

## Reply to Nozawa et al.: Complementary statistical methods support positive selection of a duplicated UV opsin gene in *Heliconius*

Statistical methods used to test for positive selection have a long history and continue to evolve (1–4). In their letter, Nozawa et al. (5) question our use of the branch-site method in our recent paper in PNAS (6). As experimental biologists, we welcome all methods that facilitate the detection of interesting parts of the genome for functional exploration. In this case, a statistically significant result using the branch-site method combined with structural modeling and the identification of a few biochemically relevant substitutions provided us with an incentive for the in vivo physiological characterization of the UV-sensitive rhodopsins in *Heliconius*. Had we not had that first hint from sequence data alone, it is unlikely that we would have made that functional discovery, which makes the eyes of *Heliconius* unique compared with all other studied butterflies.

In turn, the finding of two spectrally distinct UV-sensitive rhodopsins gave us an incentive to make an even more interesting biological observation: namely, that *Heliconius* butterflies have evolved the capacity to display UV-yellow colors on their wings in contrast to the yellow colors displayed on the wings of close relatives lacking the UV opsin duplicate. Visual modeling of how butterflies perceive color indicates this has resulted in an expansion of the number of distinctive colors on *Heliconius* wings. If *Heliconius* do indeed use the two functionally distinct UV-sensitive rhodopsins together in the context of species recognition, then this might help explain why *Heliconius* are an example of an adaptive radiation, whereas their close relatives have remained more limited in species number

and wing-color pattern variety. Last, we note that although the major findings of our paper do not rest on statistics alone, application of the small-sample method (1) to an expanded collection of sequences does indeed support the robustness of our initial result using the branch-site method.

In isolation, none of these individual observations would have much meaning, but together, they paint a fascinating picture of the biology of these beautiful butterflies. We look forward to applying statistical work by Nozawa et al. and Yang et al. (2–5) in this field to our system.

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The authors declare no conflict of interest.

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