Using More Than the Oldest Fossils: Dating Osmundaceae with Three Bayesian Clock Approaches

Guido W. Grimm1,2, Paschalia Kapli3, Benjamin Bomfleur3, Stephen McLoughlin1, and Susanne S. Renner4

1Department of Palaeobiology, Swedish Museum of Natural History, Sante Arhennius Vag 7, 10405 Stockholm, Sweden; 2Department of Palaeontology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; 3Natural History Museum of Cret and Biology Department, University of Crete, PO Box 2208, 71409 Heraklion, Certe, Greece, and Scientific Computing, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35. 69118 Heidelberg, Germany; and 4Institute of Systematic Botany and Mycology, University of Munich, Menzinger Str. 67, 80638 Munich, Germany

Received 7 October 2014; revisions returned 20 November 2014; accepted 1 December 2014

Abstract.—A major concern in molecular clock dating is how to use information from the fossil record to calibrate genetic distances from DNA sequences. Here we apply three Bayesian dating methods that differ in how calibration is achieved—“node dating” (ND) in BEAST, “total evidence” (TE) dating in MrBayes, and the “fossilized birth–death” (FBD) in FDPDiv. We inferred divergence times in the royal ferns. Osmundaceae have 16–17 species in four genera, two mainly in the Northern Hemisphere and two in South America and Australasia; they are the sister clade to the remaining leptosporangiate ferns. Their fossil record consists of at least 350 species in ~17 genera. For ND, we used the five oldest fossils, whereas for TE and FBD dating, which do not require forcing fossils to nodes and thus can use more fossils, we included up to 36 fossils and found that older fossils provide ages that overlapped those obtained from just neontological data. However, FBD estimates of speciation and extinction are sensitive to violations in the assumption of continuous fossil sampling; therefore, these estimates should be treated with caution. [Bayesian inference; fossilized birth–death dating; molecular clock calibration; node dating; total evidence dating; fossil record; royal ferns.]

Calibration is the single largest problem in molecular clock dating, influencing not only estimates of divergence times but also evaluation of the heterogeneity in substitution accumulation, since rates always derive from calibrated trees. There are many ways to calibrate genetic distances. They include fossils providing minimum divergence times (Sarich and Wilson 1967; Christin et al. 2014), oceanic islands with endemic radiations providing maximum ages of cladogenesis (Schafer et al. 2009), ancient DNA of one’s focal group (Korber et al. 2000), host ages as maximal ages for obligate parasites (Rector et al. 2007; Bellot and Renner 2014), ratios of substitution rates between hosts and parasites (Ricklefs and Outlaw 2010), published rates from other studies (e.g., Villarreal and Renner 2014), and node ages obtained in other studies, the so-called secondary calibration approach. The most widely used of these approaches is calibration with fossils. Since the introduction of Bayesian relaxed clock approaches that implement different strict and relaxed clock models, it is possible to accommodate prior notions about how tightly a fossil may fit a particular node with different prior distributions. In this framework, fossil ages can be used as point calibrations, hard minimum bounds, hard maximum bounds, soft maximum bounds, or to center a normal, lognormal, exponential, or uniform distribution. These distributions have large effects on the obtained ages (Ho and Phillips 2009; Warnock et al. 2012), and no amount of sequence data can correct the influence of incorrect prior constraints (Yang and Rannala 2006).

