LETTER

Spring predictability explains different leaf-out strategies in the woody floras of North America, Europe and East Asia

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Abstract

Intuitively, interannual spring temperature variability (STV) should influence the leaf-out strategies of temperate zone woody species, with high winter chilling requirements in species from regions where spring warming varies greatly among years. We tested this hypothesis using experiments in 215 species and leaf-out monitoring in 1585 species from East Asia (EA), Europe (EU) and North America (NA). The results reveal that species from regions with high STV indeed 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 NA than in EU and EA, and under experimentally short winter conditions NA species required 84% more spring warming for bud break, EU ones 49% and EA ones only 1%. These previously unknown continental-scale differences in phenological strategies underscore the need for considering regional climate histories in global change models.

Keywords

Biogeography, chilling, leaf-out, phenology, spring variability, temperature.

INTRODUCTION

Rising spring temperatures have advanced the onset of the growing season in many woody species of the temperate zone (Menzel & Fabian 1999; Menzel *et al.* 2006; Zohner & Renner 2014), affecting plant productivity and global carbon balance (Richardson *et al.* 2010, 2013; Keenan *et al.* 2014). However, these advances are not uniform. Experiments and monitoring data have revealed that species differ greatly in the extent to which they rely on day length, winter chilling and spring warming to regulate their leaf unfolding (Heide 1993; Caffarra & Donnelly 2011; Laube *et al.* 2014; Polgar *et al.* 2014; Zohner & Renner 2014, 2015), with day length playing overall a minor role. Thus, in experiments, only 9% of 173 temperate zone woody species used day length as an additional leaf-out signal in spring (Zohner *et al.* 2016), making the investigation of the roles of shorter winters (less chilling) and warmer springs all the more important. These two temperature signals interact, with species that need extended chilling unable to react to spring warming if winters are too short (Laube *et al.* 2014; Polgar *et al.* 2014; Zohner & Renner 2015). Hence, unfulfilled chilling requirements may halt the advance of spring leaf-out, as is already happening in a few European species analysed in this regard (Fu *et al.* 2015).

Temperate woody plants face a trade-off between early carbon gain (early leaf expansion) and avoidance of frost damage (late leaf expansion) (Cannell 1997). In regions with an unpredictable winter progression, plants should have evolved 'safe' leaf-out strategies allowing them to delay leaf unfolding until the risk of frost damage has passed (Lechowicz 1984; Körner & Basler 2010). This leads to the expectation that in such regions, chilling requirements should be high because this would help plants to avoid precocious bud development under early warm spells. In contrast, in regions with a predictable advent of spring, warming cues should be sufficient to guide leaf-out into frost-free periods (Körner & Basler 2010). Previous work on the budbreak signals of temperate species, however, has largely ignored such potential contributions of local climate history (but see Lechowicz 1984), despite the fact that regional climate histories might constrain the responses of local forest communities to ongoing climatic change.

Here we test for possible regional differences in spring temperature predictability throughout the Northern Hemisphere and their relation to leaf-out strategies. To do this, we first computed global maps of the interannual temperature variability in spring (STV) and winter (WTV). Next, we tested whether regional differences in STV explain different phenological strategies of the woody floras of NA, EU and EA. This we did by combining experimental and monitoring data for a representative set of species. Species' winter chilling requirements were inferred from twig-cutting experiments on 215 species from throughout the Northern Hemisphere. In addition, leaf-out from 2012 to 2015 was monitored in almost 500 species in the Munich Botanical Garden, including most of the 215 species used in the experiments. Lastly, we analysed the 2012 leaf-out dates of some 1500 species observed at five other Northern Hemisphere gardens. As long as trees in botanical gardens have had no chance for evolutionary adaptation (because they were not allowed to cross-pollinate, reproduce and undergo natural selection), their leaf-out times

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will reflect their native thresholds for chilling, forcing and photoperiod. Using the experimental and monitoring data, we linked species' leaf-out strategies to biogeographical region (NA, EU, EA), and tested for effects of STV on chilling requirements before leaf-out. Our expectation was that species from regions with a less predictable onset of spring conditions, would have 'safer' leaf-out strategies, meaning that they would require longer chilling periods and higher spring warming to leaf-out than species from regions with more predictable frost-free spring conditions.

