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Letters

Molecular dates require geologic testing

Reply to Wang & Mao (2016; in this issue of *New Phytologist*, pp. 1353–1358)

Wang & Mao's (2016) letter on our recent Tansley insight feature (Wilf & Escapa, 2015) included an informative summary of new molecular dating techniques and causes of temporal errors that deserves to be widely read. We concur with Wang & Mao that paleontology and molecular dating should become a powerful combination for gaining new insights into the history of life on Earth, but we found some of their specific criticisms of our article to be inaccurate. Like nearly all our paleontological colleagues, and not as Wang & Mao seemed to perceive us, we want molecular dating to work and have eagerly collaborated on productive ideas (e.g. Crisp *et al.*, 2009; Sauquet *et al.*, 2012). Our unease is not with the methodological development of molecular dating but with the downstream proliferation of sweeping, often conflicting interpretations about the history of life in the context of geologic events that are dated to geologic standards. Wang & Mao appeared to support this concern with regard to the Green Web hypothesis (de Queiroz, 2014) headlined in our paper.

Our core disagreement with Wang & Mao seems to be over the importance of empirical, geological tests of molecular dates as done in our article. We contend that to advance the collaboration of molecular dating and paleobiology, it must be possible to assess directly whether molecular dating has *intrinsic predictive value*. The scientific method demands that primary geologic data be brought to bear directly, not only incremental updates and cross-validation using other molecular methods in a semi-closed system. There are very few rigorous rock-clock comparisons (see Donoghue & Benton, 2007; Clarke & Boyd, 2014), especially for plant lineages (our article), and there is much evidence that small methodological differences cause enormous variance in divergence-age estimates (e.g. Sauquet *et al.*, 2012). Thus, we feel that our analysis, and more tests of a similar nature, are urgently needed. However, Wang & Mao did not recognize this central point and considered our empirical approach 'flawed'. Instead, they advocated for immediately ingesting new fossils into the predictive framework as calibration priors to generate new, potentially improved molecular dates. We obviously have no quarrel with doing that on its own merits. Nevertheless, simply recalculating node ages with new fossils and algorithms does not address the core issue of independent testing and its clear potential, which we demonstrated, to more rapidly correct large-scale evolutionary and biogeographic misinterpretations. Temporal errors are likely to be compounded when secondary calibrations are used (e.g. Sauquet *et al.*, 2012).

We wish to remind readers that geochronology itself represents an interdisciplinary collaboration. We feel that the ultimate goal for molecular dating should be to find the ways to join more fully in and complement that process. The scientific standards for determining the absolute ages that anchor our understanding of Earth and life history are extremely rigorous (Gradstein *et al.*, 2012). To date a fossil, the ages of associated, radioisotopically analyzed strata must be integrated with the full stratigraphic toolkit, including correlations from lithostratigraphy, biostratigraphy, paleomagnetic stratigraphy, sequence stratigraphy, and much more. We emphasize that this work is usually a collaborative effort among the various specialists involved (Fig. 1). As in the rock-clock debate, the different data sources often appear initially to be in conflict. The vital process of cross-checking and validation is what leads to new data gathering, methodological improvements, and whatever else is needed to achieve the most precise possible timeline. Several once-controversial methods, such as isotopic stratigraphy, have achieved widespread acceptance in this way, but the conversation for molecular dating has yet to begin in earnest.

The current situation, wherein molecular dates are seldom compared rigorously to geologic data, and no framework appears to exist to do so, is not sustainable in our view. Instead, there is an unprecedented proliferation of conflicting interpretations based on published 'chronograms' that currently meet none of the criteria for establishing geologic timelines. For the moment, we are not aware of any direct participation or advisory role of geochronologists in molecular dating research. Simple measures to improve this regrettable situation would include involvement across these fields in meeting sessions and research workshops. A concrete, very positive step forward that the molecular dating community could take would be to establish and maintain a set of defined benchmarks and procedures for assessing the accuracy of molecular dates and methods against the geologic timescale. As a starting point, the benchmarks could include well-dated first appearances of clades that are so abundant, widespread, and well-sampled that their oldest fossil ages are unlikely to differ significantly from their true times of origin. Several photosynthetic and heterotrophic marine planktonic groups (e.g. Berney & Pawlowski, 2006) and bivalve families (e.g. Herrera *et al.*, 2015) represent a few of the many possibilities. Involving geochronologists as well as paleontologists in this process would provide the critical feedback necessary to make the outputs useful and credible across disciplines.

