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THE BIOGEOGRAPHY, EVOLUTION, AND FUNCTION OF LEAF-OUT PHENOLOGY STUDIED WITH EXPERIMENTAL, MONITORING, AND PHYLOGENETIC APPROACHES

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München, 20. August 2016

PREFACE

Statutory declaration

Erklärung

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Susanne S. Renner betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Constantin Zohner, 20. August 2016
(Unterschrift)

1. Gutachter: Prof. Dr. Susanne S. Renner
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Note

In this thesis, I present the results from my doctoral research, carried out in Munich from January 2014 to August 2016 under the guidance of Prof. Dr. Susanne S. Renner. My thesis resulted in four manuscripts, presented in Chapters 2 to 5, of which two have been published (Chapters 2 and 5), one is accepted (Chapter 3), and one is in review (Chapter 4). I also gave the conference talks listed below. I generated all data and conducted all analyses myself except for Chapter 5 for which I contributed the phenological data and conducted some analyses. Writing and discussion involved collaboration with S.S. Renner, with input from J.-C. Svenning (Chapters 3 and 4), and J.D. Fridley (Chapter 4). L. Muffler led the writing in Chapter 5, and I wrote all parts concerned with phenology.

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List of publications

Peer-reviewed journal articles

ZOHNER, C.M., BENITO, B.M., FRIDLEY, J.D., SVENNING, J.-C., RENNER, S.S. In review. Spring predictability explains different leaf-out times in the Northern Hemisphere woody floras. *Nature.*

ZOHNER, C.M., BENITO, B.M., SVENNING, J.-C., RENNER, S.S. In press. Photoperiod unlikely to constrain climate change-driven shifts in leaf-out phenology in northern woody plants. *Nature Climate Change.*

MUFFLER, L., BEIERKUHNLEIN, C., AAS, G., JENTSCH, A., SCHWEIGER, A.H., ZOHNER, C.M., KREYLING, J. 2016. Distribution ranges and spring phenology explain late frost sensitivity of 170 woody plants from the Northern hemisphere. *Global Ecology and Biogeography*. DOI: 10.1111/geb.12466, first published 29 May 2016

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Oral presentations

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ZOHNER, C.M. (Why) do plants use the photoperiod to regulate leaf-out? Technical University Munich, Botanikertagung 2015, From molecules to the field, Freising, Germany, 2 September 2015

ZOHNER, C.M. Biogeographic history of *Limonium* (Plumbaginaceae), inferred with the first model that includes a parameter for speciation with dispersal. 16. Jahrestagung der Gesellschaft für Biologische Systematik (GfBS), Bonn, Germany, 19 March 2015

ZOHNER, C.M. The evolution and function of phenological signals and species-specific changes in leaf-out times. *Seminar series at Department of Ecology, Environment and Plant Sciences*, Stockholm, Sweden, 19 August 2014

Poster

ZOHNER, C.M., AND RENNER, S.S. Does biogeographic origin influence leaf-out phenology? *International Biogeography Society: 7th Biennial Meeting*, Bayreuth, Germany, Jan 8–12, 2015.

ZOHNER, C.M., AND RENNER, S.S. Climate-change induced shifts in leaf-out times. *Radiations 2014*, Zurich, Switzerland, June 12–15, 2014.

CONTENTS

SUMMARY

This dissertation deals with the timing of leaf unfolding in temperate woody plants, especially as regards the ongoing climate change. It particularly asks (i) if and how plants use photoperiod duration to regulate leaf-out, (ii) how adaptation to climate parameters relates to inter-specific variability in leaf-out strategies, (iii) if region-specific frost probabilities explain global biogeographic patterns in leaf-out phenology, and (iv) whether a species' frost sensitivity is linked to its phenological strategy. To address these questions, I studied the phenology of 1600 woody species grown under common conditions in temperate gardens using experimental and monitoring approaches. The experiments particularly served to disentangle the three key drivers of leaf unfolding: photoperiod, chilling, and spring warming. I investigated the role of photoperiod and the extent of bud autonomy in leaf unfolding by applying *in situ* bagging experiments to three widespread European tree species (Chapter 2). I also conducted twig cutting experiments in ~200 species to study the effects of regional climate history on species' photoperiod sensitivity (Chapter 3). I used monitoring and experimental data to study how chilling and spring warming requirements are shaped by species' phylogenetic and biogeographic history. This provided an opportunity to infer region-specific phenological responses to climate change (Chapter 4). Lastly, I assessed the link between species' leaf-out phenology and frost sensitivity by relating leaf-out observational data to information on the frost sensitivity (especially damage to their young leaves) in 170 species (Chapter 5). The experiments took me in the direction of proximate mechanisms, while most my other work focused on the ultimate (evolutionary) drivers of leaf unfolding. All my work combines experimental and statistical approaches typical of ecology with the comparative and data-mining approaches typical of systematics and macroecology.

Photoperiod control of leaf unfolding in temperate woody species is poorly understood. To investigate when, where, and how photoperiod signals are perceived by plants to trigger leaf-out, I conducted *in situ* bagging experiments in *Fagus sylvatica*, *Picea abies*, and *Aesculus hippocastanum*. Twigs of nearby branches where kept under constant 8h short days or exposed to natural day-length increase. These experiments revealed that (i) the leaf primordia in each bud autonomously react to photoperiod signals, (ii) buds only react to photoperiod in late dormancy when air temperature increases, and (iii) the phytochrome system is mediating photoperiod control of leaf unfolding.

To investigate why the relative importance of photoperiod in regulating budburst differs among species, I tested for correlations between species' photoperiod-sensitivity (as inferred from twig-cutting experiments) and their climate ranges. These analyses revealed that only 30% of temperate woody species use the photoperiod as a cue for leaf-out and these all come from regions with relatively short winter periods. In regions with long winters, increase in day length occurs too early (around the spring equinox) for frosts to be safely avoided.

Species-specific leaf-out strategies evolved as a consequence of the trade-off between early carbon gain and avoidance of late frosts to prevent tissue damage. In regions where late frost events are common one can therefore expect conservative growth strategies (i.e., late leaf display) resulting from high chilling and/or high spring warming requirements. To test for this, I modeled the spring temperature variability across the Northern Hemisphere on the basis of gridded climate data over the past 100 years and correlated these data with phenological data on 1600 Northern Hemisphere species. The results showed that especially in eastern North America the late frost risk is high and, as a result, species from east North America have late leaf out strategies and high chilling requirements compared to species from regions with low late frost risk, such as East Asia. In eastern North America, species' high chilling requirements should therefore counteract climate warming-induced advances in spring leaf unfolding, whereas opportunistic species from East Asia (that have lower winter chilling requirements) should be able to continuously track spring temperature increases.

Chapter 5 of this dissertation deals with the biogeographic and phenological importance of late frost sensitivity. With colleagues from Bayreuth, I inferred the freezing-resistance of emerging leaves in 170 species, taking advantage of a natural extreme late frost event that occurred in the Bayreuth Ecological-Botanical Garden in May 2011 (-4.3°C after bud burst of all species). Frost-tolerant species flushed on average 2 weeks earlier than species sensitive to late frosts. Species' phenological strategies therefore appear to reflect the frost sensitivity of their young leaves.

Chapter 1

GENERAL INTRODUCTION

Plant phenology

One of the founders of the science of plant phenology was Carl von Linné (1751) who described methods for compiling calendars by recording species' leaf-out times, flowering times, fruiting times, and leaf fall. The first phenological observations, however, go much further back and allowed people to predict sowing and harvesting times and also whether the climate in a particular year was different from 'normal' (Pfister, 1980). Quantification of climate parameters thus occurred long before the invention of the alcohol thermometer in 1709 and the mercury thermometer in 1714. Today's driving force behind the collection of phenological data is still their utility for agriculture and other aspects of human wellbeing (e.g., forecasting fire hazard), but increasingly also a different goal: the forecasting of the ecological and economical consequences of anthropogenic climate change.

Phenology is "the study of the timing of recurring biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species" (Lieth, 1974, p. 4). Modern phenological research is no longer restricted to the observation of life cycle events, e.g. flowering and fruiting, but aims to understand the environmental drivers underlying these observations and the relationships of different phenological events (both within and between species) to each other (Richardson *et al.*, 2013). The basis for phenological research is data on multiple phenological events, over multiple years, and from a diverse set of taxonomic groups. Such data sometimes come from observations made by one person, such as Henry David Thoreau or the Marsham family (Miller-Rushing & Primack, 2008; Sparks & Menzel, 2002), but more often from networks of institutions or researchers, such as the Pan European Phenology Project (http://www.pep725.eu/) or the International Phenological Gardens network (http://ipg.hu-berlin.de). Correlative studies have shown earlier vegetation activity in spring in response to warming, with leaf-out in woody species advancing by 3–8 days for each 1°C increase in air temperature (Menzel & Fabian, 1999; Chmielewski & Rötzer, 2001; Parmesan, 2007; Zohner & Renner, 2014). How climate change may be affecting the end of the growing season is less clear, but many species (from the temperate zone) react to warmer autumn temperatures by shedding their leaves later in the season (Menzel & Fabian, 1999; Menzel, 2000; Menzel *et al.*, 2006; Vitasse *et al.*, 2009; Vitasse *et al.*, 2011).

The ecological implications from phenological transitions in response to climate change

Shifts in the leaf-out timing of temperate species may have a huge impact on ecosystem processes, such as carbon fixation, biomass accumulation, water cycling, microclimate and -animal interactions (Richardson *et al*., 2010; Richardson *et al*., 2013). Polgar and Primack (2011) provide an example of how changes in phenology may change competition for light and water resources between trees and shrubs. Once trees produce leaves, they are retaining most of the incoming rainfall. Advanced flushing under a warming climate would thus be associated with reduced through fall in spring, perhaps leading to decreased soil water and less soil evaporation. This might have adverse consequences for understory plants and could be reinforced by decreased light intensities in early spring caused by an earlier closure of the canopy. Such effects of the presence of leaves on water retention or run off are coupled with the albedo of leaf canopies, which depends on leaf area and other properties (White *et al*., 1999; Hollinger *et al*., 2010; Zha *et al*., 2010). Phenology is playing a key role in regulating such vegetation-atmosphere feedbacks (Richardson *et al*., 2013). By influencing temperature gradients, humidity, soil temperature and moisture, solar radiation, and precipitation retention and runoff, phenological transitions exert strong effects on microclimatic conditions.

The global climate, too, is influenced by vegetation activity because phenological cycles affect water, energy, and carbon fluxes (Hogg *et al*., 2000; Schwartz & Crawford, 2001; Zha *et al*., 2010; Keenan *et al*., 2014). A lengthening of the growing season under a climate-warming scenario is unlikely to be associated with a proportional increase of carbon sequestration, however, because higher temperatures cause increased respiration. In northern ecosystems, carbon loss due to increased respiration has even been shown to exceed carbon gains from an extended growing season, thereby leading to a reduction of carbon concentration in forest ecosystems (Milyukova *et al.,* 2002; Piao *et al*., 2008). Because of such antagonistic effects, prolonged growing seasons cannot readily be equated with an increase in carbon sequestration. Furthermore, the degree to which extended growing periods influence the rates of biomass accumulation is biome-specific. For instance, coniferous forests are expected to have a lower increase in biomass than deciduous forests (Richardson *et al*., 2009, 2010).

Experimental phenology

Most phenological studies are based on correlative analyses (Sparks & Carey, 1995; Menzel & Fabian, 1999; Menzel, 2000; Cook *et al.*, 2012; Mazer *et al.*, 2013; Zohner & Renner, 2014). A shortcoming of any such study is that correlations do not offer insights into ultimate or proximate mechanisms underlying responses to climate parameters, which prevents the development of mechanistic models. To better parameterize current models, experimental studies are needed to investigate the environmental cues that trigger phenological events, such as bud break, flowering, and leaf senescence, as well as the developmental-genetic pathways and physiological mechanisms. With respect to bud break, experimental insights have come from the so-called twig cutting method, in which twigs are cut from adult trees and brought into controlled conditions to detect and quantify the environmental factors that affect dormancy release and leaf unfolding (Heide, 1993a, b; Basler & Körner, 2012; Dantec *et al.,* 2014; Laube *et al.*, 2014a, b; Polgar *et al.*, 2014; Primack *et al.*, 2015). The method relies on the assumption that dormant buds in woody species react autonomously and do not depend on the twig being connected to the stem or the root system. A carefully controlled experimental study recently confirmed that cut twigs indeed show the same phenological response as their donor tree (Vitasse & Basler, 2014), validating the approach as an appropriate method for studying budburst cues. Another approach to studying the phenological behavior of trees is to use seedlings in climate chambers (e.g., Falusi & Calamassi, 1990). However, juveniles differ from adults in their reaction to environmental cues (Vitasse *et al.*, 2014), complicating extrapolation from experiments with seedlings.

Leaf-out in woody plants and the environmental factors that regulate it

As revealed by experiments of the type described above, there are three main cues used by plants to regulate budburst: winter chilling, spring warming, and photoperiod (Falusi & Calamassi, 1990; Heide, 1993a,b; Myking & Heide, 1995; Heide, 2003; Ghelardini *et al.*, 2010; Basler & Körner, 2012; Laube *et al.*, 2014a; Polgar *et al.*, 2014). The relative role of these three factors depends on the species (Heide, 1993a, b; Körner, 2006; Körner & Basler, 2010; Polgar & Primack, 2011; Basler & Körner, 2012). ‘Chilling’ refers to an exposure of plants to cold temperatures in winter. During the winter period, buds of most temperate species are in a state of rest, a period with physiological arrested or slowed development (endodormancy), preventing bud burst regardless of the environmental conditions (Hänninen *et al.*, 2007). In chilling-sensitive species, an adequate duration of winter cooling (a sum of hours or days below a certain threshold temperature) is necessary to break the inactive phase (Körner, 2006). The molecular and physiological mechanisms underlying the perception of chilling signals are poorly understood (Cooke *et al*., 2012), and the specific temperatures adequate for the fulfillment of chilling requirements are not known, although they seem to range between zero and 12 degree Celsius (Heide, 2003). Accumulated chilling affects the amount of forcing in the spring for a plant to push out its leaves (Körner, 2006). Put simply, the less chilling in winter, the more warming is needed in spring for budburst. As an example, in a climate chamber experiment with twig cuttings of five elm species (*Ulmus* spp.), Ghelardini *et al.* (2010) found that the thermal time to budburst decreases with the number of chill days (= days with mean air temperatures below 5°C) they had experienced. This effect has since been found in at least 50 tree species (Laube *et al*., 2014a; Polgar *et al*., 2014). It predicts that, in plants employing a double control of budburst (chilling and warming requirements), the different effects of climate warming on the timing of leaf unfolding will cancel each other out: warmer springs are causing earlier leaf emergence because species' temperature requirements are fulfilled earlier. Warmer winters, by contrast, will lead to delayed budburst, because the plants experience less chilling. Therefore, a continuing linear response to spring warming is not expected (Körner & Basler, 2010; Zohner & Renner, 2014; Fu *et al*., 2015).

The relative importance of chilling and warming stimuli differs among and within species (Falusi & Calamassi, 1996; Ghelardini *et al*., 2006; Olson *et al*., 2013; Zohner & Renner, 2014), complicating the forecasting of phenological shifts in species-rich communities. Forecasting is made even more complex by additional factors that affect the timing of leaf unfolding, such as the time of dormancy induction in autumn, air humidity, and day length increase in spring (Heide, 2003; Körner, 2006; Laube *et al*., 2014b). That autumn senescence (dormancy induction) can affect leaf unfolding in the following spring was observed by Fu *et al.* (2014a), who found in *Fagus sylvatica* and *Quercus robur* that individuals that senesced earlier also leafed out earlier in the following year. The underlying mechanism of such carry-over effects is not understood, but might relate to earlier senescence allowing earlier perception of chilling signals and therefore earlier release from endodormancy (Fu *et al*., 2014a). The importance of water availability in influencing the timing of leaf unfolding in temperate woody species has rarely been analyzed (but see Fu *et al*., 2014b; Laube *et al*., 2014b; Shen *et al*., 2015). Using twigs exposed to different levels of air humidity, Laube *et al.* (2014b) showed that in some species, humidity has an effect on leaf-out, with plants exposed to drier conditions showing delayed budburst. Because relative air humidity is highly correlated with air temperature, Laube and colleagues further suggest that, instead of sensing temperature, plants might perceive winter chilling and spring warming by tracking air humidity.

Different from possible effects of autumn carry-over and air humidity, the role of day length increase for dormancy release in spring has received much attention (Heide 1993a,b; Körner & Basler, 2010; Vitasse & Basler, 2013; Laube *et al*., 2014a). While autumn-senescence of broad-leafed trees is largely determined by photoperiod signals, the role of photoperiod perception for bud break is less clear, perhaps in part reflecting experimental difficulties in adequately modifying day length when working with trees (Vitasse & Basler 2013, 2014). In a thought-provoking (and much cited) commentary, Körner and Basler (2010; also Körner, 2006 and Basler & Körner, 2012) hypothesized three main leaf-out strategies: In long-lived species, like *Fagus sylvatica* and *Celtis occidentalis*, photoperiod was thought to control both the induction and the release from dormancy, with temperature playing only a modulating role once the critical day-length has passed. The argument was that, "Because temperature is often an unreliable marker of seasonality, most long-lived plant species native to areas outside the tropics have evolved a second line of safeguarding against 'misleading' temperature conditions: photoperiodism. The significance of photoperiodism increases with latitude, not only because the annual variation of the photoperiod becomes more pronounced, but also because of its biological function. […] photoperiodism prevents phenology from following temperature as a risky environmental signal for development. […] It is an insurance against temperature-induced break of dormancy too early in the season. Thus photoperiodism constrains development to 'safe periods'." (Körner, 2006, p. 62). Shorter-lived species, like *Betula pendula* and *Corylus avellana*, were thought to be independent of day-length influences, which would allow them to respond more quickly to episodes of warm temperature in early spring, but also create more susceptibility to late frosts. Lastly, leafing out in ornamental plants from warmer climates, such as domestic cherries (*Prunus* spp.), was thought to depend almost exclusively on spring temperature, with no chilling and photoperiod requirements. Experiments in ~40 woody species from the Northern Hemisphere so far have not supported these ideas. Instead, most species studied so far show little or no response to photoperiod treatments (Laube *et al*., 2014a; Polgar *et al*., 2014). The one exception is *Fagus sylvatica*, which under short days (8-h) takes twice as long to leaf out than under long days (16-h) (Heide, 1993a). Under climate warming, the flushing times of temperature-cued species are expected to change more than those of photoperiod-sensitive species. Fu *et al.*'s (2015) Figure 1 provides an example of this effect by showing that photosensitive *F. sylvatica* is responding significantly less to temperature changes than six other European tree species with low photoperiod requirements: per 1°C increase in spring air temperature, leaf-out in *Fagus sylvatica* advanced by only 2.8 days, whereas it advanced by an average of 3.5 days in the other species.

Causes of intra- and interspecific variation in leaf-out phenology

The following paragraph will introduce the potential ultimate (evolutionary) causes of phenological differences in leaf unfolding between and within species. The timing of leaf emergence in temperate woody species can vary up to four months between species grown at the same site (Zohner & Renner 2014). Trade-offs between greater productivity and a higher frost risk may play an important role in this variation since temperate plants have to adapt to opposite selective forces: protection against the cold season and effective use of the growing season. Early-leafing species are able to replenish nutrient supplies and to start growth before late flushers do. An early-leafing strategy, however, also creates a greater susceptibility to late spring frosts.

How different species solve such trade-offs may have to do with latitudinal climate differences (Lechowicz, 1984), and studying this question is important for understanding and predicting ongoing and future changes in the phenology of forest communities, the composition of which depends on latitude. However, we are far from understanding either species' empirical behavior or the underlying climatic forces (e.g., duration of winter, spring warming, or late spring frosts) that may select for particular strategies. This is seen in contradicting results in common garden studies: In *Acer saccharum* and *Populus balsamifera*, plants originating from southern populations leaf out later than individuals from more northern populations when grown together (Kriebel, 1957; Olson *et al*., 2013). By contrast, in *Juglans nigra* and *Ulmus minor* populations of more southern origin started growth earlier in the year compared to more northern plants (Bey, 1979; Ghelardini et al., 2006).

Many insights into species-specific phenologies have come from observations conducted in botanical gardens (Panchen *et al.*, 2014; Zohner & Renner, 2014). Botanical gardens permit both observations and experiments, and constitute a common garden setup, at least to the extent that observations can also be obtained or inferred for the same plants' behavior in the wild ('non-common') situation. Botanical gardens therefore provide the opportunity to study species-specific phenological behavior and shifts in response to climate change in a representative sample of the world's temperate species (Primack & Miller-Rushing, 2009; Panchen *et al.*, 2014; Zohner & Renner, 2014). Using biannual leaf-out observations on ~500 temperate woody species grown together in the Munich Botanical Garden, I showed in my M.Sc. thesis that (under identical conditions) species from regions with cold climates leaf-out earlier than species from Southern climates because they are adapted to lower energy/temperature resources (Zohner & Renner, 2014). This led me to the prediction that advances in the timing of leaf unfolding will be counteracted by the floristic change expected under climate warming, because a northward expansion of southern species will increase the number of late flushers in the North. My study further revealed that adaptation to local climates explains a significant portion of the variation in phenological strategies between species from different geographic regions. As noticed by Lechowicz (1984), however, also within regions there is marked interspecific variability in the timing of leaf-out, and even within a forest, leaf unfolding can vary by several weeks among coexisting native trees. Lechowicz suggested that the high degree of species-specificity in the timing of leaf unfolding might be explained by phylogenetic/historical and adaptive patterns. However, it took 30 years for Lechowicz's hypothesis to be tested (Panchen *et al.*, 2014; Zohner & Renner, 2014).

Phylogeny may influence leaf unfolding because development and architecture have large genetic components and are inherited from ancestors. Phylogenetically-informed analyses of leaf-out times in woody plants from the Northern Hemisphere have found evidence of such phylogenetic inertia (Panchen *et al.*, 2014). Thus Panchen *et al.* (2014) found that Ericaceae, Fabaceae, Fagaceae, and Pinaceae tend to flush late, while Dipsacaceae and Rosaceae mostly flush early. Similarly, species from lineages with a more southern background may retain (sub)-tropical habitat requirements, such as a relatively low frost tolerance, and should therefore leaf-out late, compared to species with a mainly temperate distribution (Lechowicz, 1984). I provided support for this in my M.Sc. thesis, in which I showed that late leafing species cluster in genera that evolved primarily under subtropical conditions, such as *Carya*, *Diospyrus*, *Fagus*, *Juglans*, *Liquidambar*, *Liriodendron*, *Nyssa*, *Platanus*, and *Tilia* (Graham, 1972; Tiffney & Manchester, 2001), underscoring the phylogenetic component of leaf-out phenology.

The timing of leaf unfolding contributes in an essential way to the survival of temperate plants, yet only a few adaptive explanations for leaf unfolding strategies have been postulated or inferred (Lechowicz, 1984; Panchen *et al*., 2014). Firstly, unfolding strategies depends on growth habit, with shrubs leafing out significantly earlier than trees when grown under common conditions (Panchen *et al.*, 2014). A possible explanation for this pattern is competition for sunlight: shrubs might profit from an early flushing strategy in spring to maximize photosynthetic activity, because the light availability in the undergrowth of forests is highly reduced once canopy trees emerge their leaves. Secondly, the timing of leaf unfolding correlates with wood anatomical traits. As hypothesized by Lechowicz (1984), species with large vessels generally leaf-out late in spring (Panchen *et al*., 2014), most likely because large diameter vessels are more prone to embolisms caused by freeze-thaw events early in spring (Michelot *et al.*, 2012). Lastly, the timing of leaf unfolding correlates with leaf longevity, with deciduous species usually preceding evergreen species that can make use of past years leaves for photosynthesis early in spring (Davi *et al*., 2011; Panchen *et al*., 2014).

Research questions

To explore species-specific differences in leaf-out strategies in a biogeographic context, I made use of the broad taxonomic range of temperate woody species cultivated in the Munich Botanical Garden. My sample included species from all over the Northern Hemisphere, with North American, European, and East Asian species in roughly equal proportion. The common garden setup and the permission to carry out twig cutting experiments allowed me to study phenological adaptations to climate conditions: the leaf-out times of the studied species should still reflect their native thresholds for chilling, forcing, and photoperiod because woody plants in the Munich garden have had no opportunity for natural propagation, precluding evolutionary adaptation. I observed the leaf-out times of 498 species over five years (2012–2015) and used these observations, together with observations on another 1400 species carried out in five Northern Hemisphere gardens (see Panchen *et al*., 2014), to test if the leaf-out times of photosensitive species show less inter-annual variation than those of species flushing independent of photoperiod (Chapter 3) and to explore species-specific adaptations to climate factors such as spring temperature variability (Chapter 4). Because plants have to time their leaf unfolding to maximize carbon gain while at the same time minimizing frost damage (above, section '*Causes of intra- and interspecific variation in leaf-out phenology*'), I asked whether species from regions in which spring temperatures are highly unpredictable (implying a high late frost probability) show more conservative growth strategies than species from regions with predictable spring climates. The leaf-out data additionally allowed me to directly study the relationship between leaf-out strategy and frost sensitivity of leaves, the latter of which was inferred for 170 species from an extreme late frost event that occurred in the Bayreuth Botanical Garden in 2011 (Chapter 5).

To disentangle the relative effects of photoperiod, chilling, and spring warming on the timing of leaf unfolding, I conducted twig-cutting experiments on 144 of the 498 monitored species. Placing the results in a biogeographic context also allowed me to test for regional (climatic) differences in species' relative use of photoperiod (Chapter 3) and chilling (Chapter 4) as leaf-out triggers. I specifically asked whether photoperiod and chilling requirements are influenced by species' latitudinal occurrence, degree of continentality of the climate they are adapted to, or spring temperature variability in their native range. I also wanted to know which organs or tissues perceived photoperiod signals and at which period during dormancy plants perceive light signals. I therefore conducted *in situ* bagging experiments on three species (*Aesculus hippocastanum*, *Fagus sylvatica*, and *Picea abies*) for which previous studies have shown a high degree of photosensitivity (Chapter 2). These last experiments took me in the direction of proximate mechanisms, while most my other work focused on ultimate mechanisms.

Chapter 2

PERCEPTION OF PHOTOPERIOD IN INDIVIDUAL BUDS OF MATURE TREES REGULATES LEAF-OUT.

Zohner, C.M. and S.S. Renner

New Phytologist 208: 1023–1030 (2014)

Perception of photoperiod in individual buds of mature trees regulates leaf-out

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Summary

- Experimental data on the perception of day length and temperature in dormant temperate zone trees are surprisingly scarce.
- In order to investigate when and where these environmental signals are perceived, we carried out bagging experiments in which buds on branches of *Fagus sylvatica*, *Aesculus hippocastanum* and *Picea abies* trees were exposed to natural light increase or kept at constant 8-h days from December until June. Parallel experiments used twigs cut from the same trees, harvesting treated and control twigs seven times and then exposing them to 8- or 16-h days in a glasshouse.
- Under 8-h days, budburst in *Fagus* outdoors was delayed by 41 d and in *Aesculus* by 4 d; in *Picea*, day length had no effect. Buds on nearby branches reacted autonomously, and leaf primordia only reacted to light cues in late dormancy after accumulating warm days. Experiments applying different wavelength spectra and high-resolution spectrometry to buds indicate a phytochrome-mediated photoperiod control.
- By demonstrating local photoperiodic control of buds, revealing the time when these signals are perceived, and showing the interplay between photoperiod and chilling, this study contributes to improved modelling of the impact of climate warming on photosensitive species.

Introduction

In temperate zone trees and shrubs, winter dormancy release and budburst are mediated by temperature and photoperiod (Heide, 1993a,b; Körner & Basler, 2010; Polgar & Primack, 2011; Basler & Körner, 2012; Laube *et al.*, 2014). Although leaf senescence in autumn is usually regulated by photoperiod (Cooke *et al.*, 2012), the role of photoperiod in the regulation of bud burst varies among species (Basler & Körner, 2012; Laube *et al.*, 2014; Zohner & Renner, 2014). Of the 44 temperate zone tree species investigated, spring leaf-out is influenced by photoperiod in 18, whereas in the remaining species, winter and spring temperatures alone regulate bud burst (Heide, 1993b; Basler & Körner, 2012; Laube *et al.*, 2014). The species-specific importance of photoperiod as a leaf-out cue probably arises from the trade-off between frost prevention and selection for early photosynthesis: photoperiod tracking protects species against leafing out during brief warming periods and thus reduces the risk of frost damage. By contrast, a day length-independent leaf-out strategy allows species to use early warm days, but exposes them to damage from late frosts (Körner & Basler, 2010; Zohner & Renner, 2014).

Experimental studies focusing on the impact of day length on dormancy release in trees have used seedlings cultivated indoors (Falusi & Calamassi, 1990; Caffarra & Donnelly, 2011) or buds on cut twigs brought indoors at different times during winter/spring (Heide, 1993a,b; Ghelardini *et al.*, 2010; Basler & Körner, 2012; Laube *et al.*, 2014). A problem with these experiments is that twigs cut later experience longer chilling and longer, continuously increasing photoperiods than those cut earlier (Table 1). The change in day length between 14 December and 14 March in the temperate zone is considerable; for example, in Munich it is 3.5 h, and buds on twigs cut on these two dates and moved to an 8-h light regime indoors therefore experience vastly different jumps in photoperiod. The failure to control for this, and also for possible effects of gradual vs sudden day length increase, may have led to an under-appreciation of the effects of photoperiod on the timing of budburst (Laube *et al.*, 2014; Polgar *et al.*, 2014).

Here we experimentally study the effects of day length and chilling on leaf-out in three large, temperate tree species – *Fagus sylvatica*, *Aesculus hippocastanum* and *Picea abies*. For *Fagus sylvatica*, studies based on cut twigs or seedlings all report a day length-dependent leaf-out strategy (Heide, 1993a; Basler & Körner, 2012; Caffarra & Donnelly, 2011; Vitasse & Basler, 2013; Laube *et al.*, 2014). Evidence for the other two species is equivocal. Although Basler & Körner (2012) find a day length-dependent flushing strategy in *Picea* and no photoperiod requirements in *Aesculus*, Laube *et al.* (2014) conclude the opposite, with *Aesculus* in their study being the species with the highest photoperiod threshold of 36 species analysed.

Table 1 Experimental set-up of the experiments on leaf-out in cut twigs of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*

Start of experiment (Collection date)	21 Dec	29 Jan	11 Feb	24 Feb	10 March	21 March	4 April
Day length outside at start of experiment (h)	8	9.4	10	10.6	11.6	12	13
Chilling status: Chill days (<5°)	38	64	75	83	92	95	101
Day-degrees (> 5°C) at start of experiment	0	24	29	43	64	119	195

Different collection dates of twigs equate with different degrees of chilling. Chill days were calculated as days with mean temperature below 5°C since November 1 (following Murray *et al.*, 1989; Laube *et al.*, 2014). Photoperiod treatments for cut twigs were 8 or 16 h of light per day.

Knowledge about the underlying molecular mechanisms of photoperiodic dormancy regulation in trees is fragmentary. Phytochromes and the clock system (*LHY* and *TOC* genes) interact with the CO/FT signalling network to regulate flowering, and this pathway likely is also involved in regulating dormancy release (Cooke *et al.*, 2012). Photoreceptors and clock genes are found in all (living) plant cells, and their action can differ between organs (James *et al.*, 2008; *Arabidopsis*; Cooke *et al.*, 2012: review). In tobacco, Thain *et al.* (2000) showed that parts of single leaves can independently reset their clock systems in reaction to different light cues and that circadian rhythms in one leaf are independent of entrainment in other leaves. Cooke *et al.* (2012) therefore suggest that buds also might independently entrain to light (and/or temperature) cues. Such a mechanism would enable each bud to react autonomously to environmental cues. To our knowledge, this hypothesis has never been tested in trees.

In order to address the twin questions of the extent of bud autonomy and of the interaction between chilling and photoperiod, we conducted experiments in mature individuals of the three species mentioned above. These are the first reported *in situ* experiments on how photoperiod affects bud burst and leaf-out in adult trees. We kept some buds under constant day length, while letting others (on the same tree) experience the natural increase in day length during spring. Still using the same trees, we cut treated and untreated twigs seven times during the winter and spring and exposed them to 8- or 16-h light regimes indoors to test at which time the photoperiod signal becomes relevant as a leaf-out trigger, and to what extent photoperiod interacts with chilling status and warming temperatures. Combining the *in situ* experiment with the twig-cutting approach also allowed us to address effects of sudden vs gradual day length changes given different chilling status.

Materials and Methods

Experiments on buds on outdoor trees and buds on cut twigs brought indoors

The study took place in the botanical garden of Munich between 21 December 2013 and 1 June 2014. Cutting and bagging experiments were conducted on *Aesculus hippocastanum* L., *Fagus sylvatica* L. and *Picea abies* L. (H.Karst.) trees growing permanently outdoors. Leaf-out of individual buds was defined as the date when the bud scales had broken and the leaf had pushed out all the way to the petiole. For the bagging experiments, which ran from 1 January 2014 until the day of leaf-out in the respective species, we covered 10 branches per species with 1 m-long light-tight bags placed around the twigs every day at 17:00 h and removed the next morning at 09:00 h (Supporting Information Fig. S1). This ensured an 8-h photoperiod. Simultaneously, translucent bags of the same size and plastic thickness were placed on another 10 twigs on the same tree individuals. Climate data were obtained from Hobo data loggers (Onset Computer Corp., Bourne, MA, USA), placed inside each type of bag for each treatment, on openly exposed control twigs, and in the glasshouse (below). The percentage of leaf-out under both types of bags as well as on naturally exposed twigs was monitored every 3 d (100% leaf-out was achieved when all buds on the observed 10 branches per treatment had leafed out; Fig. 1).

For the cutting experiments, we sampled 30 replicate twigs per species on seven dates during winter/spring 2013/14 (cutting dates: 21 December, 29 January, 11 February, 24 February, 10 March, 21 March and 4 April; see Table 1). After cutting, twigs were disinfected with sodium hypochlorite solution (200 ppm active chlorine), re-cut a second time to *c.* 40 cm, and then placed in 0.5-l glass bottles filled with 0.4 l cool tap water enriched with the broad-spectrum antibiotic gentamicin sulfate ($40\ \mu g\ l^{-1}$; Sigma-Aldrich; Basler & Körner, 2012; Larcher *et al.*, 2010). Twigs were subsequently kept under short day (8 h) or long day (16 h) conditions. Temperatures in the glasshouse ranged from 18°C during the day to 14°C at night. Water was changed twice a week, and twigs were trimmed weekly by *c.* 2 cm. Additionally, on 11 February and 21 March, 16 twigs per species from each of the bagging treatments (translucent and light-tight bag) were cut

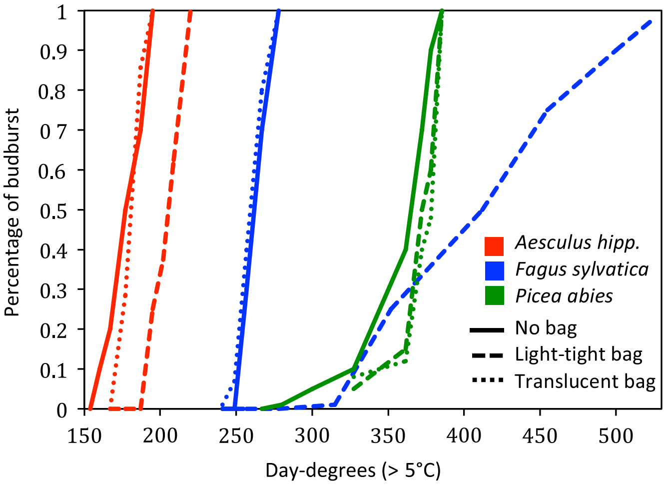

Fig. 1 Percentage of budburst per day-degree under 8-h day length (light-tight bag) and naturally increasing day length (translucent bag and without bag) for *Aesculus hippocastanum* (red), *Fagus sylvatica* (blue) and *Picea abies* (green).

and transferred to a glasshouse chamber, where they were exposed to experimental photoperiods as described above (see Fig. 2a for a scheme of treatment conditions). For all three species and all treatments, bud development was monitored every second day, and the leaf-out dates of the first 10 twigs (without bagging) or six (with bagging) that leafed out were recorded. A twig was scored as having leafed out when three buds had their leaves pushed out all the way to the petiole.

We conducted repeated-measures ANOVA to test for effects of naturally increasing day length vs constant short-day treatment

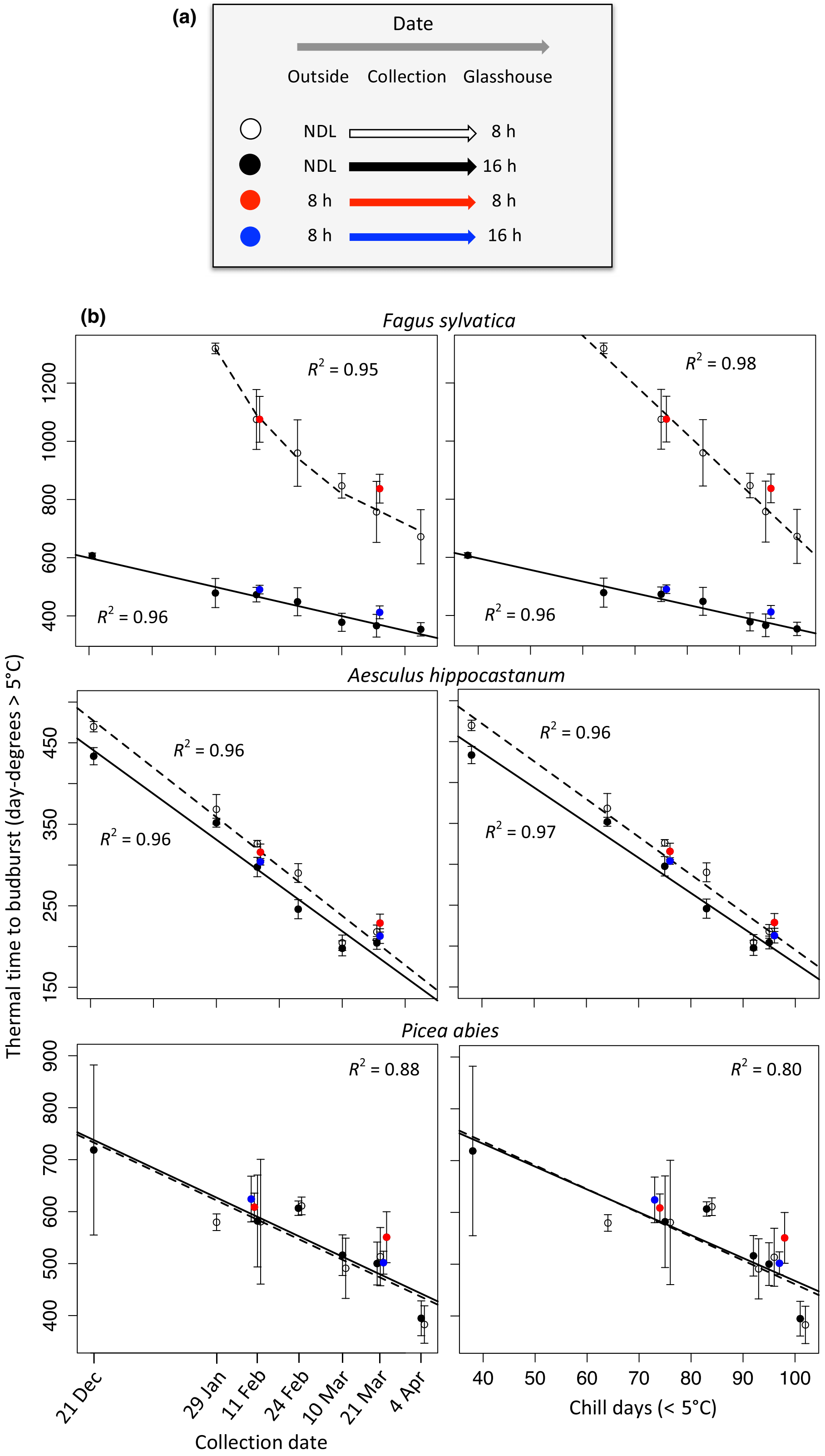

Fig. 2 (a) Explanation of treatment conditions for twig cuttings. Colour coding refers to (b). Outside, natural increase in day length (NDL) until collection date vs constant 8-h day length via bag treatment (8 h); Collection, twig collection (transfer from field to glasshouse at seven different times; see Table 1); Glasshouse, fixed day length (8 or 16 h) in glasshouse chambers. (b) Correlation between collection date (left panels), chilling (right panels) and thermal time to budburst (day-degrees > 5°C) under 8 and 16 h day length for twig cuttings of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*. For explanation of treatment conditions see (a). For statistical analysis see Table 2. Points and error bars represent the mean ± SE of thermal time to budburst. Twigs of *Fagus* and *Picea* were collected seven times during winter/spring 2013/2014; those of *Aesculus* were collected only six times because leaf-out of *Aesculus* in the field had preceded the last cutting date on 4 April. Thermal time to budburst did not increase when twigs were kept under a constant day length of 8 h in the field (light-tight bags) before collection (repeated measures ANOVA: $P > 0.1$; see coloured points).

before twigs were cut and brought indoors. ANCOVA was used to test for interactions between chilling and photoperiod treatments. Accumulated day-degrees (>5°C) until leaf-out (= sum of day-degrees accumulated outside after 1 January and in the climate chamber) were used as response variable. All statistical analyses relied on R (R Core Team, 2014).

Light perception and transmission through buds

In order to test for the light spectrum that plants use to regulate budburst, we exposed twigs of the photosensitive species *A. hippocastanum* and *F. sylvatica* to: the entire light spectrum; red light (>575 nm); and far-red light (>700 nm) (Fig. 3). Fifteen twigs were collected per species and treatment, using the same cutting procedure as above and the leaf-out dates of the first 10 twigs were recorded. The cutting date was 5 March 2015, and twigs were exposed to 16 h of light per day. Additional twigs were kept under 8- or 12-h day length (and exposed to the entire light spectrum) to test their photoperiod sensitivity. A Tukey-Kramer test was conducted to test for differences in thermal time to budburst among the treatments.

Bud scales consist of thick cuticle-like material and hardly allow for transmission of light that might be sensed by subjacent leaf tissue. To test for the quantity and quality of light they transmit, we carried out transmission analyses, using the HR4000 high-Resolution Spectrometer (Ocean Optics, Dunedin, FL, USA), which is responsive from 200 to 1100 nm. We therefore bisected the buds and removed leaf primordial tissue inside the buds of *A. hippocastanum*, *F. sylvatica* and *P. abies* and measured light transmission through all remaining bud scales (Fig. 4), and through a single bud scale (Fig. S3). For each species, we calculated the mean of the transmission spectra of 10 buds. We also measured the light transmission of the bags used in our *in situ* experiment to ensure that translucent bags transmitted across the entire spectrum while light-tight bags efficiently filtered out light across the spectrum.

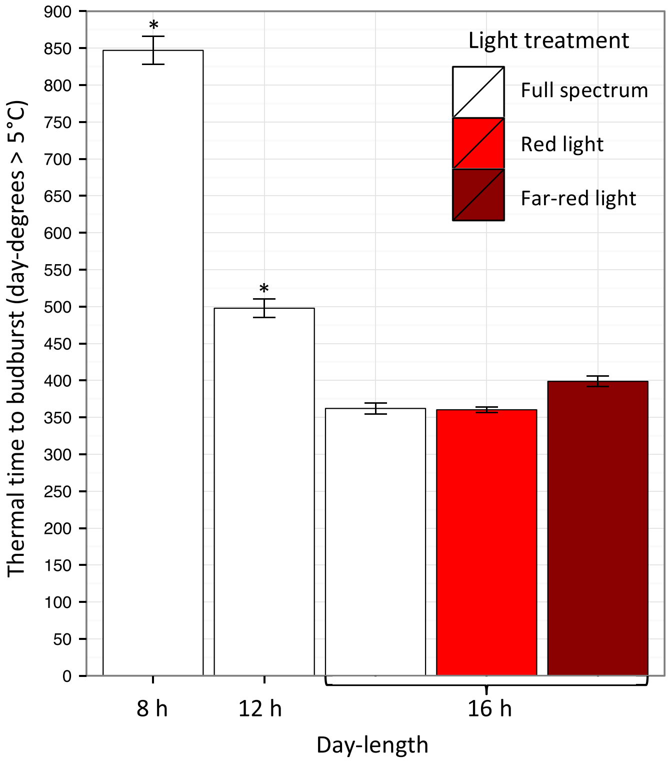

Fig. 3 Thermal time (day-degrees >5°C) to budburst under different light spectra for *Fagus sylvatica*. Twigs were collected on 5 March 2015 and exposed to 8-, 12- and 16-h day length under: the entire light spectrum; red light (>575 nm); or far-red light (>700 nm). Buds exposed to red or far-red light reacted no differently from those exposed to the entire light spectrum. Treatments differed significantly from the 16 h, full light spectrum treatment: *, $P<0.05$. Error bars represent the mean ± SE of thermal time to budburst.

Results

Effects of photoperiod on buds outdoors and on cut twigs brought indoors

Buds of *F. sylvatica* kept under constant 8-h day length (achieved by bagging twigs of outdoor trees every evening and unbagging them every morning) achieved 100% budburst 41 days later than those that experienced the natural day length increase (Figs 4, 5, S2). The same conditions delayed budburst in *A. hippocastanum* by four days and had no effect on budburst in *P. abies*. Twigs that had experienced constant 8-h days or naturally increasing day length were harvested at seven different times (Table 1) and brought into the glasshouse where they received the experimental treatments summarized in Fig. 2(a). Later cutting dates equate with plants having reached a higher chilling status and having accumulated more day-degrees (Table 1).

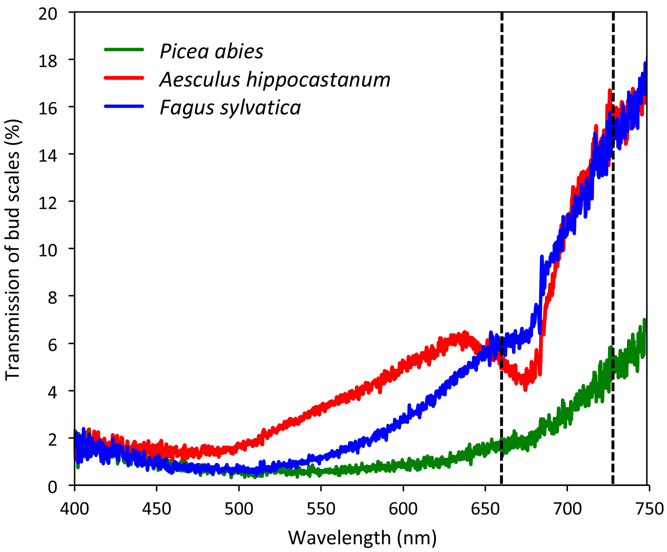

Fig. 4 Transmission spectra of buds of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*. Buds were bisected and leaf primordial tissue was removed before measurements, thus the graph reflects the quality and quantity of light that could be sensed by photoreceptors located in leaf primordia. Dashed lines indicate the absorption maxima for Phytochrome a (P_r and P_{fr}).