MATERIAL AND METHODS

Study site and methods used in phenological monitoring

Observations were carried out between January 2012 and June 2015 in the botanical garden of Munich. Leaf-out was monitored in 840 individuals from 498 species in 145 genera and 60 families, with on average two individuals per species. The monitored trees, shrubs or woody climbers are growing permanently outdoors without winter protection, and the same individuals were monitored in 2012 and 2013 (data used in Zohner & Renner 2014) and in 2014 and 2015. As in Zohner & Renner (2014), 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 4 years. 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. (2014).

Twig-cutting experiments to test the effects of chilling on forcing requirements

To study species-specific effects of winter chilling on the timing of leaf-out in a biogeographic-climatic context, we used data from twig-cutting experiments on 144 species from a parallel study that investigated latitudinal, not longitudinal differences in leaf-out strategies (Zohner et al. 2016). Experiments by Vitasse & Basler (2014) and Zohner & Renner (2015) have demonstrated that twig cuttings precisely mirror the phenological behaviour of their donor plants and therefore are adequate proxies for inferring phenological responses of adult trees to climatic changes. Data from the same type of twig-cutting experiments for 71 further species are available from Laube et al. (2014) and Polgar et al. (2014) and were added to our own data, resulting in chilling data for a total of 215 species from 92 genera in 46 families (Table S1 and Figure S1). The species-specific chilling requirements were investigated in climate chambers with three chilling treatments: collection of twigs in early winter (referred to as short chilling treatment 'C1'), mid of winter (intermediate chilling treatment 'C2') and end of winter (long chilling treatment 'C3'); see Table S2. Photoperiod had been standardised to a constant day length of 16 h (Laube et al. 2014; Zohner et al. 2016) or 14 h (Polgar et al. 2014). To test for a possible effect of short-day conditions, the same type of experiment had also been run under

a day length of 8 h in the Laube et al. (2014) and Zohner et al. (2016) studies.

To assess the effects of the chilling treatments, we quantified how much forcing (sum of degree days from 21 December until budburst using 0°C as base temperature) the respective twig needed to leaf out. To obtain a species-level chilling category for use in recursive partitioning and phylogenetic corrected analyses (see below), we grouped species into three categories: If the median forcing requirement under the C1 treatment was fewer than 75 degree days higher than under the C3 treatment, a species was assigned to the category 'no chilling requirement'. If the difference was higher than 75 degree days, a species was scored as intermediate chilling. If the forcing requirement under C2 was more than 75 degree days higher than under C3, a species was scored as high chilling. In all cases where the average treatment differences were higher than 75 degree days, the treatments also statistically differed according to an ANOVA assessment (0.05 significance level).

Computation of spring and winter temperature variability

STV was calculated as the standard deviation (SD) of mean minimum temperatures of the March–May period over the past 100 years (1901–2013). The higher the value of STV, the lower the predictability of spring air temperature. Interannual winter temperature variability (WTV) was calculated as the SD of the mean minimum temperature of the coldest month over the past 100 years (1901–2013). Gridded data on monthly minimum temperatures during this period were taken from the Climatic Research Unit (CRU) time-series data set (version 3.00 with a spatial resolution of 5-arc minutes; Jones & Harris 2008; Harris et al. 2014).

