Research article

Large-scale phylogenetic analyses reveal fagalean diversification promoted by the interplay of diaspores and environments in the Paleogene

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ABSTRACT

The order Fagales (ca. 1325 species, 32 genera) is one of the most important orders of woody angiosperms in both tropical and temperate forests. Fagalean plants have diverse diaspores and dispersal modes, and have abundant macrofossil records. Here, we present, to our knowledge, the most comprehensive phylogenetic analysis of Fagales to date based on five plastid loci. We reconstructed the phylogenetic relationships within Fagales using parsimony, likelihood and Bayesian approaches. We inferred the evolutionary shifts of diaspore types, dispersal modes, habitats, and pollination syndromes, and estimated divergence times and rates of diversification. Fossil fruit records of Fagales were also reviewed. Our results suggest that fagalean families are all monophyletic and 29 of the 32 genera are monophyletic. The majority of inter-familial and inter-generic relationships were well resolved. Our evolutionary reconstructions indicate that winged diaspore is synapomorphic, and animal dispersal system is symplesiomorphic in Fagales. Within the order, the families diverged in the mid-Cretaceous but mainly diversified after the Cretaceous–Paleogene (K–Pg) boundary. The overwhelming majority of winged and wingless fruited genera diverged or diversified during the Paleogene, reflecting adaptation to wind and animal dispersals, respectively. Correlated evolution analyses strongly supported the correlated transitions between dispersal mode and habitat. The winged fruited groups often inhabit open habitats and are commonly dispersed abiotically, whereas the wingless fruits usually inhabit closed habitats and have a vertebrate-dispersal mode. Environmental changes triggered increased diversification of Fagales in the Paleogene together with evolution of diaspores and dispersal modes. Additionally, our paleobotanically calibrated time-scale for Fagales may be useful for ecological and physiological studies.

Introduction

There are diverse species inhabiting the Earth, but species diversity among different lineages is strikingly heterogeneous, such that sister groups can markedly differ in numbers of species. High species diversity might have arisen from some combination of high speciation rate (Richardson et al., 2001) and low extinction rate (Gaston and Blackburn, 1996). Recent studies have suggested that high speciation rate has been a major contributor to high

species diversity of some groups, such as *Inga* (Richardson et al., 2001), *Lupinus* (Drummond et al., 2012), and *Tragopogon* (Bell et al., 2012). High speciation rates often result from rapid environmental changes (e.g., Benton, 2010; Hoorn et al., 2010) or differentiation of key innovative characters (e.g., Blackledge et al., 2009; Carlson et al., 2011; Biffin et al., 2012). These observations have led to the widespread belief that high species-richness may be attributed to "key opportunities" including geological and/or climatic changes or "key innovations" including morphological, behavioral, and physiological novelties (Benton, 2010; Yoder et al., 2010; Vamosi and Vamosi, 2011).

A few studies have sought causative key innovations (e.g., Blackledge et al., 2009; Simon et al., 2009), but they rarely elucidate in detail how the "key innovation" triggers increased

diversification of a group with high species richness. Along with increasing progress in paleogeographic, paleoclimatic and paleoecologic investigations (e.g., Upchurch and Wolfe, 1987; Miller et al., 2005; Friis et al., 2011), many studies about species diversification today have focused on adaptation to "key opportunities", such as climatic (e.g., Vieites et al., 2007; Arakaki et al., 2011; Nagalingum et al., 2011) and tectonic events (e.g., Benton, 2010; Hoorn et al., 2010), habitat shifts (e.g., Renaud et al., 2005; Hou et al., 2011), and extrinsic biotic factors (e.g., Whittall and Hodges, 2007; Bouchenak-Khelladi et al., 2009). However, environmental changes are extrinsic driving forces of evolutionary success. Changes of the environment that the group inhabited, as extrinsic factors, are first necessary, but the group should further respond to environmental changes by evolutionary innovation(s) and consequently can exhibit high species diversity (Benton, 2010; Drummond et al., 2012). In order to understand the evolutionary history of species diversity of a group, we need to clarify the relationship between "key innovations" and "key opportunities".

The order Fagales (*sensu* APG III, 2009) consists of 7 families, 32 genera and ca. 1325 species, and is one of the most important orders of woody angiosperms in tropical and temperate forests according to total biomass and economic use (Table S1). Many members of the order are keystone species and are dominant in their ecosystems, such as some species of *Castanopsis*, *Quercus*, and *Nothofagus* (e.g., NatureServe, 2002; Gee et al., 2003). Fagales also provide essential food and optimal habitats to many forest animals and insects. Economically, fagalean plants are widely used for lumber, firewood, and horticultural plantings, and are very important orchard crops, such as hazel (*Corylus avellana*), filbert (*Corylus maxima*), walnut (*Juglans regia*), and waxberry (*Myrica rubra*). Due to land use changes, livestock grazing and unsustainable logging, however some species of the order are under threat of extinction in the wild. Furthermore, some fagalean forests are being invaded by pine forests due to the increase in temperature, such as in the Himalayan region of India (Kala, 2012). In Fagales, 147 species are listed on the Red List as threatened (IUCN, 2012). Thus, knowing the evolutionary history of fagalean diversity is important for sustainable utilization and conservation, and will improve our understanding of evolutionary dynamics of angiosperm-dominated forests.

