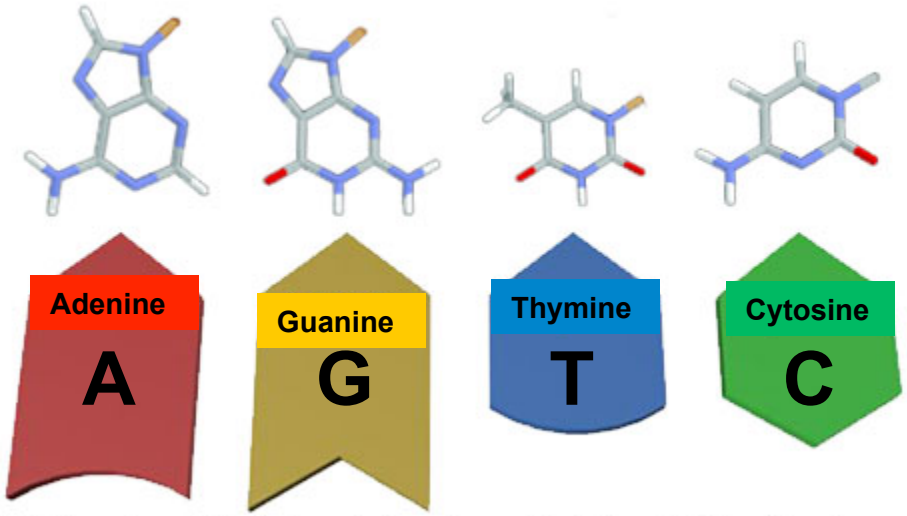
Main Challenge

- **DNA has only four characters**

Purines

Pyrimidines

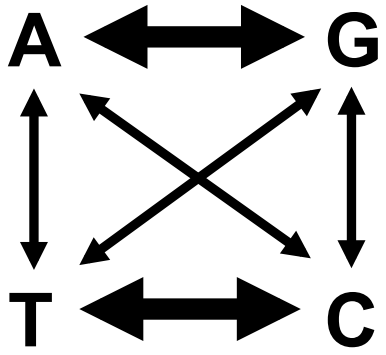
Figure B-3: The Four Nitrogenous Bases



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

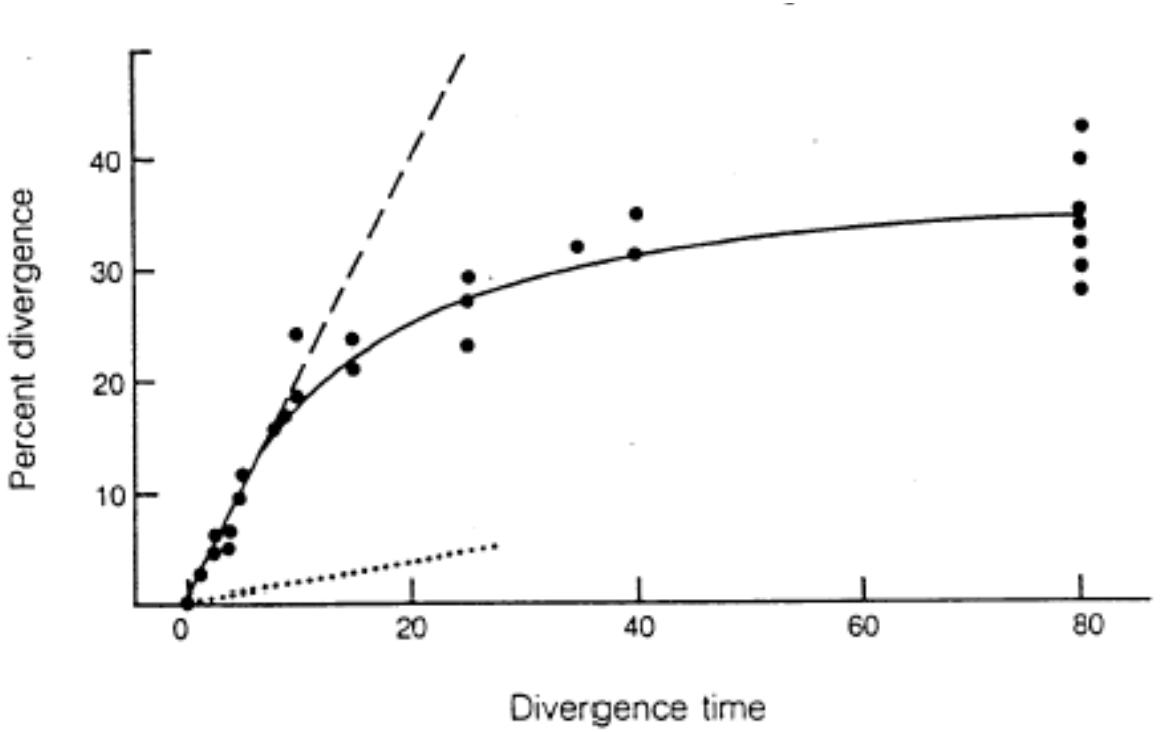
Slide by Chris Simon 2005

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

Misleading DNA evolution

Multiple substitutions hide previous changes



Brown et al. 1979. PNAS 76:1967

Slide by Chris Simon 2005

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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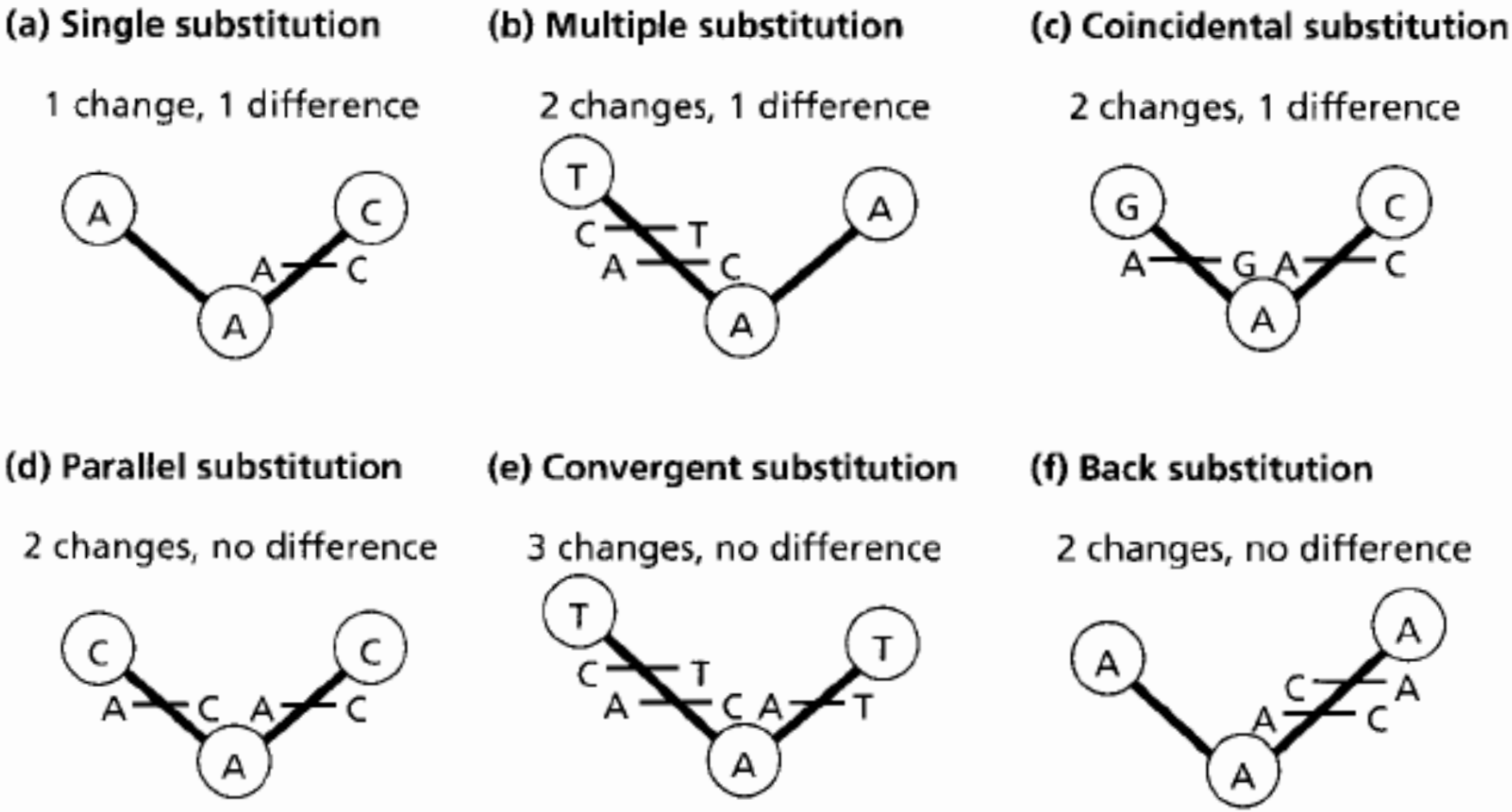
Types of Substitutions



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

Modelling evolution

- These dynamics can be modeled over a tree
- 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 - often modelled as a statistical distribution - for example a gamma distribution

Corrections for multiple substitutions

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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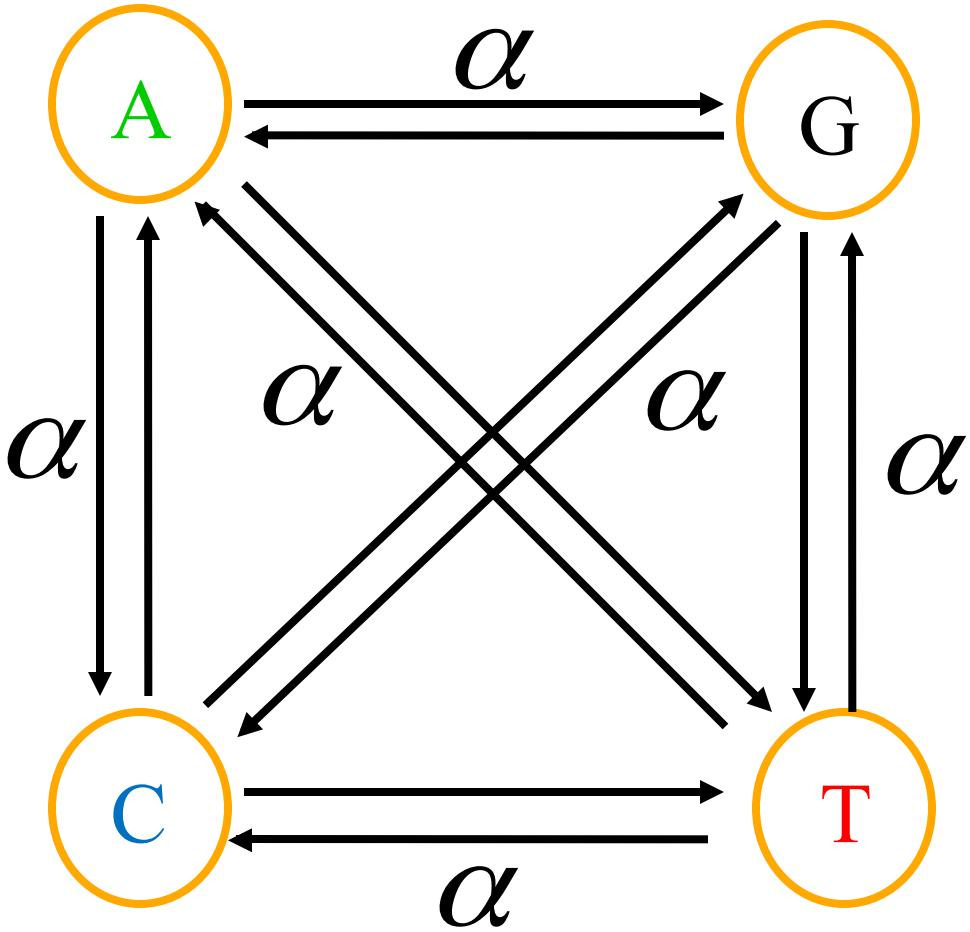
Jukes-Cantor model



