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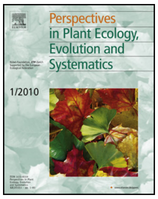

Research article

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

Xiao-Guo Xiang[a,1], Wei Wang[a,1], Rui-Qi Li[a], Li Lin[a], Yang Liu[b], Zhe-Kun Zhou[c], Zhen-Yu Li[a], Zhi-Duan Chen[a,*]

[a] State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
[b] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA
[c] Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

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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

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[*] Corresponding author. Tel.: +86 1062836434; fax: +86 1062590843.
E-mail address: zhiduan@ibcas.ac.cn (Z.-D. Chen).

[1] These authors contributed equally to this work.

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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.

Phylogenetic analysis

For DNA sequences, Clustal X v1.83 (Thompson et al., 1997) was used to obtain an initial alignment, followed by manual adjustment with BioEdit v7.1.3 (Hall, 1999). One difficult-to-align region in *trnL* intron representing 26 sites and two difficult-to-align regions in *trnL-F* spacer including 40 sites were excluded from the analyses. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods were used to conduct phylogenetic analyses in PAUP* v4.0b10 (Swofford, 2003), RAxML v7.0.4 (Stamatakis, 2006), and MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), respectively. For MP, heuristic searches were conducted with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. To assess node support, bootstrap analyses were performed using 1000 replicates with 10 random taxon additions and heuristic search options.

RAxML was conducted with GTR + Γ substitution model for each region and all model parameters estimated, executing 1000 rapid bootstrap inferences before a thorough ML search. For BI analyses, each DNA region was assigned its own best-fit model, as determined by the Akaike Information Criterion (AIC) Modeltest v3.07 (Posada and Crandall, 1998). Two independent Markov Chain Monte Carlo (MCMC) runs were conducted simultaneously, each with four linked chains, for 50,000,000 generations, sampling one tree every 1000 generations, and starting with a random tree. Stationarity was determined in Tracer v1.5 (Rambaut and Drummond, 2007). Majority rule (>50%) consensus trees were constructed after removing the burn-in samples (the first 25% of sampled trees).

Molecular age estimates

Divergence times were estimated using a Bayesian uncorrelated relaxed-clock (UCLN) model (Drummond et al., 2006) with multiple calibration points as implemented in BEAST v1.7.4 (Drummond and Rambaut, 2007). The concatenated 5-marker data set was analyzed using the GTR + Γ model for the individual marker partitions, as in the phylogenetic analyses described above. Under the UCLN model, substitution rate variation among branches is modeled using a lognormal distribution. The tree prior was set to the birth-death process.

The tree was time-calibrated by applying a prior probability on the ages of 12 internal nodes (Fig. S2 and Table S4). These 12 fossil taxa have precise ages and/or stratigraphic information and could be confidently assigned to taxonomic groups and nodes represented in our data set (Table S4). Lognormal priors were assigned to the height of 12 nodes. By how much the appearance of a clade predates the age of the first fossil is always unclear, we therefore used the 95% credibility intervals for the 12 minimum age constraints based on appropriate mean and standard deviation values (Table S4). Additionally, an age of 125 Ma was used to constrain the maximum root age of the phylogeny, which is the earliest time for eudicots pollen occurrence (Hughes, 1994), with a normal distribution and a standard deviation of five.

Four individual analyses with 50 million generations were carried out with sampling every 5000 generation, and resulted in a total of 200 million generations. The trace files were checked in Tracer v1.5 (Rambaut and Drummond, 2007) to ensure that runs had reached convergence, to decide on the appropriate number of generations to discard as burin-in, and to verify that the ESS for all parameters was above 200. The remaining trees were then combined in LogCombiner v1.7.4 (Drummond and Rambaut, 2007), and a timetree was obtained with the use of TreeAnnotator v1.7.4 (Drummond and Rambaut, 2007). BEAST analyses were performed in the Cipres Web Portal v3.1 (Miller et al., 2010).

Evolutionary inferences of diaspore types, dispersal modes, habitats, and pollination syndromes

The characters of fagalean diaspores potentially pertinent to adapting different ecological opportunities include their shapes and sizes, and accordingly dispersal systems (Tiffney, 1984, 1986). We inferred the evolutionary shifts of diaspore types, dispersal modes, and habitats, as well as pollination syndromes, while evolution of diaspore sizes was statistically analyzed by integrating extant and extinct taxa (see below). We scored fagalean diaspores into two types: winged and wingless (Hewson, 1989; Johnson and Wilson, 1993; Kubitzki, 1993; Fu et al., 1999; Huang et al., 1999; Lu et al., 1999). Based on van der Pijl (1982) and Friis et al. (2011), we coded dispersal systems of fagalean diaspores into four modes: wind, scatterhoarding and endozoochorous by vertebrates, and unassisted. According to light conditions of habitats of extant taxa, especially in relation to when the fruits are mature and dispersed, three kinds of habitats were coded: (i) closed, (ii) open, and (iii) closed and open. Open habitats comprise coastal, island swamps, bogs, borders of lakes, ponds, streams, and deciduous broad-leaved forests; closed habitats constitute evergreen broad-leaved forests, montane or submontane tropical rain forests, evergreen and deciduous mixed forests, and cloud forests (Hewson, 1989; Johnson and Wilson, 1993; Kubitzki, 1993; Fu et al., 1999; Huang et al., 1999; Lu et al., 1999; personal observation). We scored fagalean pollination syndromes into two types: anemophilous and entomophilous (Kubitzki, 1993; Manos et al., 2001).

