Lecture 3: Modelling DNA Sequence Evolution

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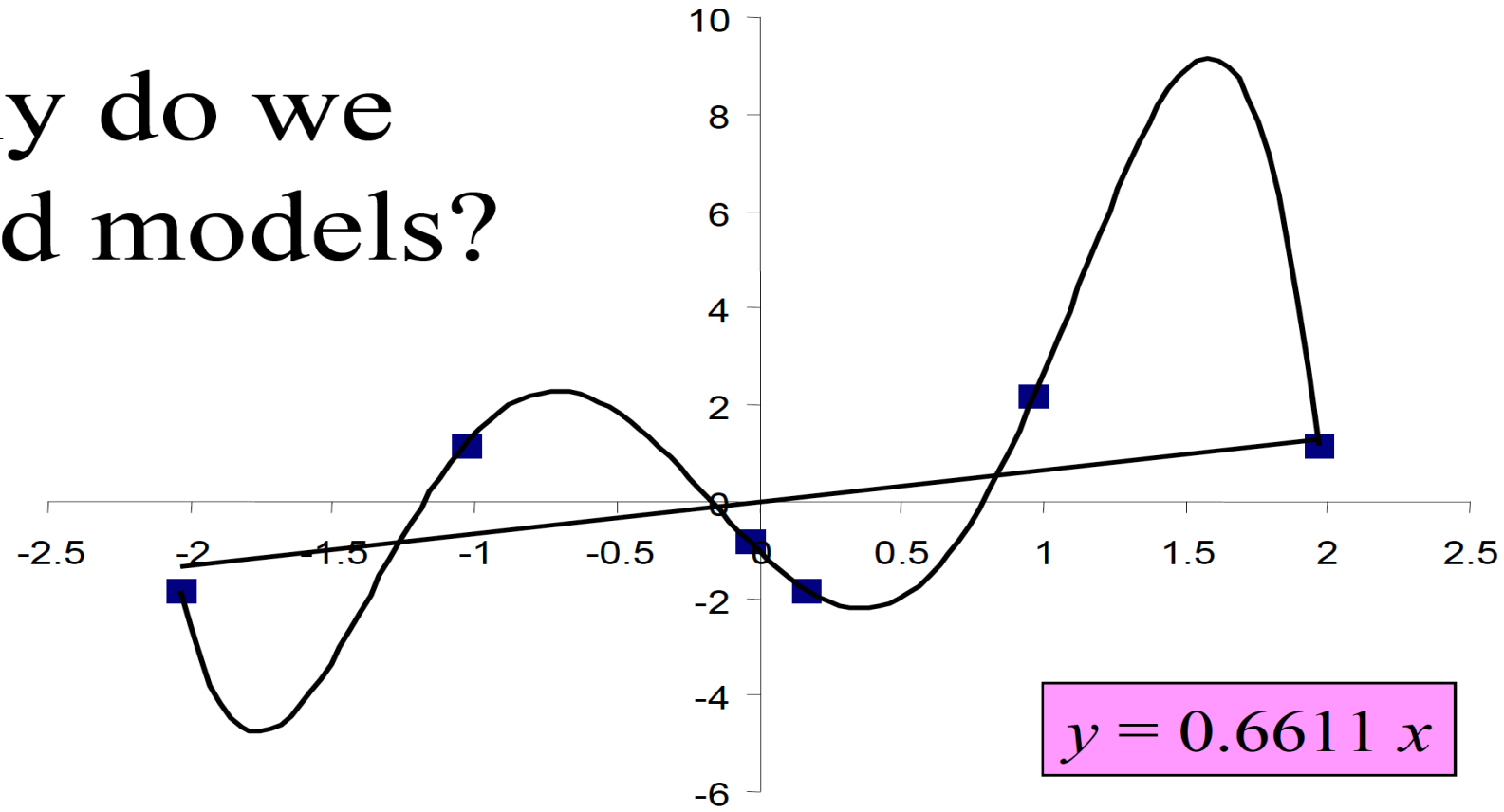
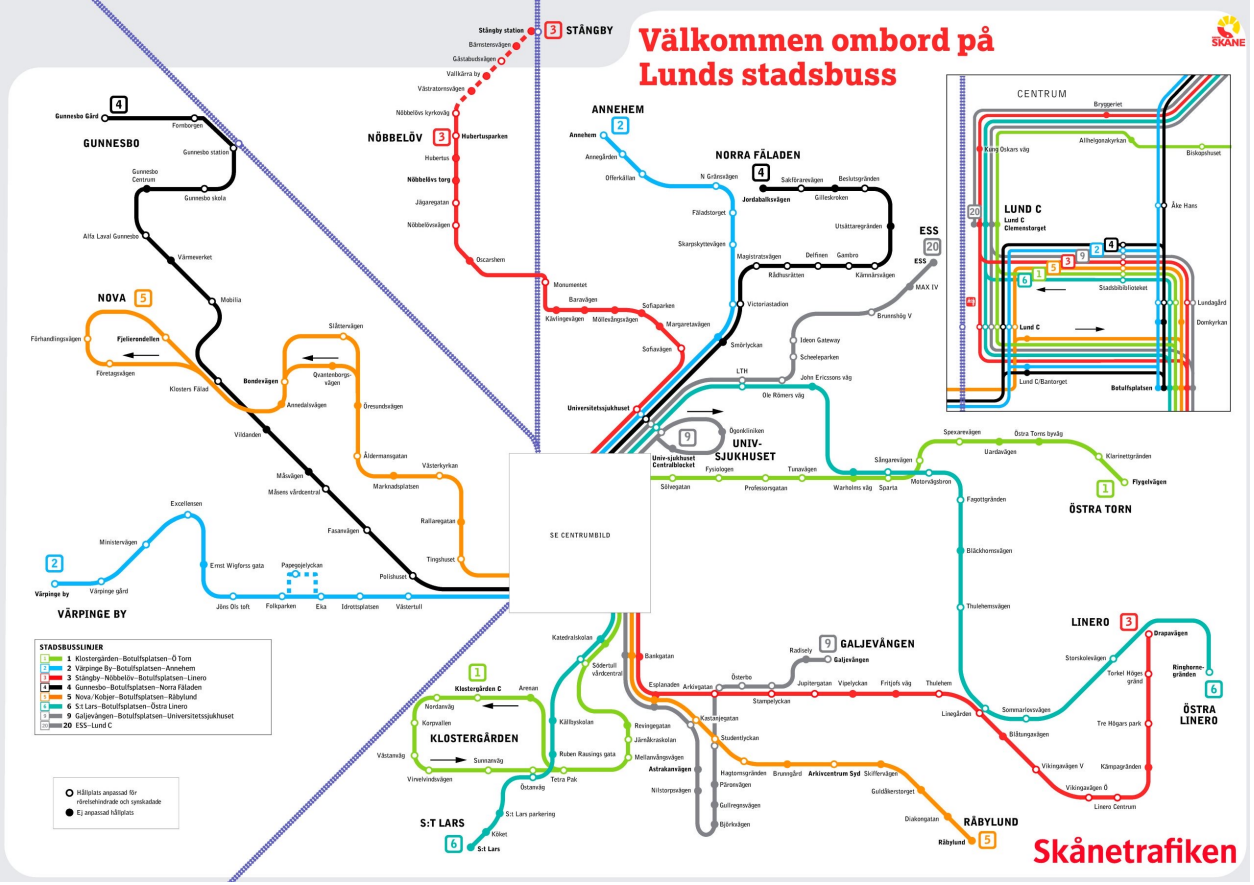
$$y = -1.5972\,x^5 + 23.167\,x^4 - 126.18\,x^3 + 319.17\,x^2 - 369.22\,x + 155.67$$

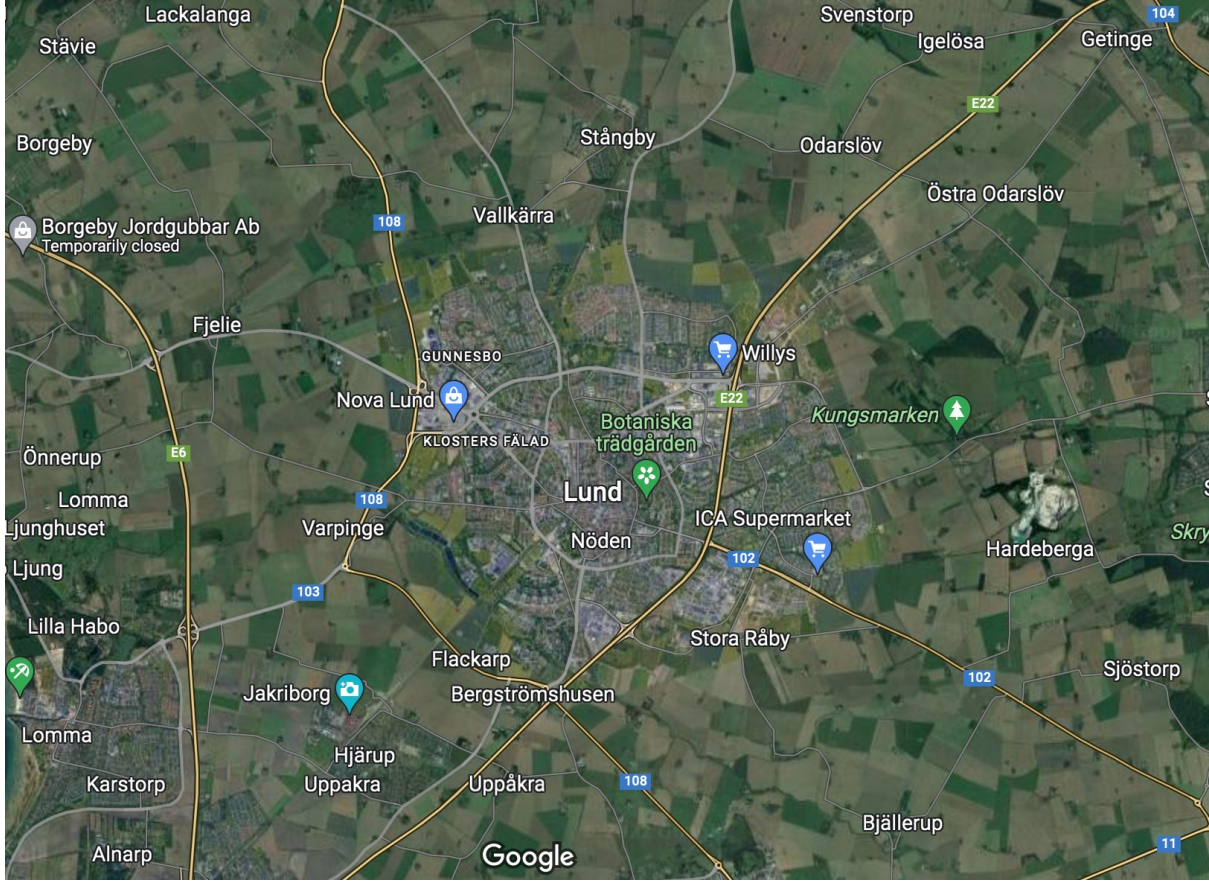
Why do we need models?



$$y = 0.6611\,x$$



A simplified map of bus routes in Lund



A realistic map of Lund

- **Which one would you use to get around Lund by bus?**

Models: an overview

- **In general, models help us predict the future based on our observations**
- **With more parameters, models have a better fit to the data (observations)**
- **Underparamaterized models: poor fit to the observed data**
- **Overparameterized models: poor prediction of future observations**
- **Choosing best models based on different criteria**
  - **Likelihood ratio tests, AIC, BIC, Bayes factors**

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**

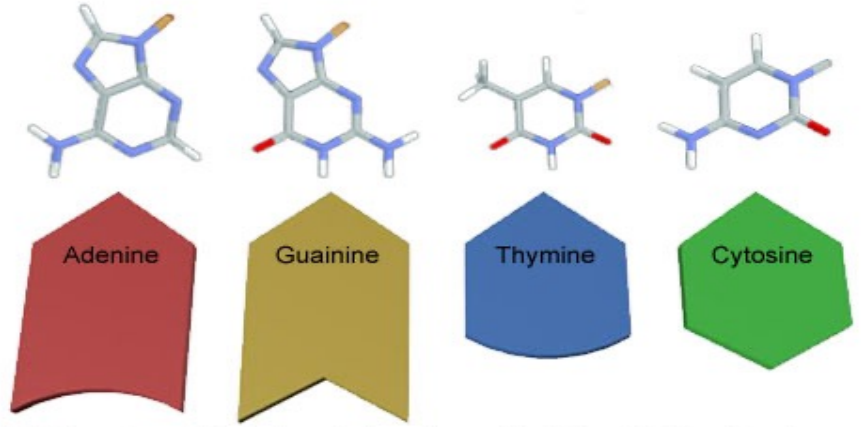
What do we model in DNA sequence evolution?

- **Nucleotide substitutions**
  - **The rate at which each nucleotide is replaced by each alternative nucleotide**

What is the challenge?