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

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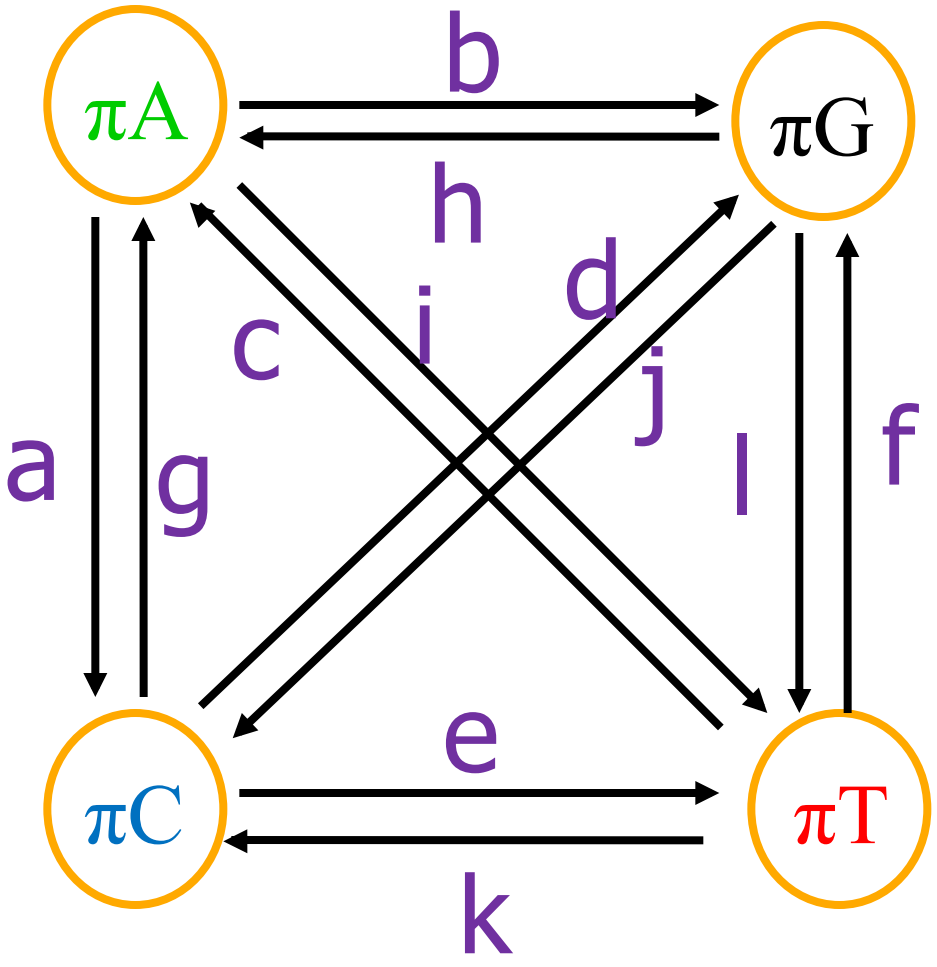
Parameters we are interested in

- 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 and branch lengths

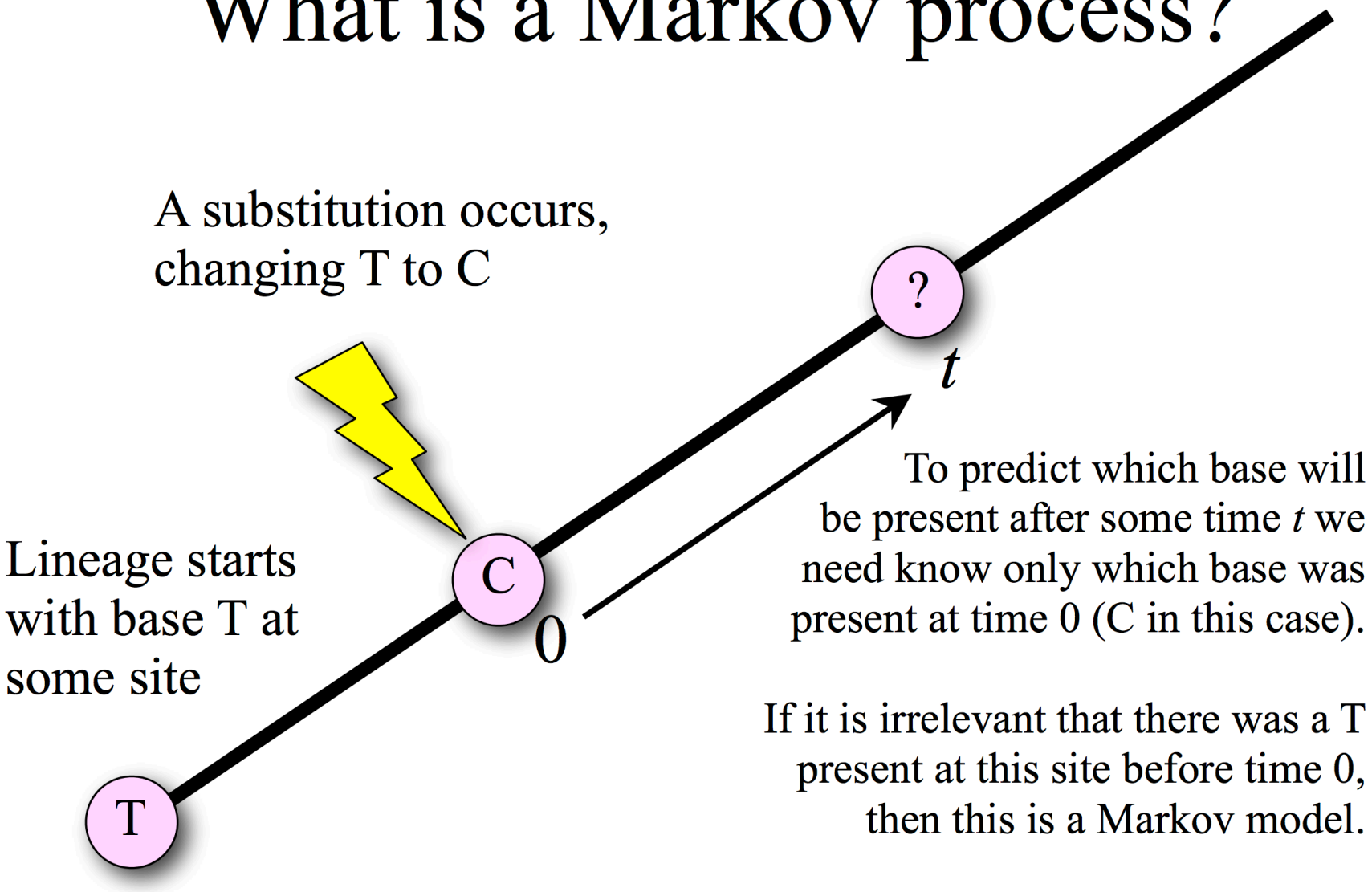
A general model of sequence evolution



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)

What is a Markov process?



Time-homogenous time-continuous stationary Markov models

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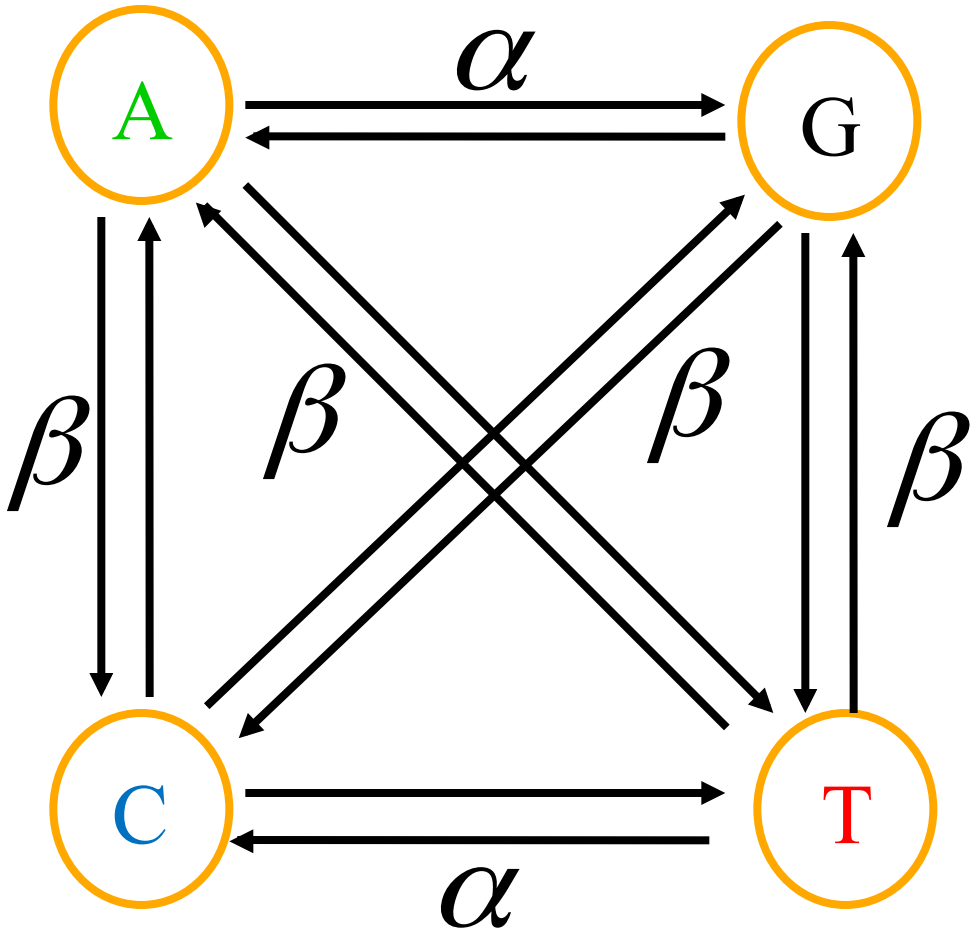
Kimura (1980) model: K2P



α = transitions β = transversions

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**

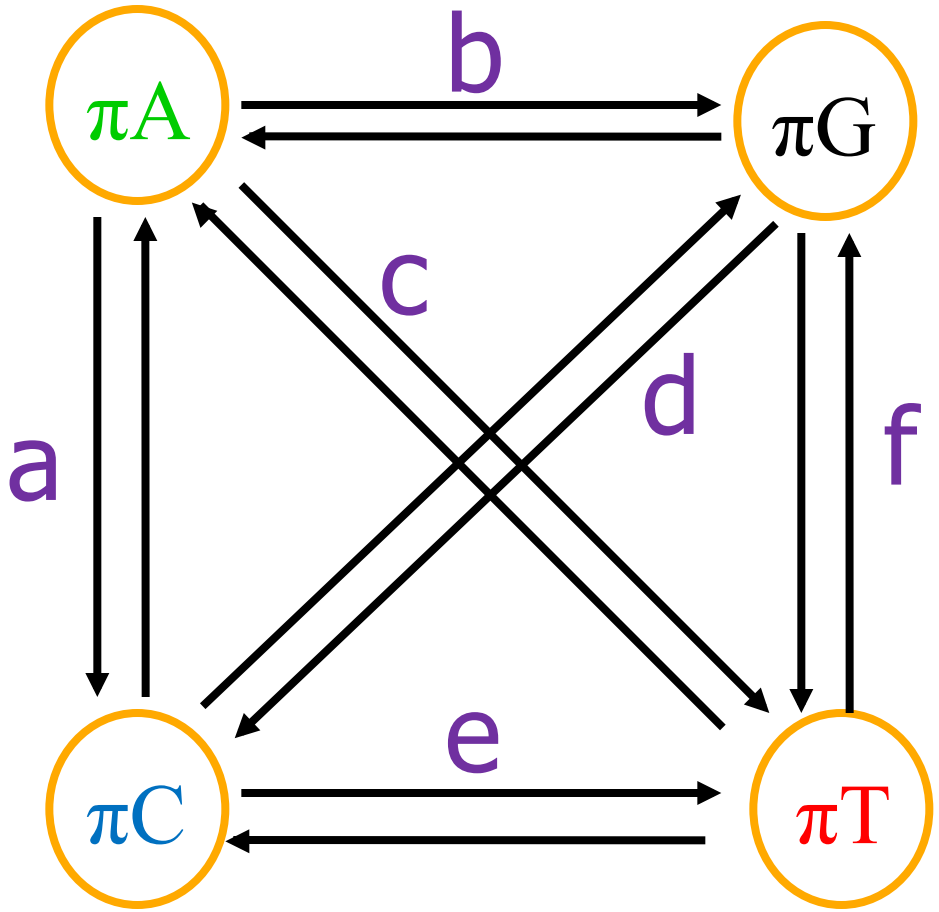
The Hasegawa-Kishino-Yano model

	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	$\pi_A c$	$\pi_C e$	$\pi_G f$	–