The evolutionary shifts of selected characters were carried out using maximum likelihood and likelihood function methods in Mesquite v2.75 (Maddison and Maddison, 2011) and Diversitree v0.9-6 (FitzJohn et al., 2012), respectively. For maximum likelihood analyses, the Mk1 model of evolution for discrete unordered characters (Lewis, 2001) was used. The procedure "trace over trees" was used to summarize reconstructions over a BEAST sample of 1000 chronograms chosen randomly (after the burn-in). For likelihood function analyses, BiSSE (Binary-State Speciation and Extinction) and MuSSE (Multi-State Speciation and Extinction) models were used for binary and multiple states, respectively. The analyses sampled 1000 BEAST trees to assess the robustness of the results against phylogenetic uncertainty. BEAST consensus tree was used to perform the reconstructions, and the mean relative probabilities of each ancestral state for all internal nodes were summarized over the 1000 replicates with the respective mean and standard deviation.

Correlated evolution analysis

To test for correlated evolution between dispersal mode and habitat, we employed Bayesian reversible-jump hyperprior (RJHP) MCMC analyses in BayesTraits v2.0 (Pagel and Meade, 2006) to estimate posterior support for dependent versus independent models for state changes between characters. Consensus BEAST tree was used. For each BayesTraits analysis, at least 3 replicate MCMC runs were conducted for 5,000,000 iterations following a burn-in of 50,000 iterations, with posterior sampling every 1000 iterations. As the independent and dependent models are estimated by MCMC, their goodness of fit is compared using the log-Bayes Factor test: 2*log[harmonic mean (dependent model)] – log[harmonic mean (independent model)]. A Bayes factor >2.0 represents positive evidence, >6.00 represents strong evidence, >10.00 represents very strong evidence (Kass and Raftery, 1995). As binary traits are required in RJHP MCMC analyses, we coded wind vs. other modes (including scatterhoarding, endozoochorous, and unassisted) for dispersal modes and open vs. other habitats (including closed and open/closed) for habitat types. Only four genera in Fagales (*Nothofagus*, *Platycarya*, *Pterocarya*, and *Rhoiptelea*), inhabit in open and closed habitats. We also coded closed vs. other habitats (including open and open/closed) for habitat types. The two results were entirely consistent with each other.

Diversification analyses

In order to mitigate the impact of incomplete taxon sampling on diversification rate analyses, diversification analyses were performed using two whole-clade methods, net diversification rates (Magallón and Sanderson, 2001) and MEDUSA (modeling evolutionary diversification using stepwise AIC; Alfaro et al., 2009). Our phylogenetic tree with 284 taxa was first pruned to include 27 terminals, each of which represents a single genus, or monophyletic groups of a few genera (Fig. 1). Missing taxa were inserted in corresponding clade based on our phylogeny and the genus-level species diversity (Table S1). Net rate of diversification (r) of Fagales and some internal clades of interest were calculated following the equation (6) of Magallón and Sanderson (2001) under two extremes of the relative extinction rate ($\varepsilon = 0.0$ and 0.9, respectively). We derived a 95% credibility interval on the expected diversity of the order through time following the equations (10a and 10b) of Magallón and Sanderson (2001). Using the mean age estimate for the stem group, standing diversities for the clades were compared with these sets of critical values, and those exceeding the upper values were regarded unexpectedly species-rich, given the estimated overall diversification rate for the order. Calculations were performed using GEIGER v1.3-1 (Harmon et al., 2008). MEDUSA fits alternative birth-death models to a phylogenetic tree taking into account taxonomic data, and can assess among-lineage variation in diversification rates and discover putative diversification rate shifts in one or more phylogenetic positions. We fit a series of alternative models of increasing complexity in the tree but present the pattern of diversification obtained using a moderate corrected AIC threshold (cut-off value = 4), which means rejecting models that do not improve the AIC scores by more than 4 (Alfaro et al., 2009). MEDUSA analysis was implemented in GEIGER v1.3-1 (Harmon et al., 2008).

