

Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate

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Summary

- While much research has focused on the timing of individual plant phenological events, the sequence of phenological events has received considerably less attention. Here we identify drivers and patterns of flower and leaf emergence sequence (FLS) in deciduous tree species of the Great Lakes region of North America.
- Five hypotheses related to cold tolerance, water dynamics, seed mass, pollination syndrome, and xylem anatomy type were compared for their ability to explain FLS. Phylogenetic and geographic patterns of FLS were also assessed. We identified additional traits associated with FLS using Random Forest models.
- Of the hypotheses assessed, those related to species' water dynamics and seed mass had the greatest support. The spatial pattern of FLS was found to be strongly related to minimum monthly temperature and the phylogenetic pattern was clustered among species. Based on results from Random Forest models, species' fruiting characteristics were found to be the most important variables in explaining FLS.
- Our results show that FLS is related to a suite of plant traits and environmental tolerances. We emphasize the need to expand phenological research to include both the timing and sequence of plant's entire phenology, in particular in relation to plant physiology and global change.

Introduction

Plant phenology, the repeated cycle of plant life-history events, interacts with ecosystem processes at multiple scales – from influencing individual plant fitness (Ehrlén & Münzbergová, 2009; Gezon *et al.*, 2016) and species' distributions (Chaine, 2010) to affecting regional and global elemental cycles (Keenan *et al.*, 2014; Wolf *et al.*, 2016). Understanding the drivers of phenological events has become a goal of multiple disciplines related to plant science (Pau *et al.*, 2011), because of the sensitivity of plant phenology to both annual climate variability and projected future shifts in baseline climatic conditions (Parmesan & Yohe, 2003). While many studies have focused on the drivers of the timing of individual phenological events (e.g. leaf emergence, flower emergence, budset; Calinger *et al.*, 2013), the sequence of phenological events has received comparatively less attention (but see Post *et al.*, 2008; Li *et al.*, 2016). Understanding the variability in the sequence of phenological events among species, especially between major vegetative and reproductive life stages, has the potential to reveal important insights into how species allocate resources to different life stages and may help to elucidate species' vulnerability to future global change.

In the present study we focus on the sequence of flower and leaf emergence in deciduous tree species. In the context used in the present study, flower–leaf sequence (FLS) can proceed in one of two ways: flowers may appear before leaf emergence (e.g. precocious flowering, hysteranthly, or proteranthly), or flowers may appear after leaf emergence. These two sequences are probably reflective of different physiological strategies within trees, in particular with respect to the use of nonstructural carbon (NSC; stored energy with the potential to contribute to basal metabolism or new tissue growth). Species that flower before leaf emergence probably rely on and utilize stored NSC (i.e. produced in previous growing seasons) to produce and maintain pollen, flowers, and early seed and fruit growth. Conversely, species that flower after leaf emergence may have greater access to newly synthesized NSC (in addition to stored NSC) for reproductive use following the seasonal onset of photosynthesis in leaves (Gough *et al.*, 2010; Hoch *et al.*, 2013).

The FLS strategy that species utilize may be indicative of different resource allocation strategies within individual trees to support reproductive and vegetative growth. Species that flower after leaf emergence must simultaneously support the development and/or maintenance of both reproductive and vegetative tissues, potentially resulting in a large resource cost to the plant. This cost may be tenable, however, if it limits the exposure of sensitive

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reproductive tissues to adverse environmental conditions (e.g. late-season frosts). Hence, FLS may represent a type of tradeoff, whereby species that flower before leaf emergence risk both flower and pollen damage, but minimize the amount of overlap in reproductive and vegetative growth. Species that flower after leaf emergence, conversely, may lessen the risk of early-season flower damage, but must utilize resources to simultaneously develop and/or maintain leaves and flowers.

Flower–leaf sequence varies among deciduous tree species, and a number of hypotheses have been proposed in the literature to attempt to explain the phenomenon of flower production before leaf emergence in relation to species' traits. In the following we outline five proposed hypotheses related to FLS:

1 Cold tolerance. Flowering before leaf emergence has been proposed to be an adaptation to cold climates as a mechanism to either extend the growing season for fruit and seed development or to attract pollinators. In a meta-analysis of flowering time studies, Munguía-Rosas *et al.* (2011) showed that early flowering tended to be strongly selected for in high-latitude (i.e. cold) climates, where individuals experience an abbreviated growing season. Earlier flowering may allow more time for fruit and seed development in these climates. This pattern was also detected by Liu *et al.* (2016), who found species that flower before leaf emergence tended to be clustered in cold-adapted genera in Magnoliaceae in China.

2 Water dynamics. Precipitation and water availability have also been proposed to be important to flowering phenology, especially in seasonably xeric systems (Peñuelas *et al.*, 2004; Shen *et al.*, 2015). The impact of precipitation on flowering and leaf phenology in more mesic environments, however, is less well understood. Janzen (1967) observed that many species in seasonally dry forests of Central America use stored 'resources' (e.g. NSC) to flower in the dry season, before the onset of vegetative growth which commences at the onset of the rainy season. Janzen (1967) suggested that reproductive growth precedes vegetative growth in many species so that, after reproduction, resources can be more completely allocated to vegetative growth to compete for light.

Water potential amongst plant organs may also be an important physiological determinant of FLS. Differences in water potential between leaves and flowers during development and maturity can lead to wilting and loss of function in tissues less adept at acquiring water during a drying event (Feild *et al.*, 2009). By flowering before leaf emergence, not only is the overall demand for water by developing tissue likely to be lessened, but the competition for water between nascent flowers and leaves is likely reduced. While water balance is inherently linked at a physiological level to reproduction, quantitative assessments and exact mechanisms that relate plant water availability and precipitation tolerances to FLS have yet to be described.

