

Leaf out times of temperate woody plants are related to phylogeny, deciduousness, growth habit and wood anatomy

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Summary

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- Leaf out phenology affects a wide variety of ecosystem processes and ecological interactions and will take on added significance as leaf out times increasingly shift in response to warming temperatures associated with climate change. There is, however, relatively little information available on the factors affecting species differences in leaf out phenology.
- An international team of researchers from eight Northern Hemisphere temperate botanical gardens recorded leaf out dates of c. 1600 woody species in 2011 and 2012.
- Leaf out dates in woody species differed by as much as 3 months at a single site and exhibited strong phylogenetic and anatomical relationships. On average, angiosperms leafed out earlier than gymnosperms, deciduous species earlier than evergreen species, shrubs earlier than trees, diffuse and semi-ring porous species earlier than ring porous species, and species with smaller diameter xylem vessels earlier than species with larger diameter vessels. The order of species leaf out was generally consistent between years and among sites.
- As species distribution and abundance shift due to climate change, interspecific differences in leaf out phenology may affect ecosystem processes such as carbon, water, and nutrient cycling. Our open access leaf out data provide a critical framework for monitoring and modeling such changes going forward.

Introduction

In many temperate and boreal regions of the world, the timing of spring leaf out is advancing due to the warming effects of climate change (Menzel & Fabian, 1999; Chmielewski & Rötzer, 2001; Menzel *et al.*, 2001, 2006; Richardson *et al.*, 2006; Wesolowski & Rowinski, 2006; Delbart *et al.*, 2008; Carroll *et al.*, 2009; Morin *et al.*, 2009; Chen & Xu, 2012; Cong *et al.*, 2012). Modelling of future leaf out phenology suggests that this trend will continue for most species, except when limited by insufficient winter chilling (Morin *et al.*, 2009; Bennie *et al.*, 2010; Cook *et al.*, 2012). Earlier leaf out will have major consequences for ecosystem processes, including carbon sequestration, hydrology, biomass accumulation and timber production (Lopez *et al.*, 2008; Vitasse *et al.*, 2009; Fridley, 2012). Changes in leaf out phenology could also influence ecological interactions, for example, spring ephemeral and bark epiphyte life cycles that synchronise with high spring light levels in the forest understory, outbreaks of insect pests that feed on leaves and numerous trophic interactions with birds, mammals and fungi (Polgar & Primack, 2011). Despite the

growing attention to phenology and its importance in climate change biology, ecosystems and physiological ecology, interspecific variation in leaf out phenology has been relatively overlooked.

Only a few phenology surveys have compared differences in the leaf out times of different species and those that have, have been restricted to between 7 and 73 tree and shrub species (Lechowicz, 1984; Sun *et al.*, 2006; Davi *et al.*, 2011; Liu *et al.*, 2011; Fridley, 2012). Despite the paucity of data, studies have revealed considerable interspecific variation in leaf out times and a consistent order in the timing of leaf out (Lechowicz, 1984; Kramer, 1995; Wesolowski & Rowinski, 2006; Bennie *et al.*, 2010). In addition, Lechowicz (1984) showed that woody species with smaller xylem vessel diameter and diffuse porous wood anatomy tended to leaf out earlier than species with larger vessel elements and ring porous wood anatomy. The difference is, perhaps, because small-vessel species have better tolerance to freezing. More recently, two additional studies in China observed that small-leaved woody species leafed out earlier and had slower leaf expansion rates than larger-leaved species (Sun *et al.*, 2006;

Liu *et al.*, 2011). The authors hypothesized that these traits were adaptations to minimise leaf herbivory (Sun *et al.*, 2006; Liu *et al.*, 2011). In a survey of seven tree species, Davi *et al.* (2011) found that deciduous species leafed out before evergreen species. Understorey species and saplings were also observed to leaf out earlier than canopy tree species, presumably to better utilise available light before canopy closure (Seiwa, 1999; Augspurger & Bartlett, 2003; Sun *et al.*, 2006; Lopez *et al.*, 2008; Richardson & O'Keefe, 2009; Liu *et al.*, 2011; Fridley, 2012; Rollinson & Kaye, 2012).

In addition to ecological factors, it has been hypothesized that differences in leaf out times might be related to the evolutionary history of species (Lechowicz, 1984). However, leaf out phenology has not been examined explicitly in a broad phylogenetic context (Lechowicz, 1984; Gazal *et al.*, 2008; Davi *et al.*, 2011; Fridley, 2012; Davies *et al.*, 2013) despite its relevance to large-scale phenological analyses (Willis *et al.*, 2008, 2010; Davis *et al.*, 2010; Wolkovich *et al.*, 2013). While previous studies have demonstrated that species vary in their leaf out phenology, research to date lacks a phylogenetic context that includes a large and diverse sample of plant species from across multiple geographical locations.

The objective of our study is to assess factors of interspecific variation in leaf out phenology by examining a broad spectrum of temperate woody plants in the Northern Hemisphere. To accomplish our objective, we address the following questions: How much interspecific variation exists in the leaf out dates of temperate woody plant species? How does leaf out phenology relate to deciduousness, growth habit, wood anatomy and phylogenetic relatedness? And finally, how does leaf out phenology vary across geographic locations? Our leaf out study utilises the broadest taxon sampling compiled to date and incorporates a rigorous phylogenetic framework to clarify leaf out patterns in northern hemisphere woody plants.

Materials and Methods

We recorded weekly leaf out dates of woody species in the spring at eight northern hemisphere temperate botanical gardens and arboreta in 2012 (Table 1). We monitored 1597 tree, shrub and vine species, representing 88 angiosperm families and seven gymnosperm families at all sites combined. Not every species was monitored at every site but on average each species was monitored at at least two sites (Table 1). In the prior year, 2011, 616 species were monitored at the Arnold Arboretum and 60 species of *Quercus* L., *Juglans* L. and *Carya* Nutt. at Morton Arboretum.

We defined the leaf out date for deciduous species as the date on which young leaves were unfolding or expanding with their final shape at least partially visible on at least three branches of an individual plant. This definition is comparable to the definitions used by the USA National Phenology Network (USA-NPN National Coordinating Office, 2012) and the International Phenological Gardens of Europe (IPGE, 1960). Our definition of leaf out date was similar for evergreen species. We defined the leaf out date for species of Pinaceae as the date on which the needles began emerging from their bundles and the needle tips were

separated from each other. New scale leaves on certain Cupressaceae species were distinguished by their distinctive lighter colour. We excluded some species of *Thuja* L., *Juniperus* L. and *Chamaecyparis* Spach. because there was no clear difference in colour or shape between new and old leaves.

We scored all species for the following traits: angiosperm vs gymnosperm, deciduous vs evergreen (deciduousness) and growth habit (tree, shrub or vine). Species that grow as trees or shrubs were classified by their dominant growth form. We additionally scored wood anatomy of 1076 angiosperm species as ring porous, semi-ring porous or diffuse porous, and 661 angiosperm species with vessel diameter classes of $\leq 100 \mu\text{m}$ or $> 100 \mu\text{m}$ based on data from FFPRI (1989), Schweingruber (1990), Schweingruber & Landolt (2010) and InsideWood (2012). Wood anatomy and vessel diameter data were not available for all species in the study.

For the statistical analysis not accounting for phylogeny, we used standard least squares modelling to determine how much variation in leaf out dates was explained by sites, angiosperm vs gymnosperm, deciduousness, growth habit, wood anatomy and vessel diameter.

In order to facilitate comparisons among sites in 2012 we calculated an adjusted leaf out date for each species so that all sites had the same mean leaf out date. This helped to deal with the fact that large numbers of species were not shared between sites and that different sites had different climates and consequent mean leaf out dates. To accomplish this, we first calculated a site adjustment factor for each site, which was the difference between the mean leaf out date of all species at the site in 2012 and the mean leaf out date of all species at all sites in 2012. Then we calculated the adjusted leaf out date for each species by adding the site adjustment factor to the leaf out dates of each species at a site and then averaged the leaf out date + site adjustment across the sites for each species. This method is similar to Primack *et al.* (2004) and Panchen *et al.* (2012). We used JMP10 (SAS Institute, Cary, NC, USA) for all statistical analyses.

