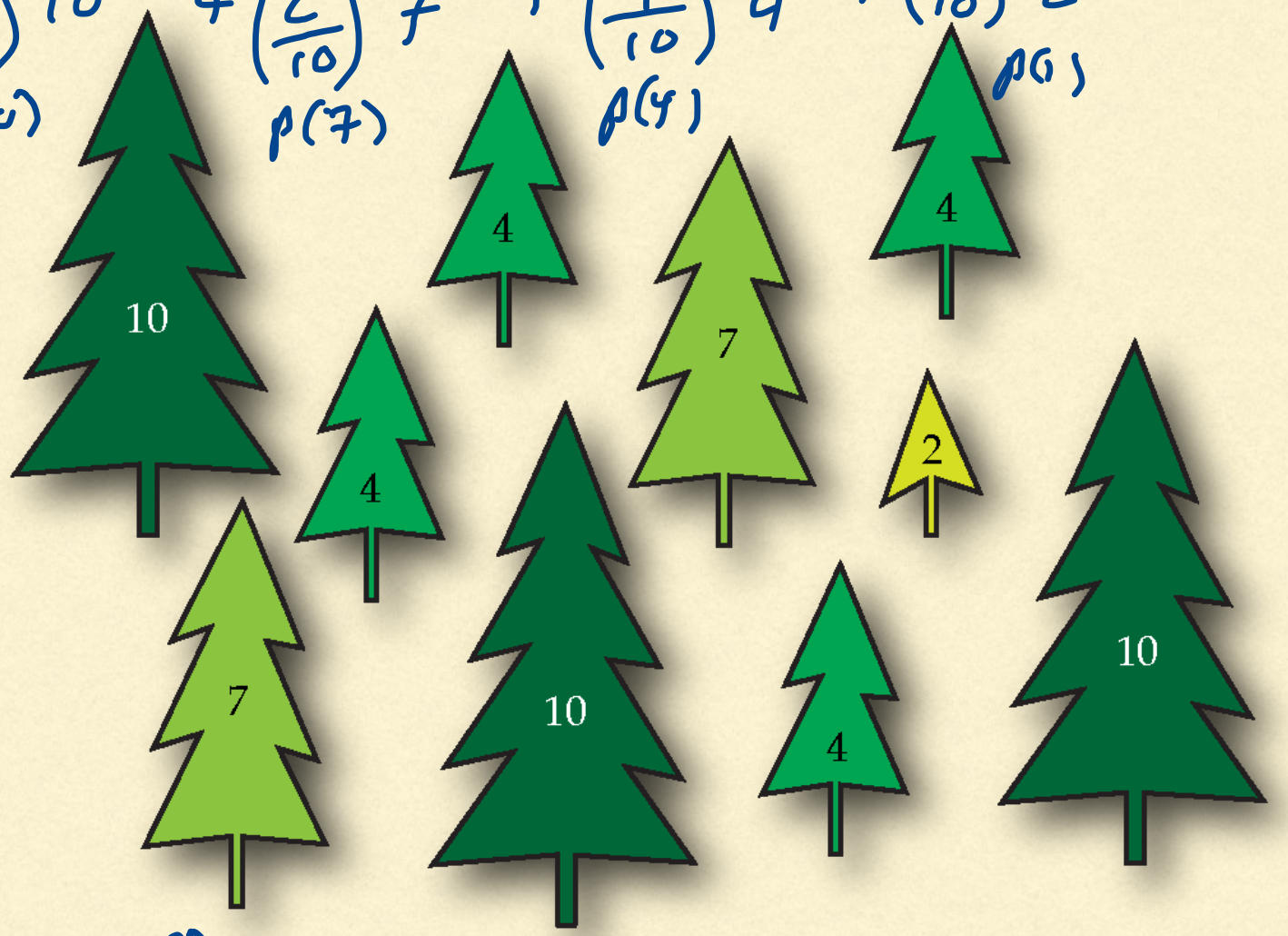
$\frac{10}{10} + \frac{10}{10} + \frac{10}{10} + \frac{7}{10} + \frac{7}{10} + \frac{4}{10} + \frac{4}{10} + \frac{4}{10} + \frac{4}{10} + \frac{2}{10} = 6.2$

# Expected value vs. average

$\left(\frac{3}{10}\right)10 + \left(\frac{2}{10}\right)7 + \left(\frac{4}{10}\right)4 + \left(\frac{1}{10}\right)2 = 6.2$

$p(10)$ $p(7)$ $p(4)$ $p(2)$




$$E[H] = \sum_{i=0}^{\infty} i\,p(i) = 0\,p(0) + 1\,p(1) + 2\,p(2) + \cdots$$

# Five common substitution models

# Jukes and Cantor (1969)

JC69 model

Parameters: $\beta$

to:

$$
\text{from:}\quad
\begin{array}{c}
\\ A \\ C \\ G \\ T
\end{array}
\begin{array}{c}
\begin{array}{cccc} A & C & G & T \end{array} \\
\begin{bmatrix}
-3\beta & \beta & \beta & \beta \\
\beta & -3\beta & \beta & \beta \\
\beta & \beta & -3\beta & \beta \\
\beta & \beta & \beta & -3\beta
\end{bmatrix}
\end{array}
$$

$v = ?$

A → anything else

rate = $3\beta$

$v = 3\beta t$

$\beta t = \frac{v}{3}$

# Kimura (1980)

## K80 (or K2P) model

Parameters: ? $\alpha, \beta$

| | A | C | G | T |
|---|---|---|---|---|
| A | $-\alpha - 2\beta$ | $\beta$ | $\alpha$ | $\beta$ |
| C | $\beta$ | $-\alpha - 2\beta$ | $\beta$ | $\alpha$ |
| G | $\alpha$ | $\beta$ | $-\alpha - 2\beta$ | $\beta$ |
| T | $\beta$ | $\alpha$ | $\beta$ | $-\alpha - 2\beta$ |

tv: transversion

ts: transition

# Kimura (1980)

## K80 (or K2P) model

rate ratio

$\kappa = \alpha/\beta$

Parameters: ?

ts : tv ratio?

| | A | C | G | T |
|---|---|---|---|---|
| A | $-\beta(\kappa+2)$ | $\beta$ | $\kappa\beta$ | $\beta$ |
| C | $\beta$ | $-\beta(\kappa+2)$ | $\beta$ | $\kappa\beta$ |
| G | $\kappa\beta$ | $\beta$ | $-\beta(\kappa+2)$ | $\beta$ |
| T | $\beta$ | $\kappa\beta$ | $\beta$ | $-\beta(\kappa+2)$ |

$$E[trs] = \frac{1}{4} K\beta t + \frac{1}{4} K\beta t + \frac{1}{4} K\beta t + \frac{1}{4} K\beta t$$

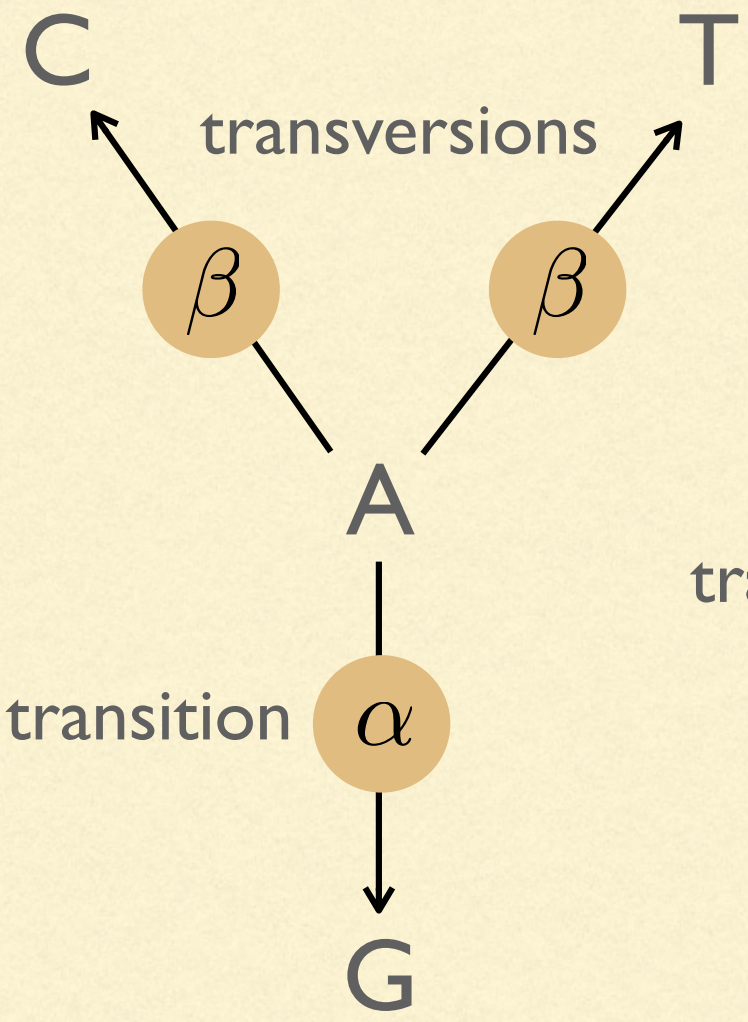
$p(A)\ A \to G \qquad p(C)\ C \to T \qquad p(G)\ G \to A \qquad p(T)\ T \to C$

$$= (4)\left(\frac{1}{4}\right) K\beta t = K\beta t$$

$$E[trv] = (8)\left(\frac{1}{4}\right) \beta t = 2\beta t$$

$$\frac{trs}{trv} \text{ ratio} = \frac{K\beta t}{2\beta t} = \frac{K}{2}$$

# Transition-transversion (rate) ratio




tratio → trs/trv ratio

kappa → trs/trv rate ratio

transition rate = $\alpha$

transversion rate = $\beta$

assume $\alpha = \beta$

transition-transversion rate ratio = 1.0

transition-transversion ratio = 0.5

# Felsenstein (1981)

$\pi_T = 1 - \pi_A - \pi_C - \pi_G$

F81 model

$\mu, \pi_A, \pi_C, \pi_G$

Parameters: ?

$$
\begin{array}{c} \\ \text{A} \\ \text{C} \\ \text{G} \\ \text{T} \end{array}
\begin{array}{c}
\begin{array}{cccc} \text{A} & \text{C} & \text{G} & \text{T} \end{array} \\
\begin{bmatrix}
-\mu(1-\pi_A) & \pi_C\mu & \pi_G\mu & \pi_T\mu \\
\pi_A\mu & -\mu(1-\pi_C) & \pi_G\mu & \pi_T\mu \\
\pi_A\mu & \pi_C\mu & -\mu(1-\pi_G) & \pi_T\mu \\
\pi_A\mu & \pi_C\mu & \pi_G\mu & -\mu(1-\pi_T)
\end{bmatrix}
\end{array}
$$