- **DNA has only four characters**

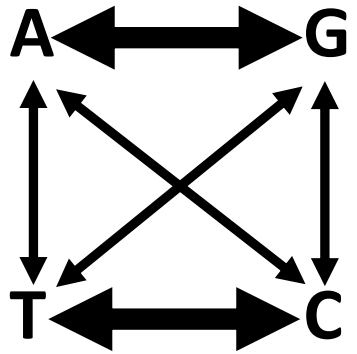
Figure B-3: The Four Nitrogenous Bases



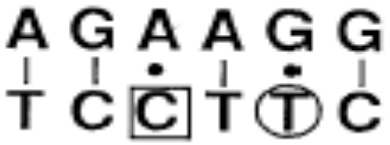
Each base has a distinct shape that can be used to distinguish it form the others. 3D representations of the four bases are shown, with the corresponding chemical structures drawn above.

Substitution types

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



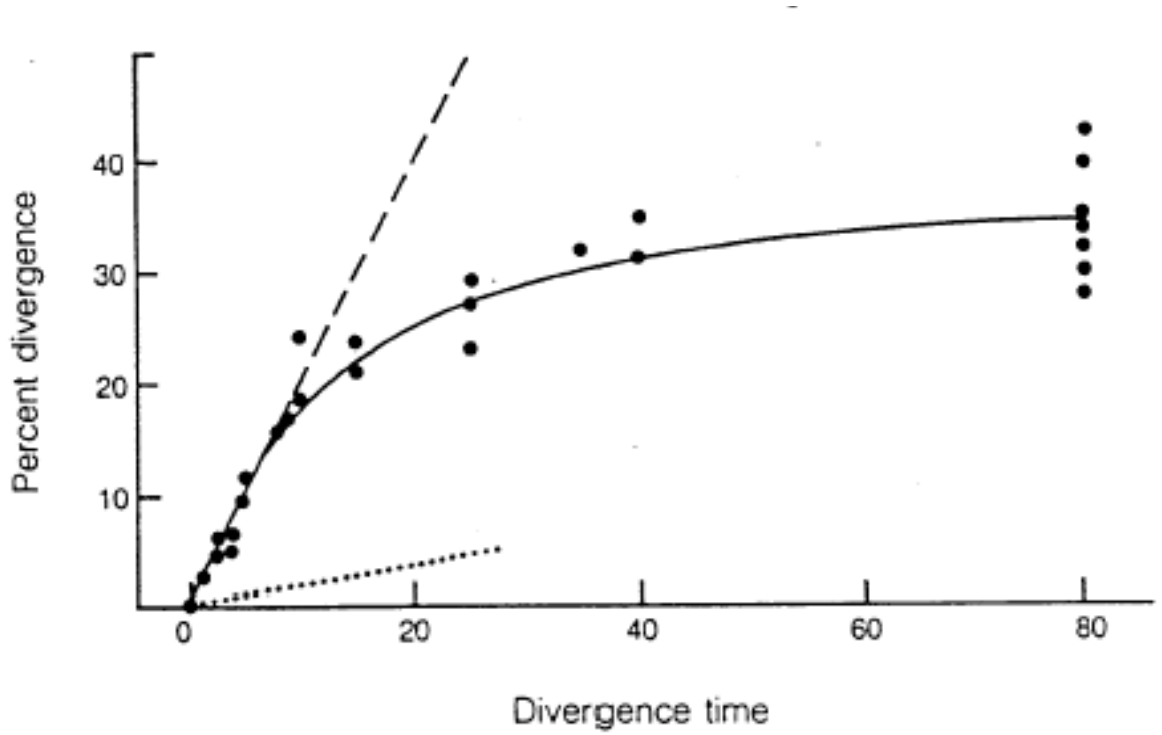
Pur - Pyr mispairs lead to transitions



In next round of replication

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

Slide by Chris Simon 2005

Types of Substitutions

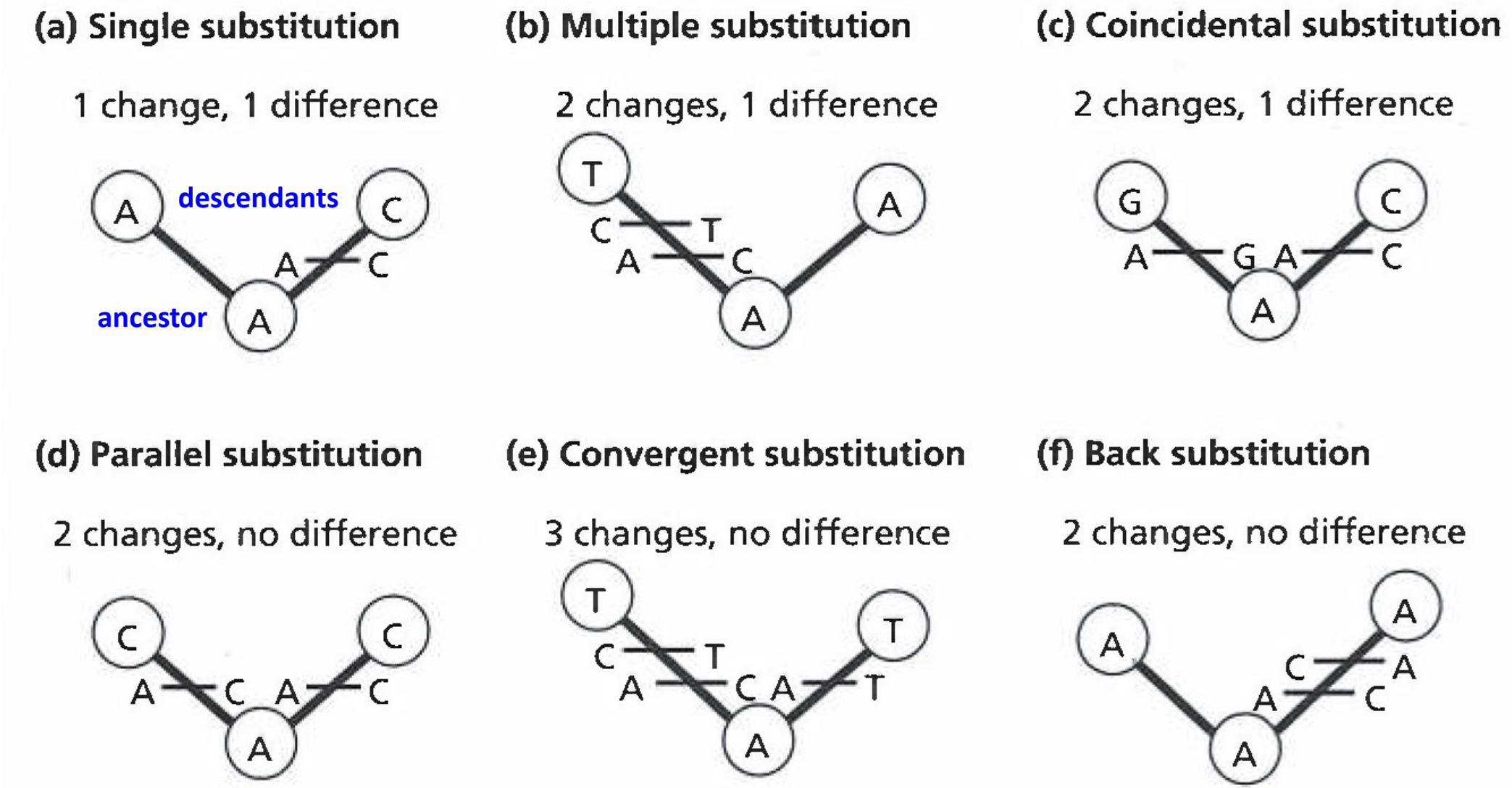
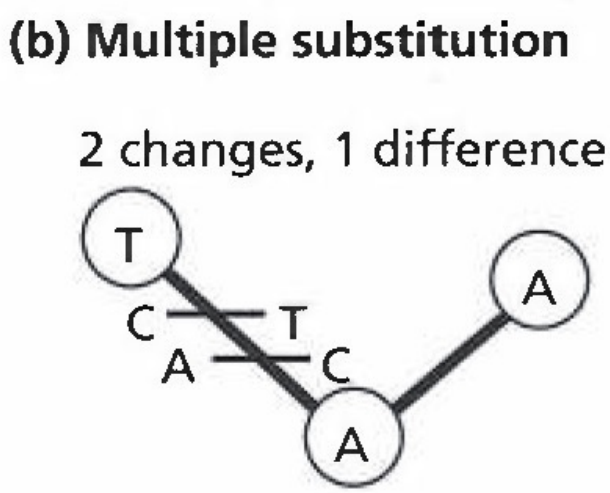


Fig. 5.9. Page, R. and E. Holmes. 1998. Molecular Evolution: A phylogenetic Approach. Blackwell.

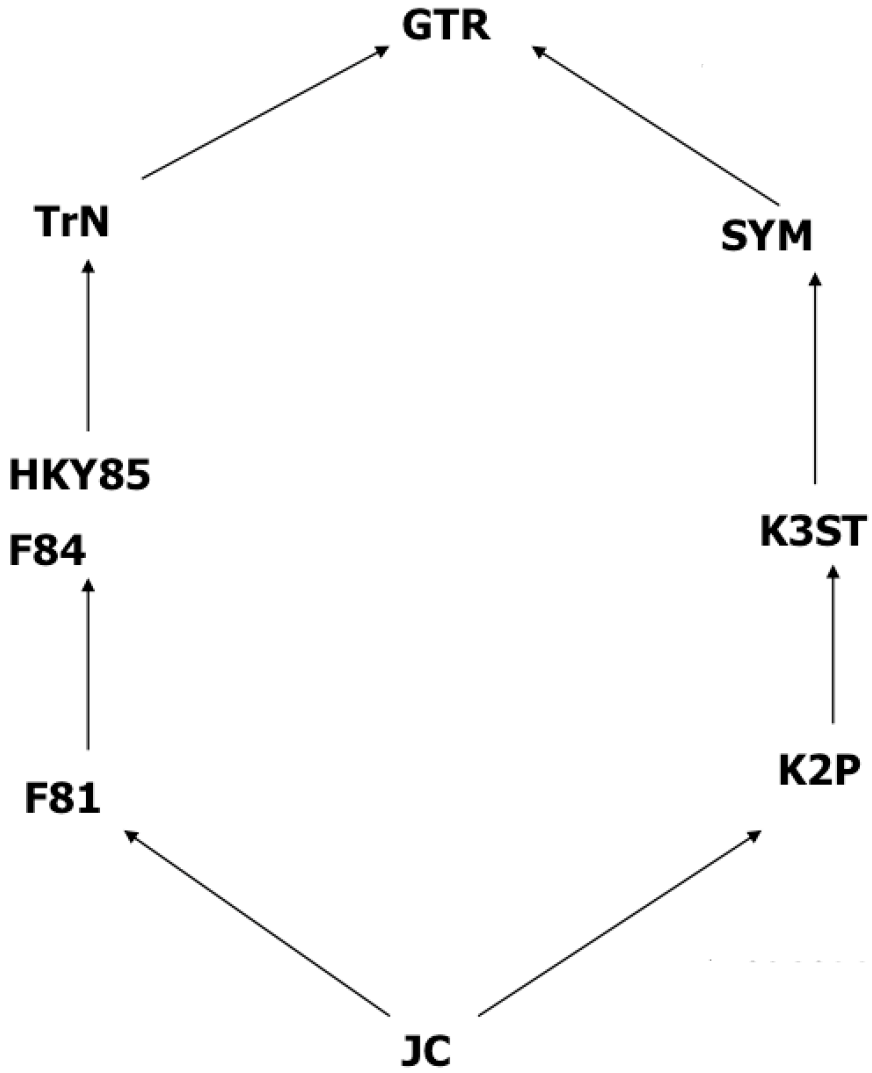
Saturation in sequence data

- **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**



Saturation in sequence data (cont.)