Fig. 5 Development of *Fagus sylvatica* buds kept under translucent (upper twig) or light-tight bags (lower twig) on 25 April 2014.

Leaf-out date in all the species was unaffected by whether twigs experienced a *gradual* (natural) day length increase (up to 12 h days) or *constant* 8-h short days (bag treatment) before being brought indoors (repeated measures ANOVA: $P > 0.1$ and Fig. 2(b), compare the blue and red dots to the black and white dots, respectively): Buds on twigs cut in February or late March, when they had already experienced quite long days outdoors, and brought into 8- or 16-h glasshouse conditions underwent budburst at the same time as buds on twigs kept under a constant 8-h day until then (see Fig. 2b).

In *F. sylvatica*, the day-degrees until leaf-out accumulated by buds kept under 8-h day length were correlated exponentially with collection date (Fig. 2b, left top panel: curve fitting white and red dots), whereas the association between day-degrees and accumulated chill days was linear (Fig. 2b, right top panel). For buds on twigs kept under 16-h day length, collection date and chill days were linearly and negatively correlated with accumulated day-degrees (Fig. 2b, top panels: curves fitting black and blue dots). The effect of day length treatment on forcing requirements was highly significant, and there was also a highly significant interaction between chilling status and day length treatment, with higher chilling reducing day length requirements and longer days reducing chilling requirements (see Table 2; Fig. 2b).

In *Aesculus*, day length barely affected forcing requirements (Table 2, $P = 0.09$), and chilling status did not affect photoperiod requirements. Collection date and chilling status were linearly correlated with required day-degrees until leaf-out (Table 2; Fig. 2b, middle panels). In *Picea,* day length had no significant effect on forcing requirements (Table 2), and collection date and chilling status were linearly correlated with day-degrees until leaf-out (Fig. 2b, lower panels).

Light perception and transmission analyses of buds

In *A. hippocastanum* and *F. sylvatica*, leaf-out date under 16-h days did not differ regardless of whether buds were exposed to the full light spectrum, only red light or only far-red light ($P > 0.15$; see Fig. 3 for *F. sylvatica*), even though in both species, under far-red conditions, leaves appeared pale due to lack of chlorophyll. Day lengths of 8 or 12 h delayed budburst in *Fagus* by 42 or 15 d and in *Aesculus* by 3 or 1 d.

Table 2 Results of ANCOVA to test for the effect of day length on species' forcing requirements, while controlling for the effect of chilling status

Explanatory factor	*Fagus* ($n = 13$)	*Aesculus* ($n = 12$)	*Picea* ($n = 12$)
Chilling	$F(1,12) = 91.3$ **$P < 0.001$**	$F(1,11) = 320.8$ **$P < 0.001$**	$F(1,11) = 25.7$ **$P < 0.001$**
Photoperiod	$F(1,12) = 1440.2$ **$P < 0.001$**	$F(1,11) = 3.8$ **$P = 0.09$**	$F(1,11) = 0.05$ $P = 0.83$
Interaction Chilling × Photoperiod	$F(1,12) = 140.6$ **$P < 0.001$**	$F(1,11) = 0.9$ $P = 0.39$	$F(1,11) = 0.03$ $P < 0.87$

n, refers to the number of treatments (Chilling (number of collection dates) × Photoperiod (8 or 16 h)); see also Fig. 2(b). *P* values < 0.1 are shown in bold.

Transmission spectra of the entire bud scale tissue were similar among species, but the relative amplitudes of transmission bands differed (Fig. 4). In the range between 600 and 800 nm, bud scales of *Aesculus* and *Fagus* transmitted two to three times more light than those of *Picea*. In all three species, light transmission increased with longer wavelengths. Between 400 and 500 nm, transmission was $< 2\%$, whereas above 500 nm it steeply increased, reaching 100% at 900 nm. In *Aesculus*, the transmission spectrum shows a local minimum *c*. 670 nm, likely due to chlorophylls located in the inner surface of bud scales in this species, whereas *Fagus* and *Picea* bud scales are dead and do not contain any chlorophyll. For transmission spectrum analysis of single bud scales, see Fig. S3.

Discussion

Photoperiod signal perceived at the local bud level

Animals have central circadian pacemakers in the brain that entrain peripheral clocks (Liu & Reppert, 2000). This leads to a close coupling between the circadian clocks of individual cells and increases the precision of timing *in vivo* (Thain *et al.*, 2000). Sessile organisms, such as most plants, by contrast have largely autonomous or weakly coupled circadian clocks that allow for independence among a plant's modules in the entrained phases of circadian rhythms. Using *in vivo* reporter gene imaging in tobacco, Thain *et al.* (2000) found that the clock systems even of sections within leaves are functionally independent. Our experiments on the effect of photoperiod on bud break on nearby twigs of single individuals of *F. sylvatica*, *A. hippocastanum* and *P. abies* provide evidence for the extent of local control (Fig. 5). The light signal likely is perceived by receptors just below the bud scales, and the genetic system involved in leaf-out regulation must therefore be located in the young leaf primordial cells. This allows each bud to react autonomously to cues by maintaining an independent circadian clock system during winter and to respond to day length increase in spring (Thain *et al.*, 2000; tobacco; James *et al.*, 2008; *Arabidopsis*; our Fig. 5 for *F. sylvatica*).

Compared to light perception, even less is known about the mechanisms of temperature sensing during bud dormancy release, although experiments on one-node cuttings prove that bud autonomy also exists for forcing and chilling requirements (Vitasse & Basler, 2014), and there is evidence that circadian clocks are involved (Rensing & Ruoff, 2002; Cooke *et al.*, 2012). Findings in *Populus* of an upregulation of the clock gene *LHY* under cold conditions and of low *LHY* expression causing delayed budburst (Ibáñez *et al.*, 2010) point to a connection between the circadian clock and chilling fulfilment. This would permit extremely fine-scale leaf-out regulation and acclimation to the microclimate differences commonly experienced by large, perennial individuals (Augspurger, 2004; Vitasse & Basler, 2013).

Interplay between chilling and photoperiod

The three tree species studied here behaved differently in terms of the extent to which chilling status and warming temperatures (degree day) interacted with day length. In *A. hippocastanum*, delayed budburst under short days probably is merely a consequence of slower growth as a result of lower light availability. By contrast, in *F. sylvatica,* day length had a huge effect on forcing requirements, and leaf-out was not possible under short days and low chilling (Fig. 2b, top panel). The correlation between cutting time and thermal time to budburst has a different slope for 8- and 16-h day length treatments (Fig. 2b). This demonstrates that the extent of chilling fulfilment influences photoperiod requirements and *vice versa*, with chilling partially substituting for unmet photoperiod requirements (see also Laube *et al.*, 2014) and increasing day length substituting for a lack of chilling. That exposure of buds to natural day length (12 h day length on 21 March) or 8-h days (bag treatment) before 21 March did not affect the leaf-out dates on twigs brought to the glasshouse (see Fig. 2b) indicates that photoperiod signals do not cause irreversible molecular responses in buds and that day length influences only the late phase of dormancy, when substantial forcing has accumulated. Long days occurring during cold periods with little accumulation of warm days therefore have no effect on subsequent forcing requirements. This can be seen in Fig. 2(b), where there is no difference in thermal requirements between buds that had experienced a gradual day length increase (up to 12 h light per day) and buds that were kept under constant 8-h day length until 21 March (compare the red or blue to the white or black points, respectively).

Our experiments also reveal that for *F. sylvatica* there is a linear, negative relationship between accumulated chill days and forcing requirements (day-degrees required), whereas collection date under short days (8 h) was nonlinearly correlated with day-degrees (Fig. 2b, top panel), probably because late in spring the number of predictably cold days varies greatly. This implies that using chill-days in leaf-out models will more accurately forecast leaf-out behaviour than will day-of-year models, although the exact temperature threshold and the precise physiological and molecular mechanisms that lead to chilling fulfilment are not yet understood (Cooke *et al.*, 2012).

For *F. sylvatica*, Vitasse & Basler (2013) put forward two hypotheses for how photoperiod may modulate the relationship between chilling status and thermal time (day-degrees) to budburst: Either, a fixed photoperiod threshold has to be reached to allow for perception of thermal time or else forcing requirements continuously decrease with increasing photoperiod. Our experiments suggest that both hypotheses are partially correct. On the one hand, insufficiently chilled buds require that a certain photoperiod threshold be exceeded before bud development (buds on twigs did not leaf-out under low chilling and 8-h day length; see Fig. 2b). Buds that had passed their chilling threshold, on the other hand, leafed out under short days, but even in these buds, longer days significantly reduced the thermal time required for budburst.

In short, *Fagus* obligatorily requires a minimal day length to allow for budburst when chilling requirements are not met, and long days partially substitute for unmet chilling requirements. *Aesculus* shows a constant delay in leaf-out under short days, does not obligatorily require a certain day length, and shows no modulating effect of day length on chilling requirements or *vice versa*. In *Picea* we found no effect of photoperiod on budburst. Our results for *Aesculus* and *Picea* are in agreement with those of Laube *et al.* (2014), but contradict Basler & Körner (2012) who found day length-dependent flushing in *Picea* and day length-independent budburst in *Aesculus*. Laube *et al.* (2014) and the present study used only individuals at low elevation, whereas Basler & Körner (2012) analysed trees along an elevational gradient of 1000 m and found that low-elevation *Picea* were less sensitive to photoperiod than high-elevation individuals. This points to ecotypic differentiation of photoperiod requirements. Intraspecific phenological plasticity or ecotypes deserve further study.

Experimental implications

Our experiments control for a possible artefact in previous studies that used buds on twigs transferred to vases in glasshouses: Twigs cut later during the winter experience increasing day lengths and higher chilling than those cut earlier (Table 1). Twigs cut at different times are thus not strictly comparable in chilling status because the photoperiod effect is not controlled for. Our experiments (in all three species), however, revealed that buds on twigs cut on 21 March and brought into a glasshouse for 16- or 8-h light treatments behaved no different regardless whether they had experienced naturally increasing day lengths (up to 12-h day length) or had been kept under a constant 8-h day (by the outdoor bagging experiments; Fig. 2b). This indirectly validates the results of earlier studies in which twigs were cut early or late in spring to study the effects of chilling, but without controlling for the day length increase experienced before they were cut (Laube *et al.*, 2014; Polgar *et al.*, 2014). That buds *in situ* and on cut twigs react similarly to similar treatments as shown here (see also Vitasse & Basler, 2014) underlines the utility of the twig cutting method for inferring woody species' responses to photoperiod.

Red light induces responses to photoperiod and bud scales filter-out nonred light

In this study we show that red light is sufficient to induce budburst as a response to day length increase (Fig. 3) and find that leaf primordial cells receive sufficient red light in spite of being tightly covered by dead bud scale tissue (Fig. 4). These data strongly suggest that phytochromes mediate the day length response of buds. Between 400 and 600 nm, bud scales filtered out light efficiently, but between 600 and 800 nm, they transmitted 2–20% of the incoming light, with far-red light transmitted three-times more than red light (Fig. 4). Bud scales thus function as optical filters, modulating the phytochrome system (Pukacki & Giertych, 1982: *P. abies* and *Pinus sylvestris*; Solymosi & Böddi, 2006: 37 woody species).

Picea abies buds transmitted the least light (Fig. 4) because of the numerous scales per bud, whereas individual *Picea* scales let through more light than those of *Aesculus* and *Fagus*; Fig. S3). Being photoperiod insensitive (Table 2, Fig. 2b), *Picea* can probably afford a higher number of bud scales, perhaps providing increased frost protection, whereas in photo-sensitive species like *Fagus* and *Aesculus* there could be a trade-off between frost resistance (more bud scales) and sufficient light transmittance (fewer bud scales).

Conclusion

This study investigated bud responses to photoperiod in adult trees growing outside, whereas earlier studies on woody species all extrapolated from bud responses on cut twigs or seedlings. We found that: dormancy release is controlled at the bud level, with light sensing (and probably also temperature sensing) occurring inside buds; leaf primordia only react to light cues during the late phase of dormancy release when they have begun accumulating warm days; in *Fagus*, but not the other species, photoperiod can partially substitute for a lack of chilling and *vice versa*; and red light triggers the day length response, with bud scales filtering-out most of the remaining light spectrum received by the primordia.

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Supporting Information

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

Fig. S1 Treatment of twigs with light-tight bags, to ensure an 8-h photoperiod and translucent bags as control.

Fig. S2 Bud development of *Aesculus hippocastanum* (on 3 April 2014) and *Fagus sylvatica* (on 22 April 2014) on mature trees kept under 8-h day length and naturally increasing day length.

Fig. S3 Transmission spectra of single bud scales of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*.

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New Phytologist Supporting Information

Article title: **Perception of photoperiod in individual buds of mature trees regulates leaf-out**

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The following Supporting Information is available for this article:

Fig. S1 Treatment of twigs with light tight bags, to ensure an 8 h photoperiod and translucent bags as control. From 1 Jan 2014 until day of leaf-out in the respective species, twigs were covered daily with bags from 17:00 h until 09:00 h

Fig. S2 Bud development of *Aesculus hippocastanum* (on 3 April 2014) and *Fagus sylvatica* (on 22 April 2014) on mature trees kept under 8h day length (left) and naturally increasing day length (right). For photoperiod treatment, light-tight and translucent (control) bags were placed around the twigs every day at 17:00 h and removed the next morning at 09:00 h from 1 Jan 2014 until day of leaf-out in the respective species.

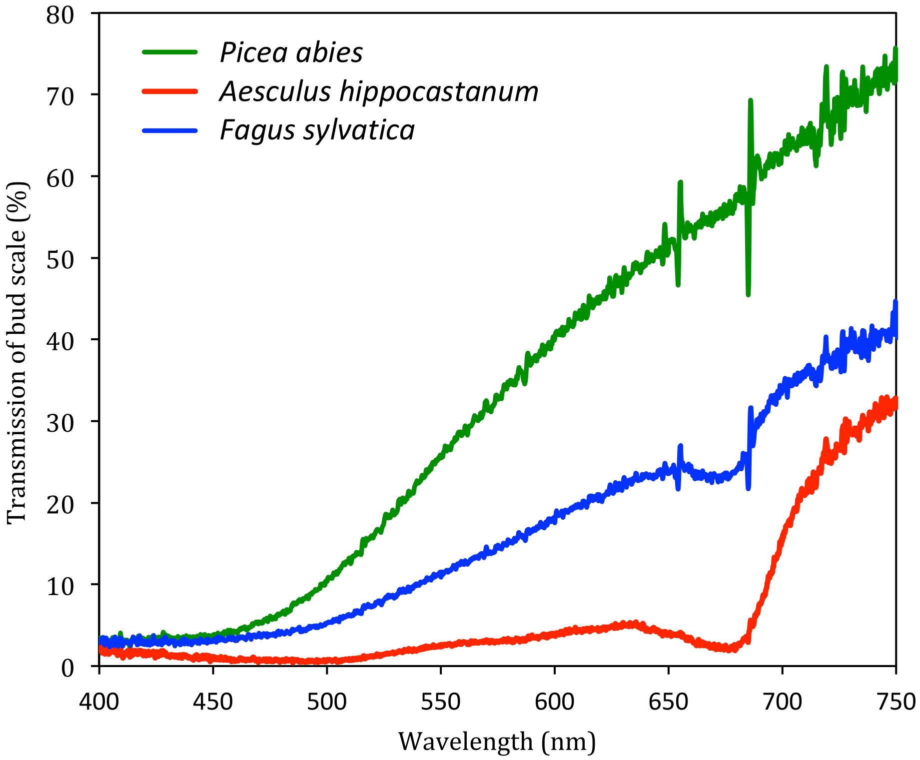

Fig. S3 Transmission spectra of single bud scales of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*.

Chapter 3

PHOTOPERIOD UNLIKELY TO CONSTRAIN CLIMATE CHANGE-DRIVEN SHIFTS IN LEAF-OUT PHENOLOGY IN NORTHERN WOODY PLANTS.

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Photoperiod unlikely to constrain climate change-driven shifts in leaf-out phenology in northern woody plants

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The relative roles of temperature and day length in driving spring leaf unfolding are known for few species, limiting our ability to predict phenology under climate warming[1,2]. Using experimental data, we assess the importance of photoperiod as a leaf-out regulator in 173 woody species from throughout the Northern hemisphere, and we also infer the influence of winter duration, temperature seasonality, and inter-annual temperature variability. We combine results from climate- and light-controlled chambers with species' native climate niches inferred from geo-referenced occurrences and range maps. Of the 173 species, only 35% relied on spring photoperiod as a leaf-out signal. Contrary to previous suggestions, these species come from lower latitudes, whereas species from high latitudes with long winters leafed out independent of photoperiod, supporting the idea that photoperiodism may slow or constrain poleward range expansion[3]. The strong effect of species' geographic-climatic history on phenological strategies complicates the prediction of community-wide phenological change.

Understanding the environmental triggers of leaf out and leaf senescence is essential for forecasting the effects of climate change on temperate zone forest ecosystems[2,3,4]. Correlation analyses suggest that warmer springs are causing earlier leaf emergence, leading to an extended growing season[5,6] and increased carbon uptake[7]. A continuing linear response to spring warming, however, is not expected because stimuli, such as photoperiod[1,8-10] and chilling[11-13], additionally trigger dormancy release.

Photoperiod limitation refers to the idea that plant sensitivity to day length protects leaves against frost damage by guiding budburst into a safe time period[1]. Experiments have shown that day length-sensitive species react to spring temperatures only once day length increases[10]. Because day length will not change under climate warming, photosensitive species may be less responsive to warmer temperatures[1,9,14,15].

Experiments addressing the relative importance of photoperiod *versus* temperature for dormancy release have been carried out in about 40 species[8-12], and among them a few species, most strikingly *Fagus sylvatica*, exhibited strong photoperiodism[8-10,12,16-19]. Results are often equivocal, perhaps in part reflecting experimental difficulties in adequately modifying day length when working with trees[9,11,12,20,21].

Why species differ in their relative reliance on photoperiod and spring temperature as leaf-out signals is largely unknown. This prevents the development of mechanistic models for predicting spring phenology under climate warming. The need to understand spring phenology in its geographic-climatic context is highlighted by studies suggesting that phenological strategies in long-lived woody species have evolved as adaptations to the climate in a species' native range[22-25]. A common garden study of 495 woody species from different climates showed that species native to warmer climates flush later than species native to colder areas, but did not investigate whether this was due to different species relying on temperature or photoperiod[25]. If photoperiod indeed provides a safeguard against leafing out too early[1,9], photoperiodism should be especially important (i) in regions with unpredictable frost events, i.e., high inter-annual variability in spring temperatures (here called 'high temperature variability' hypothesis)[26] and (ii) in regions with oceanic climates in which temperature is a less reliable signal because the change between winter and spring temperatures is less pronounced ('oceanic climate' hypothesis)[1]. A third hypothesis is that photoperiodism mirrors species' latitudinal occurrence because day-length seasonality increases towards the poles, and day length thus provides an especially strong signal at higher latitudes ('high latitude' hypothesis)[3]. Of these predicted correlates of photoperiod as a spring leaf-out signal, only the 'oceanic climate' hypothesis has been tested[12], with no significant relationship found.

We set out to (i) investigate the relative effect of photoperiod on leaf-out timing in species from different winter temperature regimes ('high latitude' hypothesis), temperature seasonality regimes ('oceanic climate' hypothesis), and between-year spring temperature variability ('high temperature variability' hypothesis) [Fig. 1a], and to (ii) test if photoperiod-sensitive species react less to spring temperatures than do photoperiod-insensitive species. We used 173 species (in 78 genera from 39 families) from the Northern Hemisphere grown in a mid-latitude (48°N) European Botanical Garden and modified the day length experienced by buds on twigs cut from these species at three different times and hence chilling levels (see Methods and Supplementary Fig. 1). To assign the species to their climate ranges, we queried geo-referenced occurrence data against climate grids for winter duration (Fig. 1b), temperature seasonality (T seasonality), and inter-annual spring temperature variability (T variability). In addition, each species was also assigned to its predominant Koeppen-Geiger climate type[25]. To achieve our second aim, we tested for correlations between species' photoperiodism (as inferred from our experiments on leaf-out in twigs under different light regimes) and their leaf-out behaviour *in situ* (as inferred from multi-annual leaf-out observations on intact trees; Fig. 2).

With low chilling (twig-collection in December), 61 (35%) of the 173 species leafed out later under short day conditions than under long days, while the remaining 112 species did not react differently regardless of short and long days. Increased chilling reduced species' sensitivity to photoperiod: Under intermediate chilling conditions (twig-collection in February), 16 (9%) of the 173 species showed delayed budburst under short days. Under long chilling conditions (twig-collection in March), only 4 (2%) species, namely *Fagus crenata, F. orientalis, F. sylvatica,* and *Carya cordiformis*, leafed out later under short days. Based on the current results, constraints on the climate-warming-driven advance of leaf-out[15] likely will be twofold in photosensitive species: (i) reduced winter chilling *per se* will cause plants to require more forcing in the spring and (ii) reduced chilling additionally will cause higher photoperiod requirements. The latter constraint will become more significant, as springs will arrive ever earlier (i.e., at ever shorter photoperiods) in the future.

Where do the species that rely on photoperiodism as a leaf-out trigger come from? Our data reject all three suggested correlates of photoperiodism (i.e., the 'high latitude', 'high temperature variability', and 'oceanic climate' hypotheses) and instead reveal that it is the species from shorter winters (i.e., lower, not higher latitudes) that rely on photoperiodism ($P < 0.05$; Table 1; Fig. 1). Of the 173 species, the 22 that come from regions with long winters (> 7 months with an average temperature below 5°C), such as alpine and subarctic regions are photoperiod-insensitive, while the 14 species with high photoperiod requirements are restricted to regions with shorter winters (not exceeding six months with an average temperature below 5°C; Fig. 1). In a hierarchical Bayesian model that controlled for possible effects of shared evolutionary history and species' growth height, winter duration remained negatively correlated with species' photoperiodism (Fig. 1a). Analyses that used the Koeppen-Geiger climate classification yielded the same results as analyses that used the climate grids, namely that most photoperiod-sensitive species are native to warm climates with mild winters (Supplementary Fig. 2).

Why is there a negative correlation between species' reliance on day length as a leaf-out signal and the winter duration in their native ranges? There are two possible mechanisms on how photoperiod perception in plants may interact with forcing requirements: (i) Either plants need to reach a fixed photoperiod threshold before they perceive forcing temperatures or (ii) forcing requirements gradually decrease with increasing photoperiod. The first mechanism would require that plants from regions with long winters have higher photoperiod thresholds because in these areas days are already long (>14-h) when minimum temperatures cross the freezing threshold (see also Way & Montgomerey[21]: Fig. 1). The second mechanism would require that the relative use of photoperiod as a budburst regulator decreases towards regions with long winters because days in spring become long before the risk of encountering freezing temperatures has passed. Experimental results from *Fagus sylvatica* show a gradual response to photoperiod independent of the latitudinal origin of the experimental plants: Forcing requirements decrease with increasing day length up to about 16-h, with further increase of daylight having little additional effect[8,10]. This supports the second mechanism. The second mechanism is also supported by *F. sylvatica* leafing out earlier at regions with long winter duration than photo-insensitive species and therefore operating at a smaller 'safety margin' against late frosts[27,28]. The hypothesis that Northern woody species evolved photoperiod-independent leaf-out strategies because at high latitudes day length increase in spring occurs too early for frost to be safely avoided needs to be tested with further experiments addressing the physiological mechanisms of photoperiod perception in different taxonomic groups.

That photosensitive species are restricted to regions with relatively short winters supports the idea that photoperiodism may slow or constrain poleward range expansion[3]. With a warming climate, however, the last day with night frost occurs ever earlier (in Germany, between 1955 – 2015, the last frost on average advanced by 2.6 days per decade; Supplementary Fig. 3), and photoperiod-sensitive species might do well at higher latitudes or elevations.

The leaf-out dates showed that those species with high photoperiod requirements had lower between-year variance in leaf-out dates than species lacking photoperiodism. Accordingly, in photoperiod-sensitive species, accumulated thermal time until budburst showed greater variation among years than that of photoperiod-insensitive species ($P < 0.01$; Fig. 2). Leaf unfolding in species that rely on day length is thus less responsive to temperature increase, and in these species photoperiod will constrain phenological responses to climate warming, with possible consequences for carbon gain, the local survival of populations and community composition[2,4]. The extent to which species' phenological strategies are influenced by their climatic histories highlights the need for a broader geographic sampling in global-change studies[29].

Our results do not support previous ideas about phenological strategies in temperate woody species (the 'high temperature variability' hypothesis; the 'oceanic climate' hypothesis; the 'high latitude' hypothesis[1,3,26]). In regions with long winters, trees appear to rely on cues other than day length, such as winter chilling and spring warming. By contrast, in regions with short winters, many species – mostly from lineages with a warm-temperate or subtropical background, e.g., *Fagus*[30] – additionally rely on photoperiodism. Therefore, only in regions with shorter winters, photoperiod may be expected to constrain climate change-driven shifts in the phenology of spring leaf unfolding.

Methods

Twig cutting experiments

We conducted twig-cutting experiments on 144 temperate woody species growing permanently outdoors without winter protection in the botanical garden of Munich to test for an effect of day length on dormancy release and subsequent leaf unfolding (see Supplementary Table 1 for species names). Twig cuttings have been shown to precisely mirror the phenology of donor trees because dormancy release is controlled at the bud level and not influenced by hormonal-signals from other parts of a tree, such as the stem or the roots[10,31]. In winter 2013/2014, c. 40 cm-long twigs were collected at three different dormancy stages (on 21 Dec, 10 Feb, and 21 Mar) for each species. After collection, we transferred the cut twigs to climate chambers and kept them under short (8 h) or long day (16 h) conditions. Temperatures in the climate chambers were held at 14°C during the night and 18°C during the day (see Supplementary Fig. 4 for a description of the temperature regime outside and in the climate chambers). Illuminance in the chambers was about 8 kLux (~100 μmol s^{-1} m^{-2}). Relative air humidity was held between 40% and 60%.

Immediately after cutting, we disinfected the twigs with sodium hypochlorite solution (200 ppm active chlorine), re-cut them a second time, and then placed them in 0.5 l glass bottles filled with 0.4 l cool tap water enriched with the broad-spectrum antibiotics gentamicin sulfate (40 microg/l; Sigma–Aldrich, Germany)[9,10]. We used 60 replicate twigs per species (10 twigs per treatment, 3x2 full factorial experiment) and monitored bud development every second day. For each treatment, we recorded the leaf-out dates of the first eight twigs that leafed out. A twig was scored as having leafed out when three buds had their leaves pushed out all the way to the petiole.

Flushing rate, i.e. the proportion of buds flushed over the total number of buds on the twigs, was not recorded. Treatment effects (long vs. short days at three different dormancy stages) on the response variable (accumulated degree days >0°C outside and in climate chamber from 21 Dec until leaf-out) were assessed in ANOVAs. We defined three categories to describe a species' photoperiodism: *none* = No response to day length, *low* = sensitivity to day length during early dormancy, *high* = sensitivity to day length also during late dormancy. Species whose twigs when cut on 21 Dec (early dormancy stage) showed no statistical difference between 8-h and 16-h photoperiod treatments were categorized as having *no* photoperiod requirements. Species whose twigs when cut on 21 Dec leafed out significantly later when they were exposed to 8-h day length compared to 16 h days were categorized as having *low* photoperiod requirements. Species whose twigs when cut on 10 Feb (advanced dormancy stage) still leafed out later under short days (8 h) than under 16-h days were categorized as having *high* photoperiod requirements. When twigs were cut on 21 March, only three *Fagus* species and *Carya cordiformis* reacted differently to 8-h and 16-h photoperiods, and we categorized them as having *high* photoperiod requirements. In addition to the ANOVA assessment, a day length effect was only considered significant if the forcing requirements under 8-h day length were >50 degree days higher than under 16-h day length and if the additional forcing requirement was >10% larger than required under long days (see Supplementary Fig. 1 for species-specific treatment effects). Information on the photoperiod requirements of 29 additional species came from a previous study[12] that used the same experimental approach to detect species' photoperiod requirements, allowing us to apply the same definition of photoperiod categories to their data. This resulted in photoperiod data for a total of 173 woody species in 78 genera from 39 families.

In-situ leaf-out observations

For 154 of the 173 species with information on photoperiod requirements (previous section), we have four years of observations of leaf-out dates, viz. 2012–2015, available from the Munich botanical garden. The 2012 and 2013 data come from our earlier study[25], and the same individuals were monitored again in 2014 and 2015. A species' leaf-out date was defined as the day when three branches on a plant had leaves pushed out all the way to the petiole. Thermal requirements of species were calculated as the sum of growing-degree days from 1 January until day of leaf-out using a base temperature of 0°C. Species names are given in Appendix Table S1.

To test if species with photoperiod requirements show lower variation in leaf-out and higher variation in thermal requirements among years than do photo-insensitive species, we applied difference-of-means tests (Fig. 2). Because vectors were not normally distributed we conducted Kruskal–Wallis H tests with a *post-hoc* Kruskalmc analysis (multiple comparison after Kruskal–Wallis)[32].

Temporal occurrence of last frost events

Weather data were downloaded from Deutscher Wetterdienst, Offenbach, Germany, via WebWerdis (https://werdis.dwd.de/werdis/ start_js_JSP.do) to gather information on the relative occurrence date and temporal shifts of the last frost (daily minimum temperature below 0°C). Information on the occurrence of the last frost from 1955 to 2015 for German locations differing in their winter duration is given in Supplementary Fig. 3. On average, across all stations, the last freezing event advanced by 2.6 days per decade.

Species ranges and climate characteristics

To obtain species' native distribution ranges, we extracted georeferenced locations from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/), using the dismo R-package[33]. Cleaning scripts in R were used to filter reliable locations and exclude species with unreliable records, using the following criteria: (i) only records from a species' native continent were included; (ii) coordinate duplicates within a species were removed; (iii) records based on fossil material, germplasm, or literature were removed; (iv) records with a resolution >10 km were removed; and (v) only species with more than 30 georeferenced GBIF records within their native continent were included. After applying these filtering criteria, we were left with distribution data for 144 of the 173 species.

We then derived species-specific climate ranges from querying georeferences against climate grids of three bioclimatic variables: T seasonality (BIO7; Temperature difference between warmest and coldest month), T variability (inter-annual spring T variability calculated as the standard deviation of March, April, and May average T from 1901 – 2013), and winter duration (defined as the numbers of months with an average T below 5°C). A grid file for the winter duration was based on global monthly weather data available at www.worldclim.org[34], from which we calculated the number of months with an average temperature below 5°C for the global land surface (see Fig. 1b). T seasonality was based on gridded information (2.5-arc minute spatial resolution data) about the annual temperature range derived from the WorldClim dataset (bioclim7)[34]. T variability was calculated as the standard deviation of spring (March, April, and May) average temperatures from 1901 to 2013 (see Supplementary Fig. 5). Data on monthly average temperatures during this period were available from the CRU database (5-arc minute spatial resolution data)[35]. For each bioclimatic variable we determined three species-specific measures: the upper and lower limits and the median which were obtained from the bioclimatic data covering a species range at the 0.95, 0.05, and 0.50 quantile, respectively.

As an alternative approach that allowed us to infer the predominant climate of 171 of the 173 species, we used the Koeppen-Geiger system[36]. Information on species-specific Koeppen-Geiger climate types was available from our earlier study[25] in which each species' natural distribution was determined using information from range maps and range descriptions: http://linnaeus.nrm.se/flora/welcome.html and http://www.euforgen.org/distribution-maps/ for the European flora, http://plants.usda.gov/java/ and http://esp.cr.usgs.gov/data/little/ for North America, and http://www.efloras.org for Asia. As a proxy for a species' native winter temperature regime, it was scored for the first Koeppen-Geiger letter (D-climate = coldest month average below -3°C, C-climate = coldest month average above -3°C). For species' summer temperature, the third Koeppen-Geiger letter was used (a-climate = warmest month average above 22°C with at least four months averaging above 10°C; b-climate = warmest month average below 22 °C but with at least four months averaging above 10 °C, c-climate = warmest month average below 22°C with three or fewer months with mean temperatures above 10 °C). The second letter in the Koeppen system refers to precipitation regime and was disregarded in the analyses. Species were scored for the predominant conditions in their native range; for example, a species occurring in 40% Cfa, 30% Dfa, and 30% Dfb climates would be scored as "D" and "a".

Data analysis

The quantiles (0.05, 0.5, and 0.95) of each climate parameter (winter duration, T seasonality, and T variability) were highly correlated among each other (Pearson correlation, $r > 0.5$). To avoid multicollinearity in our models, we included only one quantile for each climate parameter. For each climate parameter, we kept the quantile that gave the best prediction of species-level variation in photoperiodism. We fitted univariate logistic regression models to our data and, for each climate parameter, kept the variable with the lower Akaike information criterion (AIC), i.e., we kept the 0.95 quantile of winter duration, 0.95 quantile of T seasonality, and 0.5 quantile of T variability. We tested for multicollinearity among the retained predictor variables by using variance inflation factors (VIF). All VIF were smaller than 5, indicating sufficient independence of the predictor variables. ANOVA and ordinal logistic regression (OLR) were used to separately test for correlations among predictor variables and species-specific photoperiod sensitivity (see Table 1, Fig. 1c, and Supplementary Fig. 6). To examine the relative contribution of each climate variable to explain species-specific photoperiod sensitivity, we applied multivariate OLR, random forest[37,38], and hierarchical Bayesian models. The hierarchical Bayesian models allowed us to control for phylogenetic signals in our data (Supplementary Fig. 7) using the Bayesian phylogenetic regression method[39] (next section). We analysed correlations between species' native climates as inferred from the Koeppen-Geiger system[36] and their photoperiod requirements by applying contingency analyses (Fisher's test) and hierarchical Bayesian models (next section).

Data analysis including the phylogenetic structure

To account for possible effects of shared evolutionary history, we applied hierarchical Bayesian models. The phylogenetic signal in trait data was estimated using Pagel's λ[40], with the 'phylosig' function in the R package 'phytools' v0.2-1[41]. The phylogenetic tree for our 173 target species came from Panchen *et al.*[42] and was assembled using the program Phylomatic[43] (Supplementary Fig. 7). Its topology reflects the APG III phylogeny[44], with a few changes based on the Angiosperm Phylogeny Website[45]. We manually added about 10 missing species to the tree. Branch lengths of the PHYLOMATIC tree are adjusted to reflect divergence time estimates based on the fossil record[46,47]. Besides controlling for phylogenetic signal λ[40] of traits, the hierarchical Bayesian approach allowed us to control for possible effects of growth height on species-level photoperiod requirements and climate ranges, by including species' mature growth height as a fixed effect in the models. Mature growth height is a significant functional trait that is related to species' growth phenology[42] as well as climate ranges[48]. Slope parameters across traits are estimated simultaneously without concerns of multiple testing or P-value correction.

To determine which climate parameter best explains species-level differentiation in photoperiodism, we treated species' photoperiod requirements (ordinal data) as a dependent variable. Three climate variables (species-specific maximum winter duration, 0.95 quantile; max. T seasonality; 0.95 quantile; and median T variability, 0.5 quantile) and species' mature growth height were used as predictor variables (Table 1 and Supplementary Fig. 8).

Regression components are of the form:

$$\begin{aligned}\text{ordered logit}(\mathit{photoperiod}_i) = {} & \beta_{\text{max winter duration}} \text{ x } \mathit{max\ winter\ duration}_i \\ & + \beta_{\text{median T variability}} \text{ x } \mathit{median\ T\ variability}_i \\ & + \beta_{\text{max T seasonality}} \text{ x } \mathit{max\ T\ seasonality}_i \\ & + \beta_{\text{growth height}} \text{ x } \mathit{growth\ height}_i\end{aligned}$$

β refers to the estimated slopes of the respective variable. In an alternative model, we used species' Koeppen winter and summer temperature types and mature growth height as predictor variables (Supplementary Fig. 9):

$$\begin{aligned}\text{ordered logit}(\mathit{photoperiod}_i) = {} & \beta_{\text{winter temp}} \text{ x } \mathit{winter\ temp}_i \\ & + \beta_{\text{summer temp}} \text{ x } \mathit{summer\ temp}_i \\ & + \beta_{\text{growth height}} \text{ x } \mathit{growth\ height}_i\end{aligned}$$

These models do not statistically account for phylogenetic structure by allowing correlations to vary according to the phylogenetic signal λ, because λ estimation is not possible for ordinal (or logistic) models. To nevertheless account for data non-independence due to shared evolutionary history of species (see Supplementary Fig. 7), we inserted genus and family random intercept effects in the model. To examine relative effect sizes of predictor variables, we standardized all variables by subtracting their mean and dividing by 2 SD before analysis[49]. The resulting posterior distributions are a direct statement of the influence of each parameter on species-level differentiation in photoperiod requirements. The effective posterior means (EPM) for the relationships between winter duration, temperature seasonality, and spring temperature variability and species-specific photoperiodism are shown in Supplementary Fig. 8, and the EPMs for relationships between Koeppen-Geiger climates and photoperiod requirements are shown in Supplementary Fig. 9.

The hierarchical Bayesian model strongly preferred winter duration to T seasonality and T variability as an explanatory variable for species' photoperiodism. Likewise, the model using the Koeppen system preferred the Koeppen winter climate to the summer climate as a predictor of species' photoperiodism. To validate these results, instead of treating photoperiodism as dependent variable, we tested two other models. The first compared the distribution of covariates (max. winter duration, max. T seasonality, and median T variability) between the different photoperiod categories. Species' values for max. winter duration, max. T seasonality, and median T variability can be treated as continuous characters, which allowed us to incorporate phylogenetic distance matrices to control for shared evolutionary history of species (Pagel's λ values: max. winter duration = 0.40; max. temp. seasonality = 0.39; median temp. variability = 0.26; see inset Fig. 1a). This model included three dependent variables that were normally distributed with mean μ, variance $\sigma^{2,}$ and correlation structure Σ (Fig. 1a):

$$\textit{max winter duration}_i \sim N(\mu_{\textit{max winter duration i}}, \sigma^2_{\textit{max winter}\ \text{duration}}, \Sigma)$$

$$\textit{median T variability}_i \sim N(\mu_{\textit{median T variability i}}, \sigma^2_{\textit{median T variability}}, \Sigma)$$

$$\textit{max T seasonality}_i \sim N(\mu_{\textit{max T seasonality i}}, \sigma^2_{\textit{max T seasonality}}, \Sigma)$$

Regression components are of the form:

$$\mu_{\textit{max winter duration i}} = \alpha_1 + \beta_{\text{winter dur}} \text{ x } \textit{photoperiodism}_i + \beta_1 \text{ x } \textit{mature growth height}_i$$

$$\mu_{\textit{median T variability i}} = \alpha_3 + \beta_{\text{T variability}} \text{ x } \textit{photoperiodism}_i + \beta_2 \text{ x } \textit{mature growth height}_i$$

$$\mu_{\textit{max T seasonality i}} = \alpha_2 + \beta_{\text{T seasonality}} \text{ x } \textit{photoperiodism}_i + \beta_3 \text{ x } \textit{mature growth height}_i$$

The other model, based on species' Koeppen climate letters as outcome, included two binary dependent variables that capture whether species are native to regions with mild or cold winters (KW; Koeppen C or D climate) and warm or cold summers (KS; Koeppen a or b climate) [Supplementary Fig. 2]:

$$\textit{winter temp} \sim \textit{Bernoulli}(WT_i)$$

$$\textit{summer temp} \sim \textit{Bernoulli}(ST_i)$$

Regression components are of the form:

$$\text{logit}(WT_i) = \alpha_1 + \beta_1 \text{ x } \textit{photoperiodism}_i + \beta_3 \text{ x } \textit{maximum growth height}_i$$

$$\text{logit}(ST_i) = \alpha_2 + \beta_2 \text{ x } \textit{photoperiodism}_i + \beta_4 \text{ x } \textit{maximum growth height}_i$$

The term α refers to the intercept, β to the estimated slopes of the respective variable (photoperiodism and maximum growth height), and max winter duration, max temp seasonality, and median temp variability refer to species values of the respective climate parameters. The phylogenetic structure of the data was incorporated in the hierarchical Bayesian model using the Bayesian phylogenetic regression method of de Villemereuil *et al.*[39], by converting the 173-species ultrametric phylogeny into a scaled (0–1) variance–covariance matrix (Σ), with covariances defined by shared branch lengths of species pairs, from the root to their most recent ancestor[50]. We additionally allowed correlations to vary according to the phylogenetic signal (λ) of climate parameters, fitted as a multiple of the off-diagonal values of Σ[39]. Values of λ near 1 fit a Brownian motion model of evolution, while values near zero indicate phylogenetic independence. The phylogenetic variance–covariance matrix was calculated using the 'vcv.phylo' function of the ape library[51]. The resulting posterior distributions are a direct statement of the influence of spring photoperiodism on species-level differentiation in climate characteristics (i.e., species' max. winter duration, median temp. variability, and max. temp. seasonality). Effective posterior means for the respective relationships are shown in Fig. 1a.

To parameterize our models we used the JAGS[52] implementation of Markov chain Monte Carlo methods, in the R package R2JAGS[53]. We ran three parallel MCMC chains for 20,000 iterations with a 5000-iteration burn-in and evaluated model convergence with the Gelman and Rubin[54] statistic. Noninformative priors were specified for all parameter distributions, including normal priors for α and β coefficients (fixed effects; mean = 0; variance = 1000), uniform priors between 0 and 1 for λ coefficients, and gamma priors (rate = 1; shape = 1) for the precision of random effects of phylogenetic autocorrelation, based on de Villemereuil *et al.*[39].

In table 1 we summarize the statistical results. All statistical analyses relied on R 3.2.2[55].

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Author contributions

C.M.Z. and S.S.R. designed the study. C.M.Z. conducted the experiments and leaf-out observations. C.M.Z. and B.M.B. performed the analyses. C.M.Z. and S.S.R. led the writing with inputs from the other authors.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.M.Z.

Competing financial interests

The authors declare no competing financial interests.

Figures and Tables

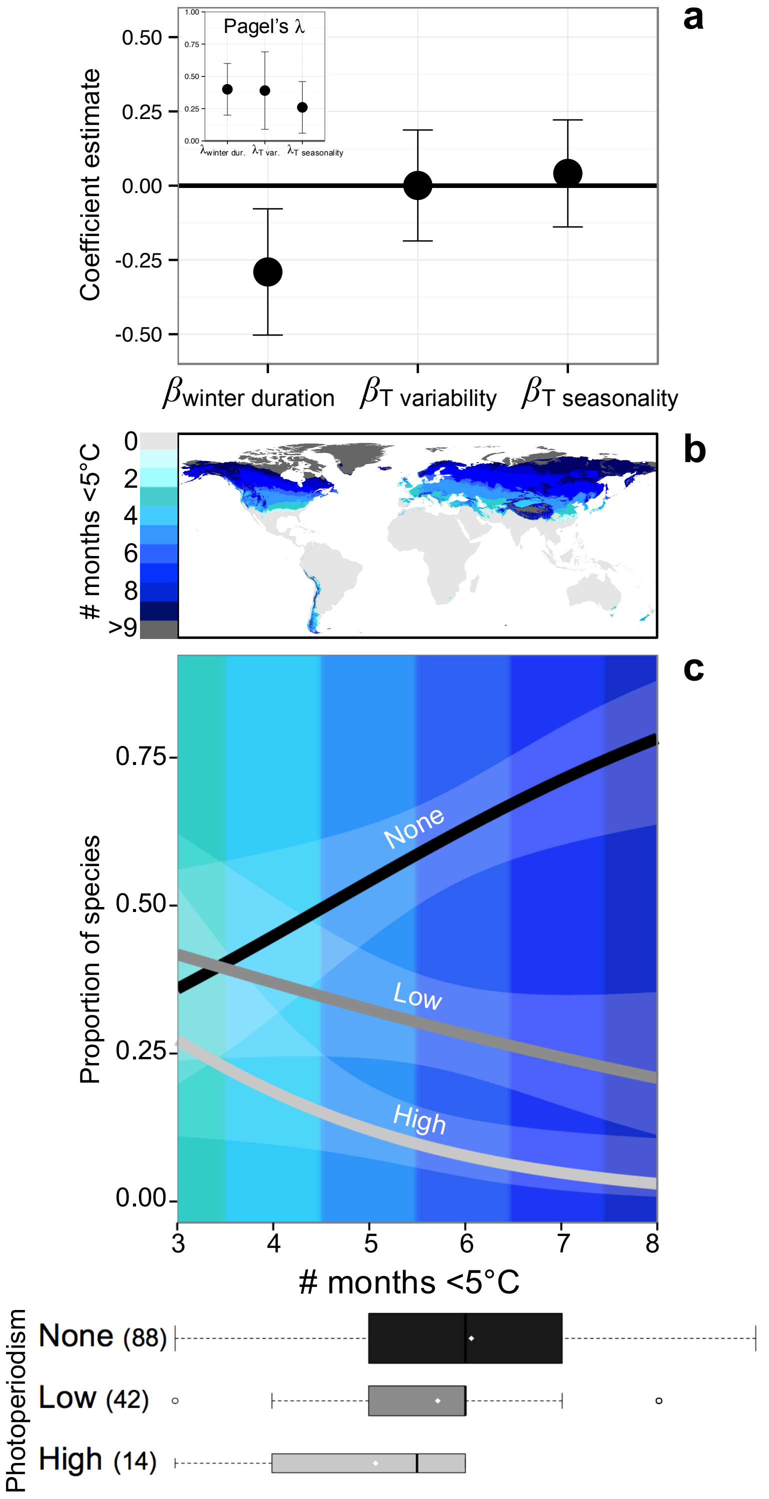


Figure 1 | Relationship between species' spring photoperiodism and the maximum winter duration in their native ranges. a, Coefficient values (effective posterior means β and 95% credible intervals) for the effect of spring photoperiodism on species' maximum winter duration, median T variability, and maximum T seasonality. Models control for phylogenetic autocorrelation and species' maximum growth height. See Supplementary Methods for a detailed description of regression components. Values reflect standardized data and can be interpreted as relative effect sizes. The inset shows fitted values of phylogenetic signal (Pagel's λ, mean and 95% CIs) for species' maximum winter duration, median T variability, and maximum T seasonality (dependent variables), respectively. **b,** Winter duration calculated as the number of months with mean air temperature below 5°C. **c,** Proportion of species with a given level of photoperiod sensitivity as a function of maximum winter duration (0.95 quantile) in a species' native range (ordinal logistic regression model; $P < 0.01$; table 1). Colours as in panel b. Envelopes around each line show 95% confidence intervals. Boxplots for species' maximum winter duration when they were grouped according to photoperiod requirements are shown below the graph. Photoperiod requirements: None = No sensitivity; Low = Sensitivity to day length during early dormancy; High = Sensitivity to day length also in late dormancy (see Supplementary Fig. 1).

