Joint evolution of mycorrhizal type, pollination, and seed dispersal mode in trees

Last revision: October 4, 2020

Authors: Akira Yamawo*, Misuzu Ohno

Affiliations:

Department of Biological Sciences, Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

*Correspondence to: yamawo.aki@gmail.com

Abstract: Mycorrhizal type, pollination, and seed dispersal mode are key drivers of tree diversity, but because they are usually studied in isolation, their evolutionary relationships are poorly understood. We investigated the evolutionary relationships among mycorrhizal type (arbuscular mycorrhizal [AM], ectomycorrhizal [EM], and AM + EM), seed dispersal mode (anemochorous, endozoochorous, and unspecialized), and pollination mode (biotic and wind) in 821 tree species. We found evidence of joint evolution of all three features. Most AM-associated trees had endozoochorous seed dispersal and biotic pollination, whereas most EM-associated trees had anemochorous seed dispersal and wind pollination. Overall, mycorrhizal type, seed dispersal mode, and pollination mode were linked, emphasizing the importance joint study of these traits for understand of tree diversity.

Introduction

Mycorrhizal symbiosis, pollination, and seed dispersal are important factors that have driven the evolution and maintained the diversity of trees in forest ecosystems (Pellmyr 1992; Seidler & Plotkin 2006; Teste et al. 2017). For example, relationships with pollinators or seed dispersers are considered to be key characteristics that led to the evolutionary success of the angiosperms beginning in the Lower Cretaceous, 130 million or 80 million years ago, respectively (Regal 1977; Crepet 1984; Pellmyr 1992; Eriksson 2014). Mycorrhizal fungi provide their host plants with nutrients from the soil or aid in defense against insects and parasitic fungi, and enhance the survival rate, growth, flower production, and fitness of their host plants (Tedersoo et al. 2020). Mycorrhizal fungi contribute to maintaining trees community diversity via plant-soil feedbacks (PSFs), and these can be either positive or negative and differ between arbuscular (AM) and ectomycorrhizal (EM) fungi (Bennett et al. 2017; Teste et al. 2017; Tedersoo et al. 2020). However, the evolutionary relationships among mycorrhizal type, pollination, and seed dispersal mode are not well understood.

Mycorrhizal, floral, and seed dispersal traits are not independent (Correia et al. 2018) but rather are connected through ecological and physiological mechanisms. For example, mutualism with AM fungi supports flower production, pollinator attraction, and fruit production (Wolfe et al. 2005). Negative and positive PSFs may favor long- and short-range seed dispersal in AM- and EM-associated trees, respectively. Because negative and positive PSFs favor low and high population density (Bennett et al. 2017), respectively, they are expected to favor biotic (Pellmyr 1992) and wind pollination (Mustajärvi et al. 2001; Hesse & Pannell 2011), respectively. Nevertheless, all three features have traditionally been studied in isolation from each other, and their combined effects remain a major gap in our understanding of tree evolution. Given the ubiquity of interactions with mycorrhizal fungi, pollinators, and seed dispersers among tree species, it is surprising how little we know about the evolutionary

relationships among these interactions.

Although there are some studies of the effects of mycorrhizal fungi on pollination, most studies are limited to experimental approaches focused on ecological effects in a few species (reviewed in Tedersoo et al. 2020). A phylogenetic approach that includes multiple species can close this gap by directly demonstrating the evolutionary patterns among these interactions (Correia et al. 2018). We investigated the evolutionary relationships among mycorrhizal type (AM, EM and both (AM + EM)), seed dispersal (by animals (endozoochorous), by wind (anemochorous) and unspecialized) and pollination (biotic and wind) mode in 821 tree species using a phylogenetic tree.

Materials and Methods

We collected information about mycorrhizal type, seed dispersal, and pollination modes from multiple databases. Tree species for which all three pieces of information were not available were excluded from the analysis. The details are as follows.

Mycorrhizal types

Five main sources were used to compile information on plant mycorrhizal types: (1) Mycoflor, a database that lists the mycorrhizal status of the tree species included in this study (Hempel et al. 2013); (2) an updated checklist of mycorrhizae of land plants (Wang & Qiu 2006); (3) a checklist of mycorrhizae of the flora of Britain (Harley & Harley 1987, 1990); (4) a database documenting mycorrhizal associations in 3000 vascular plant species from the former Soviet Union (Akhmetzhanova et al. 2012); and (5) a data set detailing the mycorrhizal types and type of plants of Europe, recently compiled by Bueno et al. (2017). We also used information on tree mycorrhizal types from Bennett et al. (2017), Brown et al. (2020), Sasaki et al. (2019), Uma et al. (2012), Seiwas et al. (2020), Liu et al. (2019) and Moyersoen et al. (2001). Other

sources of information on mycorrhizal type are included in Supplementary data. To minimize errors, the data used for all entries were individually checked in multiple databases and their cited references.

