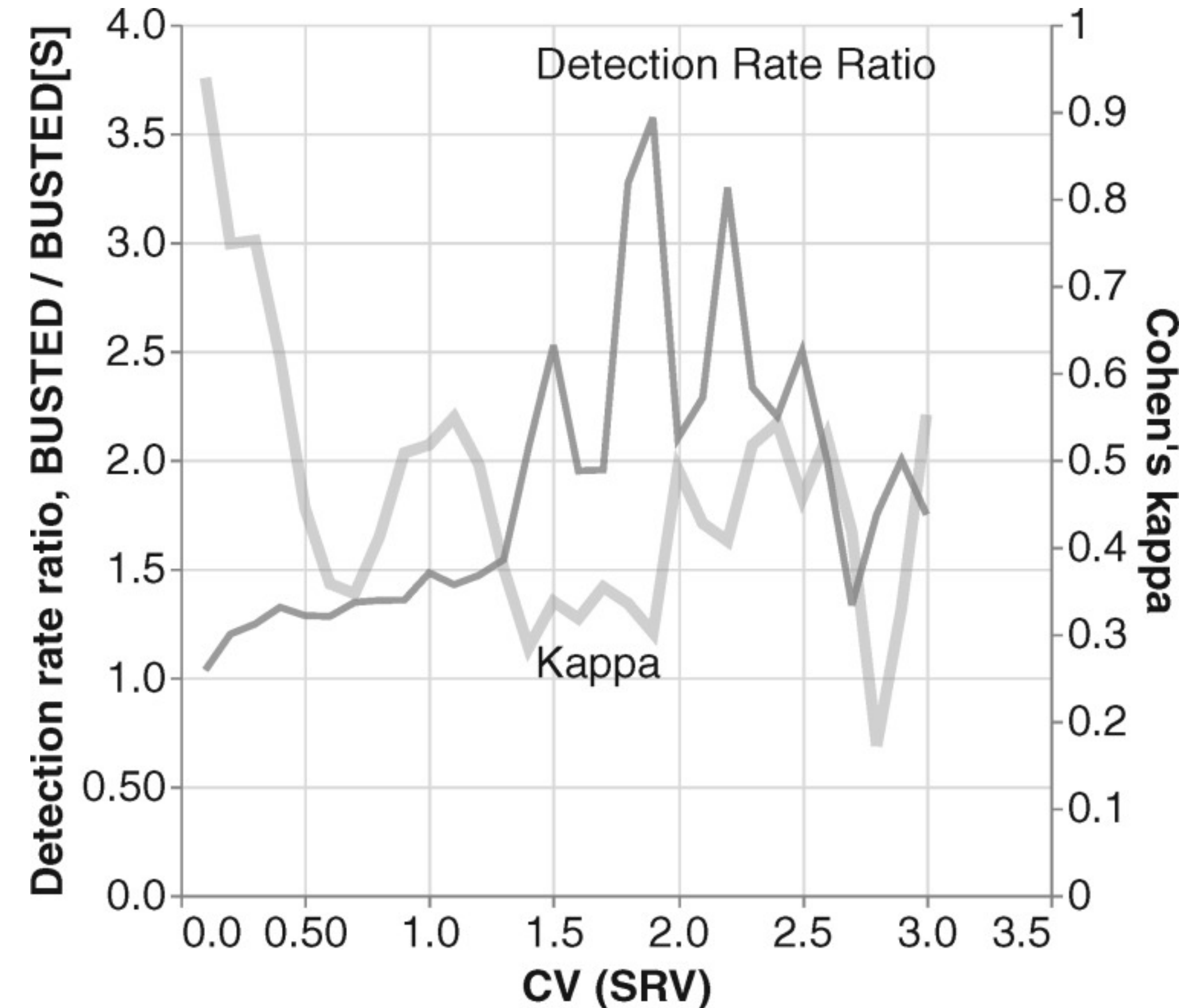


Synonymous rate variation

- **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 high 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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Affiliations + expand

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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.

JOURNAL ARTICLE

Evolutionary Shortcuts via Multinucleotide Substitutions and Their Impact on Natural Selection Analyses

Alexander G Lucaci, Jordan D Zehr, David Enard, Joseph W Thornton, Sergei L Kosakovsky Pond 

Molecular Biology and Evolution, Volume 40, Issue 7, July 2023, msad150,
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Extra base hits: Widespread empirical support for instantaneous multiple-nucleotide changes

Alexander G. Lucaci , Sadie R. Wisotsky , Stephen D. Shank, Steven Weaver, Sergei L. Kosakovsky Pond 

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[See the preprint](#)