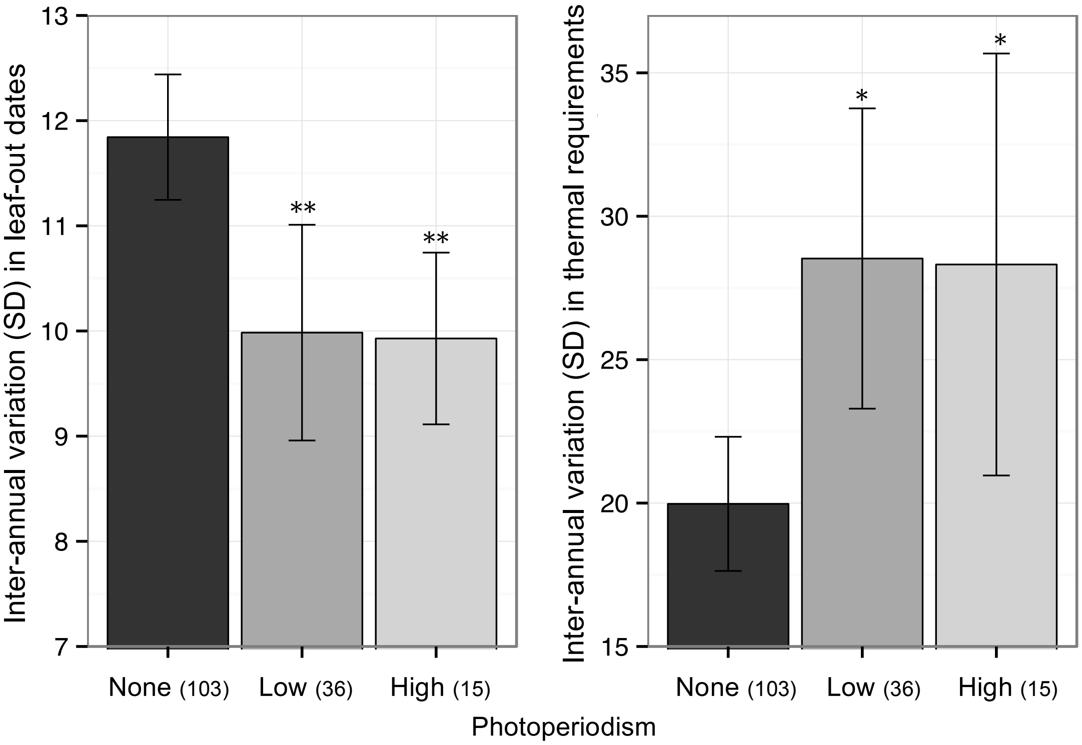


Figure 2 | Photoperiod-dependent leaf-out strategies lead to low inter-annual variability in leaf-out dates (a) and high inter-annual variability in thermal time until budburst (b). For each species (n = 154) the SD in leaf-out dates and thermal requirements was calculated on the basis of leaf-out dates available from the Munich Botanical Garden from 2012 to 2015. We show the mean ± 95% confidence interval for each group. Thermal time was calculated as the sum of growing-degree days from 1 Jan until the day of leaf-out in the respective species using 0°C as base temperature. Asterisks above bars indicate which group differed significantly from the group of species with no photoperiod requirements (*$P < 0.05$, **$P < 0.01$).

Table 1 | Global relationships between species' photoperiod requirements and duration of winter, inter-annual spring temperature variability (T variability), and T seasonality in their native range for 144 temperate woody species. Five comparative measures were used: the *F* value from univariate ANOVA, Akaike weights from bivariate regressions using ordinal logistic regression (OLR) models, parameter estimates and 95% confidence intervals (CI) based on multivariate OLR models, mean decrease in accuracy values (MDA) from random forest analysis, and coefficient values [effective posterior means (EPM) and 95% CIs] from a hierarchical Bayesian (HB) model controlling for phylogenetic autocorrelation and species' maximum growth height. For each single climatic parameter we initially considered the upper limit (0.95-quantile), median (0.5 quantile), and lower limit (0.05-quantile) across each species' range and kept the variable that yielded the lower Akaike information criterion (AIC) according to OLR models (i.e. we kept the 0.95 quantile for winter duration and T seasonality, and the 0.5 quantile for T variability). Sample size: No photoperiod requirements = 88 species; Low = 42 species; High = 14 species. *$P < 0.05$, **$P < 0.01$.

	ANOVA	**OLR**	**Multiv. OLR**	**Random forest**	**HB model**
	F values	$\text{Weight}_{\text{AIC}}$	Estimate ± CI	MDA	EPM ± CI
Winter duration	*F*(1, 142) = 9.5**	0.90**	-0.47 ± 0.28**	33.7	-1.1 ± 0.5
T variability	*F*(1, 142) = 0.3	0.05	0.99 ± 1.17	22.9	-0.3 ± 0.5
T seasonality	*F*(1, 142) = 1.9	0.05	0.00 ± 0.01	20.8	-0.2 ± 0.5

Supplementary Figures and Tables

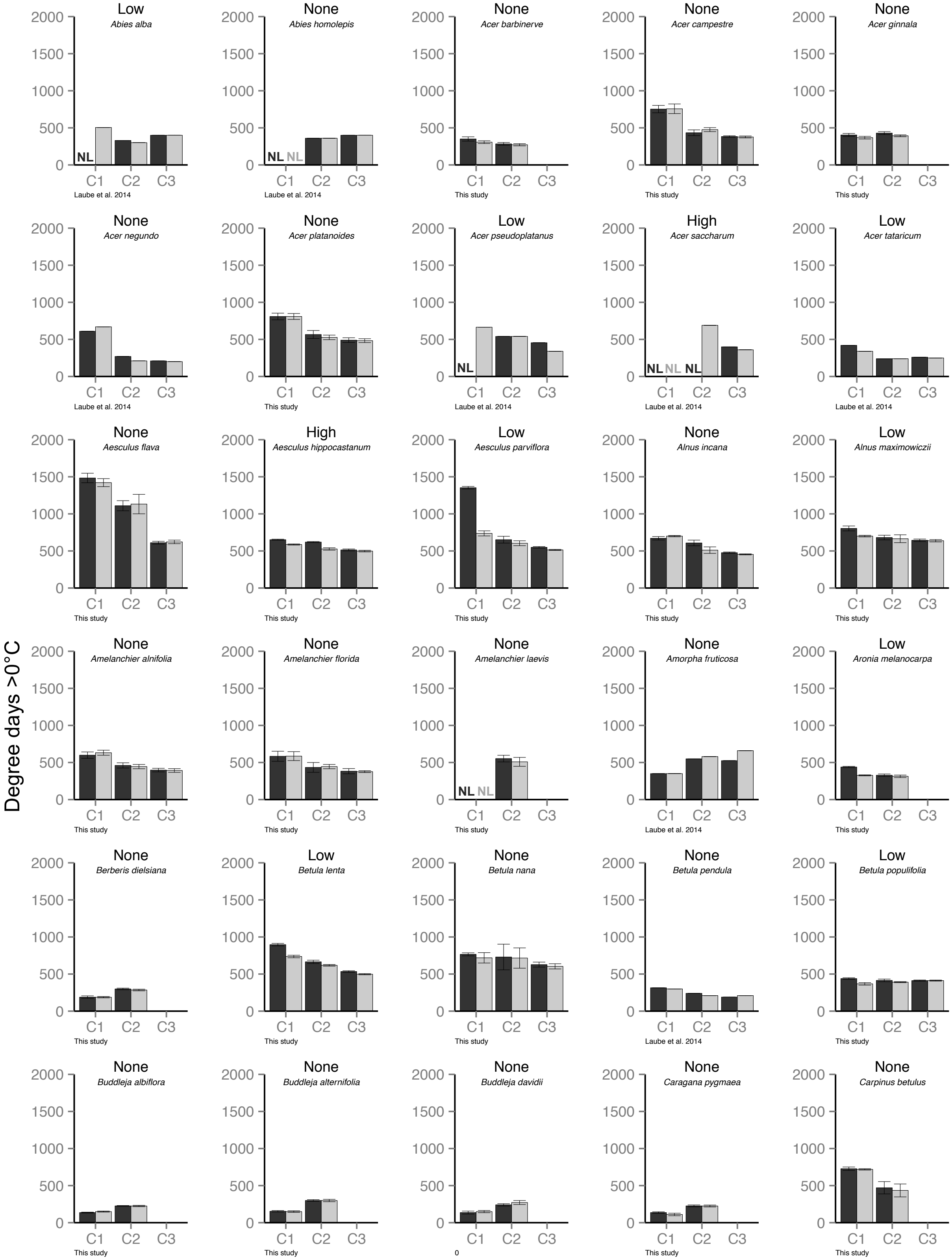
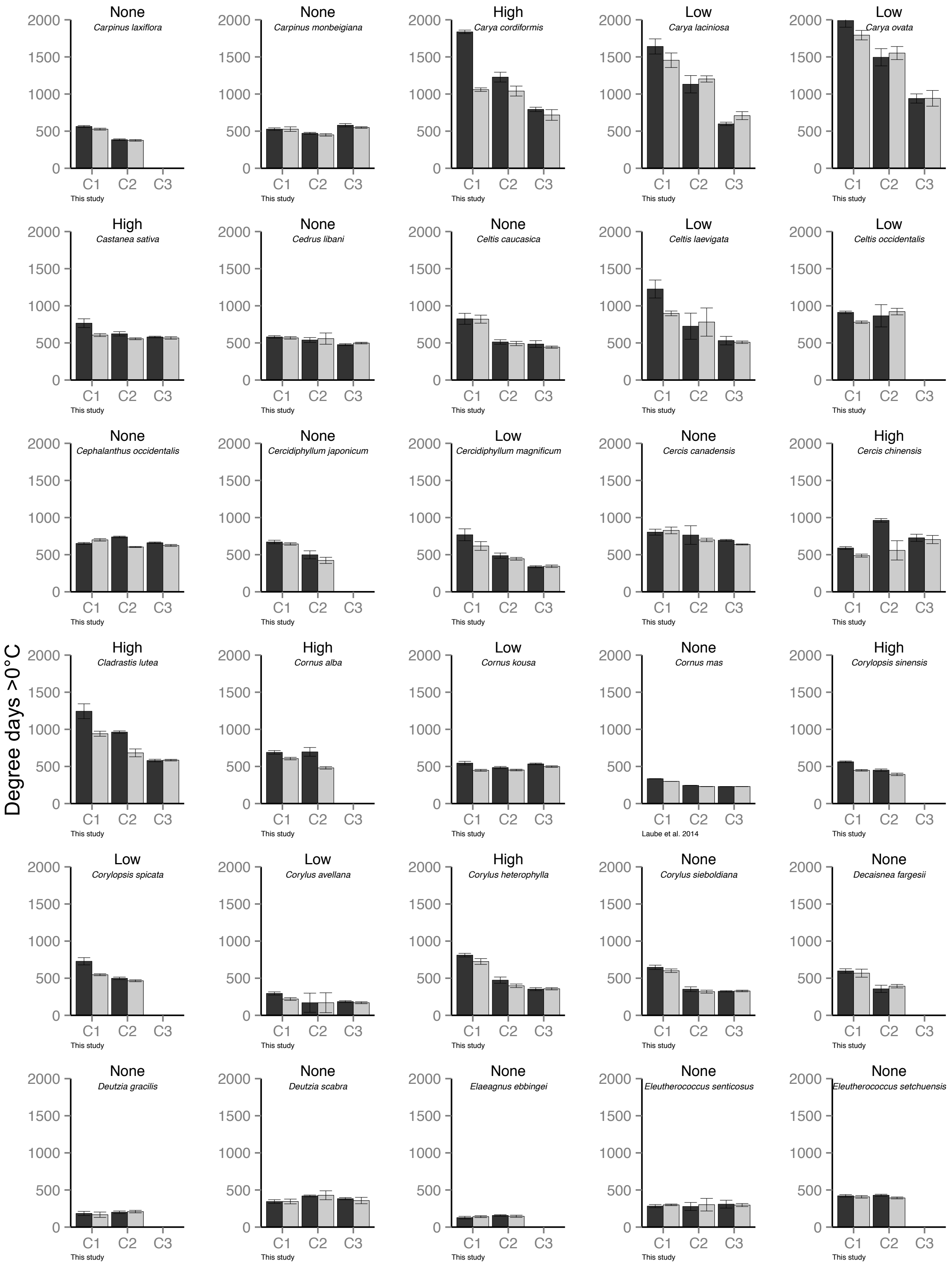


Figure S1 partial



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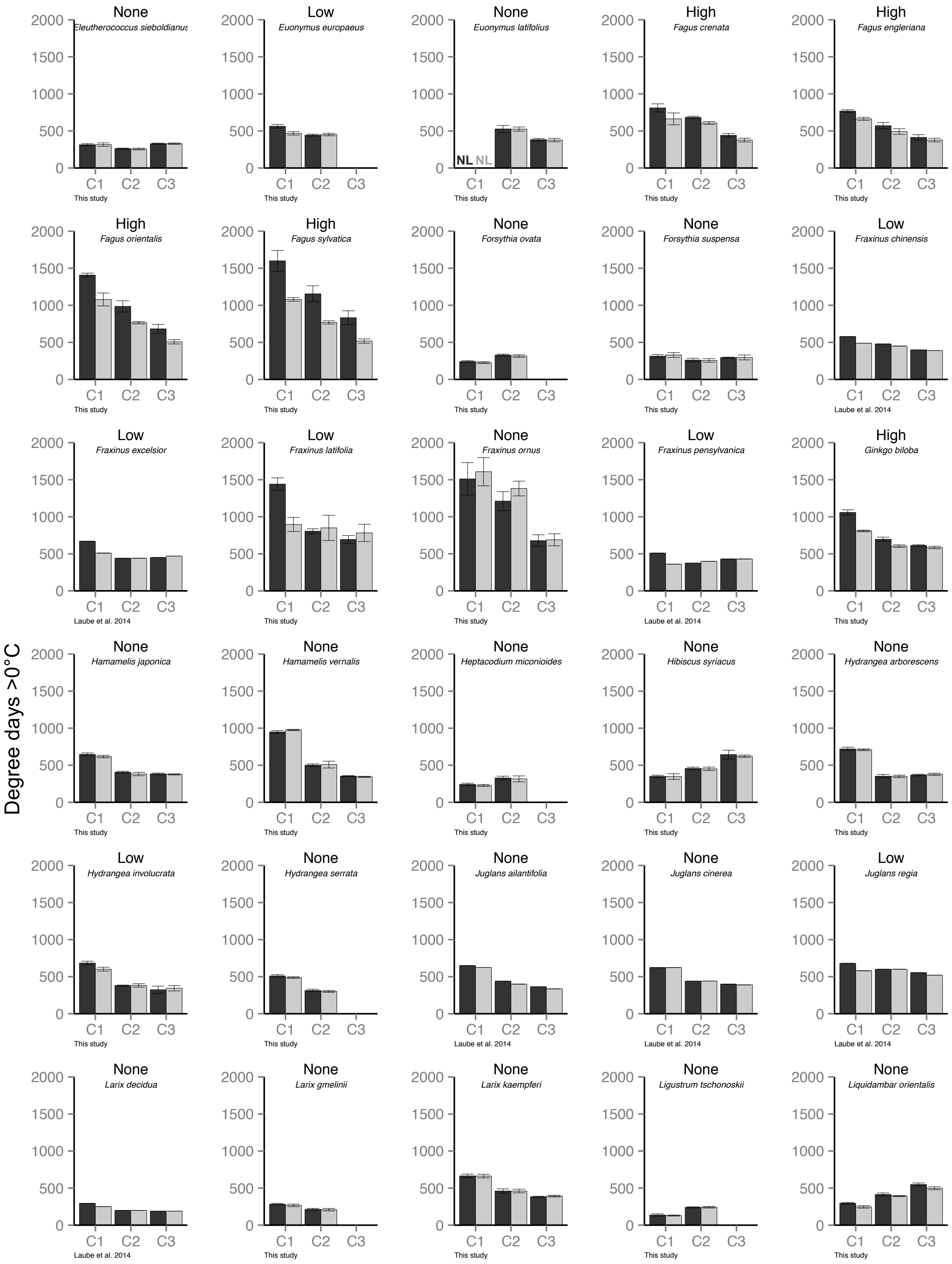

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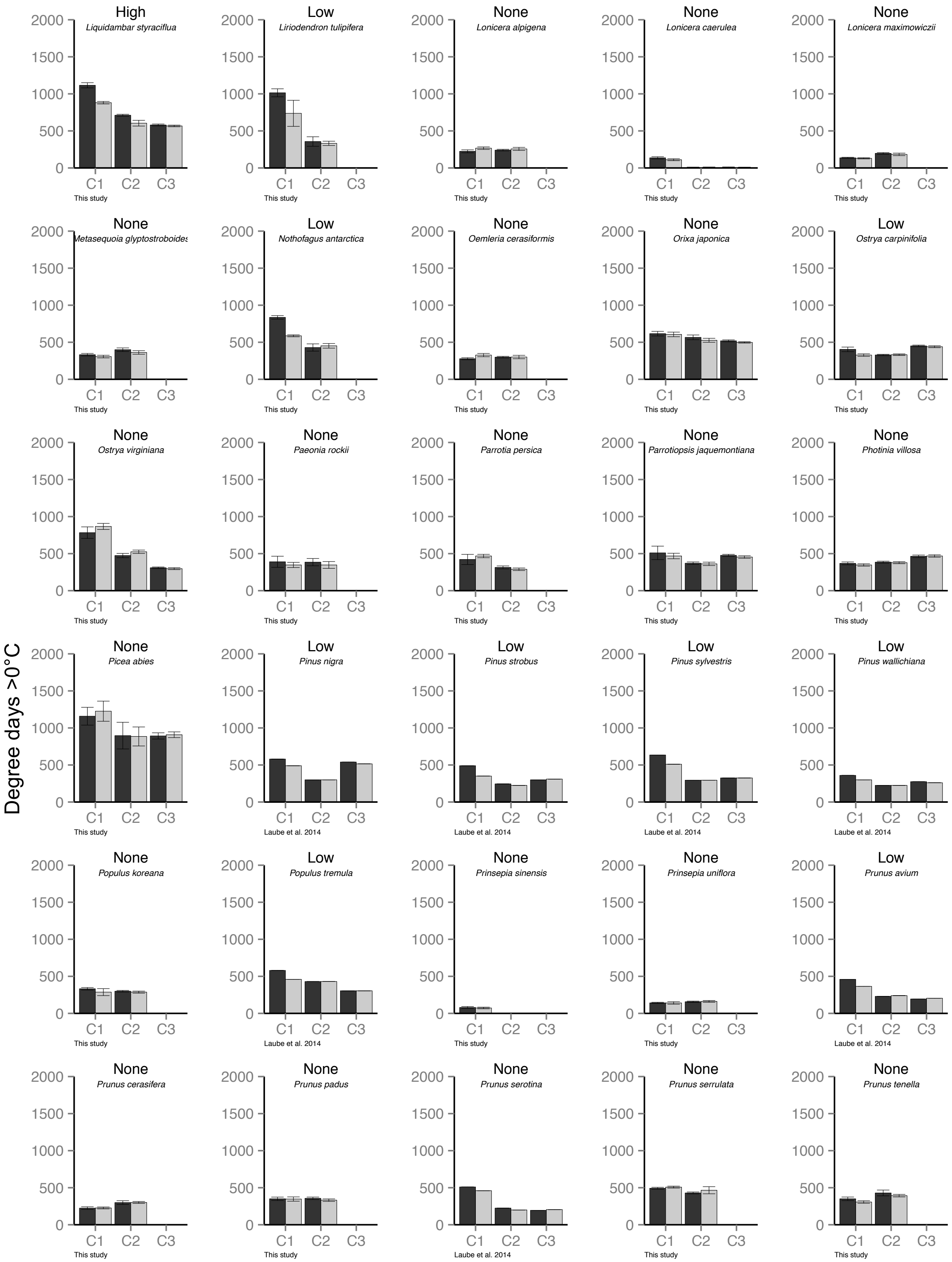

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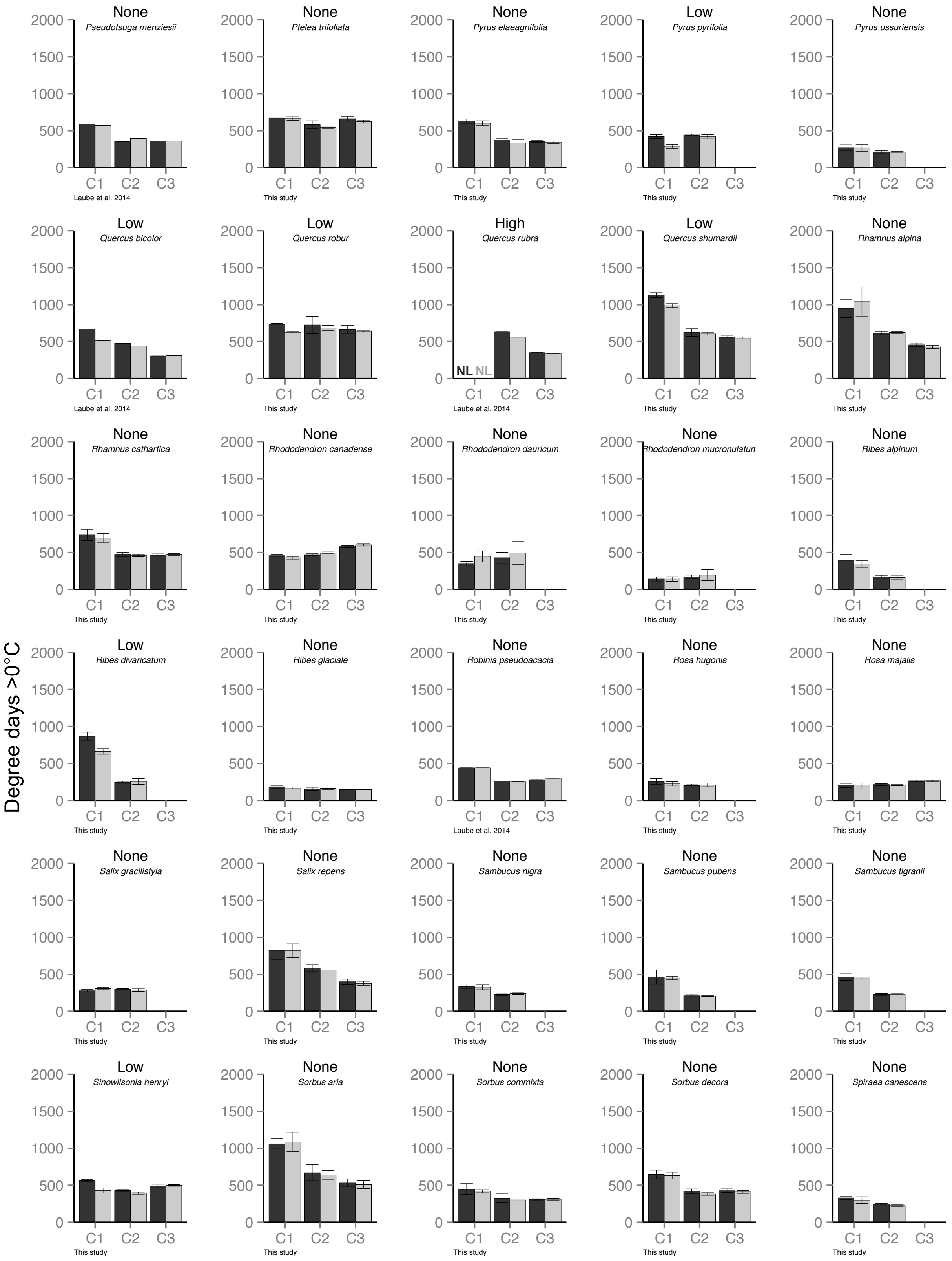

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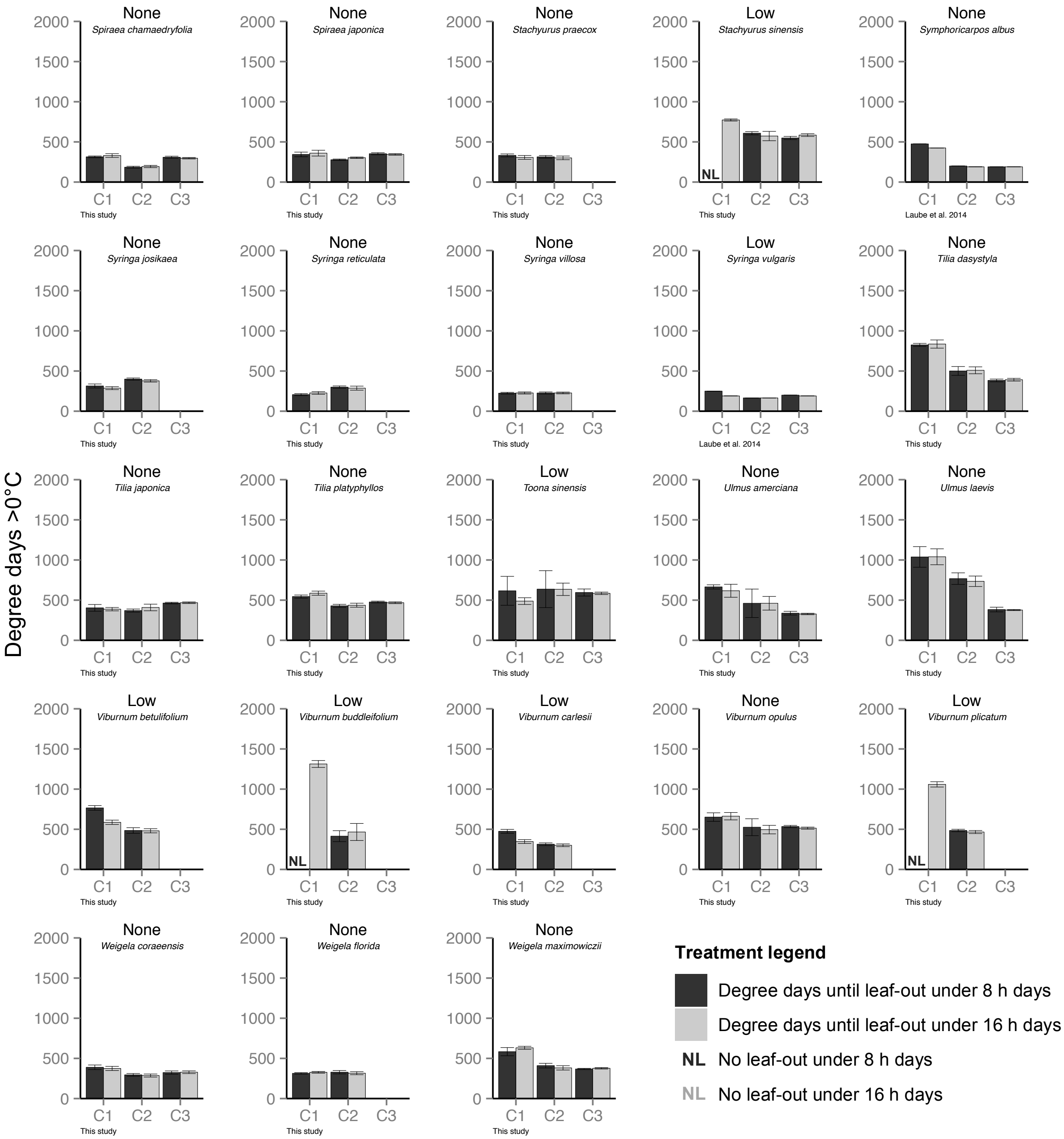
Degree days >0°C
2000
1500
1000
500
0
C1
C2
C3
None
Spiraea chamaedryfolia
This study
None
Spiraea japonica
This study
None
Stachyurus praecox
This study
Low
Stachyurus sinensis
NL
This study
None
Symphoricarpos albus
Laube et al. 2014
None
Syringa josikaea
This study
None
Syringa reticulata
This study
None
Syringa villosa
This study
Low
Syringa vulgaris
Laube et al. 2014
None
Tilia dasystyla
This study
None
Tilia japonica
This study
None
Tilia platyphyllos
This study
Low
Toona sinensis
This study
None
Ulmus amerciana
This study
None
Ulmus laevis
This study
Low
Viburnum betulifolium
This study
Low
Viburnum buddleifolium
NL
This study
Low
Viburnum carlesii
This study
None
Viburnum opulus
This study
Low
Viburnum plicatum
NL
This study
None
Weigela coraeensis
This study
None
Weigela florida
This study
None
Weigela maximowiczii
This study
Treatment legend
Degree days until leaf-out under 8 h days
Degree days until leaf-out under 16 h days
NL No leaf-out under 8 h days
NL No leaf-out under 16 h days

Figure S1 | Photoperiod requirements of 173 temperate woody species. The importance of photoperiod in regulating leaf-out (none, low, or high photoperiodism) was inferred from twig cutting experiments conducted in this study and in Laube *et al.*[12]. Graphs show forcing requirements (median growing degree days >0°C outdoors and in climate chamber ± SD) until leaf-out under short day length (8 h/d, black bars) and long day length (16 h/d, grey bars) at three different cutting dates (this study: C1 = 21 Dec 2013, C2 = 10 Feb 2014, C3 = 21 March 2014; Laube *et al.*[12]: C1 = 14 Dec 2011, C2 = 30 Jan 2012, C3 = 14 March 2012). *NL* indicates that no leaf-out occurred under 8-h (*NL* in black) or 16-h (*NL* in grey) day length. Some species leafed out before the last cutting date (C3), which is indicated by missing bars for the C3 treatment.

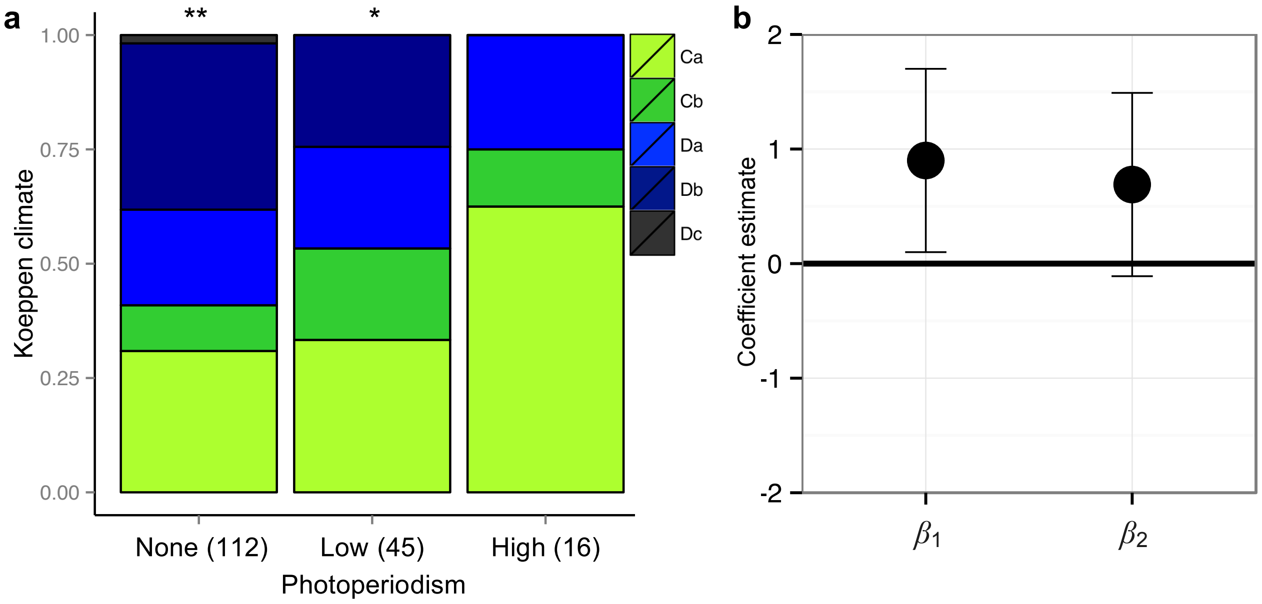

Figure S2 | Species with photoperiod requirements are native to milder climates. a, For each photoperiod category we show the relative proportion of species' Koeppen-Geiger temperature regimes (Ca = mild winter and hot summer periods, Cb = mild winter and warm summer periods, Da = cold winter and hot summer periods, Db = cold winter and warm summer periods, Dc = cold winter and cold summer periods). Asterisks above bars indicate which group differed significantly from the group containing species with high photoperiod requirements ($*P < 0.05$, $**P < 0.01$). Sample sizes are shown in brackets below the graph. **b,** Estimated coefficient values (effective posterior means and 95% credible intervals) for the effect of spring photoperiodism on species' winter (β_1) and summer (β_2) temperature regime. Winter climate and summer temperature were included as binary variables of whether the species is native to (i) mild (Koeppen letter C) or cold winter climates (Koeppen letter D); and (ii) hot (Koeppen letter a) or colder summer climates (Koeppen letters b/c). Model controls for phylogenetic autocorrelation and species' maximum growth height (see Supplementary Methods). Values reflect standardized data and can be interpreted as relative effect sizes.

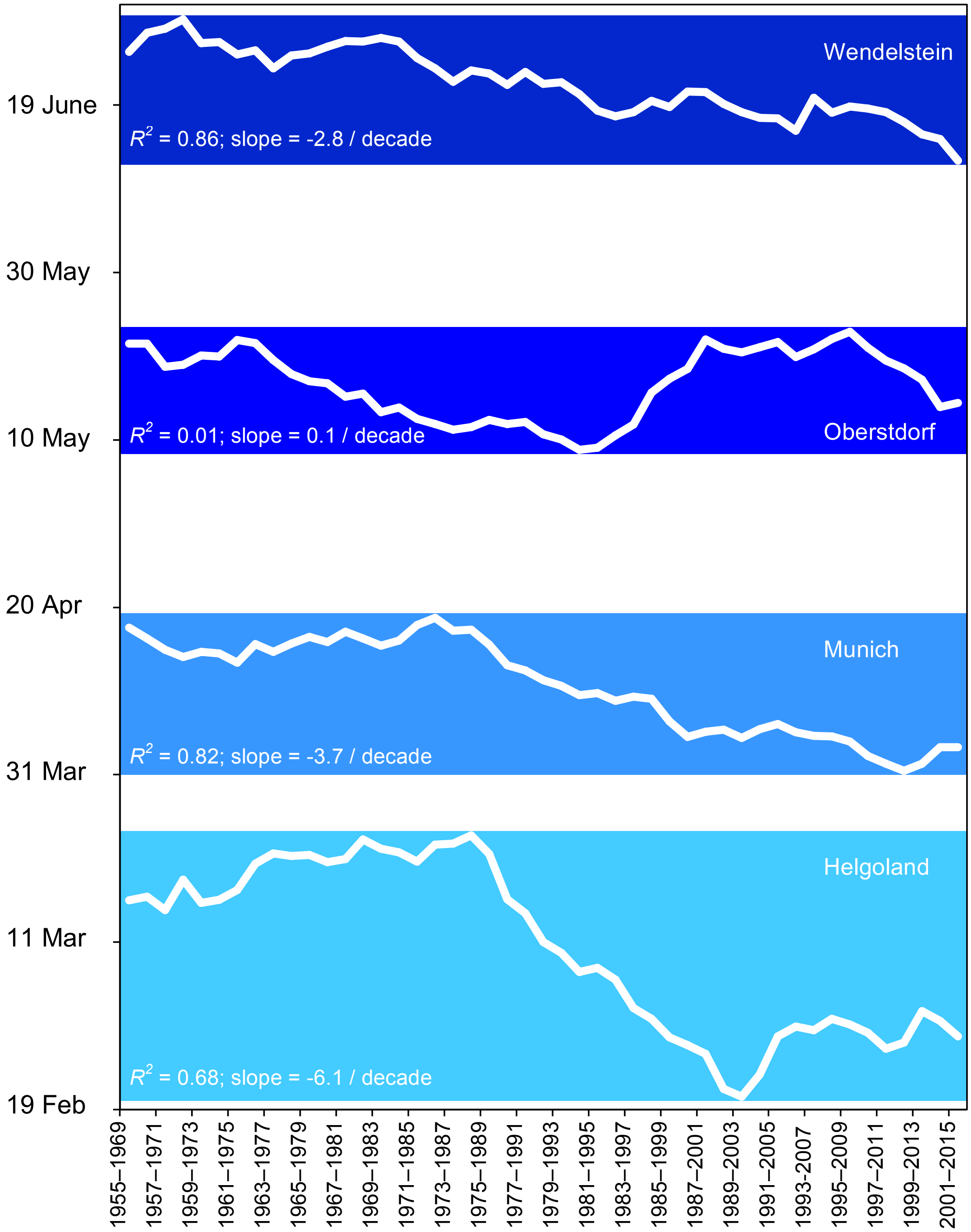

Figure S3 | Last frost events between 1955 and 2015 at four German weather stations with a 15-year moving window. Last frost events were defined as the latest day in spring with a minimum temperature below 0°C. Data for Helgoland (40 m a.s.l.; 54°10’N, 07°53’E), Munich (501 m a.s.l.; 48°08’N, 11°31’E), Oberstdorf (806 m a.s.l.; 47°25’N, 10°17’E), and Wendelstein (1832 m a.s.l.; 47°42’N, 12°00’E).

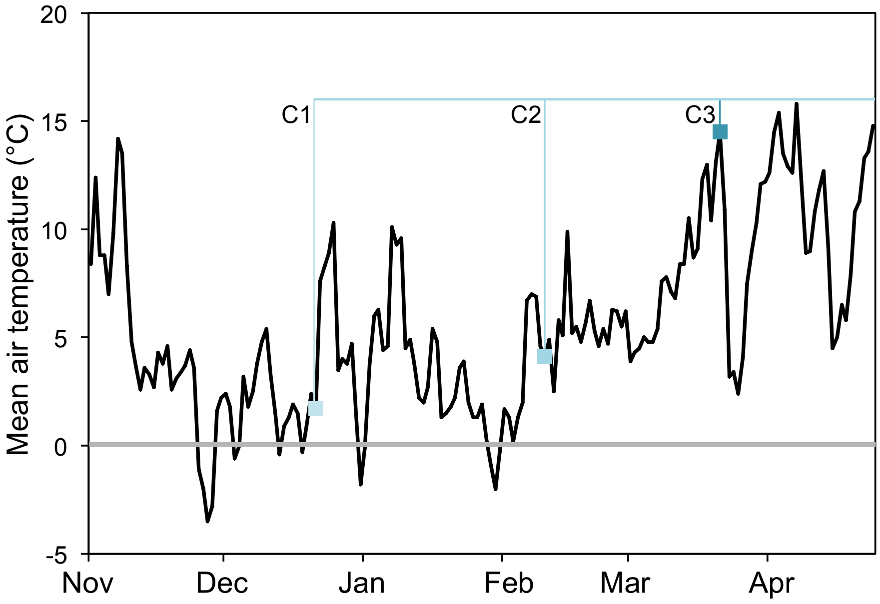

Figure S4 | Mean air temperature during the study period (Nov 2013 – Apr 2014) outside (black line) and in climate chambers (blue lines). C1 – C3: Daily mean air temperature in the climate chambers for different chilling treatments. C1: low chilling = 38 chill days, C2: intermediate chilling = 72 chill days, C3: high chilling = 88 chill days. Chill days were calculated as number of days with a mean air temperature <5°C from 1 November until start of the respective climate chamber treatment (C1, C2, C3).

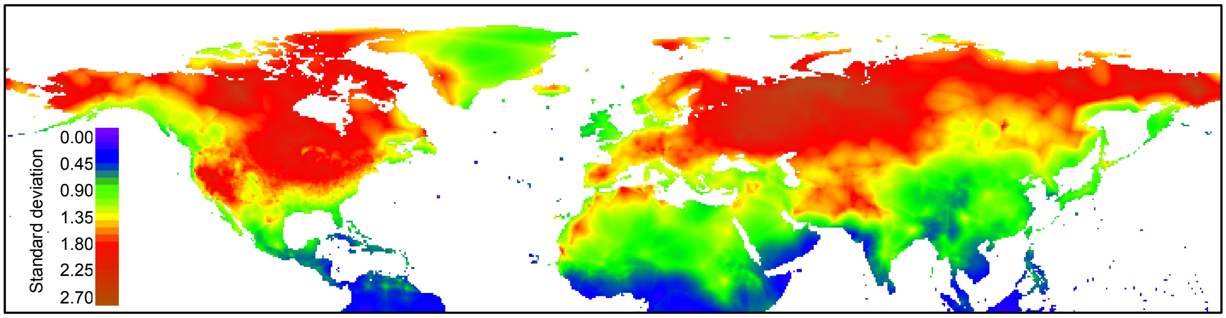

Figure S5 | Inter-annual spring temperature variability (T variability). T variability was calculated as the standard deviation of mean spring temperatures (March, April, and May) from 1901 to 2013. Data on monthly average temperatures during this period were available from the CRU database (5-arc minute spatial resolution data)[35].

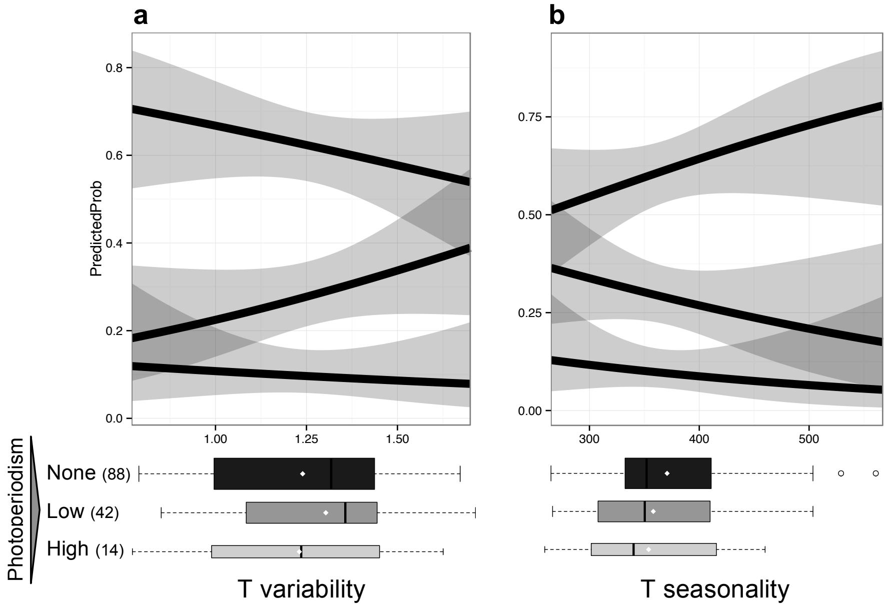

Figure S6 | Relationships between species' spring photoperiodism and the between-year spring temperature variability (a) and temperature seasonality (b) in their native ranges. **a**, Probability of species-specific photoperiod sensitivity as a function of median spring T variability in a species' native range (0.5 quantile; *P* = 0.43; univariate GLM). **b**, Probability of species-specific photoperiod sensitivity as a function of maximum T seasonality in a species' native range (0.95 quantile; *P* = 0.67; univariate GLM). Envelopes around each line show 95% confidence intervals. Boxplots for species' median T variability and maximum T seasonality when they were grouped according to photoperiod requirements are shown below the graph. Photoperiod requirements: None = No sensitivity; Low = Sensitivity to day length during early dormancy; High = Sensitivity to day length also in late dormancy (see Supplementary Fig. 1).

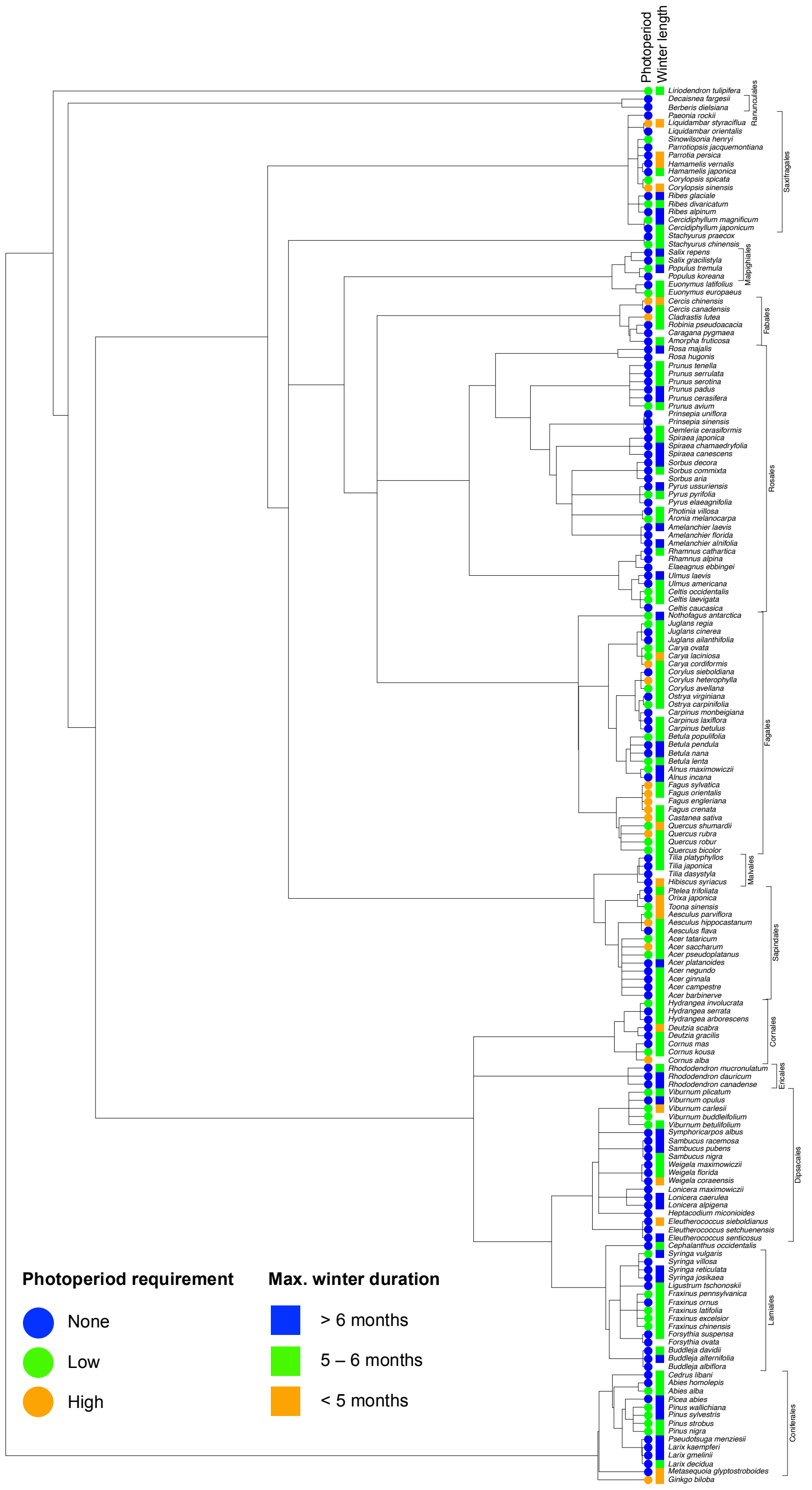

Figure S7 | PHYLOMATIC tree modified from Panchen *et al.*[42] containing the 173 woody species for which photoperiod requirements were studied. Species' photoperiod requirements and their maximum winter duration (0.95 quantile for the number of months with an average temperature below 5°C) are indicated by colored circles and squares, respectively.