Seed dispersal and pollination modes

Data on seed features, especially those that aid in long-distance dispersal (LDD), were collected from the list compiled by Heleno & Vargas (2015), the Plants of the World Online (POWO) (http://www.plantsoftheworldonline.org/), and the Global Biodiversity Information Facility (https://www.gbif.org/)(Edwards et al. 2000) (Supplementary data). The list compiled by Heleno & Vargas (2015) includes LDD characteristics of 10,792 species from 142 families that are included in the Flora Europaea (Tutin et al. 1980). POWO is a web portal that makes scientific data collected at the Royal Botanical Gardens Kew available online, including the names of over a million plants from around the globe, plus images and detailed descriptions of many of those. Seeds with characteristics that promote wind dispersal, such as wings or a pappus, were classified as anemochorous; seeds with tissues that attract animals, such as fleshy or nutritious tissues, were classified as endozoochorous (Vargas et al. 2012; Heleno & Vargas 2015). Species that lack specialized dispersal structures were classified as unspecialized.

Pollination mode was classified as either biotic or wind (Wang et al. 2019), and our classification scheme did not include other categories, such as self-pollination. Flowers with typically developed petals were classified as having a biotic pollination mode. Flower with non-petals were classified as wind pollinated. Information for classification of pollination mode was collected from the databases mentioned above (Supplementary data).

Phylogenetic tree

The Plant List 1.1 was used to standardize species names, and membership in higher taxonomic groups was standardized using the APG IV (Angiosperm Phylogeny Group IV, 2016). Our database contains information on the mycorrhizal types, seed dispersal, and pollination modes for 821 species from 397 genera and 111 families (see Supplementary data). Our database contains 93 % of the family and 83 % of the genus of major northern hemisphere tree species (Brundrett & Tedersoo 2020). The most represented families in our study are namely Leguminosae (15%), Rosaceae (10%), Pinaceae (7%), Salicaceae (4%), Fagaceae (4%).

Statistical analyses

Log–linear analysis with maximum likelihood chi-squared and post hoc Freeman–Tukey deviation tests, with Bonferroni's correction, were used to test for univariate relationships between mycorrhizal type, seed dispersal, and pollination modes (Legendre & Legendre 1988). All analyses were performed in R v. 4.0.2 software (R Development Core Team 2020). In all analyses, a phylogenetic species richness (PSR) index was used to correct for phylogenetic relatedness (Helmus et al. 2007). To calculate PSR, the number of species in a sample is multiplied by the phylogenetic species variability, a term that describes how the variance of a trait is decreased by the phylogenetic relatedness of the species being studied (Helmus et al. 2007). PSR thus represents the species richness of a sample after species relatedness has been discounted, making it more appropriate than uncorrected species richness for use in the contingency table. PSR values decrease as species relatedness increases, approaching zero (Helmus et al. 2007).

First, mycorrhizal type (AM, AM + EM, and EM) was tested against the proportion of seed dispersal mode (anemochorous, endozoochorous, and unspecialized). For each pair of mycorrhizal type and dispersal syndrome, PSR was calculated in the R package 'picante'

(Kembel et al. 2010); these values were used in the contingency tables as described in Pyšek et al. (2011) and Hempel et al. (2013).

The contingency tables were used to check for differences between categories using log-linear models with maximum likelihood chi-squared analysis. Post hoc Freeman–Tukey deviation tests (Legendre & Legendre 1998) with Bonferroni's correction were used for two-way or multiway contingency tables. In Freeman–Tukey tests, the observed and expected counts were used to calculate an approximately normalized estimate of how far each observed frequency deviates from the null hypothesis (Freeman–Tukey deviate: $\sqrt{O} + \sqrt{(O + 1)} - \sqrt{(4E + 1)}$). We also used the approach described by Sokal and Rohlf (1995) to calculate a critical value for the Freeman–Tukey deviates ($\sqrt{(v\ X_{[1,\alpha]}^{2} / (\text{no.cells})}$), with Bonferroni correction. All the analyses were performed using the statistical software R v. 4.0.2 (R Development Core Team 2020).

We used PhytoPhylo tree (Qian & Jin 2016) to construct a phylogenetic tree based on the phylogeny generated by Zanne et al. (2014) and updated by Qian & Jin (2016). Then we reconstructed this tree to incorporate the phylogenetic correction based on the PSR index by using the 'S.PhyloMaker' function implemented in R (Qian & Jin 2016). The final phylogenetic tree had 821 tip labels and 791 internal nodes (Fig. 1).