3 Pollination syndrome. A number of studies have suggested that flowering before leaf emergence may be an adaptation among wind-pollinated tree species as a way of facilitating the relatively unobstructed movement of pollen before the leaf canopy develops in the spring (Rathcke & Lacey, 1985; Niklas, 1992; Cronk *et al.*, 2015). Rathcke & Lacey (1985) suggested that many temperate and tropical deciduous tree species flower when leaves are

absent from the canopy, in the early spring and dry seasons, respectively, to facilitate pollination. Emberlin *et al.* (2002) echoed this idea in a study of the timing of *Betula* spp. pollen release in Europe, suggesting FLS to be related to species' pollination syndromes (i.e. whether species are pollinated biotically or abiotically).

4 Seed mass. Seed mass has been proposed to be related to the seasonal onset of flowering. Bolmgren & Cowan (2008) suggested that by flowering early, plants may be able to devote additional time and resources to reproduction, allowing seeds to grow larger in mass. In a test of this hypothesis with perennial and annual herbaceous species and woody plants, Bolmgren & Cowan (2008) found a negative correlation between the onset of flowering and seed mass (i.e. earlier flowering plants had larger seeds) for herbaceous species, but not woody plants. The authors suggest that stored resources in woody plants (e.g. NSC) may allow vegetative and reproductive growth to proceed independently and cite trees that flower before leaf emergence as evidence of this phenomenon.

5 Xylem anatomy type. Lechowicz (1984, 1995) noted the importance of xylem anatomy for species capable of flowering in late winter and early spring. Specifically, Lechowicz (1984, 1995) suggests that because ring-porous species are more susceptible to freeze-induced embolism of xylem vessels, they may exhibit delayed vegetative or reproductive growth compared with diffuse-porous species. Panchen *et al.* (2014) provide additional evidence across a large number of woody species that ring-porous species exhibit delayed leaf emergence in woody angiosperm species compared with other xylem anatomy types. If flower production in ring-porous species is indeed delayed until after winter because of damaged xylem vessels, it is reasonable to expect species that flower before leaf emergence to have a diffuse-porous xylem structure.

While the hypotheses discussed above fit various theoretical expectations of FLS, to our knowledge they have not been formally assessed and compared for their relative ability to predict species' FLS. To better understand the extent to which FLS represents different evolutionary strategies, there is a need to examine how FLS is distributed phylogenetically, geographically and in relation to environmental tolerances and other plant traits. In the present study we sought to compare the hypotheses described (i.e. those related to cold tolerance, water dynamics, pollination syndrome, seed mass, and xylem anatomy type) in a phylogenetic framework; to assess the phylogenetic pattern of FLS; to investigate and identify additional traits associated with FLS, including environmental tolerances and floristic/structural traits; and to map the spatial distribution of FLS to gain insights into its geographic pattern.

Materials and Methods

Species and geographic region

In the present study, we focused on native, deciduous angiosperm tree species in the Great Lakes region of North America. Coniferous trees were excluded because of their distinctly different

phenological and evolutionary strategies (Bond, 1989). The Great Lakes area is a species-rich region which encompasses multiple forest types, ranging from oak-savanna communities in the western part of the region to northern hardwood and boreal communities in the north (Barnes & Wagner, 2004). The region encompasses a transition zone between northern boreal tree species and lower-latitude eastern North American species, where a large number of tree species have a northern or southern range edge (Andersen, 2005). We used Barnes & Wagner (2004) as a guide to the trees in this region. In total, 72 tree species spanning 37 genera were included in our analyses.

Species' traits

We collected species' trait data to address five hypotheses related to FLS: cold tolerance, water dynamics, pollination syndrome, seed mass, and xylem anatomy type. Cold tolerance data were collected from the USDA Conservation Plant Characteristic (CPC) database, and defined as the coldest recorded temperature at a weather station located within the entire native range of the species. To assess the water dynamics hypothesis, we collected data on species' minimum precipitation tolerance and species' moisture use. The variables used to assess the water dynamics hypothesis were also collected from the USDA CPC database. To ensure the minimum temperature and minimum precipitation variables were reflective of species' current distributions and physiological limits, we validated these data using climate data extracted at known occurrence locations on the landscape, and, for a subset of species, compared CPC data with freezing tolerances reported in the literature (Sakai & Weiser, 1973). In all cases, the CPC data we used were significantly positively correlated with these other climatic metrics (Supporting Information Methods S1; Figs S1, S2).

Species' pollination syndromes were collected from Barnes & Wagner (2004) and were classified as 'animal'- or 'wind'-pollinated. Seed mass was collected from the Kew Seed Information Database (Royal Botanic Gardens Kew, 2017), defined as the average documented weight (g) of 1000 seeds. Xylem anatomy type was collected from the InsideWood database (InsideWood, 2004; Wheeler, 2011) and Schoch *et al.* (2004). Xylem anatomy type was classified as 'diffuse porous', 'ring porous' or 'intermediate.' Intermediate types included species that were recorded as 'semi-ring porous' or 'diffuse/ring porous.' When species-level data for xylem anatomy were not available, we used xylem anatomy reported at the genus level.