- **In severe cases the data can become random and all information about relationships can be lost**
- **Probabilistic models of sequence evolution are used to calculate expected distances**



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)**

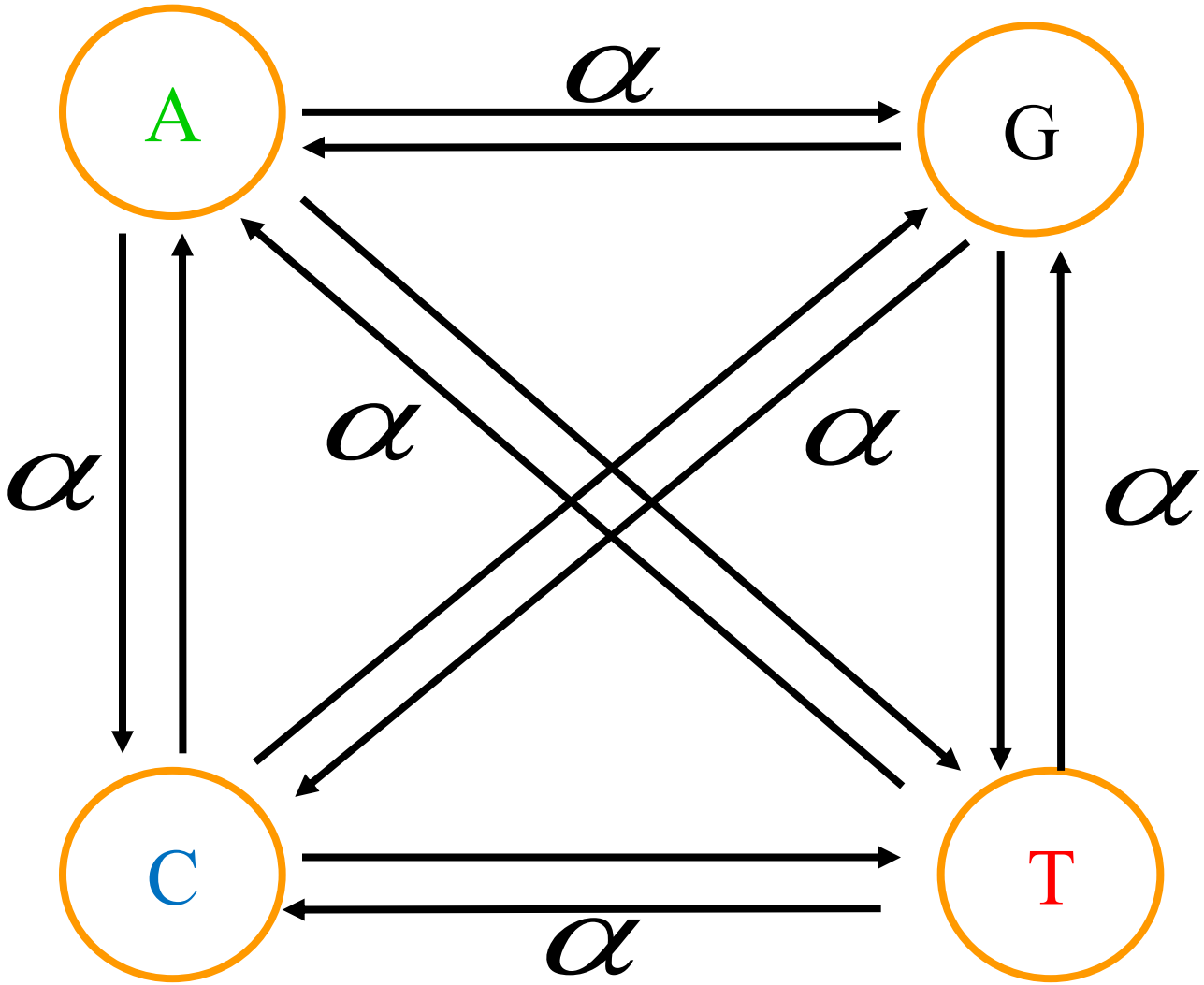
Corrections for multiple substitutions:
First DNA subtitution model

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

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 of 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 of α

Improvements on Jukes-Cantor

- **Allow base frequencies to be unequal to accommodate e.g. sequences such as these**

  AAACCTGGATTTACCGAGATTTAAGCGATATATTGCAATGC

  34% A 17% C

  29% T 20% G

- **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**

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

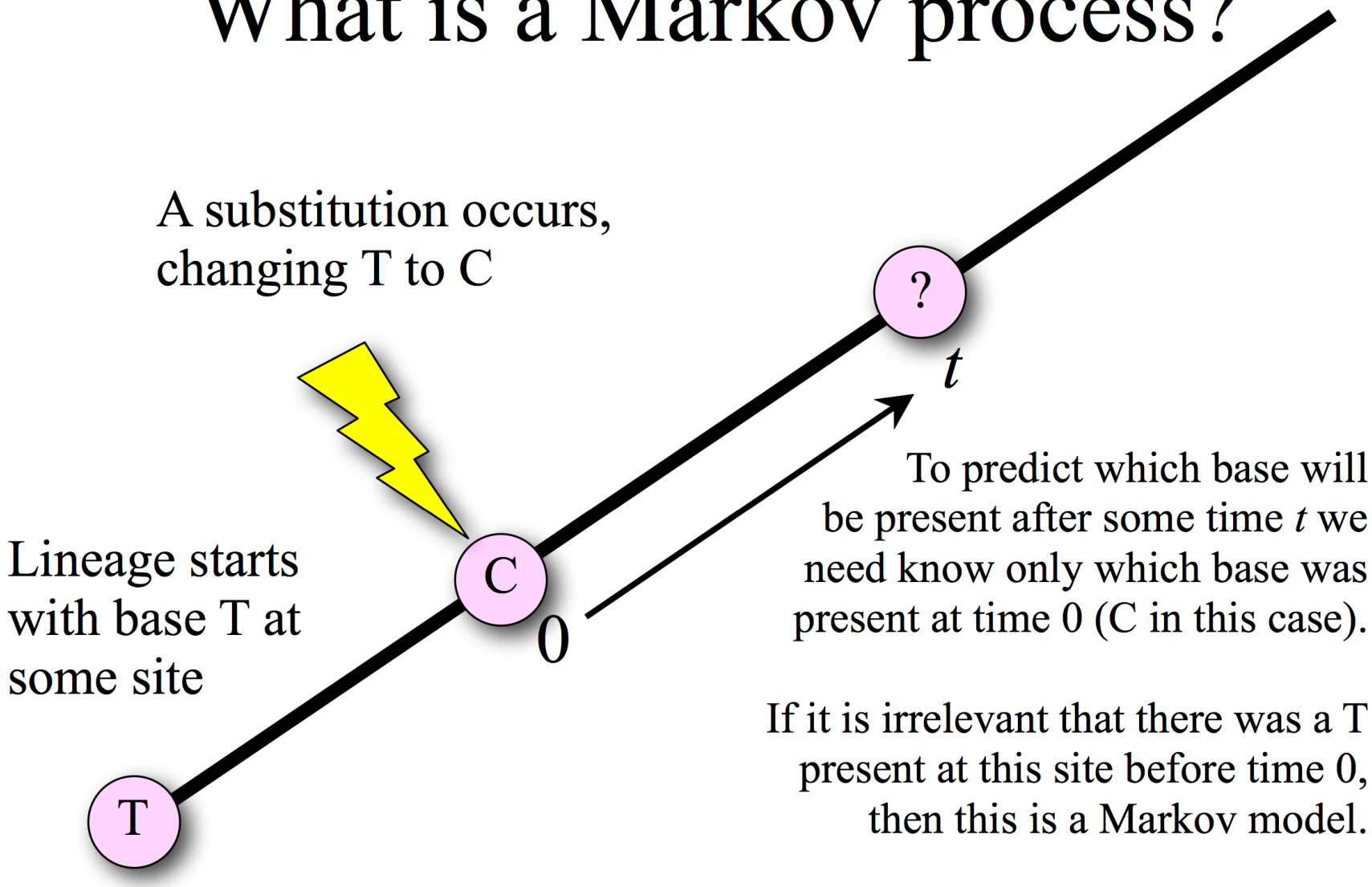
Parameters we are interested in

- **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?



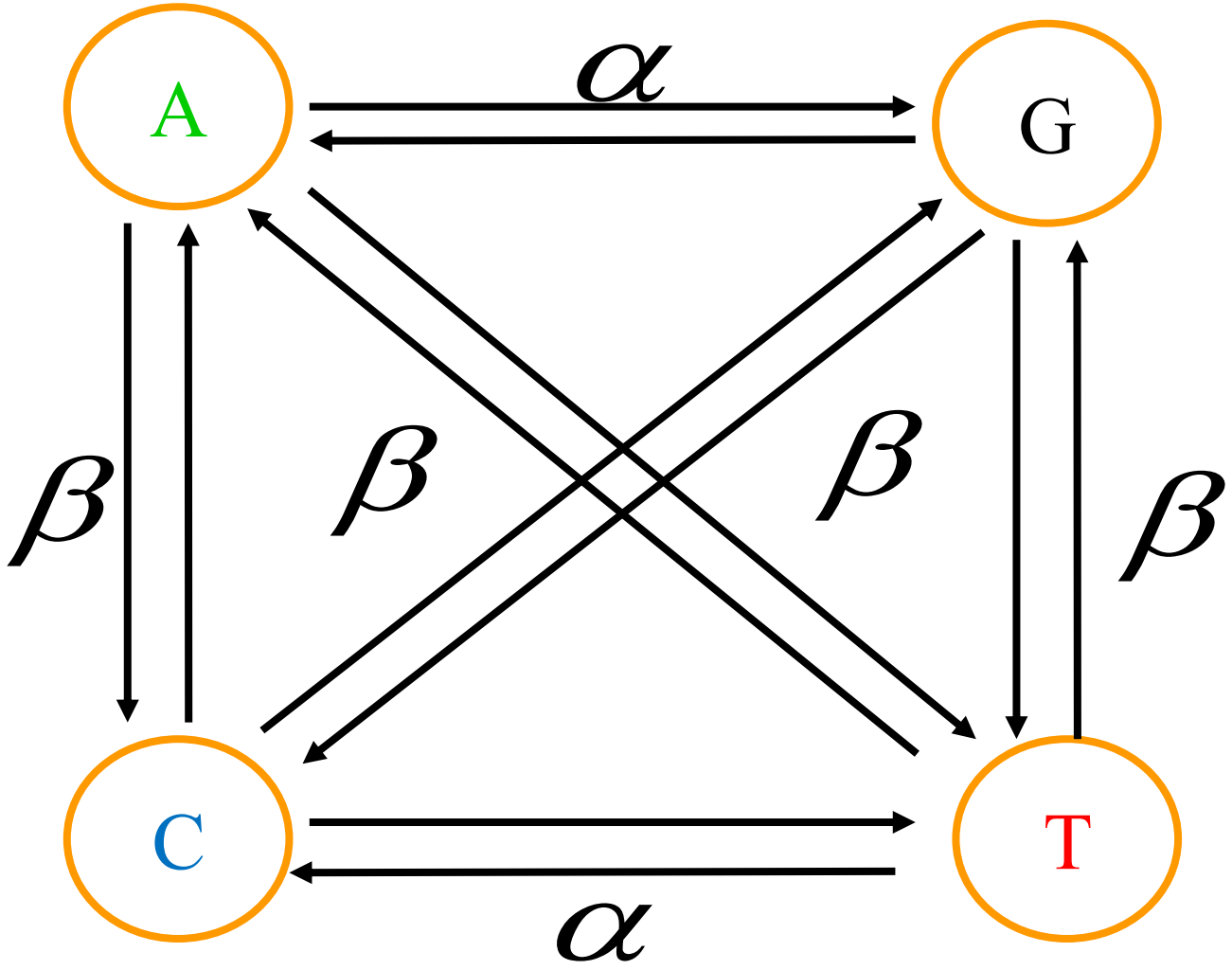
To predict which base will be present after some time *t* we need know only which base was present at time 0 (C in this case).

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



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

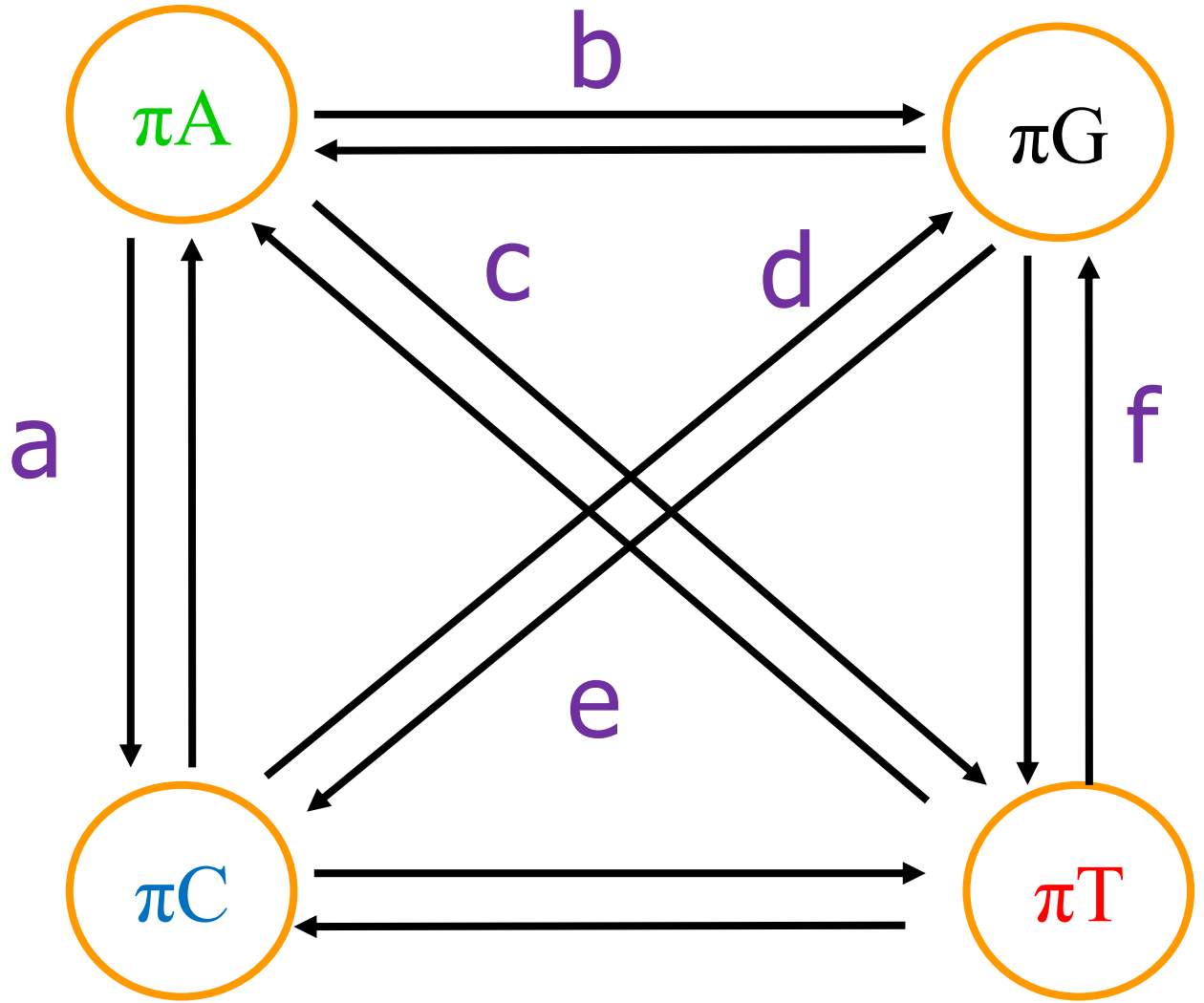
The Hasegawa-Kishino-Yano model (1985)

