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**dN/dS-based methods detect positive selection linked to trade-offs between fitness traits in the coat protein of *Potato virus Y***

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The dN/dS ratio between nonsynonymous and synonymous substitution rates is used extensively to identify codon positions involved in adaptation. However, the accuracy of this approach is questioned and few studies have attempted to validate experimentally its predictions. Using the coat protein (CP) of *Potato virus Y* (PVY), we identified several positively-selected codon positions with the softwares PAML and HyPhy. These positions differed between PVY clades. In the N clade of PVY, positive selection was detected at codon positions 25 and 68. Nonsynonymous substitutions were introduced at these positions in an infectious cDNA clone of PVY and the effect of these mutations on virus accumulation in its two major cultivated hosts, tobacco and potato, and on its efficiency of transmission from plant to plant by aphid vectors was measured. The mutation at codon position 25 significantly modified the virus accumulation in the two hosts while the mutation at codon position 68 significantly modified the virus accumulation in potato and its transmissibility by aphids. Both mutations were involved in trade-offs between different fitness traits and we suggest that detection of positive selection is particularly efficient in this case because these trade-offs impede the fixation of mutations and maintain polymorphism within populations.