We collected additional species' trait data to identify other traits associated with FLS. In total, we calculated or collected information on 30 traits from a variety of sources, including university and government databases (The PLANTS Database; USDA, 2017), field guides (Barnes & Wagner, 2004), and the peer-reviewed literature. Traits included climatic tolerances (e.g. maximum tolerable precipitation within species' ranges), edaphic tolerances (e.g. minimum/maximum tolerable soil pH), growth characteristics (e.g. height at maturity, shade tolerance), phenological characteristics (e.g. timing of fruit ripening), range characteristics (e.g. latitude of northern and southern range edges,

described later), among other traits (see Table S1 for a full list of traits, their definitions and sources). We were able to locate data for 2137 (98.94%) of the total 2160 species' traits (72 species \times 30 traits). Missing trait values were imputed using the *MISSFOREST* package (Stekhoven & Bühlmann, 2012) in R (R Core Team, 2017). *missForest* uses Random Forest models to predict missing values based on their relationship with other predictors, and has been shown to perform well in validation tests and in comparison with other imputation approaches (Penone *et al.*, 2014).

In addition to traits gathered from databases and field guides, we calculated several metrics related to species' geographic ranges, including the latitude of the northern and southern range edges, and the latitudinal range. To calculate the latitudinal edges, digitized range maps for each species were collected from the United States Geological Survey (<https://gec.cr.usgs.gov/data/little>; Little, 1971) and were overlaid on a 2.5 arcmin resolution grid. Latitude of all pixels that fell within the maps were extracted. The northern and southern range edges were calculated as the 95th and 5th percentiles of pixel latitudes, respectively. The latitudinal range was calculated as the difference between the northern and southern range edges.

Phylogenetic analyses and model comparison

For phylogenetic analyses, we used a pruned version of a phylogeny of North American trees generated by Hawkins *et al.* (2014). Briefly, Hawkins *et al.*'s phylogeny was based on the Angiosperm Phylogeny Group III (APG III) system and supplemented by phylogenies from the literature. Branch lengths were calibrated to a dated phylogeny by Bell *et al.* (2010). See Hawkins *et al.* (2014) for a full description of how the phylogenetic tree was constructed. We pruned Hawkins *et al.*'s phylogeny to include only the deciduous tree species native to the Great Lakes region.

Flower-leaf sequence was classified for each species as either flowering before leaf emergence ('before') or flowering after leaf emergence ('after'), following Barnes & Wagner (2004), yielding a binary classification. Trees noted as flowering 'during' or 'with' leaf emergence, were classified as 'after,' because we sought to accentuate the difference in the onset of reproductive and vegetative growth in our classification, and because most of the hypotheses described accentuate the unique characteristics of 'before' species that allow flowering before leaf emergence. To ensure that results of our original classification were robust, we conducted the analyses described in the following with two additional classifications: one in which species classified as flowering during or with leaf emergence were removed, and one in which these species were grouped into a third 'with' category (Methods S2; Fig. S3). Methods and Results discussed here refer to our original binary classification, as described earlier in the paper, unless specifically noted.

We tested for a phylogenetic signal of FLS using Fritz's *D* (Fritz & Purvis, 2010). Fritz's *D* quantifies the phylogenetic dispersion of binary traits by assessing the sum of sister-clade differences in trait values. Fritz's *D* is expected to be near 1 if the phylogenetic pattern of FLS is not different from random, and near 0 if FLS is clustered (i.e. as if it had evolved under Brownian

motion along the phylogenetic tree). Fritz's D may exceed these bounds depending on the strength of the pattern. Significance was determined by comparing the observed Fritz's D statistic with two simulated null distributions representing phylogenetic randomness and phylogenetic clustering under Brownian motion. We used 5000 permutations to test for significance. Fritz's D was implemented with the `phylo.d` function in the `CAPER` package (Orme *et al.*, 2012) in R.

Because FLS was treated as a binary response and was found to be phylogenetically clustered (see the Results section), we used phylogenetic generalized linear models with a logit link function (phyloGLMs; Ives & Garland, 2010) to test the five hypotheses described earlier. 'Before' species were assigned a value of 1, while 'after' species were assigned a value of 0. Phylogenetic GLMs account for the nonindependence of trait characteristics among species in the phylogeny and were implemented with the `phylglm` function in the `PHYLOLM` package (Ho *et al.*, 2014) in R. Models were compared using Akaike information criterion (AIC) and Akaike weights. Akaike weights can be interpreted as the conditional probability of each model and are recommended as a way to help interpret differences in AIC among models (Wagenmakers & Farrell, 2004). Log-transformation of continuous variables (i.e. minimum temperature, minimum precipitation, and seed mass) were necessary to ensure the phyloGLMs converged.

In addition to the GLMs, we also used phyloANOVAs (as described in Garland *et al.*, 1993) to test for differences in cold tolerance, minimum precipitation tolerance, and seed mass among trees that flower before leaf emergence and those that flower after leaf emergence. For consistency with the GLMs, minimum temperature, minimum precipitation and seed mass were each log-transformed. phyloANOVAs were limited to continuous variables, so were not conducted on species' moisture use, xylem anatomy type, or pollination syndrome. phyloANOVAs were implemented in the `PHYTOOLS` package (Revell, 2012) in R. P -values were calculated using 5000 phylogenetic simulations. By pairing phyloGLMs with phyloANOVAs we were able to both rank the support for each of the hypotheses we tested, and quantitatively test for differences in trait values among 'before' and 'after' species.