In order to account for the potential effect of the shared evolutionary history of species, we performed the same set of statistical analyses correcting for phylogenetic relationships (Davis *et al.*, 2010). We ran all phylogenetic analyses using two different phylogenies to address limitations of taxon sampling and phylogenetic resolution (see Wolkovich *et al.*, 2013 for a similar approach). The first phylogeny (hereafter referred to as the PHYLOMATIC tree) was a composite tree based on the literature and had high taxon sampling, but low phylogenetic resolution. The second phylogeny (hereafter referred to as the PHLAWD phylogeny) was based on sequence data and had low taxon sampling, but greater phylogenetic resolution.

The PHYLOMATIC tree was assembled with all 1597 species using the program Phylomatic (Webb & Donoghue, 2005). Relationships in the PHYLOMATIC tree were based on the APG III (2009) phylogeny and were further refined based on the Angiosperm Phylogeny Website (Stevens, 2012). Branch lengths of the PHYLOMATIC tree were adjusted to reflect divergence time estimates based on the fossil record (Bell *et al.*, 2010; Smith *et al.*, 2010) using the function 'bladj' implemented in Phylocom

Table 1 Mean leaf out day of year, standard deviation (SD) range of leaf out dates in days, number of species, and numbers of trees (t), shrubs (s) and vines (v) for 2012

	Latitude, longitude	Altitude (m)	Mean annual temp. (°C)	Mean March 2012 temp. (°C)	Mean day of year leaf out date	SD leaf out date (d)	Leaf out date range (d)	Number of species, t, s, v
US Nat. Arboretum	39°54'N, 76°58'W	19–50	13.7	7.6	84	10.7	46	187, 112, 74, 1
Beijing BG	39°59'N, 116°12'E	74	11.9	5.2	104	7.6	55	162, 97, 57, 8
Morton Arboretum	41°49'N, 88°03'W	223	10	3.3	89	12.2	68	416, 258, 146, 12
Arnold Arboretum	42°18'N, 71°07'W	22	10.7	3.4	101	15.6	88	1112, 548, 514, 50
Garden in the Woods	42°20'N, 71°25'W	57	10.7	3.4	111	14.5	77	162, 50, 107, 5
Ottawa Arboretum	45°23'N, 75°42'W	80	5.8	−2.7	122	16.5	59	182, 149, 31, 2
Munich BG	48°10'N, 11°30'E	523	9.2	4.5	100	15.5	71	444, 203, 230, 11
Berlin BG	52°27'N, 13°17'E	57	9.2	4.6	101	17.7	105	772, 385, 360, 27

Leaf out dates monitored at Arnold Arboretum, Boston, MA, USA; Beijing Botanical Garden, Beijing, China (Beijing BG); Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin, Germany (Berlin BG); Garden in the Woods, Framingham, MA, USA; Morton Arboretum, Lisle, IL, USA; Munich Botanical Garden, Munich, Germany (Munich BG); Ottawa Arboretum, Ottawa, Canada and US National Arboretum, Washington, DC and Beltsville, MD, USA (US Nat. Arboretum), with their respective latitude, longitude, altitude, mean annual temperature and mean March temperature over 1961–1990 for Beijing and North America and over 1971–2000 for Munich and Berlin.

4.1 (Webb *et al.*, 2008). The PHLAWD phylogeny was constructed with maximum likelihood methods using the program PHLAWD (Smith *et al.*, 2009). DNA sequence data was collected from GenBank for 451 species (see Methods in Wolkovich *et al.* (2013) for further details on markers and tree assembly).

We tested for phylogenetic signal in the adjusted leaf out dates using Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg *et al.*, 2003) using the 'phylosig' function in the package 'phytools' v0.2-1 in R v2.15.1 (R Foundation, Vienna, Austria) (Revell, 2012). To test which factors best explain leaf out dates while controlling for phylogeny, we used the phylogenetic generalized least squares function 'pgls' in the R package 'caper' v0.5 (Orme *et al.*, 2012). The adjusted leaf out date was treated as the dependent variable, while angiosperm vs gymnosperm, deciduousness (deciduous or evergreen) and growth habit (tree, shrub or vine) were treated as the predicted variables.

We used the Welch difference of means test with $\alpha = 0.05$ to determine if there were significant differences in mean adjusted leaf out dates and site mean leaf out dates for each of the factors of angiosperm vs gymnosperm, deciduousness, growth habit, wood anatomy types and vessel diameter categories. The Welch test does not assume equal variance between samples so this test was chosen because our sample sizes are unequal.

As a first step to gaining a broad overview of whether closely related species have similar leaf out phenology, we calculated the average leaf out date for each genus with three or more species and for each family with five or more species. Although taxonomic ranks are arbitrary clade identifiers, we chose to present average leaf out dates for clades that correspond to the rank of genus and family because of their prominent recognisability. Deciduous species and evergreen species for each genus or family

were averaged separately. We calculated these mean genus and family leaf out dates for the entire set of adjusted leaf out dates and separately for the 2012 leaf out dates at the Arnold Arboretum and at the Berlin Botanic Garden. As a second step we used the phylogenies to assess whether closely related species have similar leaf out phenology. Here we determined the clades that had either significantly earlier or later average leaf out dates than expected relative to a random sample of species. We used the 'aotf' function in program Phylocom v4.2 (Webb *et al.*, 2008) and 1000 randomized runs to calculate the mean adjusted leaf out date of each node under the ancestral averaging algorithm (Node.mn) and identified all nodes with significantly early average adjusted leaf out dates (Nmn.RankLow < 25, Nmn.RankHigh > 975) and significantly late average adjusted leaf out dates (Nmn.RankLow < 975, Nmn.RankHigh > 25).

We ran linear regression models to compare the order of leaf out at the Arnold Arboretum with each of the other seven sites. The Arnold Arboretum was used as the standard because this site had the most species monitored. We also ran linear regression models to compare the order of average leaf out for genera and families at the Arnold Arboretum and Berlin Botanic Garden, the two locations with the greatest number of species and genera in common. Additionally, for the species monitored in both 2011 and 2012 at the Arnold and Morton Arboreta, we ran linear regression models to determine the extent to which species order of leaf out was consistent across these 2 yr.

Results

Leaf out dates at each of the eight sites were distributed over periods of 6–15 wk (Table 1). Leaf out took place over an average of

71 d across the sites monitored in 2012, ranging from 46 d at the US National Arboretum to 105 d at Berlin Botanic Garden (Table 1). Leaf out took place over *c.* 3 months at the Arnold Arboretum (88 d) and the Berlin Botanic Garden (105 d), the two sites with the largest number of monitored species.

Much of the variation among species was explained by site, angiosperm vs gymnosperm, deciduousness, growth habit, wood anatomy and vessel diameter in the standard least squares model ($R^2 = 0.44$, $P < 0.0001$, $N = 1731$). All terms in the model were significant ($P < 0.001$) except angiosperm vs gymnosperm. The models including site and one other factor still explained some of the leaf out date (angiosperm vs gymnosperm: $R^2 = 0.29$, $P < 0.0001$, $N = 3437$; deciduousness: $R^2 = 0.31$, $P < 0.0001$, $N = 3437$; growth habit: $R^2 = 0.31$, $P < 0.0001$, $N = 3437$; wood anatomy: $R^2 = 0.33$, $P < 0.0001$, $N = 2497$ and vessel diameter: $R^2 = 0.35$, $P < 0.0001$, $N = 1747$).

There was significant phylogenetic signal in the adjusted leaf out dates for both the PHYLOMATIC ($\lambda = 0.69$, $P < 0.0001$, Blomberg's $K = 0.61$, $P = 0.011$) and PHLAWD trees ($\lambda = 0.89$, $P < 0.0001$, Blomberg's $K = 0.04$, $P = 0.001$). Evidence of phylogenetic signal in leaf out date indicates the importance of accounting for phylogenetic relatedness in our regression analysis. When controlling for phylogeny, the adjusted leaf out date had a significant relationship with deciduousness and growth habits for both the PHYLOMATIC and PHLAWD trees (Table 2).