Both winged and wingless fruits in Fagales have been considered to be responsible for species diversity in Fagales (Tiffney, 1984, 1986; Manchester, 1987, 1989; Friis et al., 2011). To test the hypothesis, we also examined the dynamic changes of rise of winged and wingless genera over geological time by integrating fossil and molecular dating data. Based on Meredith et al. (2011), sliding window analyses were conducted for winged and wingless genera. For extant genera, we used the credibility intervals of the estimated times inferred from a UCLN model. For the majority of extinct genera, the accurate time of their occurrence is unclear. We therefore used the stratigraphic periods of the fossils deposited conservatively. The time span from present to 105 Ma was divided into five-million-year windows. For each window, the number of new genera that originated during the sliding window was divided by the number of genera present prior to the start of the respective sliding window.

Statistical analyses of fossil fruits

The fruit fossil records of Fagales were investigated by the literature and the paleobiology database (http://paleodb.org/cgi-bin/bridge.pl). Referring to Tiffney (1986), we classified fagalean fossil fruits into two types: winged or large-bracted nuts (defined as “winged fruit”, usually small size, <10 mm), and wingless nuts and drupes (defined as “wingless fruit”, usually large size, ≥10 mm).

The fossil fruits reviewed are listed in Table S5. A total of 127 fossil fruits were investigated, covering a time span from the Late Cretaceous to Pliocene. We calculated the ratio of the winged and wingless fruit fossils, and we analyzed the nut size (average length) changes of all fossils, winged and wingless fossils over the geological time scale, respectively. We also divided all wingless fossil fruits through time into seven groups (Late Cretaceous, Paleocene, Eocene, Oligocene, Miocene, Pliocene and Recent), and then used linear regressions to calculate the correlation between the fraction of biotic dispersal groups and their mean nut sizes.

Results

Phylogeny of Fagales

The combined five-marker data set included 5472 characters with 1834 parsimony-informative sites (Table S6). ML analysis yielded a topology that is highly consistent with those retrieved from BI and MP analyses except for the nodes with weak support (Fig. S2). Fagales and its families were all strongly supported as monophyletic, and inter-familial relationships within the order were overall well resolved and congruent with previous studies (e.g., Manos and Steele, 1997; Soltis et al., 2000, 2011; Li et al., 2004; Herbert et al., 2006; Sauquet et al., 2012), except that our analyses supported Myricaceae as sister to the Casuarinaceae-(Ticodendraceae-Betulaceae) clade (ML BS = 58%, MP BS = 58%, PP = 83%), and are consistent with the result of Sauquet et al. (2012; ML BS = 67%), which are strongly supported by *PHYA* and *PHYC* data (unpublished data); other aforementioned studies often placed this family as sister to the Juglandaceae with poor or weak support. In addition, 29 of the 32 genera of Fagales (except *Castanopsis*, *Ostrya* and *Quercus*) were monophyletic, and the majority of inter-generic relationships in each family were well resolved except in the Quercoideae of Fagaceae.

Divergence time estimate and rates of diversification

The effective samples sizes for all BEAST runs were >200 for all parameters. Our time estimate provides for the first time a complete genus-level time scale for Fagales (Fig. S3 and Table S7). The stem group of Fagales occurred at 124.5 Ma (121.1–125.4 Ma, 95% highest posterior density, HPD) and the crown group diverged at 105.2 Ma (95% HPD: 101.3–107.3 Ma). Most families appeared during 100–75 Ma, while the genera diversified mainly from 60 Ma to 40 Ma (Figs. 1, S3, S4 and Table S7).

By calculating the net diversification rates of Fagales and its internal clades, we found that some lineages during the Paleogene have an elevated diversification rate relative to Fagales overall, such as nodes 8, 30, 41, and 43 (above the upper), nodes 27, 34, 40, 45, 48, 49, and 53 (near the upper) (Fig. S5 and Table S8). The MEDUSA analysis suggests that the current diversity of Fagales is best explained by one shift in the rates of diversification during their evolutionary history (Fig. 1). The best-fit model had a log-likelihood value of −228.2155, and it had the best-corrected AIC score (AICc = 462.43), ΔAIC of which is ≥4 (ΔAIC = 14.97) than one-rate model and <4 than three-rate model. The clade including *Castanea*, *Castanopsis*, *Chrysolepis*, *Lithocarpus*, and *Quercus* originated in 59.6 Ma (95% HPD: 50.6–66.1 Ma) and has a higher net diversification rate ($r = 0.088$, $a = 0.794$) than the background tempo for all Fagales ($r = 0.025$, $a = 0.913$).