Because the drivers of FLS are largely unknown, we also sought to identify additional traits associated with FLS, beyond those used for the formal hypothesis testing, using Random Forest models. Random Forest is a machine learning algorithm that creates many classification and regression trees (the 'forest'), using random partitions of the complete dataset, which are ensembled into a single model. Random Forest models were used to predict FLS as a function of all 30 trait variables. Variable importance (mean decrease in model accuracy) was averaged across 50 Random Forest models implemented with the `cforest` function in the `PARTY` package (Strobl *et al.*, 2007) in R.

Mapping occurrence of FLS

We used the species' range maps described earlier to map the occurrence of species that flower before leaf emergence in order

to gain some insight into the geographic distribution of FLS. To generate the map, the area within each species' range map was assigned a value of 1.0 and spatially summed to yield a map of the total number of species across space. The same procedure was then performed for only those species that flower before leaf emergence and was then divided by the total sum map. The resultant map provides the geographic pattern of the proportion of tree species that flower before leaf emergence. The range maps and approach we used to map the pattern of FLS are common ways to map the richness of tree species (e.g. Currie & Paquin, 1987; Algar *et al.*, 2007; Hawkins *et al.*, 2007; Wang *et al.*, 2009b) and the geographic pattern of species' traits (e.g. Šimová *et al.*, 2015; Ordonez & Svenning, 2016).

In total this map includes at least 67% of the species that primarily exhibit a tree growth form in the region (Nelson *et al.*, 2014) and contains 95% of the 20 most abundant angiosperm tree species by basal area found in timberlands of the state of Michigan (Pugh *et al.*, 2009). We used this map to test whether the spatial pattern of FLS was correlated with minimum temperature of the coldest month and precipitation of the driest month. Both climate variables were downloaded from the WorldClim database for current climate (centered on *c.* 1975) at a resolution of 2.5 arcmin (Hijmans *et al.*, 2005). Maps were cropped to 16 Level III Ecoregions, defined by the United States Environmental Protection Agency. Ecoregions were chosen based on either their adjacency or nearness to the Great Lakes (Table S2; Fig. S4). Although some ecoregions encompassing the Appalachian Mountains (e.g. Regions 5.3 and 8.4) are near the Great Lakes, they were excluded because of their distinctive biota.

Results

Prevalence of FLS

Of the 72 species included in our analyses, 21 (29.2%) were found to flower before leaf emergence in the Great Lakes region. FLS was found to be conserved within all genera assessed, with the exception of *Acer* and *Prunus* (Fig. 1). Within *Acer*, three species (*A. saccharinum*, *A. rubrum*, and *A. negundo*) flower before leaf emergence, and three species (*A. saccharum*, *A. pensylvanicum*, and *A. spicatum*) flower after leaf emergence. In *Prunus*, only *P. nigra* was found to flower before leaf emergence, while all other species in the *Prunus* genus (*P. pensylvanica*, *P. virginiana*, and *P. serotina*) flower after leaf emergence. All species in the *Fraxinus*, *Populus*, *Cercis*, *Ulmus*, *Alnus*, *Betula*, and *Asimina* genera included in our analyses were found to flower before leaf emergence in the Great Lakes region, while all species in all other genera flower after leaf emergence. In total, 24.3% (nine out of 37) of the genera assessed had species that flower before leaf emergence.

Flower–leaf sequence was found to be phylogenetically clustered among species. Fritz's D , a measure of phylogenetic dispersion, was -0.09 . Permutation testing revealed that the observed phylogenetic pattern of FLS was very unlikely to have been randomly generated ($P < 0.001$), and the pattern was not significantly different than if it had evolved under Brownian motion ($P = 0.607$).

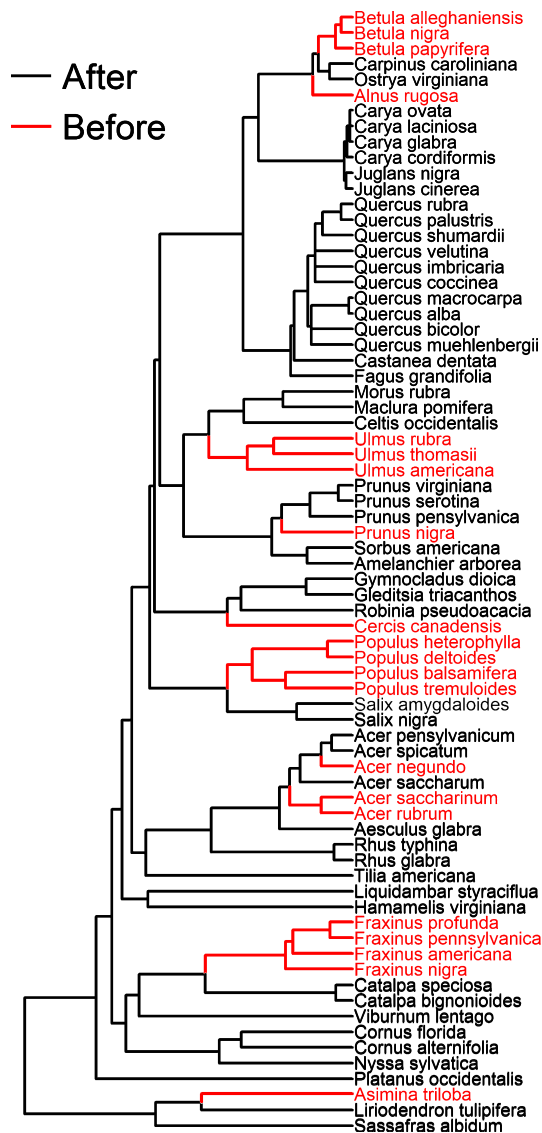


Fig. 1 Phylogenetic tree of native deciduous tree species in the Great Lakes region of North America. Species colored in red flower before leaf emergence, whereas species in black flower after leaf emergence.