Results and Discussion

All trees (821 plant species from 397 genera in 111 families) in our database were assigned to one of three mycorrhizal types: AM (573 species from 347 genera in 105 families), AM + EM (77 species from 42 genera in 23 families), or EM (171 species from 53 genera in 18 families). Association with AM fungi was widespread across the phylogenetic tree, being present in ~94% of all families and in ~87% of genera (Fig. 1). The phylogenetic tree contained two large clades, angiosperms and gymnosperms. Tree species of all three mycorrhizal types (AM,

AM + EM, and EM) were present in both clades. Likewise, seed dispersal modes (endozoochorous, 398 species from 217 genera in 91 families; anemochorous, 233 species from 80 genera in 31 families; and unspecialized, 190 species from 110 genera in 29families; Fig. 1) and pollination modes (biotic, 579 species from 327 genera in 93 families; and wind, 242 species from 73 genera in 27 families) were scattered across the phylogenetic tree.

The phylogenetically corrected log–linear analysis revealed that mycorrhizal types and seed dispersal modes were significantly linked (Table 1). Of the AM-associated trees, a greater proportion produced endozoochorous seeds than anemochorous seeds (Fig. 2A; Table S1). This result was not found in the analysis by Correia et al. (2018) that included herb species, meaning that it is specific to trees and their life history traits. In contrast, more EM-associated trees produced anemochorous than endozoochorous seeds (Fig. 2A; Table S1). Several studies have reported that within-species PSFs differ between AM- and EM-type trees (reviewd in Tedersoo et al. 2020). For EM-associated trees, PSFs are positive within species; growth and survival rate are increased in soil in which conspecifics grow (e.g. Bennett et al. 2017). On the other hand, for AM-type trees, PSFs are negative within species; growth and survival rate are reduced in soil in which conspecifics grow (e.g. Bennett et al. 2017). These contrasting effects should promote the evolution of different seed dispersal modes in AM- and EM-type trees. EM-associated trees may have evolved middle-range seed dispersal because it allows seedlings to receive positive PSFs from conspecifics but avoid competition with parental trees. In contrast, AM-associated trees may have evolved long-range seed dispersal by animals (Seidler & Plotkin 2006; Naoe et al. 2016) because it allows seedlings to avoid negative PSFs from conspecifics.

The differences in seed dispersal modes can be explained also in terms of different traits of different mycorrhizal types. Mycorrhizal interactions are largely generalist, but EM fungi are more host-specific than AM fungi (van der Heijden et al. 2015). Long-distance seed

dispersal may favor symbiosis with generalized AM fungi over that with host-specific EM fungi through higher encounter rate with generalized AM fungi around them after dispersal. These ecological conditions also favor evolution of differing seed dispersal modes between AM- and EM- type trees. These processes may have facilitated the joint evolution of mycorrhizal types and seed dispersal modes by interacting, not exclusively, with each other.

Interestingly, we found that pollination mode is also associated with mycorrhizal type (Fig. 2B, Tables 1, S2). About 87% of AM-type plants are biotically pollinated, whereas more than ~78% of EM-type trees are wind pollinated. Thus, evolution of pollination modes may have been driven by mycorrhizal mutualism types. The negative and positive PSFs promoted by AM and EM fungal associations and linked to different seed dispersal modes induce low or high tree population density, respectively (Seidler & Plotkin 2006; Bennett et al. 2017). In general, wind pollination is more effective when plants have a high population density, and the efficacy decreases as population density decreases, because the pollen concentration in the air rapidly decreases with distance from pollen-producing plants (Culley et al. 2002). In fact, many tree species that are wind pollinated, such as *Pinus* or *Fagus* species, often have high population density and are the dominant trees in the forest (Culley et al. 2002). On the other hand, the efficacy of biotic pollination may not decrease as population density decreases than wind pollination, because pollinators tend to visit conspecific flowers through foraging leaning (Pellmyr 1992). For future studies of pollination biology and its relationship to plant population density and mycorrhizal symbiosis, it would be useful to understand the evolution of pollination mode in trees.

Seed dispersal and pollination modes were also linked in the phylogenetic tree (Fig. 2C; Table S3). Most trees with biotic pollination had endozoochorous seeds. On the other hand, most trees with wind pollination had anemochorous seeds. Thus, mycorrhizal type, seed dispersal, and pollination modes appear to have evolutionary connections with each other.