# JC69 is a special case of F81

$$
\begin{array}{c} \\ \mathsf{A} \\ \mathsf{C} \\ \mathsf{G} \\ \mathsf{T} \end{array}
\begin{array}{c}
\begin{array}{cccc} \mathsf{A} & \mathsf{C} & \mathsf{G} & \mathsf{T} \end{array} \\
\begin{bmatrix}
-\frac{3}{4}\mu & \frac{1}{4}\mu & \frac{1}{4}\mu & \frac{1}{4}\mu \\
\frac{1}{4}\mu & -\frac{3}{4}\mu & \frac{1}{4}\mu & \frac{1}{4}\mu \\
\frac{1}{4}\mu & \frac{1}{4}\mu & -\frac{3}{4}\mu & \frac{1}{4}\mu \\
\frac{1}{4}\mu & \frac{1}{4}\mu & \frac{1}{4}\mu & -\frac{3}{4}\mu
\end{bmatrix}
\end{array}
\qquad
\begin{array}{cccc} \mathsf{A} & \mathsf{C} & \mathsf{G} & \mathsf{T} \end{array}
\begin{bmatrix}
-3\beta & \beta & \beta & \beta \\
\beta & -3\beta & \beta & \beta \\
\beta & \beta & -3\beta & \beta \\
\beta & \beta & \beta & -3\beta
\end{bmatrix}
$$

$$\beta = \frac{1}{4}\mu$$

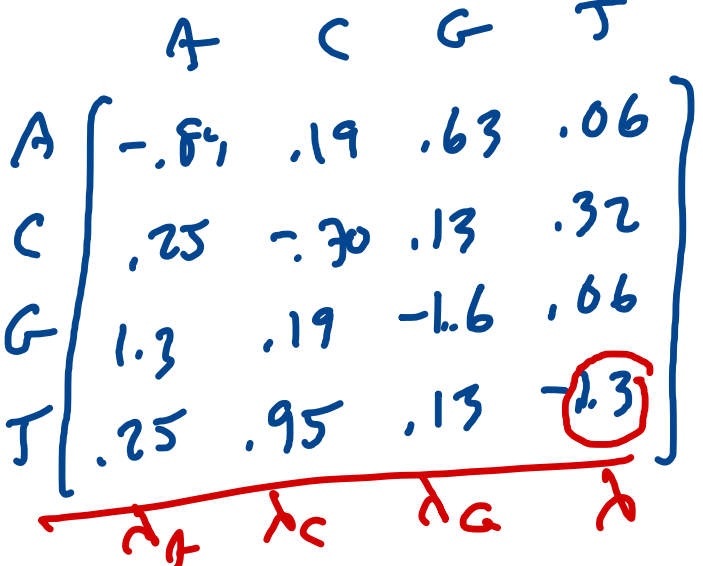
# Hasegawa, Kishino, and Yano (1985)

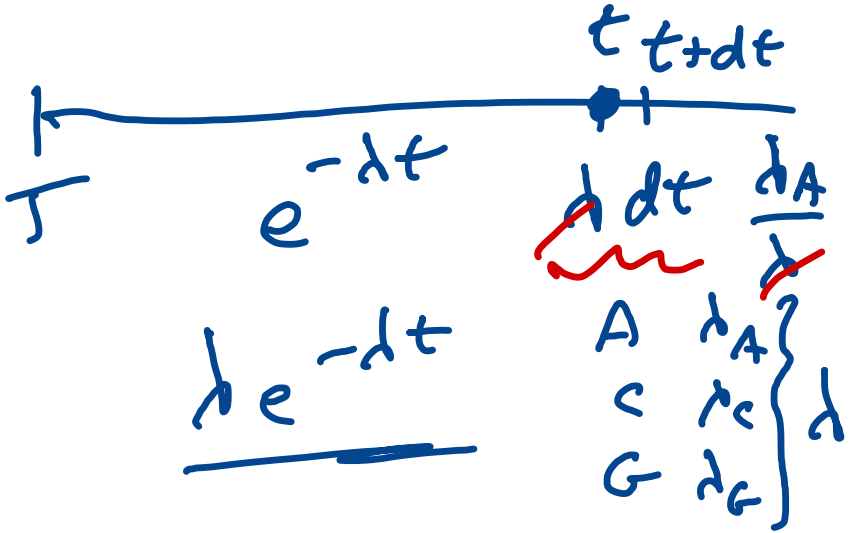
HKY85 model

$\pi_A, \pi_C, \pi_G, \kappa, \mu$

Parameters: ?

$$
\begin{array}{c|cccc}
 & A & C & G & T \\
\hline
A & -\mu\left(\pi_C + \pi_G\kappa + \pi_T\right) & \pi_C\mu & \pi_G\mu\kappa & \pi_T\mu \\
C & \pi_A\mu & -\mu\left(\pi_A + \pi_G + \pi_T\kappa\right) & \pi_G\mu & \pi_T\mu\kappa \\
G & \pi_A\mu\kappa & \pi_C\mu & -\mu\left(\pi_A\kappa + \pi_C + \pi_T\right) & \pi_T\mu \\
T & \pi_A\mu & \pi_C\mu\kappa & \pi_G\mu & -\mu\left(\pi_A + \pi_C\kappa + \pi_G\right)
\end{array}
$$




$$E[\text{no. subst. given } dt] = (0)\,p(0) + (1)\,p(1) + (2)\,p(2) + \cdots$$

$$T \to A \qquad \text{Prob}(A \mid T, t) = \frac{\lambda_A}{\lambda} \qquad \lambda = \lambda_A + \lambda_C + \lambda_G$$

$$P(A, B) = P(A)\,P(B \mid A)$$

# Tavaré (1986)

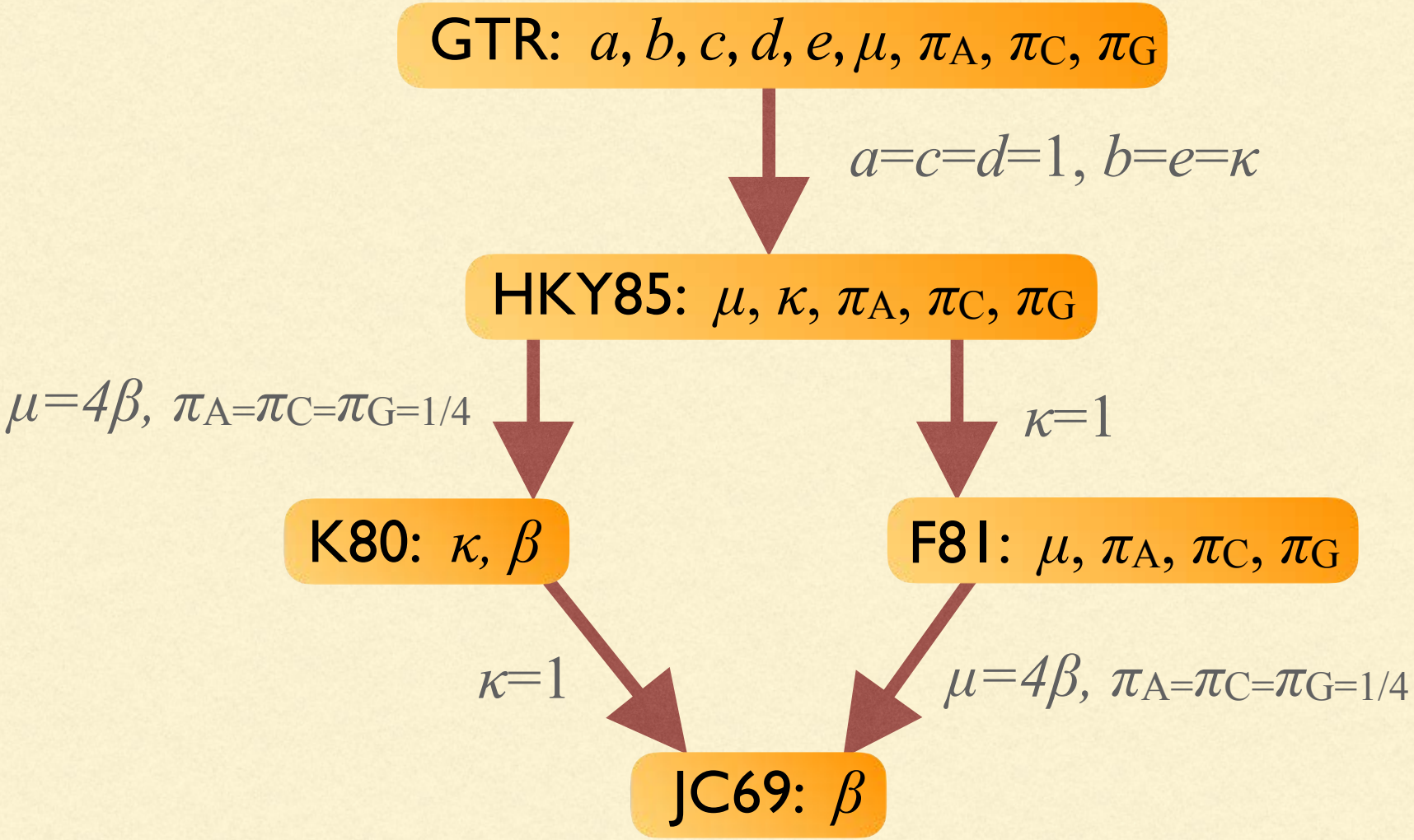
GTR model

$\pi_A, \pi_C, \pi_G, \mu, a, b, c, d, e$ Parameters: 9

| | A | C | G | T |
|---|---|---|---|---|
| A | — | $\pi_C \mu$ (a) | $\pi_G \mu$ (b) | $\pi_T \mu$ (c) |
| C | $\pi_A \mu\ a$ | — | $\pi_G \mu$ (d) | $\pi_T \mu$ (e) |
| G | $\pi_A \mu\ b$ | $\pi_C \mu\ d$ | — | $\pi_T \mu$ (f) |
| T | $\pi_A \mu\ c$ | $\pi_C \mu\ e$ | $\pi_G \mu\ f$ | — |

exchangeability parameters are circled

# GTR family


GTR: $a, b, c, d, e, \mu, \pi_A, \pi_C, \pi_G$
$a=c=d=1$, $b=e=\kappa$
HKY85: $\mu, \kappa, \pi_A, \pi_C, \pi_G$
$\mu=4\beta$, $\pi_A=\pi_C=\pi_G=1/4$
$\kappa=1$
K80: $\kappa, \beta$
F81: $\mu, \pi_A, \pi_C, \pi_G$
$\kappa=1$
$\mu=4\beta$, $\pi_A=\pi_C=\pi_G=1/4$
JC69: $\beta$