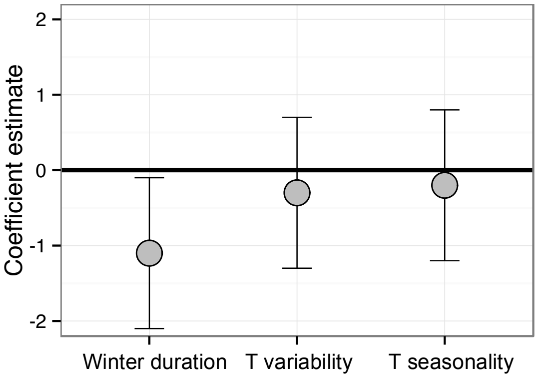

Figure S8 | Effect of species' climate parameters on variation in spring photoperiodism. Coefficient values (effective posterior means and 95% credible intervals) for relationships between species' photoperiodism and their winter duration (0.95 quantile), inter-annual spring temperature variability (T variability; 0.5 quantile), and temperature seasonality (T seasonality; 0.95 quantile). Note that in this model, photoperiod is treated as dependent variable (ordinal logistic regression). Models account for phylogenetic structure in the data and species' maximum growth height (see Supplementary Methods). Values reflect standardized data and can be interpreted as relative effect sizes. Sample sizes: N = 88 species (None), 42 (Low), 14 (High photoperiodism).

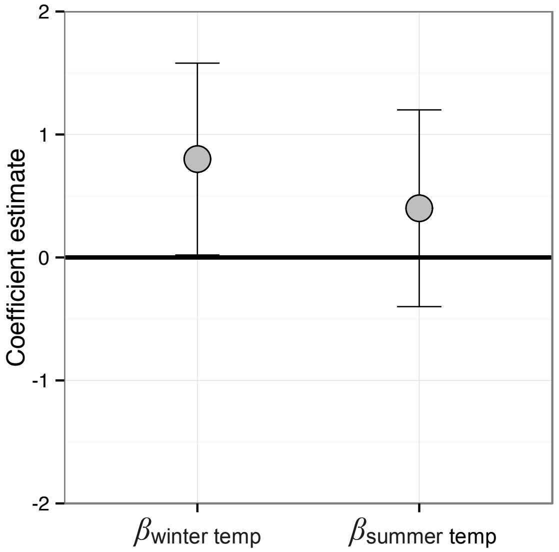

Figure S9 | The effect of winter and summer temperature regime on species-level variation in photoperiodism for 173 species using the Koeppen-Geiger climate classification. Coefficient values (effective posterior means and 95% credible intervals) for relationships between winter and summer climate and species' photoperiodism. Winter climate was included as a binary variable capturing whether a species is native to mild (Koeppen letter C) or cold winter climates (Koeppen letter D). Summer climate was included as a binary variable capturing whether a species is native to hot (Koeppen letter a) or colder summer climates (Koeppen letters b/c). The dependent variable (species' photoperiodism) was included as ordinal variable (no, low, high photoperiod requirements). To control for phylogenetic autocorrelation and a possible effect of species' growth habit, the model includes random genus and family effects and a fixed effect of species' maximum growth height (see Supplementary Methods). Values reflect standardized data and can be interpreted as relative effect sizes.

Table S1 | Photoperiod requirements, standard deviations in leaf-out dates / thermal requirements, maximum winter duration, predominant climate, and maximum growth height of 173 temperate woody species. The importance of photoperiod in regulating leaf-out (*Photo*) was inferred from twig cutting experiments conducted in this study and a previous study[12]. Species-specific standard deviations in leaf-out dates (*SD DOY*) or thermal requirements (*SD GDD*; growing degree days >0° from 1 Jan until leaf-out) were calculated on the basis of leaf-out dates available from the Munich Botanical Garden from 2012 to 2015. *Climate* refers to the predominant Koeppen-Geiger climate type in a species' native range. Maximum winter duration (*WD*) refers to species' 0.95 quantile for the number of months with an average temperature below 5°C in their native ranges. *Height* refers to the mature (maximum) recorded height of a species.

Genus	Species	Photo	SD DOY	SD GDD	Climate	WD	Height
Abies	*alba*	Low	5.56	42.35	Dfb	6	40
Abies	*homolepis*	None	-	-	Cfa	6	25
Acer	*barbinerve*	None	13.49	11.29	Dwb	5	8
Acer	*campestre*	None	10.61	21.07	Cfb	5	20
Acer	*ginnala*	None	13.96	24.22	Dfa	6	15
Acer	*negundo*	None	11.46	14.15	Dfa	5	15
Acer	*platanoides*	None	11.41	38.97	Dfb	7	30
Acer	*pseudoplatanus*	Low	-	-	Dfb	6	30
Acer	*saccharum*	High	10.34	9.17	Dfa	6	40
Acer	*tataricum*	Low	-	-	Dfa	6	15
Aesculus	*flava*	None	8.83	19.91	Cfa	5	30
Aesculus	*hippocastanum*	High	10.37	33.61	Csa	6	30
Aesculus	*parviflora*	Low	17.46	39.03	Cfa	4	4
Alnus	*incana*	None	9.6	15.64	Dfb	8	20
Alnus	*maximowiczii*	Low	9.27	42.17	Dfa	8	9
Amelanchier	*alnifolia*	None	10.18	20.57	Dfb	8	4
Amelanchier	*florida*	None	9.11	14.41	Dfb	-	4
Amelanchier	*laevis*	None	9.54	10.29	Dfb	7	8
Amorpha	*fruticosa*	None	5.1	21.67	Cfa	5	3
Aronia	*melanocarpa*	Low	12.01	22.49	Dfb	6	3
Berberis	*dielsiana*	None	14.72	10.89	Dwa	-	2
Betula	*lenta*	Low	11.73	44.74	Dfa	5	25

Table S1 continued.

Genus	Species	Photo	SD DOY	SD GDD	Climate	WD	Height
Betula	*nana*	None	11	11.24	Dfc	9	1
Betula	*pendula*	None	8.66	16.48	Dfb	7	30
Betula	*populifolia*	Low	9.18	15.06	Dfb	6	9
Buddleja	*albiflora*	None	13.03	31.09	BWk	-	4
Buddleja	*alternifolia*	None	13.15	10.98	BWk	8	5
Buddleja	*davidii*	None	-	-	Cwb	5	5
Caragana	*pygmaea*	None	14.08	25.66	Dwb	-	0.5
Carpinus	*betulus*	None	12.01	15.05	Dfb	5	25
Carpinus	*laxiflora*	None	12.12	22.2	Cfa	5	30
Carpinus	*monbeigiana*	None	12.07	22.94	Cwb	-	16
Carya	*cordiformis*	High	8.38	45.57	Cfa	5	35
Carya	*laciniosa*	Low	5.32	52.92	Dfa	4	30
Carya	*ovata*	Low	4.57	49.08	Dfa	5	27
Castanea	*sativa*	High	9.91	17.06	Cfb	5	30
Cedrus	*libani*	None	13.77	56.34	Csa	5	40
Celtis	*caucasica*	None	-	-	Csa	-	15
Celtis	*laevigata*	Low	5.68	21.43	Cfa	5	24
Celtis	*occidentalis*	Low	10.39	30.81	Dfa	5	24
Cephalanthus	*occidentalis*	None	4.19	73.28	Cfa	5	6
Cercidiphyllum	*japonicum*	None	11.76	26.9	Cfa	6	45
Cercidiphyllum	*magnificum*	Low	9.61	10.96	Dfa	8	10
Cercis	*canadensis*	None	6.38	29.72	Cfa	5	9
Cercis	*chinensis*	High	7.72	27.42	Cwa	4	3.5
Cladrastis	*lutea*	High	10.41	24.9	Cfa	5	15
Cornus	*alba*	High	11.79	10.51	Dwa	-	3
Cornus	*kousa*	Low	9.54	11.38	Cfa	5	12
Cornus	*mas*	None	9.83	14.97	Cfb	5	5
Corylopsis	*sinensis*	High	9.98	9.97	Cfa	3	1.8
Corylopsis	*spicata*	Low	10.87	11.38	Cfa	-	2.4
Corylus	*avellana*	Low	10.74	19.2	Cfb	6	8
Corylus	*heterophylla*	High	10.28	29.62	Cfa	6	7
Corylus	*sieboldiana*	None	12.12	31.29	Cfa	6	5
Decaisnea	*fargesii*	None	13.57	27.98	Cfa	-	8
Deutzia	*gracilis*	None	13.99	20.6	Cfa	5	0.6
Deutzia	*scabra*	None	11.43	19.42	Cfa	4	4
Elaeagnus	*ebbingei*	None	11.32	18.87	-	-	3
Eleutherococcus	*senticosus*	None	13.38	15.76	Dwb	7	2
Eleutherococcus	*setchuenensis*	None	11.32	15.46	Dwb	-	4
Eleutherococcus	*sieboldianus*	None	11.00	22.99	Cfa	4	2

Table S1 continued.

Genus	Species	Photo	SD DOY	SD GDD	Climate	WD	Height
Euonymus	*europaeus*	Low	11.73	16.88	Cfb	5	6
Euonymus	*latifolius*	None	9.2	16.95	Dfb	6	3
Fagus	*crenata*	High	11.7	42.67	Dfa	6	35
Fagus	*engleriana*	High	10.8	32.4	Cwa	-	17
Fagus	*orientalis*	High	-	-	Cfa	6	45
Fagus	*sylvatica*	High	6.85	30.26	Cfb	6	40
Forsythia	*ovata*	None	13.2	3.62	Dwa	-	1.5
Forsythia	*suspensa*	None	12.14	11.13	Cfa	5	5
Fraxinus	*chinensis*	Low	11.62	44.04	Dwa	6	25
Fraxinus	*excelsior*	Low	6.78	60.31	Dfb	6	35
Fraxinus	*latifolia*	Low	5.51	43.32	Csb	5	25
Fraxinus	*ornus*	None	4.99	24.87	Cfa	5	25
Fraxinus	*pennsylvanica*	Low	3.87	52.37	Dfa	6	20
Ginkgo	*biloba*	High	10.23	43.61	Cfa	4	35
Hamamelis	*japonica*	None	12.12	22.54	Dfa	6	4
Hamamelis	*vernalis*	None	11.86	22.17	Dfa	4	4
Heptacodium	*miconioides*	None	12.69	14.23	Cfa	-	8
Hibiscus	*syriacus*	None	10.42	39.63	Cfa	3	4
Hydrangea	*arborescens*	None	10.34	15.49	Dfa	5	3
Hydrangea	*involucrata*	Low	11.24	11.01	Cfa	5	1
Hydrangea	*serrata*	None	13.96	14.52	Dfb	6	1.2
Juglans	*ailanthifolia*	None	13.07	70.66	Dfa	6	20
Juglans	*cinerea*	None	-	-	Dfa	6	24
Juglans	*regia*	Low	8.74	19.63	Cfb	5	25
Larix	*decidua*	None	12.39	6.61	Dfb	6	45
Larix	*gmelinii*	None	12.92	13.89	Dwb	8	30
Larix	*kaempferi*	None	12.01	12.6	Dfa	7	40
Ligustrum	*tschonoskii*	None	12.26	19.3	Cfa	6	3
Liquidambar	*orientalis*	None	9.11	16.37	Csa		40
Liquidambar	*styraciflua*	High	8.42	18.87	Cfa	3	35
Liriodendron	*tulipifera*	Low	13.67	20.31	Cfa	5	40
Lonicera	*alpigena*	None	-	-	Dfc	7	2
Lonicera	*caerulea*	None	15.44	20.49	Dfc	8	1
Lonicera	*maximowiczii*	None	14.45	24.21	Dwb	-	4
Metasequoia	*glyptostroboides*	None	12.28	23.49	Cwa	3	45
Nothofagus	*antarctica*	Low	14.24	26.78	Cfb	7	25
Oemleria	*cerasiformis*	None	15.5	42.43	Csb	5	5
Orixa	*japonica*	None	13.4	5.72	Cwa	4	3
Ostrya	*carpinifolia*	Low	9.31	14.49	Cfb	6	20

Table S1 continued.

Genus	Species	Photo	SD DOY	SD GDD	Climate	WD	Height
Ostrya	*virginiana*	None	10.54	14.17	Dfa	6	18
Paeonia	*rockii*	None	13.77	18.67	Cwa	-	3
Parrotia	*persica*	None	12.12	18.77	Csa	3	15
Parrotiopsis	*jacquemontiana*	None	9.56	12.52	Dwa	-	6
Photinia	*villosa*	None	9.95	5.89	Cwa	5	15
Picea	*abies*	None	7.77	27.56	Dfb	8	55
Pinus	*nigra*	Low	-	-	Cfa	5	40
Pinus	*strobus*	Low	-	-	Dfb	6	50
Pinus	*sylvestris*	Low	-	-	Dfb	8	30
Pinus	*wallichiana*	Low	-	-	Dsb	7	40
Populus	*koreana*	None	14.45	10.64	Dfa	-	15
Populus	*tremula*	Low	-	-	Dfb	8	20
Prinsepia	*sinensis*	None	24.76	8.08	Dwb	-	2
Prinsepia	*uniflora*	None	11.62	21.36	Dwa	-	2
Prunus	*avium*	Low	-	-	Cfb	6	25
Prunus	*cerasifera*	None	13.5	11.87	Dfa	7	15
Prunus	*padus*	None	12.29	12.27	Dfb	8	15
Prunus	*serotina*	None	12.44	9.03	Cfa	6	30
Prunus	*serrulata*	None	11.69	16.56	Cfa	5	12
Prunus	*tenella*	None	13.52	26.2	Dfb	6	1.5
Pseudotsuga	*menziesii*	None	-	-	Csb	8	70
Ptelea	*trifoliata*	None	-	-	Cfa	5	8
Pyrus	*elaeagnifolia*	None	12.71	32.72	Csa	-	6
Pyrus	*pyrifolia*	Low	11.81	11.17	Cwa	5	15
Pyrus	*ussuriensis*	None	16.38	16.43	Dwa	7	15
Quercus	*bicolor*	Low	8.66	46.62	Dfa	5	25
Quercus	*robur*	Low	9.32	27.39	Cfb	6	40
Quercus	*rubra*	High	11.73	49.14	Dfa	6	35
Quercus	*shumardii*	Low	10.23	33.49	Cfa	3	35
Rhamnus	*alpina*	None	7.33	32.11	Dfb	-	4
Rhamnus	*cathartica*	None	7.77	25.99	Cfb	6	6
Rhododendron	*canadense*	None	9.81	11.59	Dfb	7	1.2
Rhododendron	*dauricum*	None	12.87	17.44	Dwb	9	2
Rhododendron	*mucronulatum*	None	24.79	51.45	Dwb	6	2
Ribes	*alpinum*	None	12.5	7.93	Dfb	7	1.5
Ribes	*divaricatum*	Low	7.8	17.96	Dsb	6	3
Ribes	*glaciale*	None	5.51	11.3	Cwb	8	3
Robinia	*pseudoacacia*	None	6.65	29.42	Cfa	5	25
Rosa	*hugonis*	None	12.5	20.19	-	-	2

Table S1 continued.

Genus	**Species**	**Photo**	**SD DOY**	**SD GDD**	**Climate**	**WD**	**Height**
Rosa	*majalis*	None	13.5	10.5	Dfb	8	2
Salix	*gracilistyla*	None	13.52	8.14	Cfa	5	6
Salix	*repens*	None	8.81	17.79	Dfb	7	1
Sambucus	*nigra*	None	15.26	9.94	Dfb	6	6
Sambucus	*pubens*	None	13.33	11.98	Dfb	8	6
Sambucus	*racemosa*	None	13.38	3.84	Dfb	7	3
Sinowilsonia	*henryi*	Low	8.04	17.89	Cwa	-	8
Sorbus	*aria*	None	-	-	Cfb	-	10
Sorbus	*commixta*	None	12.61	10.69	Dfb	6	10
Sorbus	*decora*	None	8.26	21.84	Dfb	8	10
Spiraea	*canescens*	None	13.5	11.87	Dwb	7	4
Spiraea	*chamaedryfolia*	None	13.53	19.07	Dfa	7	1.5
Spiraea	*japonica*	None	13.15	16.97	Cwa	6	1.8
Stachyurus	*chinensis*	Low	11.03	46.21	Cfb	5	4
Stachyurus	*praecox*	None	15.2	34.01	Cfa	5	1.5
Symphoricarpos	*albus*	None	-	-	Csb	7	2
Syringa	*josikaea*	None	13.64	5.44	Dfb	7	4
Syringa	*reticulata*	None	11.9	13.26	Dwb	7	6
Syringa	*villosa*	None	14.01	14.08	Dwa	-	4
Syringa	*vulgaris*	Low	12.48	4.83	Dfb	7	7
Tilia	*dasystyla*	None	9.95	28.13	Dfa	-	30
Tilia	*japonica*	None	9.91	11.92	Cfa	6	20
Tilia	*platyphyllos*	None	9.43	16.91	Cfb	6	30
Toona	*sinensis*	Low	12.28	47.31	Cwa	2	25
Ulmus	*americana*	None	10.44	17.14	Dfa	6	30
Ulmus	*laevis*	None	8.96	37.07	Dfb	7	30
Viburnum	*betulifolium*	Low	-	-	Cfa	6	3
Viburnum	*buddleifolium*	Low	14.18	22.55	Cfa	-	5
Viburnum	*carlesii*	Low	13.15	13.53	Cfa	4	2
Viburnum	*opulus*	None	10.75	20.61	Dfb	7	5
Viburnum	*plicatum*	Low	11.81	15.87	Cfa	5	3
Weigela	*coraeensis*	None	14.55	26.27	Dfa	4	5
Weigela	*florida*	None	10.05	14.75	Dwa	6	2.5
Weigela	*maximowiczii*	None	13.45	11.55	Dfa	6	1.5

Chapter 4

SPRING PREDICTABILITY EXPLAINS DIFFERENT LEAF-OUT TIMES IN THE NORTHERN HEMISPHERE WOODY FLORAS

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Nature (in review)

Title: Spring predictability explains different leaf-out strategies in the Northern Hemisphere woody floras

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Tables: 1

Abstract: Temperate zone trees and shrubs have species-specific requirements for winter duration (chilling) and spring warming that are thought to optimize carbon gain from leaf-out after significant frost risk has passed[1,2]. Climate-driven changes in bud break times should therefore depend on the historical frequency of frost occurrences in a given region. To date, however, regional differences in frost predictability have been largely ignored in phenology studies. We quantified continental-scale differences in spring temperature variability (STV) and species' leaf-out cues using chilling experiments in 215 species and leaf-out monitoring in 1585 species from East Asia, Europe, and North America grown under common climate conditions. The results reveal that species from regions with high STV and unpredictable frosts have higher winter chilling requirements, and, when grown under the same conditions, leaf out later than related species from regions with lower STV. Since 1900, STV has been consistently higher in North America than in Europe and East Asia, and indeed experimentally long or short winter conditions differentially affected species from the three regions, with North American trees and shrubs requiring 84% more spring warming for bud break, European ones 49%, and East Asian ones only 1% when experiencing a short winter. Such strong continental-scale differences in phenological strategies underscore the need for considering regional climate histories in global change models.

Main text: Rising spring temperatures have advanced the onset of the growing season in many deciduous species[3–5], affecting plant productivity and global carbon balance[6–8]. As shown by experiments and monitoring data, however, species differ greatly in the extent to which they rely on winter and spring temperatures to regulate leaf unfolding[9–12]. The two temperature signals interact, with species that need extended chilling unable to react to spring warming if winters are too short[11–13]. Hence, unfulfilled chilling requirements may halt the advance of spring leaf-out, as is already happening in seven European species analysed in this regard[14]. Previous work on the budbreak phenology of temperate species has largely ignored the potential contributions of local climate history (but see Lechowicz[15]), despite the fact that such histories will likely constrain the response of vegetation to ongoing climatic change.

Temperate woody plants face a trade-off between early carbon gain (early leaf expansion) and avoidance of frost damage (late leaf expansion)[1]. In regions with high spring temperature variability (STV) and unpredictable frosts, plants might have evolved 'safe' strategies and delay leaf unfolding until the risk of late frost damage has passed[15]. To test for possible regional differences in spring frost predictability we compiled STV throughout the Northern hemisphere, by computing a global map of the standard deviation of minimum spring temperatures over the past 100 years, using the Climatic Research Unit (CRU) time-series dataset[16]. Our analysis revealed marked continental-scale variation in STV, with peaks in eastern North America and northeastern Europe. STV was lowest in East Asia (EA).

To test whether regional differences in STV have led to different phenological strategies of the woody floras of North America (NA), Europe (EU), and EA, we combined experimental and monitoring data for a representative set of species. Species' winter chilling requirements were inferred from twig-cutting experiments in 215 species from 92 genera in 46 families from throughout the Northern Hemisphere. Leaf-out dates for 498 species (145 genera in 60 families) were collected over four years (2012 to 2015) in the Munich Botanical Garden, including the 215 species used in the experiments. We additionally analysed leaf-out dates from 1458 species (281 genera in 99 families) observed in 2012 at five other Northern Hemisphere gardens[17]. We first linked a species' leaf-out behaviour to its biogeographic region (NA, EU, EA), and then tested for effects of STV on leaf-out dates and chilling requirements.

Phenological traits in species from throughout the Northern Hemisphere are influenced by species' shared evolutionary history[17]. We therefore constructed a phylogeny that included all 1593 species for which experimental and monitoring data were available (Extended data Fig. 1). To estimate the phylogenetic signal in leaf-out dates (Munich data) and chilling requirements, we constructed two further phylogenies based on DNA sequences for 374 and 180 species, respectively (Extended Data Figs. 2 and 3). There was a strong phylogenetic signal in leaf-out dates (Pagel's $\lambda = 0.81$), and we therefore applied phylogenetic hierarchical Bayesian (HB) models to account for phylogenetic autocorrelation. Because trees tend to leaf-out later than shrubs and evergreen species later than deciduous species (see Panchen *et al.*[17] and our Extended Data Fig. 4b), we also included growth habit and leaf persistence in our HB models. The results showed that these two life-history traits do not statistically effect chilling requirements (Extended Data Fig. 4a,c,d).

Leaf-out strategies differed strongly by continent, with EA species having much lower requirements for winter chilling than NA species, and EU species intermediate (Figs. 1 and 2). In our experiments, 57% of the 73 NA species had high chilling requirements, whereas only 30% of the 48 EU and 5% of the 94 EA species had high chilling requirements (Extended Data Fig. 5). Under short winter conditions (C1 treatment), the forcing requirements (degree days >0°C until budburst) of NA species increased by 84% (median degree days C1/C3 treatment = 792/430), those of EU species by 49% (568/392), and those of EA species by only 1% (360/355), compared to long winter conditions (Fig. 1). An ANCOVA that included chilling treatments (C1–C3), habit (shrubs vs. trees), and continent (NA, EU, and EA) as predictor variables for species' forcing requirements revealed a significant ($P < 0.001$) interaction between species' chilling requirements and continent, i.e., chilling treatment had a greater effect on NA species than on EU and EA species (Fig. 1a, Extended Data Fig. 6, Extended Data Table 1). The effect of continent on chilling requirements remained significant when controlling for phylogenetic autocorrelation of phenological traits and when incorporating fixed effects for growth habit and leaf persistence in the HB model (Extended Data Fig. 5b). In line with this, in 12 (75%) of 16 families containing both NA and EA species, NA species had lower chilling requirements than EA species, while the opposite was true for only 2 (13%) of the 16 families (Extended Data Fig. 7a). Similarly, in 9 (53%) of 17 genera containing both NA and EA species, NA species had lower chilling requirements than EA species, while the opposite was only true for *Fraxinus* (Extended Data Fig. 7b). Results of the chilling experiment were unaffected by photoperiod treatment (Extended Data Fig. 8).

The leaf-out data for 1585 species show that across all gardens (each with a different subset of species), NA species flushed 5±2 and 9±2 (mean ± SD) days later than EU and EA species, respectively (Fig. 2a). This continent effect had a similar magnitude in shrubs, trees, evergreens, and deciduous species (Fig. 2a and Extended Data Table 2). For all gardens, our HB models controlling for shared evolutionary history, growth habit and leaf persistence revealed a significant difference between NA and EA species (Fig. 2b and Extended Data Fig. 5c). Accordingly, in 13 (46%) of 28 families containing both NA and EA species, NA species leafed out later (> 5 days) than EA species, while the opposite was true for only 2 (7%) of the 28 families (Extended Data Fig. 9).

To test our hypothesis that the observed continental-scale differences reflect species' adaptation to STV, we inferred the native climate conditions of 1137 species for which both leaf-out dates and experimental data were available, by querying over a million geo-referenced records from the Global Biodiversity Information Facility (GBIF) against climate grids for STV, mean annual temperature (MAT), and temperature seasonality (TS). We used MAT to test our expectation that species from cold climates are adapted to lower energy/temperature resources and therefore leaf-out earlier than species from more southern locations when grown together in a common garden[5] and TS to test for possible phenological differences between species from continental and oceanic climates[18,19]. To test for associations between species' leaf-out strategies and climate factors, we applied spatial and HB models (Fig. 3c,d). For the HB models, we determined the climate optimum for each species by calculating its 0.5 quantile (median) for the respective climate variable.

As expected under the hypothesis, species from areas with high STV had late bud break and high chilling requirements. In a partial correlation analysis that controlled for effects of MAT, STV was positively correlated with chilling requirements and leaf-out dates (partial $r^2 =$ 0.35 and 0.20, respectively, see Fig. 3c,d). Recursive partitioning analyses yielded similar results: of the 91 species from regions with high STV (>1.4), 50% had high chilling requirements, while only 9% of the 92 species from low STV had such requirements (Fig. 3b). The mean leaf-out date (day of the year; DOY) of the 97 tree species from regions with high STV (>1.2) was DOY 111, while the mean leaf-out date of 78 trees from regions with low STV was DOY 104—on average 7 days earlier. Similarly, in shrubs, the 158 species from regions with lower STV on average leafed out 7 days earlier than the 44 species from regions with high STV (DOY 95 and 102, resp.; Extended Data Fig. 10a). For both chilling requirements and leaf-out dates, the effect of STV remained significant when controlling for phylogenetic (HB models) and spatial autocorrelation (SAR models; Fig. 3c and Table 1). The effect of STV on leaf-out dates was consistent across all locations for which we had leaf-out data, i.e., in four gardens species from high STV leafed later than species from low STV (Extended Data Fig. 10b).

We also asked whether MAT and TS might explain the dissimilar leaf-out strategies among North American, European, and East Asian species. In accordance with earlier studies[5,20], there was a positive association between MAT and leaf-out dates (Table 1, inset Fig. 3c, and Extended Data Fig. 10b). This, however, does not explain the observed early leaf-out of East

Asian species; on average these species experience warmer MAT than European and North American species (as shown in Extended Data Fig. 11). With respect to chilling requirements, MAT had little predictive power (Table 1 and inset Fig. 3c), and the continent effect on leaf-out strategies also remained significant when controlling for MAT in HB models (Extended Data Fig. 5b,c). Another possible explanation for the continental-scale differences in leaf-out phenology could be that modern-day North America, and especially its eastern part from which most (86%) of our 419 American species originate, has a high TS (Extended Data Fig. 11). However, TS had little effect on both leaf-out dates and chilling requirements (Fig. 3, Extended Data Fig. 10b, and Table 1). This leaves STV as the best explanation for the different flushing strategies and suggests that leaf-out phenology in the modern North American woody flora is the result of high interannual fluctuations in spring temperatures that have selected for conservative growth strategies.

The west coast of North America, especially at low elevations, experiences less STV than does the eastern part (Fig. 3a). Hence, our STV hypothesis predicts that species restricted to western North America should have more opportunistic (earlier) leaf-out strategies. To test this, we contrasted the leaf-out dates of western North American species against eastern North American, European, and East Asian species. The results matched our prediction. On average, the leaf-out dates of western North American species preceded those of eastern North American species by 12 days (Extended Data Fig. 12a and Extended Data Table 3). In phylogenetic HB models, western North American species leafed out significantly earlier than eastern ones and did not differ from the leaf out times of European and East Asian species (Extended Data Figure 12b).

Previous work has emphasized the importance of *latitudinal* variation in phenological strategies[5]; this is the first study to report *longitudinal* differences in the leaf-out strategies of woody floras of the Northern Hemisphere. The finding that species from East Asia require significantly less chilling before leaf out than their North American relatives suggests that these continents' forests will react differently to continuing climate warming: earlier leaf-out in North American trees and shrubs will be constrained by unmet chilling requirements as winters get warmer, whereas East Asian woody species, lacking such winter requirements, may opportunistically benefit from increased carbon gain and nutrient uptake[6,7,21]. Hence, with continuing climate warming, the conservative growth strategies in many North American species might have adverse consequences for them and cause greater openness to invasion by pre-adapted exotics. This may help explain the invasive capacities of introduced Asian and European woody species in eastern North America[22–25]. Surprisingly little is known so far about long-term changes in spring frost damage (but see Augspurger[26]) or hail frequency[27,28], but our results underscore the need for considering regional climate histories and their evolutionary effects on species pools in global change models.

Methods

Phenological monitoring and experiments

Multi-annual observational data on leaf-out

Observations and experiments were carried out between January 2012 and June 2015 in the botanical garden of Munich. Leaf-out dates of 498 woody species (from 840 individuals; on average two individuals per species were monitored) growing permanently outdoors without winter protection in the botanical garden of Munich were monitored in spring 2014 and 2015 and combined with leaf-out data for 2012 and 2013 for the same species available from our earlier study[5]. As in Zohner and Renner[5], a plants' leaf-out date was defined as the day when at least three branches on that plant had leaves pushed out all the way to the petiole. To obtain our response variable (species leaf-out date), we first calculated the mean of all individual flushing dates for the respective species and year (2012–2015) and then calculated the average over the four years. Twig cutting experiments (next section) were conducted on 144 of the 498 species (listed in Table S4). To cross validate our results obtained from the Munich leaf-out data, we used leaf-out data from 1487 species observed at five Northern hemisphere gardens available from Panchen *et al.*[17] (Fig. 2, Extended Data Table 2).

Twig cutting experiments to test the effects of chilling on leaf-out

To study the relative importance of chilling in a broad range of temperate woody species, we carried out twig cutting experiments under controlled conditions, which can be used as adequate proxies for inferring phenological responses of adult trees to climatic changes[13,29]. Twig-cutting experiments were newly conducted on 144 of the 498 temperate woody species for which we had leaf-out data (see Extended Data Table 4 and Extended Data Fig. 13 for species selection). Data from the same type of experiments for 71 further species are available from the literature and were later added (see below). To investigate species-specific chilling requirements we implemented a climate chamber experiment with three chilling treatments. In winter 2013/2014, c. 40 cm-long twigs were collected three times for each species (10 replicate twigs per species and collection). Twigs were cut on 21 Dec (referred to as short chilling treatment 'C1'), 10 Feb (intermediate chilling treatment 'C2'), and 21 March (long chilling treatment 'C3') [Extended Data Table 5]. Temperatures in the climate chambers ranged from 18°C during the day to 14°C at night. We standardized photoperiod throughout the experiment by applying a constant day length of 16 h. To test for a possible effect of short-day conditions we also ran the experiment under a day length of 8 h (see Extended Data Fig. 8). Immediately after cutting, we cleaned twigs with sodium hypochlorite solution (200ppm active chlorine) and placed them in water bottles enriched with the broad-spectrum antibiotics gentamicin sulfate (40 microg/l; Sigma–Aldrich, Germany)[13,30]. Water was changed twice a week, and twigs were trimmed weekly by about 2 cm. Bud development was monitored every third day. The leaf-out dates of the first 8 twigs that leafed out were recorded, and a twig was scored as having leafed out when three buds had their leaves pushed out all the way to the petiole.

Assignment of species to chilling categories

Results of our own twig cutting experiments were used to categorize the 144 species in terms of their chilling requirements. We therefore assessed the effects of the treatments on the forcing requirements of species (sum of growing degree days [GDD] from 21 Dec until budburst using 0°C as base temperature). Climate data outside and in the climate chambers were obtained from Hobo data loggers (Onset Computer Corp., Bourne, MA, USA).

If the median forcing requirements under C1 treatment (collection date = 21 Dec; see Extended Data Table 5) were less than 75 GDDs higher than under C3 (Collection date = 21 March), a species was assigned to the category *no chilling* requirements. If the difference was higher than 75 GDDs, a species was scored as *intermediate chilling*. If the forcing requirements under C2 (Collection date = 10 Feb) were more than 75 GDDs higher than under C3, a species was scored as *high chilling*. Information on the chilling requirements of 71 additional species came from studies, which used the same experiment to detect species' chilling requirements[11,12], and we applied the same definition for chilling categories to their data. This resulted in chilling data for a total of 215 species (Extended Data Table 4 and Extended Data Fig. 13).

Continental effect on phenological traits

To obtain information on the native distribution area for our 1593 species, we used floristic information available from the USDA PLANTS database[31], eflora[32,33], *http://linnaeus.nrm.se/flora/welcome.html*, and *http://www.euforgen.org/distribution-maps/* and grouped species according to their main geographic region: North America (NA), South America (SA), Europe (EU), West Asia (WA) and East Asia (EA). The Ural Mountains were defined as the right border of Europe; Europe and Asia were separated by the Turgai Sea throughout the Paleocene and into the Eocene[34]. Species that do not occur in one of the defined regions were excluded from analysis.

To detect a possible continent effect on species-level chilling requirements, we tested for differential effects of chilling treatments among species from NA, EU, and EA using ANCOVA (Fig. 1a and Extended Data Table 1). SA and WA were not included in the analysis because of the few species available from these regions (chilling data for 1 SA, and 5 WA species; see Extended Data Table 4). We included chilling treatments (C1–C3), growth habit (shrubs vs. trees), and continent (NA, EU, and AS) as predictor variables of species' forcing requirements (GDD >0°C until leaf-out) and found a highly significant (P <0.001) interaction between species' chilling requirements and continent, i.e., chilling treatment had a greater effect on NA than on EU and EA species (Fig. 1, Extended Data Table 1). Extended data Fig. 6 shows the results when using days to leaf-out after collection instead of GDDs as response variable. Extended Data Fig. 8 compares the results obtained when exposing twigs to long-day (16-h) and short-day (8-h) conditions in the greenhouse.

To detect effects of biogeographic origin on species-specific leaf-out dates, for each garden, we contrasted the leaf-out dates of NA, EU, and EA species against each other, when using all available species or including only certain functional categories, i.e., trees, shrubs, deciduous, and evergreen species (Fig. 2). Contrasts with sample sizes below 20 species per continent are not shown (grey fields in heat maps). For a summary of leaf-out dates in NA, EA, and EU species monitored at six gardens see Fig. 2a and Extended Data Table 2.

To further validate the results we applied a hierarchical Bayesian (HB) approach, which accounted for the phylogenetic structure in our data and allowed us to control for the effect of growth habit (trees *vs.* shrubs), leaf persistence (evergreens *vs.* deciduous species; see Panchen *et al.*[17] and our Extended Data Fig. 4) and modern climate association (see Zohner & Renner[5] and our Fig. 3 and Extended Data Fig. 10) on species-specific leaf-out strategies; for explanation of the HB model see section on "*Trait analysis using the Phylogenetic Comparative Method in a HB model*". To additionally test if the biogeographic differences in leaf-out strategies are consistent within different phylogenetic clades, we analysed continental-scale differences in leaf-out strategies and chilling requirements on the genus and family level (Extended Data Figs. 7 and 9).

Species ranges and climate characteristics

We obtained species' native distribution ranges, by extracting species location data from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/) using the *gbif* function of the dismo R-package[35]. To exclude unreliable records and reduce spatial clustering cleaning scripts in R were applied using the following criteria: (i) Only records from a species' native continent were included; (ii) coordinate duplicates at a resolution of 2.5-arc minutes were removed; (iii) records based on fossil material, germplasm, or literature were removed; and (iv) records with a resolution >10 km were removed. After filtering only species with more than 30 records within their native continent were included, resulting in data for 1137 species (1,411,996 presence records), of which we had leaf-out data for 1130 species and chilling information for 183 species.

To estimate the climatic range of each species, georeferenced locations were queried against grid files for mean annual temperature (MAT), temperature seasonality (TS), and inter-annual spring temperature variability (STV). MAT and TS were based on gridded information (2.5-arc minute spatial resolution data) from the Worldclim dataset (BIO 1 and BIO 7)[36,37]. STV was calculated as the standard deviation of mean minimum temperatures from March until May over the past 100 years (1901 – 2013). Gridded data on monthly minimum temperatures during this period were available from the Climatic Research Unit (CRU) time-series dataset[16] (version 3.00 with a spatial resolution of 5-arc minutes[38]). For each species, we determined the climate optimum by calculating its 0.5 quantile for the respective climate variable.

Relationships between climate parameters and species-specific leaf-out times and chilling categories

We tested for multicollinearity of our predictor variables by using a variance inflation factor (VIF) analysis, implemented in the R function "vif", from the package "HH"[39]. All VIF were smaller than 5 (threshold recommended by Heiberger[39]), indicating sufficient independence among predictor variables. We then ran random forest models (randomForest R library)[40,41], applied a hierarchical Bayesian approach (see section on "*Trait analysis using the Phylogenetic Comparative Method in a HB model*") to allow for phylogenetic autocorrelation in our dependent variables, and applied Simultaneous autoregressive (SAR) models controlling for spatial autocorrelation in the residuals (see section on "*Spatial regression between leaf-out strategies and bioclimatic parameters*"; Table 1). For analysis of leaf-out times we included only gardens with more than 200 species for which both leaf-out and climate data was available, i.e., the Arnold Arboretum, the Berlin Botanical Garden, the Munich Botanical Garden, and the Morton Arboretum (see Extended Data Fig. 10b). To study the set of ecological conditions determining species' chilling requirements and leaf-out dates, we carried out recursive partitioning analyses (R library "rpart"[42]; Fig. 3b and Extended Data Fig. 10a). We allowed three climate variables (MAT, TS, and STV), growth habit (trees vs. shrubs), and leaf persistence (evergreens vs. deciduous species) as potential split points and set the minimum node size to 30 (minimum number of species contained in each terminal node).

Validation: the eastern – western North American contrast

To further validate our conclusion that conservative growth phenologies are more abundant in regions with high STV, we examined contrasts between species restricted to eastern North America and western North America. Western North America is characterised by lower STV (Fig 3a) and we therefore expected species from there to display earlier leaf-out than eastern North American species. Because there was a high bias in coniferous species in our western-eastern North American comparison (25% conifers in western and only 4% conifers in eastern North America) we excluded them in the analysis of mean leaf-out dates (see Extended Data Fig. 12a and Extended Data Table 3). In a HB model we included conifers but controlled for this bias by including a gymnosperm effect (Extended Data Fig. 12b).

Trait analysis using the Phylogenetic Comparative Method in a HB model

Generating an ultrametric phylogenetic tree

To estimate the phylogenetic signal in species-level leaf-out dates and chilling requirements we created a phylogenetic tree for our 498 target species and used Pagel's λ[43] and Blomberg's K[44], with the 'phylosig' function in the R package 'phytools' v0.2-1[45]. To build the tree we used MEGAPTERA[46] and BEAST[47]. We gathered sequence information for four plastid genes (*atpB*, *matK*, *ndhF*, and *rbcL*) and included all species for which at least one of the four genes was available from GenBank (*atpB*: 107 species available, *matK*: 353 species, *ndhF*: 145 species, and *rbcL*: 264 species). This resulted in a concatenated matrix of 377 species and a total length of 6395 bp. We performed divergence time estimation under a strict clock model of molecular substitution accumulation, the GTR+G substitution model, and the Yule process as tree prior, implemented in BEAST (v1.8.0)[47]. To calibrate our tree we set the crown age of angiosperms to 185 Ma[48]; since absolute ages are not used in this study, we did not run our analyses with alternative calibrations. The phylogeny is presented as Extended Data Fig. 2. A reduced phylogeny of 180 species illustrating the phylogenetic signal of species' chilling requirements is shown in Extended Data Fig. 3.

The initial tree used to account for shared evolutionary history when testing for associations between leaf-out dates and biogeographic/climate parameters came from Panchen *et al.*[17] and had been assembled using the program Phylomatic[49] (Extended Data Fig. 1). Its topology reflects the APG III[50] phylogeny, with a few changes based on the Angiosperm Phylogeny Website[51]. We manually added missing species, which led to a total of 1630 species included in the tree. Branch lengths of the PHYLOMATIC tree are adjusted to reflect divergence time estimates based on the fossil record[48,52].

Analysis of phenological characters (leaf-out dates and chilling requirements)

We applied a hierarchical Bayesian (HB) approach (see Fridley & Cradock[53]) for testing effects of continental origin (NA, EU, EA; Fig. 2b and Extended Data Figs. 5b,c and 12b) and climate parameters (Fig. 3c,d and Extended Data Fig. 10b) on species-level differentiation in spring leaf-out dates and chilling requirements. This approach allows estimating species-level differences in leaf-out phenology while controlling for phylogenetic signal λ[43] of phenological traits. In addition it allowed us to test for effects of continental origin (NA, EU, and EA) on species' leaf-out dates and chilling requirements while controlling for (i) species' life history strategy by including growth habit (shrubs vs. trees) and leaf persistence (evergreen vs. deciduous species; see Fig. 2b) and (ii) species' modern climate association by including variables reflecting species' native climate conditions (MAT; see Extended Data Fig. 5b,c) in the model. Slope parameters across traits are estimated simultaneously without concerns of multiple testing or P-value correction. To incorporate phylogenetic autocorrelation across all relationships a common correlation matrix (Σ) based on shared branch lengths in the PHYLOMATIC tree was incorporated in the model[54]. The resulting posterior distributions of the relationships between biogeographic/climate parameters and phenological traits are a direct statement of the influence of each parameter on species-level differentiation in chilling requirements and leaf-out dates.

To examine relative effect sizes of climate variables on species-specific leaf-out times and chilling requirements, we standardized all climate variables by subtracting their mean and dividing by 2 SD before analysis[55]. When using leaf-out times (continuous character) as response variable (Pagel's λ value of leaf-out dates = 0.81; see Extended Data Fig. 2), the phylogenetic structure of the data was incorporated in the HB model using the Bayesian phylogenetic regression method of de Villemereuil *et al.*[54], by converting the 1630-species ultrametric phylogeny into a scaled (0–1) variance–covariance matrix (Σ), with covariances defined by shared branch lengths of species pairs, from the root to their most recent ancestor[56]. We additionally allowed correlations to vary according to the phylogenetic signal (λ) of flushing dates, fitted as a multiple of the off-diagonal values of Σ[54]. The phylogenetic variance–covariance matrix was calculated using the 'vcv.phylo' function of the ape library[57]. When using chilling requirements (ordinal data) as response variable we accounted for phylogenetic structure in our data by incorporating genus and family random effects in the model because λ estimation is not possible for ordinal (or logistic) models.

We parameterized our models using the JAGS[58] implementation of Markov chain Monte Carlo methods in the R2JAGS R-package[59]. We ran three parallel MCMC chains for 20,000 iterations after a 5,000-iteration burn-in, and evaluated model convergence with the Gelman and Rubin[60] statistic. We specified non-informative priors for all parameter distributions, including normal priors for fixed effect α and β coefficients (mean = 0; variance = 1000), uniform priors between 0 and 1 for λ coefficients, and gamma priors (rate = 1; shape = 1) for the precision of random effects of phylogenetic autocorrelation[53,54].

Spatial regression between leaf-out strategies and bioclimatic parameters

To determine if between-region differences in leaf-out strategies (leaf-out dates and chilling requirements) are attributable to between-region differences in STV we carried out a spatial regression analysis. We only included cells occupied by at least five species with existing phenological data. For each cell, the mean trait value was calculated and used for subsequent analyses. (For the calculation of mean chilling requirements in each cell, the chilling categories were treated as numerical characters: no chilling requirements = 0, intermediate = 1, high = 2.) We then aggregated all response and predictor variables to a spatial resolution of 2.5° x 2.5°; initially, the resolution of climate grids and species distribution data was 2.5-arc minutes (~0.05°). Next, we regressed the aggregated response variable against aggregated predictor variables.

As a first step, we applied partial regression analysis (to remove the covariate effects of MAT) and multiple ordinary least squares regression (OLS) between each response and all predictor variables. In the OLS models there was considerable spatial autocorrelation in the residuals (Moran's I test for leaf-out dates: I = 0.38, $P < 0.001$; Moran's I test for chilling requirements: I = 0.30, $P < 0.001$), potentially biasing significance tests and parameter estimates[61]. To remove the autocorrelation we applied simultaneous autoregressive (SAR) models[62,63] using the R-package spdep[64,65]. We used a spatial weights matrix with neighbourhoods defined as cells within 3,000 km of the focal cell. For all response variables the SAR models effectively removed autocorrelation from the residuals (Moran's I test for leaf-out dates: I = 0.001, $P = 0.52$; Moran's I test for chilling requirements: I = 0.001, $P = 0.43$). See Table 1 for parameter estimates and *P*-values inferred from the OLS and SAR models. Next, we examined all subsets of the full SAR models and selected the model with the lowest AIC score (for parameter estimates of the reduced models see $\text{SAR}_{\text{reduced}}$ in Table 1). As an additional statistical measure to evaluate the SAR models we calculated Akaike weights for all predictor variables by comparing AIC scores of models containing the focal variable with models omitting the focal variable (see $\text{Weight}_{\text{AIC}}$ in Table 1).

All statistical analyses relied on R[66].

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Author contributions

C.M.Z. and S.S.R. designed the study. C.M.Z. conducted the experiments and leaf-out observations. C.M.Z. and B.M.B. performed the analyses. C.M.Z. and S.S.R. led the writing with inputs from the other authors.