Patterns in species level variation of leaf out times

The mean adjusted leaf out date was significantly earlier for: (1) angiosperms than for gymnosperms (Fig. 1, Table 3); (2) deciduous species than for evergreen species (Fig. 2, Table 3); (3) shrubs than for trees or vines (Fig. 3, Table 3); (4) semi-ring porous species than for both diffuse porous and ring porous species (Fig. 4, Table 3); and (5) species with vessel diameters $\leq 100 \mu\text{m}$ than for species with vessel diameters $> 100 \mu\text{m}$ (Fig. 5, Table 3). These differences remained significant at all sites with the following

Table 2 Results from phylogenetic generalized least squares (PGLS) examining the effects of deciduousness (deciduous vs evergreen), growth habit (shrub vs tree or vine) and angiosperm vs gymnosperm on the adjusted leaf out dates for the low resolution PHYLOMATIC tree, a composite phylogenetic tree of all woody species in our study ($n = 1597$) and the high resolution PHLAWD phylogeny, a subset of the woody species included in the study ($n = 451$)

Term	df	β	t	P
PHYLOMATIC tree	1592			
Deciduous vs evergreen		9.71	9.90	< 0.0001
Habit (shrub vs tree)		6.09	8.00	< 0.0001
Habit (shrub vs vine)		3.63	2.56	0.011
Angiosperm vs gymnosperm		-1.02	-0.25	0.806
PHLAWD tree	446			
Deciduous vs evergreen		9.71	4.51	< 0.0001
Habit (shrub vs tree)		6.59	4.39	< 0.0001
Habit (shrub vs vine)		0.987	0.33	0.740
Angiosperm vs gymnosperm		5.46	0.29	0.769

Parameter estimate (β) is equivalent to the days of difference in leaf out between the pairs.

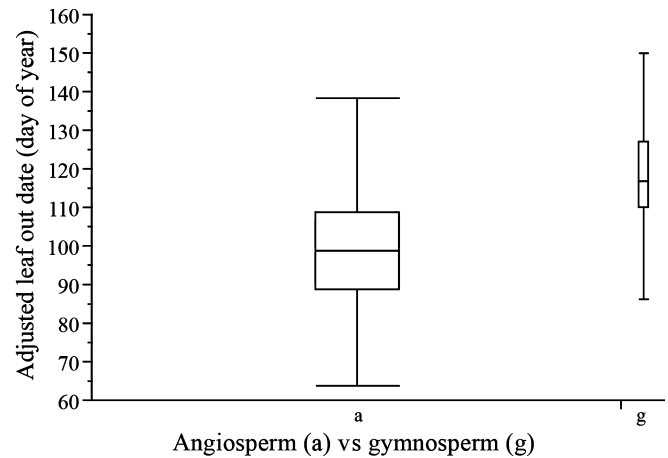


Fig. 1 The adjusted leaf out dates of angiosperms (a) vs gymnosperms (g) for 1597 species in 2012 across the eight temperate botanical garden and arboretum sites (Table 1), showing that angiosperms leaf out significantly earlier than gymnosperms ($t = 1.96$, $\alpha = 0.05$, $N = 1477$ angiosperms, 120 gymnosperms). The day of year is the number of days from 1 January 2012. The box plot shows the adjusted leaf out date range, quartiles and a mean adjusted leaf out day of year of 99 for angiosperms and 118 for gymnosperms, the box width indicates the relative sample sizes.

Table 3 Welch difference of means test for the adjusted mean leaf out date comparing angiosperm vs gymnosperm, deciduousness, growth habit, wood anatomy and vessel diameter

Comparison	Days		N_1	N_2	P
	Dif.	t			
Angiosperm vs gymnosperm	19	12.89	1477	120	< 0.001
Deciduous vs evergreen	17	15.35	1368	229	< 0.001
Shrub vs tree growth habit	10	14.32	792	736	< 0.001
Shrub vs vine growth habit	7	3.60	792	69	0.001
Diffuse vs ring porous wood anatomy	9	9.90	651	224	< 0.001
Semi-ring vs ring porous wood anatomy	12	9.75	201	224	< 0.001
Vessel diameter $\leq 100 \mu\text{m}$ vs $> 100 \mu\text{m}$	11	11.34	482	179	< 0.001
Deciduous angiosperm vs deciduous gymnosperm	5	1.37	1356	12	0.279
Evergreen angiosperm vs evergreen gymnosperm	10	5.37	121	108	< 0.001
Deciduous angiosperm vs evergreen angiosperm	15	7.23	932	77	< 0.001
Deciduous gymnosperm vs evergreen gymnosperm	27	5.18	9	94	< 0.001

Days Dif., the number of days of difference in mean leaf out dates; N_1 , the number of species that leafed out significantly earlier in the pairwise comparison (the trait listed first in the comparison column); N_2 , the number of species that leafed out significantly later (the trait listed second in the comparison column).

exceptions: the mean leaf out date for shrubs was significantly earlier than for vines at the Arnold Arboretum and the Berlin Botanic Garden ($t = 1.96$, $P < 0.001$) and there was no significant difference in mean leaf out date in the wood anatomy or vessel diameter categories at the Garden in the Woods (Supporting Information Table S1).

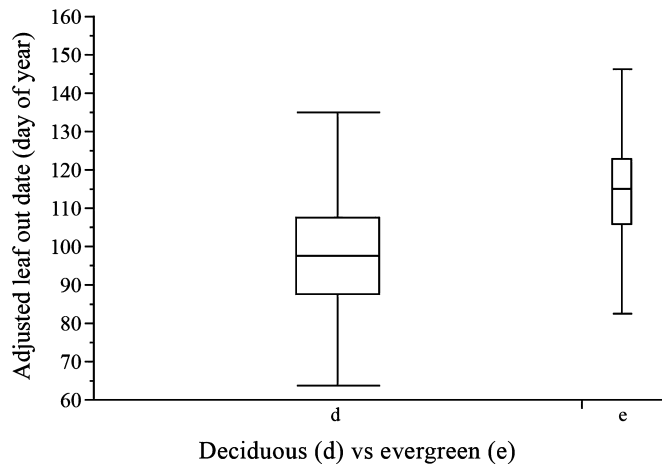


Fig. 2 The adjusted leaf out dates of deciduous species (d) vs evergreen species (e) for 1597 species in 2012 across the eight temperate botanical garden and arboretum sites (Table 1), showing that deciduous species leaf out significantly earlier than evergreen species ($t = 1.96$, $\alpha = 0.05$, $N = 1368$ deciduous species, 229 evergreen species). The day of year is the number of days from 1 January 2012. The box plot shows the leaf out date range, quartiles and a mean adjusted leaf out day of year of 98 for deciduous species and 115 for evergreen species, the box width indicates the relative sample sizes.

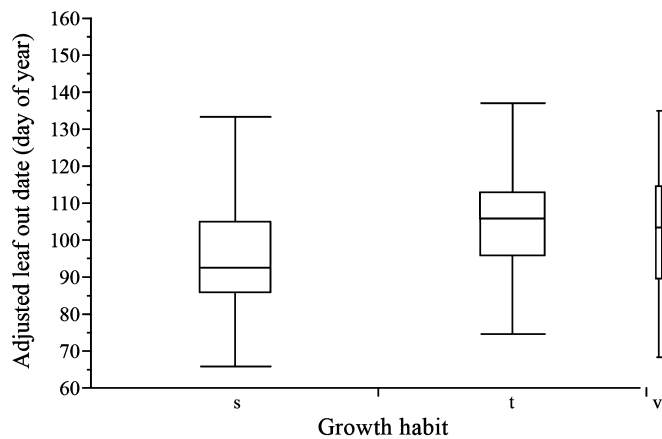


Fig. 3 The adjusted leaf out dates related to growth habit type (s, shrub; t, tree; v, vine) for 1597 species in 2012 across the eight temperate botanical garden and arboretum sites (Table 1), showing that shrubs leaf out significantly earlier than trees and vines ($t = 1.96$, $\alpha = 0.05$, $P < 0.0001$, $N = 736$ trees, 792 shrubs and 69 vines). The day of year is the number of days from 1 January 2012. The box plot shows the leaf out date range, quartiles and a mean adjusted leaf out day of year for each growth habit type (95 for shrubs, 105 for trees and 102 for vines), the box width indicates the relative sample sizes.

Furthermore, the mean adjusted leaf out date for evergreen angiosperms was significantly earlier than for evergreen gymnosperms. However, there was no significant difference in mean adjusted leaf out dates between deciduous angiosperms and deciduous gymnosperms (Table 3). When angiosperm and gymnosperm species were considered separately, the mean adjusted leaf out date for deciduous angiosperms was significantly earlier than for evergreen angiosperms and likewise for deciduous gymnosperms and evergreen gymnosperms (Table 3).