Species assignments to continent and native climate

To obtain the native distributions of the 1593 species used in experiments and/or monitoring, we gathered floristic information from the USDA PLANTS database (USDA 2015), eflora (Brach & Song 2006; eFloras 2008), http://linnaeus.nrm.se/flora/welcome.html, and http://www.euforgen.org/distribution-maps/. We then grouped species according to their native region: North America (NA), South America (SA), Europe (EU), West Asia (WA) and East Asia (EA). The eastern border of EU was set at the Ural Mountains; east of them, the Turgai Sea separated EU and Asia throughout the Paleocene and into the Eocene (Akhmetiev et al. 2012). We set the western end of the Himalayas as the border between WA and EA. The few species native to more than one of these regions, such as Betula nana, were excluded in the continent comparisons. 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. SA and WA were not included in further analyses because there were only six species from these regions. To correlate species' native ranges with the climate they experience there, we extracted species occurrences from the Global Biodiversity Information Facility (http://www.gbif.org/) using the gbif function of the dismo R-package (Hijmans et al. 2011). To exclude unreliable records

and reduce spatial clustering we included only records from a species' native continent, and removed (1) coordinate duplicates at a resolution of 2.5-arc minutes, (2) records based on fossil material, germplasm or literature and (3) records with a resolution > 10 km (see also Zohner *et al.* 2016). After filtering out species with < 30 records within their native continent, our data set comprised 1137 species (1 411 996 presence records), of which we had leaf-out data for 1130 species and experimental data for 183 species.

The georeferenced locations of the 1130 species were queried against grid files for STV, mean annual temperature (MAT) and temperature seasonality (TS). MAT and TS were based on gridded information (2.5-arc minute spatial resolution data) from the Worldclim data set (BIO 1 and BIO 7; Hijmans *et al.* 2004, 2005). TS was calculated as annual temperature range, i.e. the difference in °C between the maximum temperature of the warmest month and the minimum temperature of the coldest month. For each species, we determined the climate optimum by calculating its 0.5 quantile for the respective climate variable.

Correlations among climate parameters, leaf-out times, chilling requirements and other species traits

We tested for multicollinearity of our predictor variables (STV, MAT and TS) using a variance inflation factor (VIF) analysis, implemented in the R function 'vif', from the package 'HH' (Heiberger 2016). All VIF were smaller than 5 (threshold recommended by Heiberger [2016]), indicating sufficient independence among predictor variables. We then ran random forest models (randomForest R library; Breiman 2001; Cutler *et al.* 2007), applied a hierarchical Bayesian approach (see below, 'Accounting for phylogenetic history in the analyses') to allow for phylogenetic autocorrelation in our dependent variables, and applied Simultaneous autoregressive (SAR) models controlling for spatial autocorrelation in the residuals (see below, 'Spatial regression between leaf-out strategies and bioclimatic parameters').

To analyse the relationship between climate parameters and leaf-out times, we included only gardens with more than 200 species for which both leaf-out and climate data were available, i.e. the Arnold Arboretum, the Berlin Botanical Garden, the Munich Botanical Garden and the Morton Arboretum. For recursive partitioning analyses of climate variables (STV, MAT and TS), growth habit (trees vs. shrubs) and leaf persistence (evergreens vs. deciduous species), we used the 'rpart' option in the R library (Thernau *et al.* 2015), setting the minimum node size to 30 (minimum number of species contained in each terminal node).

Accounting for phylogenetic history in the analyses

To estimate the phylogenetic signal in species-level leaf-out dates, we created a phylogenetic tree for our 498 target species and measured phylogenetic signal with Pagel's λ (Pagel 1999) and Blomberg's K (Blomberg *et al.* 2003), using the 'phylosig' function in the R package 'phytools' v0.2-1 (Revell 2012). To build the tree we used MEGAPTERA (Heibl 2014) and BEAST (Drummond *et al.* 2012). 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 length of 6395 base pairs. 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; Drummond *et al.* 2012). To calibrate our tree, we set the crown age of angiosperms to 185 Ma (Bell *et al.* 2010); since absolute ages are not used in this study, we did not run our analyses with alternative calibrations. The 498-species phylogeny is shown in Figure S2, and the phylogenetic relationships among the 183 species used in the experiments are shown in Figure S3. The relationships among 1630 species, including the 1585 for which we have monitoring data, was built by enlarging the tree assembled by Panchen *et al.* (2014) with Phylomatic (Webb & Donoghue 2005). Its topology reflects the APG (2009) phylogeny, with a few changes based on the Angiosperm Phylogeny Website (Stevens 2012). Branch lengths reflect divergence time estimates based on the fossil record (Bell *et al.* 2010; Smith *et al.* 2010). The Phylomatic tree is shown in Figure S4.

