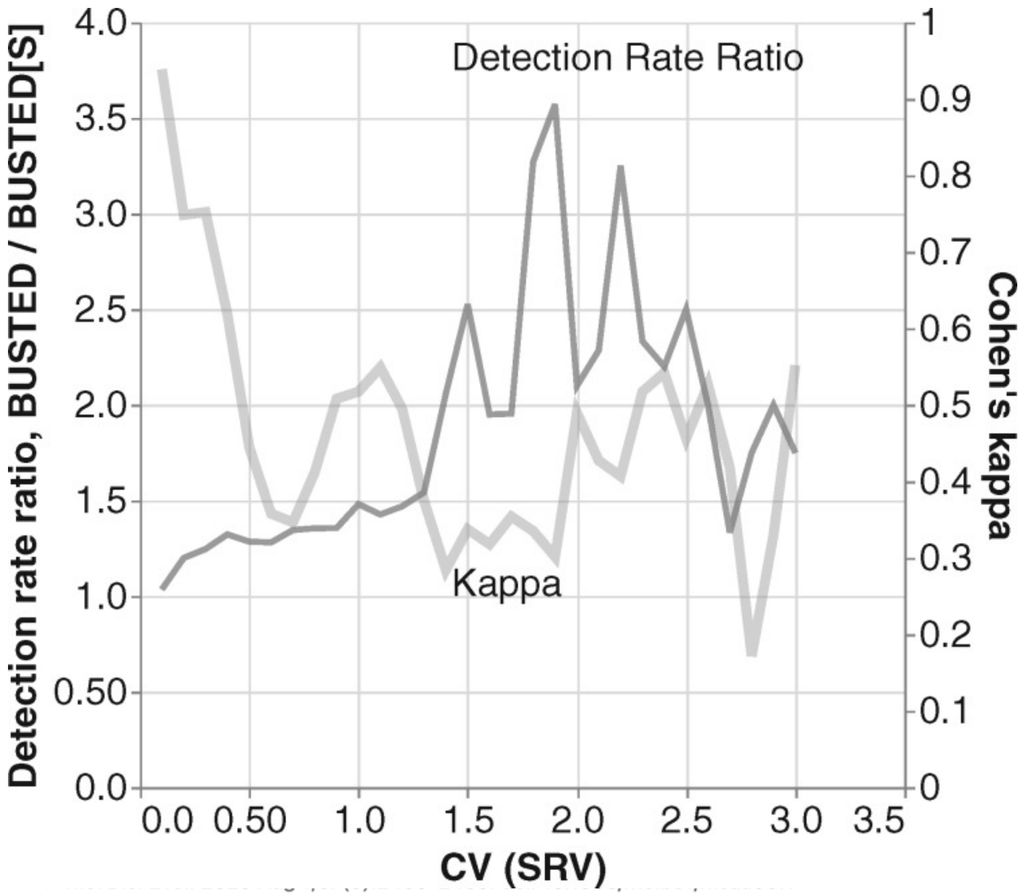
# Synonymous rate variation

- **dS** = constant for all sites (assumed by many models); this assumption appears to be nearly universally violated in biological data, due to e.g. secondary structure, localized codon usage bias, overlapping reading frames, etc.
- This can lead to, e.g. incorrect identification of relaxed constraint as selection and hight false positive rates
- Most of HyPhy methods provide support for including dS




**Synonymous Site-to-Site Substitution Rate Variation Dramatically Inflates False Positive Rates of Selection Analyses: Ignore at Your Own Peril**

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# Allowing multi-nucleotide substitutions

- Some of the methods (e.g. BUSTED, aBSREL, RELAX) can extend substitution models to allow instantaneous double- and triple-“hits” (e.g. ACC to AGG)
- Sometimes multi-nucleotide changes along short branches at a single site can drive selection signal (possible false positives?)
- HyPhy includes a simple standard analysis for estimating alignment-wide multiple-hit rates.

Extra base hits: Widespread empirical support for instantaneous multiple-nucleotide changes

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See the preprint