However, gymnosperms, which have AM-type fungal associations, did not develop biotic pollination (Fig. 1). Because gymnosperms originated before the evolution of biotic pollination (Kenrich & Crane 1997), this may indicate a phylogenetic constraint. Overall, evolution of pollination mode appears linked to evolution of mycorrhizal type via the population density of each plant species.

Although we cannot discuss the evolutionary causation of the links between mycorrhizal type, seed dispersal, and pollination mode, discussion of the evolutionary history of the relevant plants may be useful for understand of tree diversity and evolution. Plants have lived in close association with AM fungi for over 400 million years. Fossils of the earliest land plants (400 million years ago) provide evidence that fungi morphologically similar to the extant Glomales (AM mycorrhizal) lived within their cells (Remy et al., 1994; Heckman et al., 2001). Biotic pollination and endozoochory have sometimes been considered as one of the key characteristics that led to the evolutionary success of the angiosperms beginning in the Lower Cretaceous 130 million or years ago (Regal 1977; Crepet 1984; Pellmyr 1992; Eriksson 2014). We do not know whether these ancient AM-type trees had negative PSFs on conspecific individuals or not, but if they had, PSFs may have been a driving force evolution of pollination and seed dispersal mutualism in tree species. This raises a novel question: at what point in evolutionary time did PSFs appear and how did they contribute to plant diversification, together with evolution of seed dispersal and pollination?

Our findings might also better explain the latitudinal gradient of seed dispersal and pollination modes reported by previous studies of AM- and EM-type trees. The greater prevalence of endozoochory in tropical regions (Wandrag et al. 2017) and of wind pollination (Culley et al. 2002) in temperate regions has been reported or suggested independently of mycorrhizal information. Likewise, AM- and EM-type trees are more frequently found in tropical and temperature regions, respectively (Gomes et al. 2019). The latitudinal gradients

of seed dispersal and pollination may be the result of increased dependence on these interaction modes at community and ecosystem scales through the dominance of AM- and EM-type tree species in tropical and temperature regions, respectively.

In conclusion, we found evidence of joint evolution of mycorrhizal type, seed dispersal, and pollination mode in tree species. The findings strongly imply that mycorrhizal type shaped the evolution of seed dispersal and pollination mode, which have different yet physiologically and ecologically linked functions. The importance of such interactive effects highlights the need for more integrative studies to better understand how interactions shape the evolution of diversity in trees. In addition, our results highlight that most AM-associated trees, which are more common globally, are endozoochorous and biotically pollinated, and therefore depend on mammals, birds, and insects for seed dispersal and pollination. The "empty forest syndrome" describes forests in which seed dispersing animals are missing, their numbers decreased by hunting or human encroachment, and where aggregation of same species is promoted (Caughlin et al. 2015). The aggregation of conspecific AM-type trees may increase negative PSFs on conspecific seedlings. Moreover, it has been reported that the amount and species diversity of insects, many of which function as pollinators, is decreasing worldwide (Lister & Garcia 2018; Sánchez-Bayo & Wyckhuys 2019). Our findings emphasize the importance of protecting animals as seed dispersers and pollinator insects for the conservation of forest ecosystems.

Author Contributions

AY and MO developed the core idea and designed the experiments. AY and MO performed data analyses and wrote the paper. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Yudai Okuyama, for their helpful comments about statistical analysis. AY thanks Hiroshi Ikeda, Kiyoshi Ishida, Shuichi Sugiyama, Akihiro Kimura and Haruna Ohsaki for discussions during the initial stages of this project. This work was also supported by Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (KAKENHI) (grant no. 18K19353 and 19H03295 to AY).

CONFLICT OF INTEREST

The authors declare no competing financial interests.

References and Notes

1. Akhmetzhanova, A. A., Soudzilovskaia, N. A., Onipchenko, V. G., Cornwell, W. K., Agafonov, V. A., Selivanov, I. A., & Cornelissen, J. H. (2012). A rediscovered treasure: mycorrhizal intensity database for 3000 vascular plant species across the former Soviet Union: Ecological Archives E093-059. *Ecology*, *93*(3), 689-690.
2. Angiosperm phylogeny group (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc., 181, 1–20.
3. Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321), 181-184.
4. Brown, A. J., Payne, C. J., White, P. S., & Peet, R. K. (2020). Shade tolerance and mycorrhizal type may influence sapling susceptibility to conspecific negative density dependence. *Journal of Ecology*, *108*(1), 325-336.
5. Brundrett, M. C., & Tedersoo, L. (2020). Resolving the mycorrhizal status of important northern hemisphere trees. Plant and Soil., 1–32.
6. Bueno, C. G., Moora, M., Gerz, M., Davison, J., Öpik, M., Pärtel, M., ... & Zobel, M. (2017). Plant mycorrhizal status, but not type, shifts with latitude and elevation in Europe. *Global Ecology and Biogeography*, *26*(6), 690-699.
7. Caughlin, T. T., Ferguson, J. M., Lichstein, J. W., Zuidema, P. A., Bunyavejchewin, S., & Levey, D. J. (2015). Loss of animal seed dispersal increases extinction risk in a tropical tree species due to pervasive negative density dependence across life stages. Proc R Soc Lond B, 282, 20142095.
8. Chen, L., Swenson, N. G., Ji, N., Mi, X., Ren, H., Guo, L., & Ma, K. (2019). Differential