For those who may think that this sort of cooperation is not possible, we remind readers that the current superb collaboration between paleontologists and geochronologists is in no small part the result of the EarthTime initiative (<http://earth-time.org>), which began with a series of workshops more than a decade ago. The EarthTime process quickly turned the numerous misunderstandings that once existed between paleontologists and



Fig. 1 Geochronology example: the PL-1 tuff (bright white layer at arrow, 2.2 m thick) exposed in the Peñas Coloradas Formation at Palacio de los Loros, Chubut, Patagonia, Argentina (Clyde *et al.*, 2014). Zircons separated from this and two other tuffs discovered in this fossiliferous area each produced similar, high-precision U-Pb ages using laser ablation high resolution multi collector-inductively coupled plasma mass spectrometry (HR-MC-ICP-MS) at the University of Arizona Laserchron Center. Eleven zircons selected from this tuff were then analyzed using high resolution thermal ionization mass spectrometry (TIMS) at the Boise State University Isotope Geology Laboratory, producing a U-Pb age of 61.984 ± 0.041 Ma. Clyde *et al.* (2014) were able to constrain the ages of the diverse and significant plant and animal fossils in several rock formations in this area (including some mentioned in Wilf & Escapa, 2015) for the first time using these results, combined with multiple additional constraints: a new ^{40}Ar - ^{39}Ar age from a nearby Late Cretaceous basalt; new paleomagnetic data; new biostratigraphic data from dinoflagellate cysts, foraminifera, calcareous nannoplankton, and terrestrial palynomorphs; and multiple measured stratigraphic sections. Analyzing each of these sources of data required a separate effort by a dedicated specialist or specialist team.

geochronologists into productive, long-lasting collaborations. A remarkable, ongoing series of laboratory improvements followed, including significant refining of standards that enabled numerous breakthrough results (Gradstein *et al.*, 2012). The products of these efforts include the extremely high-quality calibration data that are making the explosive growth in molecular dating possible. We believe that a similar standardization and benchmarking process is now needed for better tuning chronograms (or alternative procedures) to the geologic timescale in a way that will truly gain acceptance from geologists. In the meantime, we feel that straightforward, empirical tests such as ours can play an important role.

Some additional, more specific rebuttals now follow. First, in our article and per discussion earlier, we used the geologic ages of 19 Patagonian fossil taxa to independently test the most recently published corresponding molecular dates that, to avoid circularity, did not already use the same fossils as calibrations. We note the rarity of having available a relatively large number of relatively new fossil taxa from an undersampled continent for use in this way. As such, there was no avoiding comparisons between 'outdated molecular dates and up-to-date fossil records', simply because in most cases the fossils we used had to have been published after the molecular clock studies. As Wang & Mao detailed, methods have considerably improved since some of these papers were published, and we look forward to future comparisons. However, to test

molecular dates independently, we can only use the data at hand at a given time, and we certainly see no reason not to test recently published age estimates. Our approach was necessary, not 'flawed' as Wang & Mao described it.

Second, Wang & Mao took issue with our use of mean molecular dates for comparison to fossil ages rather than reported uncertainties, and they attempted to explain away our conclusions for a majority of the analyzed taxa on this basis. Although showing those uncertainties might have aided discussion, we stated that 'we acknowledge that these uncertainties are often large, and this does not affect our conclusions'. These conclusions were based on the clear pattern, found among multiple plant lineages, of bias in the mean molecular dates toward too-young, often post-Gondwanan molecular dates. We frankly do not see any other reasonable interpretation of our results. Although we would prefer a larger sample size for a statistical analysis, Wang & Mao's comments require that we run the numbers here. There are 30 comparisons of fossils with appropriate molecular mean estimates summarized in Table 1 and Fig. 1 of our article (i.e. stem or undecided fossil to stem estimate, crown fossil to crown estimate, and not counting the additional data we showed for completeness). Fossil ages were older in 22 cases (73%) and molecular dates in eight cases (27%). An exact binomial test of this outcome vs a null hypothesis of no bias (50–50%) yielded $P=0.016$ (two-tailed; one-tailed $P=0.008$). Similarly, a Mann–Whitney U -test of the 30 comparisons rejected the null hypothesis of equal distributions at $P=0.015$ (two-tailed; one-tailed $P=0.008$; $U=285$ for both). Thus, the biased molecular dates are not only visually obvious in our original Fig. 1 but statistically significant as well. Confidence intervals do not affect this central conclusion of our article.