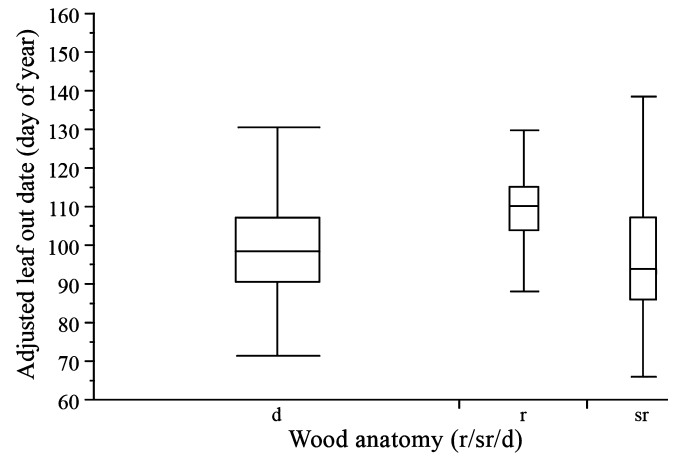


Fig. 4 The adjusted leaf out dates related to wood anatomy type (d, diffuse porous; r, ring porous; sr, semi-ring porous) for 1076 species in 2012 across the eight temperate botanical garden and arboretum sites (Table 1), showing that diffuse porous species leaf out significantly earlier than ring porous species and that semi-ring porous species leaf out significantly earlier than both diffuse and ring porous species ($t = 1.96$, $\alpha = 0.05$, $P < 0.0001$, < 0.0001 and 0.0014 , respectively, $N = 651$ diffuse porous, 224 ring porous and 201 semi-ring porous). The day of year is the number of days from 1 January 2012. The box plot shows the leaf out date range, quartiles and a mean adjusted leaf out day of year for each wood anatomy type (96 for semi-ring porous, 99 for diffuse porous and for 108 ring porous), the box width indicates the relative sample sizes.

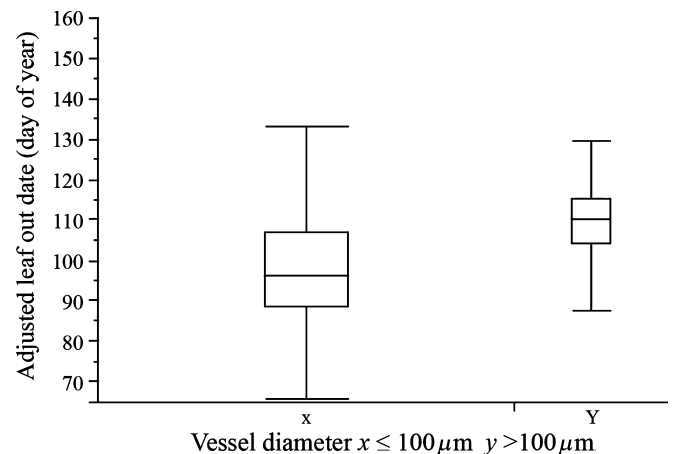


Fig. 5 The adjusted leaf out dates related to vessel diameter ($x \leq 100 \mu\text{m}$ and $y > 100 \mu\text{m}$) for 661 species in 2012 across the eight temperate botanical garden and arboretum sites (Table 1), showing that species with vessel diameters $\leq 100 \mu\text{m}$ leaf out significantly earlier than species with vessel diameters $> 100 \mu\text{m}$ ($t = 1.96$, $\alpha = 0.05$, $N = 482$ diameter $\leq 100 \mu\text{m}$, 179 diameter $> 100 \mu\text{m}$). The day of year is the number of days from 1 January 2012. The box plot shows the leaf out date range, quartiles and a mean adjusted leaf out day of year of 98 for species with vessel diameter $\leq 100 \mu\text{m}$ and 109 for species with vessel diameter $> 100 \mu\text{m}$, the box width indicates the relative sample sizes.

In order to illustrate the patterns described above, at the Arnold Arboretum, where the largest number of species was monitored, the earliest species to leaf out on 10 March 2012 were angiosperm deciduous shrubs including *Chaenomeles japonica* (Thunb.) Lindl., *Daphne mezereum* L., *Ligustrum compactum* (Wall. ex G. Don) Hook. f. & Thomson ex Decne., *Lonicera tatarica* L., *Ribes alpinum* L. and *Sorbaria sorbifolia* (L.) A. Braun. The last

species to leaf out in the spring of 2012 at the Arnold Arboretum were the gymnosperm evergreen trees *Pinus aristata* Engelm., *P. contorta* Douglas ex Loudon and *P. pumila* (Pall.) Regel and the evergreen angiosperm trees *Ilex maximowicziana* Loes. and *Magnolia grandiflora* L. on 30 May and the angiosperm evergreen shrub *Rhododendron makinoi* Tagg. on 6 June. Similarly, at Berlin Botanic Garden, where the second largest number of species was monitored, the angiosperm deciduous shrubs *Ribes alpinum* L., *Rubus idaeus* L., *Sambucus nigra* L., *Sorbaria kirilowii* (Regel) Maxim. and *S. sorbifolia* were the first to leaf out on 7 March 2012, and the last to leaf out were the gymnosperm evergreen trees *Picea torano* (Siebold ex K.Koch) Koehne, *Pinus aristata* Engelm., *P. armandii* Franch. and *P. bungeana* Zucc. ex Endl. on 6, 13 and 20 June, respectively, and the angiosperm evergreen shrub *Rhododendron fauriei* Franch. on 16 May. Most of the earliest leafing out angiosperm species have diffuse porous stems with small vessel diameters, and most of the latest leafing out angiosperm species have ring porous stems with large vessel diameters. A list of the species that leafed out first and last at each site and in the adjusted leaf out dataset is given in Table S2 and in the online dataset.

Differences among genera, families and clades

Some clades in the PHYLOMATIC and PHLAWD trees exhibited significantly conserved early or late average adjusted leaf out dates relative to a random sample of species from across the phylogenies (Tables 4, 5, Figs 6, S1, S2). For example, some of the major early leaf out clades include Rosaceae, Dipsacales (including *Viburnum* and *Lonicera*) and *Ribes* that contain predominantly deciduous species. The major late leaf out clades include gymnosperms (including *Pinus* and *Picea*) with the exception of *Larix* and *Ginkgo*, Ericaceae (including *Rhododendron*), Fagales (including *Fagus*, *Quercus*, *Carya* and *Juglans*) and *Magnolia*. There was agreement in the early and late leaf out clades between the PHYLOMATIC and PHLAWD trees (Tables 4,5).

In the nonphylogenetic comparison of families and genera, the mean leaf out date for the majority of deciduous genera was earlier than for evergreen genera (Tables S3–S5). Across the Arnold Arboretum and Berlin Botanic Garden and the adjusted leaf out datasets, the deciduous shrub genera *Chaenomeles* Lindl., *Ligustrum* L., *Lonicera* L., *Niellia* T.T. Yu & T.C. Ku, *Ribes* L. and *Sambucus* L. consistently had mean leaf out dates in the first or second week of the spring flush and the deciduous tree genera *Catalpa* Scop. and *Morus* L. were consistently among the last deciduous genera to leaf out (Tables S3–S5). Among evergreens, species of *Cotoneaster* Medik. consistently had the earliest mean leaf out date across the three datasets and *Pinus* L. the latest mean leaf out date (Tables S3–S5). For genera in which there were species of both evergreen and deciduous species, such as *Berberis* L., *Cotoneaster* Medik., *Ilex* L., *Rhododendron* L., *Lonicera* and *Viburnum* L., the deciduous species had an earlier mean leaf out date than the evergreen species. In the genera *Magnolia* L. and *Prunus* L., in which most species are deciduous, the last species to leaf out were the evergreen *M. grandiflora* L. and *Prunus laurocerasus* L.

Across the Arnold Arboretum, Berlin Botanic Garden and the adjusted leaf out datasets, the mean leaf out date for families (deciduous members only) Grossulariaceae, Caprifoliaceae, Berberidaceae and Pinaceae were consistently the earliest (Tables S6–S8). Species of Bignoniaceae, Moraceae and Vitaceae consistently had the last mean leaf out dates of deciduous species. Among evergreen species, the mean leaf out date for the family Rosaceae was notably early (due largely to many *Cotoneaster* species). Pinaceae, Taxaceae, Ericaceae families (mainly *Rhododendron* and *Erica* species) and Aquifoliaceae (mainly *Ilex* species) had late mean leaf out dates. For families with both deciduous and evergreen species such as Aquifoliaceae, Berberidaceae, Ericaceae and Pinaceae, the mean leaf out date for the deciduous species was earlier than the mean leaf out date for the evergreen species in the same family.