soil fungus accumulation and density dependence of trees in a subtropical forest. Science, 366, 124–128.

9. Correia, M., Heleno, R., Vargas, P., & Rodríguez-Echeverría, S. (2018). Should I stay or should I go? Mycorrhizal plants are more likely to invest in long-distance seed dispersal than non-mycorrhizal plants. *Ecology letters*, *21*(5), 683-691.
10. Crepet, W. L. (1984). Advanced (constant) insect pollination mechanisms: pattern of evolution and implications vis-à-vis angiosperm diversity. Ann. Mo. Bot. Gard., 607–630.
11. Culley, T. M., Weller, S. G., & Sakai, A. K. (2002). The evolution of wind pollination in angiosperms. Trends Ecol. Evol., 17, 361–369.
12. Edwards, J. L., Lane, M. A., & Nielsen, E. S. (2000). Interoperability of biodiversity databases: biodiversity information on every desktop. Science, 289, 2312–2314.
13. Gomes, S. I., van Bodegom, P. M., Merckx, V. S., & Soudzilovskaia, N. A. (2019). Global distribution patterns of mycoheterotrophy. Global Ecol Biogeogr, 28, 1133–1145.
14. Harley, J. L., & Harley, E. L. (1987). A check-list of mycorrhiza in the British flora. *The New Phytologist*, *105*(2), 1-102.
15. Harley, J. L., & Harley, E. L. (1990). A check-list of mycorrhiza in the British flora–second addenda and errata. *New Phytologist*, *115*(4), 699-711.
16. Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., Hedges, S. B. (2001). Molecular evidence for the early colonization of land by fungi and plants. Science, 293, 1129–1133.
17. Heleno, R. H. & Vargas, P. (2015). How do islands become green? Glob. Ecol. Biogeogr., 24, 518–526.
18. Helmus, M. R., Bland, T. J., Williams, C. K. & Ives, A. R. (2007). Phylogenetic measures of biodiversity. Am. Nat., 169, E68–E83.
19. Hempel, S., Götzenberger, L., Kühn, I., Michalski, S. G., Rillig, M. C., Zobel, M. et al.

(2013). Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. Ecology, 94, 1389–1399.

20. Hesse, E., & Pannell, J. R. (2011). Density-dependent pollen limitation and reproductive assurance in a wind-pollinated herb with contrasting sexual systems. J Ecol., 99, 1531–1539.

21. Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D. Blomberg, S. P. & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics, 26, 1463–1464.

22. Kenrick, P., Crane P. R. (1997) The origin and early diversification of land plants: a cladistic study. Smithsonian Institution, Washington, DC. 441 pp.

23. Legendre, P. & Legendre, L. (1988). Numerical Ecology: Developments in Environmental Modelling, 3rd edn. Elsevier, Amsterdam, the Netherland, pp. 231–235.

24. Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. Proc. Natl. Acad. Sci. USA, 115, E10397–E10406.

25. Liu, B., Li, L., Rengel, Z., Tian, J., Li, H., & Lu, M. (2019). Roots and arbuscular mycorrhizal fungi are independent in nutrient foraging across subtropical tree species. Plant and Soil, 442, 97-112.

26. Moyersoen, B., Becker, P., & Alexander, I. J. (2001). Are ectomycorrhizas more abundant than arbuscular mycorrhizas in tropical heath forests?. *New phytologist*, *150*(3), 591-599.

27. Mustajärvi, K., Siikamäki, P., Rytkönen, S., & Lammi, A. (2001). Consequences of plant population size and density for plant-pollinator interactions and plant performance. J. Ecol., 80–87.

28. Naoe, S., Tayasu, I., Sakai, Y., Masaki, T., Kobayashi, K., Nakajima, A., Sato, Y., Yamazaki, K., Kiyokawa,, H., & Koike, S. (2016) Mountain climbing bears save cherry species from global warming by their vertical seed dispersal. Curr. Biol., 26, R315–R316.