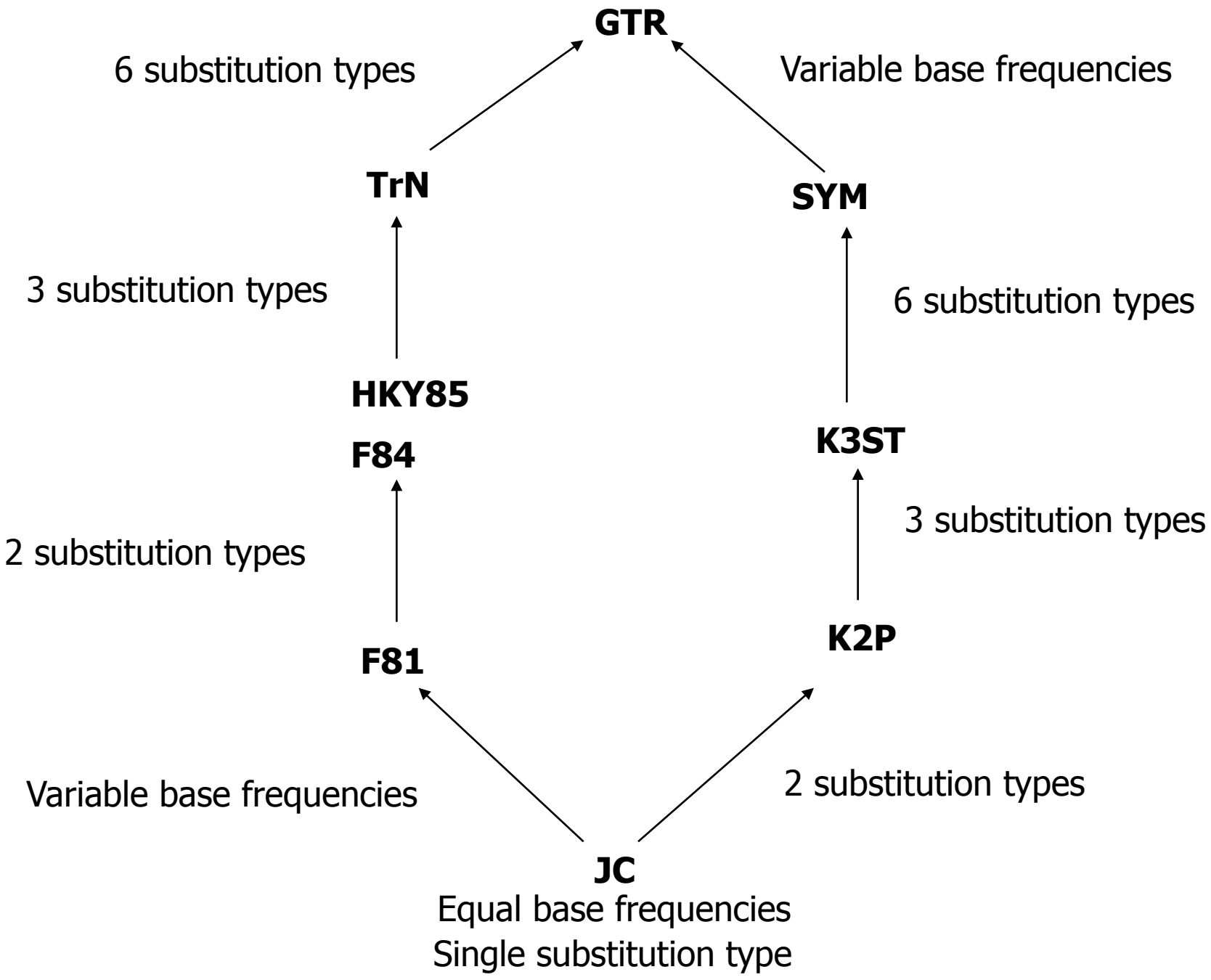
GTRmodel:

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


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

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)

- 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

Third codon position not completely degenerate: synonymous and non-synonymous changes

	AGA									UUA	
	AGG									UUG	
GCA	CGA						GGA			CUA	
GCC	CGC						GGC		AUA	CUC	
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC	CUG	AAA
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU	CUU	AAG
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys

			AGC					
			AGU					
		CCA	UCA	ACA			GUA	
		CCC	UCC	ACC			GUC	UAA
	UUC	CCG	UCG	ACG		UAC	GUG	UAG
AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA
Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Stop

Different probability of change in 2 vs. 3 vs. 4 vs. 6-codon families; "Universal" Genetic Code (nuclear)

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Typical pattern of variation among codon positions

E.g. in Collembola

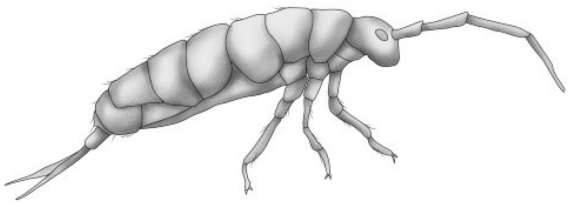
56.7% of all variable sites are located in third positions

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. JME



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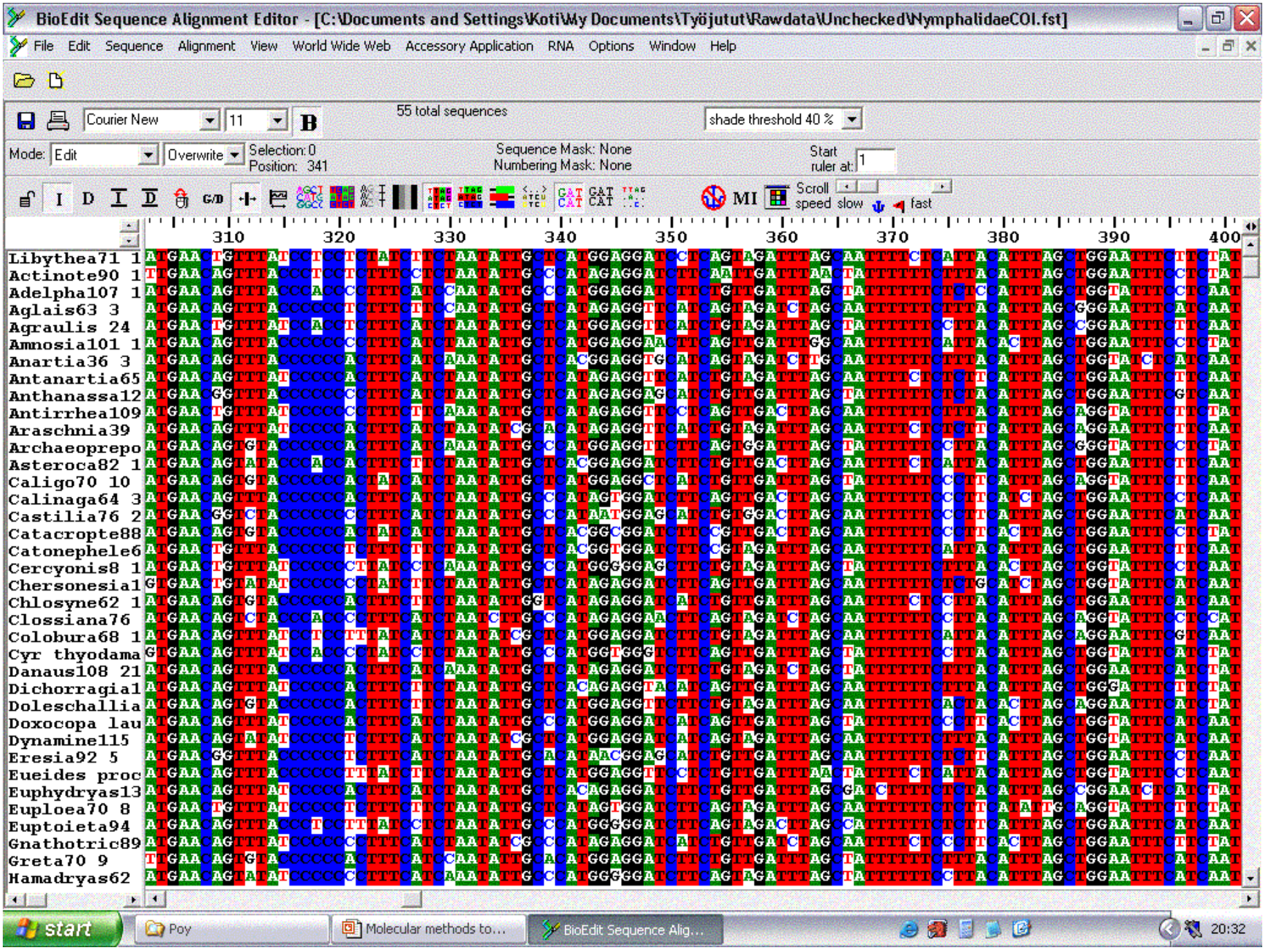
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Actinote90 1
Adelpha107 1
Aglais63 3
Agraulis 24
Amnosia101 1
Anartia36 3
Antanartia65
Anthanassa12
Antirrhea109
Araschnia39
Archaeoprepo
Asteroca82 1
Caligo70 10
Calinaga64 3
Castilia76 2
Catacropte88
Catonephele6
Cercyonis8 1
Chersonesia1
Chlosyne62 1
Clossiana76
Colobura68 1
Cyr thyodama
Danaus108 21
Dichorragia1
Doleschallia
Doxocopa lau
Dynamine115
Eresia92 5
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Euphydryas13
Euploea70 8
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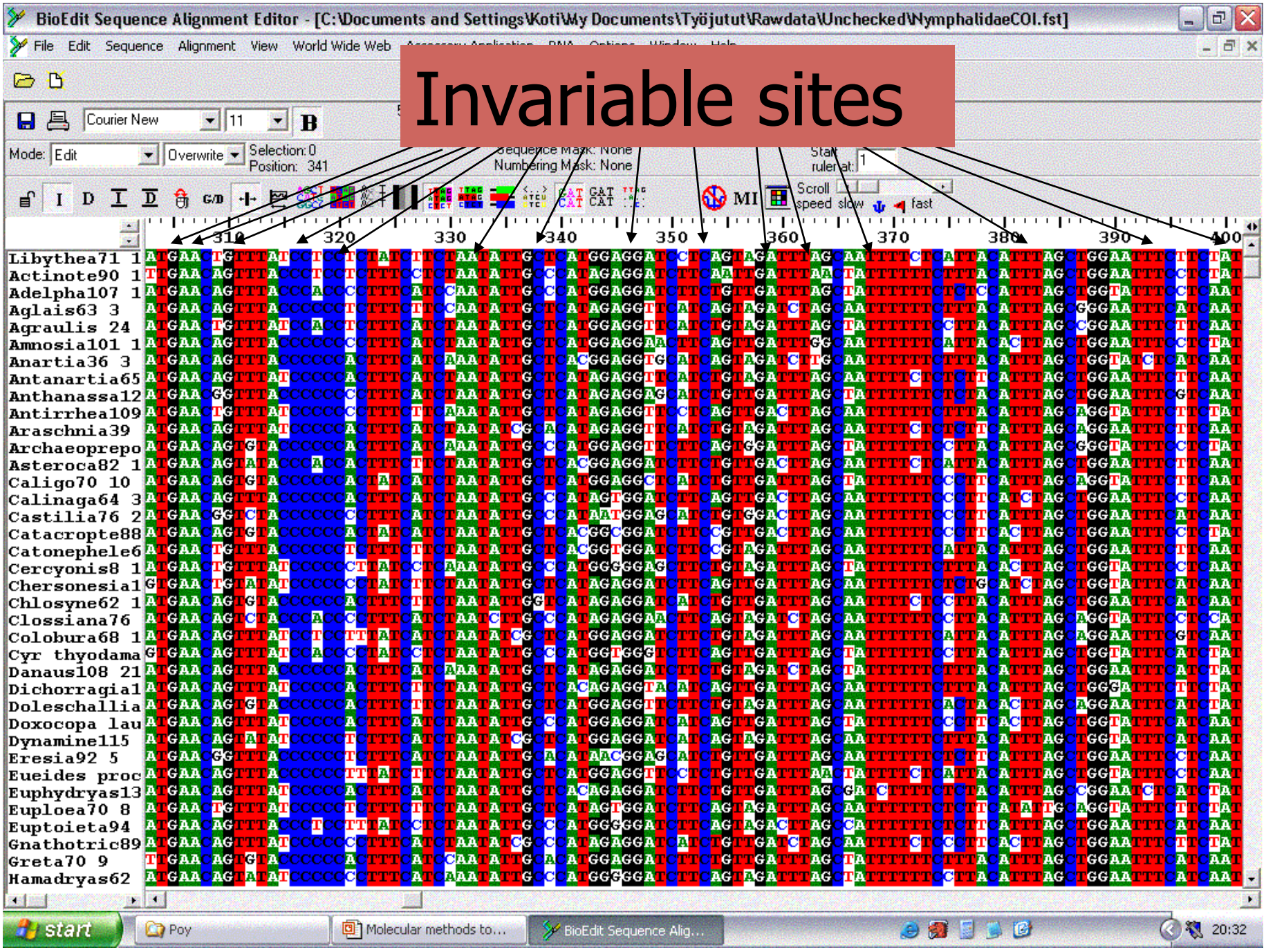
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Invariable sites
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Agraulis 24
Amnosia101 1
Anartia36 3
Antanartia65
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Antirrhea109
Araschnia39
Archaeoprepo
Asteroca82 1
Caligo70 10
Calinaga64 3
Castilia76 2
Catacropte88
Catonephele6
Cercyonis8 1
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Chlosyne62 1
Clossiana76
Colobura68 1
Cyr thyodama
Danaus108 21
Dichorragia1
Doleschallia
Doxocopa lau
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Greta70 9
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start
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Molecular methods to...
BioEdit Sequence Alig...
20:32