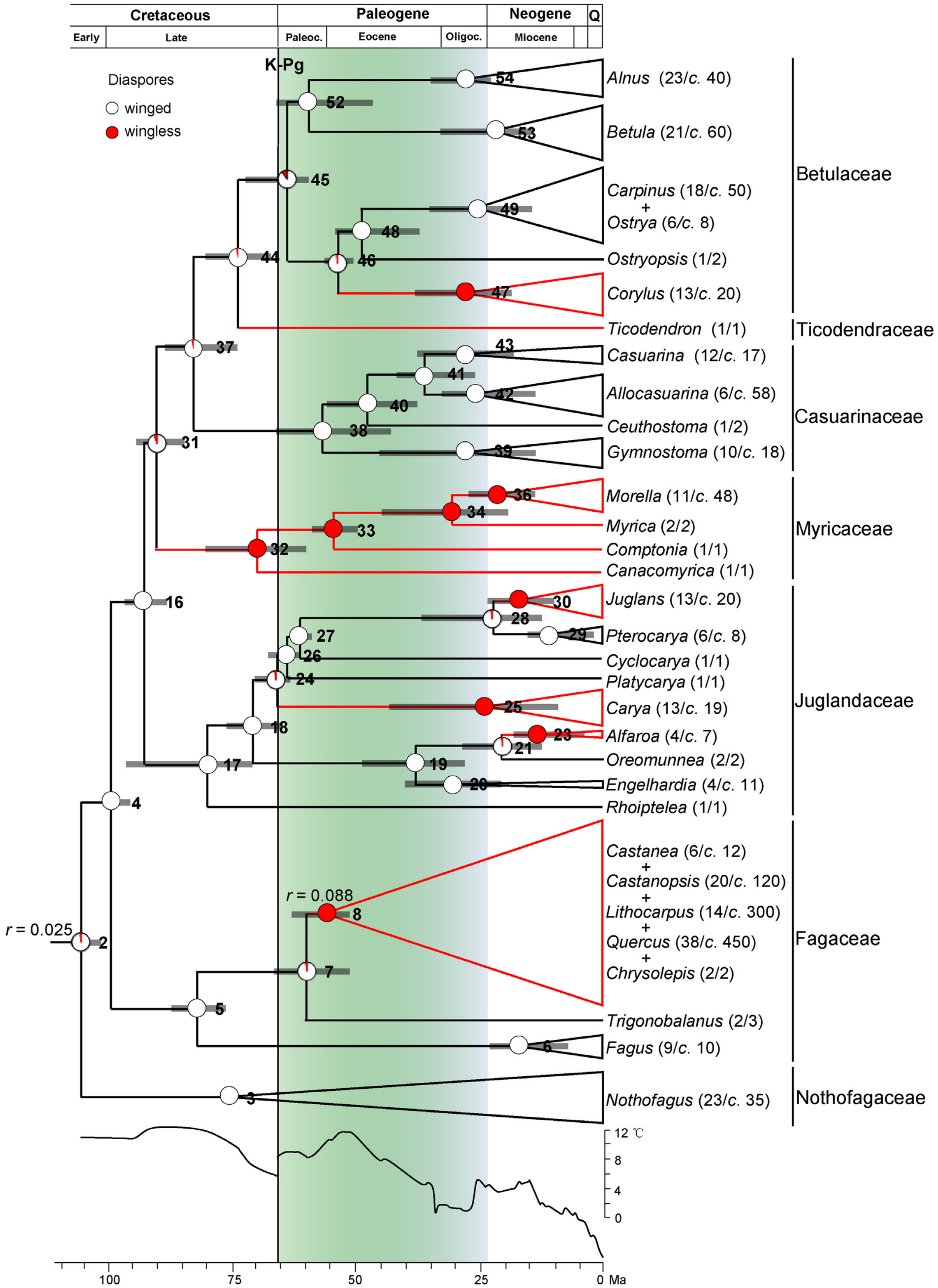

Fig. 1. Combined chronogram and reconstruction of diaspore evolution of Fagales. Numbers alongside generic names represent the proportion of species we sampled relative to the total species richness of each genus. Numbers in bold near branches indicate the node number, as referred to Fig. S3 and Table S7. The depiction of temperature changes is modified from Zachos et al. (2001) and Miller et al. (2005).