The use of several fossils to calibrate nested nodes in a tree has been suggested as a possible solution (Near et al. 2005), although this does not circumvent the problem of oldest fossils having a disproportionate effect on the outcomes (Parham and Irms 2008). A case in point is the crown age of the flowering plants (angiosperms). Numerous molecular clock studies have constrained the relevant node to maximally 135 Ma based on a few pollen grains from Israel (dated to 132.9 Ma) that are the oldest widely accepted record of flowering plants (Brenner 1996). When left unconstrained, the angiosperm crown age is usually much older, for example, 228 (193–270) Ma (Smith et al. 2010), an estimate only slightly younger than angiosperm-like pollen from the Middle Triassic, dated to 247–242 Ma (Hochuli and Feist-Burkhardt 2004, 2013). This example dramatically illustrates the problems stemming from the current need to assign oldest fossils to particular nodes, a problem worsened, not alleviated, by competing “oldest” fossils. Another problem with multiple fossils is that the effective calibrations may not resemble the specified calibrations because the various priors interact with each other, with the tree prior, and with other priors, such as monophyly constraints (Inoue et al. 2010; Heled and Drummond 2012).
Two Bayesian clock methods exist that do not rely on node dating (ND) with one or more “oldest” fossils. They are total evidence (TE) dating (Ronquist et al. 2012a) and fossilized birth–death (FBD) dating (Heath et al. 2014). TE dating combines morphological data from extant and extinct species with DNA data to infer node ages. Unlike ND, total evidence dating can be applied to a set of fossils without fixing them to specific nodes in the tree. It relies on the morphological similarity between a fossil and the reconstructed ancestors in the extant tree to infer the lengths of extinct side branches on which a fossil sits (Ronquist et al. 2012a). Total evidence dating uses a uniform prior on the clock trees, even though trees will include terminals of different ages because of extinct side branches. The FBD approach also allows the use of multiple fossils per lineage/node, both old and young, but does not require a morphological data matrix as does TE nor prior age densities on fossils as does ND. Thus far, the FBD approach has been applied to bears (Ursidae), a small clade with a fossil record from the Permian to Neogene. Most of the timing of major evolutionary events within the family, using both TE and FBD molecular clock dating. These two methods are the first to fully integrate fossils and molecular data, modeled as representing a single macroevolutionary process. They may result in older divergence times than traditional ND (Ronquist et al. 2012a; Hymenoptera, Heath et al. 2014: Ursidae) and can identify likely erroneous calibration fossils, as was the case for four of seven Hymenoptera calibration points (Ronquist et al. 2012a). To explore this possibility, we also applied traditional ND, using five oldest fossils alone or in combination.

Our phylogenies are based on ~8.6 kb of plastid DNA data for 13 extant species, 33 morphological characters scored for 19 permineralized rhizomes (data from Bomfleur et al. 2014b), and 17 frond fossilized birth–death (FBD) molecular clock dating.

Here we compare ND, TE dating, and FBD in a fossil-rich and phylogenetically pivotal group of plants, the royal ferns (Osmundaceae). The royal ferns are monophyletic and are the sister clade to all remaining leptosporangiate ferns (Pryer 2007). They comprise approximately 16–17 species in four genera, Osmunda, Osmundastrum, Leptopteris, and Todea (Metzgar et al. 2008). The first two occur mostly in the Northern Hemisphere extending into the humid tropics, the latter two in South Africa and Australasia (Kubitzki 1990). Royal ferns have an exceptional fossil record (Miller 1971; Tidwell and Ash 1994; Wang et al. 2014; Bomfleur et al. 2014b), with 150 species and 17 genera, ranging from the Permian to Neogene. Most of the fossils are foliar remains, but many are anatomically preserved (permineralized) axes.

A molecular clock model for ferns that included two Osmundaceae and that was calibrated using numerous fossils, inferred a stem age for the Osmundaceae of 206 Ma (Late Carboniferous; Pryer et al. 2004). This reappraisal (involving morphological data matrices) provided the opportunity to estimate the timing of major evolutionary events within the family, using both TE and FBD molecular clock dating. These two methods are the first to fully integrate fossils and molecular data, modeled as representing a single macroevolutionary process. They may result in older divergence times than traditional ND (Ronquist et al. 2012a; Hymenoptera, Heath et al. 2014: Ursidae) and can identify likely erroneous calibration fossils, as was the case for four of seven Hymenoptera calibration points (Ronquist et al. 2012a). To explore this possibility, we also applied traditional ND, using five oldest fossils alone or in combination.

Our phylogenies are based on ~8.6 kb of plastid DNA data for 13 extant species, 33 morphological characters scored for 19 permineralized rhizomes (data from Bomfleur et al. 2014b), and 17 frond fossilization/impression fossils, assessed on the basis of key autapomorphic features for the present study. Incorporating fossils into tree models as undertaken here in principle allows inference of speciation and extinction rates with reasonable confidence as shown with simulated data by Heath et al. (2014), and is probably an improvement over inferring these rates just from neontological data. To test how rates inferred using the FBD process would differ from those obtained with just the 13-species tree of living Osmundaceae, we carried out a TreePar analysis (Stadler 2011).