Table 4 Major clades that have either significantly earlier (day of year < 94) or later (day of year > 107) average adjusted leaf out dates than would be expected relative to a random sample of species from across the PHLAWD phylogeny

Clade	Main genera in clade	Ntaxa	Node.mn	Node.SD
<i>Paeonia</i>		2	75	0.24
<i>Ribes</i>		3	75	0.92
<i>Larix</i>		2	81	8.51
<i>Syringa</i> + <i>Ligustrum</i>		6	83	1.25
Rosaceae	<i>Spiraea</i> , <i>Cotoneaster</i> , <i>Malus</i>	67	90	6.60
Malpighiales + Celastraceae	<i>Salix</i> , <i>Hypericum</i>	17	90	1.94
Dipsacales + <i>Ilex</i> + Araliaceae	<i>Viburnum</i> , <i>Lonicera</i> , <i>Hedera</i> , <i>Eleutherococcus</i>	39	94	8.33
Fabales + Fagales	<i>Gleditsia</i> , <i>Fagus</i> , <i>Quercus</i>	73	109	1.93
Ericaceae + Cyrillaceae + Clethraceae	<i>Rhododendron</i> , <i>Cyrilla</i> , <i>Clethra</i>	27	110	6.06
<i>Magnolia</i>		6	114	18.28
Gymnosperms (excluding <i>Ginkgo</i>)	<i>Tsuga</i> , <i>Cedrus</i> , <i>Abies</i> , <i>Picea</i> , <i>Pinus</i>	34	113	4.75
<i>Fraxinus</i> + <i>Forestiera</i> + <i>Chionanthus</i>		14	114	2.01
<i>Catalpa</i>		5	117	0.57
Vitaceae	<i>Vitis</i> , <i>Ampelopsis</i>	3	118	1.27

Ntaxa, the number of taxa in the clade; Node.mn, the average adjusted leaf out date of the clade calculated using Phylocom's 'aotf' function using the ancestral averaging algorithm (Webb *et al.*, 2008); Node.SD, the standard deviation of subtending nodes' average adjusted leaf out dates.

Table 5 Major clades that have either significantly earlier (day of year < 94) or later (day of year > 112) average adjusted leaf out dates than would be expected relative to a random sample of species from across the PHYLOMATIC tree

Clade	Main genera in clade	Ntaxa	Node.mn	Nod.SD
<i>Ribes</i>		16	74	6.81
Dipsacales	<i>Viburnum, Sambucus, Lonicera, Diervilla, Abelia</i>	110	78	13.63
<i>Ligustrum</i>		11	85	13.04
<i>Deutzia</i>		15	86	8.52
Rosaceae	<i>Prunus, Spiraea, Malus, Cotoneaster</i>	371	87	7.72
<i>Philadelphus</i>		20	90	5.00
<i>Syringa</i>		11	90	10.00
<i>Salix</i>		35	95	11.43
<i>Magnolia</i>		17	109	12.19
<i>Quercus</i> + <i>Ilex</i>		61	111	6.24
<i>Rhododendron</i>		43	112	16.55
Rutaceae + Meliaceae + Simaroubaceae	<i>Phellodendron, Zanthoxylum, Picrasma, Ptelea, Melia, Toona</i>	19	113	2.87
Gymnosperms (ex. Ginkgo + Ephedra)	<i>Cedrus, Abies, Tsuga, Picea, Pinus, Taxus, Juniperus</i>	117	116	2.89
<i>Erica</i>		4	118	5.88
Moraceae + <i>Boehmeria biloba</i>	<i>Morus, Broussonetia</i>	9	118	8.08
Gentianales	<i>Gardenia, Cephalanthus, Periploca</i>	6	118	0.42
Paliureae	<i>Hovenia, Paliurus, Ziziphus</i>	3	121	8.31

Ntaxa, the number of taxa in the clade; Node.mn, the average adjusted leaf out date of the clade calculated using Phylocom's 'aotf' function using the ancestral averaging algorithm (Webb *et al.*, 2008); Node.SD, the standard deviation of subtending nodes' average adjusted leaf out dates.

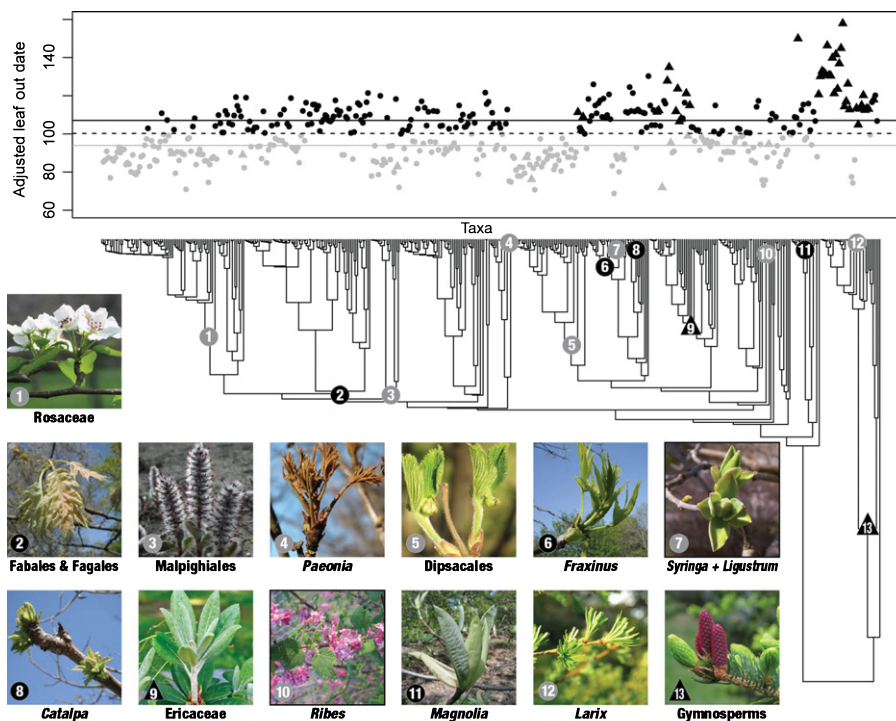


Fig. 6 Adjusted leaf out dates (day of year) for the PHLAWD phylogeny: dotted line, indicating the mean adjusted leaf out date; black line, the day of year above which species leaf out significantly later than would be expected relative to a random sample of species from across the phylogeny; grey line, the day of year below which species leaf out significantly earlier than would be expected relative to a random sample of species from across the phylogeny; circle, a deciduous species; triangle, an evergreen species. The PHLAWD phylogeny highlights major clades with significantly early average adjusted leaf out dates (in grey), significantly late average adjusted leaf out dates (in black), with numbered circles representing clades with predominantly deciduous species, and numbered triangles representing clades with predominantly evergreen species. The average adjusted leaf out date at each node was calculated using Phylocom's 'aotf' function and the ancestral averaging algorithm (Webb *et al.*, 2008).

Differences among sites and between years

There was a strong relationship between the order of leaf out of species at the Arnold Arboretum in 2012 and each of the other seven sites in 2012 indicating that species leaf out in essentially the same order at all study sites ($R^2 = 0.32\text{--}0.72$, $P < 0.0001$, $N = 100\text{--}549$, depending on site) (Table 6). There were particularly high R^2 values (0.63, 0.72 and 0.70, respectively) for the relationship of leaf out dates at the Arnold Arboretum in 2012

with the leaf out dates at the Berlin Botanic Garden (Fig. 7), Garden in the Woods and the Ottawa Arboretum (Table 6), despite the mean leaf out dates at the Garden in the Woods and Ottawa Arboretum being *c.* 1.5 and 3 wk later, respectively, than at the Arnold Arboretum (Table 1). Within this general pattern, there were also certain species that leafed out in a somewhat different sequence at separate locations. There was a strong relationship between the order of genus mean leaf out dates at Arnold Arboretum and Berlin Botanic Garden in 2012 ($R^2 = 0.68$, $N = 69$,

Table 6 Linear regression (R^2 , slope, P) of order of leaf out of species at Arnold Arboretum in 2012 with Arnold Arboretum in 2011 and with the seven other sites

