

Multiple substitutions

- The model assumes that point mutations alter one nucleotide at a time, hence most of the instantaneous rates:
 - (3134/3761 or 84.2% in the case of the universal genetic code) are 0.
- This restriction, however, does not mean that the model disallows any substitutions that involve multiple nucleotides (e.g., **ACT** \Rightarrow **AGG**).
 - This can be further relaxed with models supporting multiple nucleotide changes.
- Such substitutions must simply be realized via several single nucleotide steps, e.g., **ACT** \Rightarrow **AGT** \Rightarrow **AGG**
- In fact the (i, j) element of $\mathbf{T}(t) = \exp(\mathbf{Q}t)$ sums the probabilities of all such possible pathways of duration t , including reversions
- Compare this to the naive NG86 parsimony approach.

Alignment-wide estimates

- Using standard MLE approaches it is straightforward to obtain point estimates of $dN/dS := \beta/\alpha$
- Can also easily test whether or not $dN/dS > 1$, or < 1 using the likelihood ratio test (LRT)
- Codon models also support the concepts of synonymous and non-synonymous distances between sequences using standard properties of Markov processes (exponentially distributed waiting times)