# Rate heterogeneity

# Green plant rbcL gene

## First 88 amino acids (translation is for *Zea mays*)

```
M--S--P--Q--T--E--T--K--A--S--V--G--F--K--A--G--V--K--D--Y--K--L--T--Y--Y--T--P--E--Y--E--T--K--D--T--D--I--L--A--A--F--R--V--T--P--
Chara           (green alga; land plant lineage)   AAAGATTACAGATTAACTTACTATACTCCTGAGTATAAAACTAAAGATACTGACATTTTAGCTGCATTTCGTGTAACTCCA
Chlorella       (green alga)                       .....C...C.T.....................T..CC..C.A.....C.....T...C.T..A..G..C...A.G.....T
Volvox          (green alga)                       ........TC.T.....A.....C..A.....C...GT.GTA.....C........C.....A.........A.G......
Conocephalum    (liverwort)                        ........TC.........T........G..T...G.............G..T........A......A.AA.G.....T
Bazzania        (moss)                             ........T........C..T.....G.....A...G.G..C.....G..A..T.....G..A.........A.G.....C
Anthoceros      (hornwort)                         ........T........CC.T.....C.....T..CG.G..C..G........T.....G..A..G.C.T.AA.G.....T
Osmunda         (fern)                             ........TC....G...C..........C..T...G.G..C..G........T.....G..A.....C..AA.G.....C
Lycopodium      (club "moss")                      .GG..............C.T..C........T.....G..C.....A..C..T...C.G..A........AA.G.....T
Ginkgo          (gymnosperm; Ginkgo biloba)        ..............G.....T...........A...C....C...........T..C..G..A.....C..A........T
Picea           (gymnosperm; spruce)               ...................T...........A...C.G..C........G..T.....G..A.....C..A........T
Iris            (flowering plant)                  ..............G.....T...........T..CG....C...........T..C..G..A.....C..A........T
Asplenium       (fern; spleenwort)                 ........TC..C.G.....T..C..C..C..A..C..G..C........C..T..C..G..A..T..C..GA.G..C...
Nicotiana       (flowering plant; tobacco)         .....G....A...G.....T.............CC....C..G........T..A..G..A.....C..A........T

Q--L--G--V--P--P--E--E--A--G--A--A--V--A--A--E--S--S--T--G--T--W--T--T--V--W--T--D--G--L--T--S--L--D--R--Y--K--G--R--C--Y--H--I--E--
CAACCTGGCGTTCCACCTGAAGAAGCAGGGGCTGCAGTAGCTGCAGAATCTTCTACTGGTACATGGACTACTGTTTGGACTGACGGATTAACTAGTTTGGACCGATACAAAGGAAGATGCTACGATATTGAA
.....A..T........A........G..T..G........A........A..A........T.....G.....A.........T..T...........A.....T........TC.T..T..T..C..C..G
.....A..T..............TGT..T.....T..T.....T.....A..A..A.....T.....A.....A.........T..T.....A...C.T.....T........TC.T..T..T..C..C..G
..G.....G..A...G.A...........A..A.....T.....T.............................A.............T..TC.T....ACC.T..T..T..T.....TC.......T.G......C
.....G..A..A.................A..G...........T........A..C.....G.....C..G........C..T..GC.T..A...C.C..T..T........TC.......T..C..C...
T....A..G..G.................A..C...........T........A.............................C..T...C.T..C..CC.T.....T........TC..........C......
.....C..A..A..GG....G.....T..A..............G...........A.....G.....C.....A.....G..T...C.T..C...C.T..T..T..T..G..TC..................
....T...A..A.....C..G.....G..A..C...........T.........C.............................C..T...C.T..C...C.C..T..C........TC.G.....T..A......
.........A..G........G.....G..A.............C.........C.............C.............C..T...C.T..C...C.T..T..T.....G...........T..C..C..G
.....A..G..G..C..G.....G..A..A...........T........C..C...........C.............C..T...C.T......C.T..T..T.....G..GC.......T..C..C..G
.....C..A.....TG..........G.....C..G........C.........................A..A..G.........T...C.T..C...C.T..T..T.........C.........C.C..C..G
.....C..A..A...G..........C..A.................G..C.....A...........C.....G.....A.....G..G..C..CC.T.....T.....G..CC.............C..G
........A.....................C..G........C.....................A.....A.....C..T...C.T..C..CC.T..T..T........GC........CGC..C..G
```

All 4 bases are observed at some sites...

...while at other sites, only 1 base is observed

# Site-specific rates

Each defined subset (e.g. 1st+2nd pos. versus 3rd pos.) has its own relative rate

```
CACCGGGTCCCCGAGAGCGGGCGCGTGCGCGATCTCACGGACTGACACGTTGACGAGGTTACAGTTGACGTAAAGGAGTGTAGAATGA ATCTATAAAGTAATAATTTTAGTTTGTACATTGCACAAACCTTA
.....................................................................C..... .AT..A..GTG..A..AA..T.G.A..TT...A.T..TTTTCCG
................TG.....................................................C.....C..... .AT.....TT.TT.T.AAA.T.A.A..TT.A.T.T..TTTTCCG
.........G.....................................................C....AC.....C......G.... G.GA.A...AA.T.T........A...TTT.CTTTT.T..T..C
.....................................................C.....C.....C..... .GAA.....AG...T..AC.G.CG..CGTTA.CTT..T..TCC.
T....................................................C.....C.....C..... .AGG.....AC...T..A........C.TTCCT.T..T...C..
.........G...........................................C.....C.....C..... .CAAG.G.TA....G...A.G.C.A.G.TTC.TTTTGT......
...T.................................................C.....C.....C..... ..AA.CG.GAC...T..C........C.TTC.CTC..TG.TA..
.....................................................C.....C.....C..... ..AG..G.GA....C..C....C...C.TTC.TTT.G...TCCG
.....................................................C.....C.....C..... .AGGGCG.GAA...T..CC...C...C.TT..TTT.GG..TCCG
.........G...........................................C.....C.....C.....C..... .CA.T...G.CG..C.......AAG...TTC.TTT......CCG
.........G.....................................................C.....C..... .CAA....CA.....GC.A...C.G.AG.GCCT.T.GC....CG
.....................................................C.....C.....C.....CG.... ..A.......CG..C.......A.A.C.TTCCTTT..G...CCG
```

$r_1$ applies to subset 1

1st+2nd codon positions

(sites 1 - 88)

$r_2$ applies to subset 2

3rd codon positions

(sites 89-132)

Relative rates have mean 1.0: $r_1\ p(r_1) + r_2\ p(r_2) = 1$

2/3 1/3

# The genetic code

First 12 nucleotides at the 5' end of the *rbc*L gene in corn:

```
5'-ATG|TCA|CCA|CAA-3'   coding strand
3'-TAC|AGT|GGT|GTT-5'   DNA
                        template strand
```

```
5'-AUG|UCA|CCA|CAA-3'   mRNA
```

```
N-Met|Ser|Pro|Gln-C     polypeptide
```

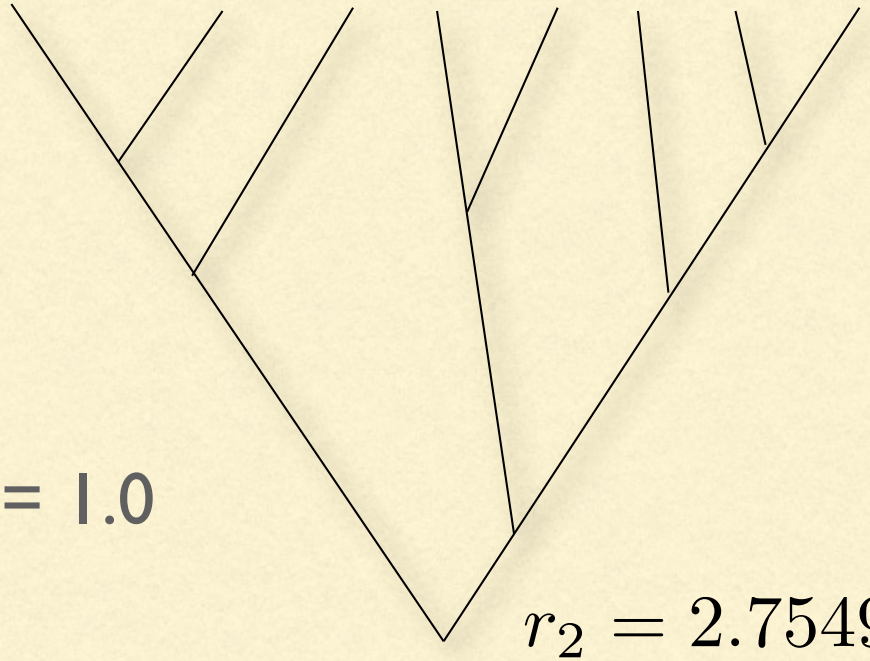
## Codon Table

| | U | C | A | G |
|---|---|---|---|---|
| **U** | UUU Phe<br>UUC Phe<br>UUA Leu<br>UUG Leu | UCU Ser<br>UCC Ser<br>UCA Ser<br>UCG Ser | UAU Tyr<br>UAC Tyr<br>UAA Stp<br>UAG Stp | UGU Cys<br>UGC Cys<br>UGA Stp<br>UGG Trp |
| **C** | CUU Leu<br>CUC Leu<br>CUA Leu<br>CUG Leu | CCU Pro<br>CCC Pro<br>CCA Pro<br>CCG Pro | CAU His<br>CAC His<br>CAA Gln<br>CAG Gln | CGU Arg<br>CGC Arg<br>CGA Arg<br>CGG Arg |
| **A** | AUU Ile<br>AUC Ile<br>AUA Ile<br>AUG Met | ACU Thr<br>ACC Thr<br>ACA Thr<br>ACG Thr | AAU Asn<br>AAC Asn<br>AAA Lys<br>AAG Lys | AGU Ser<br>AGC Ser<br>AGA Arg<br>AGG Arg |
| **G** | GUU Val<br>GUC Val<br>GUA Val<br>GUG Val | GCU Ala<br>GCC Ala<br>GCA Ala<br>GCG Ala | GAU Asp<br>GAC Asp<br>GAA Glu<br>GAG Glu | GGU Gly<br>GGC Gly<br>GGA Gly<br>GGG Gly |

# Site-specific rates

$$L = \underbrace{p(\mathbf{y}_1|r_1) \cdots p(\mathbf{y}_{88}|r_1)}_{\text{1st+2nd codon positions}} \; \underbrace{p(\mathbf{y}_{89}|r_2) \cdots p(\mathbf{y}_{132}|r_2)}_{\text{3rd codon positions}}$$

$$r_1 = 0.12255$$




mean relative rate:

(0.12255)(2/3) + (2.75490)(1/3) = 1.0

# Site-specific rates

JC69 transition probabilities that would be used for every site if rate *homo*geneity were assumed:

$$P_{ii}(t) = \frac{1}{4} + \frac{3}{4}e^{-4\beta t}$$

C —— C
identity

$$P_{ij}(t) = \frac{1}{4} - \frac{1}{4}e^{-4\beta t}$$

C —— T
difference

# Site specific rates

JC69 transition probabilities that would be used for sites in **subset 1**:

$$P_{ii}(t) = \frac{1}{4} + \frac{3}{4}e^{-4r_1\beta t}$$

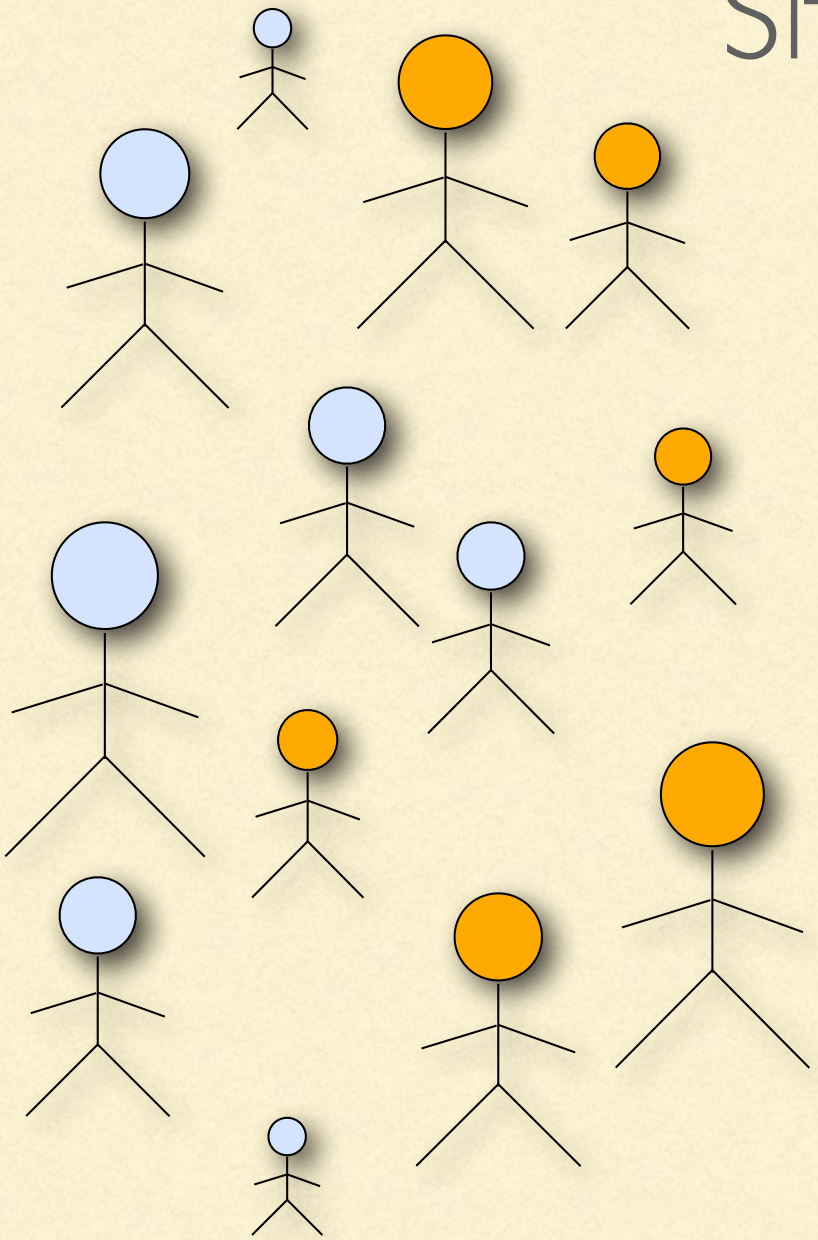
$$P_{ij}(t) = \frac{1}{4} - \frac{1}{4}e^{-4r_1\beta t}$$

JC69 transition probabilities that would be used for sites in **subset 2**:

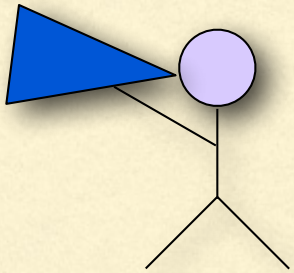
$$P_{ii}(t) = \frac{1}{4} + \frac{3}{4}e^{-4r_2\beta t}$$

$$P_{ij}(t) = \frac{1}{4} - \frac{1}{4}e^{-4r_2\beta t}$$

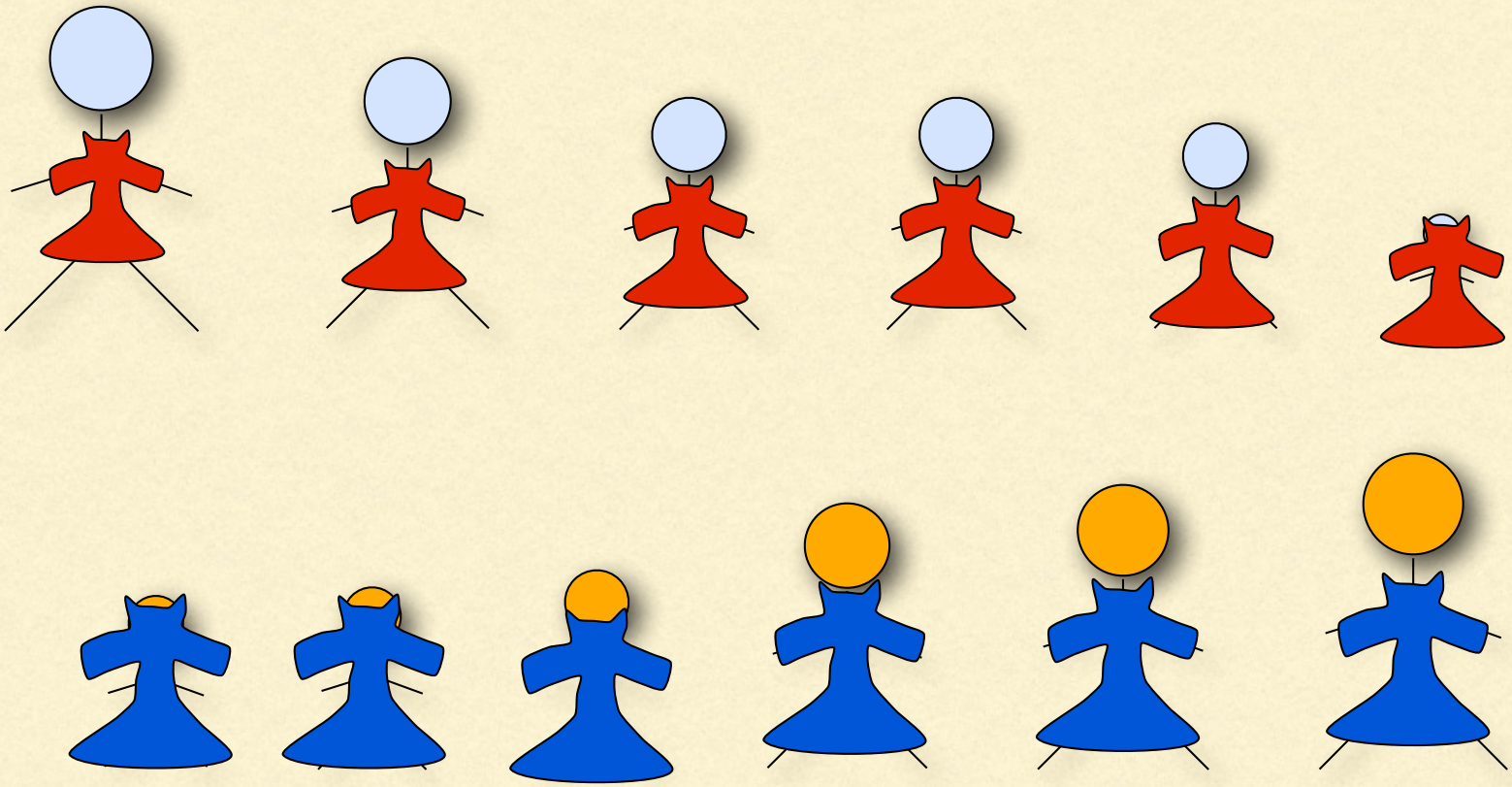
# Site-specific approach



OK, I am going to divide you into 2 groups based on the color of your head, and everyone in each group will get a coat of the average size for their group. Very sorry if this does not work well for some people who are unusually large or small compared to their group.



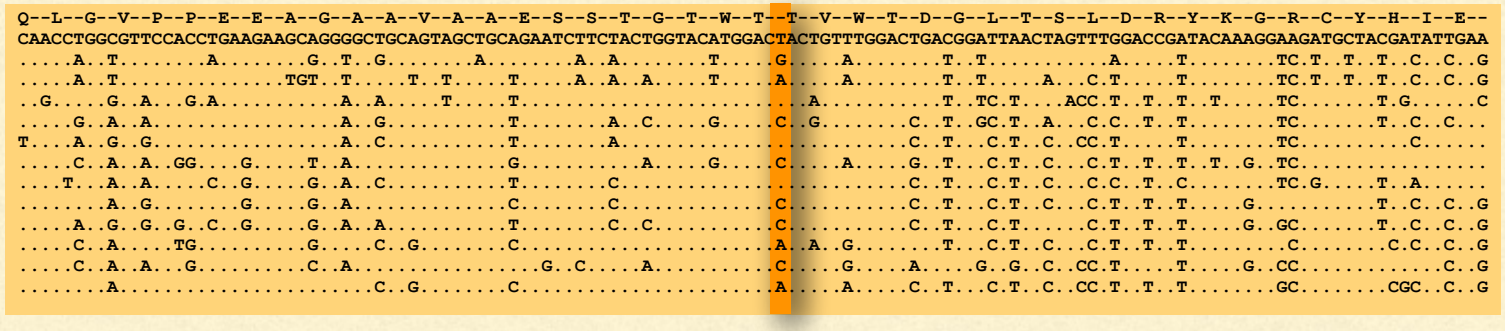
# Site-specific approach



Good: costs less: need to buy just one coat for every person
Bad: every person in a group has to wear the same size coat