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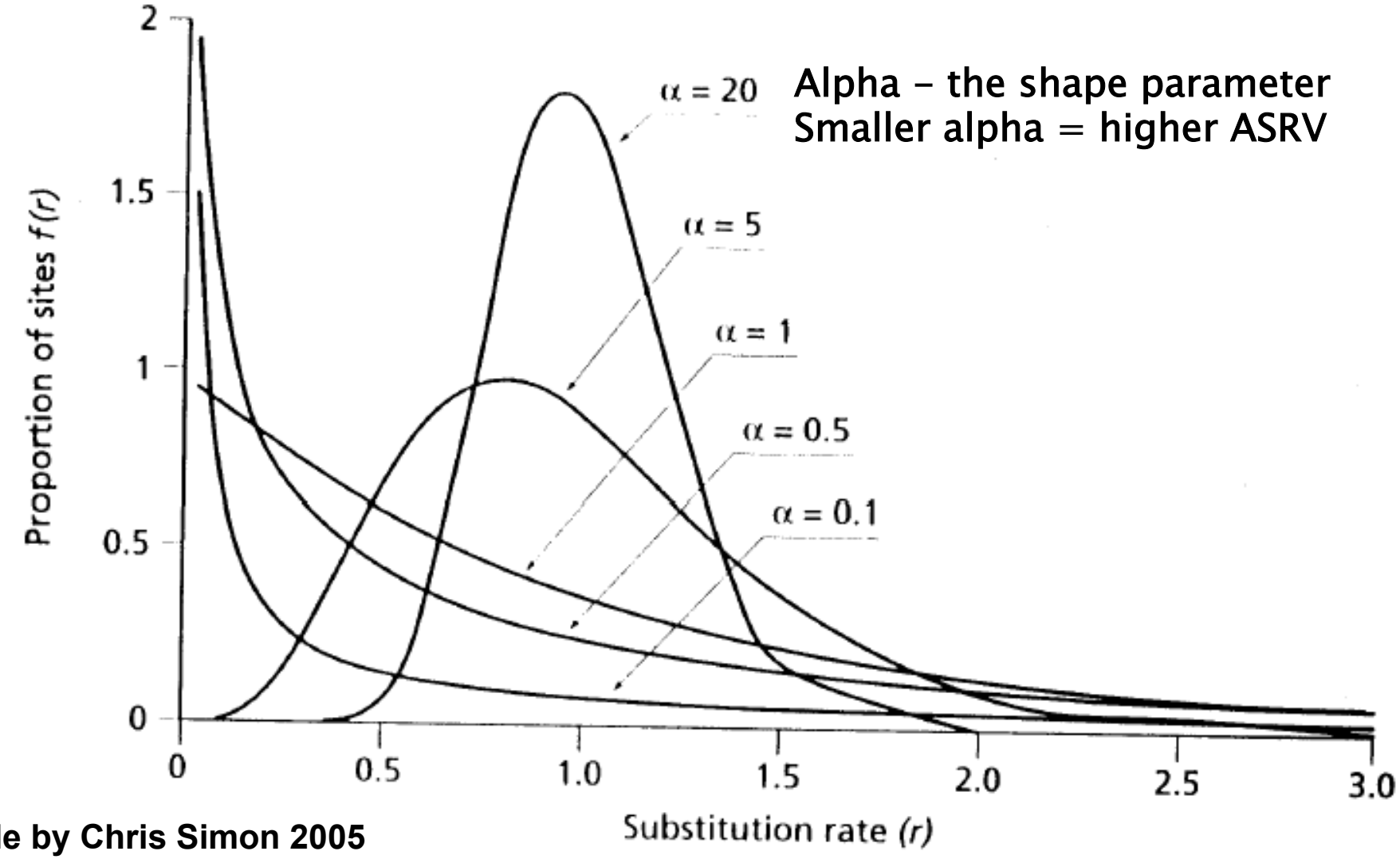
ASRV, Yang 1996, TREE 11(9):367-372

Gamma distribution:

Relative substitution rates for different α values



Alpha – the shape parameter
Smaller alpha = higher ASRV

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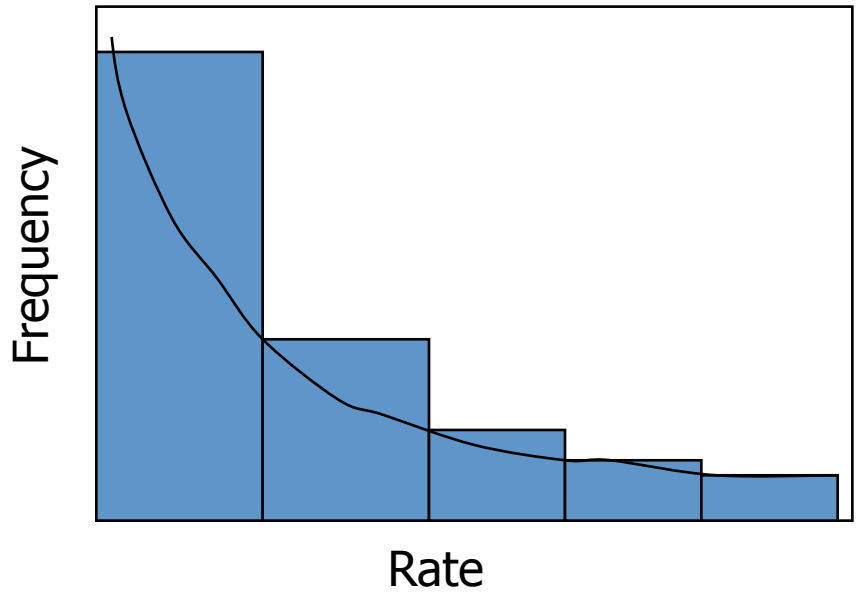
Gamma distribution computationally costly

- 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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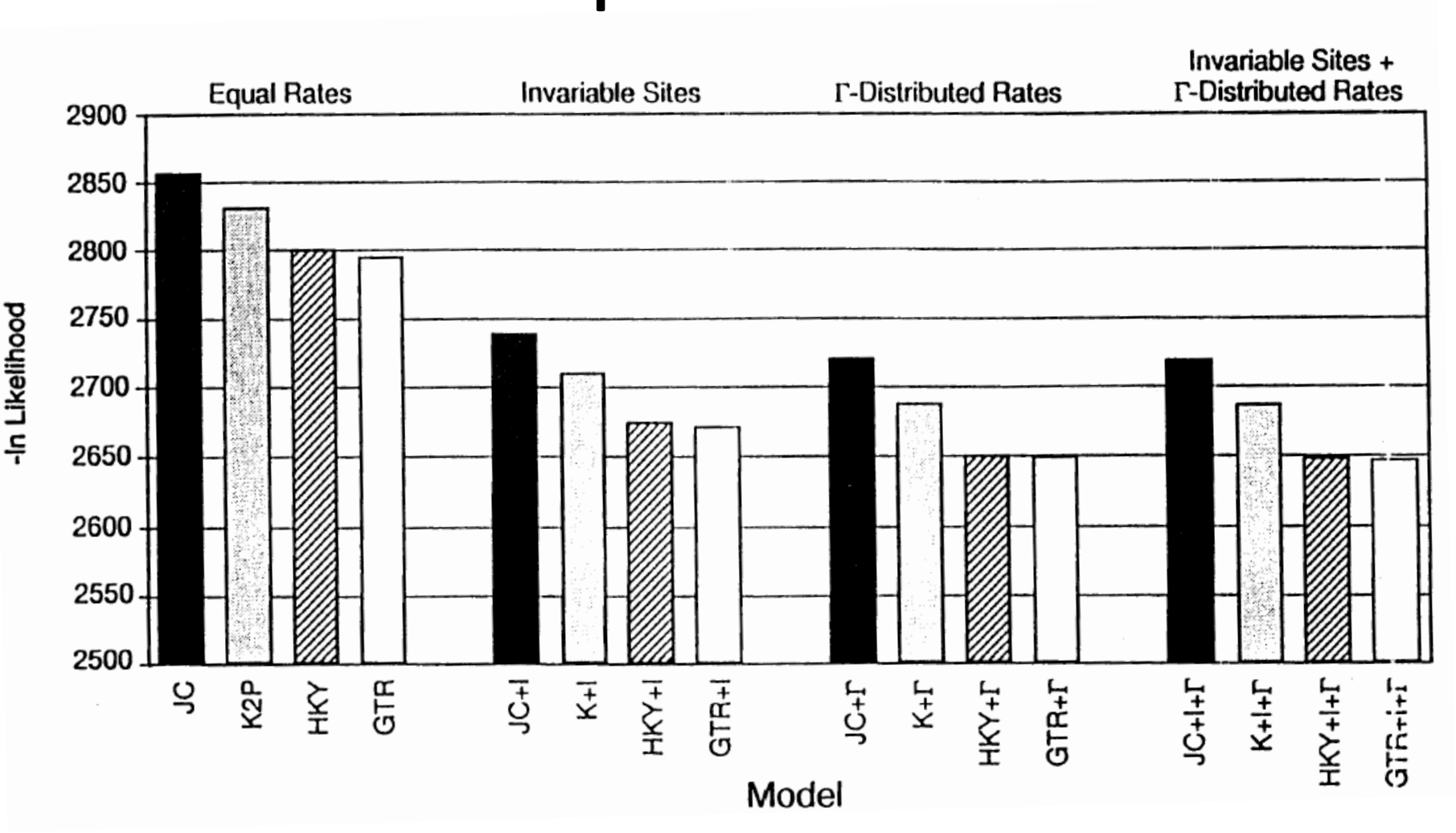
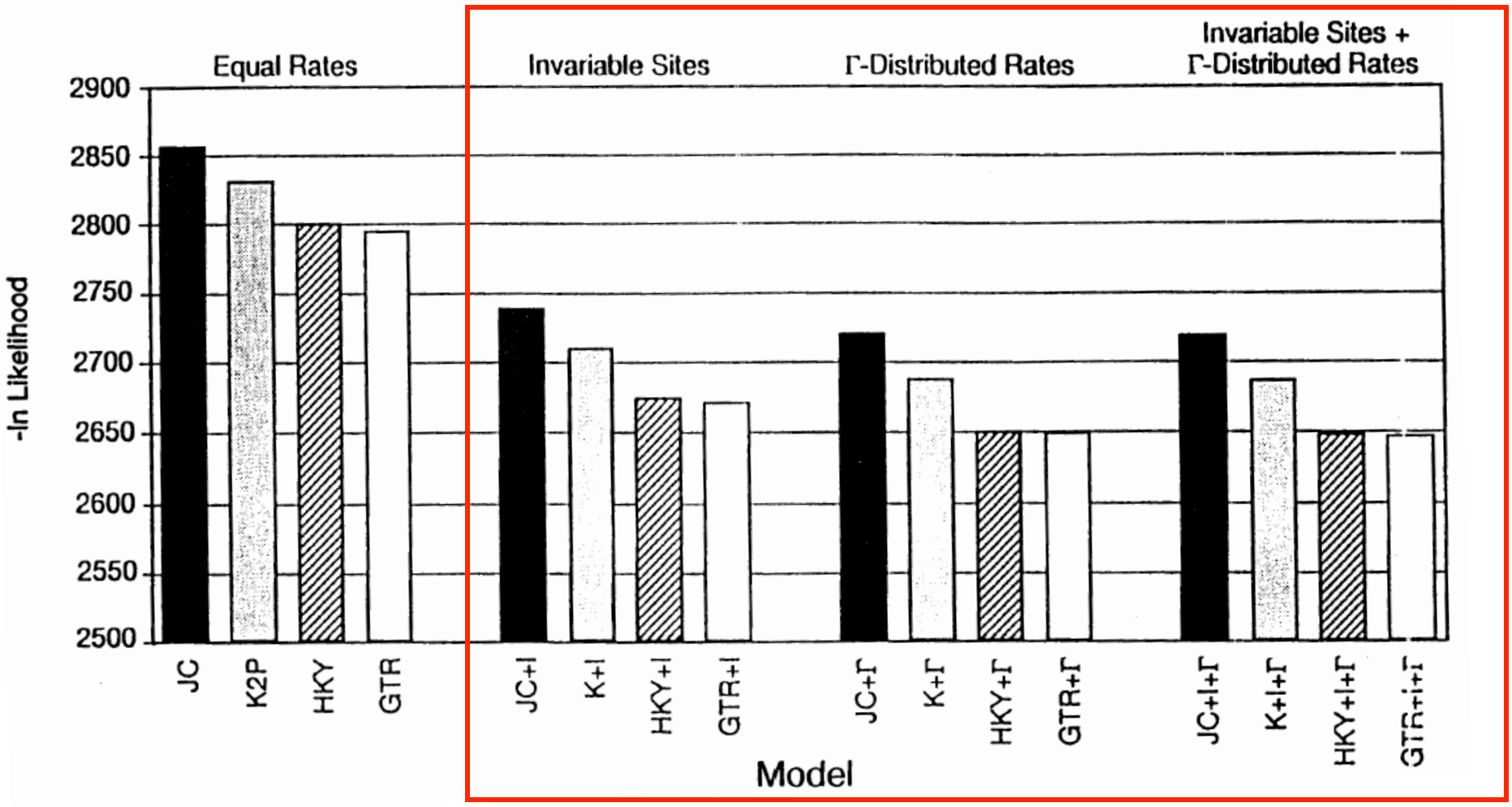
ASRV >> fit improvement than by other parameters