### MATERIALS AND METHODS

#### DNA Sampling, Alignment, and Phylogenetic Analyses

We relied on the plastid DNA matrix of Metzgar et al. (2008), which consists of the protein coding rbcL, atpB, and rps4 genes and the spacers rbcL-accD, atpB-rbcL, rps4-trnS, trnG-trnR, and trnL-trnF. A few regions were excluded due to ambiguities in the alignment (29 bp in rbcL-accD, 129 bp in rcl-atpB, 51 bp in rps4-trnS, 59 bp in trnC-trnR, and 146 bp in trnL-trnF) or missing data (rps4), resulting in a matrix of 15 species and 8616 nucleotide positions. We excluded the outgroup, Gleicheniaceae, because of long-branch attraction (Bomfleur et al. 2014b) and follow these authors in rooting Osmundaceae between Todea/Leptopteris and Osmunda/Osmundastrum instead of between Osmundastrum and the remaining three genera (Yatabe et al. 2005; Metzgar et al. 2008). Sampling covers the seven species of Osmunda from the three subgenera Claytosmunda, Osmunda, and Pleniasium; the single species of Osmundastrum, three of the approximately six species of Leptopteris, and both species of Todea (the second species lacked morphological data and was therefore excluded from some analyses). Osmundaceae generic and subgeneric classification schemes proposed by Miller (1971) and Yatabe et al. (2005) are summarized in Fig. S1 (available on Dryad at http://dx.doi.org/10.5061/dryad.mf81m). Species names and authors, herbarium vouchers, deposition in herbaria, and geographic origin of material are provided by Metzgar et al. (2008).

Phylogenetic analyses relied on maximum likelihood (ML) as implemented in RAxML 8.0.22 (Stamatakis

Downloaded from http://sysbio.oxfordjournals.org/...
398 SYSTEMATIC BIOLOGY VOL. 64

FIGURE 1. Fossil record of the modern Osmundaceae mapped on a maximum likelihood tree from a plastid DNA matrix of 8616 aligned positions analyzed under the GTR + Γ model with two data partitions. Rhizomes in red, fronds in green, with fossils either assigned to a branch (stippled lines) or a tree portion (shaded areas). Nodes (A–E) for which minimum age constraints were used in node datin runs are shown in the inset. Stratigraphic ranges of fossils abbreviated as follows: LT = Late Triassic; EJ, MJ, LJ = Early, Middle, and Late Jurassic, respectively; LC = Late Cretaceous; PG = Paleogene; NG = Neogene.

2014), using the GTR + Γ substitution model. We ran both unpartitioned and partitioned analyses in which RAxML found separate substitution rates for the two genes (rbcL and atpA) and the five spacers. Support for the ML topology was assessed with the rapid bootstrap/automatic bootstop implementation in RAxML (Stamatakis et al. 2008; Pattengale et al. 2009).

ND with Oldest Fossils, TE Dating, and FBD Dating

The 36 fossils included in our study (listed in Table S1 with supporting references, localities, ages, and other details available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m) are unambiguous members of modern Osmundaceae. The 19 rhizomes of Jurassic to Neogene age were included in a morphological matrix by Bombliez et al. (2014b). The 17 Triassic to Neogene fronds, including 14 Osmunda spp., two Osmundopsis spp., and Todea amissa, are placed within modern Osmundaceae based on lineage-diagnostic fertile or sterile features (references in Tables S1 and S2, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m).

The species-rich fossil genera Cladophlebis (including more than 80 described fossil species) and Todites (including more than 35 fossil species), usually identified as osmundaceous foliage fossils (Tidwell and Ash 1994), possess insufficient diagnostic characters for unambiguous assignment; some may represent one of the numerous extinct lineages of Osmundales.