	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 (1986)



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	$\pi_A c$	$\pi_C e$	$\pi_G f$	—

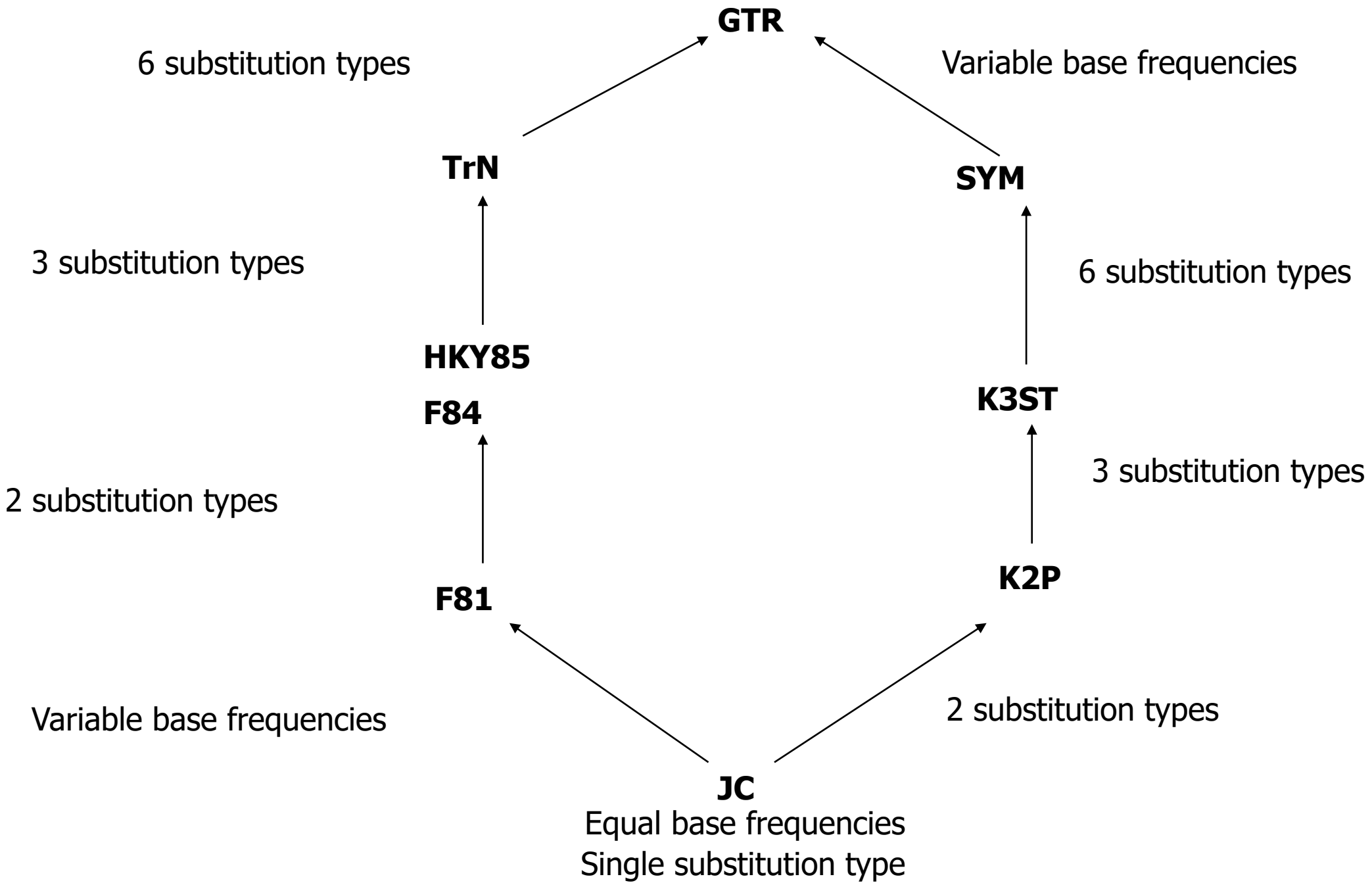
GTR model:

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

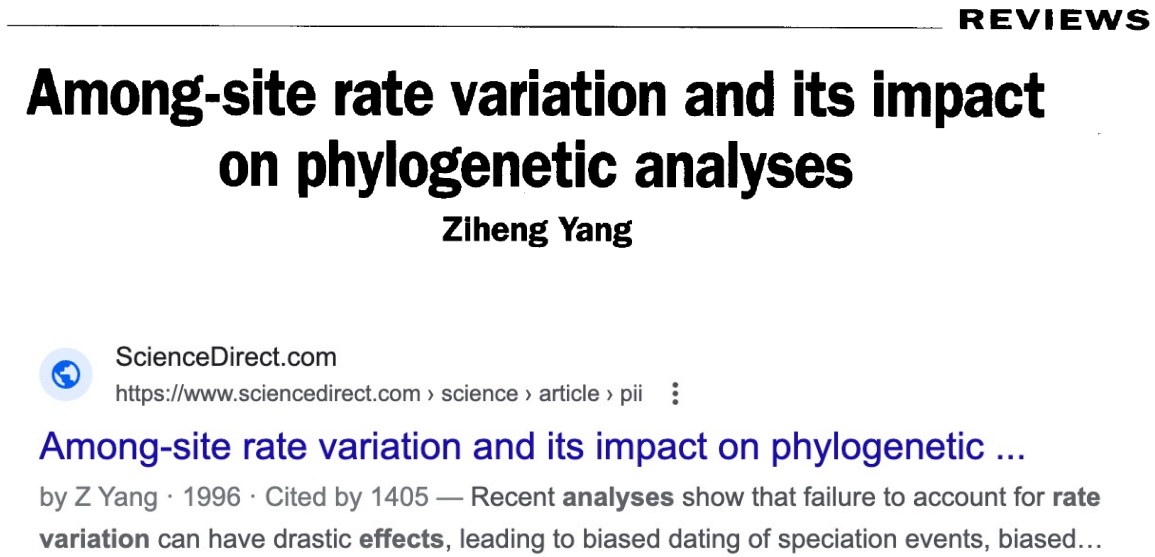

GTR
6 substitution types
Variable base frequencies
TrN
SYM
3 substitution types
6 substitution types
HKY85
K3ST
F84
3 substitution types
2 substitution types
K2P
F81
Variable base frequencies
2 substitution types
JC
Equal base frequencies
Single substitution type

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)

- **All of the models so far assume that the rate of change is the same for every position in the alignment**
- **Variable vs. 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**


REVIEWS

Among-site rate variation and its impact on phylogenetic analyses

Ziheng Yang

ScienceDirect.com
https://www.sciencedirect.com › science › article › pii

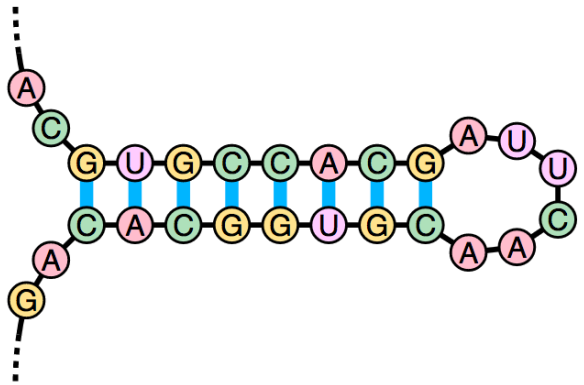
Among-site rate variation and its impact on phylogenetic ...

by Z Yang · 1996 · Cited by 1405 — Recent **analyses** show that failure to account for **rate variation** can have drastic **effects**, leading to biased dating of speciation events, biased…

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

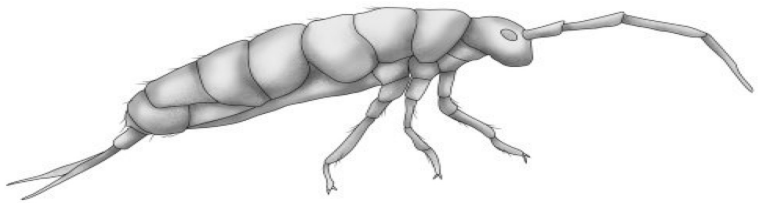
56.7% of all variable sites are located in third positions

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.



Slide by Chris Simon 2005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Courier New
11
55 total sequences
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Mode: Edit
Overwrite
Selection: 0
Position: 341
Sequence Mask: None
Numbering Mask: None
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310 320 330 340 350 360 370 380 390 400
Libythea71 1
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
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Danaus108 21
Dichorragia1
Doleschallia
Doxocopa lau
Dynamine115
Eresia92 5
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Euploea70 8
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Gnathotric89
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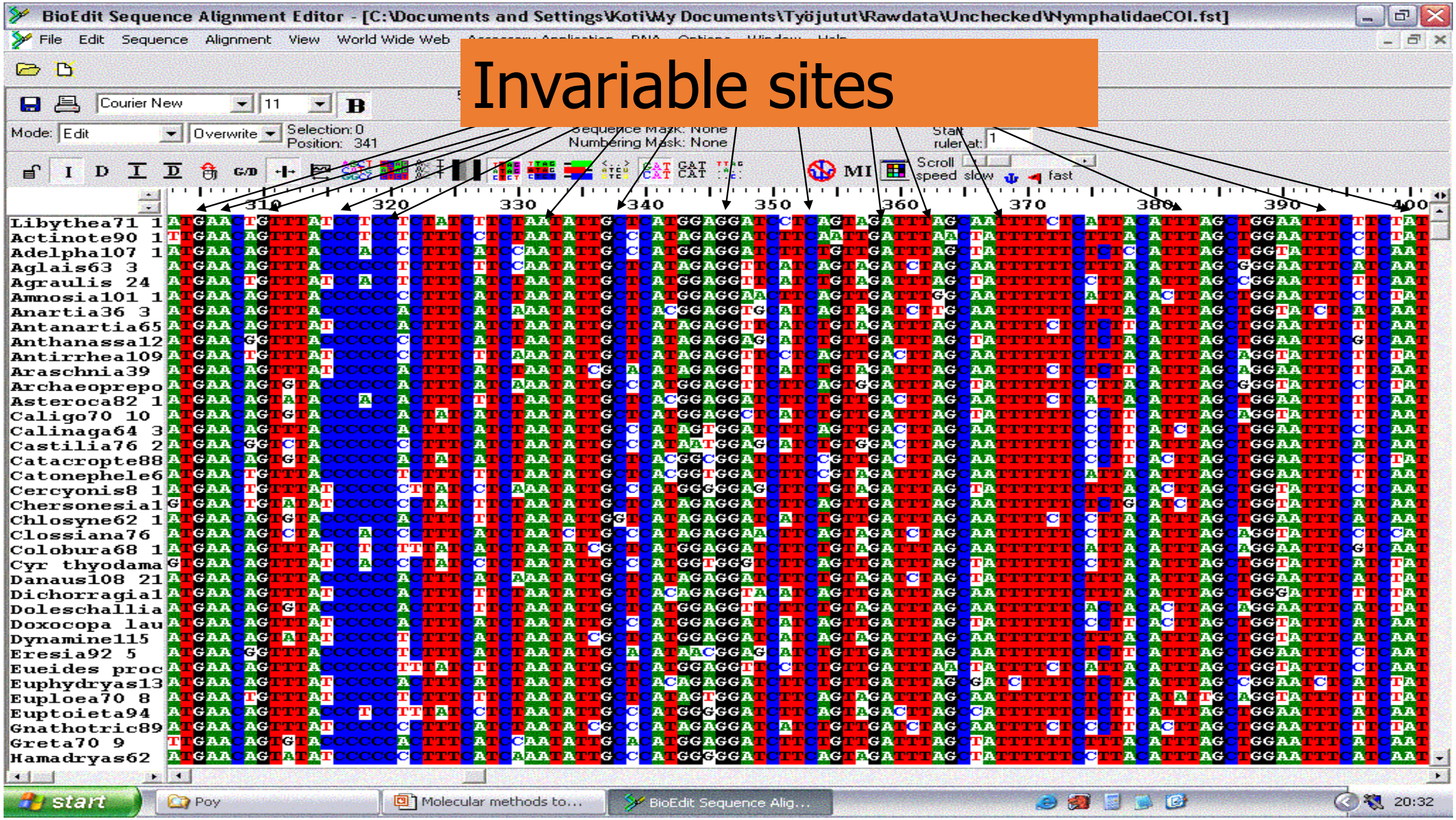
Invariable sites
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File Edit Sequence Alignment View World Wide Web
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11
Mode: Edit
Overwrite
Selection: 0
Position: 341
Sequence Mask: None
Numbering Mask: None
Scroll speed slow fast
310 320 330 340 350 360 370 380 390 400
Libythea71 1
Actinote90 1
Adelpha107 1
Aglais63 3
Agraulis 24
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Antanartia65
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Antirrhea109
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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
Eueides proc
Euphydryas13
Euploea70 8
Euptoieta94
Gnathotric89
Greta70 9
Hamadryas62
start
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Molecular methods to...
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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 among-site rate variation with Gamma distribution