We applied a hierarchical Bayesian (HB) approach (following Fridley & Craddock 2015 and Zohner *et al.* 2016) for testing effects of continental origin (NA, EU, EA) and climate on species-level differentiation in spring leaf-out dates and chilling requirements. When using leaf-out time (a continuous character) as a response variable (Pagel's λ value of leaf-out dates = 0.81; see Figure S2), we used the Bayesian phylogenetic regression method of de Villemereuil *et al.* (2012) to incorporate the phylogenetic structure of the data in the HB model. This allowed us to test species-level differences in leaf-out times while controlling for phylogenetic signal, measured by λ. When using chilling requirement (ordinal data) as a 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 also controlled for (1) species' life-histories (trees tend to leaf-out later than shrubs and evergreen species later than deciduous species; see Panchen *et al.* [2014] and our Figure S5b) by including growth habit (shrubs vs. trees) and leaf persistence (evergreen vs. deciduous species) and for (2) species' latitudinal distribution (see Zohner & Renner 2014) by including the MAT that species experience in their native ranges in the models. The resulting posterior distributions are a direct statement of the relative effect size of each biogeographic and climatic parameter on species-level differentiation in chilling requirements and leaf-out dates. See Fridley & Craddock (2015) and Zohner *et al.* (2016) for details on phylogenetic regression in a HB model, model parameterisation and prior choice.

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 for which we had 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 $2.5° \times 2.5°$; initially, the resolution of climate grids and species distribution data was 2.5-arc minutes (*c.* 0.05°). Next, we regressed the aggregated response variable against aggregated predictor variables.

We applied partial regression analysis (to remove the covariate effects of MAT; see Partial r^2 in Table 1) and multiple ordinary least squares regression (OLS) between each response and all predictor variables (see OLS in Table 1). The OLS models showed 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 (Dormann 2007). To remove the autocorrelation, we applied simultaneous autoregressive (SAR) models (Borcard & Legendre 2002; Griffith & Peres-Neto 2006) using the R-package 'spdep' (Bivand *et al.* 2013; Bivand & Piras 2015). We used a spatial weights matrix with neighbourhoods defined as cells within 3000 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, $P = 0.43$). 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 SARreduced 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 WeightAIC in Table 1).

All statistical analyses were conducted in R (R Core Team 2016).

RESULTS

Continental-scale differences in spring temperature variability and leaf-out strategies

Our analysis revealed that spring temperature variability (STV) differs markedly among continents, with peaks in eastern NA and northeastern EU (Fig. 1a). STV was lowest in EA. A map for the interannual winter temperature variability (WTV) showed similar biogeographical variation (Pearson correlation coefficient between STV and WTV = 0.85; Figure S6). The regional differences in STV were unaffected when we compared only regions with similar climates (similar MAT and TS; Figure S7).

Leaf-out strategies differed markedly by continent, with NA species (1) having much higher requirements for winter chilling than EA species, and EU species intermediate (Fig. 2) and (2) leafing out later than EA and EU species in the field when grown under the same conditions (Fig. 3). Thus, in the climate chamber 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 (Figure S8a). 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 1% (360/355), compared to long winter conditions (Fig. 2).