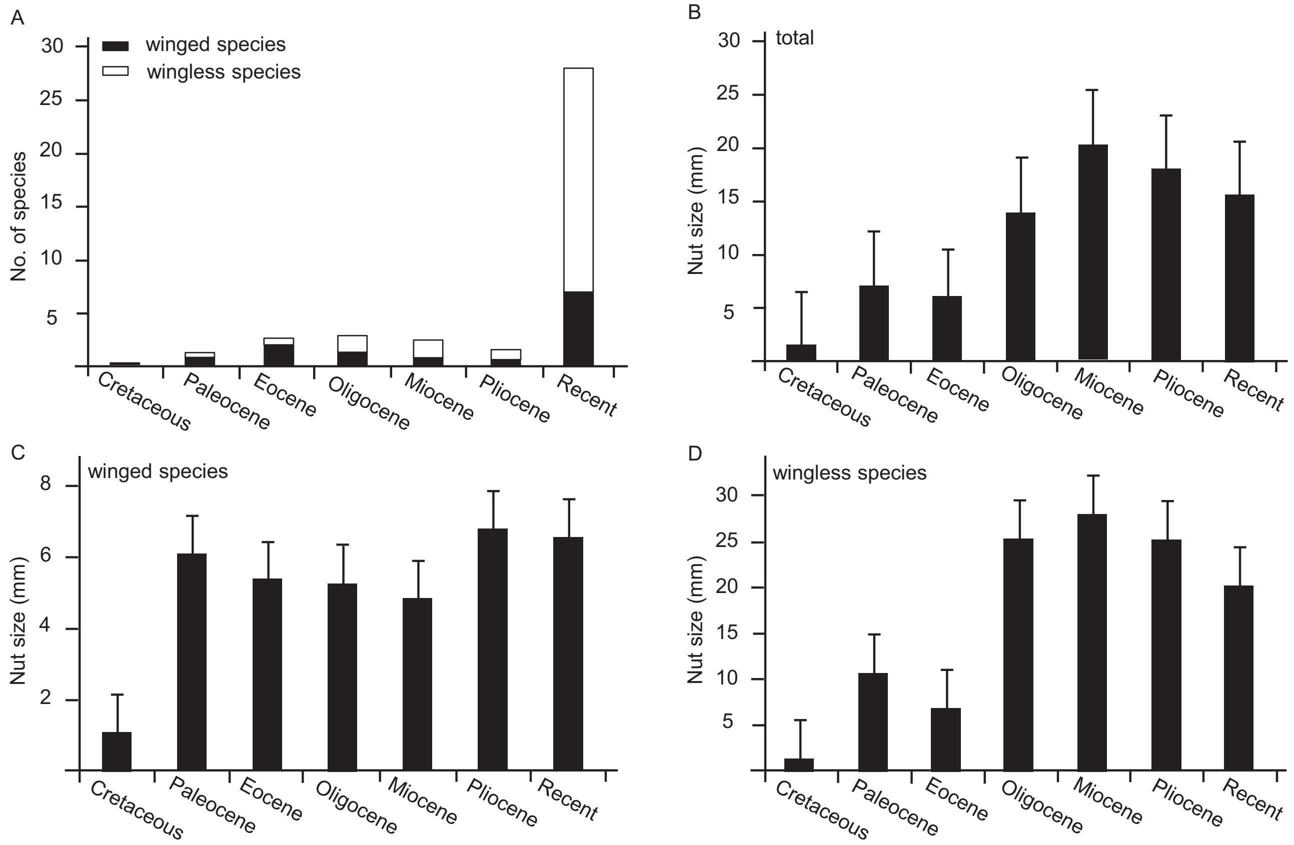

Fig. 2. Statistical analyses of fruit types and sizes through time. (A) The ratio of winged and wingless groups since the late Cretaceous. (B) Nut size changes of the whole Fagales since the late Cretaceous. (C) Nut size changes of winged groups since the late Cretaceous. (D) Nut size changes of wingless groups since the late Cretaceous.

Evolutionary inferences of selected characters

Two likelihood methods generated highly congruent results about evolutionary inferences of the selected four characters. In Fagales, our reconstructions indicate that winged diaspores are symapomorphic, and the wingless ones are derived and have independently evolved at least seven times (Figs. 1, S6 and S7); biotic dispersal was symplesiomorphic, whereas abiotic dispersal had independently evolved in Betulaceae and Juglandaceae (Figs. S8 and S9). Anemophilous is inferred to be the ancestral state, whereas entomophilous is usually found in a few more derived genera (Table S1 and Figs. S10, S11). The ancestor of Fagales inhabited in closed environments, and then migrated to open habitats time after time (Figs. S12 and S13).

Statistical analyses of fossils

The statistical analysis of 127 fossil taxa in Fagales shows that the winged groups first occurred in the Late Cretaceous, while wingless groups appeared later (Table S5). The statistical analyses of more than 50 fossil fagalean fruit records indicate that ~66% of those with wings occurred during the Paleocene and Eocene (Fig. 2A). The fagalean taxa with wingless fruits have few records during the Late Cretaceous, but the number of fossils rapidly increased since the late Paleocene (Figs. 2A, S14 and Table S5). Fruit sizes in Fagales were small during the Cretaceous, and became larger in the Paleogene (Fig. 2B). The average size of the winged fruits did not increase apparently during the Cenozoic (Fig. 2C). Also, there are 57 wingless fruit fossil records through the Cenozoic, and the fruit sizes greatly increase in the wingless groups, such as Fagaceae and Juglandaceae (Fig. 2D).

Discussion

Early diversification of Fagales in the mid-Cretaceous

The resulting chronogram suggests that the diversification of extant Fagales started in the mid-Cretaceous (node 2) (Fig. 1 and Table 1), which was a little older than that of Magallón and

Table 1
Estimated ages (million years ago, Ma) for the nodes of interest in Fagales using BEAST.