Assessment of hypotheses

Phylogenetic GLMs representing the five hypotheses varied in their ability to explain FLS. The water dynamics model had the lowest AIC (67.38) and highest Akaike weight (0.92). Although the difference in AIC of the next highest ranked model was relatively small (seed mass model; $\Delta\text{AIC} = 6.29$), the Akaike weight was substantially smaller (0.04), indicating that among the five models tested, the water dynamics model had the greatest support. The level of support for the seed mass model was followed by the cold tolerance, pollination syndrome, and xylem anatomy type models (Table 1).

phyloANOVAs revealed a number of trait differences between species that flower before leaf emergence and species that flower after leaf emergence (Fig. 2a–f). Species that flower before leaf emergence had lower tolerable minimum log-transformed

temperatures, lower tolerable log-transformed precipitation, and smaller log seed mass compared with species that flower after leaf emergence (minimum temperature, $F = 8.414$, $P = 0.037$; minimum precipitation, $F = 8.95$, $P = 0.029$; seed mass, $F = 13.09$, $P = 0.010$) (Fig. 2a,b,d).

Identification of additional traits related to flower–leaf sequence

Random Forest models revealed a number of additional traits associated with FLS. Not surprisingly, the timing of flower onset was the most important variable explaining FLS in the Random Forest models (Fig. 3). The next three highest ranked variables (i.e. fruit type, seed disperser, fruit begin month) in the Random Forest models were each associated with species' fruiting and seed characteristics. Closer inspection of these variables revealed that species that flower before leaf emergence tended to be wind-dispersed and have earlier fruit maturation compared with species that flower after leaf emergence (data not shown). Additionally, 13 of the 21 (61.9%) tree species that flower before leaf emergence were found to produce samaras (i.e. a winged fruit type), although 'before' species were also represented in six of the nine fruit types found in the region (Fig. S5). In general, edaphic variables (e.g. pH limits and soil texture adaptations) and growth characteristics (e.g. height at 20 yr/maturity) tended not to be important in the Random Forest models, with the exception of adaptation to fine-textured soils (sixth most important variable).

To understand how our classification of species' FLS influenced our results, we assessed two additional classifications, one in which species that flower with leaf emergence were removed from the analysis, and another where 'with' species were considered as their own group, in addition to 'before' and 'after' species. In general, Random Forest results for the two additional classifications were similar to our original classification (Figs S6, S7). For each classification, the timing of flower onset was the most important variable, and fruit and seed characteristics (e.g. fruit type, seed disperser, fruit begin month) tended to be highly ranked. Curiously, for both additional classifications, pollination syndrome was among the top four most important variables. Inspection of this variable revealed that most 'after' species were insect-pollinated, while most 'before' and 'with' species were wind-pollinated. The phyloGLMs also indicated increased support for the pollination hypothesis, when conducted with these additional classifications (Table S3), although Akaike weights were still low.

Because of the overall importance of fruiting traits in Random Forest models in our analyses, we developed an additional 'fruit characteristics' phyloGLM model to test against the models generated based on hypotheses identified in the literature. This model included the beginning month of fruit maturation and seed disperser variables. The 'fruit characteristics' model substantially outperformed all other models tested. When compared against models based on hypotheses found in the literature, this model had an Akaike weight of 0.95, while all other models had weights < 0.05 (Table 1).

Spatial pattern and analyses

When mapped, the geographic pattern of FLS showed a clear latitudinal trend, with the proportion of ‘before’ species being greatest at higher latitudes (Fig. 4a). Correlation analyses between the geographic pattern of ‘before’ species and climate variables revealed a strong negative relationship with minimum temperature of the coldest month ($r = -0.89$, $P < 0.01$) and a weak, though significant, negative relationship with precipitation of the driest month ($r = -0.17$, $P < 0.01$) (Fig. 4b,c), indicating that the proportion of ‘before’ species tended to be highest in cold and dry climates.

Discussion

The sequence of phenological events in tree species is an understudied phenomenon that has important implications for understanding how species allocate resources to reproductive and vegetative growth. Quantifying the drivers of phenological sequences is likely to be an important and rewarding area of future research, especially when linking species’ physiological processes to global change scenarios. To our knowledge, our study is among the first to quantify the prevalence of a phenological sequence (flower and leaf emergence) among tree species, assess its phylogenetic and spatial pattern, and formally compare

Table 1 Summary statistics of phylogenetic generalized linear models (GLMs)

Model name	Coefficient	Estimate	P-value	Model AIC	Weight-all ¹	Weight-hypo ¹
Cold tolerance	Intercept	148.32	< 0.01	74.45	0.00	0.03
	Log minimum temperature	−27.30	< 0.01			
Pollination syndrome	Intercept	−1.90	< 0.01	76.31	0.00	0.01
	Pollination – wind	2.10	< 0.01			
Seed mass	Intercept	0.12	0.82	73.66	0.00	0.04
	Log seed mass	−0.26	0.03			
Water use	Intercept	10.49	< 0.01	67.38	0.04	0.92
	Log minimum precipitation	−2.43	< 0.01			
	Moisture use – low	−2.61	0.01			
	Moisture use – medium	−1.59	< 0.01			
Xylem anatomy	Intercept	−0.92	0.16	77.39	0.00	0.01
	Xylem – diffuse	0.10	0.89			
	Xylem – ring	0.29	0.60			
Fruit characteristics ²	Intercept	6.45	0.04	61.25	0.95	–
	Log fruit month	−3.97	< 0.01			
	Seed dispersal – wind	1.44	0.07			

¹Akaike weights were calculated with respect to models based on hypotheses identified from the literature (Weight-hypo) and with respect to all models (Weight-all).
²Variables for this model were chosen based on their importance in Random Forest models.