# Mixture models

All *k* relative rates applied to every site

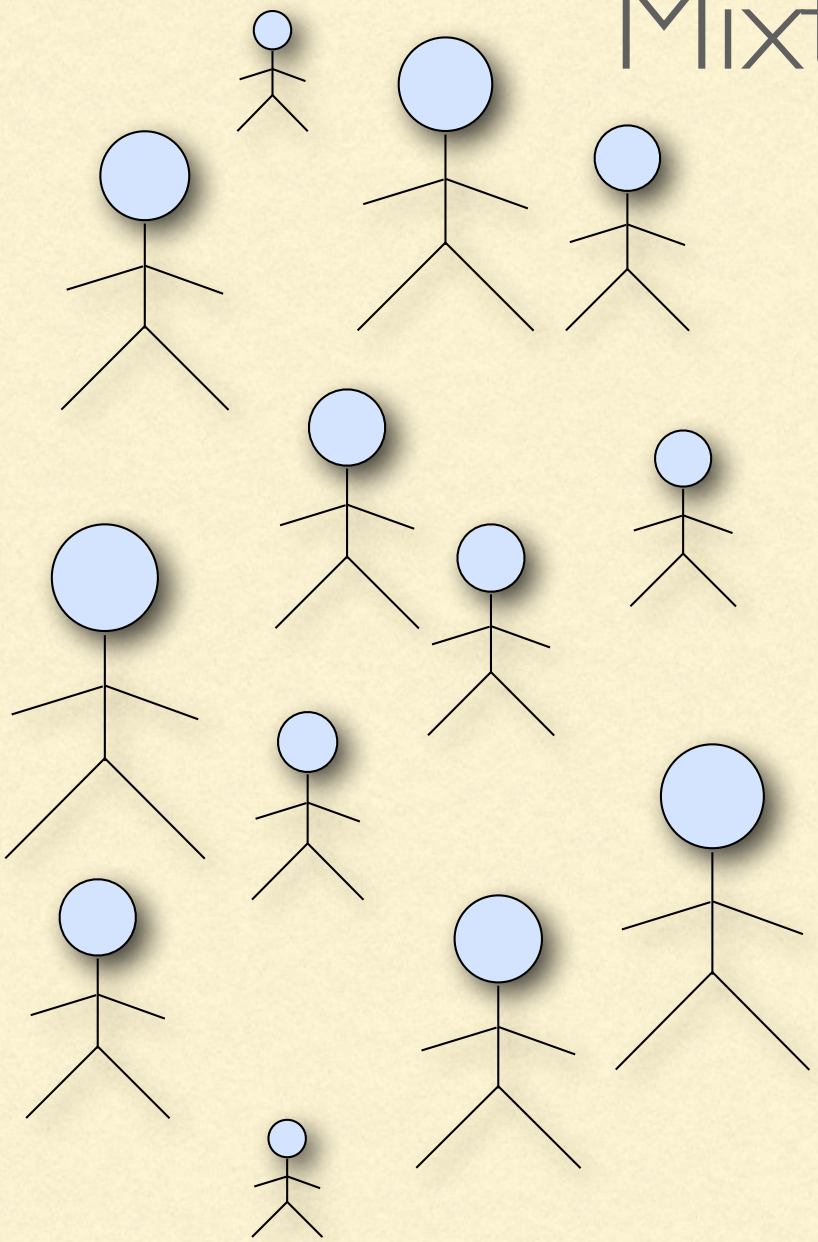


site $i$

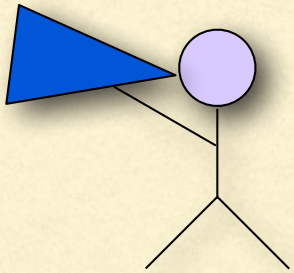
$$L_i = p(\mathbf{y}_i|r_1)p(r_1) + p(\mathbf{y}_i|r_2)p(r_2) + \cdots + p(\mathbf{y}_i|r_k)p(r_k)$$

Common examples
- Invariable sites (I) model
- Discrete Gamma (G) model

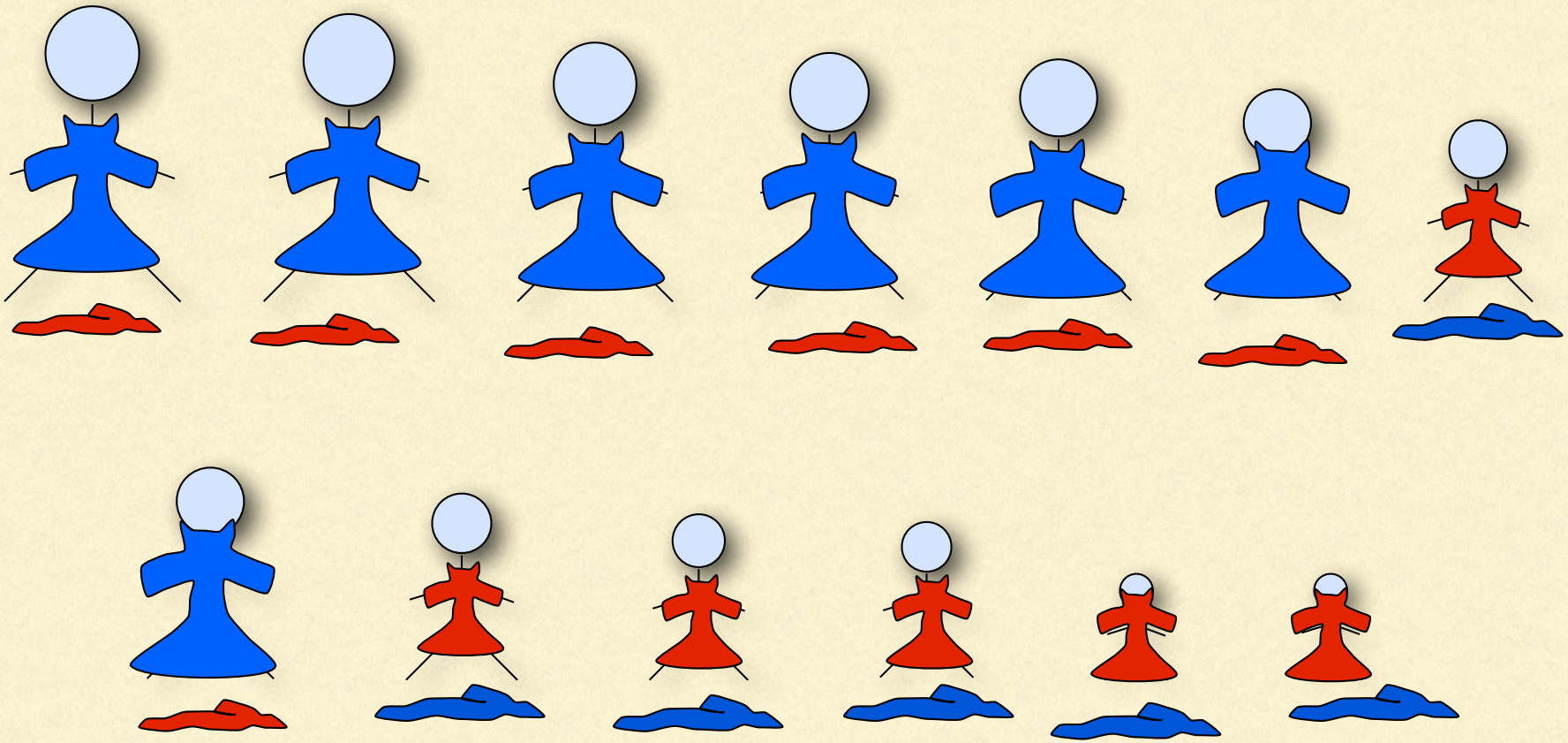
# Mixture model approach



OK, I am going to give each of you 2 coats: use the one that fits you best and throw away the other one. This costs twice as much for me, but on average leads to better fit for you. I have determined the two sizes of coats based on the distribution of your sizes.



# Mixture model approach



Good: every person experiences better fit because they can choose the size coat that fits best

Bad: costs more because two coats much be provided for each person

# Invariable sites model (Reeves 1992)

A fraction $p_{invar}$ of sites are assumed to be invariable (i.e. rate = 0.0)

$$L_i = p(\mathbf{y}_i|r_1)p_{\text{invar}} + p(\mathbf{y}_i|r_2)(1 - p_{\text{invar}})$$

$$r_1 = 0.0$$

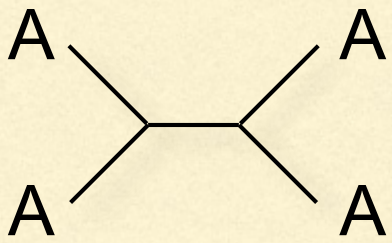
$$r_2 = \frac{1}{1 - p_{\text{invar}}}$$

Allows for the possibility that any given site could be variable or invariable

$$\bar{r} = \cancel{p_{\text{invar}}(0.0)} + \cancel{(1 - p_{\text{invar}})}\cancel{\left(\frac{1}{1 - p_{\text{invar}}}\right)} = 1.0$$

# Invariable sites model

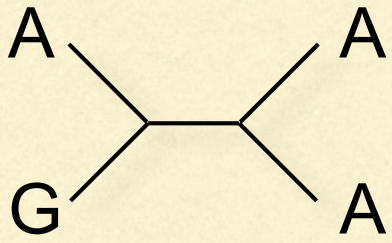
If site *i* is a *constant* site, both terms will contribute to the site likelihood:




$$L_i = p(\mathbf{y}_i|r_1)p_{\text{invar}} + p(\mathbf{y}_i|r_2)(1 - p_{\text{invar}})$$

---

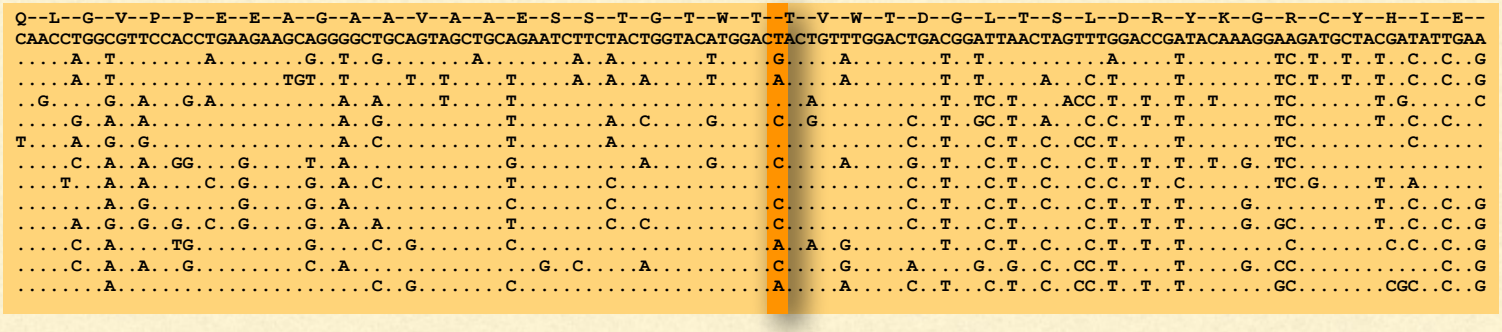
If site *i* is a *variable* site, there is no way to explain the data with a zero rate, so the likelihood in the first term equals zero:




$$L_i = \cancel{p(\mathbf{y}_i|r_1)}^{0.0}p_{\text{invar}} + p(\mathbf{y}_i|r_2)(1 - p_{\text{invar}})$$