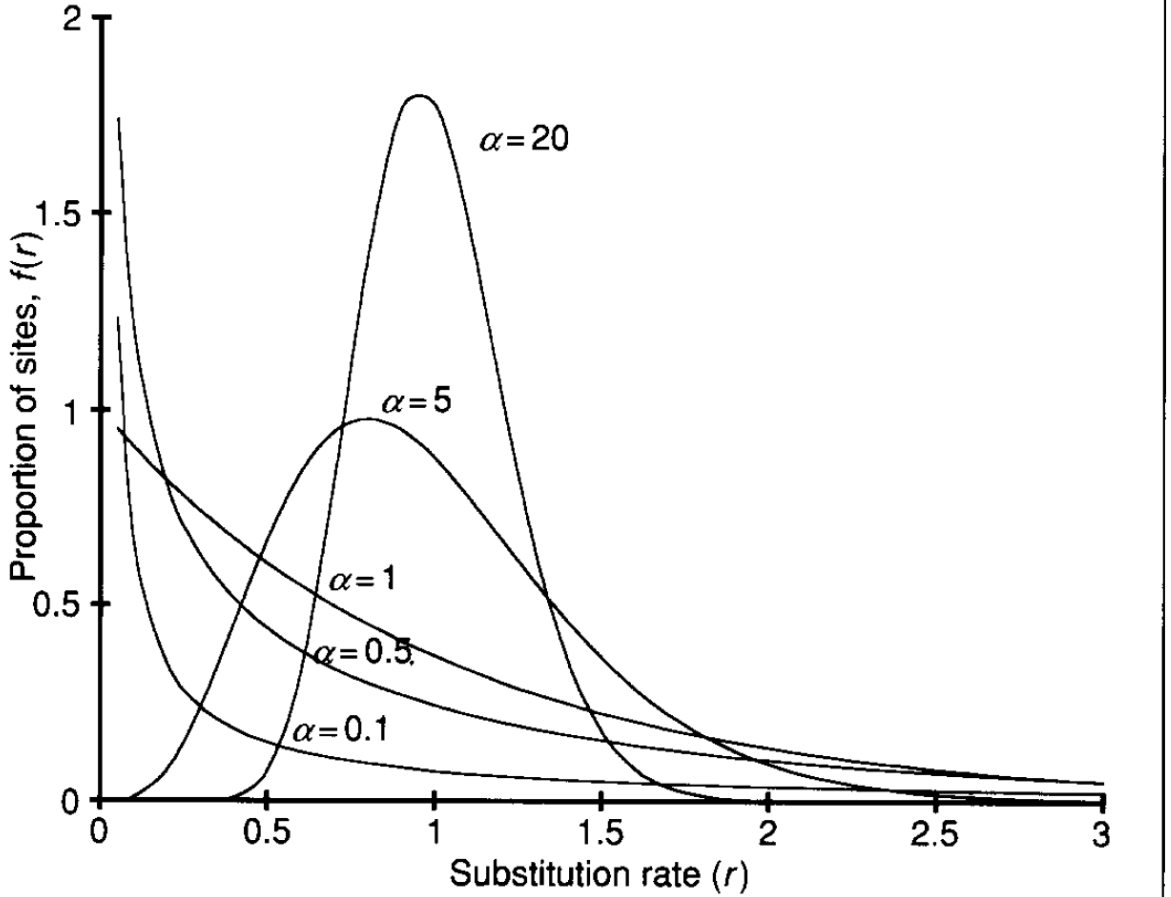


Fig. 1. The density function, $f(r)$, of the gamma distribution of substitution rates at sites (r). The gamma distribution has a shape parameter α and a scale parameter β, with mean α/β and variance α/β^2. Since the rate is a proportional factor,

Gamma distribution:

Relative substitution rates for different α values

Fig. 1 from Yang 1996:

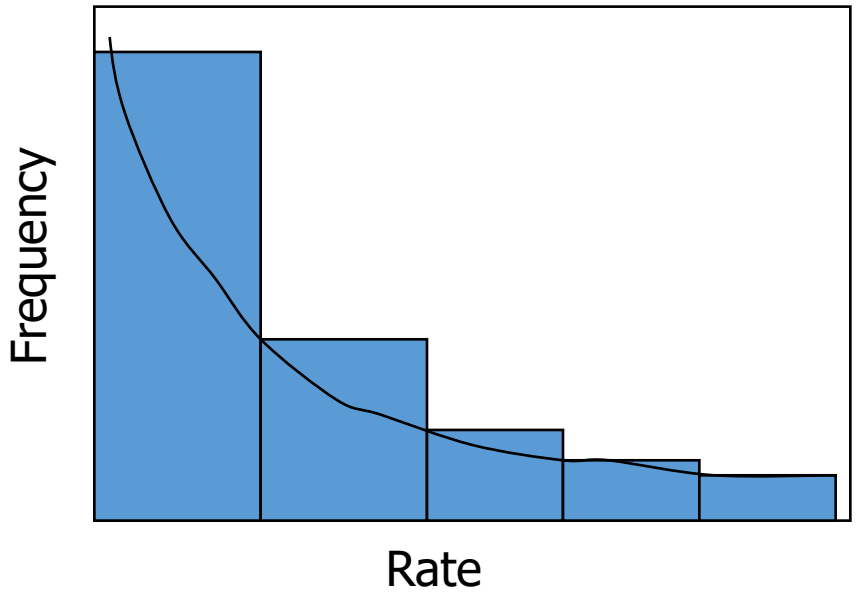
Alpha – the shape parameter of the gamma distribution

