Estimating the ages of extant Gnetales

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Based on 125 my-old seeds that resemble a living species nested high up in a molecular phylogeny of *Ephedra*, it has been suggested that extant species of this genus are older than 125 my (Yang et al., Am. J. Bot. 92: 231-241. 2005; Rydin et al., Ann. Bot. 98: 123-140. 2006). We evaluated this hypothesis in several ways using GenBank *rbcL* and *matK* sequences, which represent 50% of the 1050 gymnosperm species, including 42 of the 45-50 species of *Ephedra*, 26 of the 31 species of *Gnetum*, the single species of *Welwitschia*. As reported in earlier studies, *rbcL* shows high divergence between the three genera, but almost none within. Molecular clocks therefore yield young (i.e., Oligocene and Miocene) crown-group ages for the deepest splits within *Ephedra* and *Gnetum* (as found in earlier studies: Huang and Price, Mol. Biol. Evol. 20: 435-440. 2003; Ickert-Bond and Wojciechowski, Syst. Bot. 29: 834-849. 2004; Won and Renner, Syst. Biol. 55: 610-622, 2006). (i) To address the hypothesis of a slowdown in *rbcL* substitution rates at the onsets of speciation in *Gnetum* and *Ephedra*, we are carrying out standard statistical tests comparing all Cycadales, *Ginkgo*, Coniferales, and Gnetales. (ii) Modeling of *rbcL* diversity in Gnetales under different substitution rates indicates that the rate within *Ephedra* is about ten times slower than that in *Gnetum*, making *Ephedra* genetic distances especially short. (iii) A lineage-through-time plot for Gnetales, a survival analysis comparing three speciation/extinction models, and a statistical test for diversification rate change all point to an abrupt increase in diversification, with a break point at two-thirds of the total time depth of extant Gnetales. This break point is dated to 20 my ago if the chronogram is calibrated with the *Welwitschia*-like fossil *Cratonia cotyledon* (Rydin et al., Biol. Letters 270: 29-32. 2003), which constrains the Gnetales crown age to minimally 120 my old. A different calibration does not change the evidence for an abrupt strong increase in Gnetales diversification rates and the signal for major prior extinction. Such information cannot usually be obtained from the fossil record and provides an example of the insights gained by combining data and approaches across fields. (iv) Chromosome numbers in *Ephedra* and *Gnetum* indicate polyploidy and possibly hybridization in both Old World and New World species complexes, raising the possibility that plastid genomes may have been exchanged among species quite recently, which would contribute to short genetic distances. (v) To evaluate whether the 125 my-old seed fossils uniquely match one extant species we studied seeds of six random species of *Ephedra* under the SEM. Results show that the uniqueness of testa protuberances may be overrated. Based on the combined evidence (excluding the still running tests for an *rbcL* slowdown) we conclude that extant species of Gnetales are the result of recent radiations in *Ephedra* and *Gnetum* that followed massive extinction in the Oligocene and Miocene. Fieldwork on hybrid zones and investigation of the karyotypes of sister species pairs appear the most promising avenues for estimating the ages of the extant species of Gnetales.