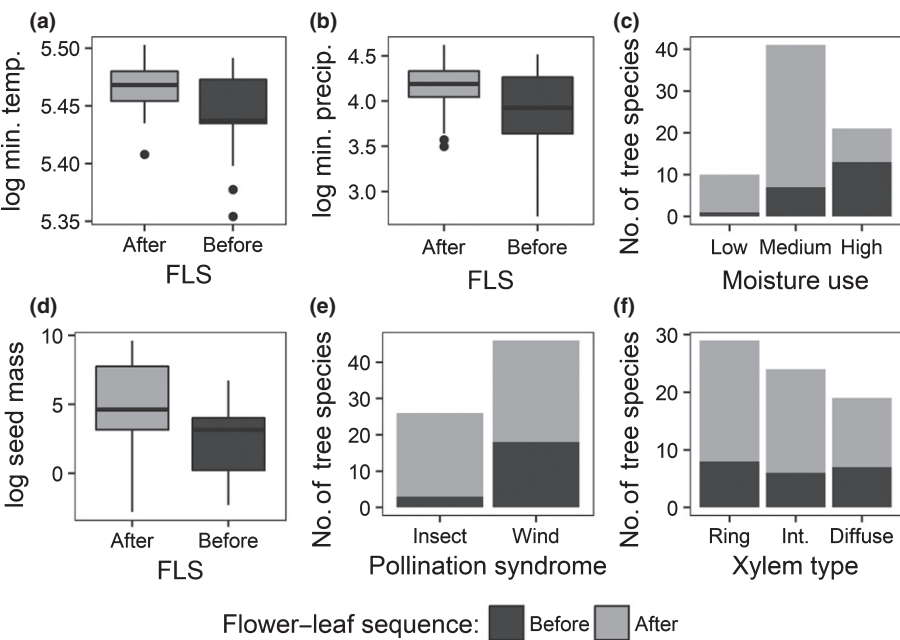


Fig. 2 Distribution of flower–leaf sequences (FLS) among species: (a) log minimum tolerable temperature (K); (b) log minimum tolerable precipitation (cm); (c) moisture use; (d) log seed mass (g); (e) pollination syndrome; and (f) xylem anatomy type. For boxplots, horizontal bars represent the median value, the box extends from the 25th to the 75th percentiles, vertical lines extend to the furthest values no greater than 1.5 × interquartile range, and individual dots represent data that fall outside 1.5 × interquartile range.

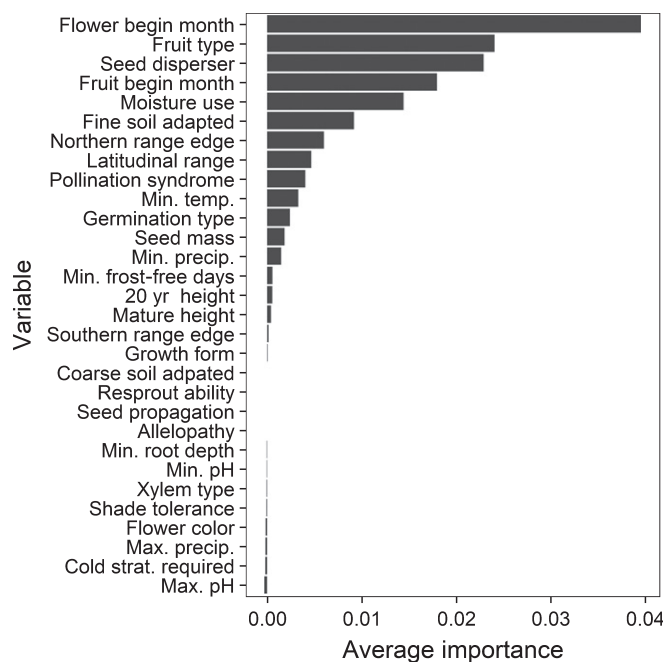


Fig. 3 Average variable importance (mean decrease in accuracy) of 50 Random Forest models predicting flower–leaf sequence in deciduous tree species.

hypotheses describing its occurrence. By combining formal comparisons of hypotheses that have been proposed in the literature with Random Forest models, we show that FLS is closely related to species' climatic tolerances and fruiting/dispersal characteristics. Our results illustrate the wide-ranging effects that phenological sequences can have on species throughout their entire reproductive cycle. Further, our finding that the geographic pattern of FLS is related to multiple climate variables suggests FLS may be an important trait to study in the context of future climate change.

FLS occurrence

Approximately 29% of tree species in the Great Lakes region, and a similar percentage of genera (24%) were found to flower before leaf emergence. Like other studies that have quantified the phylogenetic pattern of phenological events (e.g. Kochmer & Handel, 1986; Bolmgren & Cowan, 2008; Willis *et al.*, 2008; Davis *et al.*, 2010; Davies *et al.*, 2013), we found FLS to be phylogenetically clustered among tree species, indicating that closely related species tended to have the same FLS (Fig. 1). Interestingly, the phylogenetic pattern of FLS revealed that the trait seems to have primarily differentiated at the genus level or higher, similar to other traits also conserved within genera (e.g. leaf orientation, fruit type, dispersal mechanism). Of the 37 genera assessed, only *Acer* and *Prunus* were found to have both species that flower before and some that flower after leaf emergence. While we cannot be certain that this is not simply an artefact of the number of species included in these genera, as *Acer* and *Prunus* were two of the six most species-rich genera in our study, these genera may warrant further study to elucidate the drivers of FLS among closely related species.