The oldest fronds (O. claytoniites) with features diagnostic of modern members of Osmunda/Osmundastrum are of Late Triassic age and were associated with the root node (Node A in Fig. 1). Jurassic fronds were also associated with the root because they exhibit only symplesiomorphies of the Osmunda/Osmundastrum clade or parallelisms limited to its subclades. Fronds with apomorphic characters diagnostic of Osmunda or its subgenera were associated with Nodes C and D (Fig. 1). They are of Early Cretaceous and younger ages. Todea amissa (early Eocene, Argentina) has fronds characteristic of Todea and was thus linked to Node B (Carvalho et al. 2013).

For ND in BEAST v1.8 (Drummond et al. 2012), we used the five oldest fossils as constraints. We ran

<table>
<thead>
<tr>
<th>Classic age constraints</th>
<th>FBD-inferred age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 227+ Ma</td>
<td>244 Ma (263–233)</td>
</tr>
<tr>
<td>B: 52+ Ma</td>
<td>117 Ma (144–82)</td>
</tr>
<tr>
<td>C: 153+ Ma</td>
<td>234 Ma (246–208)</td>
</tr>
<tr>
<td>D: 84+ Ma</td>
<td>104 Ma (126–87)</td>
</tr>
<tr>
<td>E: 12+ Ma</td>
<td>14 Ma (6–13)</td>
</tr>
</tbody>
</table>
Each analysis was run twice for $2 \times 10^7$ generations, on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m. Based on Carvalho et al. (2013; Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m), one frond fossil was assigned a point age of 52 Ma, and the age of the stratum was included in the 97.5% quantile. Three constraints were employed, and the tree prior was set to the minimum age of the stratum comprising the respective fossil and the mean so that the maximum age of the stratum was included in the 97.5% quantile. One frond fossil was assigned a point age of 52 Ma based on Carvalho et al. (2013; Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m); FDPPDiv assumes a constant rate model, so we fitted only the priors on the posterior values was evaluated by running an additional analysis without the data (the DNA alignment). The parameters of all runs were evaluated using Tracer v1.5 (Rambaut and Drummond 2009) to confirm that (i) each Markov chain reached stationarity, (ii) the Effective Sample Sizes (ESS) were >200 for all optimized parameters, and (iii) independent runs produced convergent results. In each Markov chain Monte Carlo (MCMC) run, the first 10% of the samples were discarded as burn-in; the remaining samples were summarized in TreeAnnotator (part of the BEAST package) and visualized using FigTree (Rambaut 2014). For total evidence dating in MrBayes 3.2 (Ronquist et al. 2012b) we used the same DNA matrix as before (except that Todea papuana was excluded because many rhizome characters were unavailable) plus a morphological matrix with 33 characters for the 19 rhizome fossils (Bomfleur et al. 2014b). We used two data partitions, with GTR+$\Gamma$ for the DNA matrix and Mr + model for the morphological matrix (Lewis 2001), as implemented in MrBayes. No topological constraints were employed, and the tree prior was uniform (Ronquist et al. 2012a). Two rhizomes were assigned a point age of 16 Ma based on Pigg and Rothwell (2001; details shown in Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m); all other fossils were assigned uniform distributions based on min/max ages of the fossil occurrences. Three independent MCMC runs, with eight heated chains each running an additional analysis without the data (the DNA alignment), were run for $2 \times 10^7$ generations, sampling trees every 2000th generation. Convergence was checked as for the BEAST run, and in each MCMC run, the first 2 million generations of the sampled trees were discarded as burn-in and the remainder summarized and visualized.

For FBD dating we relied on FDPPDiv (available at https://github.com/tracyc7/FDPPDiv, commit v.3f1b6ed704d29f0b0f278f9489981e2df4b3b32a4f), with the ML topology as input tree (Fig S1, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m). FDPPDiv treats the topology and branch lengths of the extant species tree as given and does not permit assignment of separate substitution rates to specific data partitions. As required for FDPPDiv, the age of each fossil was drawn before analysis from a uniform distribution of its age range (ranges are given in Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m). We performed two MCMC runs of $2 \times 10^7$ generations, sampling every 1000th steps. Log files were then compared in Tracer to check that convergence had been reached.