29. Pellmyr, O. (1992). Evolution of insect pollination and angiosperm diversification. Trends Ecol. Evol., 7, 46–49.
30. Pyšek, P., Jarošík, V., Chytrý, M., Danihelka, J., Kühn, I., Pergl, J., Tichy, L., Biesmeijer, J. C., Ellis, W. E., Kunin, W. E., Settele, J. (2011). Successful invaders co-opt pollinators of native flora and accumulate insect pollinators with increasing residence time. Ecol. Monog., 81, 277–293.
31. Qian, H. & Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. J. Plant Ecol., 9, 233–239.
32. R Development Core Team (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
33. Regal, P. J. (1977). Ecology and evolution of flowering plant dominance. Science, 196, 622–629.
34. Remy, W., Taylor, T.N., Hass, H., Kerp, H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA, 91, 11841–11843.
35. Sasaki, T., Konno, M., Hasegawa, Y., Imaji, A., Terabaru, M., Nakamura, R., ... & Seiwa, K. (2019). Role of mycorrhizal associations in tree spatial distribution patterns based on size class in an old-growth forest. Oecologia, 189, 971–980.
36. Seidler, T. G., & Plotkin, J. B. (2006). Seed dispersal and spatial pattern in tropical trees. PLoS Biol., 4, e344.
37. Seiwa, K., Negishi, Y., Eto, Y., Hishita, M., Masaka, K., Fukasawa, Y., ... & Suzuki, M. (2020). Successful seedling establishment of arbuscular mycorrhizal-compared to ectomycorrhizal-associated hardwoods in arbuscular cedar plantations. Forest Ecol. Manag., 468, 118155.
38. Sokal, R.R. & Rohlf, F.J. (1995). Biometry – The principles and practice of statistics in

biological research. 3rd edition. W. H. Freeman, New York.

39. Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. Biol. Conserv, 232, 8–27.

40. Tedersoo, L., Bahram, M., & Zobel, M. (2020). How mycorrhizal associations drive plant population and community biology. Science, 367.

41. Teste, F. P., Kardol, P., Turner, B. L., Wardle, D. A., Zemunik, G., Renton, M., & Laliberté, E. (2017). Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. Science, 355, 173–176.

42. Tutin, T. G., Heywood, V. H., Burges, N. A. & Moore, D. M. (1980). Flora Europaea. Cambridge University Press, Cambridge, UK.

43. Uma, E., Sathiyadash, K., Loganathan, J., & Muthukumar, T. (2012). Tree species as hosts for arbuscular mycorrhizal and dark septate endophyte fungi. J Forest Res., 23, 641–649.

44. Vargas, P., Heleno, R., Traveset, A. & Nogales, M. (2012). Colonization of the Galápagos Islands by plants with no specific syndromes for long-distance dispersal: a new perspective. Ecography, 35, 33–43.

45. Wandrag, E. M., Dunham, A. E., Duncan, R. P., & Rogers, H. S. (2017). Seed dispersal increases local species richness and reduces spatial turnover of tropical tree seedlings. Proc. Natl. Acad. Sci. USA, 114, 10689–10694.

46. Wang, B. & Qiu, Y.L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16, 299–363.

47. Wang, T. N., Clifford, M. R., Martínez-Gómez, J., Johnson, J. C., Riffell, J. A., & Di Stilio, V. S. (2019). Scent matters: differential contribution of scent to insect response in flowers with insect vs. wind pollination traits. Ann. Bot., 123, 289–301.

48. Wolfe, B. E., Husband, B. C., & Klironomos, J. N. (2005). Effects of a belowground

mutualism on an aboveground mutualism. Ecol. Lett,. 8, 218–223.

49. Zanne, A.E., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., FitzJohn, R.G. et al. (2014). Three keys to the radiation of angiosperms into freezing environments. Nature, 506, 89–92.

50. van der Heijden, M. G., Martin, F. M., Selosse, M. A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. New phytol., *205*, 1406-1423.