# Discrete Gamma model (Yang 1994)

No relative rate is exactly 0.0, and all are equally probable



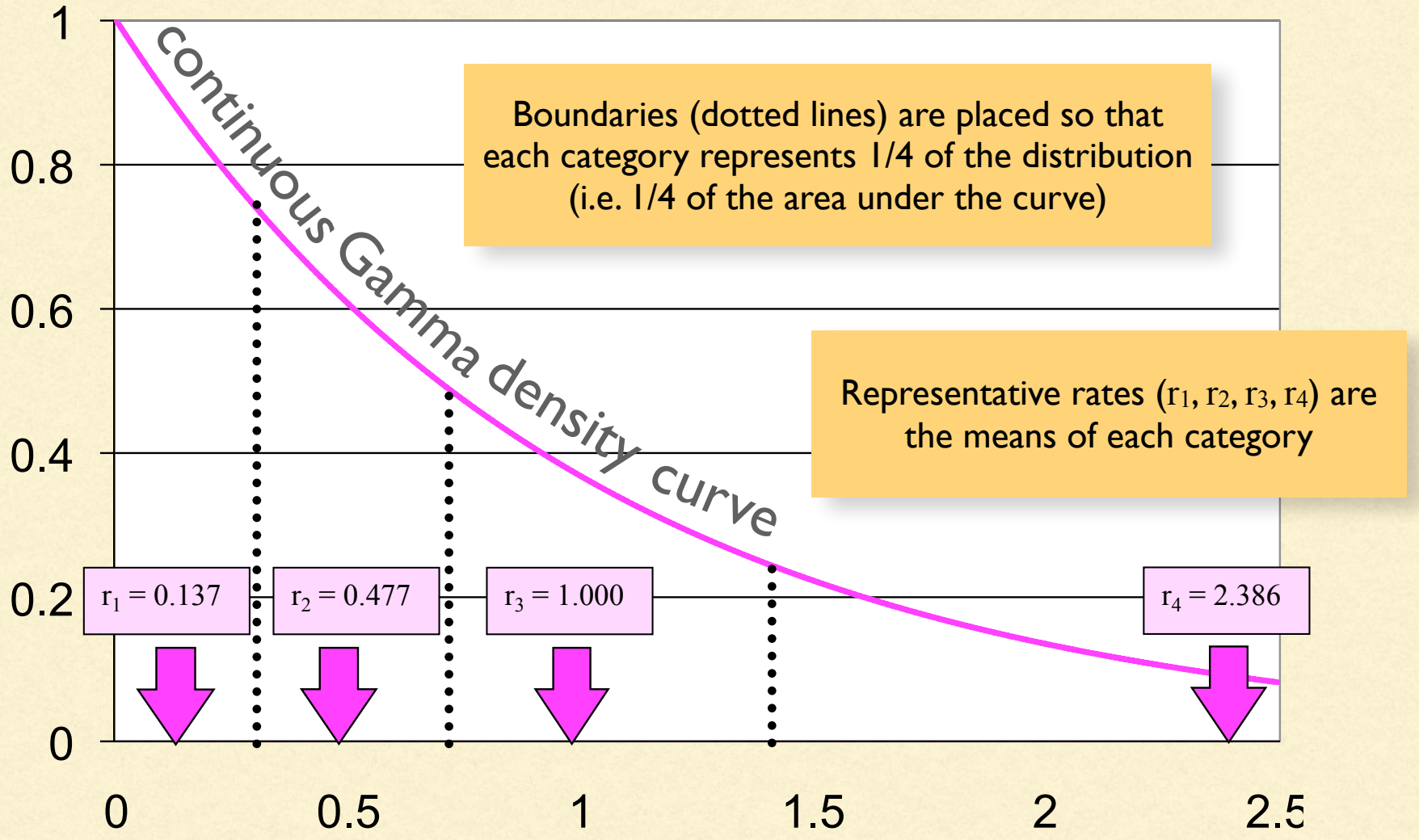
site $i$

$$L_i = p(\mathbf{y}_i|r_1)\left(\frac{1}{4}\right) + p(\mathbf{y}_i|r_2)\left(\frac{1}{4}\right) + p(\mathbf{y}_i|r_3)\left(\frac{1}{4}\right) + p(\mathbf{y}_i|r_4)\left(\frac{1}{4}\right)$$

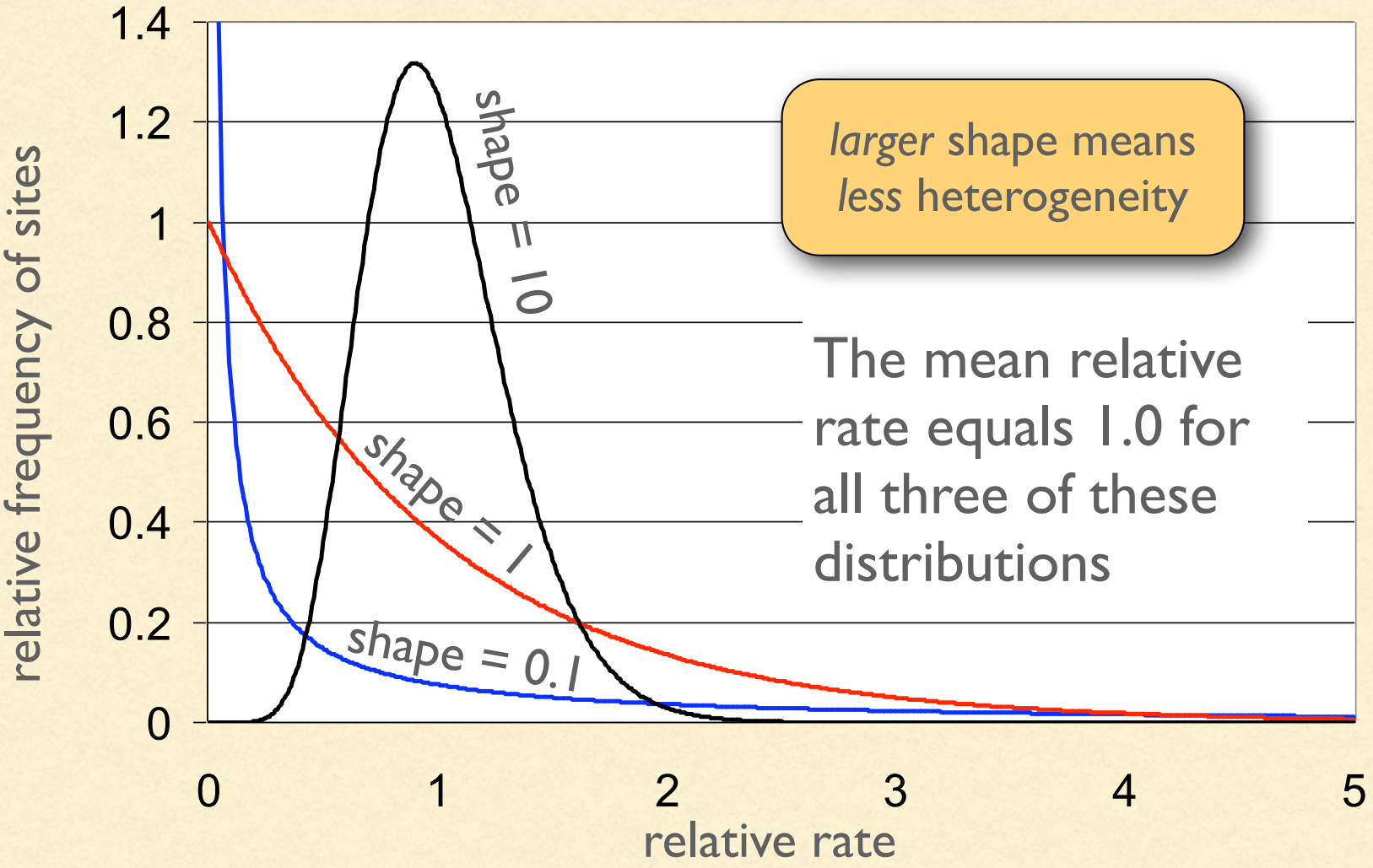
Relative rates are determined by a discrete gamma distribution

Number of rate categories can vary (4 used here)

# Relative rates in 4-category case



# Gamma distributions



# Codon models

# Muse & Gaut (1994); Goldman & Yang(1994)

| | TTT (Phe) | TTC (Phe) | TTA (Leu) | TTG (Leu) | CTT (Leu) | CTC (Leu) | … | GGG (Gly) |
|---|---|---|---|---|---|---|---|---|
| TTT (Phe) | — | $\alpha\,\pi_C$ | $\beta\,\pi_A$ | $\beta\,\pi_G$ | $\beta\,\pi_C$ | 0 | ⋯ | 0 |
| TTC (Phe) | $\alpha\,\pi_T$ | — | $\beta\,\pi_A$ | $\beta\,\pi_G$ | 0 | $\beta\,\pi_C$ | ⋯ | 0 |
| | | $\pi_C$ | — | $\alpha\,\pi_G$ | 0 | 0 | ⋯ | 0 |
| | | $\pi_C$ | $\pi_A$ | — | 0 | 0 | ⋯ | 0 |
| CTT (Leu) | | | 0 | 0 | — | $\alpha\,\pi_C$ | ⋯ | 0 |
| CTC (Leu) | | | | 0 | $\alpha\,\pi_T$ | — | ⋯ | 0 |
| ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | | | |
| GGG (Gly) | 0 | 0 | 0 | 0 | 0 | | | |

synon. subst.
Phe →Phe
T→C

nonsynon. subst.
Phe→Leu
C→A

rate = 0 if more than one nucleotide change is required

# Interpreting codon model results

$\omega = \beta/\alpha$ is the nonsynonymous/synonymous rate ratio

| omega | mode of selection | example(s) |
|---|---|---|
| $\omega < 1$ | stabilizing selection<br>(nucleotide substitutions rarely change the amino acid) | functional protein coding genes |
| $\omega = 1$ | neutral evolution<br>(synonymous and nonsynonymous substitutions occur at the same rate) | pseudogenes |
| $\omega > 1$ | positive selection<br>(nucleotide substitutions often change the amino acid) | envelope proteins in viruses under active positive selection |

$$\mathbf{P}(t) = e^{\mathbf{Q}t}$$

# JC69 revisited

$$v = 3\beta t$$

## Q matrix
(instantaneous rates)

$$\begin{array}{c} \\ \text{A} \\ \text{C} \\ \text{G} \\ \text{T} \end{array} \begin{array}{c} \begin{array}{cccc} \text{A} & \text{C} & \text{G} & \text{T} \end{array} \\ \begin{bmatrix} -3\beta & \beta & \beta & \beta \\ \beta & -3\beta & \beta & \beta \\ \beta & \beta & -3\beta & \beta \\ \beta & \beta & \beta & -3\beta \end{bmatrix} \end{array}$$