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: Chilling treatment had a greater effect on NA species than on EU and EA species (Fig. 2a, Figure S9 and Table S3). The effect of continent on chilling requirements remained significant when controlling for phylogenetic autocorrelation of phenological traits and when incorporating fixed effects for species' growth habit, leaf persistence and median MAT in the HB model (Figure S8b). In line with this, in 12 (75%) of 16 families containing both NA and EA species, NA species had higher chilling

Table 1 Relationships between climate variables and global patterns of leaf-out times and chilling requirements

	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

MAT, mean annual temperature; TS, temperature seasonality; STV, spring temperature variability. Seven comparative measures were used: the coefficient of determination from bivariate partial regression (partial r^2), standardised regression coefficients from multivariate ordinary least-squares regression (OLS), standardised regression coefficients from simultaneous autoregressive models (SAR), the reduced SAR model with the lowest AIC score (SAR$_{reduced}$), Akaike weights based on SAR models, mean decrease in accuracy values (MDA) from random forest analysis and coefficient estimates (means and 95% confidence intervals [CIs]) from a hierarchical Bayesian (HB) model controlling for phylogenetic autocorrelation, growth habit and leaf persistence. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
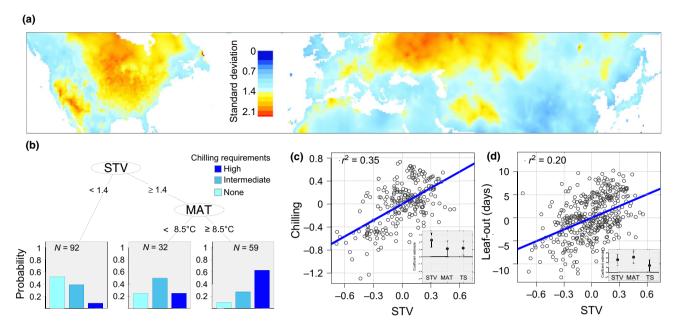
Figure 1 The effect of STV on leaf-out strategies in Northern Hemisphere woody plants. (a) STV calculated as standard deviation of minimum temperatures between 1 March and 31 May from 1901 to 2013. (b) Recursive partitioning tree for the relationship between climate parameters and species-specific chilling requirements in temperate woody species. Median STV, MAT and TS in a species' native range, growth habit (shrub/tree) and leaf persistence (deciduous/evergreen) were evaluated as potential split points. Number of species contained in each terminal node shown within graphs. (c and d) The relationship between 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. Insets show coefficient values (means and 95% CIs) 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) estimated from HB models including phylogenetic autocorrelation and fixed tree and evergreen effects. Values reflect standardised data and can be interpreted as relative effect sizes (see Table 1).

requirements than EA species, while the opposite was true for only 2 (13%) of the 16 families (Figure S10a). Similarly, in 9 (53%) of 17 genera containing both NA and EA species, NA species had higher chilling requirements than EA species, while the opposite was only true for *Fraxinus* (Figure S10b). Photoperiod treatment (in the experiments) had little effect on forcing requirements, and the biogeographical patterns of species-specific chilling requirements were unaffected by photoperiod treatment (Figure S11).

Growth habit (trees vs. shrubs) and leaf persistence (broad-leafed species vs. evergreens, mostly conifers) did not statistically affect chilling requirements (Figure S5a, c and d). As explained in Material and Methods, our experimental data (Zohner *et al.* 2016) were augmented by results from similar experiments carried out by Polgar *et al.* (2014) and Laube *et al.* (2014). When their data were excluded, results did not differ (Figure S11).

In the common garden observations, NA species flushed 5 ± 2 and 9 ± 2 (mean ± SD) days later than EU and EA species, respectively, when using all 1585 species (Fig. 3a). Analyses of subsets of the data showed that this continent effect had a similar magnitude in different functional categories (shrubs, trees, evergreens and deciduous species; Fig. 3a and Table S4). Contrasts with sample sizes below 20 species per continent are not shown (grey fields in heat maps). For a summary of the leaf-out dates of all NA, EA and EU species monitored at the six gardens see Table S4. For all gardens, our HB models revealed a significant difference between NA and EA species (Fig. 3b and Figure S8c). In line with this, in

13 (46%) of 28 families containing both NA and EA species, NA species leafed out later (> 5 days) than EA species, whereas the opposite was true for only 2 (7%) of the 28 families (Figure S12).

