NG86 example

- The observed **`N/S`** ratio (**`1.0`**) is **lower** than the expected **`EN/ES`** ratio (**`4.05`**).
- The ratio of the ratios **`(N:S)/(EN:ES)`** yields **`dN/dS = 1/4.05 ~ 0.25.`**
- This ratio quantifies the ***excess*** or ***paucity*** of non-synonymous substitutions and is near **`dN/dS = 1`** for neutrally evolving sequences/sites.
- Because there are **fewer** non-synonymous substitutions than expected under neutrality, we conclude that most non-synonymous mutations are **removed by natural selection**, i.e., the sequences are under **negative selection**
- **If there were more** non-synonymous substitutions than expected, we would conclude that many non-synonymous mutations are **fixed due to natural selection**, i.e., the sequences are under **positive selection**

NG86 limitations: multiple substitutions

- How many synonymous and how many non-synonymous substitutions does it take to replace **`CCA`** with **`CAG`**?
- **Assume** the shortest path (minimum of 2 substitutions)
  - **`CCA (Proline)`** ⟹ **`CAA (Histidine)`** ⟹ **`CAG (Glutamine)`**
  - **`CCA (Proline)`** ⟹ **`CCG (Proline)`** ⟹ **`CAG (Glutamine)`**
- Average over the two possible paths: `0.5` synonymous and `1.5` non-synonymous substitutions.
- Intuitively, paths should **not** be equiprobable, e.g., because it should be more expensive to route evolution through (presumably) suboptimal intermediate amino-acids.