## P matrix (transition probabilities)

$$\begin{bmatrix} \frac{1}{4} + \frac{3}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} \\ \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} + \frac{3}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} \\ \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} + \frac{3}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} \\ \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} - \frac{1}{4}e^{-4\beta t} & \frac{1}{4} + \frac{3}{4}e^{-4\beta t} \end{bmatrix}$$

# 2-state version

## Q matrix (instantaneous rates)

$$\begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta & \beta \\ \beta & -\beta \end{bmatrix} \end{array}$$

$$v = \beta t$$

## P matrix (transition probabilities)

$$\begin{bmatrix} \frac{1}{2} + \frac{1}{2}e^{-2\beta t} & \frac{1}{2} - \frac{1}{2}e^{-2\beta t} \\ \frac{1}{2} - \frac{1}{2}e^{-2\beta t} & \frac{1}{2} + \frac{1}{2}e^{-2\beta t} \end{bmatrix}$$

# 2-state version

$$\mathbf{P}(t) = e^{\mathbf{Q}t}$$

$$\mathbf{Q} = \begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta & \beta \\ \beta & -\beta \end{bmatrix} \end{array}$$

# 2-state version

$$\mathbf{P}(t) = e^{\mathbf{Q}t}$$

$$\mathbf{Q}t$$

$$\begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta t & \beta t \\ \beta t & -\beta t \end{bmatrix} \end{array}$$

# 2-state version

$$\mathbf{P}(t) = e^{\mathbf{Q}t}$$

$$\mathbf{Q}t$$

$$\begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta t & \beta t \\ \beta t & -\beta t \end{bmatrix} \end{array}$$

$$\begin{bmatrix} \frac{\sqrt{2}}{2} & -\frac{\sqrt{2}}{2} \\ \frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \end{bmatrix} \begin{bmatrix} 0 & 0 \\ 0 & -2\beta t \end{bmatrix} \begin{bmatrix} \frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \\ -\frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \end{bmatrix}$$

eigenvector matrix | eigenvalue matrix | inverse eigenvector matrix

# 2-state version

$$\mathbf{P}(t) = e^{\mathbf{Q}t}$$

$$e^{\mathbf{Q}t}$$

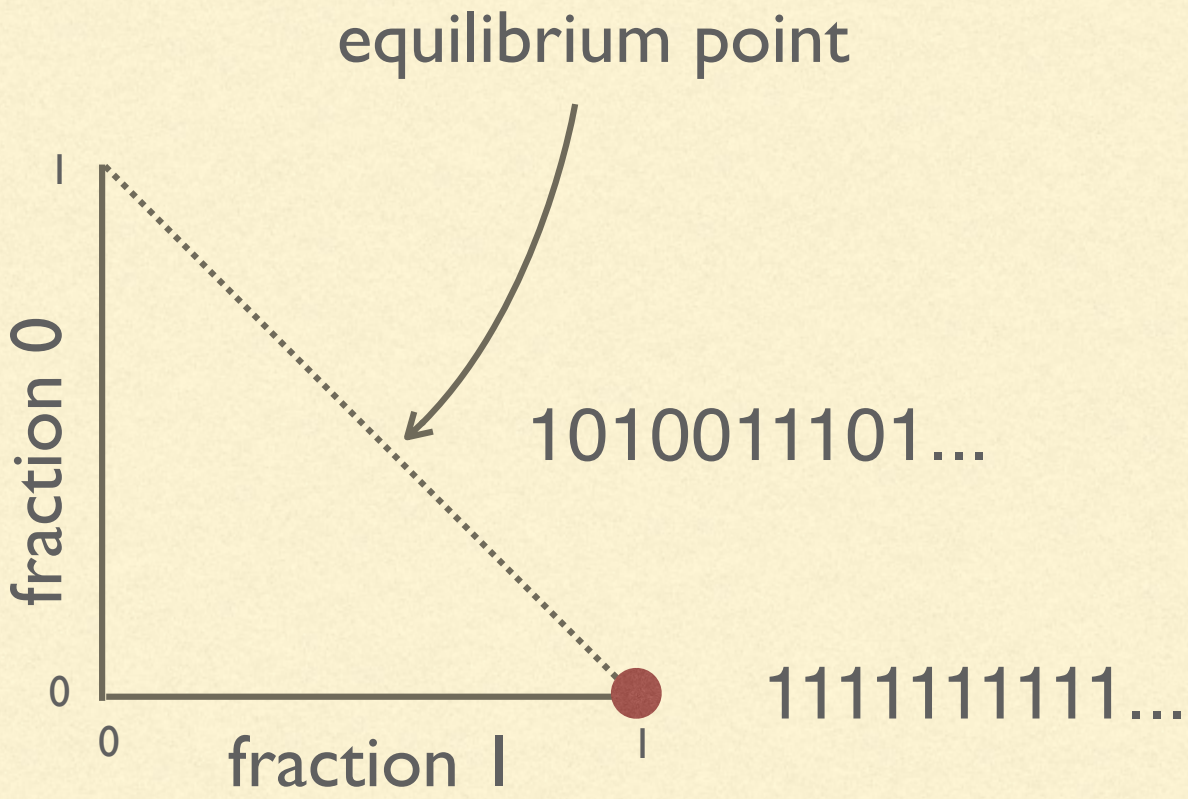
$$\begin{bmatrix} \frac{1}{2} + \frac{1}{2}e^{-2\beta t} & \frac{1}{2} - \frac{1}{2}e^{-2\beta t} \\ \frac{1}{2} - \frac{1}{2}e^{-2\beta t} & \frac{1}{2} + \frac{1}{2}e^{-2\beta t} \end{bmatrix}$$

$$\begin{bmatrix} \frac{\sqrt{2}}{2} & -\frac{\sqrt{2}}{2} \\ \frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \end{bmatrix} \begin{bmatrix} e^{0} & 0 \\ 0 & e^{-2\beta t} \end{bmatrix} \begin{bmatrix} \frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \\ -\frac{\sqrt{2}}{2} & \frac{\sqrt{2}}{2} \end{bmatrix}$$

# 2-state version

## Q matrix

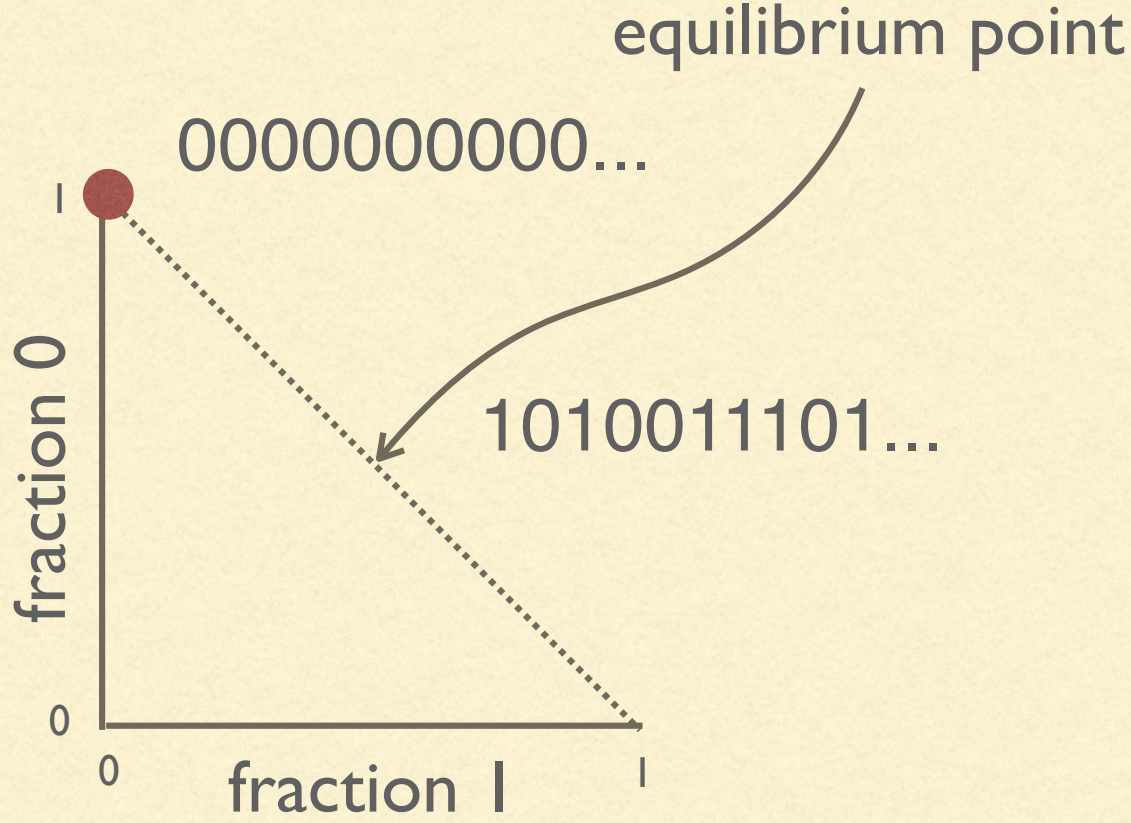
$$\begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta & \beta \\ \beta & -\beta \end{bmatrix} \end{array}$$




## P matrix

$$\begin{bmatrix} \frac{1}{2} + \frac{1}{2}e^{-2\beta t} & \frac{1}{2} - \frac{1}{2}e^{-2\beta t} \\ \frac{1}{2} - \frac{1}{2}e^{-2\beta t} & \frac{1}{2} + \frac{1}{2}e^{-2\beta t} \end{bmatrix}$$

# 2-state version




## Q matrix

$$\begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} -\beta & \beta \\ \beta & -\beta \end{bmatrix} \end{array}$$

## P matrix

$$\begin{bmatrix} \frac{1}{2} + \frac{1}{2}e^{-2\beta t} & \frac{1}{2} - \frac{1}{2}e^{-2\beta t} \\ \frac{1}{2} - \frac{1}{2}e^{-2\beta t} & \frac{1}{2} + \frac{1}{2}e^{-2\beta t} \end{bmatrix}$$