**Effects of Using Rhizomes versus Fronds and Different Fossil Percentages**

To be scored for the morphological matrix required for TE dating, fossils need sufficient characters. The frond and rhizome fossils used here differ greatly in this respect, with rhizome fossils having many more codable characters. This meant that for TE dating, only rhizome fossils could be used. To be able to compare TE results with FBD results, we carried out additional FBD runs that used only the 19 rhizomes or only the 37 fronds. We also ran FBD analyses for which we randomly drew 10% (4 fossils), 25% (9 fossils), or 50% (18 fossils) of the 36 fossils, with each drawing repeated 10×. Otherwise, these runs relied on the same settings as used for the full data sets.

**Inferring Speciation and Extinction with TreePar versus the FBD Approach**

To quantify diversification through time we estimated tree-wide speciation and extinction rates using the R package TreePar (Stadler 2011) on just the extant species tree and the FBD process on the tree with all 36 fossils. FBD assumes a constant rate model, so we fitted only this model of the many available in TreePar.

**Documentation**

Input and output files (used matrices, trees, and calibration results) and OSM files are included in an archive hosted at www.dryad.org (doi:10.5061/dryad.m7s5).
TABLE 1. Divergence ages (median values and 95% highest posterior density intervals) and optimized substitution rates obtained with FBD dating, TE dating, or ND with just the five oldest fossils as minimum age constraints (nodes A–E in Fig. 1).

<table>
<thead>
<tr>
<th>Node</th>
<th>FBD</th>
<th>TE</th>
<th>ND unpartitioned</th>
<th>ND partitioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root node</td>
<td>243 (264–233)</td>
<td>185 (187–183)</td>
<td>229 (234–227)</td>
<td>228 (231–227)</td>
</tr>
<tr>
<td>Todea/Leptopteris</td>
<td>116 (132–100)</td>
<td>113 (150–66)</td>
<td>143 (171–130)</td>
<td>107 (129–91)</td>
</tr>
<tr>
<td>Osmunda/Osmundastrum</td>
<td>235 (283–235)</td>
<td>182 (186–176)</td>
<td>156 (164–153)</td>
<td>176 (201–153)</td>
</tr>
<tr>
<td>Osmunda crown</td>
<td>138 (150–119)</td>
<td>143 (171–130)</td>
<td>107 (129–91)</td>
<td>104 (115–95)</td>
</tr>
<tr>
<td>Subgenus Plenasium/Osmunda split</td>
<td>111 (126–98)</td>
<td>(see above)</td>
<td>84 (86–84)</td>
<td>84 (86–84)</td>
</tr>
<tr>
<td>Todea crown</td>
<td>13 (23–4)</td>
<td>12 (27–2)</td>
<td>9 (20–3)</td>
<td></td>
</tr>
<tr>
<td>Subgenus Leptopteris crown</td>
<td>27 (54–21)</td>
<td>49 (105–9)</td>
<td>25 (58–12)</td>
<td>19 (27–12)</td>
</tr>
<tr>
<td>Subgenus Plenasium</td>
<td>9 (13–5)</td>
<td>84 (123–62)</td>
<td>12 (27–4)</td>
<td>7 (10–4)</td>
</tr>
<tr>
<td>Subgenus Osmunda</td>
<td>13 (15–13)</td>
<td>21 (58–2)</td>
<td>12 (13–12)</td>
<td>12 (13–12)</td>
</tr>
<tr>
<td>Substitution rate</td>
<td>1.33*10^{-4} (1.19–1.46)</td>
<td>1.59*10^{-4} (1.56–1.62)</td>
<td>1.46*10^{-4} (1.44–1.48)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: The DNA matrix, substitution model, and partitioning schemes are described in Materials and Methods, Fig. 2 visualizes the FBD results, and the TE chronogram is shown in Fig. S2 (available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m). Note that TE dating only relied on the rhizome fossils, whereas FBD and ND also relied on the frond fossils.

