NG86 example

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Seq1	**ACA**	**ATA**	**ATC**	**TTT**	**AAT**	**CAA**
Syn	1	2/3	2/3	1/3	1/3	1/3
NonSyn	2	7/3	7/3	8/3	8/3	7/3
Seq2	**ACA**	**ATA**	**ACC**	**TTT**	**AAC**	**CAA**
Syn	1	2/3	1	1/3	1/3	1/3
NonSyn	2	7/3	2	8/3	8/3	7/3
Syn	**1**	**2/3**	**5/6**	**1/3**	**1/3**	**1/3**
NonSyn	**2**	**7/3**	**13/6**	**8/3**	**8/3**	**7/3**

Mean

ES = 3½, EN = 14⅙: under neutrality, we **expect** the ratio of non-synonymous to synonymous substitutions of **EN/ES ~ 4.05**

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**