Figures

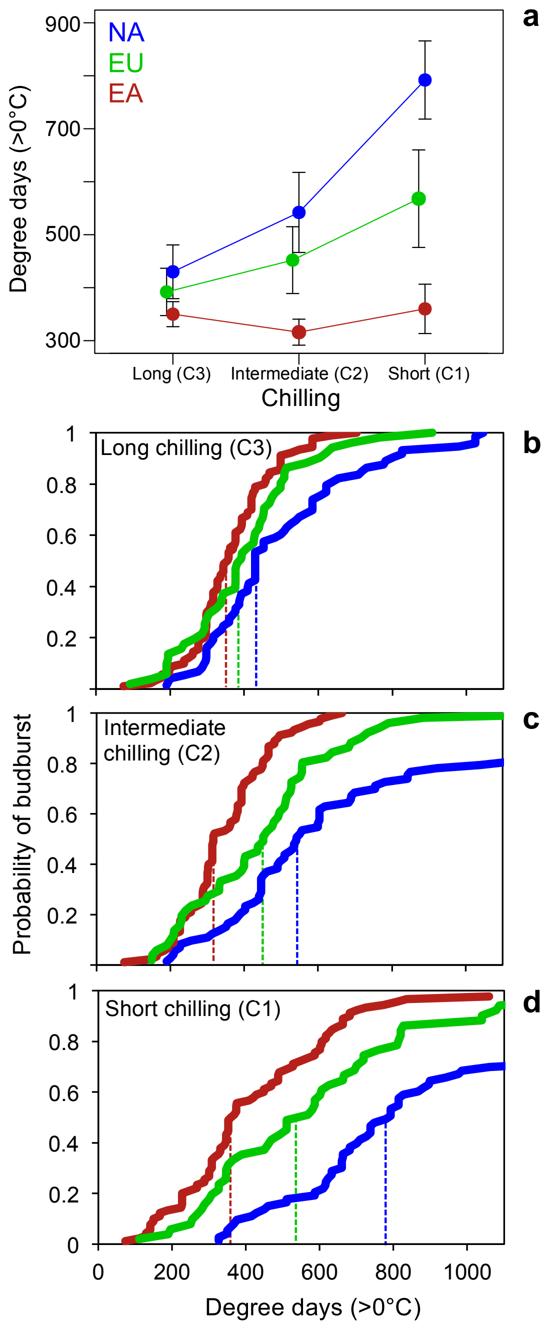

a
NA
EU
EA
Degree days (>0°C)
900
700
500
300
Long (C3)
Intermediate (C2)
Short (C1)
Chilling
b
Long chilling (C3)
c
Intermediate chilling (C2)
d
Short chilling (C1)
Probability of budburst
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
Degree days (>0°C)

Figure 1 | Contrasting responses of North American (NA), European (EU), and East Asian (EA) species to experimentally reduced winter chilling. a, Median forcing requirements (accumulated degree days >0°C outdoors and in a climate chamber) ± 95% CI until leaf-out under different chilling levels for NA (N = 72 species), EU (N = 48), and EA (N = 88) species. **b–d,** Leaf-out probability curves for NA, EU, and EA species calculated as their forcing requirements until leaf-out under different chilling treatments: (**b**) long chilling, (**c**) intermediate chilling, and (**d**) short chilling. Dashed lines indicate median forcing requirements for NA, EU, and EA species.

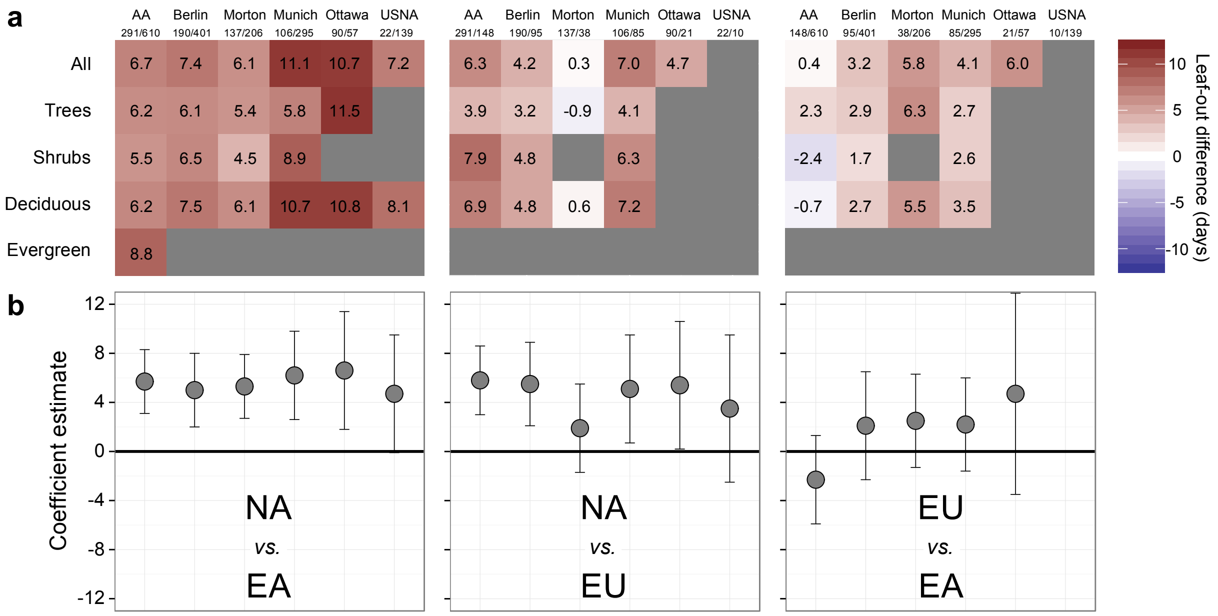


Figure 2 | Contrasting leaf-out dates of North American (NA), European (EU), and East Asian (EA) species. a, Heat maps for the difference in species-level leaf-out dates between NA and EA species (left panel), NA and EU species (middle panel), and EU and EA species (right panel) monitored at six gardens when all species, or only trees / shrubs / deciduous / evergreen species were included (see Extended Data Table 2). *AA*: Arnold Arboretum, Boston, MA, USA; *Berlin*: Botanical Garden and Botanical Museum Berlin-Dahlem, Berlin, Germany; *Morton*: Morton Arboretum, Lisle, IL, USA; *Munich*: Munich Botanical Garden, Munich, Germany; *Ottawa*: Ottawa Arboretum, Ottawa, Canada; and *USNA*: US National Arboretum, Washington, DC and Beltsville, MD, USA. Sample sizes for each continent at the respective garden are shown below garden names. Contrasts with sample sizes below 20 species per continent are not shown (grey fields in heat map). **b,** Coefficient values (effective posterior means and 95% credible intervals) for differences in leaf-out dates between NA and EA species, NA and EU species, and EU and EA species. Models include phylogenetic autocorrelation and fixed tree and evergreen effects. Values reflect standardized data and can be interpreted as relative effect sizes.

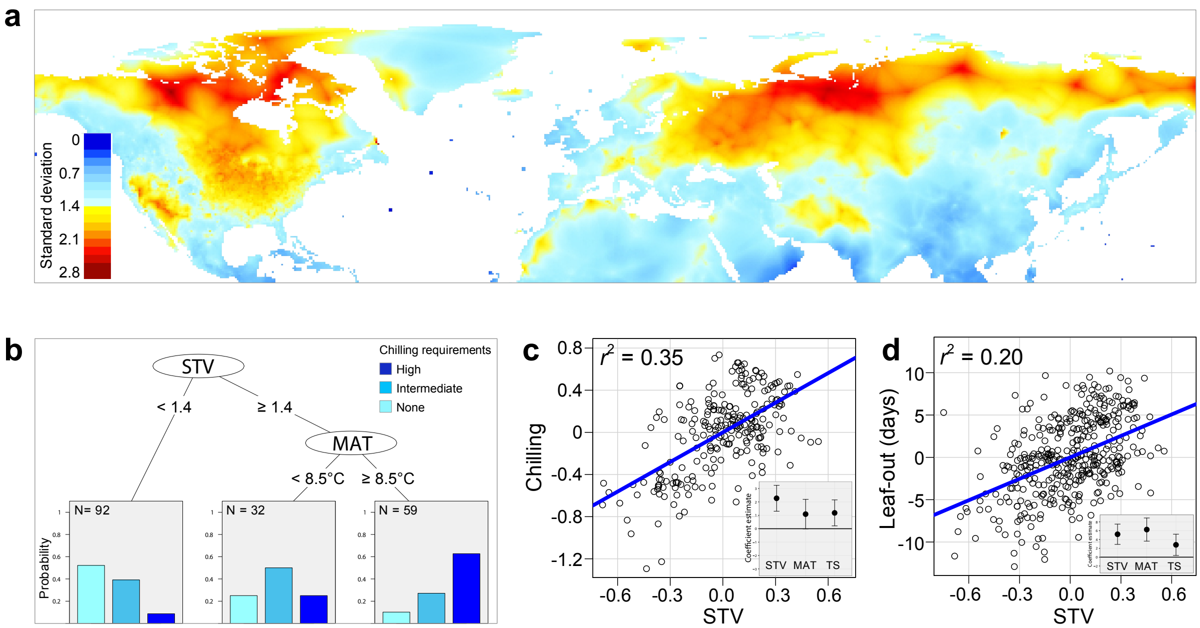
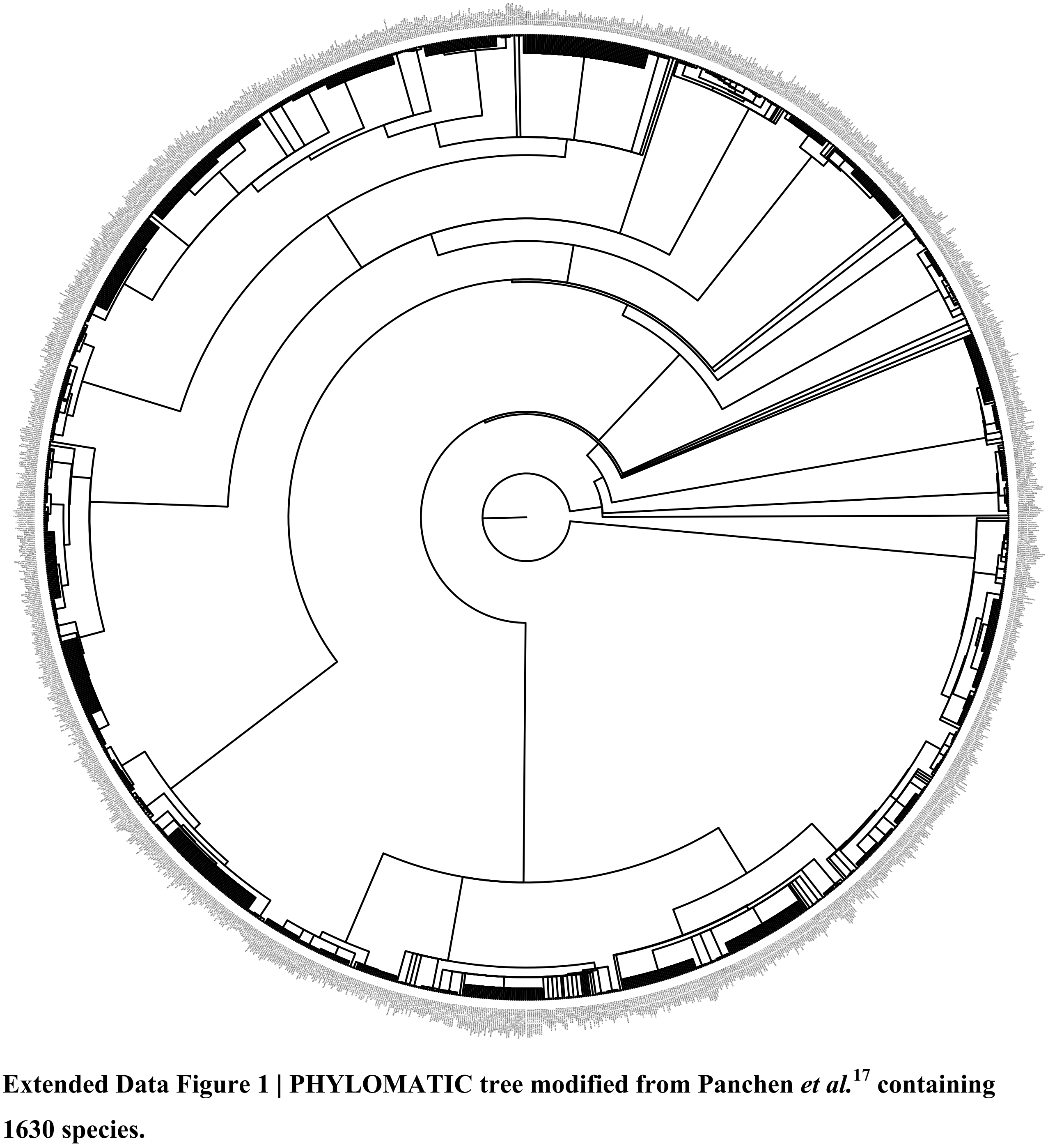


Figure 3 | The effect of spring temperature variability on leaf-out strategies in Northern Hemisphere woody plants. a, Inter-annual spring temperature variability (STV) calculated as SD of minimum temperatures between March and May from 1901 to 2013. **b**, Recursive partitioning tree for the relationship between climate parameters and species-specific chilling requirements in temperate woody species. STV**,** mean annual temperature (MAT), temperature seasonality (TS), growth habit, and leaf persistence were evaluated as potential split points. Number of species contained in each terminal node shown below graphs. **c**,**d**, The relationship between global STV and proportional mean chilling requirements (**c**) and mean Munich leaf-out times (**d**) within 2.5° × 2.5° regions as shown by partial-regression plots after controlling for MAT (see Table 1). Insets show estimated coefficient values (means and 95% credible intervals) from phylogenetic hierarchical Bayesian models for relationships between three climate variables (STV, MAT, and TS) and species' (**c**) chilling requirements (N = 183 species) and (**d**) Munich leaf-out dates (N = 366 species). Values reflect standardized data and can be interpreted as relative effect sizes.

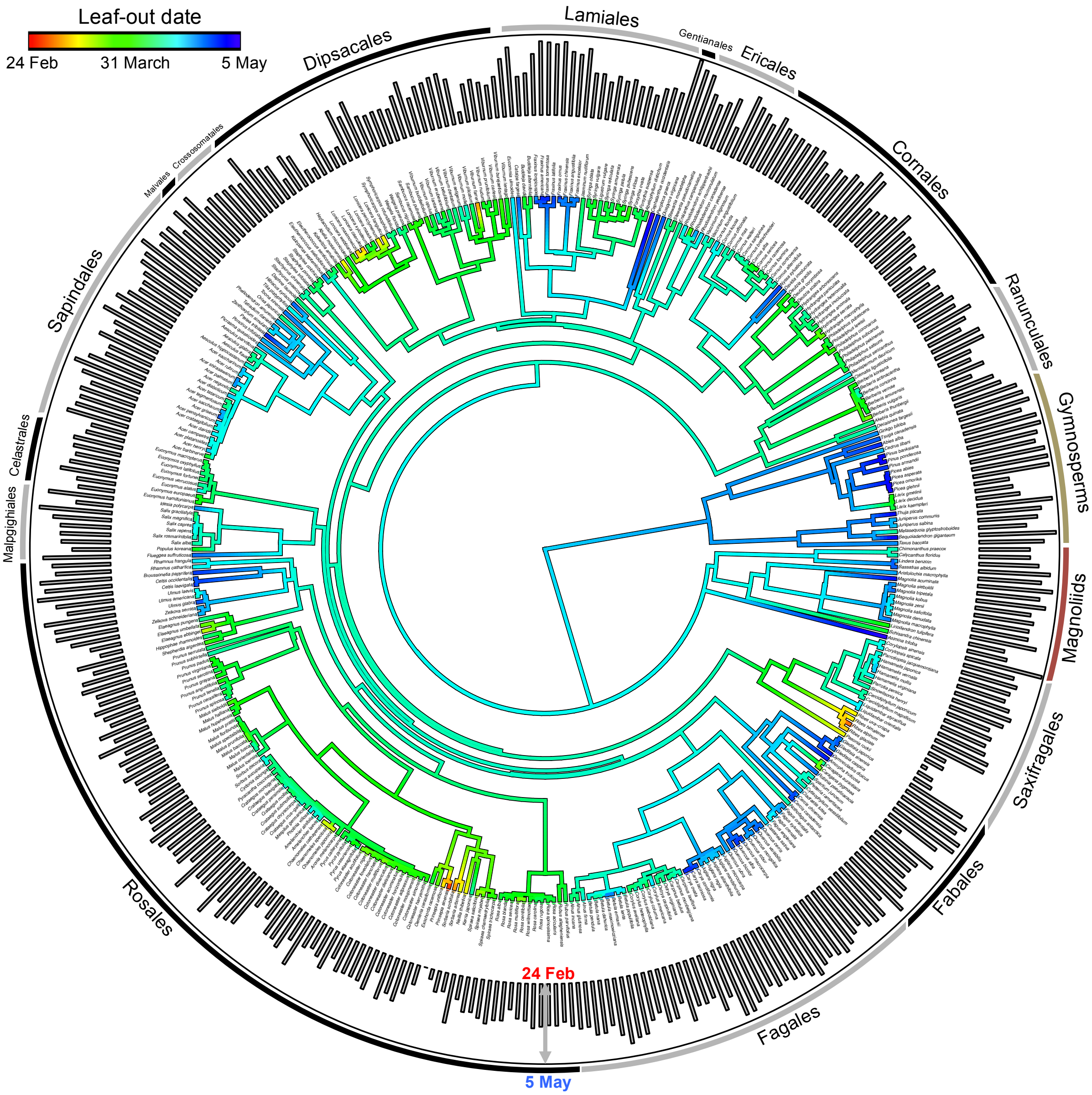
Table 1 | Relationships between climate variables and global patterns of leaf-out times and chilling requirements. MAT, mean annual temperature; TS, temperature seasonality; STV, spring temperature variability. Five comparative measures were used: the coefficient of determination from bivariate partial regression (partial r^2), standardized regression coefficients from multivariate ordinary least-squares regression (OLS), standardized regression coefficients from simultaneous autoregressive models (SAR), Akaike weights based on SAR models, mean decrease in accuracy values (MDA) from random forest analysis, and coefficient estimates (effective posterior means and 95% credible intervals) from a hierarchical Bayesian (HB) model controlling for phylogenetic autocorrelation.

	partial r^2	**OLS**	**SAR**	**SAR$_{reduced}$**	**Weight$_{AIC}$**	**MDA**	**HB**
Leaf-out times (Munich, N = 366 species)							
MAT	0.19***	0.43***	0.37***	0.39***	1.00	40.2	6.3 ± 1.3
TS	0.01	-0.01	-0.08		0.34	23.0	2.8 ± 1.2
STV	0.20***	0.51***	0.36***	0.33***	1.00	42.9	5.2 ± 1.2
Chilling (N = 183 species)							
MAT	0.07***	0.22***	0.06		0.49	14.5	1.1 ± 1.1
TS	0.01*	-0.37***	-0.18**	-0.22***	0.97	39.0	1.2 ± 1.0
STV	0.35***	0.70***	0.28***	0.29***	0.99	85.0	2.3 ± 0.9

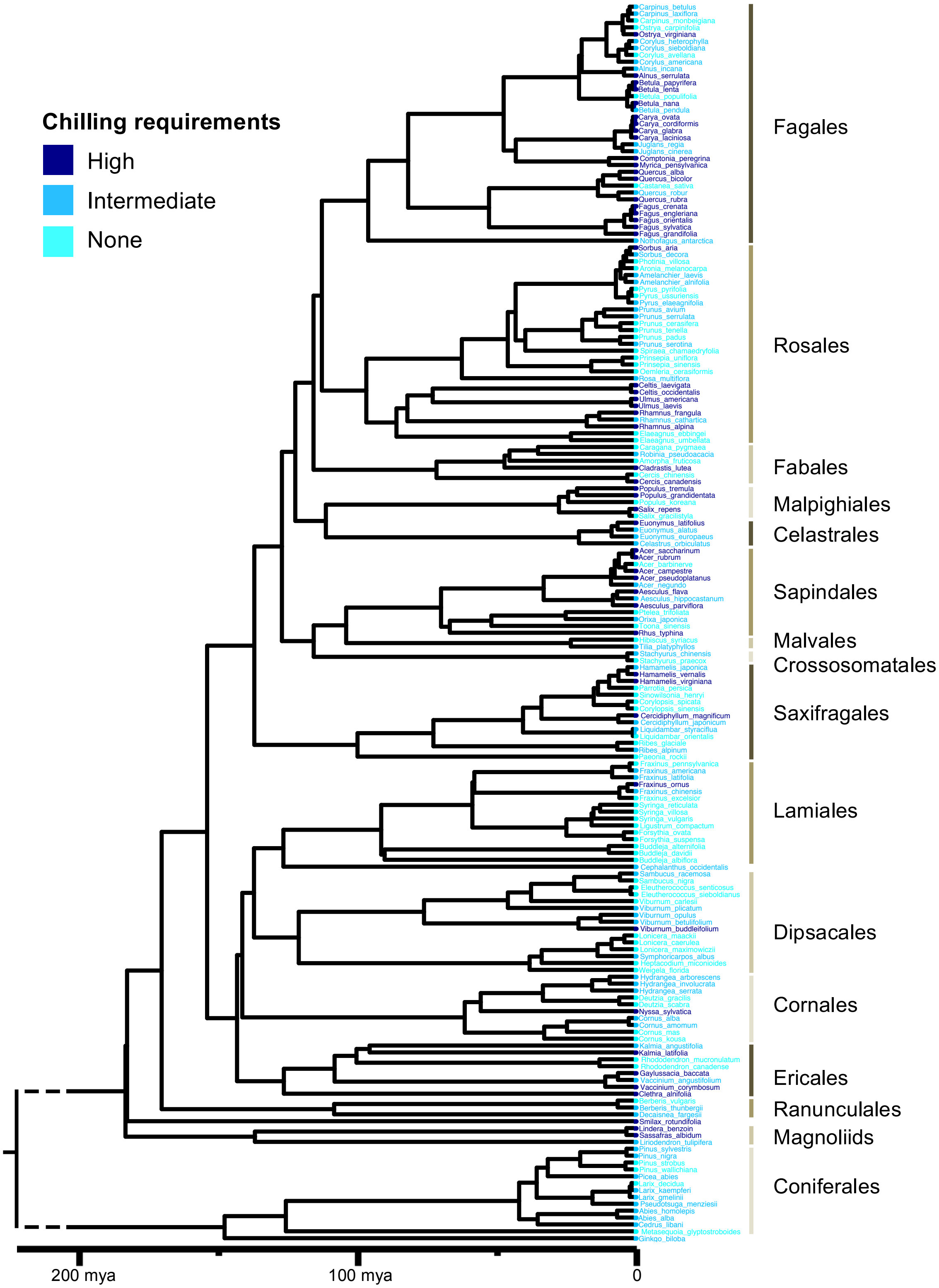
Extended data



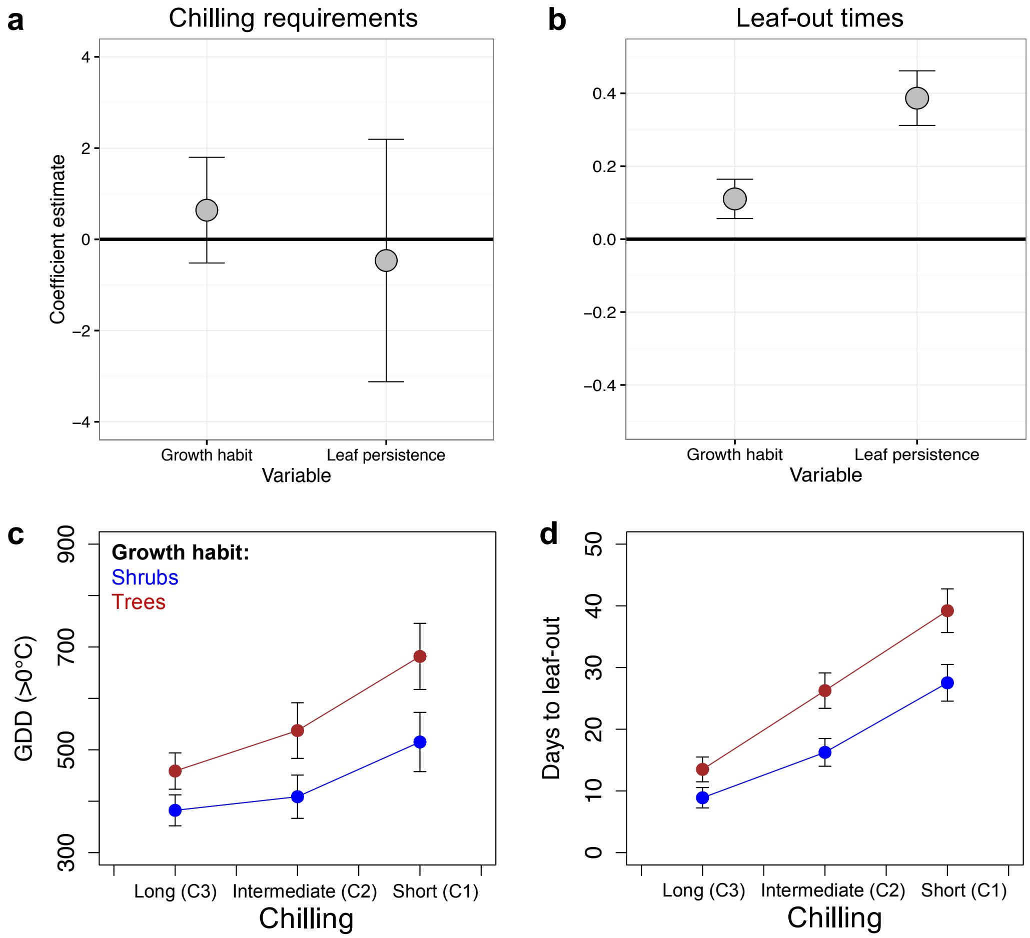
Extended Data Figure 1 | PHYLOMATIC tree modified from Panchen *et al.*[17] containing 1630 species.



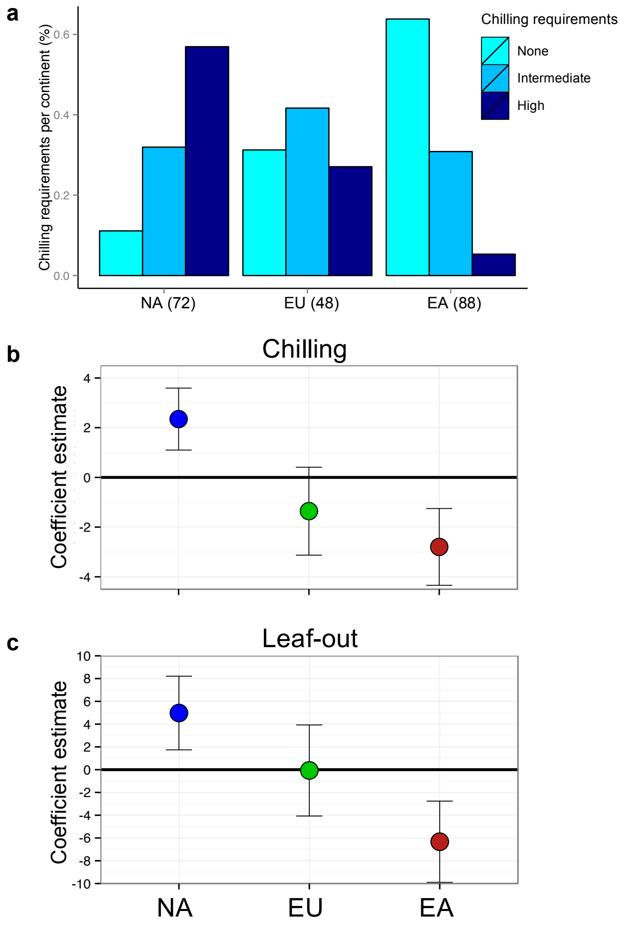
Extended Data Figure 2 | Phylogeny of 374 woody temperate species with mean leaf-out dates observed in the Munich Botanical Garden (between 2012 and 2015) indicated by colours and the outermost bars. Pagel's $\lambda = 0.81$, $P < 0.001$; Blomberg's $K = 0.06$, $P < 0.001$.



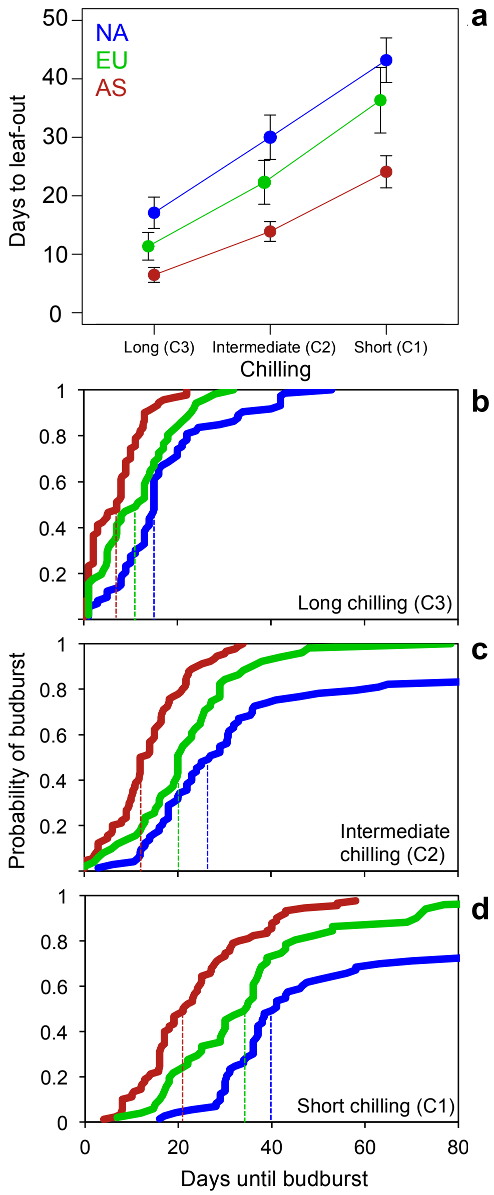
Extended Data Figure 3 | Phylogeny of 180 woody temperate species with their chilling requirements indicated by the colours.



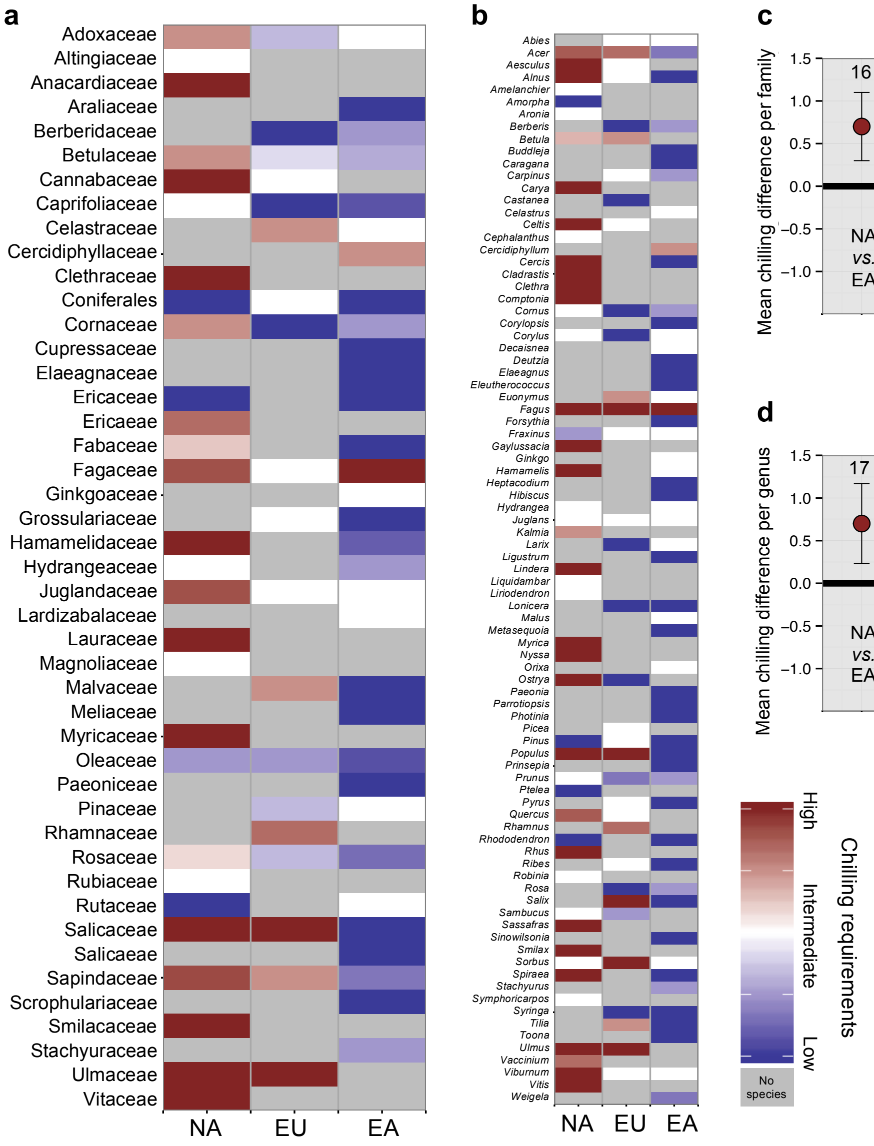
Extended Data Figure 4 | The effect of growth habit and leaf persistence on species' leaf-out strategies. a, **b**, Coefficient values (effective posterior means and 95% credible intervals) for the effect of growth habit (shrubs vs. trees) and leaf persistence (deciduous vs. evergreen species) on species-specific (**a**) chilling requirements and (**b**) leaf-out dates (Munich data). Chilling data: 108 shrubs and 107 trees, 202 deciduous and 13 evergreen species; Leaf-out data: 295 shrubs and 203 trees, 470 deciduous and 28 evergreen species. To account for phylogenetic autocorrelation, we inserted genus and family random effects for ordinal chilling categories or incorporated the phylogenetic structure using the Bayesian phylogenetic regression method for leaf-out dates. Values reflect standardized data and can be interpreted as relative effect sizes. **c, d,** Median forcing requirements (accumulated degree days >0°C outdoors and in a climate chamber) ± 95% CI (**c**) and median days until leaf-out in a climate chamber ± 95% CI (**d**) under different chilling levels for trees (red curve, N = 107 species) and shrubs (blue curve, N = 108 species).



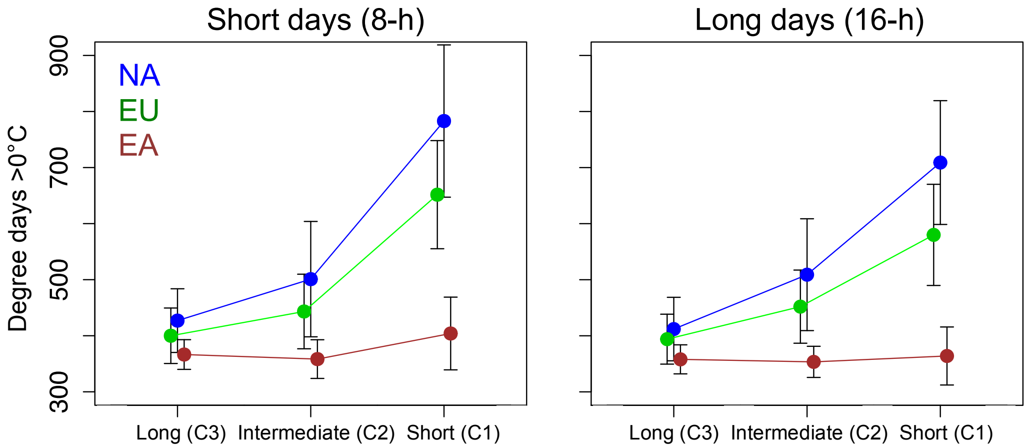
Extended Data Figure 5 | Contrasting leaf-out strategies in North America (NA), Europe (EU), and East Asia (EA). a, Effect of continental origin on species-specific chilling categories. Number of species with available data shown in brackets. **b**, Estimated coefficient values (effective posterior means and 95% credible intervals) from phylogenetic models for differences in chilling requirements between NA, EU, and EA species (N = 66 NA, 43 EU, and 68 EA species). The model includes genus and family random effects to account for shared evolutionary history of species. **c**, Estimated coefficient values including phylogenetic autocorrelation for differences in Munich leaf-out times between NA, EU, and EA species (N = 100 ENA, 74 EU, and 173 EA species). To control for species' life history strategy and native climate (mean annual temperature), both models (**b** + **c**) include fixed effects for growth habit (shrubs vs. trees), leaf persistence (evergreens vs. deciduous species), and species' 0.5 quantiles for mean annual temperature in their native ranges. Values reflect standardized data and can be interpreted as relative effect sizes.



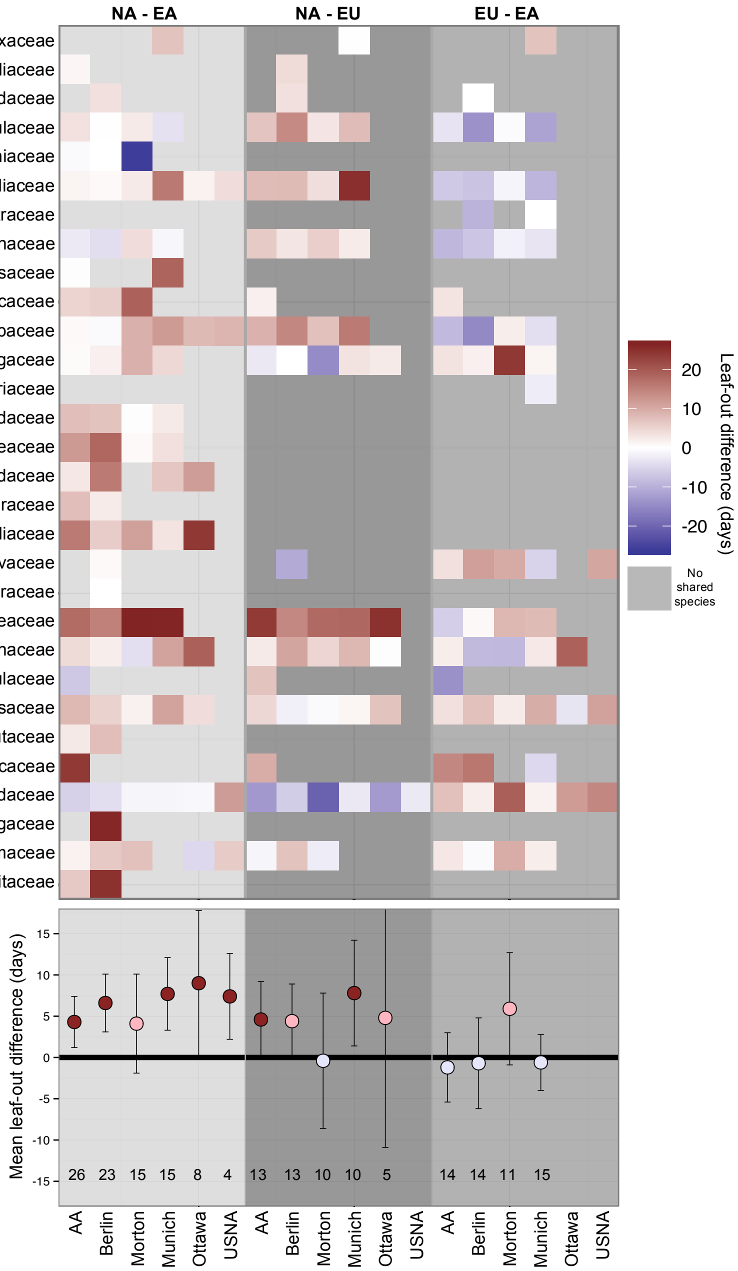
Extended Data Figure 6 | Days to leaf-out for North American (NA), European (EU), and East Asian (EA) species under three different chilling levels. a, Median days until leaf-out in a climate chamber ± 95% CI under different chilling levels for NA (N = 72 species), EU (N = 48), and EA (N = 88) species. **b–d,** Leaf-out probability curves for NA, EU, and EA species calculated as the number of days required until budburst under different chilling treatments: **(b)** long chilling, **(c)** intermediate chilling, and **(d)** short chilling. Dashed lines indicate median forcing requirements for NA, EU, and EA species.



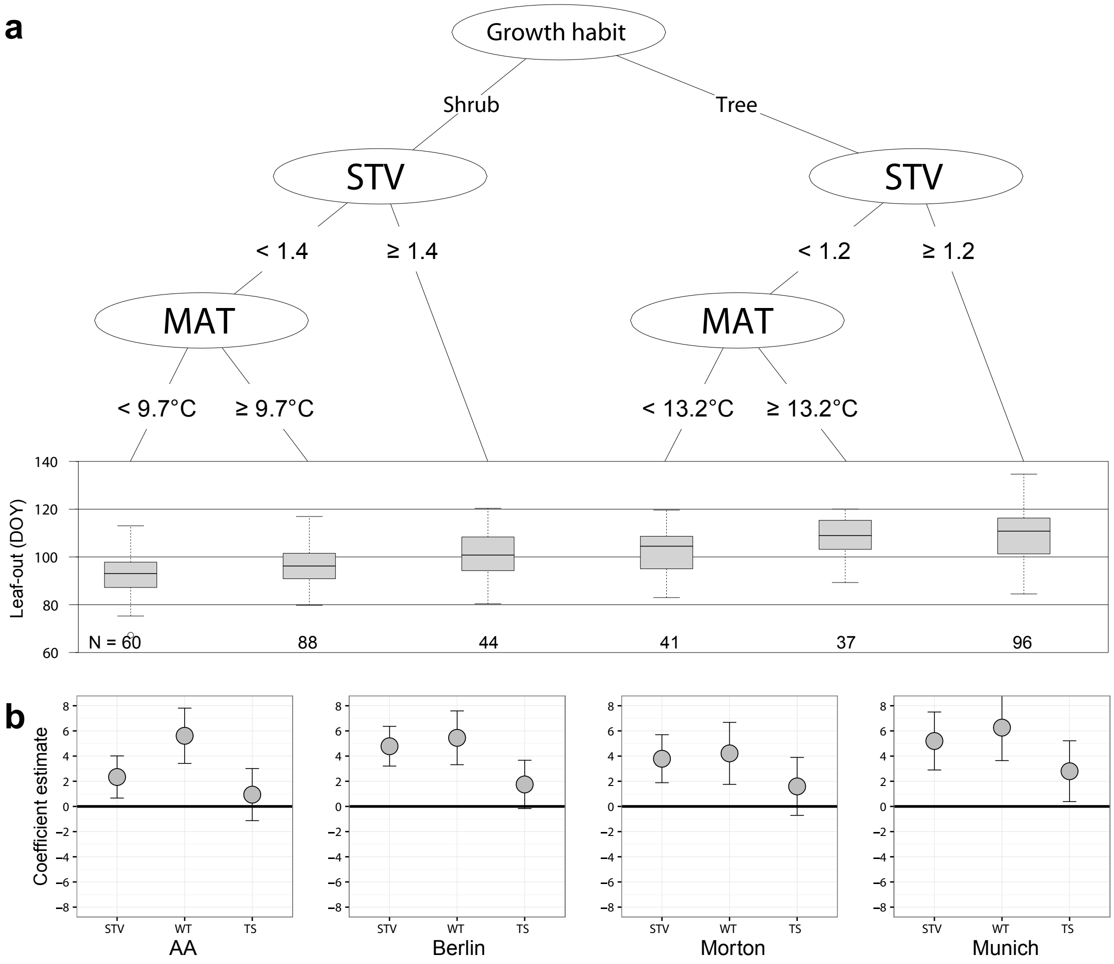
Extended Data Figure 7 | Family- and genus-level differences in chilling requirements between North America (NA), East Asia (EA), and Europe (EU). a, b, Heat maps showing mean chilling requirements per family (**a**) and genus (**b**) for NA, EU, and EA species. **c, d,** Mean within-family (**c**) and within-genus (**d**) differences in chilling requirements (± confidence intervals) between NA and EA species (left bar), NA and EU (middle), and EU and EA species (right). Note that for the calculation the chilling categories were treated as numerical characters (no chilling requirements = 0, intermediate = 1, high = 2). Numbers of within-family / within-genus contrasts are shown above bars.



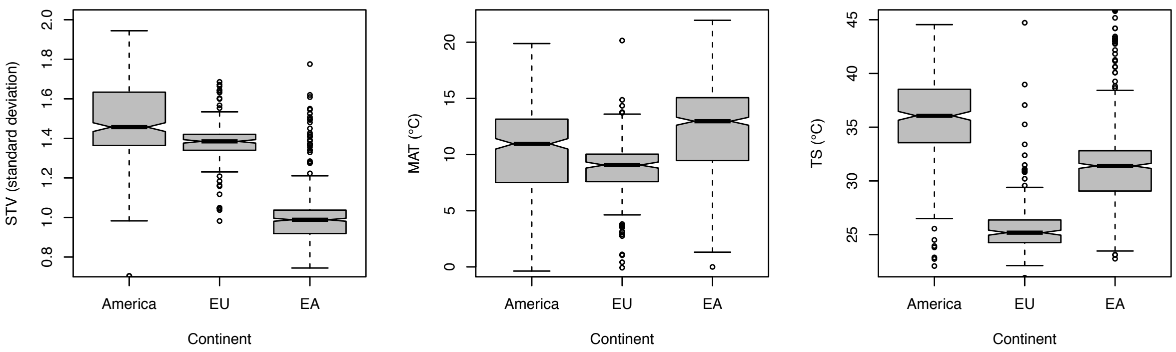
Extended Data Figure 8 | Region-specific responses to reduced chilling are not sensitive to photoperiod treatment. Contrasting responses of North American (NA), European (EU), and East Asian (EA) species to experimentally reduced winter chilling under short (left panel) and long day conditions (right panel). We show the median forcing requirements (accumulated degree days >0°C outdoors and in a climate chamber) ± 95% CI until leaf-out under three different chilling levels for NA (N = 49 species), EU (N = 34), and EA (N = 78) species, when twigs were exposed to 8h (left panel) or 16 h day length (right panel) in the climate chamber.



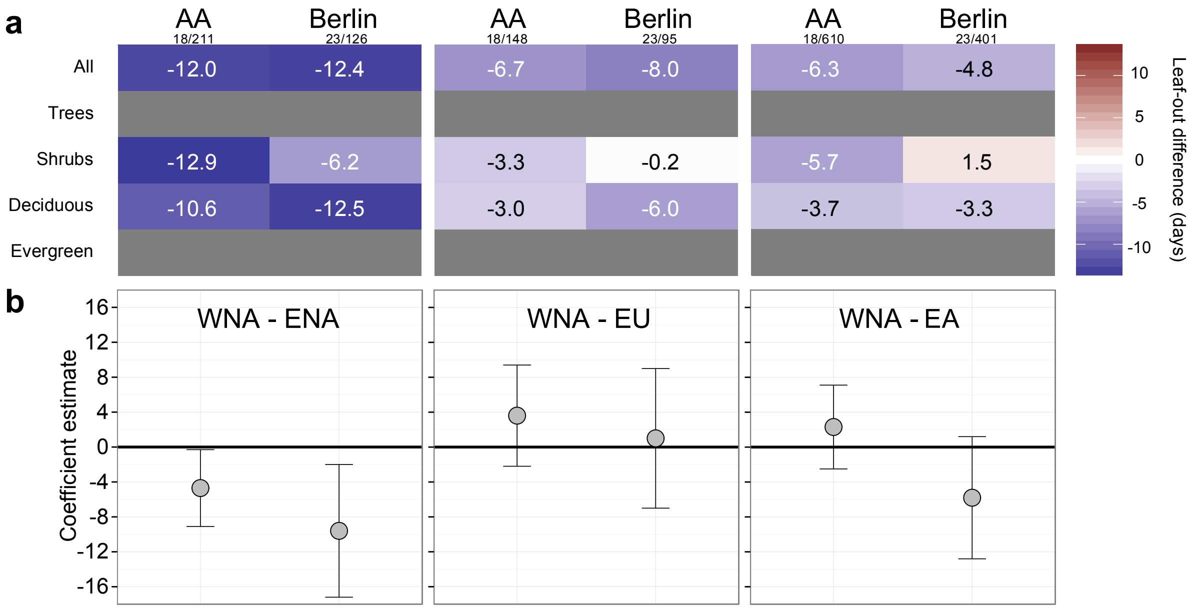
Extended Data Figure 9 | Family-level differences in the timing of spring leaf unfolding between North America (NA), East Asia (EA), and Europe (EU). a, Heat maps showing within-family differences in leaf out times monitored at six gardens (see Fig. 2) between NA and EA species (left panel), NA and EU (middle panel), and EU and EA species. Each contrast contains at least two species per continent. **b,** Mean within-family differences in leaf-out dates (± confidence intervals) between NA and EA species (left panel), NA and EU (middle panel), and EU and EA species. Number of within-family contrasts available for each garden is shown above garden names.



