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Response to Comments on "Molecular Phylogenies Link Rates of Evolution and Speciation"

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TECHNICAL COMMENT

Response to Comments on "Molecular Phylogenies Link Rates of Evolution and Speciation"

Neither Witt and Brumfield (*1*) nor Brower (*2*) reanalyzed any of our data, nor did they investigate our statistical methods mathematically. Had they done so, they would have appreciated that our results (*3*) are not expected to be either systematically affected by the node density artifact or by sparse taxon sampling. Our inclusion of a wide range of taxa demonstrates that the results we found are not confined to the limited range of taxa in previous studies, but rather appear to apply generally.

The node-density effect produces a curvilinear relationship between nodes n and path lengths x in the phylogenetic tree of the form $n = x^{\delta}$, such that $\delta > 1$. Contrary to remarks by Witt and Brumfield (*1*), values of $\delta = 1$ are not compatible with the artifact. We explained this point in (*3*) (supplementary online text), and it is easily proven mathematically. The paper by Fitch and Bruschi (*4*) cited in (*1*), which attempted to correct for the node density effect by adjusting paths using a linear rule of thumb (equivalent to $\delta = 1$), may have given the impression that the node density effect is expected to produce a linear relationship. It is not expected to, and Fitch and Bruschi did not suggest that it did. In a later paper, Fitch and Beintema (*5*) showed a clearer example of the curvilinear form of the relationship [see also (*6*)]. As stated in (*3*), we excluded all trees with $\delta > 1$, regardless of whether the value of δ was statistically significant. Thus, we applied a conservative inclusion criterion and there is no reason to believe that the node density effect may have biased our results.

Witt and Brumfield propose that error in specifying phylogenetic trees systematically biases towards finding a relationship between rates of evolution and speciation. There is no reason to expect error to affect all of our trees in the same direction. Rather, error in specifying trees will, in general, add noise to our results. Nevertheless, we asserted in (*3*) that a significant association between speciation and genetic evolution could arise in any one of our trees from chance events, including misspecifiying a tree. We used the Kolmogorov-Smirnov test to show that our set of results was highly unlikely if the null hypothesis were true. Brower (*2*) argues that a correlation in 30 to 50% of cases is not surprising. As stated in (*3*), the Kolmogorov-Smirnov statistic shows that our set of results is only expected to arise by chance five times in a million. We therefore remain confident that this indicates that there is an important phenomenon to explain.

Both comments assert that sparsely sampled phylogenies cannot be used to test our hypothesis. However, our results do not depend on measuring the rate of speciation per se. Rather, they rest upon whether differences in the net speciation rate amongst lineages influence the net amount of genetic change. Sparse taxon sampling acts to mask any punctuated molecular clock, and thus, inclusion of sparsely sampled trees makes our results more conservative. Biased sparse taxon sampling produces the node density effect, and so would have been detected by our δ-test. We discussed these issues in the fourth and final paragraphs in (*3*). The recommendation by Witt and Brumfield to use thoroughly sampled phylogenies, though desirable in that they lend greater statistical power, is not necessary for studying the phenomenon we reported.

In a similar vein, Brower remarks that branch lengths among families and orders "have little to do with the rate of genetic evolution attending individual speciation events." These branch lengths, like all others on a phylogenetic tree, represent the accumulated changes over many historical speciation events and so are just as directly related to our hypothesis as later events among contemporary species. All of our trees have branch lengths estimated from genetic data where the length of the branch is a measure of the expected amount of evolutionary change. Brower is correct to say that, of our 56 trees, two use allozyme data and one is based upon microsatellites—but this makes no difference to our interpretations or conclusions. Measures of rates of evolution need not be confined to gene sequences per se, as allozyme and microsatellite differences also accumulate as a function of time and rate of underlying evolution.

Brower also questions our inclusion of the Zamudio study (*7*) of 37 mtDNA haplotypes among three closely related species of horned lizard because it includes intraspecific data. Intraspecific data is not necessarily irrelevant, and Brower acknowledges that mtDNA can exhibit phylogenetic structure among populations. Our point in using this tree was that intraspecific phylogenetic structure arising from diverging populations may reveal incipient speciation events—and where this happens, we are justified in examining rates of genetic change. There is no reason to sideline the early intraspecific genetic differentiation as different in kind from the later interspecific differentiation; they form a continuum. Witt and Brumfield question our inclusion of bacterial and viral phylogenies on grounds that the species concept is less clear for them. This may or may not be true, but it should not preclude asking whether differences in lineage or species branching rates are related to the amount of genetic change.

We deliberately included partially overlapping data sets to ensure that our results were not particular to a given tree or set of gene sequences. This proved to be true and, contrary to the suggestion by Witt and Brumfield, it did not inflate our estimates of the link between speciation and genetic change. Thus, for example, two artiodactyl trees agree on no effect, and the two plant data sets agree that there is no effect for two different measures of genetic change on the same species. Two similar mammal-wide trees agree that there is an effect using different concatenated gene-sequence data sets.

We estimated the branch lengths of the Barraclough *et al*. (*8*) phylogeny from their sequence data using a GTR model of evolution [supplementary online text for (*3*)]. R. Baker supplied branch lengths for the Baker *et al*. tree (*9*), and we estimated branch lengths for the Jerminn *et al*. data (*10*) using a log-Det model. The log-Det model is useful when analyzing data with compositional heterogeneity of base frequencies, such as these data show. Brower argued that our analytical methods were not sufficiently described. On the contrary, the supplementary online text for (*3*) includes all of the equations for the statistical model, the NEXUS tree files for all of our analyses, and a reference for the computer program to do the all of the calculations.

In summary, we used a detailed statistical model and strict exclusion criteria to identify data sets in which rates of evolution are linked to rates of speciation in a statistically significant way. Our results appear to apply generally across a range of taxa. Whether different taxa exhibit the effect to different degrees will be an interesting topic for future studies.

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References

1. C. C. Witt, R. T. Brumfield, *Science* **303**, 173 (2004); www.sciencemag.org/cgi/content/full/303/5655/173b
2. A. V. Z. Brower, *Science* **303**, 173 (2004); www.sciencemag.org/cgi/content/full/303/5655/173c
3. A. J Webster, R. J. H. Payne, M. Pagel, *Science* **301**, 478 (2003).
4. W. Fitch, M. Bruschi, *Mol. Biol. Evol.* **4**, 381 (1987).
5. W. Fitch, J. J. Beintema, *Mol. Biol. Evol.* **7**, 438 (1990).
6. R. D. M. Page, E. Holmes, in *Molecular Evolution: A Phylogenetic Approach* (Blackwell Science, Oxford, UK, 1998), pp. 168-169.
7. K. R. Zamudio, *Evolution* **52**, 1821 (1998).
8. T. G. Barraclough, J. E. Hogan, A. P. Vogler, *Proc. R. Soc. London Ser. B* **266**, 1061 (1999).
9. R. H. Baker, G. S. Wilkinson, R. DeSalle, *Syst. Biol.* **50**, 87 (2001).
10. L. S. Jermiin *et al.*, *J. Mol. Evol.* **39**, 160 (1994).

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