FLS hypotheses

Of the five hypotheses identified in the literature, those related to species' water dynamics and seed mass had the greatest support (Table 1). The importance of precipitation and moisture use to FLS was somewhat surprising, as precipitation, in general, is not often considered an important driver of phenology in temperate, nonwater-limited ecosystems (Polgar & Primack, 2011), such as the Great Lakes region. Our results, however, indicate that species that flower before leaf emergence can tolerate significantly lower precipitation than species that flower after leaf emergence.

In habitats with low water availability, it may be advantageous for trees to minimize overlap in flower production and vegetative growth/maintenance, in order to limit large transpiration losses and to conserve water. Likewise, the potential for 'before' species to minimize transpiration losses may help to explain how these species, which mostly have medium to high moisture use, persist in environments that receive low precipitation (Fig. 4b,c). Interestingly, many of the 'before' species in our analysis are also characteristic of riparian and bottomland forests (Barnes & Wagner, 2004) and as a result may be able to directly access the water table below ground or surface waters. Thus, landscape position may be important in explaining how 'before' species have the tendency to be found in areas with lower precipitation (Figs 2b, 4c), despite their relatively high soil moisture use (Fig. 2c). It remains unclear to what extent there is a mechanistic relationship between water availability and FLS in our study region and species, but our results suggest that future studies aimed at identifying these mechanisms would be informative and could help to explain the pattern observed in the present study.

The importance of precipitation could also be reflective of a broader climatic effect on FLS. While we did not find great support for the cold tolerance hypothesis in the phyloGLMs (Akaike weight = 0.03) relative to other variables, our spatial analyses revealed a very strong significant negative correlation ($r = -0.89$; Fig. 4b) between the proportion of 'before' species and minimum temperature of the coldest month. This finding may be indicative of a climatic control on the occurrence of FLS at the site or community level, whereby sites that are cooler and have lower precipitation may harbor a greater proportion of 'before' species than sites that are warmer and have higher precipitation. Taking the phyloGLMs and spatial analyses together, it is clear that climate plays a role in FLS, and suggests that FLS may be an important trait to study in the context of future climate change, and in relation to recent changes in tree abundances in North America (Fei *et al.*, 2017).

Similar to other studies, our work showed a significant relationship between plant seed characteristics and phenology (Seiwa & Kikuzawa, 1991; Castro-Díez *et al.*, 2003; Du & Qi, 2010). The significant difference in seed mass among 'before' and 'after' species is consistent with our findings that fruiting and dispersal characteristics were important to FLS (Table 1). When assessed against the five hypotheses identified in the literature, the fruit characteristics model substantially outperformed all other models, indicating a link between species' FLS and dispersal strategies. Generally, species that flower before leaf emergence ripen their

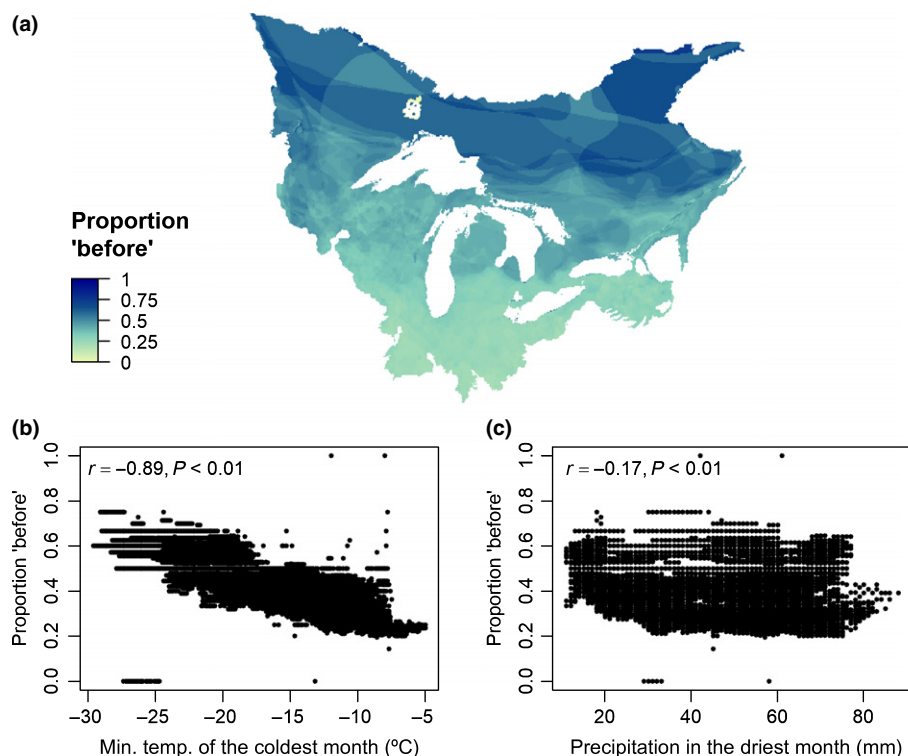


Fig. 4 (a) Map showing the proportion of species that flower before leaf emergence in the Great Lakes region of North America. Relationship between the proportion of species that flower before leaf emergence and: (b) minimum temperature of the coldest month; (c) precipitation in the driest month.

fruit earlier, produce smaller massed seeds, and tend to be wind-dispersed. These findings may indicate that the FLS 'before' strategy evolved as a way for trees to disperse seeds quickly, allowing seeds more time during the growing season to disperse to suitable habitat and/or germinate and become cold-hardy before first frost. Interestingly, several of these dispersal characteristics are also known to vary by latitude (e.g. seed size, dispersal mechanism; Moles *et al.*, 2007), further suggesting a role for climate in driving these patterns.