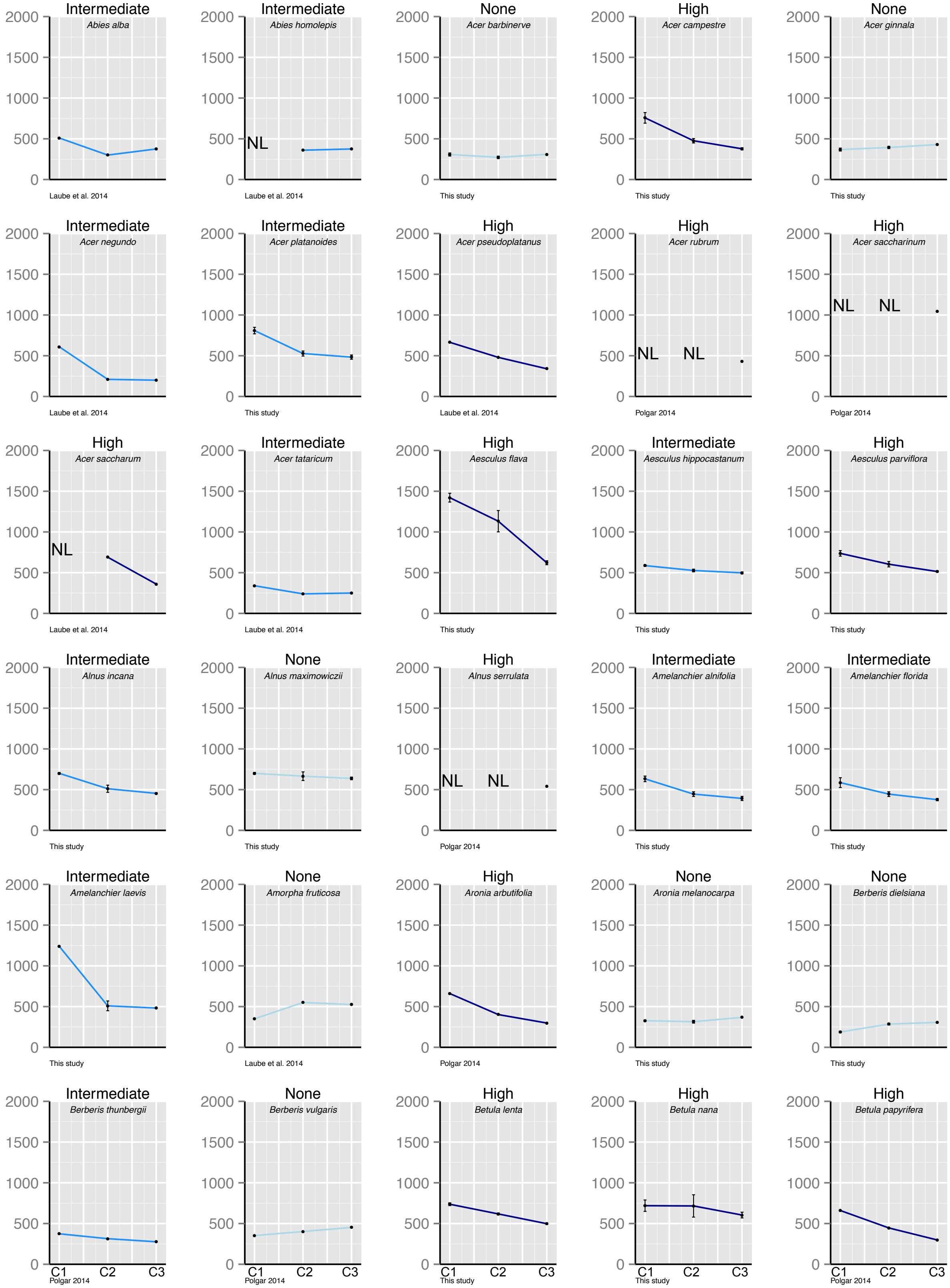
Extended Data Figure 10 | The effect of climate on species-specific leaf-out times. a, Recursive partitioning tree for the relationship between climate parameters and species-specific leaf-out dates (day-of-year, DOY) observed in Munich in 366 temperate woody species. Sprig temperature variability (STV), mean annual temperature (MAT), temperature seasonality (TS), growth habit, and leaf persistence were evaluated as potential split points. Number of species contained in each terminal node shown below boxplots. **b,** Coefficient values (effective posterior means and 95% credible intervals) for the relationship between three climate parameters and leaf-out times monitored at four gardens. Sample sizes: Arnold Arboretum (AA), 822 species; Berlin Botanical Garden (Berlin), 627 species; Morton Arboretum (Morton), 354 species; Munich Botanical Garden (Munich), 366 species. Models include phylogenetic autocorrelation and fixed tree and evergreen effects. Values reflect standardized data and can be interpreted as relative effect sizes.



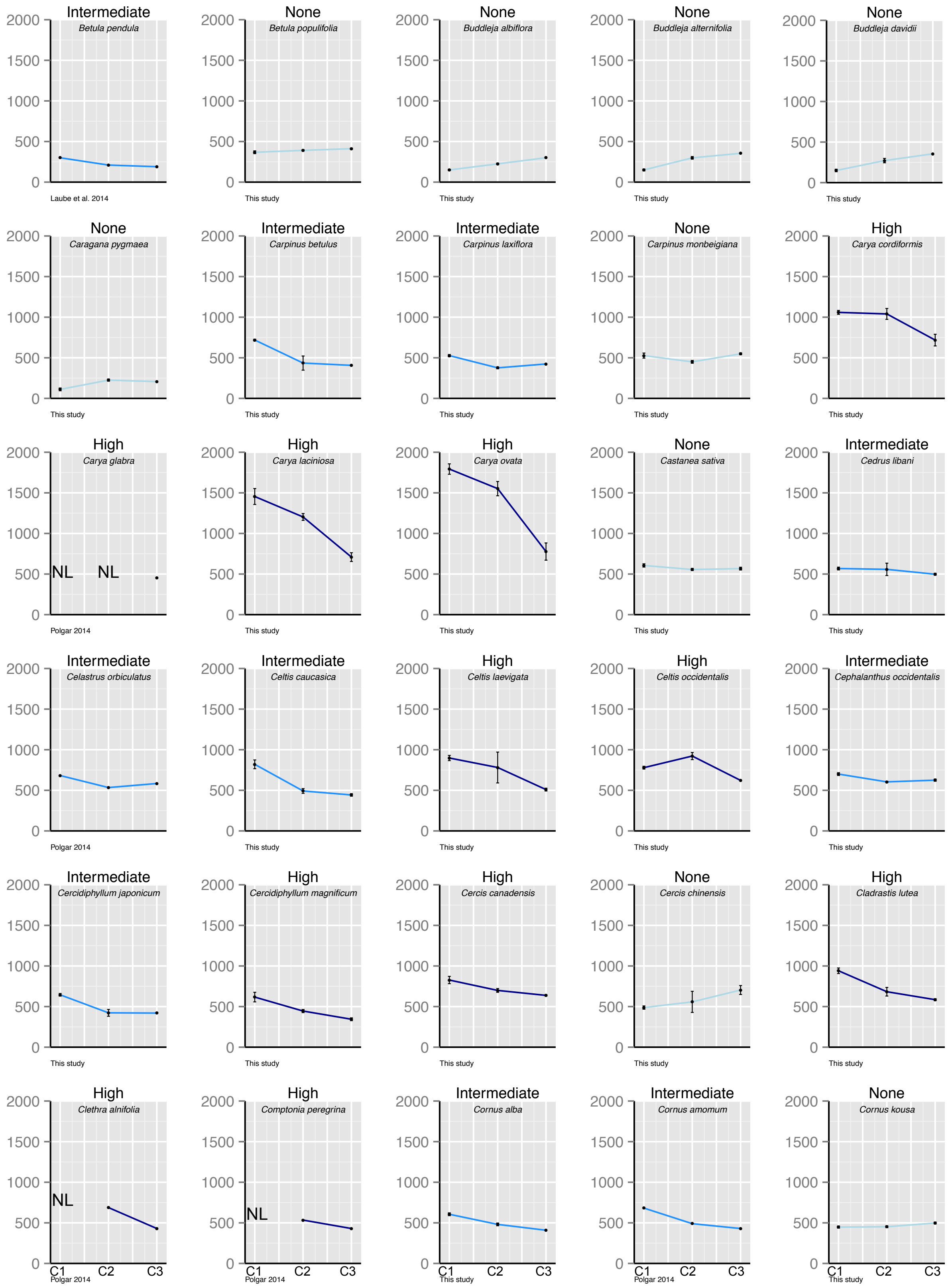
Extended Data Figure 11 | The temperature regimes experienced by species native to North America (NA, N = 431), Europe (EU, n= 237), or East Asia (EA, n = 929). Boxplots show 50% quantiles of species' spring temperature variability (STV), mean annual temperature (MAT), and temperature seasonality (TS). STV as standard deviation of minimum spring temperatures from 1901 to 2013, MAT in °C (BIO1), TS as temperature difference between the warmest and coldest month in °C (BIO7).



Extended Data Figure 12 | Leaf-out contrasts between western North American (WNA) and eastern North American (ENA), European (EU), and East Asian (EA) species. a, Heat maps for the difference in species-level leaf-out dates between WNA and ENA species (left panel), WNA and EU species (middle panel), and WNA and EA species (right panel) monitored at two gardens when all species, or only shrubs / deciduous species were included. Sample sizes for each continent at the respective garden are shown below garden names. Contrasts with sample sizes below 10 species per continent (trees and evergreen species) are not shown (grey fields in heat map). Leaf-out dates were observed in 2012 and came from Panchen *et al.*[17] (see Extended Data Table 3). AA: Arnold Arboretum, Boston, MA, USA; Berlin: Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin, Germany. **b**, Coefficient values (effective posterior means and 95% credible intervals) for differences in leaf-out dates between WNA and ENA species, WNA and EU species, and WNA and EA species. Models include phylogenetic autocorrelation and fixed tree, evergreen, and gymnosperm effects. Values reflect standardized data and can be interpreted as relative effect sizes.



Extended Data Figure 13 partial



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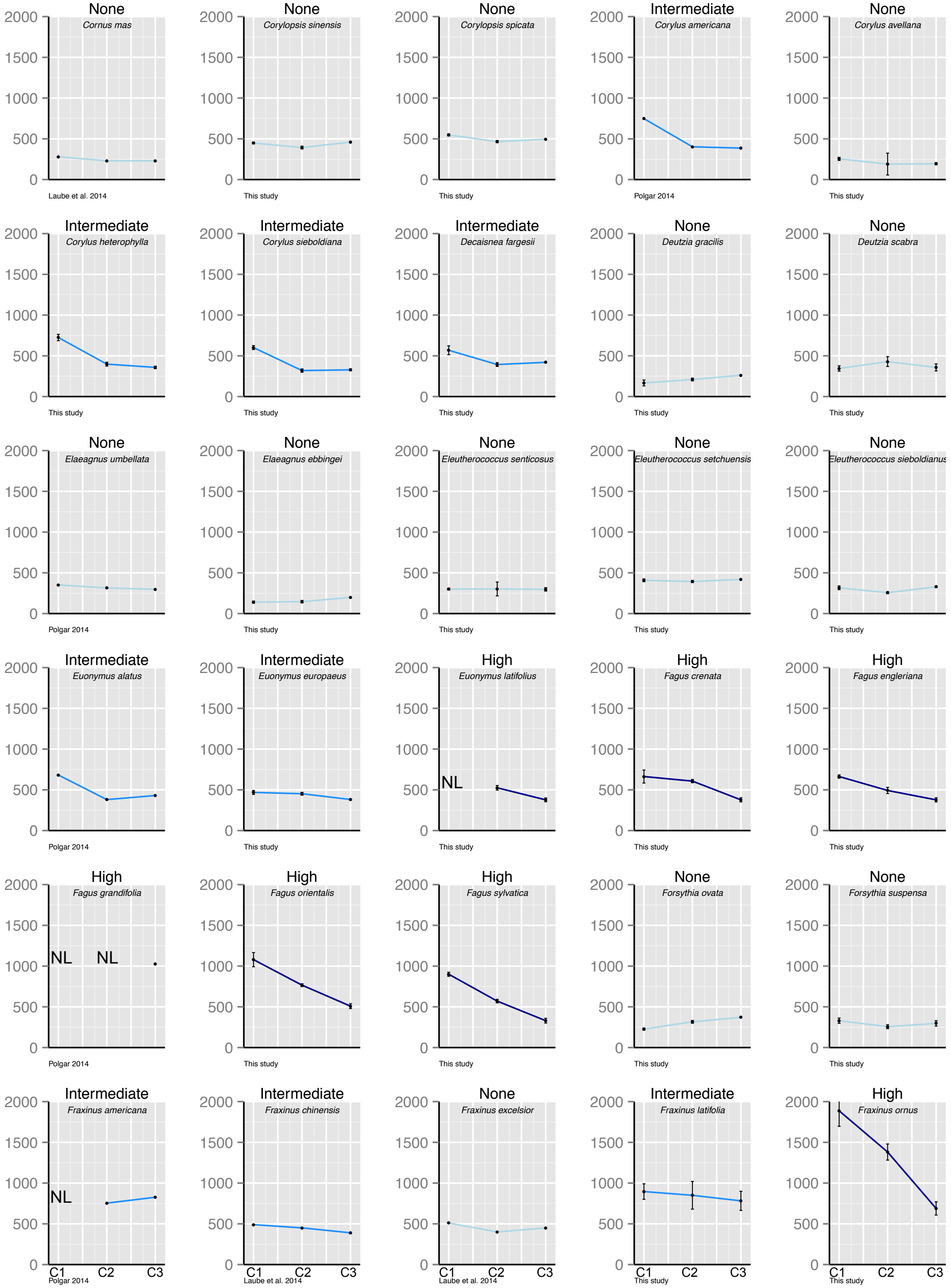

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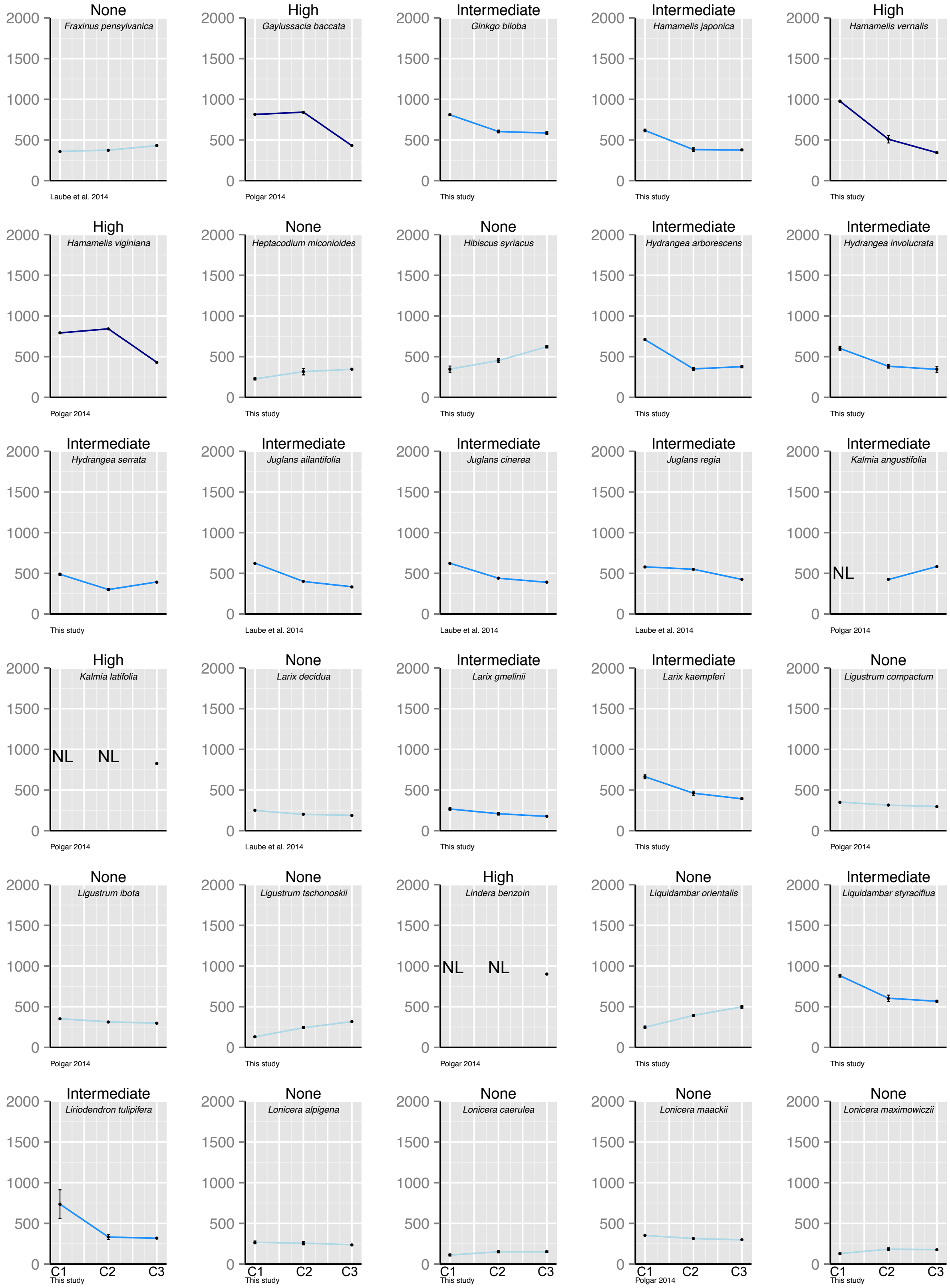

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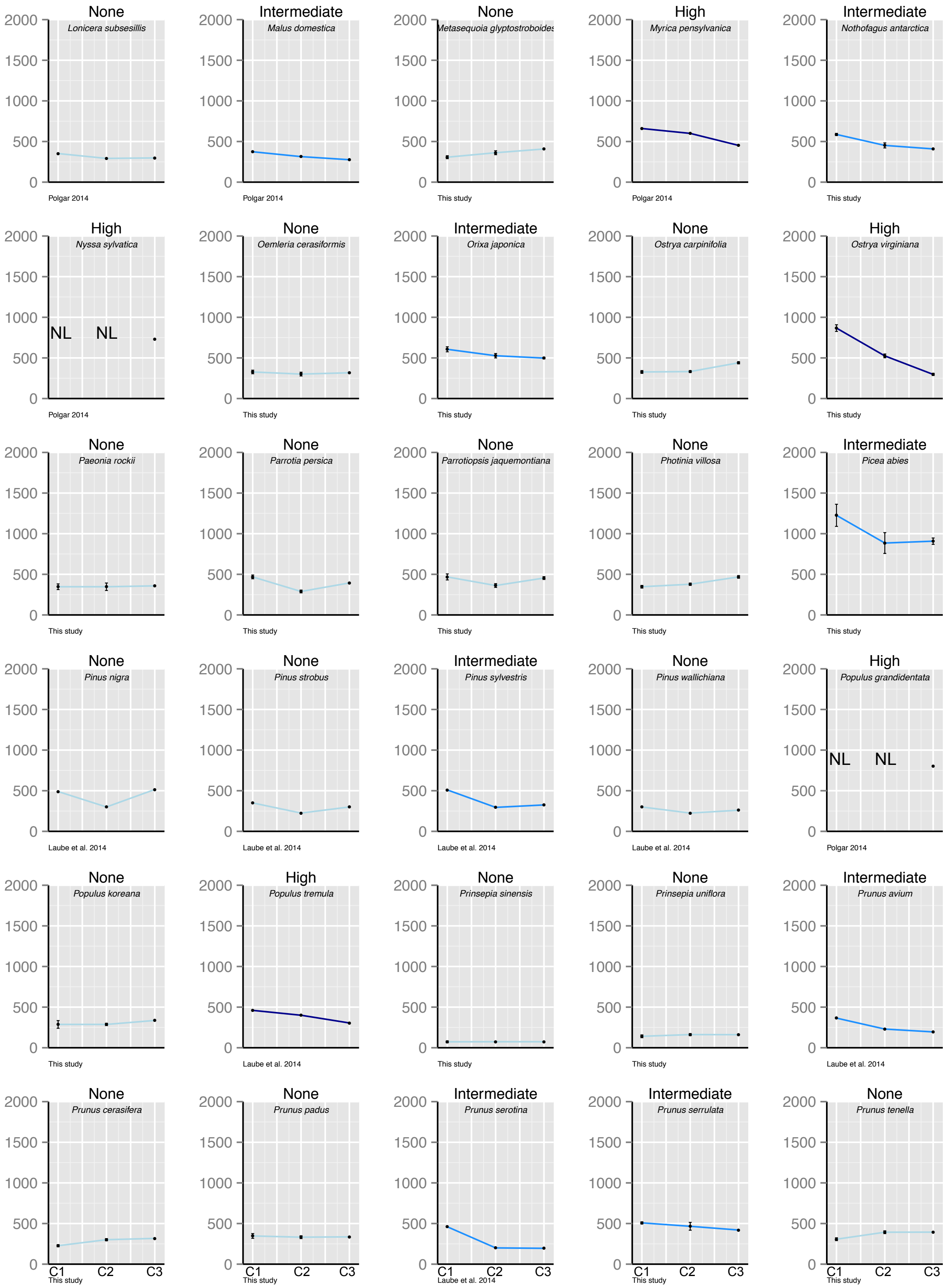

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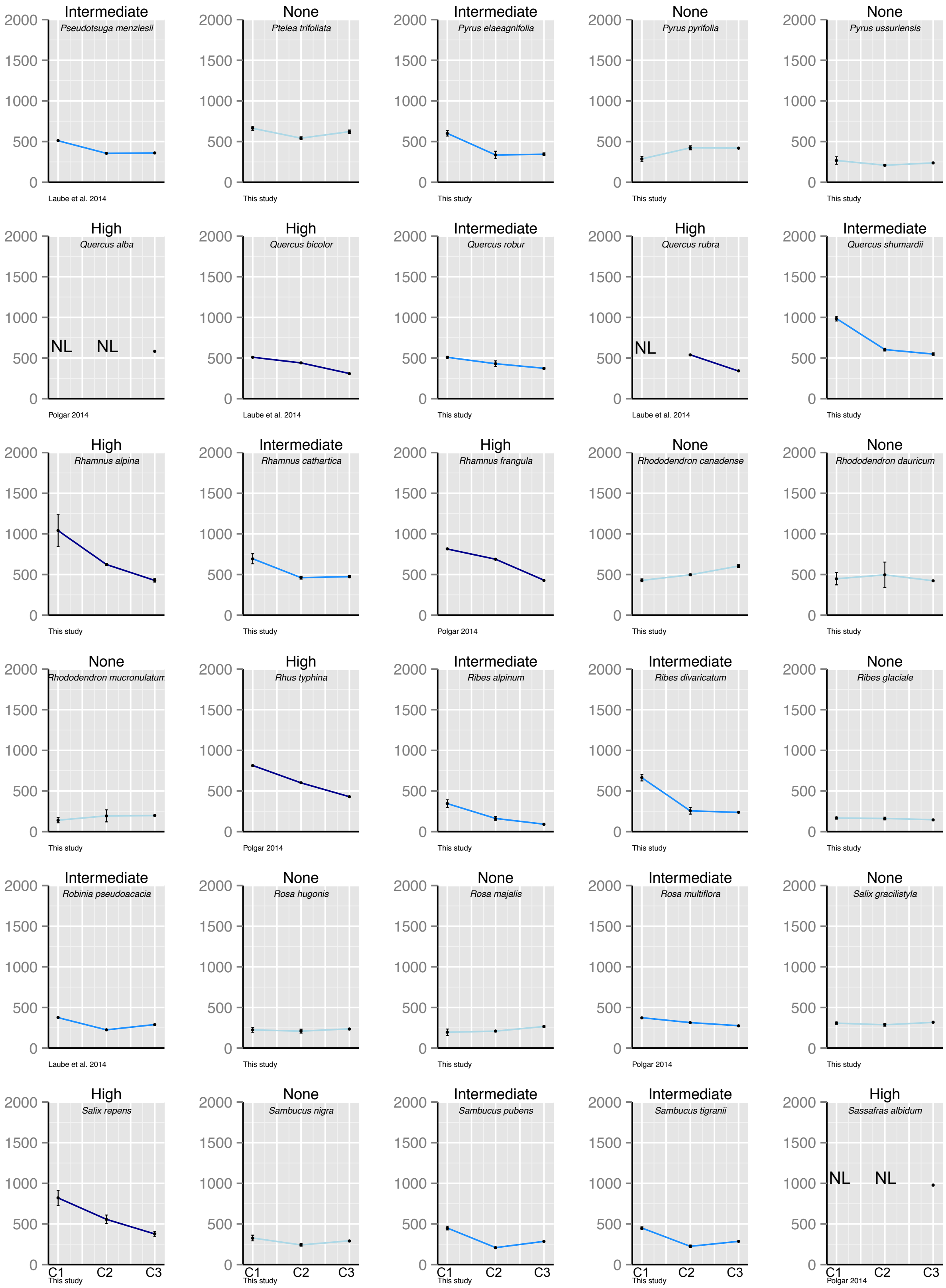

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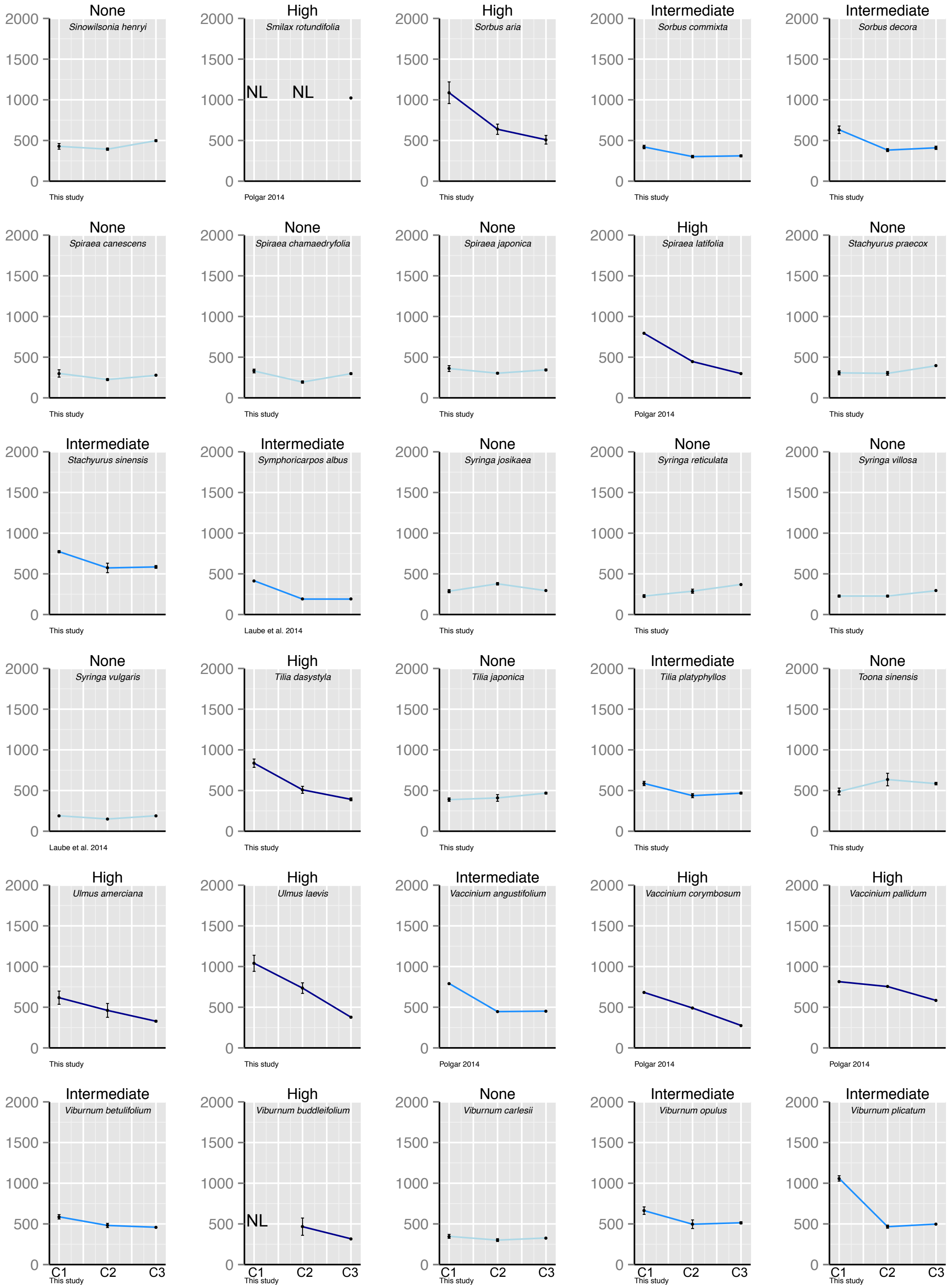

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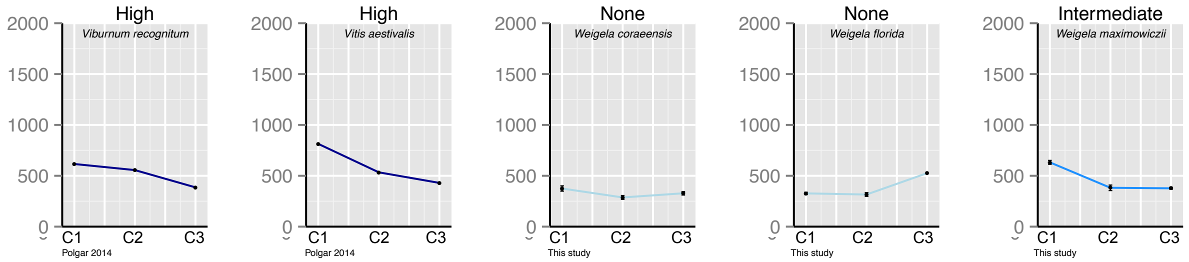

Extended Data Figure 13 | Chilling requirements of 215 temperate woody species. The importance of chilling for subsequent bud development (none, intermediate, or high chilling requirements) was inferred from twig cutting experiments conducted in this study, Laube *et al.*[11], and Polgar *et al*.[12] (see label below each graph). Graphs show forcing requirements (median growing degree days >0°C outdoors and in climate chamber ± SD) until leaf-out in 215 woody species at three different cutting dates (this study: C1 = 21 Dec 2013, C2 = 10 Feb 2014, C3 = 21 March 2014; Laube *et al.*[11]: C1 = 14 Dec 2011, C2 = 30 Jan 2012, C3 = 14 March 2012; Polgar *et al.*[12]; C1 = 9 Jan 2013, C2 = 17 Feb 2013, C3 = 22 March 2013). Lines in dark blue: species assigned to the category *high chilling* requirements; blue: species assigned to the category *intermediate chilling* requirements; light blue: species assigned to the category *no chilling* requirements. *NL* indicates that no leaf-out occurred at the repective cutting date. Some species leafed out before the last cutting date (C3), and in this case we show the degree days required until leaf-out in the field for the same individuals.

Extended Data Table 1 | ANCOVA for the relationship between forcing requirements and continent (North America, Europe, East Asia), chilling treatment (C1 – C3), and habit (shrub, tree) [see Figs. 1 and S10]. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

N = 208 species	***F*-value**
Continent	*F*(2) = 86.4***
Chilling	*F*(1) = 65.8***
Habit	*F*(1) = 23.1***
Continent x Chilling	*F*(2) = 12.4***
Continent x habit	*F*(2) = 0.4
Chilling x habit	*F*(1) = 1.4

Extended Data Table 2 | Leaf-out times of North American (NA), European (EU) and East Asian (EA) species. Mean leaf-out dates monitored at six gardens of species restricted to NA, EU, and EA, when all species or only trees / shrubs / deciduous / evergreen species were included. 95% confidence intervals and sample sizes shown in brackets, respectively. *AA*: Arnold Arboretum, Boston, MA, USA; *Berlin*: Botanical Garden and Botanical Museum Berlin-Dahlem, Berlin, Germany; *Morton*: Morton Arboretum, Lisle, IL, USA; *Munich*: Munich Botanical Garden, Munich, Germany; *Ottawa*: Ottawa Arboretum, Ottawa, Canada; *USNA*: US National Arboretum, Washington, DC and Beltsville, MD, USA.

		AA	Berlin	Morton	Munich	Ottawa	USNA
NA	**All**	106.1 (1.6) (291)	106.8 (2.4) (190)	92.5 (2.2) (137)	105.5 (2.2) (106)	126.7 (2.7) (90)	89.6 (3.9) (22)
	Trees	109.9 (1.9) (166)	113.1 (2.7) (106)	94.8 (2.9) (96)	110.1 (2.6) (63)	128.4 (2.8) (73)	92.8 (4.7) (12)
	Shrubs	101.3 (2.8) (125)	98.8 (3.6) (84)	87.1 (2.5) (41)	98.7 (2.9) (43)	119.6 (7.2) (17)	85.8 (6.0) (10)
	Deciduous	103.0 (1.4) (249)	105.4 (2.3) (175)	91.1 (2.0) (125)	104.7 (2.1) (101)	125.0 (2.7) (78)	89.7 (4.1) (21)
	Evergreen	125.2 (5.2) (42)	126.5 (11.4) (14)	106.8 (11.5) (12)	121.8 3.9 (5)	141.7 (3.1) (11)	89.0 (-) (1)
EU	**All**	99.8 (3.1) (148)	102.6 (3.3) (95)	92.2 (3.6) (38)	98.5 (2.8) (85)	122.0 (8.1) (21)	91.9 (5.2) (10)
	Trees	106.0 (4.4) (46)	109.9 (4.3) (51)	95.7 (4.4) (26)	106.0 (2.9) (38)	126.6 (7.3) (17)	93.5 (5.9) (8)
	Shrubs	93.4 (3.6) (45)	94.0 (4.0) (44)	84.5 (3.6) (12)	92.4 (3.7) (47)	102.2 (21.7) (4)	85.5 (6.8) (2)
	Deciduous	96.1 (2.7) (74)	100.6 (3.5) (81)	90.5 (3.6) (32)	97.5 (2.8) (78)	115.6 (8.5) (16)	91.9 (5.2) (10)
	Evergreen	115.8 (8.2) (17)	114.0 (8.6) (14)	101.0 (10.6) (6)	110.3 (10.0) (7)	142.2 (3.5) (5)	- (-) (0)
EA	**All**	99.4 (1.2) (610)	99.4 (1.7) (401)	86.4 (1.5) (206)	94.4 1.4 (295)	116.0 (4.4) (57)	82.4 (1.7) (139)
	Trees	103.7 (1.7) (283)	107.0 (2.2) (193)	89.4 (2.4) (115)	103.3 (2.0) (100)	116.9 (4.8) (48)	83.9 (2.2) (81)
	Shrubs	95.8 (1.7) (327)	92.3 (2.1) (208)	82.6 (1.1) (91)	89.8 (1.6) (195)	111.0 (11.4) (9)	80.3 (2.7) (58)
	Deciduous	96.8 (1.2) (525)	97.9 (1.6) (367)	85.0 (1.2) (190)	94.0 (1.4) (279)	114.2 (4.4) (53)	81.6 (1.7) (132)
	Evergreen	116.4	115.3	103.5	100.2	140.2	97.0

Extended Data Table 3 | Contrasting leaf-out times of eastern and western North American species. Mean leaf-out dates monitored at two gardens of species restricted to western North America and eastern North America, when all species or only trees / shrubs / deciduous / evergreen species were included. 95% confidence intervals and sample sizes shown in brackets, respectively. *AA*: Arnold Arboretum, Boston, MA, USA; *Berlin*: Botanical Garden and Botanical Museum Berlin-Dahlem, Berlin, Germany.

		AA	**Berlin**
Western North America	**All**	93.1 (5.5) (18)	94.6 (6.1) (23)
	Trees	103.5 (7.7) (4)	97.8 (12.7) (5)
	Shrubs	90.1 (5.9) (14)	93.8 (7.1) (18)
	Deciduous	93.1 (5.5) (18)	94.6 (6.1) (23)
	Evergreen	- (-) (0)	- (-) (0)
Eastern North America	**All**	105.1 (1.7) (211)	107.0 (2.6) (126)
	Trees	106.8 (1.7) (115)	112.8 (2.5) (70)
	Shrubs	103.0 (3.1) (96)	100.0 (4.4) (56)
	Deciduous	103.7 (1.5) (194)	107.1 (2.7) (124)
	Evergreen	121.2 (9.5) (17)	105.5 (48.1) (2)

Extended Data Table 4 | Results of twig cutting experiments to study the relative importance of chilling in 215 temperate woody species. Continent: continent a species is native to (NA = North America, SA = South America, EU = Europe, EA = East Asia, WA = West Asia). Chilling: classification of species according to chilling requirements (see Methods for classification rules). Species for which the results of the twig cutting experiments come from previous studies are indicated by superscripts: (1) data from Laube *et al.*[11] and (2) data from Polgar *et al.*[12]. C1 / C2 / C3: median forcing requirements (growing degree days >0°C outdoors and in climate chamber) until leaf-out for the three different chilling treatments C1 (short chilling), C2 (intermediate chilling), and C3 (long chilling). NL: no leaf-out within study period. For graphic representation see Extended Data Fig. 13.

Genus	Species	Continent	Chilling	C1 / C2 / C3
Abies	*alba*	EU	Intermediate[1]	510 / 300 / 375
Abies	*homolepis*	EA	Intermediate[1]	NL / 360 / 375
Acer	*barbinerve*	EA	None	307 / 272 / 309
Acer	*campestre*	EU	High	757 / 476 / 377
Acer	*ginnala*	EA	None	368 / 393 / 429
Acer	*negundo*	NA	Intermediate[1]	610 / 210 / 200
Acer	*platanoides*	EU	Intermediate	809 / 527 / 483
Acer	*pseudoplatanus*	EU	High[1]	665 / 480 / 340
Acer	*rubrum*	NA	High[2]	NL / NL / 430
Acer	*saccharinum*	NA	High[2]	NL / NL / 1046
Acer	*saccharum*	NA	High[1]	NL / 690 / 360
Acer	*tataricum*	EA	Intermediate[1]	340 / 240 / 250
Aesculus	*flava*	NA	High	1421 / 1132 / 622
Aesculus	*hippocastanum*	EU	Intermediate	587 / 527 / 498
Aesculus	*parviflora*	NA	High	737 / 604 / 514
Alnus	*incana*	EU	Intermediate	700 / 511 / 454
Alnus	*maximowiczii*	EA	None	700 / 665 / 638
Alnus	*serrulata*	NA	High[2]	NL / NL / 540
Amelanchier	*alnifolia*	NA	Intermediate	632 / 445 / 392
Amelanchier	*florida*	NA	Intermediate	586 / 445 / 377
Amelanchier	*laevis*	NA	Intermediate	1240 / 509 / 482
Amorpha	*fruticosa*	NA	None[1]	350 / 550 / 525
Aronia	*arbutifolia*	NA	High	660 / 402 / 298
Aronia	*melanocarpa*	NA	None	327 / 314 / 368
Berberis	*dielsiana*	EA	None	188 / 285 / 306
Berberis	*thunbergii*	EA	Intermediate[2]	374 / 314 / 276
Berberis	*vulgaris*	EU	None[2]	352 / 402 / 452
Betula	*lenta*	NA	High	737 / 618 / 498
Betula	*nana*	EU	High	719 / 716 / 604
Betula	*papyrifera*	NA	High[2]	660 / 446 / 298
Betula	*pendula*	EU	Intermediate[1]	300 / 210 / 190
Betula	*populifolia*	NA	None	368 / 391 / 412
Buddleja	*albiflora*	EA	None	150 / 225 / 300
Buddleja	*alternifolia*	EA	None	150 / 299 / 355
Buddleja	*davidii*	EA	None	155 / 300 / 354
Caragana	*pygmaea*	EA	None	111 / 225 / 207
Carpinus	*betulus*	EU	Intermediate	719 / 435 / 407
Carpinus	*laxiflora*	EA	Intermediate	527 / 376 / 424
Carpinus	*monbeigiana*	EA	None	527 / 450 / 549
Carya	*cordiformis*	NA	High	1059 / 1040 / 718
Carya	*glabra*	NA	High[2]	NL / NL / 452
Carya	*laciniosa*	NA	High	1455 / 1203 / 709
Carya	*ovata*	NA	High	1793 / 1552 / 777
Castanea	*sativa*	EU	None	606 / 556 / 567
Cedrus	*libani*	WA	Intermediate	574 / 558 / 498
Celastrus	*orbiculatus*	EA	Intermediate[2]	682 / 534 / 584
Celtis	*caucasica*	EU	Intermediate	820 / 492 / 443
Celtis	*laevigata*	NA	High	898 / 781 / 509
Celtis	*occidentalis*	NA	High	779 / 922 / 622
Cephalanthus	*occidentalis*	NA	Intermediate	700 / 604 / 624
Cercidiphyllum	*japonicum*	EA	Intermediate	645 / 423 / 420

Extended Data Table 4. Continued

Genus	Species	Continent	Chilling	C1 / C2 / C3
Cercidiphyllum	*magnificum*	EA	High	617 / 445 / 344
Cercis	*canadensis*	NA	High	827 / 699 / 638
Cercis	*chinensis*	EA	None	488 / 558 / 704
Cladrastis	*lutea*	NA	High	941 / 683 / 585
Clethra	*alnifolia*	NA	High[2]	NL / 688 / 430
Comptonia	*peregrina*	NA	High[2]	NL / 534 / 430
Cornus	*alba*	EA	Intermediate	606 / 481 / 409
Cornus	*amomum*	NA	Intermediate[2]	682 / 490 /430
Cornus	*kousa*	EA	None	448 / 452 / 498
Cornus	*mas*	EU	None[1]	280 / 230 / 230
Corylopsis	*sinensis*	EA	None	448 / 393 / 459
Corylopsis	*spicata*	EA	None	547 / 466 / 496
Corylus	*americana*	NA	Intermediate[2]	748 / 402 / 386
Corylus	*avellana*	EU	None	254 / 190 / 194
Corylus	*heterophylla*	EA	Intermediate	725 / 398 / 358
Corylus	*sieboldiana*	EA	Intermediate	601 / 319 / 328
Decaisnea	*fargesii*	EA	Intermediate	568 / 393 / 420
Deutzia	*gracilis*	EA	None	167 / 209 / 262
Deutzia	*scabra*	EA	None	345 / 429 / 358
Elaeagnus	*ebbingei*	EA	None	141 / 146 / 198
Elaeagnus	*umbellata*	EA	None	352 / 314 / 298
Eleutherococcus	*senticosus*	EA	None	300 / 302 / 297
Eleutherococcus	*setchuenenis*	EA	None	408 / 393 / 420
Eleutherococcus	*sieboldianus*	EA	None	316 / 256 / 328
Euonymus	*alatus*	EA	Intermediate[2]	682 / 380 / 430
Euonymus	*europaeus*	EU	Intermediate	468 / 452 / 381
Euonymus	*latifolius*	EU	High	NL / 525 / 377
Fagus	*crenata*	EA	High	663 / 607 / 377
Fagus	*engleriana*	EA	High	663 / 492 / 377
Fagus	*grandifolia*	NA	High[2]	NL / NL / 1024
Fagus	*orientalis*	EU	High	1079 / 766 / 508
Fagus	*sylvatica*	EU	High	900 / 570 / 330
Forsythia	*ovata*	EA	None	227 / 316 / 371
Forsythia	*suspensa*	EA	None	330 / 256 / 297
Fraxinus	*americana*	NA	Intermediate[2]	NL / 754 / 826
Fraxinus	*chinensis*	EA	Intermediate[1]	490 / 450 / 390
Fraxinus	*excelsior*	EU	None[1]	510 / 400 / 450
Fraxinus	*latifolia*	NA	Intermediate	896 / 850 / 782
Fraxinus	*ornus*	EU	High	1887 / 1381 / 689
Fraxinus	*pennsylvanica*	NA	None[1]	360 / 375 / 430
Gaylussacia	*baccata*	NA	High[2]	814 / 842 / 430
Ginkgo	*biloba*	EA	Intermediate	809 / 604 / 585
Hamamelis	*japonica*	EA	Intermediate	617 / 382 / 377
Hamamelis	*vernalis*	NA	High	976 / 509 / 344
Hamamelis	*virginiana*	NA	High[2]	792 / 842 / 430
Heptacodium	*miconioides*	EA	None	227 / 316 / 345
Hibiscus	*syriacus*	EA	None	347 / 452 / 622
Hydrangea	*arborescens*	NA	Intermediate	709 / 350 / 377
Hydrangea	*involucrata*	EA	Intermediate	601 / 382 / 344
Hydrangea	*serrata*	EA	Intermediate	488 / 301 / 394
Juglans	*ailantifolia*	EA	Intermediate[1]	625 / 400 / 335
Juglans	*cinerea*	NA	Intermediate[1]	624 / 440 / 390
Juglans	*regia*	EU	Intermediate[1]	580 / 550 /426
Kalmia	*angustifolia*	NA	Intermediate[2]	NL / 424 / 584
Kalmia	*latifolia*	NA	High[2]	NL / NL / 826
Larix	*decidua*	EU	None[1]	250 / 200 / 190
Larix	*gmelinii*	EA	Intermediate	267 / 209 / 176
Larix	*kaempferi*	EA	Intermediate	663 / 461 / 392
Ligustrum	*compactum*	EA	None[2]	352 / 314 / 298
Ligustrum	*ibota*	EA	None[2]	352 / 314 / 298
Ligustrum	*tschonoskii*	EA	None	130 / 242 / 317
Lindera	*benzoin*	NA	High[2]	NL / NL / 900
Liquidambar	*orientalis*	WA	None	247 / 393 / 498
Liquidambar	*styraciflua*	NA	Intermediate	881 / 604 / 567
Liriodendron	*tulipifera*	NA	Intermediate	737 / 332 / 317
Lonicera	*alpigena*	EU	None	267 / 257 / 238
Lonicera	*caerulea*	EU	None	111 / 151 / 151
Lonicera	*maackii*	EA	None[2]	352 / 314 / 298
Lonicera	*maximowiczii*	EA	None	130 / 183 / 177
Lonicera	*subsessilis*	EA	None[2]	352 / 292 / 298
Malus	*domestica*	-	Intermediate[2]	374 / 314 / 276
Metasequoia	*glyptostroboides*	EA	None	307 / 362 / 408
Myrica	*pensylvanica*	NA	High[2]	660 / 600 / 452