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

Slide by Chris Simon 2005

ASRV >> fit improvement than by other parameters



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

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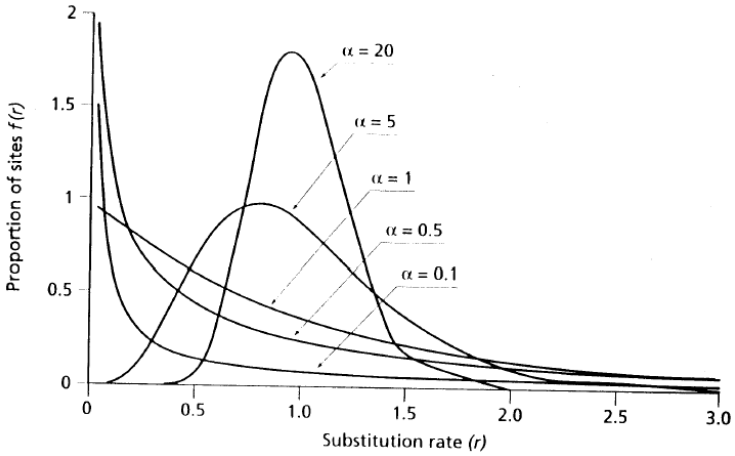
Difficulties in estimating ASRV

- **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**
  - **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 estm.)**
  - **Base composition (1 fixed, 3 estm.)**
  - **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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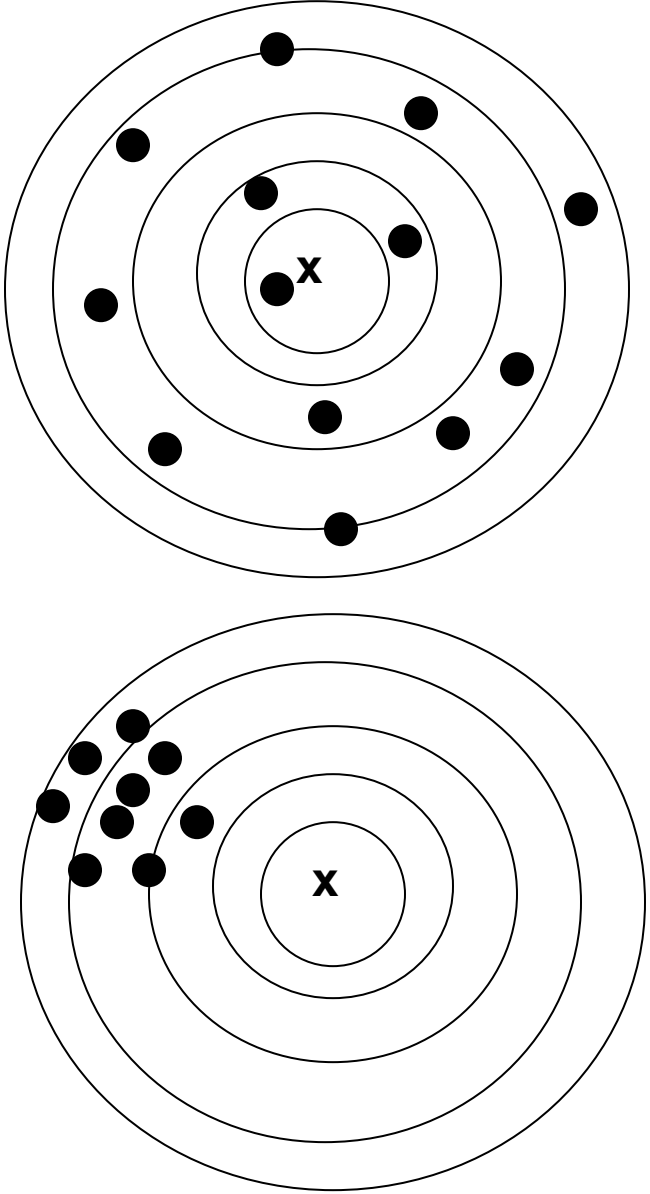
Models can be made more parameter rich to increase their realism

- 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



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)
- Few parameters, lower error variance but may not be centered around the mean (lower accuracy)

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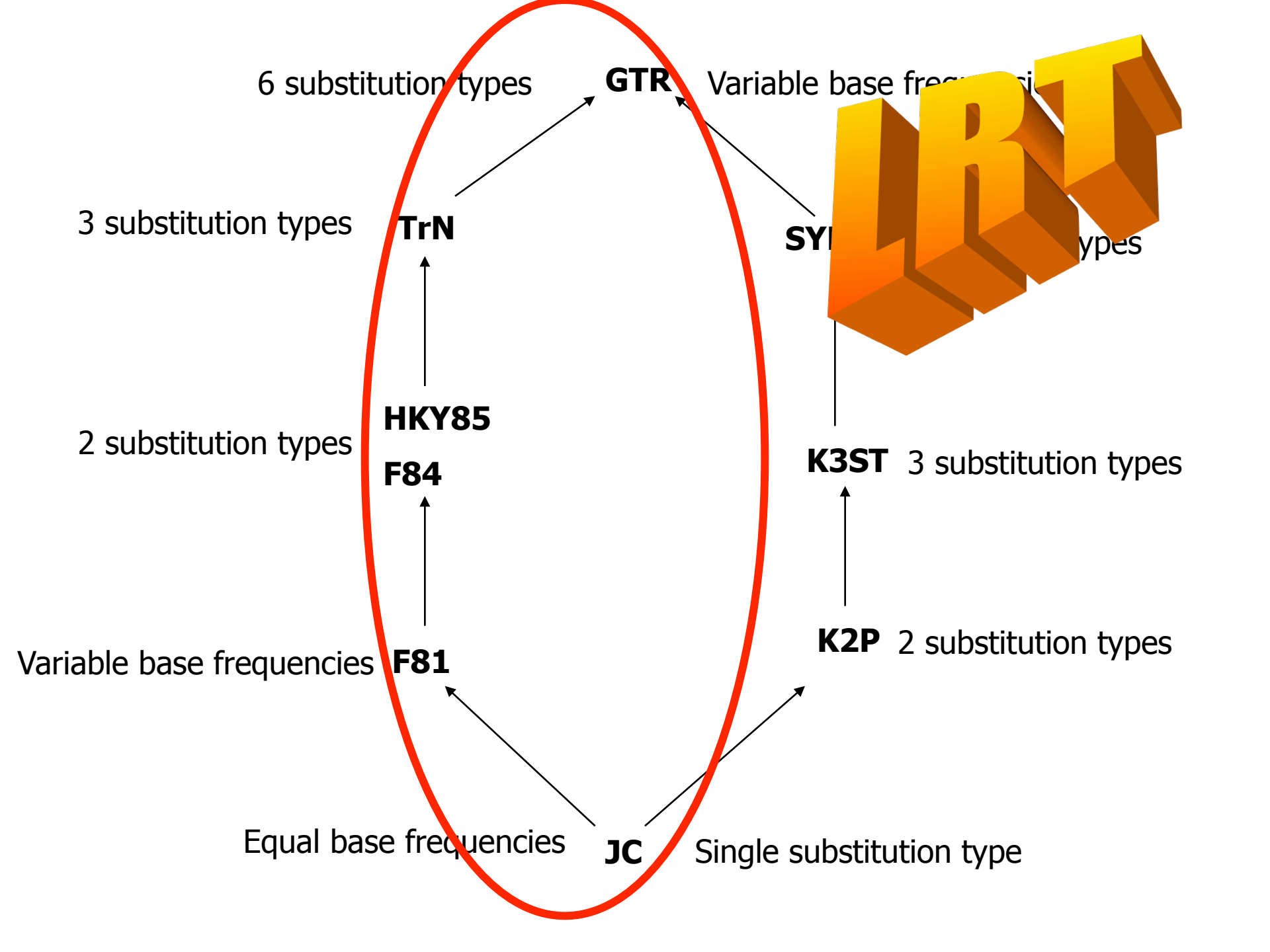
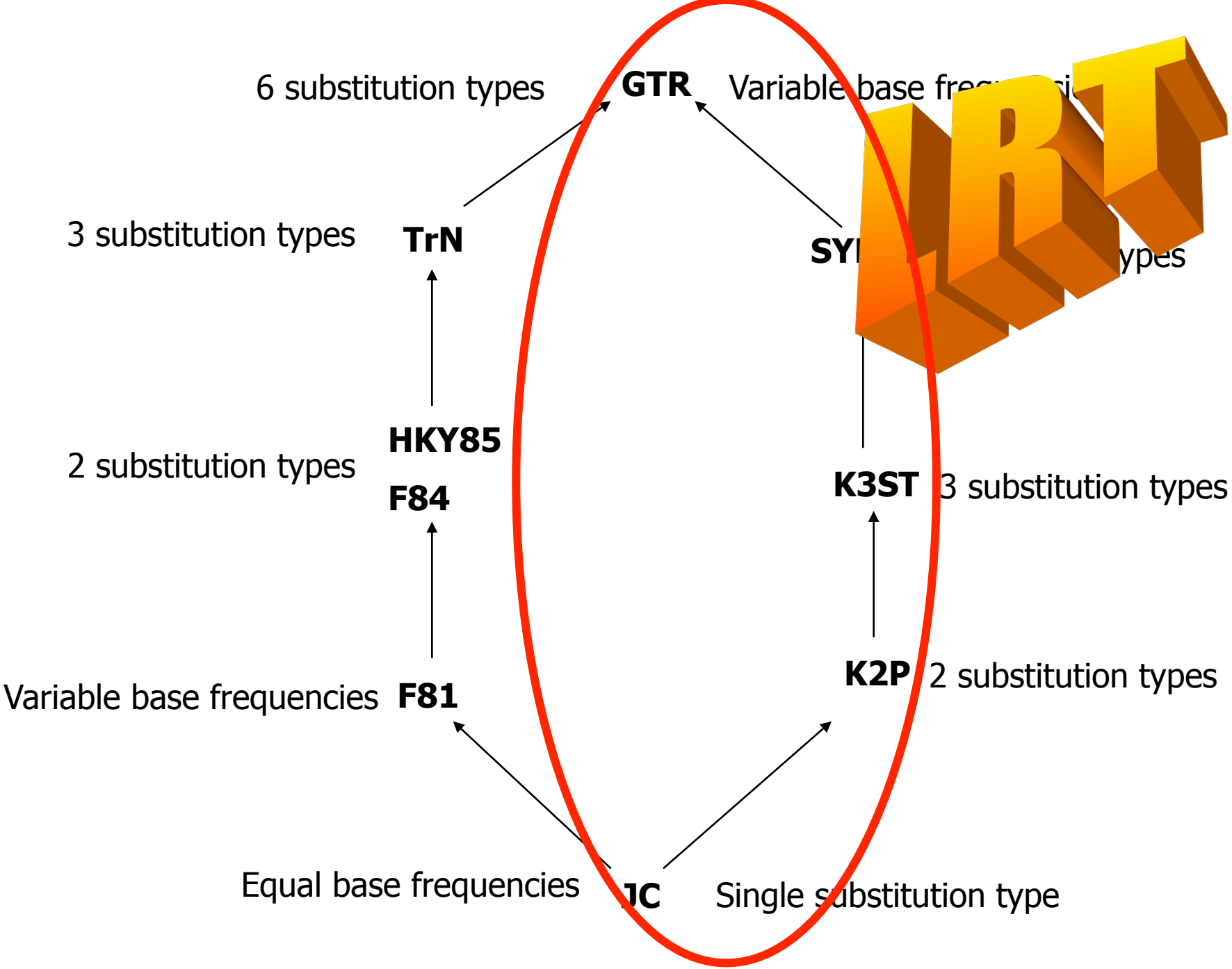
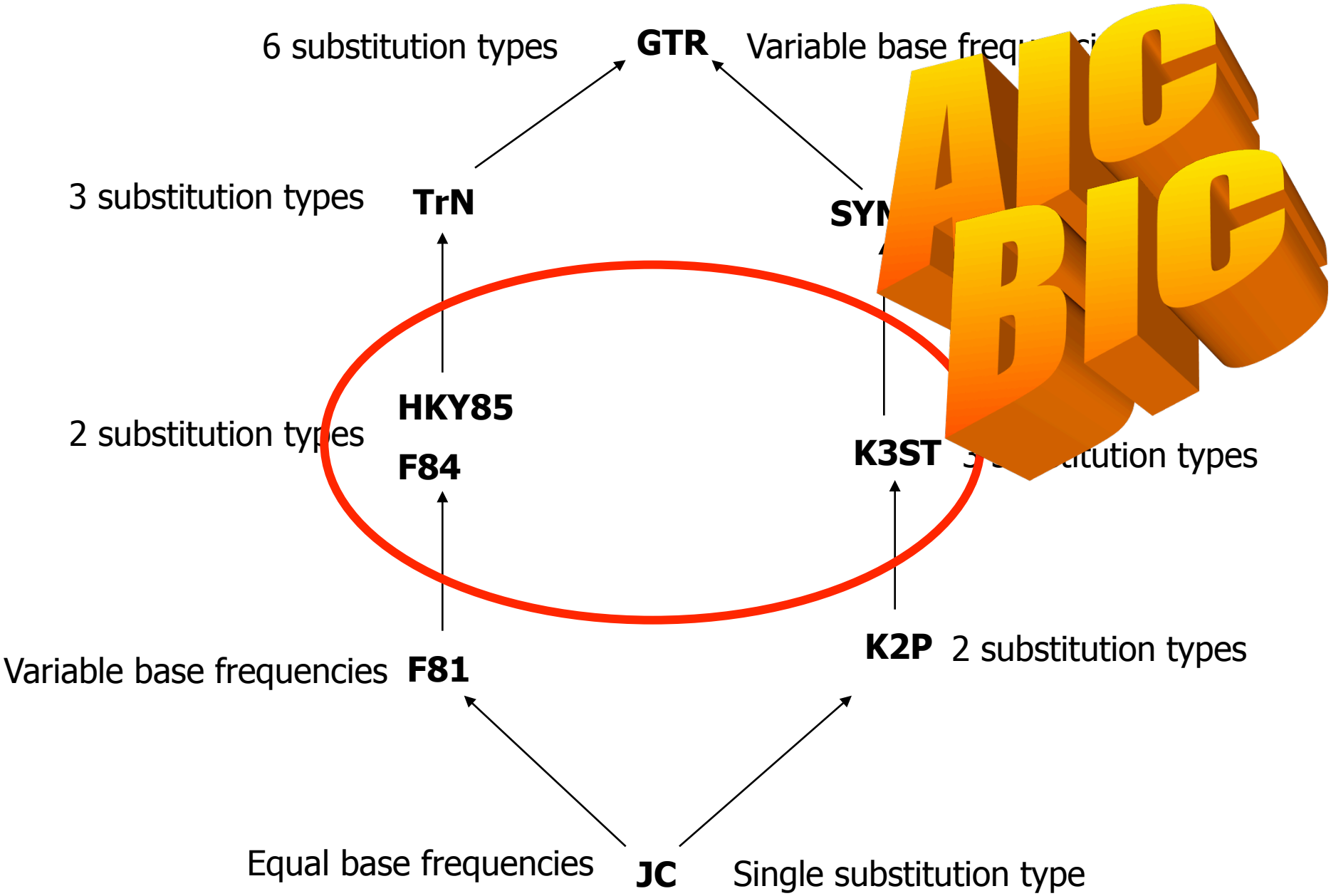
Choosing between models