Site	R^2	Slope	P	N	Mean	SD	Min	Max	AA12 mean	AA12 SD	AA12 min	AA12 max
AA2011	0.69	0.66	<0.0001	549	26-Apr	12.52	26-Mar	4-Jun	6-Apr	15.70	9-Mar	29-May
USNA	0.39	0.50	<0.0001	110	20-Mar	9.84	5-Mar	15-Apr	5-Apr	12.29	12-Mar	2-May
Munich	0.52	0.81	<0.0001	304	9-Apr	15.95	6-Mar	9-May	4-Apr	14.15	9-Mar	14-May
Berlin	0.63	0.94	<0.0001	543	9-Apr	16.66	6-Mar	19-Jun	6-Apr	14.31	9-Mar	29-May
Beijing	0.32	0.34	<0.0001	100	12-Apr	8.13	16-Mar	10-May	8-Apr	13.50	9-Mar	14-May
Morton	0.38	0.48	<0.0001	366	27-Mar	12.25	14-Mar	21-May	5-Apr	15.82	9-Mar	29-May
GitW	0.72	0.80	<0.0001	108	19-Apr	14.48	14-Mar	30-May	14-Apr	15.29	9-Mar	18-May
Ottawa	0.70	0.88	<0.0001	142	30-Apr	16.13	25-Mar	23-May	11-Apr	15.79	9-Mar	29-May

AA2011, Arnold Arboretum in 2011; USNA, US National Arboretum; Munich, Munich Botanical Garden; Berlin, Botanic Garden and Botanical Museum Berlin-Dahlem; Beijing, Beijing Botanical Garden; Morton, Morton Arboretum; GitW, Garden in the Woods; Ottawa, Ottawa Arboretum. N , Mean, SD, Min and Max are, respectively, the number of species, mean leaf out date, earliest leaf out date and last leaf out date at each site for species common to that site and Arnold Arboretum and AA12 Mean, AA12 SD, AA12 Min, AA12 Max are, respectively, the number of species, mean leaf out date, earliest leaf out date and last leaf out date at Arnold Arboretum in 2012 for species common to Arnold Arboretum and each site.

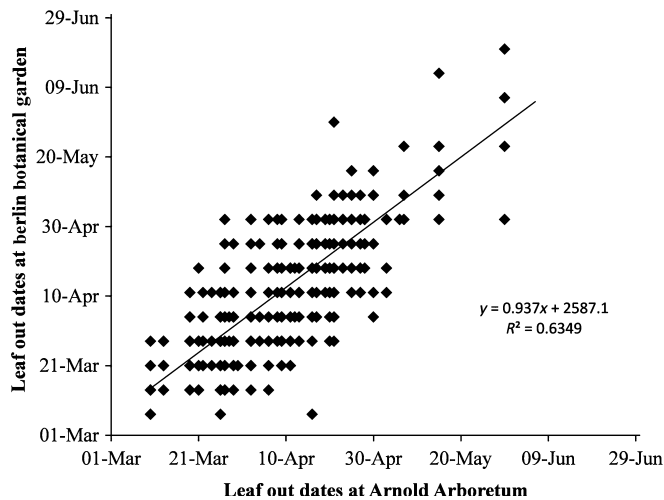


Fig. 7 Order of leaf out of species at Arnold Arboretum and Berlin Botanical Garden in 2012, indicating that species leaf out in roughly the same order ($R^2 = 0.63$, $P < 0.0001$, $N = 549$). Many of the individual points are difficult to see due to their overlapping values.

$P < 0.0001$) and between the order of family mean leaf out date at these two sites in 2012 ($R^2 = 0.83$, $N = 33$, $P < 0.0001$); that is, genera and families leafed out in the same order at the two locations, even though the species represented in each genus and family differed.

The order of leaf out dates of the species at the Arnold Arboretum in 2012 and 2011 were highly correlated ($R^2 = 0.69$, $P < 0.0001$, $N = 551$) (Table 6). The high R^2 value indicates that the order of leaf out of species remains very much the same in consecutive years, despite the Arnold Arboretum mean leaf out date being significantly earlier, by 20 d, due to a substantially warmer spring in 2012 than 2011 ($t = 1.96$, $N = 616$). The order of leaf out dates of *Quercus*, *Juglans* and *Carya* species at the Morton Arboretum in 2012 and 2011 were also correlated ($R^2 = 0.36$, $P < 0.0001$, $N = 60$).

We have made the dataset available as supplemental data for other researchers to use in other research projects (Tables S9–S11). We request that scientists using this data acknowledge its source and inform us of its use.

Discussion

Overall, leaf out dates were spread over a long period during spring, with specific timing related to multiple aspects of a species' biology. Interspecific variation in leaf out dates was associated with ecological and anatomical factors, even when corrected for phylogenetic nonindependence. First, angiosperms, on average, leafed out earlier than gymnosperms. This difference in mean leaf out date held true when evergreen species were analysed separately but not when deciduous species were analysed separately, possibly because of the small sample size of deciduous gymnosperms surveyed (nine species) and because the evergreen and deciduous gymnosperm samples were dominated by groups of closely related plants in single clades. Of the species monitored in this study, half of the deciduous gymnosperms were in one genus (*Larix*) and 2/3 of the evergreen gymnosperms were in Pinaceae, so it is possible that the results reflect, at least in part, characteristics of these clades rather than gymnosperms as a whole.

A second factor related to leaf out phenology is deciduousness. Across all comparisons, deciduous species leafed out on average before evergreen species. This was true when analysing angiosperms, gymnosperms, trees and shrubs separately. In general, for a particular group, deciduous species leafed out 1–2 wk earlier than evergreen species. A likely explanation is that leaves from past years on evergreen species can photosynthesise during the period in the spring before the risk of frost has passed while deciduous species must produce new leaves as soon as possible to replenish nutrient supplies (Davi *et al.*, 2011; Michelot *et al.*, 2012). Also, the leaves of evergreen species are energetically more expensive to produce and are therefore more carefully protected from potential frost damage (Villar & Merino, 2001). For deciduous species, the first process at the end of dormancy is recovery of photosynthetic capacity by producing new leaves. Evergreen leaves change physiologically in autumn to protect the leaf photosystem from winter damage and then change back in the spring (Öquist & Huner, 2003). Evergreen plants repair their existing leaves before beginning new leaf production, and this may explain the tendency of evergreen species to leaf out later than deciduous species (Lundmark *et al.*, 1988).

The third key factor is that mean leaf out dates differed significantly for different growth habits, with shrubs leafing out before trees, as was described for a much smaller sample size of species in China by Sun *et al.* (2006) and Liu *et al.* (2011). In our study, shrubs leafed out earlier on average than trees in angiosperms and gymnosperms and in deciduous and evergreen species. Early leaf out may be particularly advantageous for shrubs in allowing them to become photosynthetically active before the canopy trees produce leaves and reduce the supply of direct sunlight (Seiwa, 1999; Augspurger & Bartlett, 2003; Lopez *et al.*, 2008; Richardson & O'Keefe, 2009; Rollinson & Kaye, 2012). Shrubs may also be better able to tolerate the loss of their first crop of leaves to frost damage because replacing damaged leaves may be less expensive for shrubs than for trees due in part to shrubs having smaller leaf size than trees (Sun *et al.*, 2006; Liu *et al.*, 2011). Differences in early spring microclimates at different heights may expose trees and shrubs to different selective pressures (Geiger *et al.*, 2009; Vitasse, 2013). This will require further research to determine. Vines tended to leaf out later than shrubs and earlier than trees, though the pattern is not as clear, possibly due to both the small number of vine species (69 species) surveyed and to vines' heterogeneous growth forms and ecology.

Another important characteristic affecting leaf out times is wood anatomy, also noted in a previous study (Lechowicz, 1984). Species with diffuse porous stems and semi-porous stems leafed out 1–2 wk earlier on average than species with ring porous wood anatomy. Species with smaller vessel diameters leafed out earlier on average than species with larger vessel diameters. In our study, species with the narrower diameter vessels associated with diffuse and semi-ring porous anatomy are presumably less likely to be damaged by embolisms caused by freeze–thaw events than are the large diameter vessels of ring porous species (Essiamah & Eschrich, 1986; Michelot *et al.*, 2012) and hence can leaf out earlier in the spring. Diffuse and semi-ring porous species are also more likely to retain functioning undamaged vessels from the previous season that can facilitate early spring growth (Essiamah & Eschrich, 1986; Suzuki *et al.*, 1996; Michelot *et al.*, 2012). Ring porous species may need to initiate new wood growth in the spring before bud break can occur (Aloni & Peterson, 1997). There are likely to be other traits beyond wood anatomy that could be important drivers of leaf out times and warrant further research, including leaf area, leaf mass, leaf thickness and twig thickness (Sun *et al.*, 2006; Liu *et al.*, 2011).