(a) Similar age from the three methods in bold.
(b) FDPPDiv does not allow data partitioning.
(c) Age for the MRCA of Osmunda and Osmundastrum = MRCA of all extant Osmundaceae because of suspected misplacement in TE run (Fig. S2, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m).
(d) MRCA of subgenera Osmunda and Plenasium = MRCA of all three subgenera of Osmunda (inter-subgeneric relationships not resolved in the TE consensus tree).
(e) Averaged over all branches and partitions.
FIGURE 2. Chronogram for the modern Osmundaceae as inferred with the FBD approach, with the inferred placement of the 36 used fossils (red = rhizomes; green = fronds). Blue bars represent 95% highest posterior density intervals. The paleogeographic locations of the fossils are shown on the maps to the right. Geological periods and epochs abbreviated: G = Guadalupian; L = Lopingian (Permian); ET, MT, LT = Early, Middle, and Late Triassic, respectively; EJ, MJ, LJ = Early, Middle, and Late Jurassic, respectively; EC, LC = Early and Late Cretaceous, respectively; PG = Paleogene; NG = Neogene; Q = Quaternary.
FBD time tree. The Osmunda/Osmundastraum lineage had established in the Southern Hemisphere (Antarctica) by the Late Triassic, but the small genera (lineages) surviving today apparently date to the late Mesozoic and Paleogene of the Northern Hemisphere. The Todea/Leptopteris lineage, today confined to southern Africa (Todea) and Australasia (both genera), is represented in the fossil record by one Northern Hemisphere Early Cretaceous rhizome and one frond from the Paleogene of South America.

Speciation and Extinction Rates from Neontological Data versus with Fossils Included

With the FBD method, the speciation rate ($\lambda$) was estimated as 0.0299 (0.0099–0.0549), the extinction rate ($\mu$) as 0.0240 (0.0039–0.0495), indicating a high turnover and relatively slow diversification rate (0.006 per myr). The fossil recovery rate, which models how many lineages (extinct and extant) are covered by the fossil sample was $\psi = 0.01531$, meaning that there is a 31% probability that a species will be represented in the fossil record. Inference (using TreePar) of speciation and extinction rates from just neontological data gave slightly higher speciation ($\lambda = 0.0314$) and extinction rates ($\mu = 0.0339$; Table 3), but both values fell within the confidence intervals of the FBD-inferred rates.

**TABLE 2.** Stability of FBD-dating inferences using subsets of 4, 9, or 18 fossils, drawn randomly from the total set of fossils (10%, 25%, 50% resampling), with each subsampling repeated 10×.

<table>
<thead>
<tr>
<th>Nodes</th>
<th>BAR-PAP</th>
<th>HYM-WIL</th>
<th>FRA-WIL</th>
<th>BAR-WIL</th>
<th>BAN-VAC</th>
<th>BAN-JAV</th>
<th>JAP-LAN</th>
<th>JAP-REG</th>
<th>JAP-VAC</th>
<th>CLA-JAP</th>
<th>CIN-JAP</th>
<th>Root node</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBDabs (%)</td>
<td>2.1</td>
<td>4.1</td>
<td>5.7</td>
<td>24.6</td>
<td>1.3</td>
<td>2.0</td>
<td>1.2</td>
<td>8.8</td>
<td>24.8</td>
<td>30.1</td>
<td>52.6</td>
<td>53.9</td>
</tr>
<tr>
<td>FBDrel (%)</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>33</td>
<td>15</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Notes: Shown are the absolute (FBDabs)—and relative differences (FBDrel)—to the divergence ages as inferred using all 36 fossils and the FBD approach (Fig. 2).

with the FBD approach (Heath et al. 2014).

The FBD approach does not require a morphological matrix and has proven its power in unifying the model of branching times on the tree (Heled and Drummond 2012; Warnock et al. 2012; Heath et al. 2014; Silvestro et al. 2014). This problem is avoided by applying a birth–death process to uncalibrated nodes conditioned on the calibrated nodes (Yang and Rannala 2006), which seems a more realistic representation of the lineage-diversification process and is achieved in the FBD approach (Heath et al. 2014).