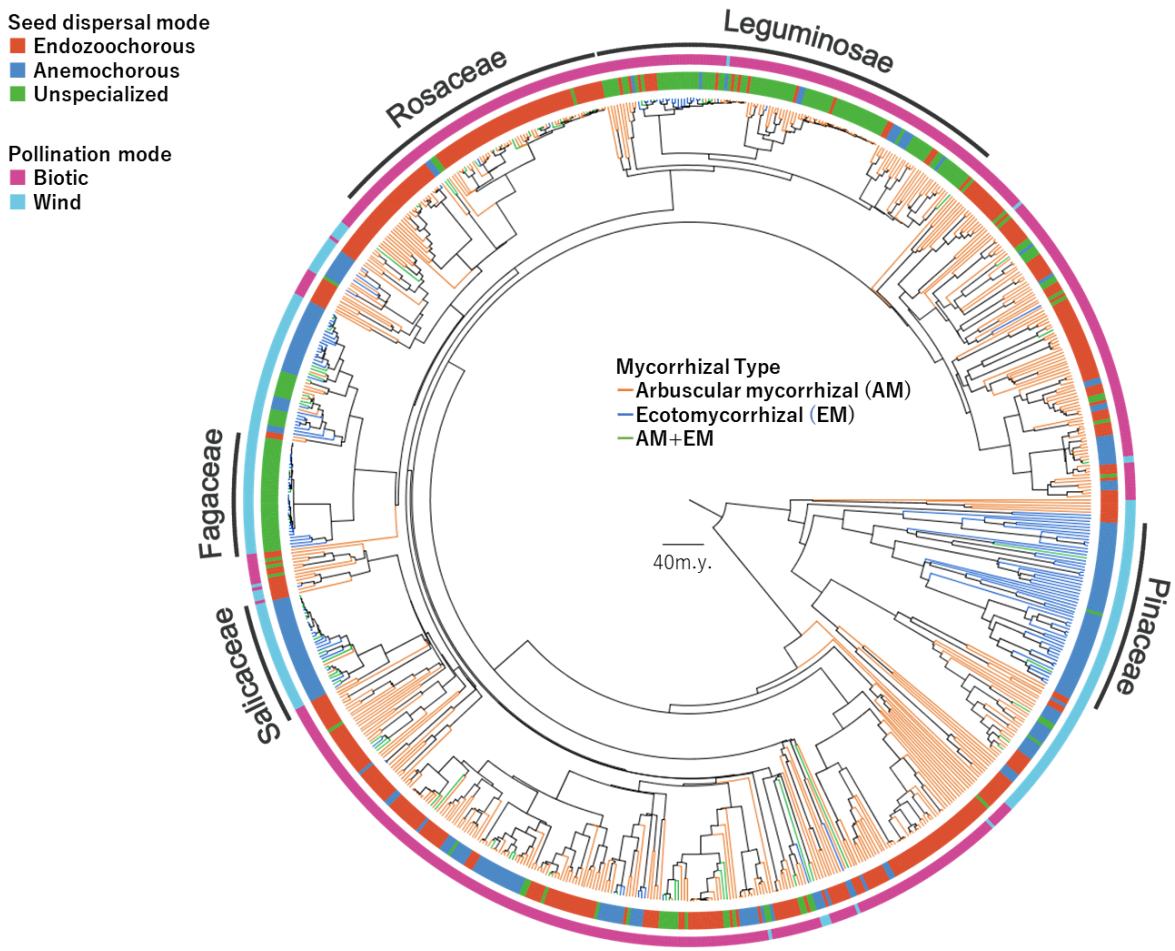

Fig. 1. A time-calibrated phylogeny of the 821 plant species used in this study. Tip color denotes mycorrhizal type (orange, arbuscular mycorrhizal; blue, ectomycorrhizal; green, arbuscular + ectomycorrhizal). Colors of inner and outer labels ringing the tree indicate seed dispersal mode (red, endozoochorous; blue, anemochorous; green, unspecialized) and pollination mode (pink, biotic; blue, wind).