Smaller alpha = higher ASRV

Yang (1996) TREE 11(9): 367–372

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**

Slide by Chris Simon 2005

Modelling ASRV leads to greater improvement in fit than other parameters

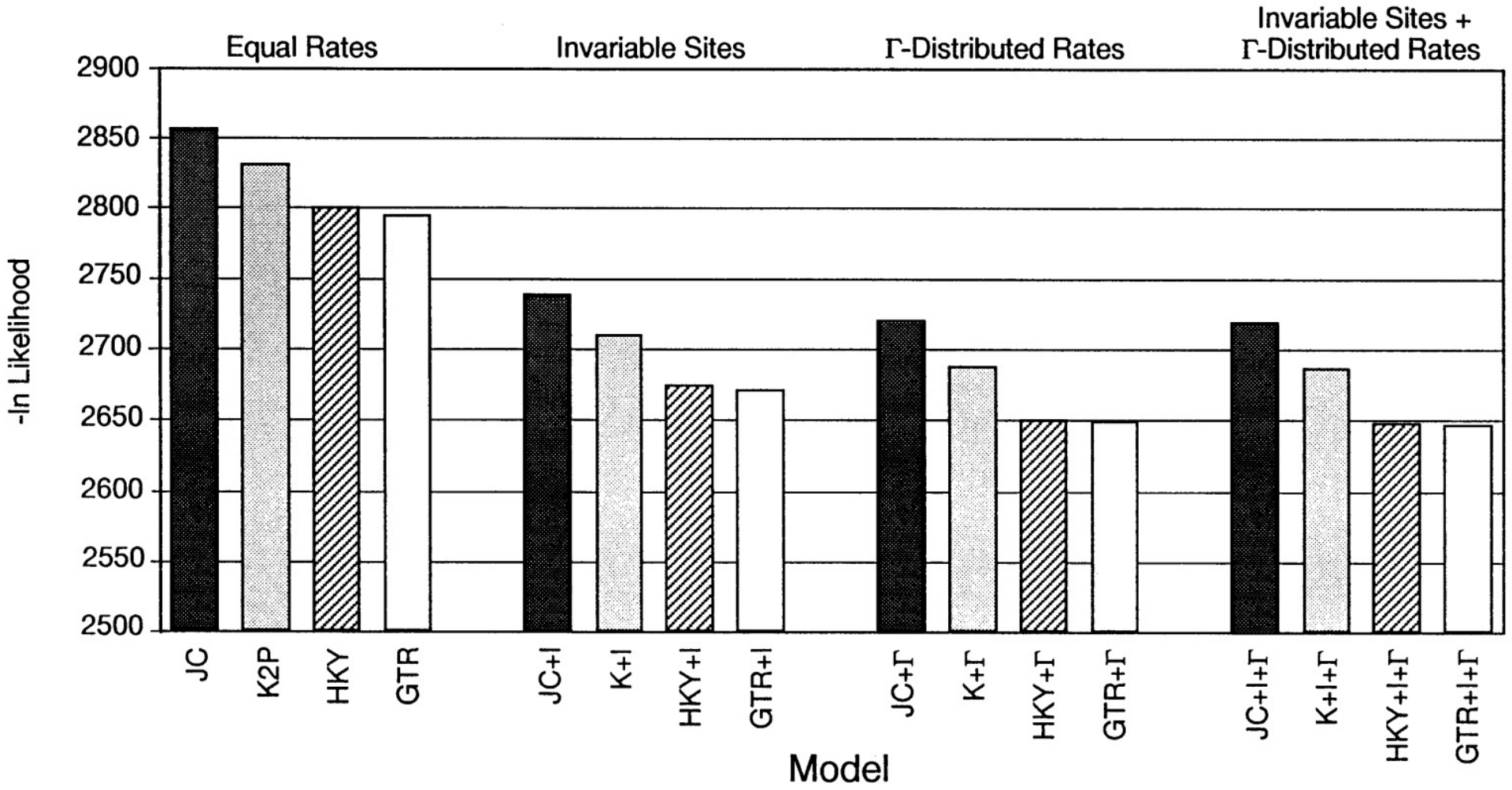


Fig. 4 from Frati et al. 1997. J. Mol. Evol. 44:145-158

Modelling ASRV leads to greater improvement in fit than other parameters

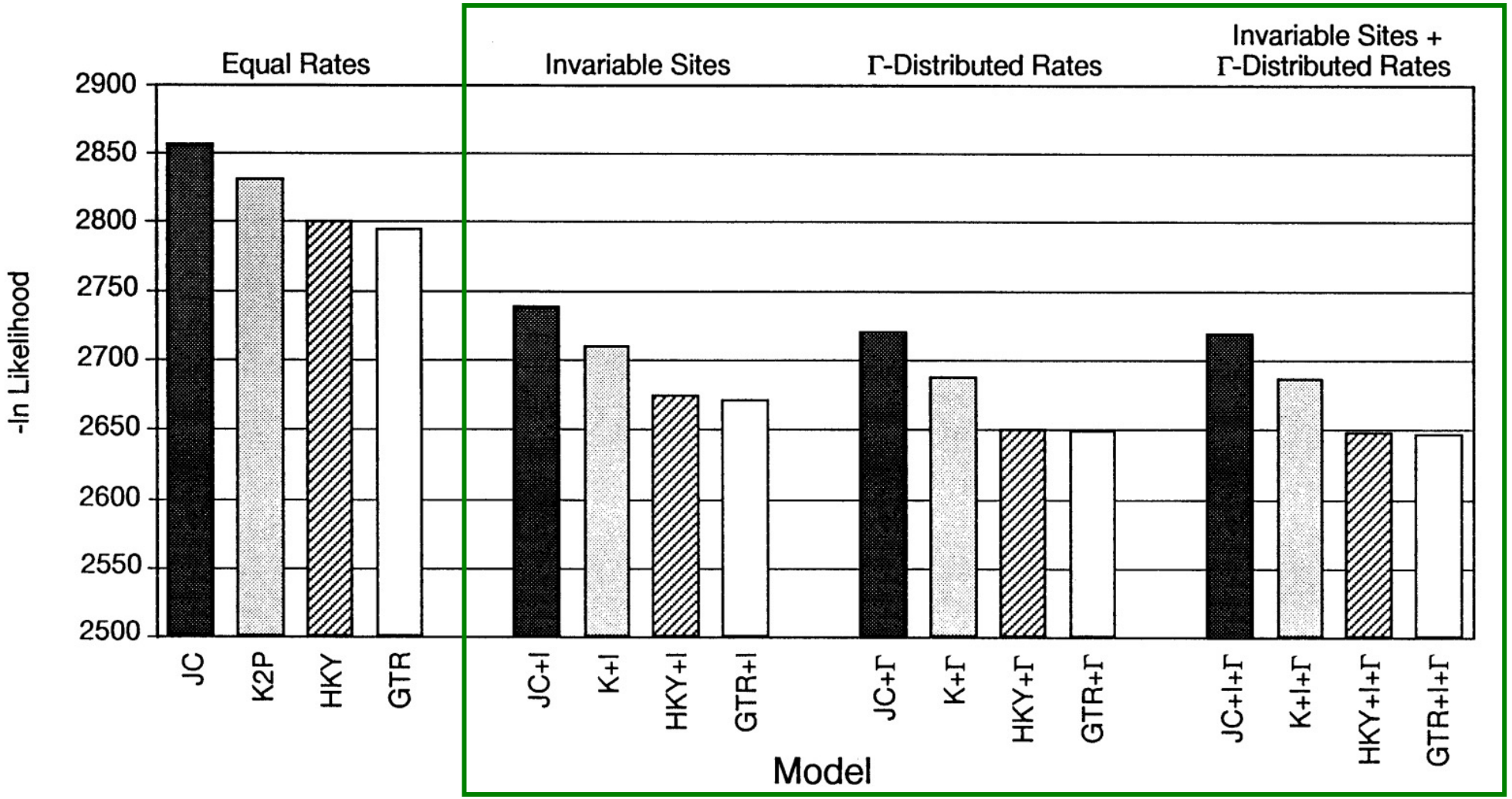


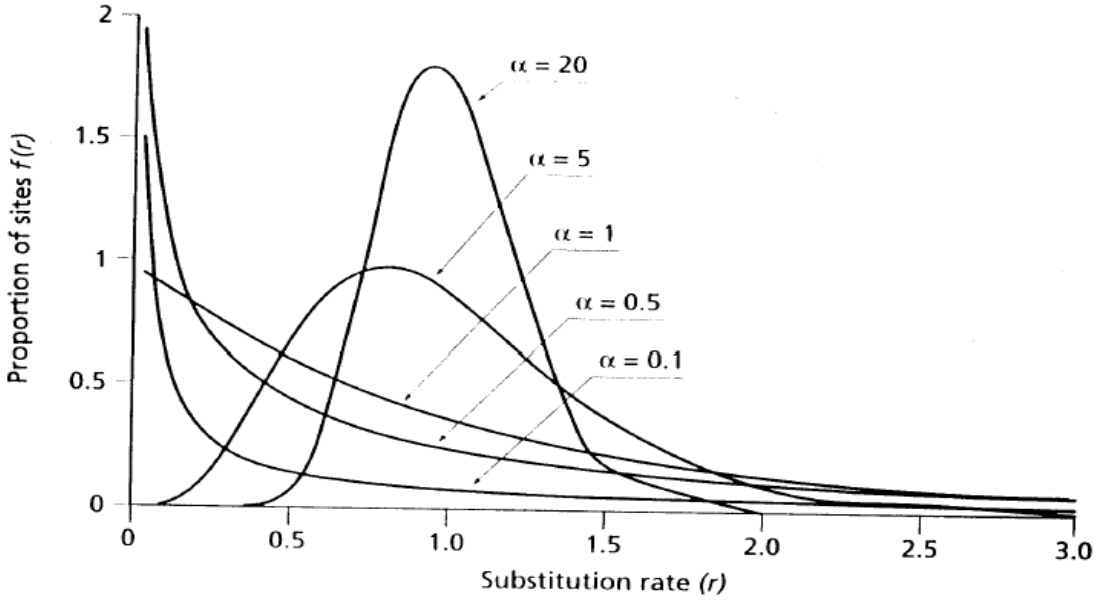
Fig. 4 from Frati et al. 1997. J. Mol. Evol. 44:145-158

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

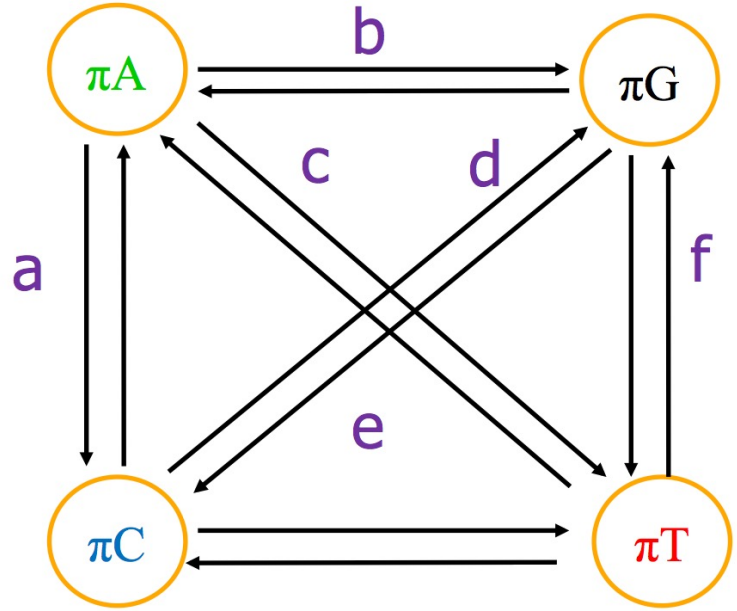
- **Gamma distribution is always unimodal**
  - **Not necessarily the case in our dataset!**
- **Flexible rate heterogeneity across sites model**
  - **Probability distribution free model so that you can find the distribution that fits your data (FreeRate Model)**
  - **Implemented in IQ-TREE**

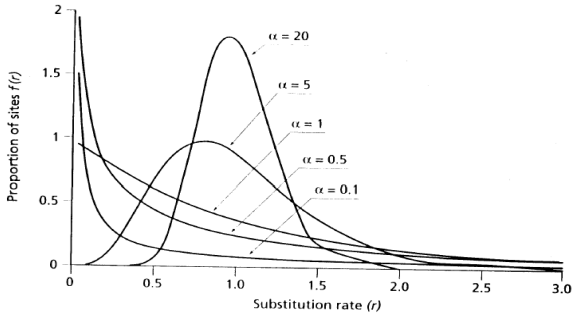


Kalyaanamoorthy et al. 2017 (Nature Methods)
doi:10.1038/nmeth.4285

Parameters in models of DNA evolution

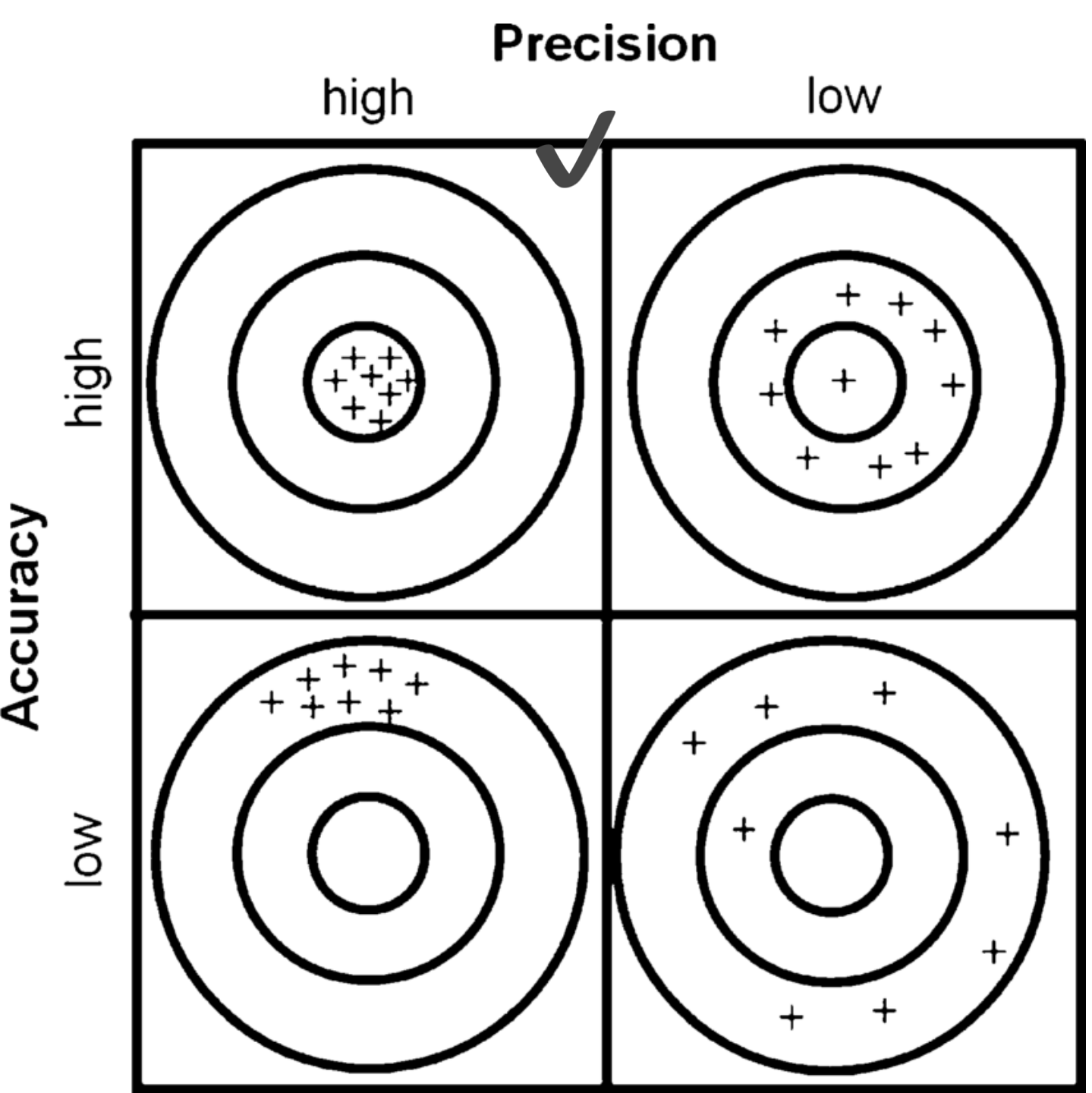
- **Numbers of parameters estimated:**
  - **Base composition**
    - **1 fixed, 3 estimated**
  - **Substitutions**
    - **up to 5; 1 fixed, 5 estimated**
  - **Among-site-rate variation**
    - **Gamma shape parameter = 1 parameter**
    - **Invariant sites = 1 parameter**
    - **Gamma + I = 2 parameters**



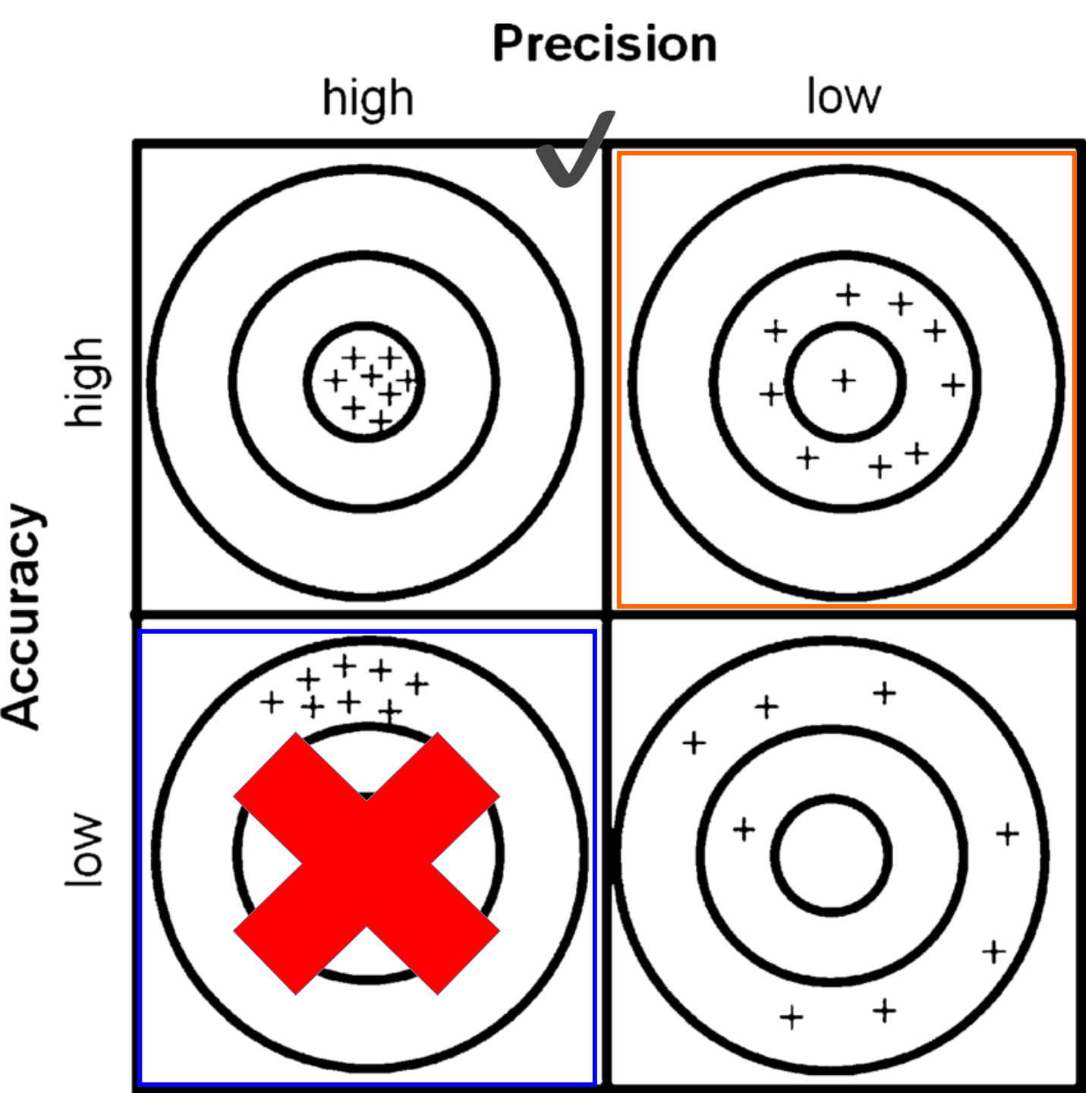


Models can be made more parameter rich to increase their realism

- **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**



Target analogy: Trade-off between highly parameterized models & model error variance