The depth of phylogenetic signal in leaf out phenology varied widely across the phylogeny

In several instances, clades with significantly earlier or later leaf out times corresponded to recognized taxonomic ranks. For example, Rosaceae species, on average, leafed out significantly earlier in the year, while the gymnosperms (excluding *Ginkgo*, *Ephedra* and *Larix*) leafed out significantly later. However, several clades do not conform to traditional taxonomic ranks, but represent a range of evolutionary depths. An example would be the Fagales + Fabales clade, which leafed out significantly later relative to the rest of species in the study. The presence of clades that do

not have formally designated taxonomic ranks emphasizes the importance of taking a phylogenetic perspective, as opposed to a traditional taxonomic perspective. The phylogenetic signal in leaf out date may be due in part to its correlation with other phylogenetically conserved traits. For instance, of the major conserved clades, those that leafed out significantly early contain predominantly deciduous species while the late leaf out clades can be either predominantly deciduous or evergreen.

We found that the order of leaf out times is consistent at different locations and shows little inter-annual variation. This consistent ordering of species leaf out has been found in other studies of woody plants (Lechowicz, 1984; Wesolowski & Rowinski, 2006). Some of the leaf out differences of individual species between different locations could be due to different microsites or to geographic variation in the plant material source. Determining growing degree days for each species at each site would be another approach to confirm that leaf out order is consistent across years and sites. However, both approaches give similar results and each has advantages and disadvantages (Polgar & Primack 2011; Archetti *et al.*, 2013; Laube *et al.*, 2014). We did not distinguish between native and non-native species in our analysis. Non-native species growing in botanical gardens outside of their native range, in conditions of novel temperature, photoperiodic and chilling cues may introduce additional variance that needs to be considered (Alberto *et al.*, 2013; Polgar *et al.*, 2014; Zohner & Renner, 2014). Native species at each garden may be represented by more individuals and therefore greater genetic variation than non-native species, again potentially influencing observations. We are now collecting more years of observations at these sites, data that will clarify the extent to which warmer temperatures associated with climate change will impact different species. In the current study, we used weekly observations, which was practical given the large number of species under observation. The accuracy of leaf out timing could be improved with more frequent visits, but weekly observation should be adequate (Miller-Rushing *et al.*, 2008).

Our results have a variety of implications for forest and ecosystem ecology. There is currently great interest in determining how leaf out and flowering phenology will be affected by climate change. Many studies have demonstrated that warming spring temperatures have already resulted in earlier leaf out phenology and may cause even earlier leaf out in coming decades (Menzel & Fabian, 1999; Menzel, 2000; Chmielewski & Rötzer, 2001; Menzel *et al.*, 2001, 2006; Delbart *et al.*, 2008; Carroll *et al.*, 2009; Jeong *et al.*, 2011; Cong *et al.*, 2012; Fridley, 2012). A warming climate will affect species composition of forests as well, with complicated effects on ecosystem processes. Climate change projections predict that by the end of this century, the characteristic maple–birch–beech forests of the northeastern United States will be replaced by the oak–hickory forests more typical of the southeastern United States and pine forests in the southeastern United States displaced by oak–pine forests (Karl *et al.*, 2009). If species composition is changing with climate change, predicting leaf out times in the future for a particular geographical area becomes more complicated. In New England, a warming climate resulting in early leaf out birch and maple species being replaced

by later leaf out oak and hickory species will make it difficult to predict community-wide phenological changes and ecological interactions. Climate change models are also predicting that the warming climate will delay leaf out times in certain species if they are unable to meet their winter chilling requirements (Morin *et al.*, 2009; Bennie *et al.*, 2010; Cook *et al.*, 2012; Rollinson & Kaye, 2012). Understanding such species differences in winter chilling requirements for leaf out times is a clear priority for forest ecology research linked to climate change (Laube *et al.*, 2014; Polgar *et al.*, 2014). Climate change models sometimes assume all woody plants leaf out at the same time while our results show that woody plants behave differently depending on phylogenetic affinities, growth habit, deciduousness and wood anatomy. While warming conditions will tend to cause some trees to leaf out earlier, the changing composition of the forest and different responses in interspecific leaf out times will also have implications on ecosystem processes and need to be factored into climate change model predictions.

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References

- Alberto FJ, Aitken SN, Alia R, Gonzalez-Martinez SC, Hanninen H, Kremer A, Lefevre F, Lenormand T, Yeaman S, Whetten R *et al.* 2013. Potential for evolutionary responses to climate change evidence from tree populations. *Global Change Biology* 19: 1645–1661.
- Aloni R, Peterson CA. 1997. Auxin promotes dormancy callose removal from the phloem of *Magnolia kobus* and callose accumulation and earlywood vessel differentiation in *Quercus robur*. *Journal of Plant Research* 110: 37–44.
- Ang III. 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Archetti M, Richardson AD, O'Keefe J, Delpierre N. 2013. Predicting climate change impacts on the amount and duration of autumn colors in a New England forest. *PLoS ONE* 8: e57373.
- Augsburger C, Bartlett E. 2003. Differences in leaf phenology between juvenile and adult trees in a temperate deciduous forest. *Tree Physiology* 23: 517–525.
- Bell CD, Soltis DE, Soltis PS. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.
- Bennie J, Kubin E, Wiltshire A, Huntley B, Baxter R. 2010. Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: the implications of local adaptation to climate. *Global Change Biology* 16: 1503–1514.
- Blomberg SP, Garland T Jr, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745.
- Carroll E, Sparks T, Donnelly A, Cooney T. 2009. Irish phenological observations from the early 20th century reveal a strong response to temperature. *Biology and Environment-Proceedings of the Royal Irish Academy* 109B: 115–126.
- Chen X, Xu L. 2012. Phenological responses of *Ulmus pumila* (Siberian Elm) to climate change in the temperate zone of China. *International Journal of Biometeorology* 56: 695–706.
- Chmielewski FM, Rötzer T. 2001. Response of tree phenology to climate change across Europe. *Agricultural and Forest Meteorology* 108: 101–112.
- Cong N, Wang T, Nan H, Ma Y, Wang X, Myneni RB, Piao S. 2012. Changes in satellite-derived spring vegetation green-up date and its linkage to climate in China from 1982 to 2010: a multi-method analysis. *Global Change Biology* 19: 881–891.
- Cook BI, Wolkovich EM, Parmesan C. 2012. Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences, USA* 109: 9000–9005.
- Davi H, Gillmann M, Ibanez T, Cailleret M, Bontemps A, Fady B, Lefevre F. 2011. Diversity of leaf unfolding dynamics among tree species: new insights from a study along an altitudinal gradient. *Agricultural and Forest Meteorology* 151: 1504–1513.
- Davies TJ, Wolkovich EM, Kraft NJB, Salamin N, Allen JM, Ault TR, Betancourt JL, Bolmgren K, Cleland EE, Cook BI *et al.* 2013. Phylogenetic conservatism in plant phenology. *Journal of Ecology* 101: 1520–1530.
- Davis CC, Willis CG, Primack RB, Miller-Rushing AJ. 2010. The importance of phylogeny to the study of phenological response to global climate change. *Philosophical Transactions of the Royal Society B* 365: 3201–3213.
- Delbart N, Picard G, Le Toans T, Kergoat L, Quegan S, Woodward I, Dye D, Fedotova V. 2008. Spring phenology in boreal Eurasia over a nearly century time scale. *Global Change Biology* 14: 603–614.
- Essiamah S, Eschrich W. 1986. Water uptake in deciduous trees during winter and the role of conducting tissue in spring reactivation. *International Association of Wood Anatomists Bulletin* 7: 31–38.
- FFPRI. 1989. *Microscopic identification of Japanese wood*. Forestry and Forest Product Research Institute (FFPRI). [WWW document] URL <http://f030091.ffpri.affrc.go.jp/IDB-E/home.php> [accessed 1 August 2012].
- Fridley JD. 2012. Extended leaf phenology and the autumn niche in deciduous forest invasions. *Nature* 485: 359–362.
- Gazal R, White MA, Gillies R, Rodemaker E, Sparrow E, Gordon L. 2008. GLOBE students, teachers, and scientists demonstrate variable differences between urban and rural leaf phenology. *Global Change Biology* 14: 1568–1580.
- Geiger R, Aron RH, Todhunter P. 2009. *The climate near the ground, 7th edn*. Lanham, MD, USA: Rowman & Littlefield.
- InsideWood. 2012. *Inside wood*. [WWW document] URL <http://insidewood.lib.ncsu.edu/> [accessed 1 August 2012].
- IPGE. 2013. *Phenological observation guide of the International Phenological Gardens*. International Phenological Gardens of Europe, Berlin, Germany. [WWW document] URL http://www.agrar.hu-berlin.de/fakultaet-en/departments/dntw-en/agrarmer-en/phaenologie/ipg/IPG_ObsGuide.pdf/view [accessed 1 August 2013].
- Jeong S, Ho C, Gim H, Brown ME. 2011. Phenology shifts at start vs. end of growing season in temperate vegetation over the Northern Hemisphere for the period 1982–2008. *Global Change Biology* 17: 2385–2399.
- Karl TR, Melillo JM, Peterson TC. 2009. *Global climate change impacts in the United States*. Cambridge, UK: Cambridge University Press.
- Kramer K. 1995. Phenotypic plasticity of the phenology of 7 European tree species in relation to climatic warming. *Plant, Cell & Environment* 18: 93–104.
- Laube J, Sparks TH, Estrella N, Hoefler J, Ankerst DP, Menzel A. 2014. Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology* 20: 170–182.
- Lechowicz M. 1984. Why do temperate deciduous trees leaf out at different times – adaptation and ecology of forest communities. *American Naturalist* 124: 821–842.
- Liu Z, Li K, Cai Y, Fang Y. 2011. Correlations between leafing phenology and traits: woody species of evergreen broad-leaved forests in subtropical China. *Polish Journal of Ecology* 59: 463–473.

