Based on the likelihood ratio test, there **is** evidence of *episodic diversifying* selection in this dataset (p=0.01901).

BUSTED analysis (v4.0) was performed on the alignment from /Users/sergei/Dropbox/Talks/VEME-current/data/spike.fas using HyPhy v2.5.40. This analysis **did not include** site-to-site synonymous rate variation.

Suggested citation: Gene-wide identification of episodic selection, Mol Biol Evol. 32(5):1365-71, Synonymous Site-to-Site Substitution Rate Variation Dramatically Inflates False Positive Rates of Selection Analyses: Ignore at Your Own Peril, Mol Biol Evol. 37(8):2430-2439

threshold	10	Update			
118 sequences in the alignment	•	1273 codon sites in the alignment	•••	1 partitions	^
162 median branches/pa used for testing	rtition	3 classes non-synonymous rate variation		None synonymous rate variation	
0.019 p-value for episodic diversityfing selection		11 Sites with ER≥10 for positive selection	4	N/A:N/A Multiple hit rates (2H:3H)	Ф

Alignment-wide results

Evidence ratio

Model AIC-c Params. Rate distribution Rate plot Log (L) Unconstrained model -9287.35 18937.1 181 **Tested ω** 0.000 (0.45262%) 0.2561 (97.532%) 14.88 (2.0156%) Mean = 0.5497, CoV = 3.740-9290.62 18941.7 Constrained model 180 Tested ω 0.000 (46.919%) 1.000 (2.2974%) 1.000 (50.784%) Mean = 0.5308, CoV = 0.94020.0 0.2 0.4 0.60.81.0

Gene-wide selection analysis using a branch-site method (BUSTED), **SARS-CoV-2** spike

hyphy busted --srv No --alignment data/spike.fas --tree data/ spike.tree --starting-points 5

Produces HIV-sets.fas.BUSTED.json file View in http://vision.hyphy.org/BUSTED or https://observablehq.com/@spond/busted

BUSTED analysis

West Nile Virus NS3 protein

- No statistical support for selection; ML point estimate allocates a small proportion of sites (~1%) to the selected group (dN/dS ~ 2)
- The rest of the gene is very strongly conserved (dN/dS = 0.004)

HIV-1 transmission pair

Very strong evidence of strong episodic diversification (dN/dS ~ 100) on a small proportion of sites (2%)

• The rest of the gene evolves with weak purifying selection (dN/dS = 0.6-0.7)

SARS-CoV-2 spike

- Evidence of episodic diversification (dN/dS ~ 15) on a small proportion of sites (~2%)
- Most of the rest of the gene evolves with purifying selection (dN/dS = 0.2)