Constraining fossil calibrations for molecular clocks

Sir,

In a recent paper, Müller and Reisz(1) proposed how fossil calibrations should be selected for application in molecular clock studies. The topic is of interest to those, like ourselves, who are actively engaged in reconstructing the tree of life using molecules. Nonetheless, we believe that these authors have erred both in their proposal of fossil calibration constraints and in their characterization of debates in the field. Their paper is an extension of one such debate that took place in another journal, where we responded(2) to some of their initial criticisms(3) of our work. Here, we respond to their additional criticisms(1) and to their proposal for constraining fossil calibrations.

First, Müller and Reisz(1) defined two opposing camps in this field: researchers favoring multiple fossil dates versus those "preferring to use only a single fossil date." They placed themselves and others, including Conroy and van Tuinen(4) in the former and only Hedges and Kumar(2) in the latter. But this distinction is false and unfortunate. Of course, one should use as many reliable calibrations as are available. At the same time, there is nothing inherently wrong with using a single calibration point when no other robust calibrations are available or useable due to a lack of DNA sequence data. Like others, we have used both single and multiple calibrations(5,6) and we stated previously: "we never advocated the use of only a single calibration point."(2) In fact, our comment here is authored by members of the two purported camps defined by Müller and Reisz to further demonstrate that their claim is erroneous.

Müller and Reisz(1,3) have also argued that the split of birds and mammals, which we used in several studies,(7,8) is a poor calibration point. This is not because they disagree with the date used—their own estimate of 313 Million years ago (Ma)(3) as the minimum time is virtually identical to the date used by us (310 Ma)—but because they believe that the maximum constraint on that date is difficult to assess and could be tens of millions of years older. But Müller and Reisz also fail to point out that we were even more conservative in our proposal of that calibration point when we stated that the transition from fishes to tetrapods at 360–370 Ma provided a maximum constraint.(7,8) When we pointed this out previously in our response to Reisz and Müller,(2) they responded, surprisingly, by criticizing the general notion of "transitions" in the fossil record,(9) a term and concept well-established in paleontology.(10,11) Also, they claimed that we were making non-scientific inferences (e.g. literally, fossils on lineages) despite our citation of scientific literature showing otherwise.

Müller and Reisz(1,3) propose that molecular evolutionists should abandon the bird–mammal calibration and instead use four other calibrations: lungfish–tetrapod (408–419 Ma), bird–lizard (252–257 Ma), bird–crocodilian (251–243 Ma), and alligator–caiman (71–66 Ma). This proposal is unusual in two regards: (1) the time ranges are extraordinarily narrow for inferring evolutionary events in the fossil record, and (2) molecular evolutionists have for years been using a great diversity (hundreds) of fossil calibration points from the tree of life, depending on needs of each study, and were never wedded (exclusively) to a single point, such as the bird–mammal split. While their minimum calibration times are essentially identical (within 3%) to those already proposed by others,(12) it is their maximum times that we address here.

We contend that the maximum calibration points of Müller and Reisz(1) are the result of flawed methods and should not be used. As we elaborated elsewhere,(2) minimum times can be determined by a single fossil, with a high probability of being correct, whereas maximum times are much more difficult to establish and will always be associated with a probability—potentially high—of being incorrect. Our proposal of using evolutionary transitions is one example of how a maximum calibration constraint can be established, but that requires a large number of fossils in a phylogenetic and morphological sequence,(2,7) something not demonstrated by Müller and Reisz.

The flaw in Müller and Reisz's proposal(1,3) is their assumption that the oldest fossil of the most closely related group provides a maximum estimate of the splitting event. In reality, it is only a minimum for its own lineage and the true maximum—and splitting event—could be much older. Their corollary that a maximum time is also supported by the absence of fossils from older sedimentary strata is likewise untenable because discovery of fossils from unexpected places and times is a major activity of paleontologists. Therefore, anyone using the maximum calibration times proposed by Müller and Reisz, in a molecular clock study, will likely be underestimating times of divergence of their study organisms.

Finally, the plea by Müller and Reisz(1,3,9) for "closer interactions" between paleontologists and molecular biologists, while commendable, implies two things in the context of their critiques: (1) that molecular evolutionists have not interacted with paleontologists, and (2) that all paleontologists are in agreement with Müller and Reisz. On the contrary, molecular evolutionists often coauthor articles with paleontologists(13,14) and participate in symposia and volumes together.(15) Furthermore, it is divisive to construct a non-existing debate and ascribe it to the lack of cross-over between fields. We believe a better approach is to focus objectively on the arguments made by original authors and present all of the evidence clearly.

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