- Lopez OR, Farris-Lopez K, Montgomery RA, Givnish TJ. 2008. Leaf phenology in relation to canopy closure in southern Appalachian trees. *American Journal of Botany* 95: 1395–1407.
- Lundmark T, Heden J, Hallgren J. 1988. Recovery from winter depression of photosynthesis in pine and spruce. *Trees* 2: 110–114.
- Menzel A. 2000. Trends in phenological phases in Europe between 1951 and 1996. *International Journal of Biometeorology* 44: 76–81.
- Menzel A, Estrella N, Fabian P. 2001. Spatial and temporal variability of the phenological seasons in Germany from 1951 to 1996. *Global Change Biology* 7: 657–666.
- Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* 397: 659.
- Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-kübler K, Bissolli P, Braslavská O, Briede A *et al.* 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* 12: 1969–1976.
- Michelot A, Simard S, Rathgeber C, Dufrene E, Damesin C. 2012. Comparing the intra-annual wood formation of three European species (*Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiology* 32: 1033–1043.
- Miller-Rushing AJ, Inouye D, Primack RB. 2008. How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *Journal of Ecology* 96: 1289–1296.
- Morin X, Lechowicz MJ, Augspurger C, O'Keefe J, Viner D, Chuine I. 2009. Leaf phenology in 22 North American tree species during the 21st century. *Global Change Biology* 15: 961–975.
- Öquist G, Huner NPA. 2003. Photosynthesis of over-wintering evergreen plants. *Annual Review of Plant Biology* 54: 329–355.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012. *Comparative analyses of phylogenetics and evolution in R*. [WWW document] URL <http://cran.r-project.org/web/packages/caper/> [accessed 15 June 2013].
- Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.
- Panchen Z, Primack R, Aniško T, Lyons R. 2012. Herbarium specimens, photographs and field observations show Philadelphia area plants are responding to climate change. *American Journal of Botany* 99: 751–756.
- Polgar C, Gallinat A, Primack RB. 2014. Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord. *New Phytologist* 202: 106–115.
- Polgar CA, Primack RB. 2011. Leaf-out phenology of temperate woody plants: from trees to ecosystems. *New Phytologist* 191: 926–941.
- Primack D, Imbres C, Primack RB, Miller-Rushing AJ, Deltredici P. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *American Journal of Botany* 91: 1260–1264.
- Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223. [WWW document] URL <http://cran.r-project.org/web/packages/phytools> [accessed 15 June 2013].
- Richardson A, Bailey A, Denny E, Martin C, O'Keefe J. 2006. Phenology of a northern hardwood forest canopy. *Global Change Biology* 12: 1174–1188.
- Richardson AD, O'Keefe J. 2009. Phenological differences between understory and overstory: a case study using the long-term Harvard forest records. In: Noormets A, ed. *Phenology of ecosystem processes*. Dordrecht, the Netherlands: Springer Science + Business Media, 87–117.
- Rollinson CR, Kaye MW. 2012. Experimental warming alters spring phenology of certain plant functional groups in an early successional forest community. *Global Change Biology* 18: 1108–1116.
- Schweingruber FH. 1990. *Anatomie europäischer Bölzer. – anatomy of European woods*. Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft, Bern, Switzerland and Stuttgart, Germany: Haupt.
- Schweingruber FH, Landolt W. 2010. *The xylem database*. Swiss federal research institute WSL. [WWW document] URL <http://www.wsl.ch/dendro/xylem/db/index.php> [accessed 1 August 2012].
- Seiwa K. 1999. Changes in leaf phenology are dependent on tree height in *Acer mono*, a deciduous broad-leaved tree. *Annals of Botany* 83: 355–361.
- Smith SA, Beaulieu J, Donoghue MJ. 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. *BMC Evolutionary Biology* 9: 37.
- Smith SA, Beaulieu JM, Donoghue MJ. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences, USA* 107: 5897–5902.
- Stevens PF. 2012. *Angiosperm phylogeny website*. [WWW document] URL <http://www.mobot.org/MOBOT/research/APweb/> [accessed 1 August 2012].
- Sun S, Jin D, Li R. 2006. Leaf emergence in relation to leaf traits in temperate woody species in East-Chinese Quercus fabri forests. *Acta Oecologica* 30: 212–222.
- Suzuki M, Yoda K, Suzuki H. 1996. Phenological comparison of the onset of vessel formation between ring-porous and diffuse-porous deciduous trees in a Japanese temperate forest. *International Association of Wood Anatomists Journal* 17: 431–444.
- USA-NPN National Coordinating Office. 2012. *USA-NPN plant and animal phenophase definitions*. USA-NPN Technical Series 2012-004. [WWW document] URL www.usanpn.org [accessed 1 August 2012].
- Villar R, Merino J. 2001. Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytologist* 151: 213–226.
- Vitasse Y. 2013. Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. *New Phytologist* 198: 149–155.
- Vitasse Y, Porte AJ, Kremer A, Michalet R, Delzon S. 2009. Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. *Oecologia* 161: 187–198.
- Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and character evolution. *Bioinformatics* 24: 2098–2100.
- Webb CO, Donoghue MJ. 2005. PHYLOMATIC: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5: 181–183.
- Wesolowski T, Rowinski P. 2006. Timing of bud burst and tree-leaf development in a multispecies temperate forest. *Forest Ecology and Management* 237: 387–393.
- Willis CG, Ruhfel B, Primack RB, Miller-Rushing AJ, Davis CC. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences, USA* 105: 17 029–17 033.
- Willis CG, Ruhfel BR, Primack RB, Miller-Rushing AJ, Losos JB, Davis CC. 2010. Favorable climate change response explains non-native species' success in Thoreau's woods. *PLoS ONE* 5: e8878.
- Wolkovich EM, Davies TJ, Schaefer H, Cleland EE, Cook BI, Travers SE, Willis CG, Davis CC. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407–1421.
- Zohner C, Renner S. 2014. Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records forecasts long-term change. *Ecology Letters*, in press. doi: 10.1111/ele.12308

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 The PHLAWD phylogeny highlighting nodes with significantly early and significantly late average adjusted leaf out dates.

Fig. S2 The PHYLOMATIC tree highlighting nodes with significantly early and significantly late average adjusted leaf out dates.

Table S1 Mean leaf out date of each site in 2012

Table S2 Woody species that leafed out first and last at each site in 2012

Table S3 Mean leaf out dates of genera at Arnold Arboretum in 2012

Table S4 Mean leaf out dates of genera at the Berlin Botanic Garden in 2012

Table S5 Mean leaf out dates of genera for the adjusted leaf out dataset in 2012

Table S6 Mean leaf out dates of families at the Arnold Arboretum in 2012

Table S7 Mean leaf out dates of families at the Berlin Botanic Garden in 2012

Table S8 Mean leaf out dates of families for the adjusted leaf out dataset in 2012

Table S9 Leaf out dates of each species at each site

Table S10 PHYLOMATIC tree

Table S11 PHLAWD phylogeny

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