Target analogy: Trade-off between highly parameterized models & model error variance

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

In summary, 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**
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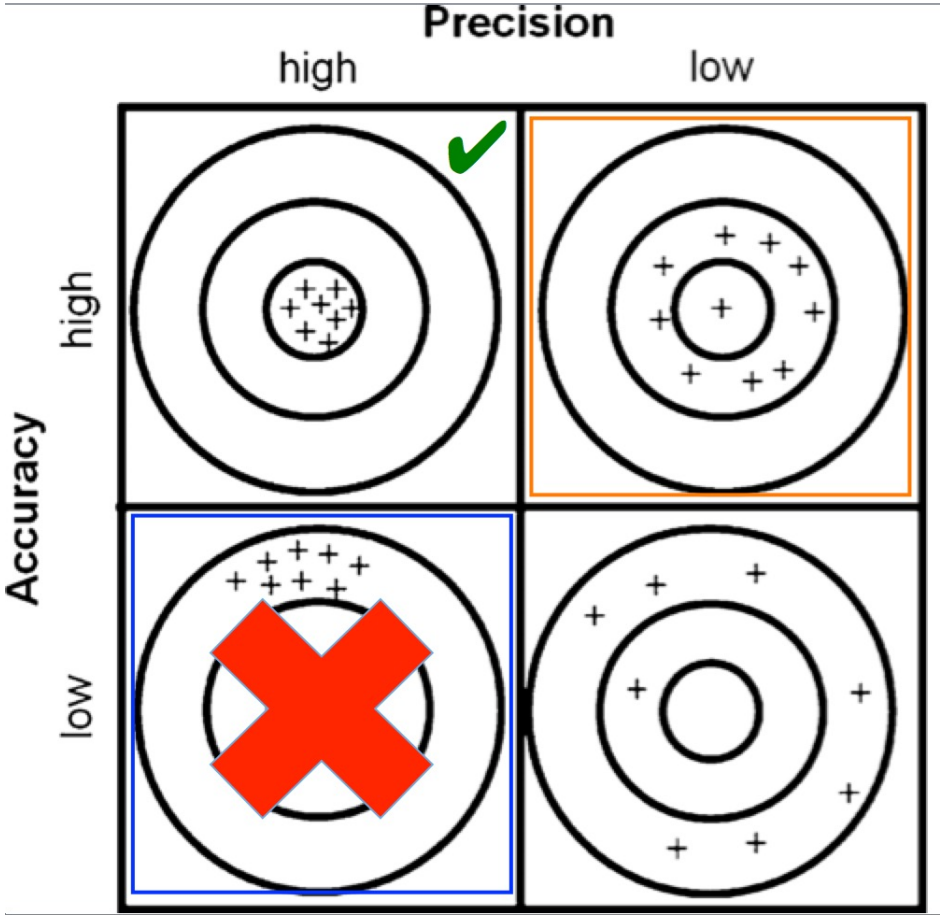
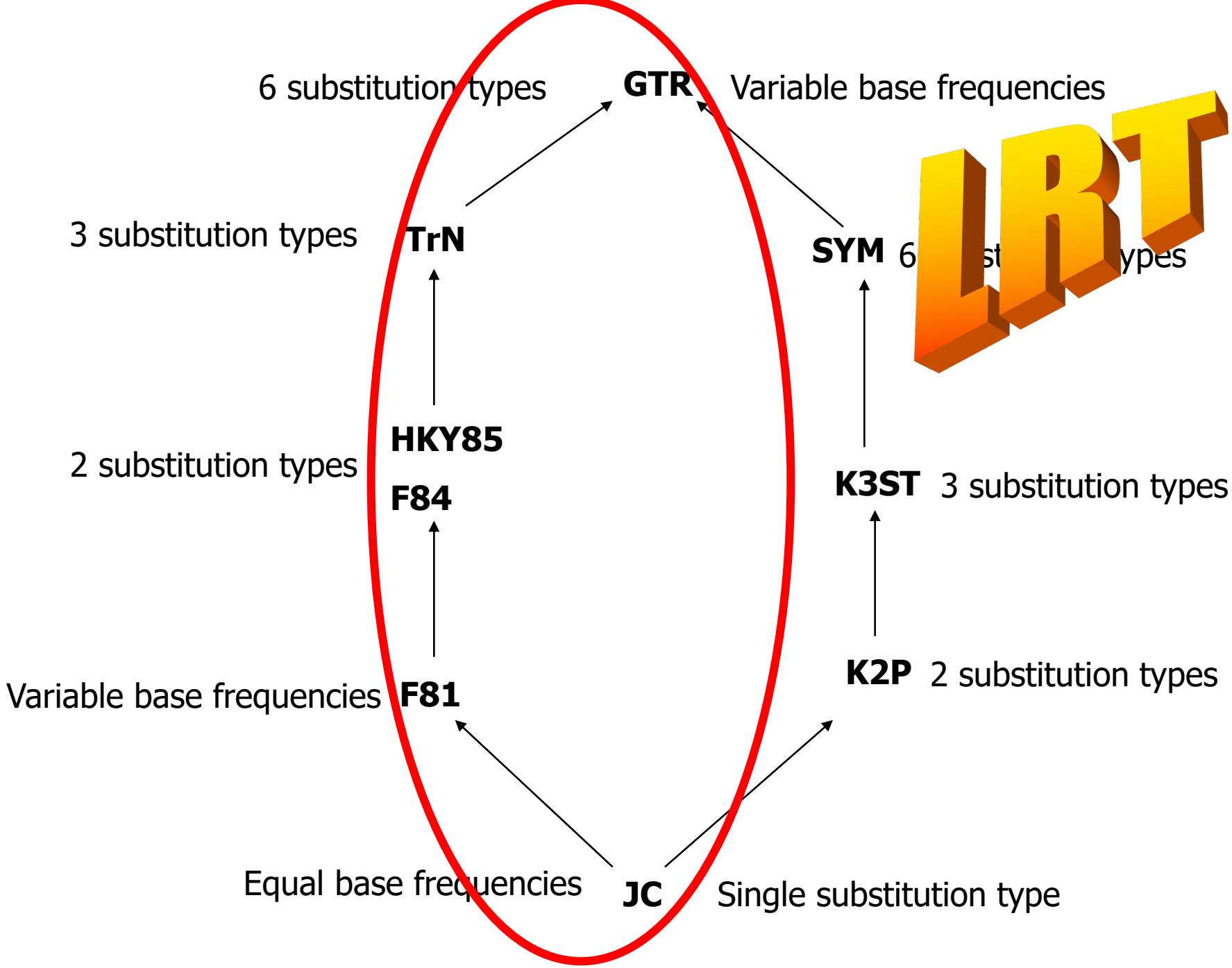


Choosing between models

- **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)**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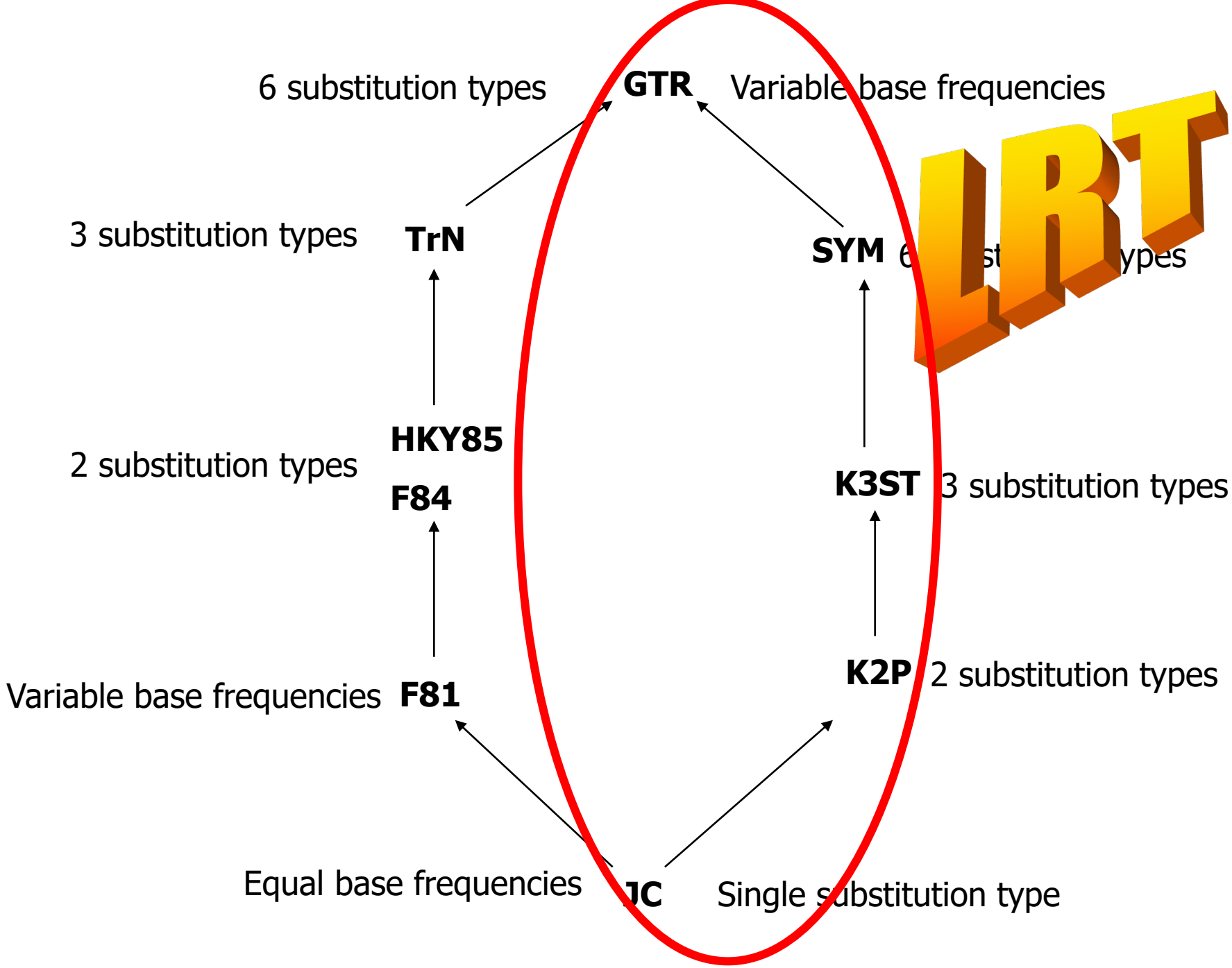
GTR
Variable base frequencies
3 substitution types
TrN
SYM
LRT
HKY85
2 substitution types
F84
K3ST
3 substitution types
K2P
2 substitution types
Variable base frequencies
F81
Equal base frequencies
JC
Single substitution type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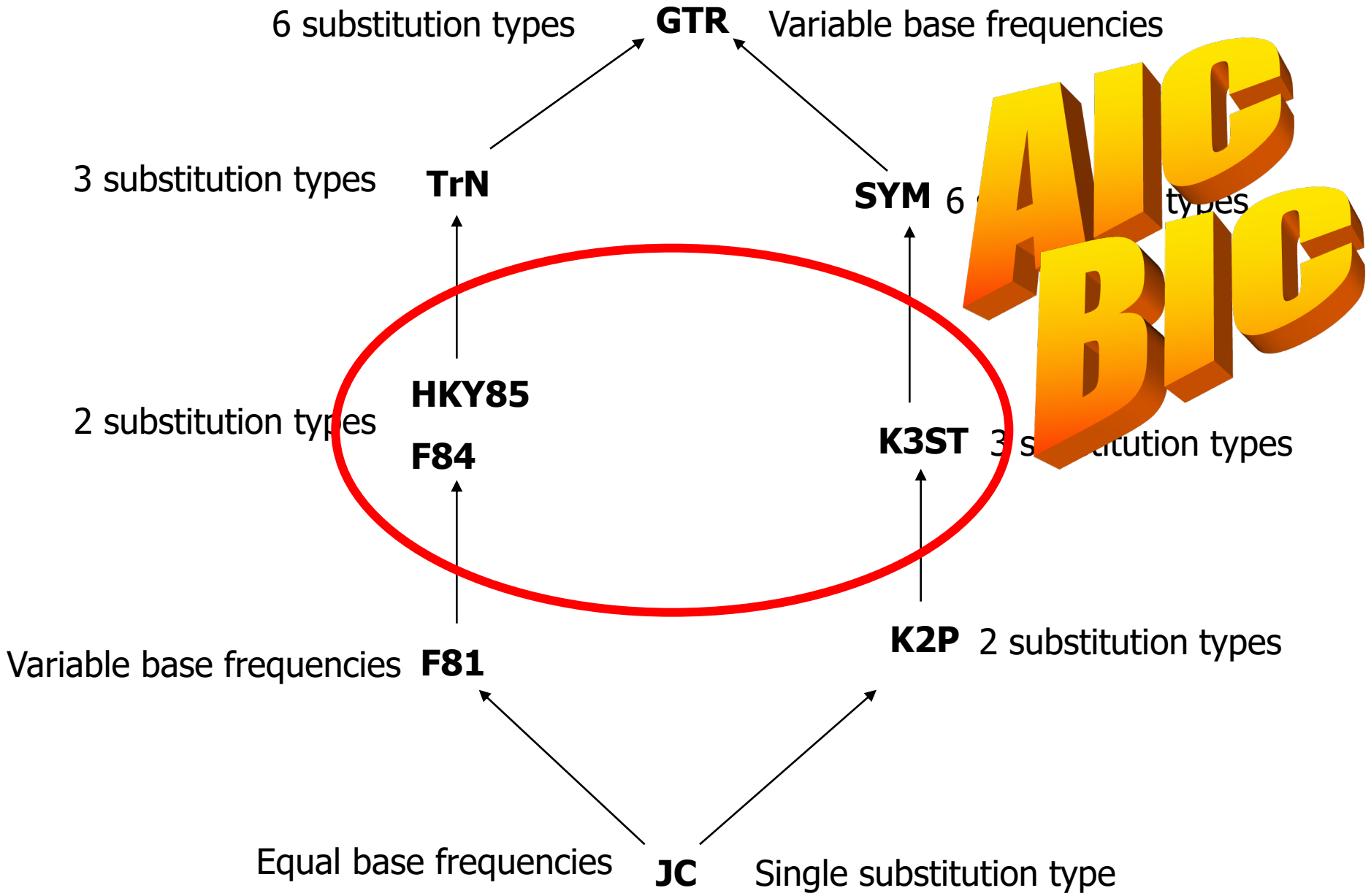
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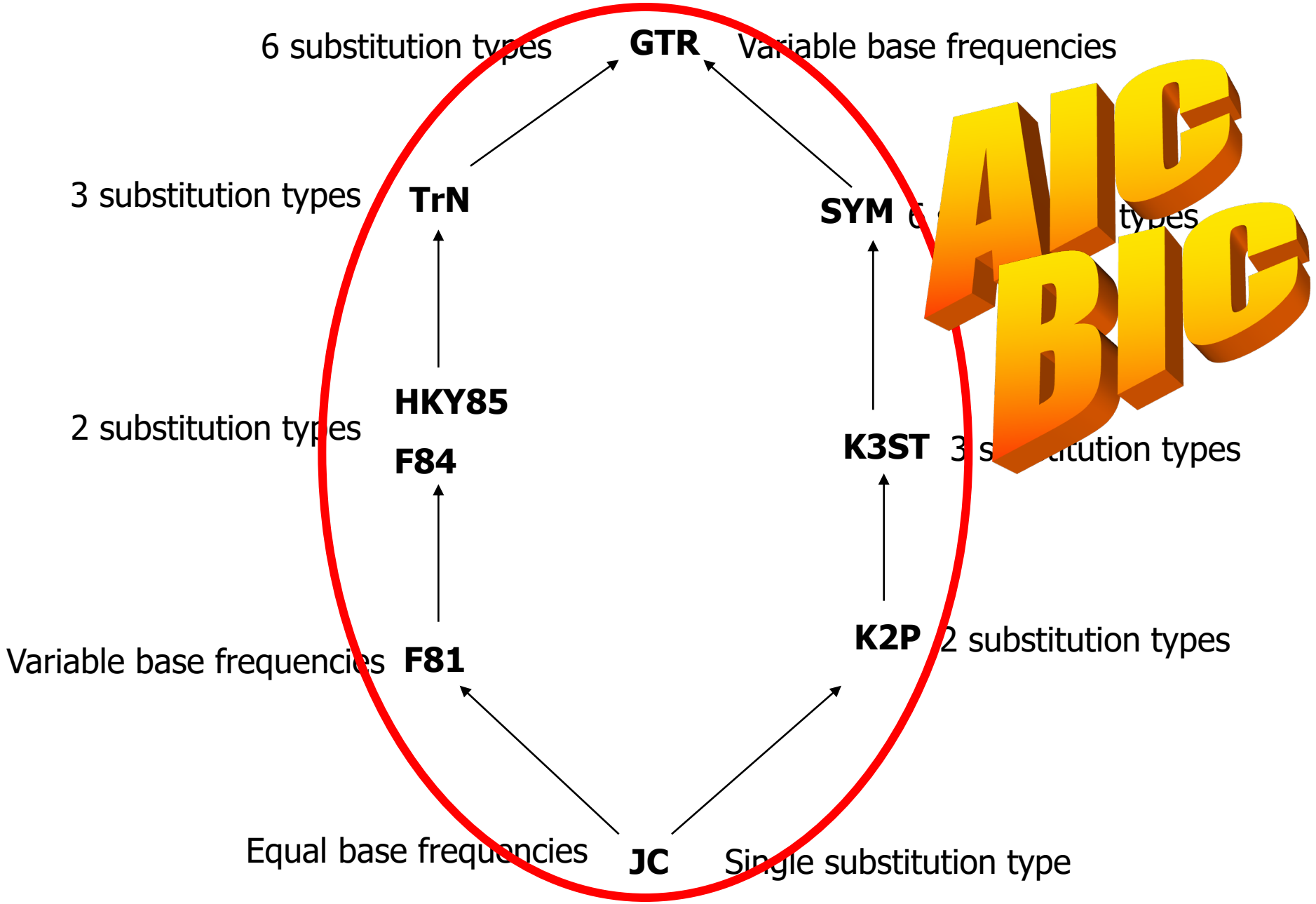
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Estimation of substitution model parameters