Extended Data Table 4. Continued

Genus	Species	Continent	Chilling	C1 / C2 / C3
Nothofagus	*antarctica*	SA	Intermediate	587 / 452 / 409
Nyssa	*sylvatica*	NA	High^2	NL / NL / 730
Oemleria	*cerasiformis*	NA	None	327 / 301 / 317
Orixa	*japonica*	EA	Intermediate	606 / 527 / 498
Ostrya	*carpinifolia*	EU	None	327 / 332 / 440
Ostrya	*virginiana*	NA	High	867 / 525 / 297
Paeonia	*rockii*	EA	None	347 / 347 / 358
Parrotia	*persica*	WA	None	468 / 289 / 394
Parrotiopsis	*jacquemontiana*	EA	None	468 / 362 / 454
Photinia	*villosa*	EA	None	347 / 378 / 468
Picea	*abies*	EU	Intermediate	1226 / 885 / 908
Pinus	*nigra*	EU	None^1	490 / 300 / 515
Pinus	*strobus*	NA	None^1	350 / 225 / 300
Pinus	*sylvestris*	EU	Intermediate^1	510 / 295 / 325
Pinus	*wallichiana*	EA	None^1	300 / 225 / 260
Populus	*grandidentata*	NA	High^2	NL / NL / 804
Populus	*koreana*	EA	None	287 / 287 / 336
Populus	*tremula*	EU	High^1	460 / 400 / 305
Prinsepia	*sinensis*	EA	None	73 / 74 / 74
Prinsepia	*uniflora*	EA	None	141 / 162 / 161
Prunus	*avium*	EU	Intermediate^1	365 / 230 / 195
Prunus	*cerasifera*	WA	None	227 / 301 / 317
Prunus	*padus*	EU	None	347 / 332 / 336
Prunus	*serotina*	NA	Intermediate^1	460 / 200 / 195
Prunus	*serrulata*	EA	Intermediate	508 / 466 / 420
Prunus	*tenella*	EU	None	307 / 393 / 394
Pseudotsuga	*menziesii*	NA	Intermediate^1	510 / 355 / 360
Ptelea	*trifoliata*	NA	None	663 / 542 / 622
Pyrus	*elaeagnifolia*	EU	Intermediate	601 / 335 / 344
Pyrus	*pyrifolia*	EA	None	287 / 423 / 420
Pyrus	*ussuriensis*	EA	None	267 / 209 / 237
Quercus	*alba*	NA	High^2	NL / NL / 584
Quercus	*bicolor*	NA	High^1	510 / 440 / 310
Quercus	*robur*	EU	Intermediate	509 / 430 / 374
Quercus	*rubra*	NA	High^1	NL / 540 / 340
Quercus	*shumardii*	NA	Intermediate	985 / 604 / 549
Rhamnus	*alpina*	EU	High	1040 / 623 / 427
Rhamnus	*cathartica*	EU	Intermediate	694 / 461 / 474
Rhamnus	*frangula*	EU	High^2	814 / 688 / 430
Rhododendron	*canadense*	NA	None	428 / 496 / 604
Rhododendron	*dauricum*	EA	None	448 / 496 / 423
Rhododendron	*mucronulatum*	EA	None	141 / 194 / 198
Rhus	*typhina*	NA	High^2	814 / 600 / 430
Ribes	*alpinum*	EU	Intermediate	345 / 162 / 95
Ribes	*divaricatum*	NA	Intermediate	663 / 256 / 237
Ribes	*glaciale*	EA	None	167 / 162 / 147
Robinia	*pseudoacacia*	NA	Intermediate^1	375 / 225 / 290
Rosa	*hugonis*	EA	None	224 / 209 / 237
Rosa	*majalis*	EU	None	195 / 209 / 266
Rosa	*multiflora*	EA	Intermediate^2	374 / 314 / 276
Salix	*gracilistyla*	EA	None	307 / 287 / 317
Salix	*repens*	EU	High	820 / 557 / 377
Sambucus	*nigra*	EU	None	327 / 242 / 293
Sambucus	*pubens*	NA	Intermediate	450 / 209 / 286
Sambucus	*tigranii*	EU	Intermediate	450 / 225 / 286
Sassafras	*albidum*	NA	High^2	NL / NL / 980
Sinowilsonia	*henryi*	EA	None	428 / 393 / 498
Smilax	*rotundifolia*	NA	High^2	NL / NL / 1024
Sorbus	*aria*	EU	High	1087 / 638 / 509
Sorbus	*commixta*	EA	Intermediate	420 / 302 / 311
Sorbus	*decora*	NA	Intermediate	632 / 382 / 410
Spiraea	*canescens*	EA	None	300 / 225 / 276
Spiraea	*chamaedryfolia*	EA	None	330 / 194 / 297
Spiraea	*japonica*	EA	None	360 / 303 / 344
Spiraea	*latifolia*	NA	High^2	792 / 446 / 298
Stachyurus	*chinensis*	EA	Intermediate	773 / 573 / 585
Stachyurus	*praecox*	EA	None	307 / 301 / 394
Symphoricarpos	*albus*	NA	Intermediate^1	415 / 190 / 190
Syringa	*josikaea*	EU	None	287 / 378 / 293
Syringa	*reticulata*	EA	None	227 / 287 / 370
Syringa	*villosa*	EA	None	227 / 227 / 293
Syringa	*vulgaris*	EU	None^1	190 / 150 / 190
Tilia	*dasystyla*	WA	High	836 / 509 / 392

Extended Data Table 4. Continued

Genus	Species	Continent	Chilling	C1 / C2 / C3
Tilia	*japonica*	EA	None	388 / 409 / 468
Tilia	*platyphyllos*	EU	Intermediate	587 / 437 / 468
Toona	*sinensis*	EA	None	488 / 635 / 585
Ulmus	*americana*	NA	High	617 / 461 / 328
Ulmus	*laevis*	EU	High	1040 / 735 / 377
Vaccinium	*angustifolium*	NA	Intermediate^2	792 / 446 / 452
Vaccinium	*corymbosum*	NA	High^2	682 / 490 / 276
Vaccinium	*pallidum*	NA	High^2	814 / 754 / 584
Viburnum	*betulifolium*	EA	Intermediate	587 / 481 / 459
Viburnum	*buddleifolium*	EA	High	NL / 466 / 317
Viburnum	*carlesii*	EA	None	347 / 301 / 327
Viburnum	*opulus*	EU	Intermediate	663 / 496 / 514
Viburnum	*plicatum*	EA	Intermediate	1059 / 466 / 498
Viburnum	*recognitum*	NA	High^2	616 / 556 / 386
Vitis	*aestivalis*	NA	High^2	814 / 534 / 430
Weigela	*coraeensis*	EA	None	375 / 287 / 328
Weigela	*florida*	EA	None	327 / 316 / 526
Weigela	*maximowiczii*	EA	Intermediate	632 / 382 / 377

Extended Data Table 5 | Experimental setup of the twig cutting experiment addressing species' chilling requirements. C1, C2, C3 = Different collection dates of twigs resulting in different levels of chilling. Chill days were calculated as days with a mean air temperature below 5°C between 1 November and the respective collection date.

	C1	C2	C3
Start of experiment (Collection date)	21 Dec 2013	11 Feb 2014	21 March 2014
Chilling status	Low	Intermediate	High
Chill days (below 5°C) from 1 November until collection date	38	72	88

Chapter 5

DISTRIBUTION RANGES AND SPRING PHENOLOGY EXPLAIN LATE FROST SENSITIVITY IN 170 WOODY PLANTS FROM THE NORTHERN HEMISPHERE.

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RESEARCH PAPER

Distribution ranges and spring phenology explain late frost sensitivity in 170 woody plants from the Northern Hemisphere

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ABSTRACT

Aim Cold events determine the distributional range limits of woody species. Despite global warming, the magnitude of late frost events in boreal and temperate regions is not expected to change. Hence, the risk for late spring frost damage of woody species may increase with an earlier onset of the growing season. Here, we investigated biogeographical, phenological and phylogenetic effects on late frost sensitivity.

Location Ecological-Botanical Gardens Bayreuth, Germany (49°55′45″ N, 11°35′10″ E).

Methods We inspected 170 woody species in the Ecological-Botanical Gardens from across the entire Northern Hemisphere for frost damage after an extreme late frost event in May 2011 (air temperature −4.3 °C after leaf unfolding of all species). Distribution range characteristics, climatic parameters of place of origin and phenological strategy were linked to sensitivity to the late frost event.

Results The northern distribution limit and the range in continentality across the distributional ranges correlated negatively with a taxon's late frost sensitivity (pseudo-$R^2 = 0.42$, pseudo-$R^2 = 0.33$, respectively). Sensitivity to the late frost event was well explained by the climatic conditions within species' native ranges (boosted regression trees; receiver operating characteristic 0.737). Average (1950–2000) May minimum temperature in species' native ranges was the main explanatory variable of late frost sensitivity (51.7% of explained variance). Phylogenetic relatedness explained additional variance in sensitivity to the late frost event. Sensitivity to the late frost event further correlates well with species phenological strategy. Frost-tolerant species flushed on average 2 weeks earlier than frost-sensitive species.

Main conclusions Range characteristics and the prevalent climatic parameters across species native ranges are strongly related to their susceptibility to late spring frost damage. Further, more late frost-sensitive species unfolded their leaves later than more tolerant species and late frost tolerance is phylogenetically conserved. Thus, late frost sensitivity may challenge natural and human-assisted migration of woody species under global warming.

Keywords

Assisted colonization, assisted migration, common garden experiment, distribution limit, extreme events, frost damage, leaf-out, leaf unfolding, spring freeze.

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INTRODUCTION

Due to climate warming, extreme cold events are generally expected to occur less frequently (IPCC, 2012), but their magnitude is likely to persist (Kodra *et al.*, 2011). Further decrease in wintertime sea ice in the Barents–Kara seas could even increase the likelihood of extreme cold events in Europe (Petoukhov & Semenov, 2010). Such extreme cold events can cause considerable damage to plants with significant ecological and also economic consequences (Gu *et al.*, 2008; Jalili *et al.*, 2010).

In Central Europe, the start of the growing season has advanced over the last decades (Menzel & Fabian, 1999; Badeck *et al.*, 2004). Over three decades, leaf-out has started 6 days earlier (Menzel & Fabian, 1999). However, extreme cold events (spring frosts) after an earlier onset of the growing season are increasing the risk of frost damage in the temperate zone (Inouye, 2008; Martin *et al.*, 2010; Hufkens *et al.*, 2012; Augspurger, 2013).

Trees are less able to cope with rapid climate changes compared with other plant functional types due to their conservative dispersal strategies and their longevity (Petit & Hampe, 2006). An important factor limiting adaptation to global change in temperate tree species might be late frost sensitivity (Kollas *et al.*, 2014). Knowledge on the role of late spring frost sensitivity in controlling range limits is therefore essential for understanding current and future natural and human-assisted range shifts.

In general, the probability of frost damage differs between tree species and is modified by their phenological phase (Augspurger, 2009). Directly after bud burst, temperate woody plants respond sensitively to frost events starting around −3 to −5°C (Sakai & Larcher, 1987; Inouye, 2008; Martin *et al.*, 2010; Kreyling *et al.*, 2012b; Lenz *et al.*, 2013). Recent studies suggest that the susceptibility of species to late frosts is influenced by their phenological strategy, i.e. the leaves of early flushing species tend to withstand lower temperatures than species with a late spring phenology (Lenz *et al.*, 2013; Vitasse *et al.*, 2014a). Species with a longer dormancy period avoid late frost damage at the price of a shorter growing season (Lockhart, 1983; Leinonen & Hänninen, 2002; Basler & Körner, 2012). In contrast, species with a short dormancy period should profit from a prolonged vegetation period, but have to invest more in frost resistance mechanisms.

In addition, drought tolerance of plant species can modify the impact of frost events due to the physiologically comparable mechanisms aimed at preventing dehydration of cells (Blödner *et al.*, 2005; Beck *et al.*, 2007). Similar to drought stress, frost leads to dehydration of plant tissues and cells by crystallization of water (Sakai & Larcher, 1987). Hence, the water balance across a species' native range can have an impact on its late frost sensitivity due to cross-stress tolerance between drought and frost (Walter *et al.*, 2012). In consequence, differing drought tolerance between woody species is likely to also be reflected in varying late frost sensitivity.

Sudden late frost events can affect large areas and can cause widespread damage (Gu *et al.*, 2008; Hufkens *et al.*, 2012; Kreyling *et al.*, 2012a; Lenz *et al.*, 2013). A strong late frost event in spring 2007 caused severe damage to woody species and crops across the eastern United States, and led to the loss of young foliage, shoots and fruits as well as to widespread necrosis and desiccation of leaves (Gu *et al.*, 2008). Another large-scale cold event during the early vegetation period occurred in May 2011, where large parts of Germany experienced an extreme late frost event. This frost event led to frost damage such as severe leaf damage and a shortened vegetation period, and meant that additional investment of resources in second leaf-out across species was necessary for recovery (Kreyling *et al.*, 2012a).

Minimum temperatures in winter are assumed to limit the native ranges of woody species (Sakai & Weiser, 1973). Likewise, cold tolerance of tree species is closely related to the climate of their native ranges, with a study focusing solely on cold tolerance over winter and before bud burst (Kreyling *et al.*, 2015) finding the strongest correlations in late winter and early spring. In general, it has long been acknowledged that late frost events pose a risk for woody species in temperate regions (Gayer, 1882; Ellenberg, 1963).

However, for a long time there were no studies quantifying the effect of late frost events on the distribution ranges of woody species. Just recently, Kollas *et al.* (2014) pointed out that it is not the absolute minimum temperature in winter that controls the native range limits but rather the low-temperature extremes during bud burst in springtime, which is the phenological stage where woody plants respond most sensitively to sudden freezing events. This is in line with Lenz *et al.* (2013), who found that freezing temperatures in spring might be one of the main driving factors for range limits due to the selective pressure controlling the beginning of the growing season. Given this potentially strong effect of late frost sensitivity on distribution ranges, the increased risk of late frost damage (Inouye, 2008; Martin *et al.*, 2010; Hufkens *et al.*, 2012; Augspurger, 2013) opposes the poleward and upward range shifts expected with global warming (Parmesan *et al.*, 1999; Lenoir *et al.*, 2008). However, studies quantifying the effect of distributional and underlying climatic characteristics of species native ranges on late frost sensitivity across a large spatial scale and multiple species are missing.

Here, we tested if late frost sensitivity of woody species can be explained by the climatic conditions in their native distributional ranges, in particular spring minimum temperature. In particular we hypothesized that woody species whose native ranges are characterized by low temperatures (spring, winter, annual) and low amounts of precipitation (summer, growing season, annual), are well adapted to late frost events. In addition, we tested if frost sensitivity is related to the order in which species leaf out each year. We expected early leafing species to develop high frost resistance, while phenologically late species should afford lower frost resistance to their leaves. Finally, we checked if phylogeny (members of certain genera) contributed additional power for explaining

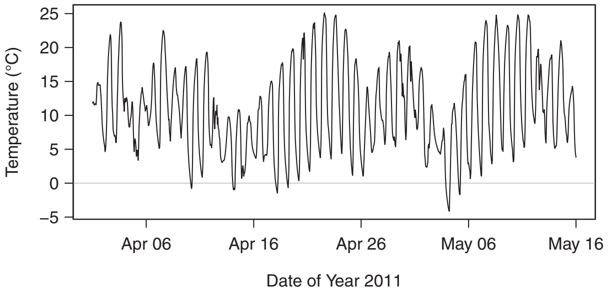

Figure 1 Air temperature (hourly means at +2m) from 1 April to 16 May 2011 showing the warm April preceding the late frost event on 4 and 5 May at the Ecological-Botanical Gardens of the University of Bayreuth, Germany. Data courtesy of Th. Foken, Department of Micrometeorology, University of Bayreuth.

the sensitivity to a late spring frost event. For this, we inspected 170 adult and established woody species growing in the Ecological-Botanical Gardens (EBG) of the University of Bayreuth for damage after one extreme frost event (air temperature below −4.3°C). This late frost event occurred naturally in May 2011 after the start of the growing season (Kreyling *et al.*, 2012a). We then tested if the observed frost damage could be explained by distributional and underlying climatic characteristics of these species native ranges.

METHODS

Ecological-Botanical Gardens Bayreuth and the late frost event in May 2011

The EBG of the University of Bayreuth, Germany (49°55′45″ N, 11°35′10″ E, 16 ha) is located at an elevation of 355 to 370 m a.s.l. The local climate represents a transition between oceanic and continental influences, with a long-term mean annual temperature of 8.2 °C and mean annual precipitation of 724 mm (Foken, 2007). As the EBG was founded in 1978, all tree specimens are of comparable age and have reached tree size with considerable growth in height. Thus, the EBG offers an implicit common garden setting to study late frost sensitivity of even-aged woody plant species.

Late frost events, i.e. frost events after the end of winter in spring or summer, occur occasionally. Such frost events can appear after bud burst of trees. The late frost event in May 2011 was the most extreme since the start of temperature recording on the site in 1997 and at the nearest station of the German Weather Service in 1961 (distance about 10 km; the second coldest event in 1976 reached −3.7 °C). Temperatures dropped to −10 °C close to the surface (+5 cm) and −4.3 °C at a height of 2 m on the early morning of 4 May (meteorological station at the EBG, coordinates as above; data courtesy of Th. Foken, Department of Micrometeorology, University of Bayreuth) (Fig. 1). This frost event happened after an extraordinarily warm April during which all studied species had started greening (Fig. 1). Bud burst was completed when the late frost event took place. Frost damage became clearly visible over the following days. On 16 May we checked the new foliage and new needles of adult plant individuals of 170 woody species in the EBG (with heights between 1 and 15 m) – one to ten individuals per species – for visible frost damage (0 = no frost damage, 1 = at least one individual showing frost damage measured by leaf browning as an indicator).

Species distributional characteristics and underlying climatic conditions

For each species we obtained the native distribution range from various sources (data are given in Appendix S1 in Supporting Information). Based on the species distribution ranges, for each species we calculated the following distributional characteristics: southernmost occurrence, latitudinal and longitudinal distribution centroid as well as northernmost occurrence. For the climatic characterization of the distribution ranges, the current climatic conditions (averages over the time period 1950–2000) with a spatial resolution of 10 arcmin (obtained from WorldClim, http://worldclim.org; Hijmans *et al.*, 2005), were intersected with the native ranges. Continentality was chosen as a further parameter because a strong continental climate within a species' native range might lead to a higher frost tolerance due to required protection against cold winters, a higher risk of extreme late frost events and drought during summer (Czajkowski & Bolte, 2006). Continentality within a species' distribution range was quantified by using a simplified continentality index (high values equal high continentality; Iwanow, 1959 in Hogewind & Bissolli, 2011):

$$\text{continentality} = \frac{260 \times \text{annual temperature range}}{\text{latitude}}.$$

Spatial information about the annual temperature range was derived from Bioclim variable 7 (BIO7) from the WorldClim dataset, which is calculated as the difference between the maximum temperature of the warmest month (BIO5) and the minimum temperature of the coldest month (BIO6). Based on this gridded information about the annual temperature range and the latitude of the corresponding grid cells we calculated minimum, mean and maximum continentality as well as the range of continentality (maximum – minimum) for the distribution range of each species. All spatial analyses were conducted with the GIS software ArcGIS 10 (ESRI 2011, Redlands, CA, USA).

To test the influence of phylogenetic relatedness on the sensitivity to the late frost event, we pooled species-specific distributional characteristics for the 69 different genera under investigation. For all genera we calculated: northernmost and southernmost occurrence, maximum and minimum continentality, the average range of continentality (average of the species-specific ranges) as well as the average and variation (standard deviation) of species latitudinal and longitudinal distribution centroids. Of the 69 genera, only those genera (16 genera, 105 species) with more than three species were included in the genera-specific analyses (Appendix S1).

Table 1 Climatic parameters and their univariate (generalized linear model, GLM) and multivariate (boosted regression tree, BRT) relationship with the sensitivity of 170 woody species to the late frost event in May 2011 in the Ecological-Botanical Gardens, Bayreuth.

Climatic parameter	Aggregation across species range	P_{GLM}	Expl. var.$_{\mathrm{BRT}}$
May minimum temperature	Mean	<0.001	51.7%
Temperature annual range	Standard deviation	<0.001	14.4%
Annual precipitation sum	Standard deviation	0.157	
Mean warmest month temperature	Standard deviation	0.025	18.7%
Sum of monthly precipitation (May–September)	Maximum	0.007	15.1%
De Martonne aridity index	Standard deviation	0.555	

The current climatic conditions (averages over the period 1950–2000) at 10-arcmin spatial resolution from WorldClim (http://worldclim.org; Hijmans *et al.*, 2005) were used for the analyses. Each single climatic parameter was assessed as the maximum (0.95 quantile), the mean, the minimum (0.05 quantile) and the standard deviation over all grid cells occupied by each respective species. After excluding collinearity (see Methods), six candidate climatic parameters were kept for further statistics in the stated aggregation across each species range. P_{GLM} provides their univariate *P*-value according to a binomial GLM. Expl. var.$_{\mathrm{BRT}}$ provides the explained variance of those parameters, which showed significant univariate relations to late frost damage (P_{GLM} <0.001) and have thus been used in the binomial BRT model (ROC = 0.737).

To understand the underlying climatic processes that shape the general relationships between late frost sensitivity and distributional characteristics, we analysed climatic parameters of the species distribution ranges at the species level. Here, we initially considered nine climatic parameters. The six parameters which have been used for further analyses are shown in Table 1. Three climatic parameters (annual mean temperature, minimum temperature of the coldest quarter, and precipitation of the warmest quarter) were removed due to autocorrelation with the six remaining parameters (see below). For each of the climatic parameters we considered the maximum (0.95 quantile), mean, minimum (0.05 quantile) and standard deviation across each species' native range (resulting in 36 parameters). Minima and maxima were used to take extreme values into account. Extreme values might characterize the absolute limits of species occurrences more precisely than mean conditions (Zimmermann *et al.*, 2009). Standard deviations were chosen to characterize the spatial heterogeneity across species ranges and to investigate the potential impact of climatic variability. Such variability can be expected to lead to more conservative phenology, with strategies to avoid spring frost risk (e.g. later onset of leaf unfolding at the price of a shorter growing season) and higher investment in protection (Wang *et al.*, 2014). Dehydration tolerance of plants plays an important role not only during drought but also during frost events (Sakai & Larcher, 1987; Blödner *et al.*, 2005; Beck *et al.*, 2007). Therefore, precipitation of the warmest quarter, sum of monthly precipitation from May to September and the aridity index according to De Martonne (1926) were considered in addition to temperature and annual precipitation parameters [aridity index = mean annual precipitation sum (mm)/(mean annual temperature (°C) + 10)].

Species leaf-out strategies

Data on leaf-out dates for 110 of the 170 species were available from observational studies on woody species conducted in the Munich Botanical Garden from 2012 to 2015 (see Zohner & Renner, 2014 for methodological details). The sampling included a broad range of woody species from the Northern Hemisphere. Individuals grown in the garden are mostly wild collections that are acclimated, but not evolutionarily adapted. Hence, their leaf-out times reflect native phenological strategies. For analysis, the mean of a species' leaf-out date (from 2012 to 2015) was used. Leaf-out was defined as the day when three to four branches of a plant unfolded leaves and pushed out all the way to the petiole.

Statistical analysis

The effects of species distributional characteristics on late frost tolerance (at species as well as genus level) were tested by simple and mixed generalized linear models based on a quasi-binomial distribution. To estimate goodness of fit for the generalized linear models, we calculated a pseudo-R^2 according to Nagelkerke (1991) using the NagelkerkeR2()-function of the fmsb-R-package (version 0.5.1).

The influence of the climate within a species' native range on sensitivity to the late frost event was quantified by boosted regression trees (BRT) (Elith *et al.*, 2008). Before fitting BRTs, a reduction in dimensionality was applied by removing autocorrelated parameters. Candidate climate parameters were tested for collinearity with each other using Spearman's nonparametric correlation. Where pairs of variables were highly correlated ($q > 0.7$), a univariate binomial generalized additive model (GAM) was fitted to the data using each highly correlated variable. In order to obtain less correlated variables and a final minimal model, the variable within each pair that yielded the higher Akaike information criterion (AIC) value was omitted. For the six resulting climate parameters, we ran univariate binomial generalized linear models (GLMs) which resulted in four climate parameters that were significantly related to sensitivity to the late frost event: (1) mean over the species' range of the minimum temperature in May, (2) standard deviation over the species' range of the mean temperature of the warmest month, (3) maximum over the species' range of the sum of

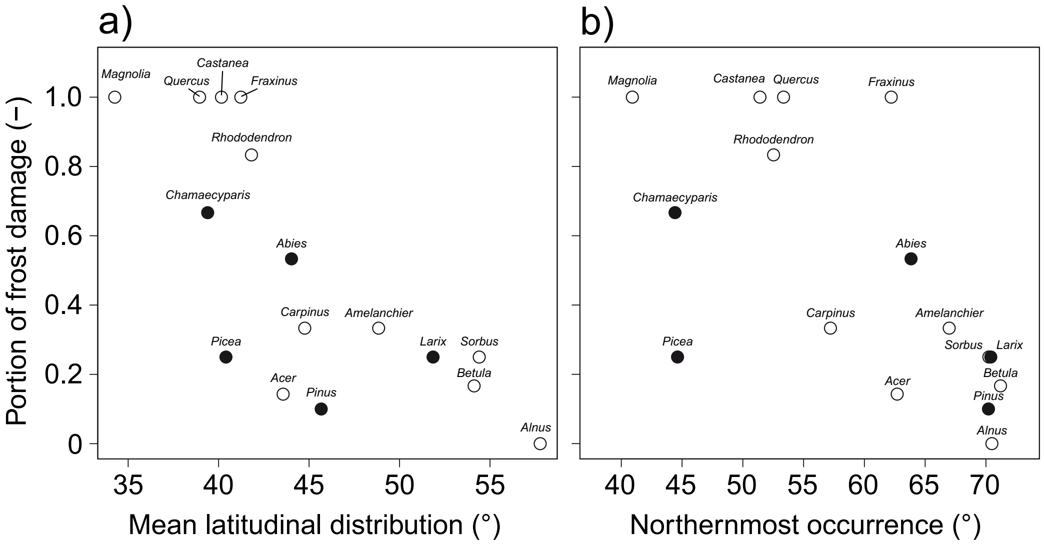

Figure 2 Relation between the probability of late frost damage for 16 Northern Hemisphere genera of 105 woody plant species and (a) the mean latitudinal distribution (species-specific latitudinal distribution centroid averaged for each genus, $P = 0.005$, pseudo-$R^2 = 0.59$) and (b) the northernmost occurrence (maximum latitudinal occurrence for each genus, $P = 0.022$, pseudo-$R^2 = 0.42$). The probability of late frost damage is depicted as the portion of species within each genus with visible late frost damage during May 2011. Filled symbols refer to coniferous species and open symbols to broad-leaved species.

precipitation from May to September, and (4) standard deviation over the species' range of the annual temperature range (Table 1). Only these four significantly explaining climatic parameters ($P < 0.05$) were considered in the subsequent BRT models.

Binomial BRTs were fitted according to Elith *et al.* (2008) with the selection of the final model being based on minimal estimated cross-validated deviance. This was obtained by setting the tree complexity to 5, the learning rate to 0.001 and the bag fraction to 0.9. The cross-validated receiver operating characteristic (ROC) score was used to express the correlation between climate within a species' native range and late frost damage. For each climatic parameter, its relative importance for explained variance was provided.

In addition to the climatic parameters of species native ranges the role of phylogenetic relatedness on late frost tolerance was tested with ANOVA analyses paired with post hoc multiple comparison tests. To omit statistical biases caused by small sample sizes we focused on a comparison of seven genera for which at least five species were investigated (*Abies*, *Acer*, *Betula*, *Fraxinus*, *Pinus*, *Quercus*, *Rhododendron*; see Appendices S1 & S2 for detailed information). Differences in the geographical distribution of these genera were tested by using Tukey honestly significant difference tests for multiple comparisons. Differences in late frost sensitivity were tested pairwise by using Wilcoxon rank sum tests for independent samples because of the binomial character of the tested variable. The level of significance was adjusted for these multiple tests by applying the Bonferroni–Holm correction.

All statistical analyses were executed with the software R 3.0.2 (R Development Core Team, 2013) and the additional packages mgcv v.1.7-26, gbm v.2.1, sciplot v.1.1-0, and popbio v.2.4.

RESULTS

The probability of leaf damage of the observed 170 woody plant species and 16 genera due to the studied late frost event significantly decreased with increasing latitudinal distribution centre (at species level, $P = 0.005$, pseudo-$R^2 = 0.15$, Appendix S2; at genus level $P = 0.005$, pseudo-$R^2 = 0.59$, Fig. 2a). This pattern was consistent for broad-leaved as well as coniferous genera as the effect of leaf morphology on late frost sensitivity was not significant in a generalized linear mixed effect model ($P = 0.09$). The same positive effect on sensitivity to the late frost event was found for the northernmost occurrence (species level, $P = 0.001$, pseudo-$R^2 = 0.12$; genus level, $P = 0.022$, pseudo-$R^2 = 0.42$; Fig. 2b), again with no significant difference between broad-leaved and coniferous genera ($P = 0.17$), but not for the southernmost occurrence (species level, $P = 0.13$, pseudo-$R^2 = 0.02$; genus level, $P = 0.48$, pseudo-$R^2 = 0.04$).

Besides the significant effects detected for the geographical ranges (distribution centre and northernmost occurrence), phylogenetic relatedness showed a strong effect on the frost tolerance of the investigated species. Species-specific frost tolerance was significantly better explained when including 'genus' as an additional explanatory variable besides the distributional variables (pseudo-$R^2 = 0.15$ vs. 0.86 for latitudinal distribution centre and pseudo-$R^2 = 0.12$ *vs.* 0.87). For instance, observed frost damage differed significantly between the genera *Quercus* (frost damage in all observed species) and *Pinus* (no frost damage in any observed species), despite their largely overlapping geographical distribution ranges (Appendices S2 & S3). Likewise, *Pinus* and *Acer* (frost damage in only 2 out of 14 species) differed significantly from *Fraxinus* (frost damage in all observed species) and *Rhododendron* (frost damage in four out of five observed species) despite the distributional characteristics (latitudinal distribution centre, northernmost as well as southernmost occurrence) not differing significantly among these genera in our dataset (Appendix S3).

Longitude had no significant effect on sensitivity to the late frost event, neither the mean nor the variation of the longitudinal centroids ($P = 0.42$ and $P = 0.10$, respectively). The same was true for maximum and minimum continentality experienced by a species over its range within each genus ($P = 0.20$ and $P = 0.30$, respectively). Also species-specific mean continentality averaged for each genus showed no significant effect on sensitivity to the late frost event ($P = 0.23$). However, the probability of late frost damage significantly decreased with increasing species-specific range of continentality averaged for each genus ($P = 0.045$, pseudo-$R^2 = 0.33$). This means that the wider the range of continentality experienced by the species of a certain genus in their distribution ranges, the lower was the probability of late frost damage within this genus.

The species-specific probability of being damaged by the late frost event was well explained by the climatic conditions within the native distribution ranges (BRT cross-validated ROC score = 0.737). The probability of frost damage was best explained by the mean over the species' range of the May minimum temperature (51.7%) followed by the standard deviation over the species' range of the mean temperature of the warmest month (18.7%), the maximum over the species' range of the sum of precipitation from May to September (15.1%) and by the standard deviation over the species' range of the annual temperature range (14.4%) (Table 1). The probability of being damaged by the late frost event increased with increasing mean over the species' range of the May minimum temperature ($P < 0.001$), with decreasing standard deviation over the species' range of the mean temperature of the warmest month ($P = 0.025$), with increasing maximum over the species' range of the sum of precipitation from May to September ($P = 0.007$), and with decreasing standard deviation over the species' range of the annual temperature range ($P < 0.001$) (Fig. 3).

Species leaf-out dates (mean for 2012 to 2015) were highly correlated with sensitivity to the late frost event ($P < 0.001$) (Fig. 4). On average, the leaf-out dates of frost-resistant species preceded those of frost-sensitive species by 10 days.

DISCUSSION

The sensitivity of the 170 woody species studied to the late frost event in May 2011 was found to be significantly related to species distributions (latitude of species distributional centres, northern range limit). Furthermore, genera with wider ranges in their latitudinal distribution and in continentality turned out to be less vulnerable to the late frost event in May 2011. In addition to the biogeographical patterns, we found that the phenological strategy of species was highly adapted to sensitivity to the late frost event, with early leafing species being less susceptible to late frost events. These patterns were consistent for broad-leaved and coniferous species.

Notably, many of the woody species studied here grew outside their native range. Thus, climate, community composition, photoperiod and soil conditions may not be at their preferred values. Still, frost sensitivity as well as phenological strategy of leaf-out can be assumed to be rather conservative traits so that our results bear implications beyond the single study site. We did observe clear geographical patterns by only considering the natural species distributions without further information on the precise origin of the studied ecotypes. To address this limitation, not just the mean of the climatic parameters within the native ranges but also the maxima, minima and the standard deviation have been used to characterize the distribution ranges. Hence, extreme values and spatial heterogeneity across species ranges are taken into account.

Species sets in botanical gardens represent a subjective sample of species able to tolerate the conditions at the specific garden. Despite this obvious bias, our results indicate that sensitivity to the studied late frost event could be significantly better explained by including genus as an additional explanatory factor besides the distributional variables. Further, the observed frost damage differed significantly between genera, even if the distributional characteristics did not due to the given subset of species within the genera. Hence, our study hints at phylogenetic relatedness having strong effects on the late frost tolerance, i.e. phylogenetic conservatism of late spring frost tolerance.

Up to now, more attention has been paid to the role of extreme cold events in winter and winter frost sensitivity as limiting factors for the ranges of tree species (Sakai & Weiser, 1973; Jalili *et al.*, 2010; Kreyling *et al.*, 2015). 'Winter hardiness zones' have been classified, reflecting distribution patterns related to the extreme minimum temperatures in tree species ranges (Roloff & Bärtels, 2006; Daly *et al.*, 2012). However, Lenz *et al.* (2013) and Kollas *et al.* (2014) found extreme frost events during bud burst in spring rather than minimum winter temperature to be the factor that was most limiting for species distribution. Focusing on the underlying climatic drivers, the probability of frost damage in our study was well explained by the climatic characteristics of species native ranges (BRT ROC = 0.737). Concerning specific climate parameters, late frost sensitivity was most strongly related to the May minimum temperature within the native range (> 50% of explained variance), which is at the beginning of the growing season of most species considered. Species with higher May minimum temperatures in their native range responded more sensitively to this particular late frost event. This tight link across 170 species from all over the Northern Hemisphere supports the conclusion of Lenz *et al.* (2013) and Kollas *et al.* (2014) that late frost sensitivity is an important consideration in projections of range shifts of woody species in the face of climate change.

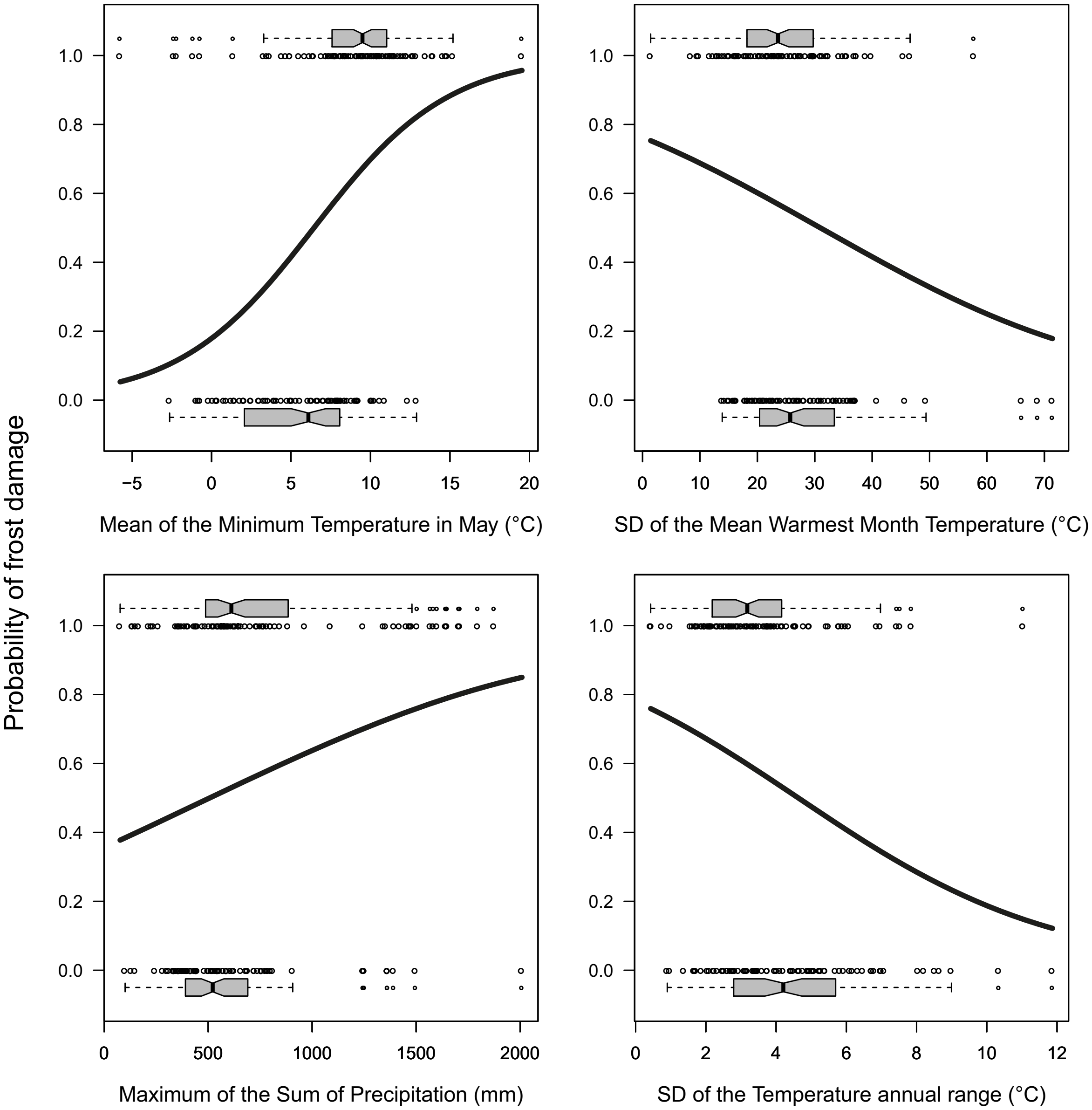

Figure 3 The univariate probability of species-specific late frost damage (no damage = 0, damage = 1) in relation to climatic characteristics of the species ranges of 170 woody species (only those parameters with $P < 0.05$ in univariate generalized linear models are shown; see Table 1).

In addition to species differences in sensitivity to the late frost event, phenological adaptation to climatic conditions in the native range of woody species could play an important role with regard to their response to late frost events. The timing of bud burst, which is a sensitive phase in the phenological cycle, is crucial for the risk of frost damage in respect to cold events in the temperate latitudes during the spring (Sakai & Larcher, 1987; Inouye, 2008; Martin *et al.*, 2010; Kreyling *et al.*, 2012b; Augspurger, 2013; Vitasse *et al.*, 2014b). By investigating the leaf-out strategies of a broad range of taxonomically distinct temperate woody species in relation to their sensitivity to the late frost event, we found that leaf unfolding dates were highly related to the frost sensitivity of the leaves: species resistant to the late frost event leafed-out as much as 10 days earlier than susceptible species. This demonstrates that species finely adjust the time of leaf appearance – the most freezing-sensitive phenological phase – to their susceptibility to late frosts. By using a broad range of woody temperate species from various climates, our study thereby confirms similar patterns found for smaller and more regional subsets of species (Lenz *et al.*, 2013; Vitasse *et al.*, 2014a).

According to Lenz *et al.* (2013) freezing tolerance within species differs among phenological stages. Here, the strongest changes in frost sensitivity occurred before bud burst and there were none or only slight changes in both possible directions after leaf unfolding, depending on the individual species. Thus, a possible caveat of our approach is that not all

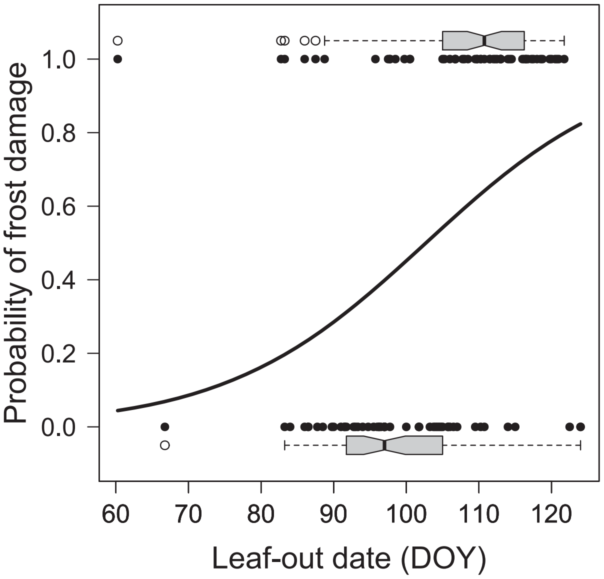

Figure 4 The univariate probability of species-specific late frost damage (no damage = 0, damage = 1) in relation to the leaf-out strategy of 110 woody species ($P < 0.001$; univariate generalized linear model). Species leaf-out dates (recorded as day of the year, DOY) are the average dates from 2012 to 2015 observed in the Munich Botanical Garden.

species were at exactly the same phenological stage when they were exposed to the freezing event. However, bud burst and leaf unfolding were completed in all studied species when they were hit by the late frost. Therefore, it is unlikely that contrasting phenological stages at the time of the frost event would be responsible for the observed pattern of early leafing species being more frost tolerant. Unfortunately, we lack phenological data for the 2011 study year, but infer species phenological strategies from leaf-out data collected from 2012 to 2015 (including the warmest recorded spring in Bavaria in 2014). The order of species-level leaf-out dates was found to be highly conserved over time (Panchen *et al.*, 2014; Zohner & Renner, 2014) and therefore our leaf-out data can be assumed to reflect also the sequence of leaf unfolding in the year 2011.

Which phenological safety mechanisms do frost-sensitive species use to avoid precocious bud development? Current studies suggest that chilling requirements are the main drivers to limit advanced budburst (Laube *et al.*, 2014). According to Fu *et al.* (2015), reduced chilling over winter potentially leads to an increased heat requirement in spring and consequently to a delayed tracking of climate warming in spring phenology. However, the impact of photoperiod as well as temperature cues on phenology have to be kept in mind, especially in times of global warming (Körner & Basler, 2010; Basler & Körner, 2012): late successional tree species have been observed to be photoperiod sensitive. Photoperiodic control of phenology can limit the phenological responses of late successional species to warming, particularly when a warm spring temperature would suggest promoted development. Savage & Cavender-Bares (2013) found that northern species within the family Salicaceae were more strongly constrained by photoperiod as a cue for bud burst than southern species. The role of photoperiodic constraints on spring phenology indicated by their example clearly requires further attention, as it is of great importance for understanding the impacts of climate change on species migrations.

In our study, high spatial (and ecological) heterogeneity within a species' natural range was found to be linked to reduced observed damage as a response to the spring frost event. For a species as a whole, a high spatial heterogeneity of annual air temperature and climatic continentality within its range necessitates, among other things, protection against cold winters, extreme late frost events and summer drought (Czajkowski & Bolte, 2006). Moreover, species that tolerate high heterogeneity in terms of the warmest mean monthly temperature also need to be adapted to drought, as high temperatures during summer are likely be connected to a higher evapotranspirational demand and hence can cause drought stress (Dai *et al.*, 2004).

Likewise, low precipitation during the growing season (the maximum, i.e. 95% quantile, of the sum of monthly precipitation from May to September across the species' native range) in the native range reduced the probability of being damaged by the late frost event. Thus, water shortage experienced during evolution can play a role with regard to late frost sensitivity. This, again, can potentially be explained by cross-stress tolerance in the face of drought or frost (Walter *et al.*, 2012). Plant species that are adapted to drought are often also adapted to frost-induced water stress via physiological responses, such as accumulation of non-structural carbohydrates, to protect phenological, morphological or physiological traits (Inouye, 2000; Beck *et al.*, 2007).

In conclusion, the individual sensitivity of the 170 woody species observed to a late frost event after leaf unfolding can be explained by the species' natural latitudinal range, the spectrum of continentality and by specific climatic conditions, in particular the mean minimum temperature in May across the species' distribution.

Thus, late frost sensitivity appears to be a factor controlling species' distribution limits and is an important consideration in projections of range shifts of tree species or in concepts of assisted migration. Furthermore, we reveal in this study that late frost sensitivity appears to be synchronized with the species' phenological strategy.

Implications and outlook

Species are expected to respond to global warming with upward or poleward shifts of their distribution limits (Parmesan *et al.*, 1999; Lenoir *et al.*, 2008). In particular, tree species are found to lag behind the rapidity of warming, a fact commonly explained by their conservative dispersal strategies and long regeneration cycles (Petit & Hampe, 2006). Therefore, assisted migration is discussed as an option to support the adaptation of forest stands to future climate conditions. However, the success of actively shifted populations might be jeopardized by extreme winter frost (Jalili *et al.* 2010) and late frost events (our results, but see also Kollas *et al.*, 2014), as these appear to be two of the most important factors controlling species native range limits.

Even though the late frost event in May 2011 was extreme (the most severe late frost event since records began on site in 1997 and locally in 1961), it was generally not lethal to any tree species in this study. However, late spring frost events can have strong ecological implications as they can reduce growth performance. For instance, tree ring widths dropped by up to 90% in *Fagus sylvatica* in the Alps in years with spring temperatures below −3 °C (Dittmar *et al.*, 2006) due to reduced growing season length and loss of resources like stored carbon and other nutrients (Lockhart, 1983; Gu *et al.*, 2008; Augspurger, 2009; Martin *et al.*, 2010). This can scale up to extreme late frost events altering biogeochemical cycles (Mulholland *et al.*, 2009). Resilience, however, appears remarkably high with tree rings in *F. sylvatica* in the Alps in the years after the frost events reaching equal increments as before (Dittmar & Elling, 2006). We therefore assume that the tight link between species distributions and late frost sensitivity observed in our study is not due to lethal effects but rather to loss of storage and a shortened growing season. In consequence, the link could be due to the reduced competitive power and potential carry-over effects on build-up of dormancy and winter hardening in autumn.