Plasticity of flower and leaf emergence sequence

Our work provides insights into the drivers of FLS in forest trees, but there remain a number of important uncertainties related to FLS. First, it remains unknown whether FLS is plastic within species, similar to other phenological traits (e.g. Kramer, 1995; Vitasse *et al.*, 2010). It is conceivable that FLS varies across species' ranges or in response to environmental gradients, similar to patterns observed for the timing of flower and leaf emergence (Polgar & Primack, 2011). While it has been reported that some herbaceous flowering plant species may be able to switch their FLS in response to the climate individuals experience (Debussche *et al.*, 2004), additional research is needed to determine if deciduous tree species have this same sort of plasticity. Conversely, work showing a genetic basis for the transition between reproductive and vegetative life stages in plants (Wang *et al.*, 2009a) may suggest that FLS is consistent across species' ranges and in relation to environmental gradients. Borchert (1998), for instance, showed that two tropical, deciduous species growing along elevational and precipitation gradients consistently had the same sequence of

flowering, leaf emergence, and leaf drop at each location along the gradient. Furthermore, our finding that FLS is mostly conserved within genera may also be indicative of some genetic control on FLS. Further study of the environmental and genetic drivers of FLS, in particular in genera where FLS is not conserved (i.e. *Acer* and *Prunus* in the present study), may yield particularly insightful findings.

Linking phenology to tree resource allocation

There also remains a need to better understand the strategies utilized by plants to overcome the potential energetic and physiological burden associated with flowering before leaf emergence. It is conceivable that trees that flower before leaf emergence are able to do so by a number of different mechanisms. For example, 'before' trees may accumulate and store a larger reserve of NSC (i.e. relative to 'after' trees) before the end of the growing season which is then accessed in late winter and early spring for the production and maintenance of reproductive tissues. This type of buffering would permit NSC reserves in both 'before' and 'after' trees to be at similar levels at the time of budburst. While it has been documented that starch (a type of NSC) concentrations in tree stems reach a maximum in late summer and a minimum by late winter (Martínez-Vilalta *et al.*, 2016), it remains unknown how FLS influences the magnitude of such trends.

Alternatively, 'before' and 'after' trees may accumulate similar amounts of NSC reserves by the end of the growing season, and 'before' species may simply make greater use of reserves at the start of the growing season. Such a strategy may be energetically risky for 'before' trees. This risk may be especially apparent if

nascent leaves are damaged (e.g. by a late frost) as the energy required to flush a new set of leaves would probably lead to sizeable depletion of NSC reserves that have already been accessed for flower production and maintenance. However, it remains difficult to assess just how vulnerable trees are to NSC depletion, because little is known about the mobility and accessibility of NSC reserves for new growth and respiratory demands within trees following a disturbance (Gessler & Treydte, 2016; Hartmann & Trumbore, 2016). In addition to the hypotheses we have addressed, a number of other explanations related to how 'before' and 'after' tree physiologies differ and are sustained are also plausible, and demand further research (e.g. photosynthesis in nonleaf tissues, such as flowers and immature fruit; Bazzaz *et al.*, 1979; Guzmán-Delgado *et al.*, 2017).

Finally, based on our finding that FLS is linked to a number of important ecological traits, we propose that the sequence of other, less studied, phenological events (e.g. production and turnover of fine roots or twigs) warrants further study, especially in the context of NSC dynamics and general plant performance. A more integrated view of plant phenology that incorporates both the timing and sequence of multiple phenological events will probably help to reveal important functional differences between and within species related to both energy allocation and ecological and reproductive strategies. Understanding these functional differences and their sensitivities will be crucial to accurately assessing how species will respond to future global change.

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Author contributions

A.V.G. and S.W.G. planned and designed the research, collected and interpreted the data, and wrote the manuscript. A.V.G. performed the data analyses. A.V.G. and S.W.G. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Comparison between minimum tolerable temperature from the USDA Conservation Plant Characteristic (CPC) database and: (a) minimum temperature of the coldest month extracted at geographic occurrences; (b) freezing tolerances from the literature.

Fig. S2 Comparison between minimum tolerable precipitation from the USDA Conservation Plant Characteristic (CPC) database and precipitation of the driest month extracted at geographic occurrences.

Fig. S3 Distribution of FLS, including a separate category for species that flower with leaf emergence, among six traits.

Fig. S4 Map of EPA Level III ecoregions around the Great Lakes region.

Fig. S5 Distribution of FLS among fruit types.

Fig. S6 Random Forest results when species that flower with leaf emergence are included as a separate group.

Fig. S7 Random Forest results when species that flower with leaf emergence are removed.

Table S1 Description of traits used in Random Forest models

Table S2 EPA Level III Ecoregions used in mapping analyses

Table S3 Summary statistics from phylogenetic GLMs. Species that flower with leaf emergence were removed from these analyses

Methods S1 Description of how temperature and precipitation data were validated with occurrences and physiological limits estimated in the literature.

Methods S2 Description of how analyses for two additional FLS classifications were conducted.

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