

NG86 example

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Seq1	<u>ACA</u>	<u>ATA</u>	<u>ATC</u>	<u>TTT</u>	<u>AAT</u>	<u>CAA</u>
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	<u>ACA</u>	<u>ATA</u>	<u>ACC</u>	<u>TTT</u>	<u>AAC</u>	<u>CAA</u>
Syn	1	2 / 3	1	1 / 3	1 / 3	1 / 3
NonSyn	2	7 / 3	2	8 / 3	8 / 3	7 / 3
Mean	Syn	1	2 / 3	5 / 6	1 / 3	1 / 3
	NonSyn	2	7 / 3	13 / 6	8 / 3	7 / 3

ES = $3\frac{1}{2}$, **EN** = $14\frac{1}{6}$: under neutrality, we expect the ratio of non-synonymous to synonymous substitutions of **EN/ES** ~ 4.05

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- 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 $dN/dS = 1/4.05 \sim 0.25$.
- 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**