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Response to Comment on “Large-Scale Sequence Analysis of Avian Influenza Isolates”

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We repeated the dN/dS analysis as described by Holmes *et al.* using the methods and data as described in our original report. We obtained essentially the same results and agree that it cannot be concluded from dN/dS calculations whether PB1-F2 is under positive, negative, or neutral selection.

We are delighted that Holmes *et al.* (1) have conducted a comprehensive analysis of the nonsynonymous/synonymous substitution ratios (dN/dS) for PB1-F2. As a result of their efforts, we have repeated our analysis of dN/dS for avian influenza PB1-F2 proteins by dividing the PB1

sequence alignment into four gene regions as described in (1) but using the same data and analysis method [phylogenetic analysis by maximum likelihood (PAML)] used in our study (2). Our results are essentially the same as those reported by Holmes *et al.* (Table 1). Thus, our reported high positive selection value for PB1-F2 is indeed likely to be an artifact due to a strong purifying selection by PB1 and a high tolerance for substitutions in PB1-F2. We are pleased that this has been brought to the community's attention, because other publications reporting positive selection for PB1-F2

may also be in error for this same reason. Those doing this type of analysis on transcripts in which the same mRNA is used to code for multiple products in alternate reading frames need to exercise caution as, to our knowledge, none of the programs in general use provide information about positive selection for overlapping genes. We agree that it cannot be concluded from dN/dS calculations whether PB1-F2 is under positive, negative, or neutral selection in this context.

References

1. E. C. Holmes, D. J. Lipman, D. Zamarin, J. W. Yewdell, *Science* **313**, 1573 (2006); www.sciencemag.org/cgi/content/full/313/5793/1573b.
2. J. C. Obenauer *et al.*, *Science* **311**, 1576 (2006).

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Table 1. Nonsynonymous/synonymous ratios in avian influenza PB1 coding regions.

Data sets	Number of codons	dN/dS
PB1-F1 single coding	668	0.025
PB1-F1 double coding	91	0.023
PB1-F2	90	9.365
PB1-F2 control	95	14.188

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