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`**⟹**`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`**⇒**`AGT`**⟹**`AGG`**
- In fact the **`(i,j)`** element of **`T(t) = exp(Qt)`** 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 := β/α`**
- 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)

$$E[subs] = -\sum_i \pi_i \hat{q}_{ii}, \quad E[subs] = E[syn] + E[nonsyn] = -\sum_i \pi_i \hat{q}^s_{ii} - \sum_i \pi_i \hat{q}^{ns}_{ii}.$$