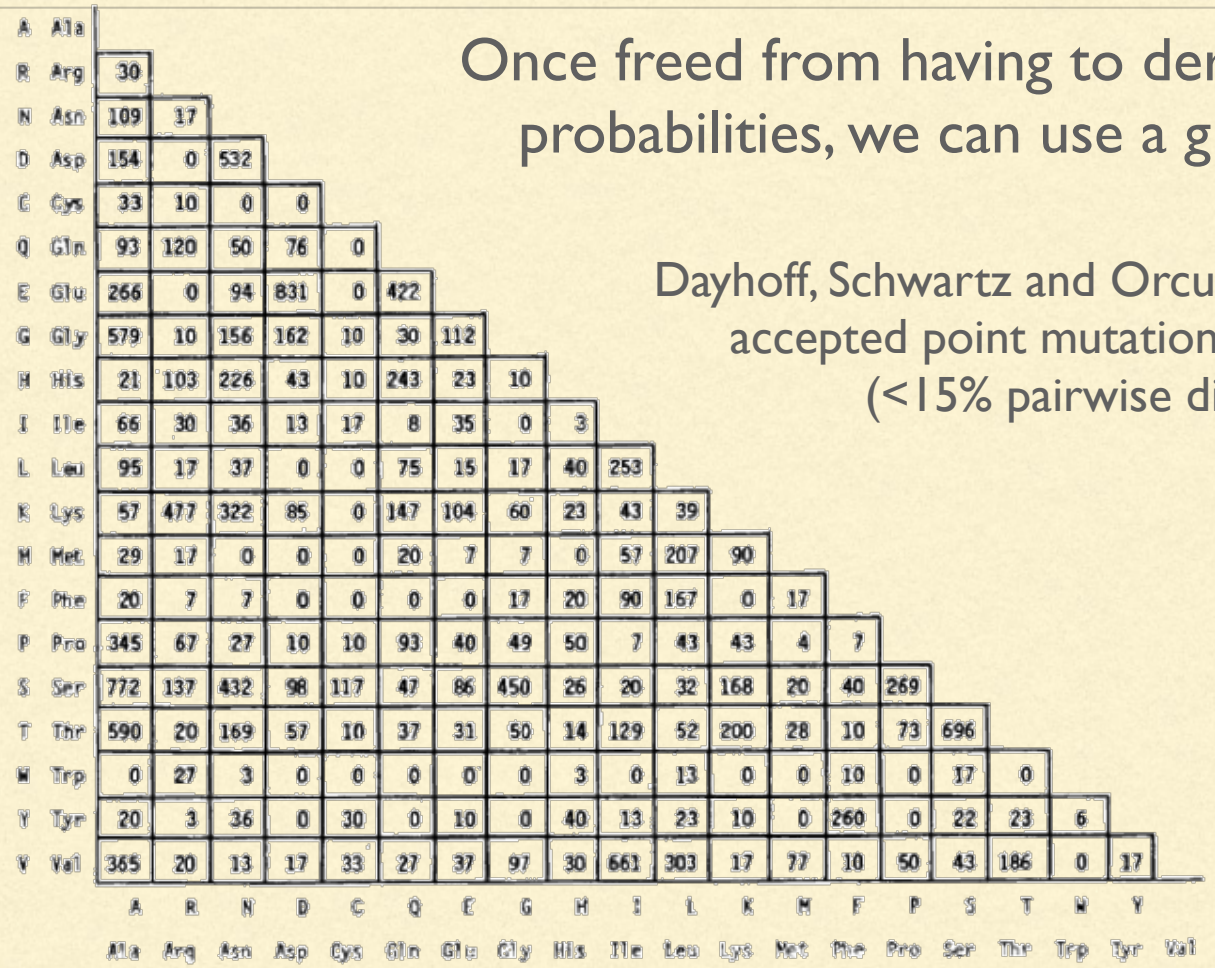
# Diagonalization demo

https://plewis.github.io/applets/diagonalization/

(inspired by Grant Sanderson's visual explanations at https://www.3blue1brown.com)

# Empirical amino acid models

# A different path from Q to P



| | | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Ala | | | | | | | | | | | | | | | | | | | |
| R | Arg | 30 | | | | | | | | | | | | | | | | | | |
| N | Asn | 109 | 17 | | | | | | | | | | | | | | | | | |
| D | Asp | 154 | 0 | 532 | | | | | | | | | | | | | | | | |
| C | Cys | 33 | 10 | 0 | 0 | | | | | | | | | | | | | | | |
| Q | Gln | 93 | 120 | 50 | 76 | 0 | | | | | | | | | | | | | | |
| E | Glu | 266 | 0 | 94 | 831 | 0 | 422 | | | | | | | | | | | | | |
| G | Gly | 579 | 10 | 156 | 162 | 10 | 30 | 112 | | | | | | | | | | | | |
| H | His | 21 | 103 | 226 | 43 | 10 | 243 | 23 | 10 | | | | | | | | | | | |
| I | Ile | 66 | 30 | 36 | 13 | 17 | 8 | 35 | 0 | 3 | | | | | | | | | | |
| L | Leu | 95 | 17 | 37 | 0 | 0 | 75 | 15 | 17 | 40 | 253 | | | | | | | | | |
| K | Lys | 57 | 477 | 322 | 85 | 0 | 147 | 104 | 60 | 23 | 43 | 39 | | | | | | | | |
| M | Met | 29 | 17 | 0 | 0 | 0 | 20 | 7 | 7 | 0 | 57 | 207 | 90 | | | | | | | |
| F | Phe | 20 | 7 | 7 | 0 | 0 | 0 | 0 | 17 | 20 | 90 | 167 | 0 | 17 | | | | | | |
| P | Pro | 345 | 67 | 27 | 10 | 10 | 93 | 40 | 49 | 50 | 7 | 43 | 43 | 4 | 7 | | | | | |
| S | Ser | 772 | 137 | 432 | 98 | 117 | 47 | 86 | 450 | 26 | 20 | 32 | 168 | 20 | 40 | 269 | | | | |
| T | Thr | 590 | 20 | 169 | 57 | 10 | 37 | 31 | 50 | 14 | 129 | 52 | 200 | 28 | 10 | 73 | 696 | | | |
| W | Trp | 0 | 27 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 13 | 0 | 0 | 10 | 0 | 17 | 0 | | |
| Y | Tyr | 20 | 3 | 36 | 0 | 30 | 0 | 10 | 0 | 40 | 13 | 23 | 10 | 0 | 260 | 0 | 22 | 23 | 6 | |
| V | Val | 365 | 20 | 13 | 17 | 33 | 27 | 37 | 97 | 30 | 661 | 303 | 17 | 77 | 10 | 50 | 43 | 186 | 0 | 17 |

| A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ala | Arg | Asn | Asp | Cys | Gln | Glu | Gly | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Trp | Tyr | Val |

Figure 80. Numbers of accepted point mutations (×10) accumulated from closely related sequences. Fifteen hundred and seventy-two exchanges are shown. Fractional exchanges result when ancestral sequences are ambiguous.

Once freed from having to derive formulas for transition probabilities, we can use a great variety of Q matrices.

Dayhoff, Schwartz and Orcutt (1978; DSO78) identified 1572 accepted point mutations using closely-related sequences (<15% pairwise divergence), producing this matrix.

Kosiol and Goldman (2005) discussed ways of estimating a Q matrix from these numbers.

# The elements of Q

The Q matrix is often presented in the following form, factored into a symmetric matrix exchangeabilities and a set of state frequencies.

```
Ala
Arg  0.267828
Asn  0.984474  0.327059
Asp  1.199805  0.000000  8.931515
Cys  0.360016  0.232374  0.000000  0.000000
Gln  0.887753  2.439939  1.028509  1.348551  0.000000
Glu  1.961167  0.000000  1.493409 11.388659  0.000000  7.086022
Gly  2.386111  0.087791  1.385352  1.240981  0.107278  0.281581  0.811907
His  0.228116  2.383148  5.290024  0.868241  0.282729  6.011613  0.439469 ...
Ile  0.653416  0.632629  0.768024  0.239248  0.438074  0.180393  0.609526 ...
Leu  0.406431  0.154924  0.341113  0.000000  0.000000  0.730772  0.112880 ...
Lys  0.258635  4.610124  3.148371  0.716913  0.000000  1.519078  0.830078 ...
Met  0.717840  0.896321  0.000000  0.000000  0.000000  1.127499  0.304803 ...
Phe  0.183641  0.136906  0.138503  0.000000  0.000000  0.000000  0.000000 ...
Pro  2.485920  1.028313  0.419244  0.133940  0.187550  1.526188  0.507003 ...
Ser  4.051870  1.531590  4.885892  0.956097  1.598356  0.561828  0.793999 ...
Thr  3.680365  0.265745  2.271697  0.660930  0.162366  0.525651  0.340156 ...
Trp  0.000000  2.001375  0.224968  0.000000  0.000000  0.000000  0.000000 ...
Tyr  0.244139  0.078012  0.946940  0.000000  0.953164  0.000000  0.214717 ...
Val  2.059564  0.240368  0.158067  0.178316  0.484678  0.346983  0.367250 ...
         Ala       Arg       Asn       Asp       Cys       Gln       Glu ...
```

exchangeabilities

(only values below diagonal shown)

```
Freq 0.087127  0.040904  0.040432  0.046872  0.033474  0.038255  0.049530 ...
```

frequencies

# GTR revisited

$$\mu \begin{bmatrix} - & \pi_C a & \pi_G b & \pi_T c \\ \pi_A a & - & \pi_G d & \pi_T e \\ \pi_A b & \pi_C d & - & \pi_T f \\ \pi_A c & \pi_C e & \pi_G f & - \end{bmatrix}$$

The off-diagonal elements of the GTR Q matrix can similarly be obtained by multiplying a symmetric exchangeability matrix and a diagonal matrix of frequencies.

$$\mu \begin{bmatrix} - & a & b & c \\ a & - & d & e \\ b & d & - & f \\ c & e & f & - \end{bmatrix} \begin{bmatrix} \pi_A & 0 & 0 & 0 \\ 0 & \pi_C & 0 & 0 \\ 0 & 0 & \pi_G & 0 \\ 0 & 0 & 0 & \pi_T \end{bmatrix}$$

exchangeabilities frequencies

# What does all this accomplish?

- An empirical Q matrix can be constructed from many closely-related pairwise comparisons
- A Q matrix can be extrapolated to any desired value of *t* using P=exp(Qt) to generate a P matrix
- Models generic features of protein evolution; Q matrix does not necessarily reflect your particular sequences
- Frequencies can be swapped with more appropriate set (locally estimated)

# Successive improvements

- JTT model (Jones et al. 1992)

  Based on a much larger protein database

- WAG model (Whelan & Goldman 2001)

  Avoids need to use closely-related sequence pairs by obtaining ML estimate of Q matrix

- LG model (Le & Gascuel 2008)

  Add rate heterogeneity to ML estimation of Q matrix

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