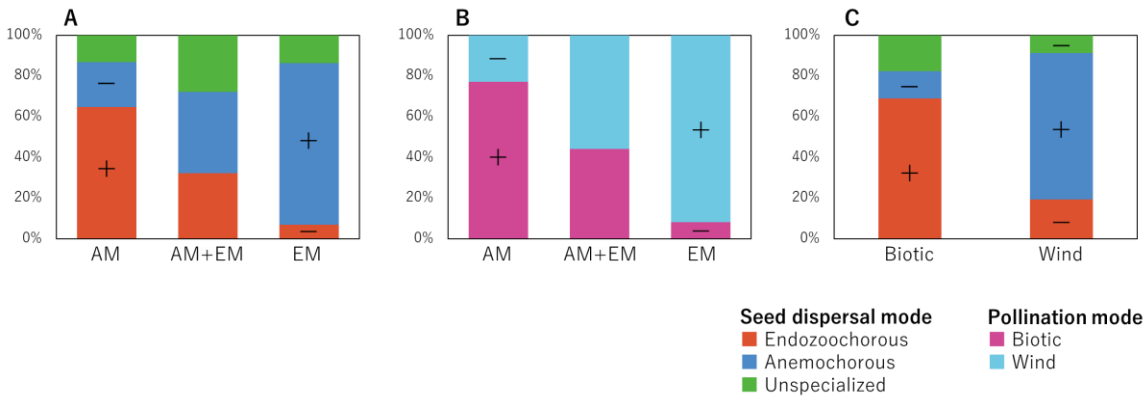

Fig. 2. (A) Relative proportions (% of total) of specialized structures for seed dispersal in trees with different mycorrhizal types: AM, arbuscular mycorrhizal; EM, ectomycorrhizal; AM + EM, arbuscular + ectomycorrhizal. (B) Relative proportions (% of total) of pollination modes in trees with different mycorrhizal types. (C) Relative proportions (% of total) of specialized structures for seed dispersal in trees with different mycorrhizal status in trees with different pollination modes.

Table 1 Results of phylogenetically corrected log-linear analyses examining the relationship between mycorrhizal type, seed dispersal, and pollination mode in 821 tree species.

	Seed dispersal mode	Pollination mode
Mycorrhizal type	N = 821, df = 4, X^2 = 115.95, P < 0.001	N = 821, df = 2, X^2 = 144.67, P < 0.001
Seed dispersal mode		N = 821, df = 2, X^2 = 157.01, P < 0.001

Table S1 Results of the phylogenetically corrected Freeman–Tukey test for relationship between mycorrhizal type and seed dispersal mode.

Mycorrhizal type	Seed dispersal mode	Observed phylogenetic species richness (PSR)	Expected phylogenetic species richness (PSR)	Freeman–Tukey deviate (FTD)	Freeman–Tukey significance test
AM	endozoochorous	154.07785	113.51671	3.533539948	*P*<0.01 (+)
	anemochorous	52.66734	90.24478	-4.44272251	*P*<0.01 (−)
	unspecialized	31.25713	34.240821	-0.475433577	ns
AM+EM	endozoochorous	8.694485	12.83284	-1.17180374	ns
	anemochorous	10.731603	10.20199	0.2351478	ns
	unspecialized	7.479595	3.870856	1.586880915	ns*
EM	endozoochorous	6.061223	42.48401	-7.954999731	*P*<0.01 (−)
	anemochorous	70.822242	33.77441	5.224306258	*P*<0.01 (+)
	unspecialized	12.189693	12.814741	-0.105900395	ns

AM: arbuscular mycorrhizal; EM: ectomycorrhizal

"+" Significantly higher and "−" significantly lower number of species than expected by the log-linear model (FTD critical value = 2.305705); ns: not significant; *no test because expected PSR < 5.

Table S2 Results of the phylogenetically corrected Freeman–Tukey test for relationship between mycorrhizal type and pollination mode.

Mycorrhizal type	Pollination mode	Observed PSR	Expected PSR	FTD	Freeman-Tukey significance test
AM	biotic	174.8576	126.32244	3.983620881	*P*<0.01 (+)
	wind	51.749	100.28416	-5.596816398	*P*<0.01 (−)
AM+EM	biotic	12.02023	15.20654	-0.787585359	ns
	wind	15.25839	12.07208	0.917804231	ns
EM	biotic	7.765638	53.11449	-8.862829096	*P*<0.01 (−)
	wind	87.515086	42.16624	5.737645175	*P*<0.01 (+)

AM: arbuscular mycorrhizal; EM: ectomycorrhizal

"+" Significantly higher and "−" significantly lower number of species than expected by the log-linear model (FTD critical value = 1.996799); ns: not significant.

Table S3 Results of the phylogenetically corrected Freeman–Tukey test for relationship between seed dispersal mode and pollination mode.

Pollination mode	Seed dispersal mode	Observed PSR	Expected PSR	FTD	Freeman-Tukey significance test
biotic	endozoochorous	208.75972	159.50021	3.653137639	*P*<0.01 (+)
	anemochorous	40.91489	99.01433	-7.055651398	*P*<0.01 (−)
	unspecallized	53.40774	44.56781	1.294999144	ns
wind	endozoochorous	28.33318	77.59269	-6.906797374	*P*<0.01 (−)
	anemochorous	106.26733	48.16789	6.749005939	*P*<0.01 (+)
	unspecallized	12.84115	21.68108	-2.062301558	*P*<0.01 (−)

AM: arbuscular mycorrhizal; EM: ectomycorrhizal

"+" Significantly higher and "–" significantly lower number of species than expected by the log-linear model (FTD critical value = 1.996799); ns: not significant.