The FBD approach does not require a morphological matrix and can, in principle, use the entire fossil record of a focal group, which greatly reduces the impact of unrepresentative (or misinterpreted) oldest fossils. Not having to compile a morphological matrix will be welcome to phylogeneticists interested in...
divergence times, yet without expertise and resources for morphological work on living and fossil taxa. For many groups, perhaps especially plants, building morphological matrices including fossil and extant taxa may not be feasible (as was the case here for Osmundaceae fronds). Weaknesses of the FBD approach are that it does not directly incorporate uncertainties around the tree topology and the fossil ages; cannot use morphological data even if available; and does not permit assignment of separate substitution rates to separate data partitions. Partitioning of substitution models, however, can cause statistical problems in clock dating (Dos Reis et al. 2014). In our data set, there were no strong differences between ages inferred from unpartitioned BEAST clock dating runs and partitioned ones (Table 1).

Implications of the Inferred Divergence Scenario for the Evolution of Osmundaceae

Initial radiations in the ferns took place in the mid-Paleozoic (Taylor et al. 2009). Monophyly, the group incorporating whisk ferns (Psilotales), adder’s tongue ferns (Ophioglossales), horsetails (Equisetales), and leptosporangiate ferns (Polypodiales), have a fossil record extending back to at least the Late Devonian (Taylor et al. 2009). Osmundaceae in the broad sense (including the primitive Thamnopteroideae) were established in the Permian, with many well-preserved (permineralized) rhizomes from the Middle to Upper Permian of Australia and Russia (Gothe 1970; McLoughlin 1992) and members of their sister family Guaiaceae recorded from coeval strata in South America and China (Herbst 1981; Wang et al. 2014). The Guaiaceae and Osmundaceae emerged during a phase of stepwise global warming in the wake of the Late Paleozoic Ice Age, with Thamnopteroideae then becoming extinct during the end-Permian biotic crisis. The core Osmundaceae persisted in moist temperate to tropical climates to the present and extended into high latitudes during phases of greenhouse climates in the Mesozoic and Paleogene (Collinson 2002).

Given this phylogenetic and fossil background, we prefer the older Osmundaceae divergence times inferred with the FBD method (and also partly the TE approach; Table 1) over the mostly younger ages inferred from node-dating-using-oldest-fossils. Osmundaceous fronds (of poorly understood affinity; Table S2, available on Dryad at http://dx.doi.org/10.5061/dryad.m6t1m: Todites, Rootodites, Biroditae, and Elantodites) are common from the Late Triassic onwards. The FBD-inferred Middle to Late Triassic stem ages of the lineages Osmunda/Osmundastrum and Leptopteris/Todea are consistent with these paleontological dates; these lineages apparently beginning to diverge in the aftermath of the end-Permian mass extinction (Tidwell and Ash 1994, Taylor et al. 2009; Fig. 2). The preferred chronogram (Fig. 2) further indicates segregation of Todea and Leptopteris and of the three subgenera of Osmunda during the mid-Cretaceous and radiation and establishment of extant species in the Neogene. Modern Osmundaceae appear to have originated in the humid temperate belt of southern Gondwana (see maps in Fig. 2). The modern distribution of the single species of Osmunda cinnamomea includes humid climate tracts of South America, eastern North America, and East Asia. Fossil evidence places Osmundastrum in Canada by the Late Cretaceous (Serbet and Rothwell 1999), so range expansion to both hemispheres may have occurred during the more humid phases of the mid-Mesozoic.

An implication of the inferred Late Triassic crown age of Osmunda/Osmundastrum is that Early to Middle Jurassic rhizomes, which are intermediate between Osmundastrum and Osmunda, represent stem group taxa of either Osmundastrum or Osmunda. For the recently described 182–190 myr-old Korsøråd fern rhizome from Sweden (Bromfleur et al. 2014a), the divergence times obtained here suggest that it represents an early precursor of the Osmundastrum lineage. The “Osmundastrum precursor” hypothesis is one of three alternatives that can be inferred from the set of analyses conducted by Bromfleur et al. (2014b). Our molecular (FBD) dating also provides a time frame for the morphological innovations in the Osmundastrum lineage,