A remarkable aspect of Fagales at the generic level is the diversity of diaspore morphologies ranging from winged to wingless (Fig. S1). The shapes and developments of wings are diverse (Lu et al., 1999; unpublished data), but all of those fruits with wings are specialized for wind dispersal (Tiffney, 1986; Friis et al., 2011). Wingless diaspores have occurred in some taxa, such as Myricaceae, some genera of Juglandaceae and Betulaceae, which are usually dispersed by birds or mammals (Stone, 1973; Tiffney, 1986; Friis et al., 2011). It is hypothesized that the rise of most of Fagales, such as Juglandaceae, Fagaceae and Myricaceae, were related to the diversity of diaspores and dispersal systems (Tiffney, 1984, 1986; Manchester, 1987, 1989). The fossil record of fagalean fruits is extensive, with representation of many extant and extinct genera (e.g., Manchester, 1987; Elliott et al., 2006; Mindell et al., 2009; Friis et al., 2011). Moreover, those fossil fruits also displayed diverse morphology despite that a comprehensive review of the worldwide fossil occurrences in Fagales is still lacking. Both the fossil record and molecular dating studies indicate that Fagales are an ancient angiosperm lineage. The oldest putative fruit fossil of Fagales was discovered from the Late Cretaceous (∼83 Ma; Herendeen et al., 1995). Molecular clock estimates suggest a stem age of ca. 102 million years ago (Ma) (Magallón and Castillo, 2009) or 103 Ma (Wang et al., 2009) for the order. It is well-known that the mass extinctions extensively occurred at the Cretaceous–Paleogene (K–Pg) boundary 65.5 Ma, which led to many new ecological niches (e.g., Wolfe and Upchurch, 1986; McElwain and Punyasena, 2007). After the K–Pg boundary, global climates and vegetation types have dramatically changed (Wolfe, 1985; Zachos et al., 2001). Thus, Fagales present a remarkable opportunity to investigate the relationship between key opportunities and key innovations for understanding species diversity.

Here, we first reconstruct a large-scale phylogenetic tree for Fagales using five plastid DNA regions with the most comprehensive taxon sampling to date. Within the large-scale phylogenetic framework, we then infer the evolutionary shifts of diaspore types, dispersal modes, habitats, and pollination syndromes, and estimate divergence times and rates of diversification. We also review the fossil fruits and statistically analyze their variation on geological time scales. Finally, we investigate the interplay of diaspores and environments on the evolution of fagalean diversity at the generic level.

Materials and methods

Taxon sampling

A total of 284 species of Fagales were sampled in the present study, including 82 species from ca. 180 of the six genera of Betulaceae, 29 species from 95 of the four genera of Casuarinaceae, 89 species from ca. 897 of the seven genera of Fagaceae, 44 species from 64 of the nine accepted genera of Juglandaceae (Lu et al., 1999; APG III, 2009), 15 species from ca. 52 of the four accepted genera of Myricaceae (Herbert et al., 2006), 23 species from 35 of the mono-generic Nothofagaceae, and one species of the monotypic Ticodendraceae. Our taxon sampling scheme included all 32 accepted genera of Fagales and covered almost all subgenera or sections of each genus. Following the results of Soltis et al. (2011), our outgroups included twelve species representing other three orders of the nitrogen-fixing clade and COM clade: *Anisophyllea fallax*, *Coriaria nepalensis* and *Cucumis sativus* (Cucurbitales); *Celtis philippensis*, *Morus alba*, *Rhamnus cathartica* and *Urtica urens* (Rosales); *Polygala vulgaris*, *Quillaja saponaria*, *Stylobasium rownie* (Fabales); *Brexia madagascariensis* and *Humiria balsaminifera* (COM clade). Voucher information and GenBank accession numbers are listed in Table S2.

DNA extraction, PCR amplification and sequencing

Five chloroplast markers were used in this study: *rbcL* and *matK* genes, *trnL* intron, and *trnL* (UAA) 3′ exon-*trnF* (GAA) and *atpB-rbcL* spacers. The majority of species have at least three of the five markers. Total genomic DNA was extracted from silica gel-dried leaves or herbarium specimens using the modified CTAB protocol of Doyle and Doyle (1987). Amplification of DNA regions was performed using the conventional polymerase chain reaction (PCR). PCR amplification and sequencing were performed followed Li et al. (2004). The primers used in this study are listed in Table S3. PCR products were purified using a QIAGEN PCR-DNA Purification Kit. Sequencing reactions were conducted using the ABI Prism Bigdye Terminator Cycle Sequencing Kit (Applied Biosystems, ABI). Products were analyzed on ABI 3730xl automated DNA sequencers.

Sixty-nine new sequences of *rbcL*, thirty-nine of *matK*, forty-three of *trnL* intron, fifty-two of *trnL-F* spacer, and sixty of *atpB-rbcL* were generated in this study and have been deposited at GenBank. The combined five-marker data set included 296 taxa, in which the amount of missing data was: *matK* = 13.5%, *rbcL* = 22%, *trnL* = 37.5%, *trnL-F* = 28.7%, and *atpB-rbcL* = 34.5%. Based on Wiens (2005) and Wiens and Moen (2008), the proportion of our missing data is not enough to affect the performance of the phylogenetic analysis.