- **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 these tests, one needs to compute the likelihood of the model**
- **Covered in 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 parameters that can be estimated in 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 also takes into account 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**

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 (covered in tutorials)**

Partitioned models (1/2)

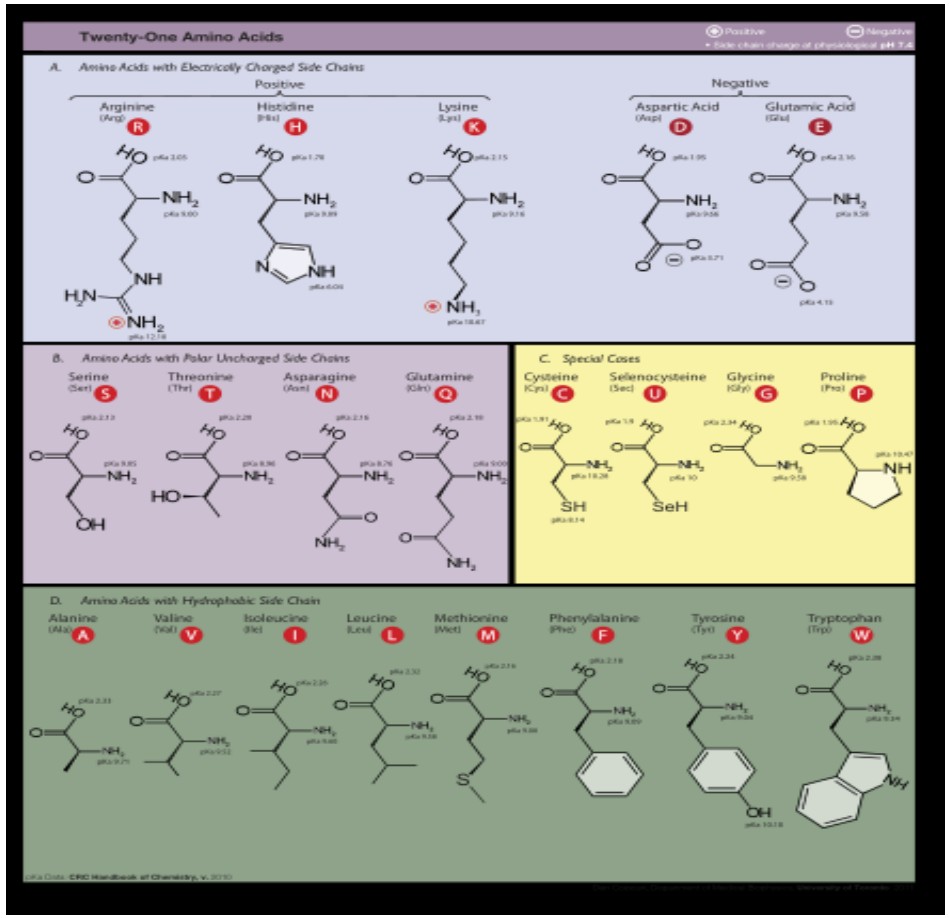
- **Today's datasets tend to be large, including hundreds or thousands of genes**
- **Unrealistic to have the same model for the whole dataset (underparameterization)**
- **Modelling DNA substitution for separate sections of the data (partitions)**
  - **E.g. different genes, codon positions, introns/exons, 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 (covered in tutorials)**

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**



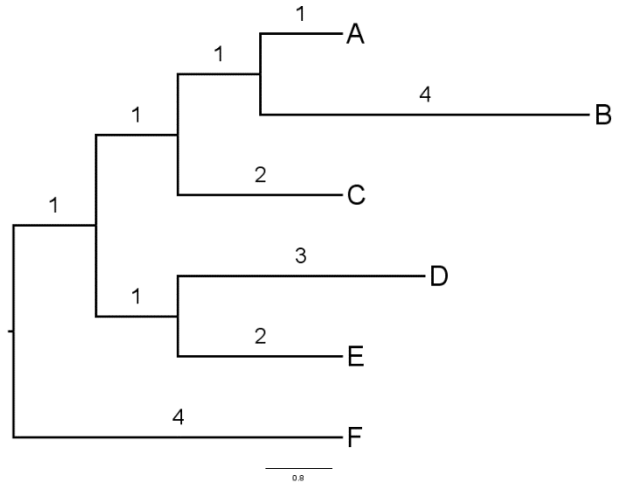
Inferring phylogenies: methodological overview

- **Distance methods**
  - **A clustering method using pairwise distances between sequences (e.g. neighbour joining)**
- **Discrete characters**
  - **Using an optimality criterion to choose the best tree**
    - **Maximum parsimony (Occam's razor)**
      - **Best explanation is the simplest one (the one that minimizes the number of substitutions)**
      - **Doesn't perform as well as model-based methods on molecular data**
      - **Still used for morphological characters**
    - **Maximum likelihood**
    - **Bayesian inference**

Distance methods involve two stages

- **Stage 1: calculate the evolutionary distance between pairs of sequences (using a DNA substitution model)**
- **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 base pairs (bp), 15 bp differ, or D = 15/98 = 0.153 or 15.3%

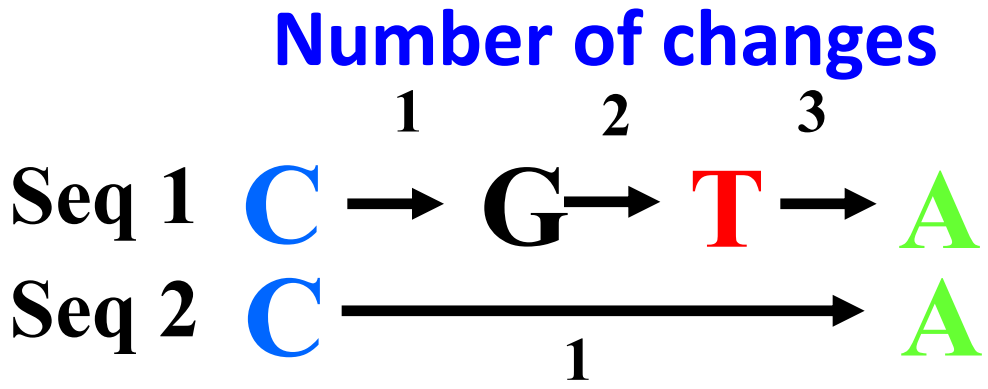
Distance matrix:

	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 (among site rate variation)**



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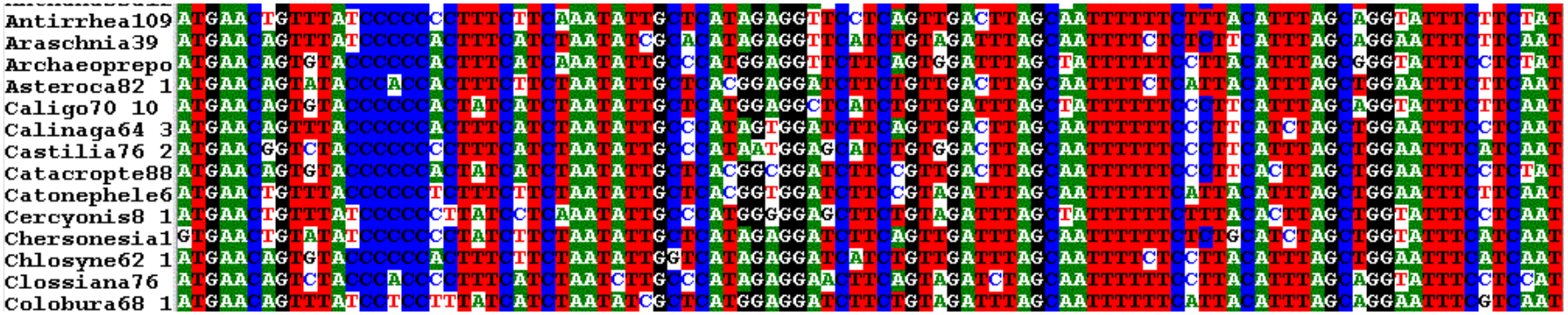

Dissimilarities matrix:

	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

- **Computationally fast**
- **A large number of models are available with many parameters - improves estimation of distances**
- **Great for getting a quick tree in data checking/exploration phase**

Distance – disadvantages

- **Prone to systematic errors**
- **Problems with missing data**
- **Generally outperformed by Maximum Likelihood and Bayesian 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)**

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**