---

**Table 3.** Optimized FBD parameters for the Osmundaceae; for comparison, the speciation, and extinction rates estimated from the unpartitioned neontological data with TreePar (Stadler 2011) under a model of density dependence or a constant rate model of diversification

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median value</th>
<th>95% HPD interval</th>
<th>ESS(^a)</th>
<th>TreePar constant rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speciation rate (λ)</td>
<td>0.0299</td>
<td>0.0099-0.0549</td>
<td>10923</td>
<td>0.0314(^b)</td>
</tr>
<tr>
<td>Extinction rate (µ)</td>
<td>0.0240</td>
<td>0.0039-0.0495</td>
<td>10890</td>
<td>0.0339(^b)</td>
</tr>
<tr>
<td>Fossil recovery rate (α)</td>
<td>0.0153</td>
<td>0.0065-0.0253</td>
<td>8475</td>
<td>NAV</td>
</tr>
<tr>
<td>Extant species sampling</td>
<td></td>
<td>(=1)(^d)</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

---

\(^a\) Effective sample size (rounded to full numbers).
\(^b\) With few tips (in our case 13), TreePar sometimes infers negative diversification.
\(^c\) Nonapplicable.
\(^d\) In the most recent release of the FDPFDiv code that we used...
which occupies similar humid habitats as Osmanda, through a broad range of latitudes (previous paragraph). The post-Cretaceous increase in shaded, moist niches in broad-leaved angiosperm-dominated forests may have provided improved ecological conditions for Osmundaceae, particularly for a lineage such as Leptopteris, which cannot survive without permanent moisture (Brownsey and Perrie 2012).

Inferring Speciation and Extinction Rates with the FBD Approach

Incorporating many fossils allows confident inference of speciation and extinction rates as shown with simulated data by Heath et al. (2014). In our Osmundaceae data, speciation and extinction rates from the FBD approach and the neoentological data (with TreePar) were similar (Table 3), with the confidence intervals around the FBD rate bracketing the TreePar rates. With very few tip species, TreePar sometimes infers higher extinction than speciation rates, which seems to have been the case here (13 tip species). A parameter that may influence (and distort) the inferred speciation and extinction rates is the sampling rate of the living species, which is set to $I$ in FDPDiv. Future within-species sampling may reveal that some Osmanda forms include multiple species. For instance, the extremely widespread O. (Osmundastrum) cinnamomea (approximately 5 common synonyms) and O. regalis (approximately 10 synonyms) show intra-specific morphological variation (not covered in our molecular data) that is comparable with inter-specific diversity in other Osmundaceae. This would mean that species sampling in this study might not have been complete. Estimates of speciation and extinction are also sensitive to violations in the assumption of continuous fossil sampling (T. Heath, personal communication, September 2014), and the fossil sampling rate that the FBD method inferred from our data, $\psi = 0.01531$, which implies that a species has a 31% probability of being represented in the fossil record, seems extremely high. All this cautions against attaching too much weight to our estimates of diversification and turnover.

CONCLUSIONS

It is now feasible to analyze molecular and fossil data together, to jointly estimate speciation and extinction dates of fossil species and branching times of the phylogeny, assuming a common underlying birth–death process (Ronquist et al. 2012a; Slater et al. 2013; Silvestro et al. 2014; Heath et al. 2014). As shown by our experiments with just 10%, 25%, and 50% of the total 36 fossils, the FBD approach is relatively insensitive to fossil sampling density (as found by Heath et al. 2014), although the reconstructed tree ages are fairly sensitive to the oldest fossil. It is, however, extremely memory intensive; our data required 8 CPU processors and MCMC lengths of 20 million generations, taking about 7 h/run. The TE approach, at least for Osmundaceae, yielded an unexpected mix of young and old divergence times, and also an odd tree topology, whereas the FBD approach resulted in much older divergence times than did traditional ND.

SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.m61f1m.

FUNDING

This research was funded by the Swedish Research Council (VR) grant 2010-3931 to S.McL.

ACKNOWLEDGMENTS

The authors thank the editor, Frank Anderson, the Associate Editor Thomas Near, and the reviewers Tracy Heath and Daniele Silvestro for constructive criticism.

REFERENCES