Node	Age (LHPD–UPHD)
2 – Fagales crown	105.2 (101.3–107.3)
7 – Origin of wingless group in Fagaceae	59.5 (50.6–66.1)
8 – Diversification of wingless group in Fagaceae	56.4 (50.5–63.2)
17 – Diversification of Juglandaceae	79.9 (71.2–96.4)
21 – Origin of wingless *Alfaroa*	20.5 (12.4–29.3)
24 – Origin of wingless *Carya*	66.6 (62.6–69.7)
28 – Origin of wingless *Juglans*	22.6 (12.4–37.3)
31 – Origin of wingless Myricaceae	90.4 (85.0–94.6)
32 – Diversification of Myricaceae	69.7 (60.4–81.7)
38 – Diversification of Casuarinaceae	56.2 (45.3–65.7)
44 – Origin of wingless Ticodendraceae	74.0 (66.9–80.3)
45 – Diversification of Betulaceae	64.4 (59.4–72.5)
46 – Origin of wingless *Corylus*	54.0 (50.6–56.4)

The node numbers correspond to those in Figs. 1 and S3. UHPD and LHPD are the upper and lower highest posterior densities, respectively.

Castillo (2009; 93.5 Ma) and Wang et al. (2009; 92–98 Ma), but younger than one of the calibration scenarios of Sauquet et al. (2012; 112.7–124.8 Ma). Based on our time estimates, most families appeared during 100 Ma to 75 Ma (Figs. 1, S3, S4 and Table S7). Fossil evidence also suggested that all major fagalean lineages were present at the latest by the Cenomanian (Friis et al., 2006). The distribution of various fagalean fossils, including flowers, fruits, pollen grains, leaves, and woods (Manchester, 1987; Friis et al., 2011), from Europe and North America suggests that fagalean plants were already distributed widely in the mid-Cretaceous. Based on evolutionary inference of pollination syndromes, the early-diverging lineages in Fagales are anemophilous (Fig. S10). Wind-pollinated plants can have an adaptation to open vegetation like that proposed for the mid-Cretaceous (Upchurch and Wolfe, 1987; Friis et al., 2011). Fagalean plants are very important trees or shrubs in tropical and temperate forests (Table S1). Thus, our results support the viewpoint that the angiosperm-dominated forests began to rise during the mid-Cretaceous (Wang et al., 2009).

Increased diversification of Fagales in the Paleogene

Given the inferred credibility intervals of the estimated times of divergence, our estimates indicate that more than 87% of the extant genera within Fagales occurred or diverged in the Paleogene (Fig. S4 and Table S7). Our review of fossil fruits indicates that 28 extinct genera and 17 extant genera have so far been documented in its fossil history, and ~84% of them appeared during the Paleocene and Eocene (Fig. S14). The range of these fossil fruits covered Eurasia, North America and Oceania (Table S5) suggests that the plants belonging to the order were already distributed widely during this time. Thus, we supposed that Fagales experienced a burst of diversification at the generic level during the Paleogene.

It is well known that land vegetation and ecosystems on the planet were profoundly devastated at the K–Pg boundary (Wolfe and Upchurch, 1986; Vajda et al., 2001; Nichols and Johnson, 2008). After the mass extinction interval, the global ecosystems gradually recovered and modern tropical rainforests and temperate forests developed (Upchurch and Wolfe, 1987; Wing et al., 2009; Field et al., 2011; Wang et al., 2012). Fagales, with various diaspores, as one of the important components in tropical and temperate forests, could make use of many new ecological niches generated by the K–Pg extinction event. Global warming climate during the Paleogene (Upchurch and Wolfe, 1987; Zachos et al., 2001; Miller et al., 2005) could also have facilitated fagalean diversification.

Based on our time estimates, about 83% of extant winged genera (15/18 genera) occurred or diverged during the Paleogene (Figs. 1, S4 and Table S7). Sliding window analyses indicate that the winged genera rapidly rose during both the Late Cretaceous and Paleogene (Fig. 3), but the majority of extant and extinct genera occurred during Paleocene (37/45 = 75.6%). Moreover, net diversification rate analyses indicate that eight winged clades, viz. nodes 27, 40, 41, 43, 45, 48, 49, and 53, have an elevated diversification rate relative to Fagales overall during Paleogene (Fig. S5A and Table S8). Fruit sizes of the winged fruits was small during the Cretaceous and Cenozoic (Fig. 2C), but the shape and number of wings shows a marked increase in diversity since the Paleogene. The wings of fagalean fruits, reflecting adaptation to wind dispersal (Tiffney, 1986; Friis et al., 2011), are developed from the involucre (e.g., *Carpinus* and *Ostrya*; Li and Sbrovtso, 1999), bracts (*Pterocarya* and *Engelhardia*; Lu et al., 1999), or perianths (*Platycarya*; Lu et al., 1999). Based on our evolutionary reconstructions, winged diaspores in Fagales are dispersed by wind or may be simply gravity-dispersed (unassisted) (Figs. 4A and S8). Except several genera in Juglandaceae, most of the extant winged groups of Fagales inhabit various open habitats (Figs. 4B and S12). Some plants possess a tree habit (e.g., *Alnus*, *Betula* and *Pterocarya* in temperate forests), however such species are often fast-growing and short-lived, and tend not to form time-stable, closed communities (Tiffney, 1984, 1986; Friis et al., 2011). Winged taxa in Juglandaceae are high trees and occupy canopies of temperate forests (e.g., *Platycarya* and *Pterocarya*) and tropical rainforests (e.g., *Oreomunnea*), so that winged fruits may stay aloft longer than in the case of small trees or shrubs, allowing them to disperse greater distances. The RJHP MCMC analysis indicates a strong support for correlated transitions between wind dispersal mode and open habitat (BF = 34.40). We thus suggest that a diversification of fruits with wings, may have been favored by relatively open habitats after the K–Pg boundary (Tiffney, 1984, 1986; Friis et al., 2011), allowing them to spread over more extensive regions.