CONCLUSIONS

The sensitivity of 170 boreal and temperate tree species in the EBG of the University of Bayreuth to the late frost event in May 2011 was well explained by species geographical distributions and the underlying climatic conditions in species native ranges, in particular by spring minimum temperatures. Sensitivity to the late frost event was generally greater for species with lower northern range limits, lower variability in continentality and higher May minimum temperatures as well as higher precipitation during the growing season in their native ranges. Species phenological strategies appear to be well adjusted to late frost sensitivity. Early leafing species were more tolerant against the late frost event than species that started their development later in spring. Hence, our study emphasizes the ecological and evolutionary importance of late frost damage in tree species. Single extremes such as late frost events can potentially jeopardize natural and anthropogenic range shifts as a response to global warming and should therefore be acknowledged in further research, nature conservation or forestry.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Species list with information on distribution and frost damage.
Appendix S2 Distributional characteristics and late frost sensitivity of the investigated woody species.
Appendix S3 Differences in late frost sensitivity and geographical distributions between the investigated tree genera.

BIOSKETCH

Lena Muffler is interested in global change and the impact of extreme weather events, such as frost, late frost and drought, on ecosystems. Here, species responses to global warming are investigated by statistical modelling (species distribution modelling, multiple regression analysis), ecological experiments (reciprocal transplantation, climate manipulation) and field observations.

Editor: Thomas Hickler

SUPPORTING INFORMATION

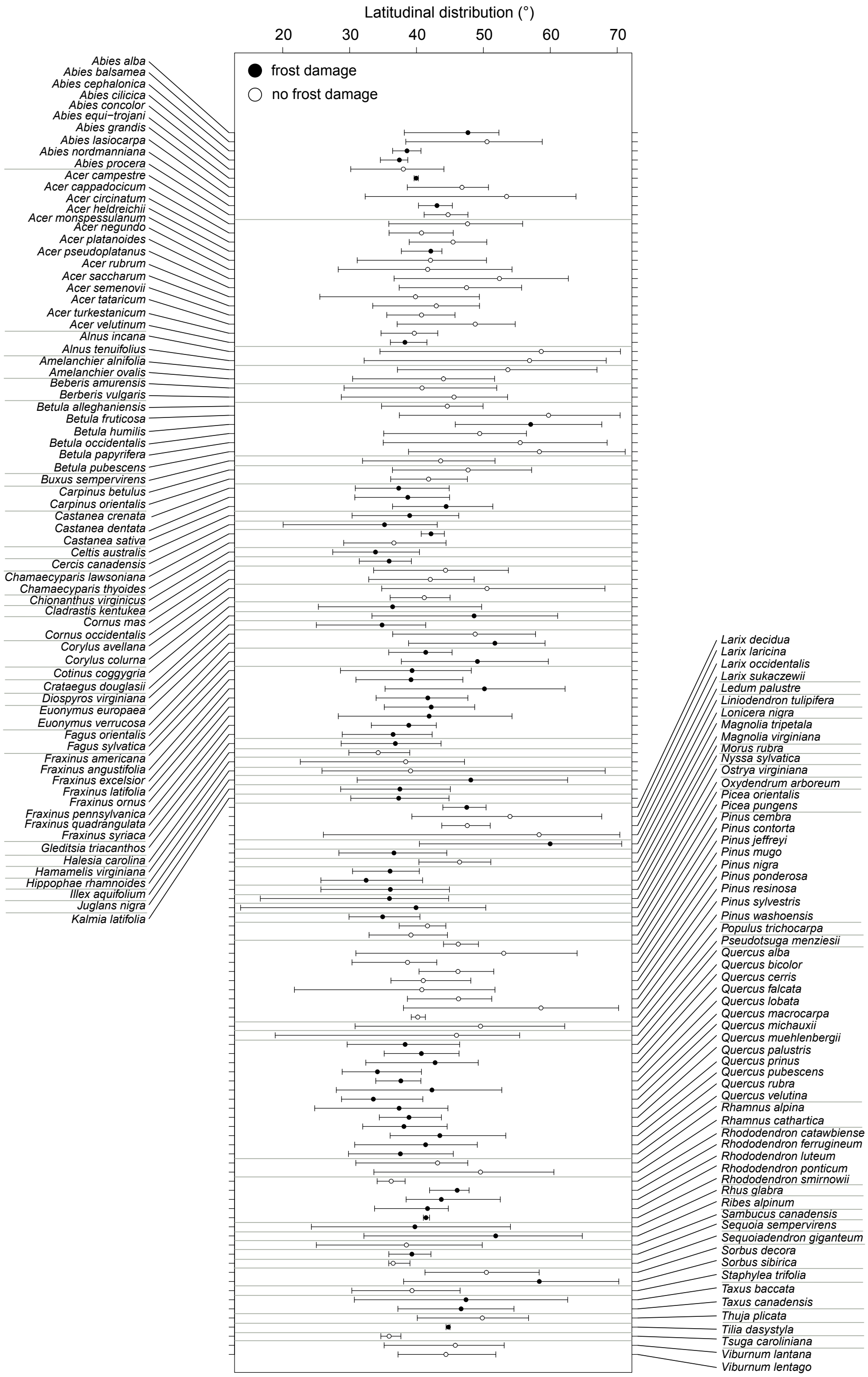
Appendix S1: 170 species were inspected for frost damage (1 = if at least one individual showed frost damage measured by leaf browning as indicator, 0 = no damage) in the Ecological Botanical Gardens in Bayreuth after a late frost event in May 2011. Source shows the reference of the species' native ranges (1 = Bioversity International (2013) European Forest Genetic Resources Programme. Distribution maps. http://www.euforgen.org/distribution_maps.html, 2 = U.S. Department of the Interior & U.S. Geological Survey (2013) Digital representations of tree species range maps from „Atlas of United States Trees“ by Elbert L. Little, Jr. http://esp.cr.usgs.gov/data/little/., 3 = Horikawa, Y. (1976) Atlas of the Japanese Flora II: An Introduction to Plant Sociology of East Asia, Gakken Co. Ltd., Tokyo., 4 = Ying, T.-S., Chen, M.-L. & Chang, H.-C. (2003) Atlas of the Gymnosperms of China, China Science and Technology Press, Beijing., 5 = Jalas, J. & Suominen, J. (1973) Atlas florae Europaeae. Distribution of vascular plants in Europe: Gymnospermae (Pinaceae to Ephedraceae), Committee for Mapping the Flora of Europe, Helsinki., 6 = Meusel, H., Jäger, E. & Weinert, E. (1992) Vergleichende Chorologie der zentraleuropäischen Flora - Karten, VEB Gustav Fischer Verlag, Jena., 7 = Little, E.L. (1971) Atlas of United States Trees. Conifers and Important Hardwoods, United States Government Printing Office, Washington D.C., 8 = Little, E.L. (1977) Atlas of United States Trees. Minor Eastern Hardwoods, United States Government Printing Office, Washington D.C., 9 = Little, E.L. (1976) Atlas of United States Trees. Minor Western Hardwoods Washington, United States Government Printing Office, Washington D.C., 10 = Interactive agricultural ecological atlas of Russia and neighboring countries (2009) Economic plants and their diseases, pests and weeds. http://www.agroatlas.ru/en/content/related/).

Species	Number of individuals	Frost damage	Source
Abies alba Mill.	5	1	1
Abies balsamea (L.) Mill.	1	0	2
Abies cephalonica Loud.	8	1	5
Abies chensiensis Tiegh.	1	0	4
Abies cilicica (Ant. & Kotschy) Carr.	5	1	6
Abies concolor (Gord. & Glend.) Lindl. ex Hildebr.	3	0	2
Abies delavayi Franch.	3	1	4
Abies equi-trojani (Asch. & Sint. ex Boiss.) Coode & Cullen	1	1	6
Abies grandis (Dougl. ex D.Don) Lindl.	2	0	2
Abies holophylla Maxim.	2	1	4
Abies lasiocarpa (Hook.) Nutt.	6	0	2
Abies nordmanniana (Stev.) Spach	4	1	6
Abies procera Rehd.	2	0	2
Abies sibirica Ledeb.	1	1	4
Abies veitchii Lindl.	5	0	3
Acer campestre L.	1	0	1
Acer cappadocicum Gled.	1	0	6
Acer circinatum Pursh	2	0	2
Acer heldreichii Orph. ex Boiss.	2	1	6
Acer monspessulanum L.	2	0	6
Acer negundo L.	4	0	2
Acer platanoides L.	1	0	6
Acer pseudoplatanus L.	1	0	1
Acer rubrum L.	6	0	2
Acer saccharum Marshall	1	0	2

Acer semenovii Regel & Herder	1	0	6
Acer tataricum L.	4	0	6
Acer turkestanicum Pax	1	0	6
Acer velutinum Boiss.	2	1	6
Actinidia kolomikta (Rupr. & Maxim.) Maxim.	1	1	10
Alnus incana (L.) Moench	1	0	6
Alnus maximowiczii Callier	1	0	3
Alnus tenuifolia Nutt.	1	0	2
Amelanchier alnifolia (Nutt.) Nutt. ex M.Roem.	2	0	2
Amelanchier asiatica (Sieb. & Zucc.) Endl. ex Walp.	1	1	3
Amelanchier ovalis Medik.	2	0	10
Aralia cordata Thunb.	1	1	3
Aralia elata (Miq.) Seem.	3	1	3
Berberis amurensis Rupr.	1	0	6
Berberis vulgaris L.	1	0	6
Betula alleghaniensis Britton	1	0	2
Betula fruticosa Pall.	5	0	6
Betula humilis Schrank	1	1	6
Betula occidentalis Hook.	1	0	2
Betula papyrifera Marshall	1	0	2
Betula pubescens Ehrh.	1	0	6
Buxus sempervirens L.	1	0	6
Callicarpa japonica Thunb.	2	1	3
Carpinus betulus L.	1	0	6
Carpinus orientalis Mill.	3	0	6
Carpinus turczaninovii Hance	1	1	3
Castanea crenata Sieb. & Zucc.	1	1	6
Castanea dentata (Marshall) Borkh.	1	1	2
Castanea sativa Mill.	3	1	1
Celtis australis L.	1	1	6
Cercis canadensis L.	2	1	8
Chamaecyparis lawsoniana (A.Murr.) Parl.	2	1	2
Chamaecyparis obtusa (Sieb. & Zucc.) Endl.	1	1	3
Chamaecyparis thyoides (L.) Britton, Sterns & Poggenb.	1	0	2
Chionanthus virginicus L.	3	1	2
Cladrastis kentukea (Dum.Cours.) Rudd	1	1	2
Clethra barbinervis Sieb. & Zucc.	1	1	3
Cornus mas L.	1	0	6
Cornus occidentalis (Torr. & A.Gray) Coville	1	0	2
Corylus avellana L.	1	0	6
Corylus colurna L.	1	0	6
Cotinus coggygria Scop.	6	1	6
Crataegus douglasii Lindl.	1	1	8
Diospyros virginiana L.	2	1	2
Euonymus europaeus L.	1	0	6
Euonymus verrucosus Scop.	1	1	6
Fagus orientalis Lipsky	1	1	1
Fagus sylvatica L.	1	1	1
Fraxinus americana L.	3	1	2
Fraxinus angustifolia Vahl	3	1	6
Fraxinus excelsior L.	5	1	1
Fraxinus latifolia Benth.	1	1	2
Fraxinus ornus L.	5	1	6
Fraxinus pennsylvanica Marshall	1	1	2
Fraxinus quadrangulata Michx.	1	1	2
Fraxinus syriaca Boiss.	1	1	6
Ginkgo biloba L.	3	1	4
Gleditsia triacanthos L.	3	1	2
Halesia carolina L.	1	0	2
Hamamelis virginiana L.	2	0	2
Hippophae rhamnoides L.	3	0	6
Ilex aquifolium L.	2	1	6
Juglans ailantifolia Carr.	1	1	3
Juglans nigra L.	1	1	2
Kalmia latifolia L.	1	1	2
Larix decidua Mill.	3	1	1
Larix laricina (Du Roi) K.Koch	1	0	7
Larix occidentalis Nutt.	1	0	2

Larix sukaczewii Dylis	3	0	6
Ledum palustre L.	4	1	6
Lindera obtusiloba Blume	1	1	3
Liriodendron tulipifera L.	2	1	2
Lonicera nigra L.	2	0	6
Magnolia kobus DC.	2	1	3
Magnolia tripetala (L.) L.	1	1	2
Magnolia virginiana L.	1	1	2
Metasequoia glyptostroboides Hu & Cheng	1	1	4
Morus australis Poir.	1	1	3
Morus rubra L.	1	1	2
Nyssa sylvatica Marshall	2	1	2
Ostrya virginiana (Mill.) K.Koch	2	1	2
Oxydendrum arboreum (L.) DC.	1	1	2
Photinia villosa (Thunb.) DC.	1	1	3
Picea jezoensis (Sieb. & Zucc.) Carr.	2	0	4 & 3
Picea orientalis (L.) Link	2	0	6
Picea pungens Engelm.	2	0	2
Picea smithiana (Wall.) Boiss.	4	1	4
Pinus cembra L.	2	0	1
Pinus contorta Dougl. ex Loud.	1	0	2
Pinus jeffreyi Balf.	1	0	2
Pinus mugo Turra	5	0	6
Pinus nigra J.F.Arnold	3	0	1
Pinus ponderosa Dougl. ex P. & C.Lawson	7	0	2
Pinus resinosa Aiton	1	0	2
Pinus sylvestris L.	1	0	6
Pinus wallichiana A.B.Jacks.	2	1	4
Pinus washoensis Mason & Stockw.	1	0	2
Populus trichocarpa Torr. & A.Gray ex Hook.	1	0	2
Pseudotsuga menziesii (Mirb.) Franco	4	0	2
Pterocarya rhoifolia Sieb. & Zucc.	3	1	3
Quercus acutissima Carruth.	1	1	3
Quercus alba L.	1	1	2
Quercus bicolor Willd.	2	1	2
Quercus cerris L.	2	1	6
Quercus dentata Thunb.	2	1	3
Quercus falcata Michx.	6	1	2
Quercus lobata Née	1	1	2
Quercus macrocarpa Michx.	1	1	2
Quercus michauxii Nutt.	1	1	2
Quercus muehlenbergii Engelm.	1	1	2
Quercus palustris Münchh.	1	1	2
Quercus prinus L.	1	1	2
Quercus pubescens Willd.	6	1	6
Quercus rubra L.	1	1	2
Quercus serrata Thunb.	1	1	3
Quercus velutina Lam.	1	1	2
Rhamnus alpina L.	1	0	6
Rhamnus cathartica L.	1	0	6
Rhododendron catawbiense Michx.	3	0	2
Rhododendron ferrugineum L.	3	1	6
Rhododendron japonicum (A.Gray) Sur.	10	1	3
Rhododendron luteum Sweet	10	1	6
Rhododendron ponticum L.	3	1	6
Rhododendron smirnowii Trautv.	3	1	6
Rhus glabra L	1	1	2
Ribes alpinum L.	1	1	6
Rubus phoenicolasius Maxim.	1	0	3
Sambucus canadensis L.	1	0	2
Sciadopitys verticillata (Thunb.) Sieb. & Zucc.	3	0	3
Sequoia sempervirens (D.Don) Endl.	1	1	2
Sequoiadendron giganteum (Lindl.) J.Buchh.	4	0	2
Sorbus alnifolia (Sieb. & Zucc.) K.Koch	1	0	3
Sorbus decora (Sarg.) C.K.Schneid.	1	0	9
Sorbus matsumurana (Makino) Koehne	3	0	3
Sorbus sibirica Hedl.	1	1	6
Staphylea trifolia L.	1	0	2

Taxus baccata L.	1	1	6
Taxus canadensis Marshall	1	1	6
Thuja plicata Donn ex D.Don	2	0	2
Thuja standishii (Gordon) Carr.	1	0	3
Tilia dasystyla Steven	1	1	6
Tsuga caroliniana Engelm.	2	0	2
Viburnum lantana L.	1	0	6
Viburnum lentago L.	1	0	2
Zelkova serrata (Thunb.) Makino	2	1	3



Appendix S2: Distributional characteristics and late frost sensitivity of woody species investigated in this study. Shown is the distributional centre (points) as well as the southernmost and northernmost occurrence of each investigated species. Species-specific information about frost damage is indicated by point color (black: frost damage was detected; white: no frost damage was detected). Genera are separated by grey horizontal lines. Without species where just single occurrence points could be found in the literature.

Appendix S3: Differences in late frost sensitivity and geographical distributions between the tree genera investigated in this study. Bold printed p-values depict significant differences. Differences in frost damage were tested by using pairwise Wilcoxon Rank Sum test in combination with Bonferroni-Holm correction of the level of significance. Differences in geographical distributions are tested by Tukey HSD tests for multiple comparison tests. Only genera with at least 5 species were included (for detailed information see Appendix S1 and Appendix S2)

	Wilcox-test Frost damage	Tukey HSD Centr Lat	Tukey HSD Max Lat	Tukey HSD Min Lat
Genus	**p**	**p**	**p**	**p**
Quercus-Pinus	**5.54E-06**	**0.0286465**	0.3716428	0.2054556
Quercus-Acer	**1.26E-05**	0.1696174	0.6970641	0.3285181
Pinus-Fraxinus	**8.00E-05**	0.4715257	0.9666129	0.534572
Fraxinus-Acer	**0.0001732**	0.9220777	0.9998628	0.7360812
Quercus-Betula	**0.000238145**	**0.0000005**	**0.0000398**	**0.028745**
Fraxinus-Betula	**0.002481524**	**0.0000988**	**0.0034449**	0.1222686
Rhododendron-Pinus	**0.002935353**	0.7690877	0.5107114	0.9492052
Quercus-Abies	0.005516333	0.1629635	0.9867465	**0.0229243**
Rhododendron-Acer	0.009772931	0.9916618	0.7872328	0.7422515
Pinus-Abies	0.01867785	0.988373	0.8641217	0.9867807
Fraxinus-Abies	0.0257534	0.8735566	0.9999595	0.1386153
Rhododendron-Betula	0.05777957	**0.0013111**	**0.0005748**	1
Acer-Abies	0.06866261	0.9999843	0.9930786	0.8083061
Rhododendron-Quercus	0.136641	0.910126	0.9999755	**0.0474949**
Betula-Abies	0.2200314	**0.0022374**	**0.0008253**	0.9996843
Rhododendron-Fraxinus	0.2683816	0.9999902	0.943574	0.1613587
Pinus-Acer	0.2730348	0.9427577	0.9920683	0.9986046
Pinus-Betula	0.2763029	**0.0209967**	**0.0318206**	0.9374512
Rhododendron-Abies	0.3133992	0.9781886	0.9798797	0.9997301
Betula-Acer	0.9469029	**0.0005165**	**0.0023826**	0.688668
Quercus-Fraxinus	1	0.934884	0.9484493	0.9997928

Chapter 6

GENERAL DISCUSSION

6.1 Do temperate woody species use the photoperiod in spring as a cue for leaf-out?

Determining the environmental cues that trigger the timing of plant growth and development is of vital importance for climate change research (Körner & Basler, 2010; Richardson *et al*., 2013; Way & Montgomerey, 2015). While temperature-dependent processes should be affected by global warming, processes that are mediated by day length should not change in the future because photoperiod will not change with climate warming. The topic is complex because phenological events can be triggered by a combination of different external cues. This is the case for the timing of leaf unfolding in temperate woody species: cues from chilling, warming, and photoperiod interact, leading to a situation in which leaf emergence is the result of multiple environmental forces operating at different times during dormancy (Sanz-Perez *et al*., 2009; Caffarra & Donnelly, 2011; Cooke *et al*., 2012; Laube *et al*., 2014a; Polgar *et al*., 2014; Zohner & Renner, 2014). Hence, to forecast future responses, we need to broaden our understanding of the physiological and molecular mechanisms of leaf unfolding.

A main goal of my doctoral research was to answer the following questions about photoperiod-control of leaf unfolding: (i) Where (in which tissues and organs) do plants perceive photoperiod signals and how? (ii) When during dormancy are photoperiod signals perceived? (iii) Which species rely on photoperiod to time leaf-out? And finally, what is the evolutionary advantage of using photoperiod to trigger dormancy release? The first two questions were addressed in Chapter 2 of this thesis, questions three and four in Chapter 3.

Since "photoreceptors and the clock system are, in principal, found in all plant cells" (Cooke *et al*., 2012, p. 1715), there are many ways how photoperiod could trigger leaf unfolding. To answer where (and how) photoperiod is perceived I conducted *in situ* bagging experiments, in which branches of trees were kept under short day conditions, while the remaining parts of the same trees experienced the natural day length increase during spring (Chapter 2). Principally, light signals could be perceived by all parts of the tree exposed to solar radiation (leaving the root system as the most unlikely organ to be involved in light perception), with two mechanisms for how light signals could be perceived, either systemic or local. A systemic response predicts that buds kept under constant short days will not differ in their reaction from uncovered buds of the same tree because (hormonal or other) signaling processes should lead to a uniform reaction. Alternatively, there might be localized responses only in certain parts of a tree. The latter was the case in my experiments: in *Fagus sylvatica*, buds kept under constant 8-h days leafed out 41 days later than buds on the same branch exposed to natural photoperiod, ruling out a systemic response and instead suggesting that each bud autonomously perceives and reacts to day length. Additional experiments in which I exposed buds to different light spectra revealed that it is the leaf primordial cells inside the dead bud scales that react to far-red light to activate the signal cascade ultimately inducing leaf unfolding (Figs. 3 and 4 in Chapter 2).

Another question I aimed to answer was when photoperiod signals are perceived during the dormant winter period. Related to this is the question if photoperiod signals at some threshold value induce an irreversible reaction or if instead a continuous long-day signal is required that can be interrupted, then causing a slower or arrested reaction? Using a combination of *in* situ bagging and twig cutting experiments, I found that buds of *F. sylvatica* perceive photoperiod signals only in the late phase of dormancy, while long days experienced concurrent with cold air temperatures do not affect dormancy. Therefore it can be concluded that, in photosensitive species, long-days do not *per se* cause an irreversible reaction, but are required concurrent with spring warming to allow for bud burst.

Which particular day-length is required to allow for bud development and in which way does photoperiod interact with chilling and warming temperatures? According to Vitasse and Basler (2013), there are two possibilities: (i) Either a fixed photoperiod threshold has to be met before buds are able to respond to warming signals or (ii) warming requirements continuously decrease with increasing day-length. To test this I exposed dormant twigs of *F. sylvatica* to different photoperiods (8-h, 12-h, and 16-h light per day) and found strong support for the second mode of action, i.e., the longer the days, the less warming was required to induce leaf unfolding (see Fig. 3 in Chapter 2).

Having answered where and when photoperiod perception takes place in trees, I focused on inter-specific variability in photoperiodism and the underlying adaptive mechanisms fostering it. According to Körner and Basler (2010), long-lived species, especially those from regions with unpredictable temperature regimes (oceanic climates), might rely on photoperiod signals to time their leaf unfolding. In addition, species from higher latitudes might be more sensitive to photoperiod, first, because the annual variation in photoperiod increases with latitude, and second, because of its hypothesized function to act as an insurance against being misguided by unpredictable spring temperatures (Körner, 2006; Saikkonen *et al.*, 2012). Prior to my work a single study on 36 temperate woody species had addressed Körner's hypotheses and had failed to find any significant correlation between species' native climates or successional strategies and their relative use of photoperiod, instead suggesting that most temperate species have evolved a photoperiod-independent leaf-out strategy (Laube *et al*., 2014a). I conducted photoperiod experiments on another 144 species, which together with the results obtained by Laube *et al.* (2014a) now provides information on 173 species (in 78 genera from 39 families) from throughout the Northern Hemisphere. The results contradict the view that photoperiodism (reliance on day-length increase in spring) is especially pronounced in species from high-latitude regions with unpredictable weather systems. Instead, they show that it is the species from more Southern regions with relatively short winter periods that use day-length signals to time leaf unfolding (Chapter 3).

Why is the relative importance of photoperiod as a bud burst signal decreasing towards high-latitude regions with long winters? As I show in Chapter 2 for *F. sylvatica* (also Heide 1993a), photoperiod signals interact with warming requirements, such that longer days continuously reduce the amount of warming required for budburst. The period in spring when days are getting long, however, is not changing with latitude (the spring equinox around which day length is maximally increasing occurs on 21/22 March all over the World). Therefore it should be riskiest for a population to rely on photoperiod in regions with long winters because day-length is increasing too early for guiding leaf-out into a frost-free period, probably explaining why especially genera with a subtropical history, such as *Fagus*, show photoperiodism, whereas genera with a mainly Northern distribution history such as *Betula* leaf-out independent of photoperiod. These results suggest that photoperiod will not constrain leaf-out phenology in northern woody plants with continuously warmer springs. Much more likely is a constraint coming from chilling requirements and spring frost risks (Chapter 4 and 6.2; below).

6.2 Geographic variation in leaf-out strategies

The following section of my general discussion focuses on the adaptive mechanisms leading to geographic variation in winter chilling and spring warming requirements. For a discussion of geographic variation in photoperiod requirements see Chapters 3 and paragraph 6.1 above.

Theoretically it should benefit deciduous plants to delay leaf unfolding until tissue-damaging frosts have passed. On the other hand, delaying leaf-out should decrease an individual's fitness because it would forego the opportunity to use available energy resources for carbon fixation. Leaf unfolding in locally adapted genotypes should therefore occur as soon as possible after the last occurrence of damagingly low temperatures for the respective genotypes. A study on five European tree species reported exactly such a convergence of leaf-out towards the time of minimum risk of freezing damage (Lenz *et al*., 2016). In all five species, and irrespective of climatic conditions (leaf-out was monitored at different altitudes and over multiple years), species leafed out soon after the probability to encounter freezing damage had approached zero, with the specific times differing among species because of differences in freezing resistance of emerging leaves. Given the stochasticity of spring temperatures, how do species know when the probabilistically safe period has arrived? My results suggest that the timing of budburst depends on the interplay between chilling and spring warming requirements (Laube *et al*., 2014a; Polgar *et al*., 2014; Chapter 4). With increasing winter duration, forcing requirements decrease towards a minimum value, allowing species to track the progression of the winter season and to 'predict' its probable end.

On the basis of these findings it can be argued that regional differences in leaf-out strategies of temperate woody plants reflect the different late frost probabilities that plants have experienced (during evolutionary times) in their native ranges. To test this, experimental and observational data for a broad range of temperate woody species are required, allowing for inferring species-specific chilling and spring warming requirements. In addition, global climate data are needed to compute regional frost probabilities. The calculation of such maps, however, is difficult because, to infer when the risk of a plant to experience frost damage has passed, information on a species' specific frost sensitivity is required, emphasizing the need of further studies addressing frost sensitivity in leaves (e.g., Chapter 5). In Chapter 4 of this dissertation, I worked around this problem by calculating a global map of inter-annual spring temperature variability (see Fig. 3a in Chapter 4) as a proxy for late frost probability. The map shows that especially the eastern part of North America has a highly variable spring climate, matching my experimental results that species from this region have high chilling and spring warming requirements, resulting in late leaf-out compared to species from regions with low spring temperature variability (such as East Asia) when grown in a common garden.

6.3 Phenology and invasive success

Geographic variation in the timing of leaf unfolding should influence species' invasion success. With the ever-warmer winter and spring conditions that are expected under climate change, opportunistic phenological strategies might enable species to take advantage of rising air temperatures by extending their growing season. Regions, such as eastern North America, with many species with conservative leaf-out strategies – due to historically high temperature variability favoring conservative growth strategies (see Chapters 4 and 6.2) – might be especially vulnerable to invasions by species with opportunistic leaf-out strategies. By contrast, regions in which opportunistic strategies were already favored in the past, such as Eastern Asia, might be less prone to invasions.

Four studies have addressed possible links between phenological strategy and invasive success. They have found that, by adjusting flowering times (Willis *et al*., 2010; Wolkovich *et al*., 2013), leaf-out times (Polgar *et al*., 2014), or leaf senescence times (Fridley, 2012), eastern North American invasives are better able to track climatic changes than are natives. While focusing on invasive/native comparisons, these studies also point towards a biogeographic pattern, with East Asian species phenologically behaving more opportunistic than their North American brethren.

While I did not focus on invasive/non-invasive contrasts *per se*, my results (Chapters 4 and 6.2) show that there are, in fact, large differences in the leaf-out strategies among continental woody floras, which might well explain the above discussed asymmetric invasion pattern in the Northern hemisphere. Most shrubs and trees invading eastern North America are from East Asia, and invasions of East Asian species in North America are much more common than *vice versa* (Fridley, 2008, 2013). Because their opportunistic leaf-out strategies allow them to make use of soil and light resources in spring, in a way occupying a "vacant niche" (Elton, 1958; Wolkovich & Cleland 2011, 2014), species from East Asia should have a competitive advantage over species from North America. Effects of current climate change are likely to further separate the "phenological niches" between native and invasive species. This can be predicted from the lack of chilling requirements revealed in Chapter 4 for East Asian 'candidate invaders'. These species will linearly track climate warming while warmer winters will constrain temperature tracking, especially in highly chilling-sensitive North American species (see Fig. 1 in Chapter 4).

6.4 Herbarium phenology

Analyzing long-term phenological data (i) allows to predict species' responses to climate warming and (ii) to draw conclusions about constraints that photoperiod and chilling requirements may place on temperature-driven phenological changes. Data on the flowering times of hundreds of species in North America and Europe are now available from long-term observations (Fitter *et al.*, 1995; Bradley *et al.*, 1999; Fitter & Fitter, 2002, Miller-Rushing & Primack, 2008; Amano *et al.*, 2010; Dunnell & Travers, 2011, Iler *et al.*, 2013; Mazer *et al.*, 2013) or herbarium specimens (Borchert, 1996; Primack *et al.*, 2004; Lavoie and Lachance, 2006). However, prior to my work, herbarium data had never been used for assessing long-term leaf-out times, because botanists normally collect fertile plants (with flowers or fruits) and the potential of herbarium material for judging leaf-out times was therefore not seen or at least underestimated. Numerous species, such as *Acer platanoides*, *Carpinus betulus* and *Fagus sylvatica*, however, flush and flower simultaneously, and for these, herbarium records can be used to provide data on spring flushing times (Fig. 1). The Munich herbarium with 3 million specimens is among the World's largest, and already in my M.Sc. thesis (Zohner & Renner, 2014) I used label data on specimen collecting times to create long-term series of local leaf-out times (as far back as 140 years) for native woody species (*Acer platanoides*, *Carpinus betulus*, and *Fagus sylvatica*, see Fig. 2). Using this approach, I have since obtained data for 17 additional species, with the results showing species-specific climate tracking correlated with species' photoperiod and chilling requirements (Zohner & Renner 2014; Zohner & Renner, unpublished data gathered for a DFG funding application).

The utility of herbarium specimens for inferring data on budburst times (at least in species that flower and leaf out simultaneously) suggests further studies, using what I call the 'herbarium approach'. The approach opens up the possibility to (i) investigate species-specific and even within-species phenological variation along latitudinal gradients (ecotypic phenological differentiation) and (ii) to compare the inter-annual variation in budburst dates between photoperiod-sensitive and insensitive species to discover the limitations day-length dependency sets to climate-change induced phenological shifts. Knowing the extent to which photoperiod-sensitive species are able to track temperature might give important ecological implications for future warming scenarios, because day-length independent species are thought to gain a competitive advantage over photoperiod-sensitive species due to a greater potential to lengthen the growing season (Körner & Basler 2010).

Fig. 1. The spring phenology of *Acer platanoides* as seen in herbarium records.

The herbarium method also opens up the possibility to study phenological evolution: by comparing phenological behavior along latitudinal gradients it is possible to assess how long-lived plant genotypes respond to climatic niches. Evolution requires heritable traits that differ among individuals and eventually populations. Documenting population-level traits in natural history collections is often difficult because collecting (i.e., sampling) is not always sufficiently dense for addressing micro-evolutionary questions. Historic collections sometimes allow observing trait change directly, for example, over 100-, 50- or 25-year periods. Among the plant traits in which changes over time and space (in different populations) has been inferred from herbarium collections are leaf width (Guerin *et al*., 2012), leaf-out times (Zohner & Renner, 2014; Everill *et al*., 2014), and flowering times (Borchert, 1986, 1996; Primack *et al*., 2004; Lavoie & Lachance, 2006; Miller-Rushing *et al*., 2006; Panchen *et al*., 2012; Calinger *et al*., 2013). Typically, this type of study involves plotting the days of the year when flowering or flushing specimens of a particular species were collected over time or, alternatively, against the accumulated chill days or spring warming days of the respective years. The method has been used since the mid-1980s and has been tested against actual observations in the field for the same species also studied in the herbarium (Borchert, 1986, 1996; Robbirt *et al*., 2011; Davis *et al*., 2015). With the except of the earliest such studies (Borchert, 1986, 1996), all authors have related earlier flowering or leaf-out times today compared to those in the past (back to 130 years; Zohner

& Renner, 2014) to the earlier arrival of spring in the North American and European continent from where the respective herbarium material came, using linear regression. Based on this work, species-specific phenological behavior obtained from sufficiently large numbers of herbarium specimens is similar to behavior observed in field studies. The herbarium method is unique in containing a time and space component. Hence, it allows for studying how plant genotypes have adapted to climatic niches by comparing the phenological behavior (over time) between different populations. In combination, all this means that herbarium specimens represent a great resource for a targeted enlargement of our understanding of species' phenological behavior. So far, leaf-out phenology has been studied in a tiny proportion of the World's woody species, and many potentially important factors are just beginning to be analyzed (see Chapter 5).

6.5 Future research questions

6.5.1 Intraspecific variability of leaf-out phenology studied along latitudinal gradients

Analyses of intraspecific phenological variation in naturally wide-ranging species' along N-S gradients will allow assessing how long-lived plant genotypes respond to changing climatic niches (Olson *et al*., 2013). Up to now, few studies have applied this idea (but see Borchert *et al*., 2005), most likely because gathering phenological data along wide-ranging latitudinal gradients is very time consuming. To tackle this problem I am planning to combine two approaches that will allow for fast and inexpensive generation of multi-species phenological data covering Northern to Southern Europe: (i) the use of herbarium specimens to obtain the timing of leaf unfolding and (ii) the use of the twig-cutting method to study regional effects on species' phenological cueing mechanisms (photoperiod, chilling, and spring warming). I have already selected about 20 widespread tree species from seven families that are well represented in herbaria and whose leaf-out I plant to study (i) along latitude, (ii) over time, and (iii) between species. This will give me long time series for local leaf-out times to study the degree of site-specificity in the magnitude and direction of the responses to global climate warming.

In summer 2014, I visited the herbaria of Aarhus, Copenhagen, and Stockholm to gather phenological data for Northern Europe, and I now have in hand photos of about 3000 specimens from the 20 species, all geo-referenced, at the stage of leaf-out as defined with consistent criteria. Phenological data for Southern Germany is available for the same species from my earlier work

(Zohner & Renner, 2014). To enlarge the sampling, especially for Southern locations, I am planning to visit the herbaria of Berlin, Florence, Istanbul, and Paris.

To study and compare leaf-out dates, specimens collected over large areas need to be geo-referenced and related to the local climate and photoperiodic conditions. For example, a specimen sampled on 25 April may represent a very early-flushing event at the northern limit of the area, but a mid- or late-flushing case at the southern limit. Consequently, each date has to be adjusted according to the prevailing climate conditions occurring at a plant's sampling location. This will allow comparing climate tracking in temperature-sensitive and photoperiod-sensitive species. Because day-length changes consistently each year, plants using these signals to time their leaf unfolding can be expected to show low variation from year to year. Preliminary evidence supports this (see Fig. 2 in which temperature sensitivity is compared between photosensitive *Fagus sylvatica* and insensitive *Acer platanoides* and *Carpinus betulus*).

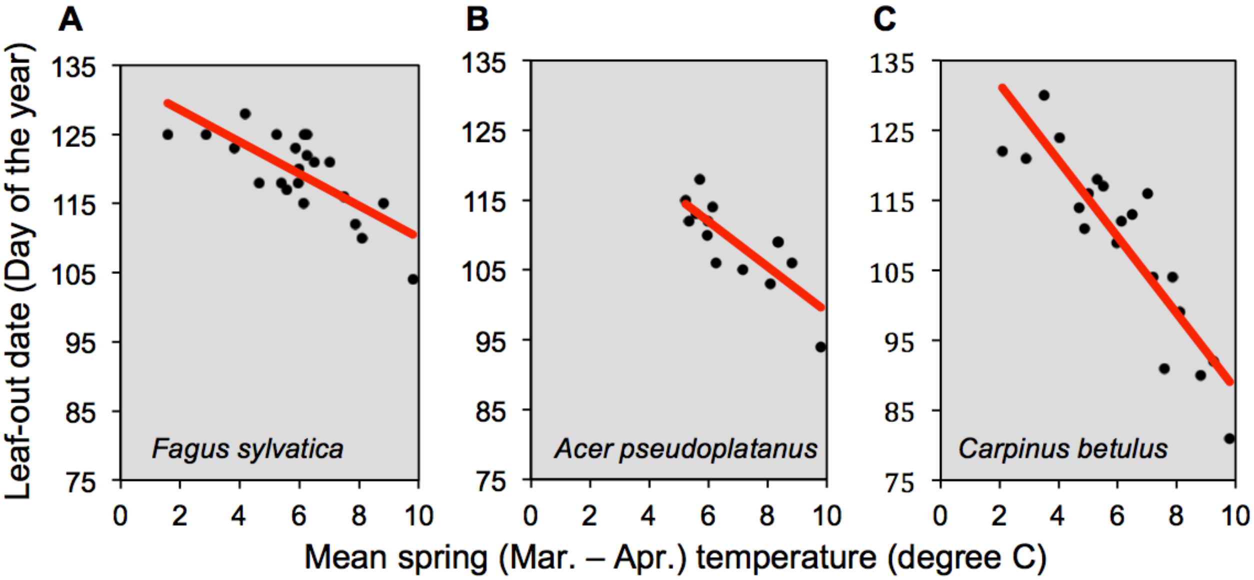


Fig. 2. Using herbarium specimens to study the influence of spring air temperature on leaf-out date in three common European tree species modified from Zohner and Renner (2014). **(A)** *Fagus sylvatica,* showing a leaf-out advance of only 2.3 days per each 1°C increase in spring temperature. **(B)** *Acer platanoides,* showing a leaf-out advance of 3.2 days/°C. **(C)** *Carpinus betulus,* showing a leaf-out advance of 5.4 days/°C.

In addition, because the fastest increase in day length occurs at the same time all over the Northern Hemisphere, photosensitive species are expected to show low spatial variation (along their latitudinal distribution gradient) compared to photo-insensitive species. Up to now, no study has investigated this, however, because phenology is usually monitored locally so as to reduce confounding effects from genetic or phenotypic geographic variation within species. To test the twin hypotheses of low inter-annual variation and predictable spatial variation along latitude (with spring day length change become steeper further north) in photosensitive species, I will compare their phenological responses to that of species that do not rely on photoperiod. The expectation is that photoperiod-sensitive plants should be less affected by spring warming *at all sites* and will show lower *between-site variation*.

Because the temporal occurrence of photoperiod and temperature signals changes with latitude, I expect change in species' strategies with latitude. Using herbarium data, I can infer species' order of leaf-out at different latitudes. By combining this information with climate information, I will also be able to answer the question if the Northern distribution limit of certain species is constrained by the probabilities of young leaves to suffer frost damage (Chapter 5 provides examples of spring frost damage and how it relates to species' native ranges). The pilot data show that leaf unfolding in *Acer platanoides* on average occurs 2.4 days later per each degree increase in Northern latitude (Fig. 3).

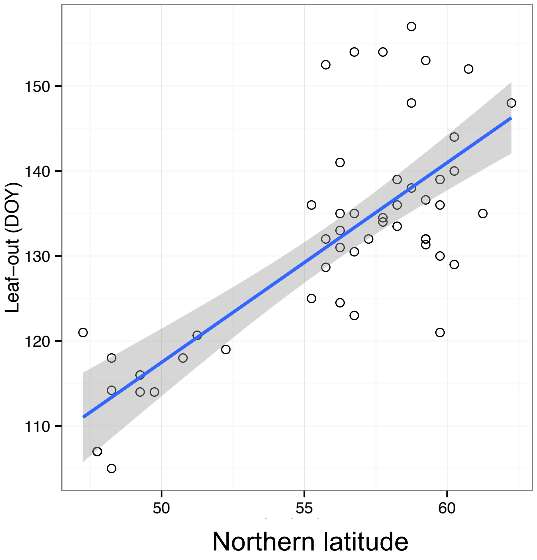


Fig. 3. Mean leaf-out dates (day-of-the-year, DOY) of *Acer platanoides* as a function of latitude. Information on leaf-out came from label information on 80 herbarium specimens photographed in the herbaria of Aarhus, Copenhagen, Munich, and Stockholm. $R^2 = 0.59$, $P < 0.001$, Slope = 2.4 days / degree latitude.

A shortcoming of the herbarium method is that it does not offer direct insights into the environmental (photoperiod and temperature) cues triggering leaf unfolding. To address the question how species' relative requirements of external leaf-out cues change along latitude, greenhouse experiments are necessary because they allow disentangling the effects of local temperature and day length. I am planning to study intraspecific differentiation of photoperiod and chilling requirements in *Fagus sylvatica* and other species featuring similar distribution by collecting twigs from 40°N to 60°N (complete latitudinal range of *F. sylvatica*). Therefore twigs of the same individuals (in Botanical Gardens or private gardens of colleagues) will be collected twice during the winter period (to allow different chilling treatments), brought to Munich and kept under different light conditions to test for latitudinal differentiation in photoperiod requirements. The question I want to answer is if Northern populations are less sensitive to photoperiod signals because day length increases occur too early for late frosts to be safely avoided (see Chapter 3 for a species-level analysis of photoperiod sensitivity).

6.5.2 Leaf senescence and vegetation periods

In 2014 and 2015, I gathered leaf-senescence dates for the 450 species that have already been monitored for leaf-out in the Munich Botanical Garden. Sandra Petrone Mendoza, a M.Sc. student in the lab of Susanne Renner whom I co-advised, conducted the 2014 observations (see Petrone Mendoza, 2014). The data in hand allow for studying the adaptive mechanisms leading to interspecific differentiation in the timing of leaf senescence. One expectation is that warm-adapted species lose their leaves earlier than species originating from colder climates because they have higher temperature thresholds necessary to induce senescence (Zohner & Renner, 2014). The opposite expectation arises under a photoperiod-driven senescence scenario: species from cold climates should rely on high photoperiod thresholds because in their native ranges days are still long when temperatures begin to drop. Therefore, when grown together in the Munich Botanical Garden, the senescence times of species from cold regions should precede those of species from more southern climates because the critical short-day threshold to induce senescence is met earlier in Northern species. As shown in Chapter 4, species-specific frost resistance in combination with regional frost probabilities should also have major influences on leaf-fall times. Hence, one can expect conservative growth strategies (in this case early leaf senescence) in species from regions with high inter-annual temperature fluctuations and frost risk. Preliminary analyses support this hypothesis: North American species on average senesced their leaves two weeks earlier than East Asian species, with European species intermediate (see Petrone Mendoza, 2014).

Combining data on senescence and leaf-out times will also allow studying the duration of the vegetation period in a global species sample. This might help forecasting future phenological transitions, such as changes in the growing season length, in the floristically changed communities expected from climate warming.

6.5.3 The molecular basis of dormancy release

Understanding the molecular mechanisms underlying plant's responses to environmental forces is essential for predicting plant behavior under global climate change. However, the genetic mechanisms underlying the transition from winter dormancy to photosynthetic activity in temperate woody species are poorly understood. In a review about dormancy in temperate trees, Cooke *et al*. (2012, p. 1708) state that "[...] dormancy is remarkably difficult to quantify in buds, and we do not yet have any validated molecular or non-destructive physiological markers to demarcate bud dormancy." A major issue when dealing with temperate tree species is that laboratory research on developmental processes in tall plant species is difficult, restricting studies to young trees. In addition, genomic data are still scarce. Hence, most developmental molecular studies are focusing on annual model plants that do not undergo dormancy (but see Böhlenius *et al*., 2006; Ibanez *et al.*, 2010).

In *Populus tremula* x *tremuloides*, Ibanez *et al.* (2010) showed that the expression of clock genes (e.g., LHY and TOC1) changes as bud burst progresses, suggesting that genes involved in photoperiodism might be adequate indicators for developmental stages during dormancy and subsequent bud development. The *CONSTANS* (*CO*) and *FLOWERING LOCUS T (FT)* genes function downstream of the circadian clock system. The *FT* gene has been shown in *Populus* to control growth cessation and bud set in autumn induced by short days (Böhlenius *et al*., 2006). Down-regulation of *FT* in *Populus* resulted in early bud set under short days as well as under long days; while overexpression of *CO* or *FT* resulted in continuing growth even under short days, suggesting that *FT* acts as an inhibitor of growth cessation (Olsen, 2010). Different members of the family of *FT*-like genes have different roles in dormancy-related processes, and further gene characterization and functional studies are needed to detect their specific roles (Hsu *et al*., 2011; Olsen, 2010).

A first step towards understanding the molecular nature of the transition between winter dormancy and spring development would be the quantification of a plant's state of dormancy. Therefore, marker genes (that are up-regulated during dormancy or dormancy release) have to be established by performing gene expression analyses in buds. These analyses will give deeper insights in the molecular mechanisms behind budburst and help to describe the complex process of leaf-out more precisely rather than the simple noting of budburst dates. I am planning to use some of the genes described above and analyze their transcription rates via qRT-PCR. By studying the expression of such genes during bud development within trees for which the photoperiod response has been experimentally studied, one would link molecular with experimental data allowing for studying how gene expression controls bud phenology. In photoperiod-sensitive species one would assume circadian-clock genes to be up-regulated during dormancy release in buds, while day-length independent species should lack such a response. A review of the molecular mechanisms suggests that *PRR5* is a candidate marker gene for depth of bud dormancy in *Populus* (Cooke *et al*., 2012).

A pilot study carried out by Sandra Petrone, the M.Sc. student whom I co-advised, on five temperate tree species (*Aesculus hippocastanum*, *Fagus crenata*, *F. orientalis*, *F. sylvatica*, and *Populus tremula*), tested for RNA isolation out of leaf primordial tissue in buds, and established two candidate genes (*CO* and *FT*) to quantify their expression during dormancy. Sufficient amounts of RNA from leaf buds could be obtained for all selected species. Therefore, leaf bud collection and grinding protocol as described in Petrone Mendoza (2014) can be used for further studying the expression profiles of other woody, non-model species. The quality of the PCR sequences, however, was poor, possibly due to amplification of more than one gene of the same family. To obtain clean sequences, it will be necessary to clone the PCR products to make sure that only one copy is amplified for each species by designing specific primers. In future studies, the expression levels of the selected genes will be studied via qRT-PCR using the isolated RNA obtained. This pilot study represents a first step towards establishing a standard method for RNA isolation of leaf buds within non-model, woody species, with the ultimate goal of studying the molecular basis of dormancy in a broad range of temperate woody species.

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