

NG86 example

Mean	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

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 $(\mathbf{N:S}) / (\mathbf{EN:ES})$ yields $\mathbf{dN/dS} = 1/4.05 \sim 0.25$.
- This ratio quantifies the **excess** or **paucity** of non-synonymous substitutions and is near $\mathbf{dN/dS} = 1$ 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**