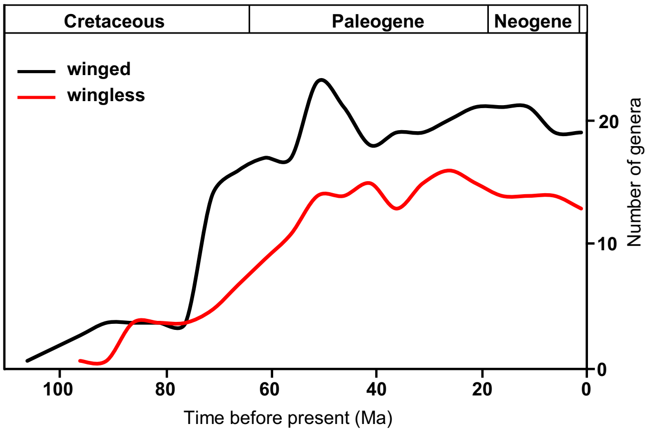

Fig. 3. Dynamic changes of rise of winged and wingless genera over geological time.

Based on our sliding window analysis, the majority of wingless genera occurred during Paleogene (Fig. 3). Net diversification rate analyses indicate that three wingless clades, viz. nodes 8, 30, and 34, have an elevated diversification rate relative to Fagales overall during this period, and 85% of extant wingless genera (12/14 genera) occurred during the Paleogene including more than 70% of the fagalean species at that time (Figs. S4, S5B and Table S8). The RJHP MCMC analysis showed very strong support for correlated transitions between scatterhoarding, endozoochorous and unassisted dispersal systems and closed and open/closed habitats (BF = 34.40). Wingless diaspores in Fagales are typically dispersed by mammals or birds (Figs. 4A and S8; Stone, 1973; Tiffney, 1986; Friis et al., 2011), and many of the extant groups with wingless diaspores inhabit closed canopy habitats, such as evergreen forests (e.g., *Castanopsis* and *Lithocarpus*) (Figs. 4B and S12). During the Late Paleocene to early Eocene, modern tropical and temperate forests began to rise, and their communities became more and more closed (Wing and Tiffney, 1987; Morley, 2000; Upchurch et al., 2002; Jacobs, 2004). Previous studies have indicated that, in closed habitats, fruit dispersal by vertebrates hoarding and burying was an important mechanism (van der Pijl, 1982; Tiffney, 1984, 2004). Wang and Chen (2009) used different sizes of artificial seeds to observe and track the behaviors of scatterhoarding rodents in a natural pine forest in Northwest Yunnan, China. They found that rodents consistently consumed small seeds in situ, but removed medium-sized seeds (1.2–2.5 cm in diameter) and transported bigger seeds farther (Wang and Chen, 2009). Based on the statistical analysis of the nuts and their size through successive stages of the Cretaceous and Tertiary, increase of nut size in the Fagales was

