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	ACA	<u>ATA</u>	ACC	<u>TTT</u>	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 = $3\frac{1}{2}$, EN = $14\frac{1}{6}$: under neutrality, we <u>expect</u> 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