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


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


6 substitution types
GTR
Variable base frequ
3 substitution types
TrN
SYM
AIC
BIC
HKY85
2 substitution types
F84
K3ST
titution types
K2P
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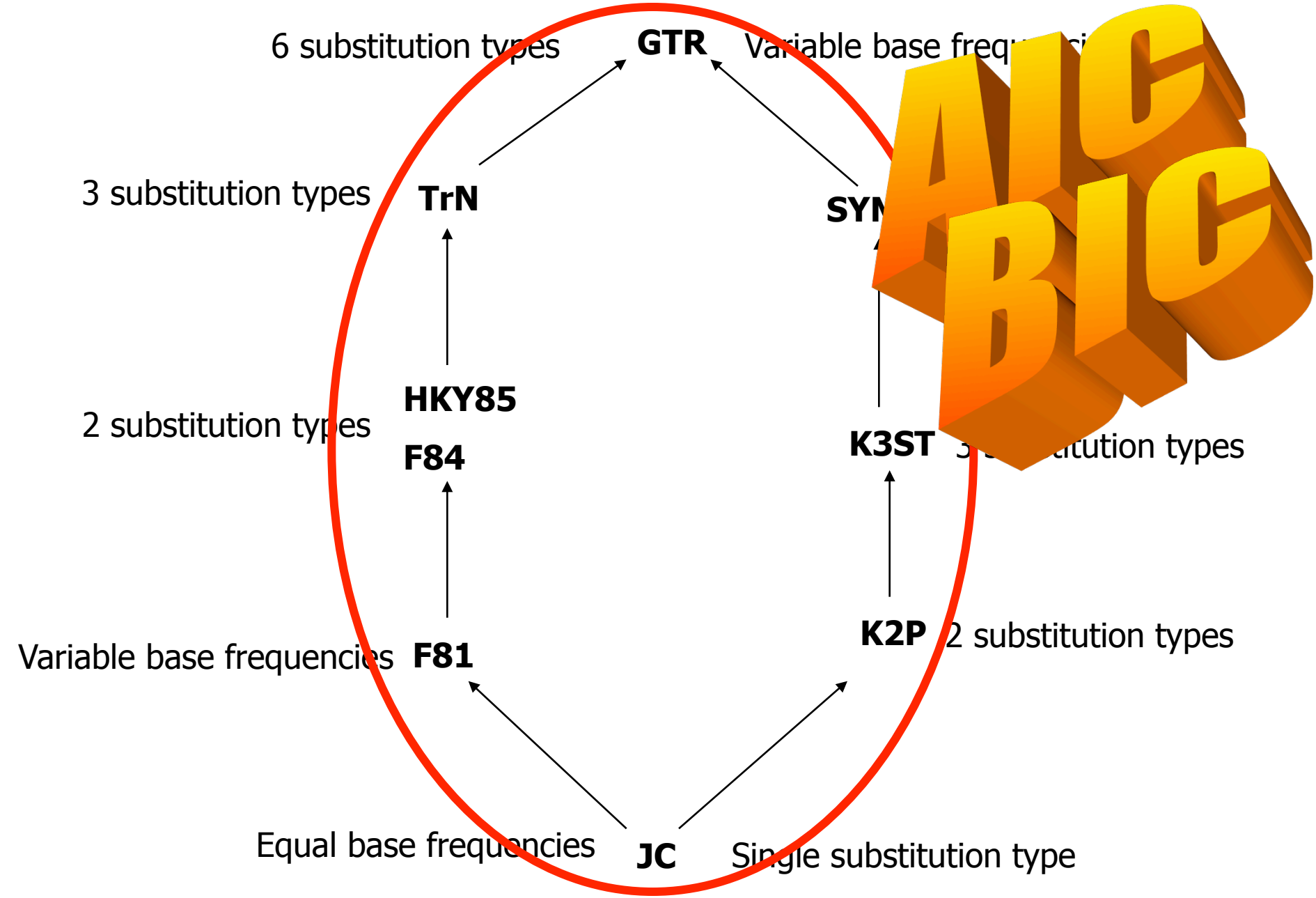
Single substitution type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2 substitution types
Equal base frequencies
JC
Single substitution type
AIC
BIC

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

Need to know the likelihood of a model

- For both tests, one needs to compute the likelihood of the model
- This will be covered in the next lecture
- For now, assume we know the likelihood of the models we want to compare
- Comparison tools:
  - Likelihood ratio test (LRT)
  - Akaike information criterion (AIC) and corrected AIC (AIC_c)
  - Bayesian information criterion (BIC)

Likelihood ratio test (LRT)

$$LR = 2*(\ln L_1 - \ln L_0)$$

Alternative hypothesis

More parameter-rich

Null hypothesis

Less parameter-rich

- **LRT statistic approximately follows a chi-square distribution**
- **Degrees of freedom equal to the number of extra parameters in the more complex model**

Akaike Information Criterion

- A measure of the relative quality of statistical models for a given dataset (Wikipedia definition)
  - It deals with the trade-off between the goodness of fit and the complexity of the model
- AIC(M) = - 2*Log(Likelihood(M)) + 2*K(M)
  - K(M) is the number of estimable parameters of model M
- Given a dataset, models can be ranked according to their AIC
- The model with the lowest AIC is selected
- AIC_c – correction for finite sample size – usually used

Bayesian Information Criterion

- **BIC takes into account also sample size *n***
- **BIC(M) = - 2*Log(Likelihood(M)) + *K*(M)*Log(*n*)**
  - **K(M) is the number of estimable parameters of model M and *n* is the number of characters**
- **The model with the lowest BIC is selected**