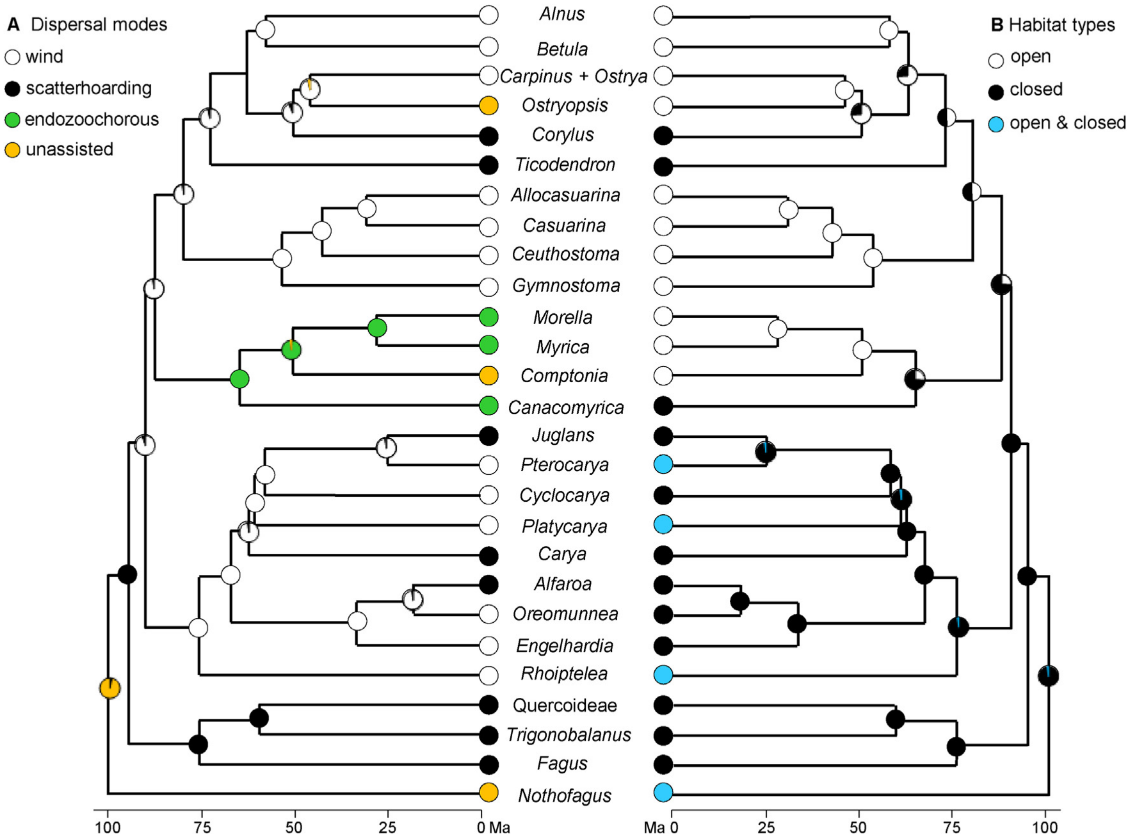

Fig. 4. Summaries of evolutionary reconstructions of dispersal modes (A) and habitat types (B) at the generic level. A and B are from Figs. S8 and S12, respectively.

found in the wingless fruits (Fig. 2B–D). Moreover, there is a strong positive relationship between the fraction of biotic fruit dispersal and the nut size (average diaspore length) over geological time ($r = 0.904$, $P < 0.05$; Fig. 5). The rapid diversification of mammals and birds after the K–Pg boundary (Feduccia, 1995, 2003; Hedges et al., 1996; Adkins et al., 2003; O'Leary et al., 2013) would have facilitated dispersal of wingless fruits. Thus, the activities of vertebrate dispersers might have played a key role in the diversification of wingless fagalean groups during the Paleogene.

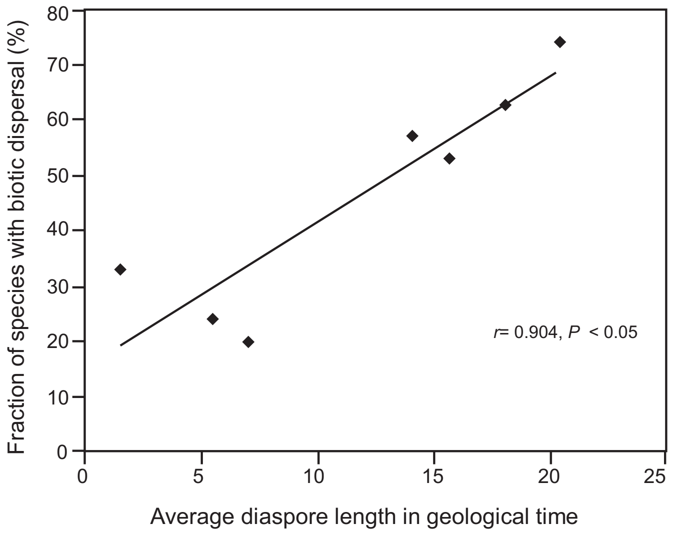

Fig. 5. Correlation between the fraction of species with biotic dispersal and average diaspore length in geological time.

Conclusion

A burst of diversification of Fagales at generic level occurred during the Paleogene, and it appears to be the result of ecological opportunity, combined with diaspore diversification that followed the evolution of dispersal patterns. The K–Pg extinction event and subsequently global warming climate provided extrinsic environment prerequisites for diversification of Fagales during the Paleogene. The winged diaspores with diverse wings and/or small size fruits could disperse easily by abiotical strategies in the open habitats, and the wingless diaspores could have been dispersed by vertebrate in the closed habitats. Thus, diaspores of Fagales by positively responding to the environmental changes led to its diversity. The Fagales provides an empirical study to explain lineage trends in species diversity, as a result of the interplay of ecological changes and key innovations. It is clearly advantageous to integrate key character evolution into diversity explanations in a dated-phylogeny context.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ppees.2014.03.001.

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