Third, Wang & Mao suggested without evidence that an additional four of the 19 fossil taxa we used, namely three *Araucaria* species and a cycad (Triassic *Kurtziana*), may be 'outliers', apparently because they thought we interpreted some as belonging to crown rather than stem nodes. However, the status of Middle Jurassic *Araucaria mirabilis* as a crown Araucariaceae is established from extremely abundant and detailed anatomical evidence and phylogenetic analyses (Escapa & Catalano, 2013), as we discussed. In addition, Escapa & Catalano (2013) resolved two Cretaceous (one Albian, one Albian–Cenomanian) fossil genera in the stem of the agathoid lineage, which is sister to *Araucaria* and contains the living genera *Agathis* and *Wollemia*. Thus, there is overwhelming support for at least Middle Jurassic (> 163.5 million yr ago (Ma)) origins of crown Araucariaceae and divergence by the early Late Cretaceous (Cenomanian, > 93.9 Ma) of the *Araucaria* and agathoid clades, in sharp contrast to the considerably younger molecular dates of these lineages in the papers we cited. For *Araucaria grandifolia* and *Kurtziana*, Wang & Mao's point is misplaced because we considered these as stem (not crown) representatives of their lineages (*A.* Section *Araucaria* and *Zamiaceae*, respectively). Our provisional placement of Eocene *A. pichileufensis* in the crown of *A.* Section *Eutacta* may be disputed until a phylogenetic analysis is done. However, this species is known from abundant, well-preserved cone scales and leafy branches that are virtually indistinguishable from several extant New Caledonian species in the section, as long noted (Florin, 1940).

Fourth, Wang & Mao contested our comparatively mundane suggestion, made by others before us (e.g. Bell & Donoghue, 2005), that the standard practice of forcing incompletely preserved fossils to calibrate minimum ages of stem rather than crown nodes may be a cause of young bias if their true evolutionary position was near or in the crown. Wang & Mao correctly pointed out that the reverse is true as well, namely that forcing lineages to a crown node will lead to overestimates; however, they also seemed to indicate that we advocated for default crown placements. We wish to clarify here that we did not do so. We only pointed out the problem caused by the standard practice, which seems almost certain to cause young bias, and we called for a methodological innovation to address the issue as an example of a problem that seems eminently solvable in the near term. Indeed, the community seems well on its way toward improving this situation, as Wang & Mao described.

Finally, our original conclusions stand. Diverse plant fossils from Gondwana directly refute the Green Web idea for their respective lineages and clearly show young-bias on corresponding molecular dates. The broader Green Web hypothesis (de Queiroz, 2014) may well be based on a considerably larger number of biased molecular age estimates for lineages that lack adequate fossil records for comparison. We note that in the short time since our article was accepted, new data have reinforced the relevance of these ideas. A comprehensive study of fossil-rainforest floras from Antarctica, South America, Australia, and New Zealand found that the Gondwanan plant associations are continuously recognizable from the Late Cretaceous to the present day: before, during, and after Gondwanan breakup (Kooyman *et al.*, 2014). Also, new fossils from Gondwana continue to be much older than previous molecular dates indicated. A spectacular new example is the Late Cretaceous (>66 Ma) pollen grains of basal Asteraceae from Antarctica reported by Barreda *et al.* (2015). In the meantime, molecular dates continue to be used in support of large-scale historical interpretations involving Gondwanan floras and geological events (and many other topics; e.g. Merckx *et al.*, 2015).

We look forward to the day when we will agree with Wang & Mao's assertions that molecular dating 'enables the testing of hypotheses about the distribution and evolution of plants across time and space'; provides 'a reliable complement to timescales based solely on fossils'; and 'will be compatible with fossil records by integrating up-to-date fossil calibrations'. However, Wang & Mao rejected nearly all our attempts to test these ideas empirically, and they attempted to dismiss a great deal of relevant and high-quality geologic and paleontological data that we presented. Nevertheless, Wang & Mao apparently agreed with our general conclusions regarding Gondwanan plant distributions and the Green Web hypothesis. We call on the molecular dating community to recognize the need for a vast increase in geologic tests of molecular dates and to develop the benchmark tests that will better validate the method against the geologic timescale, in consultation with

geochronologists and paleontologists. The scientific rewards will be considerable.

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Author contributions

Both authors contributed substantially to interpretation of the relevant issues and to writing the manuscript. Statistical tests were done by P.W. Otherwise, no new research design or performance, nor data analysis and interpretation, were necessary to compose this letter.

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