Modeltesting programs

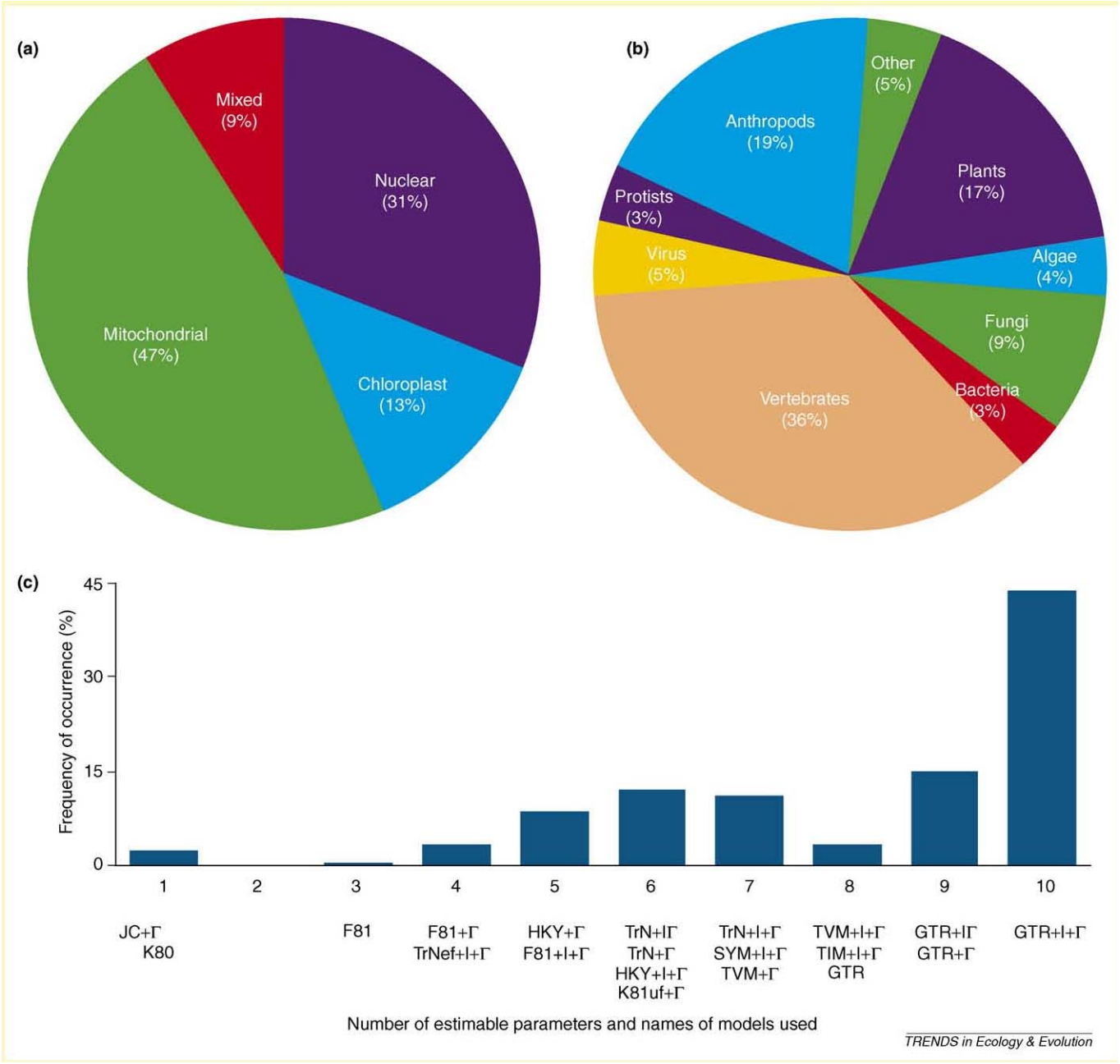
- **Modeltest**
  - **Posada & Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9): 817-818.**
- **jModeltest**
  - **Darriba et al. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8), 772.**
- **PartitionFinder**
  - **Lanfear et al. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. MBE 34(3), 772 − 773.**

Output from jModelTest

```
---------------------------------------------------------------
*                                                             *
*        CORRECTED AKAIKE INFORMATION CRITERION (AICc)        *
*                                                             *
---------------------------------------------------------------

 Sample size: 7705.0

 Model selected:
   Model = GTR+I+G
   partition = 012345
   -lnL = 124050.4448
   K = 299
   freqA = 0.3118
   freqC = 0.1859
   freqG = 0.1721
   freqT = 0.3302
   R(a) [AC] =  1.8584
   R(b) [AG] =  8.1223
   R(c) [AT] =  3.8422
   R(d) [CG] =  1.9446
   R(e) [CT] =  13.2353
   R(f) [GT] =  1.0000
   p-inv = 0.5420
   gamma shape = 1.0570
```

Best model

Model	-lnL	K	AICc	delta	weight	cumWeight
GTR+I+G	124050.44479	299	248723.116454	0.000000	1.000000	1.000000
HKY+I+G	124507.54811	295	249628.667552	905.551098	2.30e-197	1.000000
SYM+I+G	124637.30469	296	249890.343721	1167.227268	0.00e+000	1.000000
GTR+G	125146.28705	298	250912.636212	2189.519758	0.00e+000	1.000000
SYM+G	125604.64648	295	251822.864292	3099.747838	0.00e+000	1.000000
HKY+G	125827.80977	294	252267.028447	3543.911993	0.00e+000	1.000000
K80+I+G	126897.82784	292	254402.741487	5679.625033	0.00e+000	1.000000
K80+G	127883.70157	291	256372.328272	7649.211818	0.00e+000	1.000000
GTR+I	128411.14327	298	257442.348652	8719.232198	0.00e+000	1.000000
SYM+I	128662.58435	295	257938.740032	9215.623578	0.00e+000	1.000000
HKY+I	129622.81043	294	259857.029767	11133.913313	0.00e+000	1.000000
F81+I+G	129925.12106	294	260461.651027	11738.534573	0.00e+000	1.000000
F81+G	130912.74863	293	262434.744325	13711.627871	0.00e+000	1.000000
K80+I	131130.71024	291	262866.345612	14143.229158	0.00e+000	1.000000
JC+I+G	131880.87114	291	264366.667412	15643.550958	0.00e+000	1.000000
JC+G	132773.35353	290	266149.472099	17426.355645	0.00e+000	1.000000
F81+I	143289.73580	293	287188.718665	38465.602211	0.00e+000	1.000000
JC+I	144715.21061	290	290033.186259	41310.069805	0.00e+000	1.000000
GTR	146171.15092	297	292960.199774	44237.083321	0.00e+000	1.000000
SYM	146261.55346	294	293134.515827	44411.399373	0.00e+000	1.000000
HKY	148503.72377	293	297616.694605	48893.578151	0.00e+000	1.000000
K80	149762.98834	290	300128.741719	51405.625265	0.00e+000	1.000000
F81	155229.75311	292	311066.592027	62343.475573	0.00e+000	1.000000
JC	156259.70450	289	313120.014529	64396.898076	0.00e+000	1.000000



Kelchner & Thomas 2007, TREE 22:87-94

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 - ModelFind implemented**

Partitioned models

- ***A priori*** **separation of characters into different partitions**
  - **E.g.: different codon position, RNA, introns/exons, etc.**
- **Each partition analyzed with a different model**
- **Allows for heterogeneity across data subsets in overall rate and in substitution model parameters, plus in some programs also possible to unlink topology and branch lengths**
- **"Different data subsets can thus have independent branch lengths or even different topologies." (Ronquist and Huelsenbeck, 2003:1573)**

Output from PartitionFinder

STRATEGY 1

```
Best partitioning scheme

Scheme Name       : step_10
Scheme lnL        : -118126.425049
Scheme BIC        : 239403.118009
Number of params  : 352
Number of sites   : 7705
Number of subsets : 8

Subset | Best Model | # sites    | subset id                        | Partition names
1      | GTR+G      | 714        | d117c135876d3e868828f25d0953a9bd | Partition_11, Partition_14, Partition_8, Partition_12, Partition_10
2      | F81+I      | 4290       | 4a675e7e540ecb009621eb52d7552b70 | Partition_1
3      | GTR+G      | 440        | 5d196738faf5467fb2f903ee5b3d1bb3 | Partition_13, Partition_16, Partition_17, Partition_15
4      | GTR+G      | 637        | 065fff28d8d1b017945d1d664ee88c2e | Partition_6, Partition_9, Partition_7
5      | SYM        | 252        | 428b6c6e493caaddb383eeadddfdaf08 | Partition_2
6      | SYM+G      | 413        | c4bdbe6a705db90b2dcd3ea803712b39 | Partition_4, Partition_5
7      | HKY+G      | 228        | 88f604a648ae5dceb0d58a600bec5ca5 | Partition_3
8      | GTR+G      | 731        | 69ca434e659726db47f5700ecf6cdee5 | Partition_18
```

BIC score

Best model for each partition

STRATEGY 2

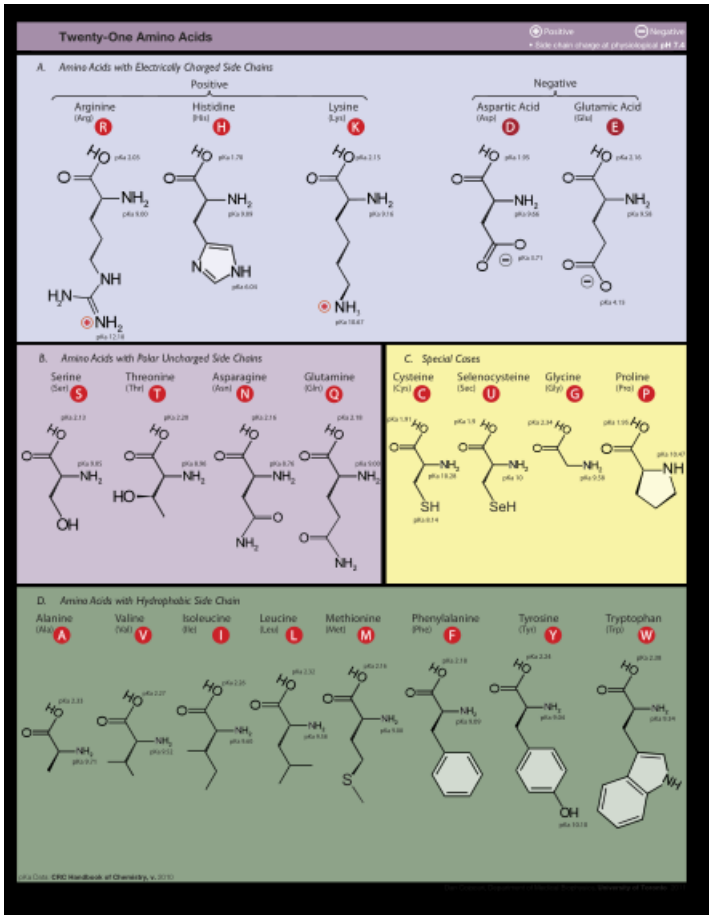
```
Best partitioning scheme

Scheme Name       : separate
Scheme lnL        : -119493.545898
Scheme BIC        : 242200.007081
Number of params  : 359
Number of sites   : 7705
Number of subsets : 7

Subset | Best Model | # sites    | subset id                        | Partition names
1      | GTR+G      | 485        | 88a99148e17429cfbf7c84b3c6ad405b | Partition_3
2      | GTR+I+G    | 4940       | 6147b066b9346034e59c2738ca8adab6 | Partition_1
3      | GTR+G      | 407        | 207a8ca99fe345782ac3e9b948ae6783 | Partition_4
4      | GTR+G      | 342        | a9de06c1f843a93e4545a0c53c47027b | Partition_5
5      | GTR+G      | 733        | 0c045f1386af47731957496eda7faeed | Partition_2
6      | GTR+G      | 368        | 067651d33be225f5683a0ee6ce036017 | Partition_6
7      | GTR+G      | 430        | 6ecc87f27a392fcae101b8b0c99c64e0 | Partition_7
```

BIC score comparison

Strategy 1 BIC = 239403 ✔
Strategy 2 BIC = 242200

Protein models

- 21 amino acids – 21x21 matrix too big for estimation
- Models are based largely on empirical aa replacement with matrices from different taxa
- Examples: JTT, WAG, MtREV, Blosum62



Recommended reading

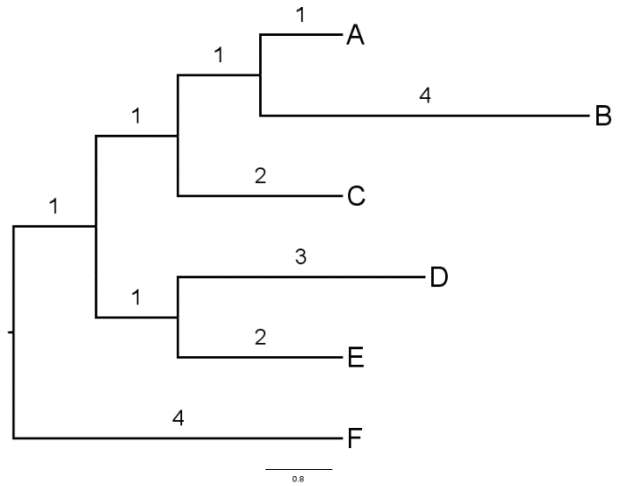
- **Hoff et al. 2016. Does the choice of nucleotide substitution models matter topologically? BMC Bioinformatics 17: 143. doi.org/10.1186/s12859-016-0985-x**
- **Kainer & Lanfear. 2015. The Effects of Partitioning on Phylogenetic Inference. Molecular Biology and Evolution, 32(6), 1611–1627. doi.org/10.1093/molbev/msv026**

Distance methods

Distance methods involve two stages

- **Stage 1: calculate the evolutionary distance between pairs of sequences**
- **Stage 2: use the distances to construct a tree that describes those evolutionary distances**

	A	B	C	D	E
B	5				
C	4	7			
D	7	10	7		
E	6	9	6	5	
F	8	11	8	9	8



Distance Methods

Distance Estimates: estimation of the divergence between two sequences deriving from a common ancestor.

- it is a measure of (dis)similarity between sequences

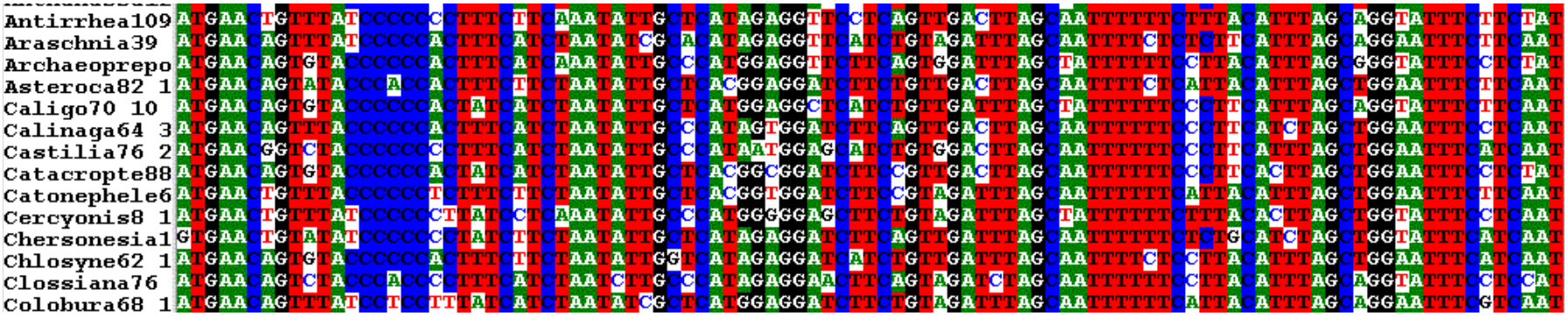
- branch lengths are proportional to the distance

- if we assume a molecular clock the distance is directly proportional to time

Distance can be expressed as a proportion of sites that differ between two sequences:

98 bp of which 15 differ, or D = 15/98 = 0.153

Distance can be expressed as a proportion of sites that differ between two sequences:



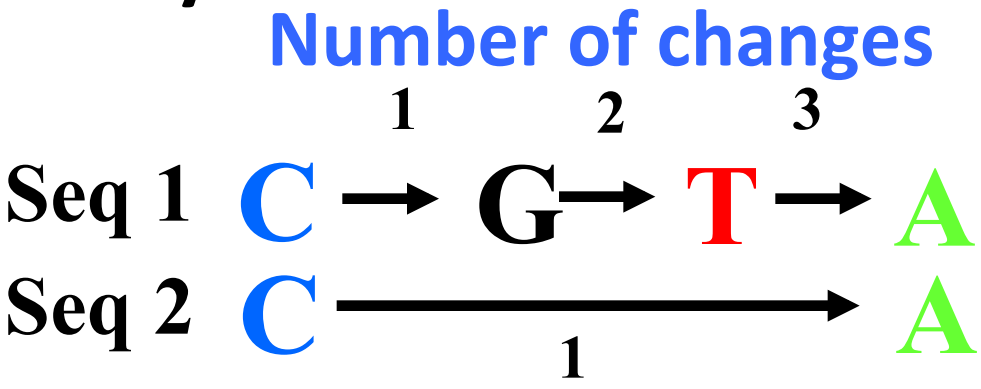
Distance matrix:

	1	2	..	n
1				
2	0,33			
:				
n	0,23	0,63		

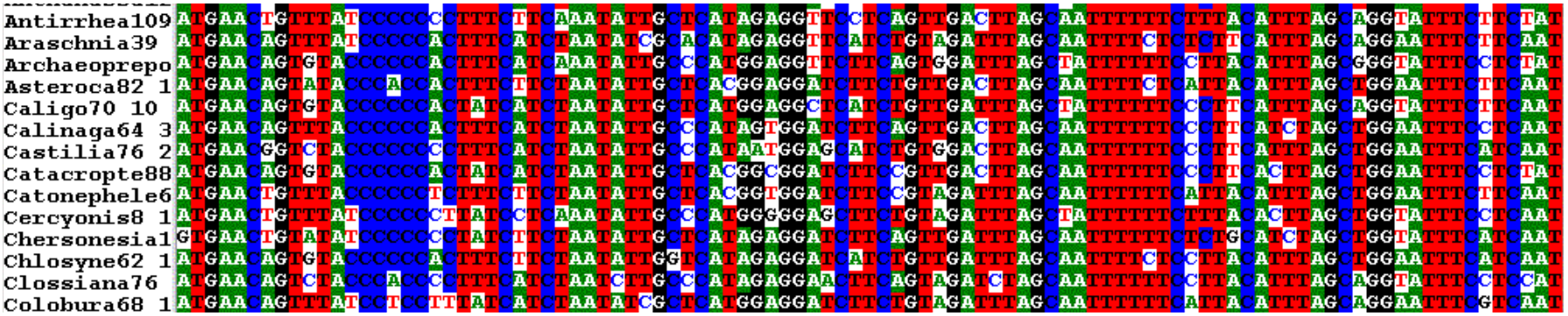
-> Direct measure of distance underestimates the true distance
- Remember multiple hits!

Models correct for unobserved changes

- All models include a correction for multiple substitutions at the same site
- All (except Logdet distances) can be modified to include a gamma correction for site rate heterogeneity



Distance can be expressed as a proportion of sites that differ between two sequences:



Dissimilarities matrix:

	1	2	..	n
1				
2	0.33			
:				
n	0.23	0.63		

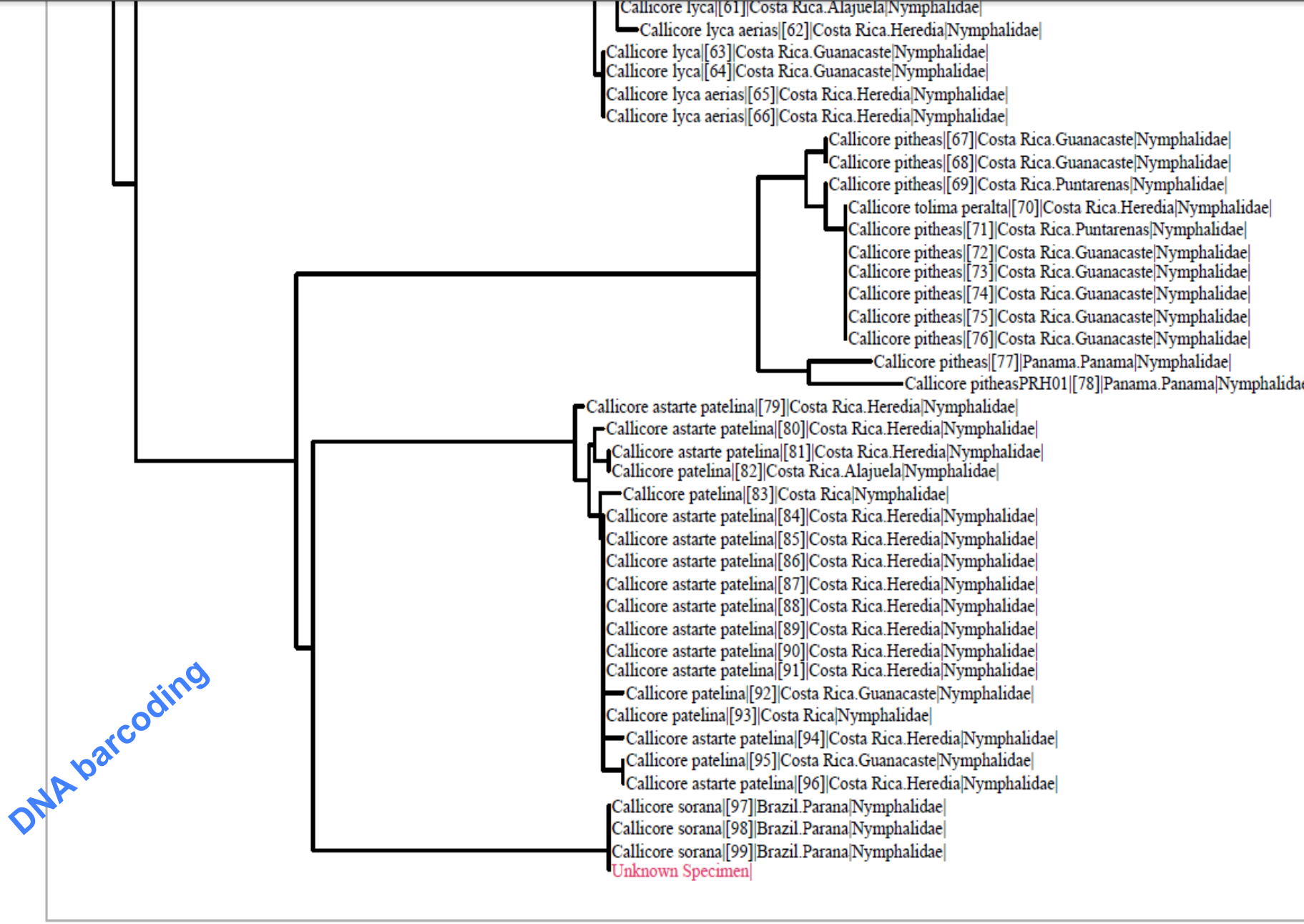
Correction for multiple substitutions →

Evolutionary distance matrix:

	1	2	..	n
1				
2	0.35			
:				
n	0.24	0.66		

Distances - advantages

- **Fast when using clustering algorithms**
- **A large number of models are available with many parameters - improves estimation of distances**


Callicore lyca|[61]|Costa Rica.Alajuela|Nymphalidae|
Callicore lyca aerias|[62]|Costa Rica.Heredia|Nymphalidae|
Callicore lyca|[63]|Costa Rica.Guanacaste|Nymphalidae|
Callicore lyca|[64]|Costa Rica.Guanacaste|Nymphalidae|
Callicore lyca aerias|[65]|Costa Rica.Heredia|Nymphalidae|
Callicore lyca aerias|[66]|Costa Rica.Heredia|Nymphalidae|
Callicore pitheas|[67]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[68]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[69]|Costa Rica.Puntarenas|Nymphalidae|
Callicore tolima peralta|[70]|Costa Rica.Heredia|Nymphalidae|
Callicore pitheas|[71]|Costa Rica.Puntarenas|Nymphalidae|
Callicore pitheas|[72]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[73]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[74]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[75]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[76]|Costa Rica.Guanacaste|Nymphalidae|
Callicore pitheas|[77]|Panama.Panama|Nymphalidae|
Callicore pitheasPRH01|[78]|Panama.Panama|Nymphalidae
Callicore astarte patelina|[79]|Costa Rica.Heredia|Nymphalidae|
Callicore astarte patelina|[80]|Costa Rica.Heredia|Nymphalidae|
Callicore astarte patelina|[81]|Costa Rica.Heredia|Nymphalidae|
Callicore patelina|[82]|Costa Rica.Alajuela|Nymphalidae|
Callicore patelina|[83]|Costa Rica|Nymphalidae|
Callicore astarte patelina|[84]|Costa Rica.Heredia|Nymphalidae|
Callicore astarte patelina|[85]|Costa Rica.Heredia|Nymphalidae|
Callicore astarte patelina|[86]|Costa Rica.Heredia|Nymphalidae|
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Callicore patelina|[92]|Costa Rica.Guanacaste|Nymphalidae|
Callicore patelina|[93]|Costa Rica|Nymphalidae|
Callicore astarte patelina|[94]|Costa Rica.Heredia|Nymphalidae|
Callicore patelina|[95]|Costa Rica.Guanacaste|Nymphalidae|
Callicore astarte patelina|[96]|Costa Rica.Heredia|Nymphalidae|
Callicore sorana|[97]|Brazil.Parana|Nymphalidae|
Callicore sorana|[98]|Brazil.Parana|Nymphalidae|
Callicore sorana|[99]|Brazil.Parana|Nymphalidae|
Unknown Specimen|
DNA barcoding

Distances - disadvantages

- **Distance estimates are only correct if model used is correct**
  - **But that's also the case for ML and BI**
  - **Rate variations in different parts of a tree are intractable for distance measures**
  - **Information on variation in characters is lost once sequence differences are converted to distances**

Distances - disadvantages

- **Generally outperformed by Maximum Likelihood methods in choosing the correct tree in computer simulations**
  - **See e.g. Ogden & Rosenberg (2006) Multiple Sequence Alignment Accuracy and Phylogenetic Inference. Syst. Biol. 55(2): 314–328 (DOI: 10.1080/10635150500541730)**