The link between leaf-out strategies and STV

To test whether the observed continental-scale differences in leaf-out strategy reflect species' adaptation to STV, we applied recursive partitioning, spatial and HB models (Fig. 1b–d). In these models, we included MAT to test our expectation that species from cold climates should be adapted to lower temperatures and therefore should leaf-out earlier than species from more southern locations when grown together in a common garden (Zohner & Renner 2014), and we also included TS to test for possible phenological differences between species from continental and oceanic climates (Körner & Basler 2010; Vitasse *et al.* 2014). 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. 1c and d and Partial r^2 in Table 1). Recursive partitioning analyses yielded similar results: of the 91 species from regions with high STV (> 1.4), 50% had high chilling requirements, whereas only 9% of the 92 species from low STV had such requirements (Fig. 1b). 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, whereas the mean leaf-out date of
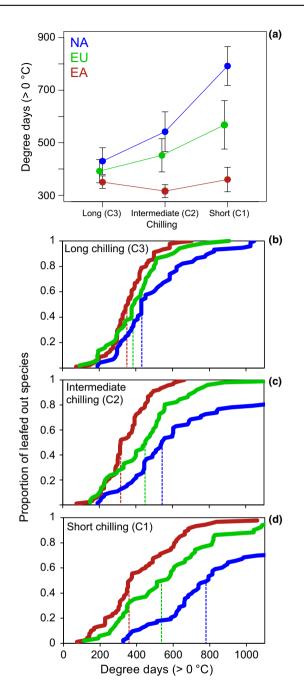
Figure 2 Contrasting responses of North American (NA), European (EU) and East Asian (EA) species to experimentally reduced winter chilling. (a) Median forcing requirements (accumulated degree days >0°C outdoors and in a climate chamber) ± 95% CI until leaf-out under three chilling treatments for NA ($n = 72$ species), EU ($n = 48$) and EA ($n = 88$) species. (b–d) Leaf-out probability for NA, EU and EA species as a function of accumulated degree days for the three chilling treatments: (b) long chilling, (c) intermediate chilling and (d) short chilling. Dashed lines indicate median forcing requirements for NA, EU and EA species, i.e. the accumulated degree days when 50% of species had leafed out.

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, respectively; Figure S13a). For both chilling requirements

and leaf-out dates, the effect of STV remained significant when controlling for phylogenetic (HB models; insets Fig. 1c and d and HB in Table 1) and spatial autocorrelation (SAR models; Fig. 1c and d and SAR in 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 (Figure S13b).

Regarding the effects of MAT and TS, we found a positive association between MAT and leaf-out dates (Table 1, inset Fig. 1d and Figure S13b), but MAT had little predictive power with respect to chilling requirements (Table 1 and inset Fig. 1c). TS had little effect on both leaf-out dates and chilling requirements (Fig. 1, Figure S13b).

Using within-North America climate differences as a test of the effect of STV on leaf-out strategy

The west coast of NA, especially at low elevations, experiences less STV than does the eastern part (Fig. 1a), and species restricted to western NA might therefore have more opportunistic (earlier) leaf-out strategies than species from eastern NA. Indeed, when grown together, the leaf-out dates of western NA species preceded those of eastern NA species by an average of 12 days (Figure S14a and Table S5). In HB models, western NA species leafed out significantly earlier than eastern ones and did not differ from EU and EA species (Figure S14b). Because our western-eastern NA comparison was biased by the 25% conifer species in the west vs. only 4% conifers in the east, we excluded conifers in a direct comparison (Figure S14a). In a HB model, we included conifers but controlled for this bias by including a gymnosperm effect (Figure S14b).

DISCUSSION

Previous work alluded to, but never tested, the possible importance of *longitudinal* phenological differences. For example, Körner & Basler (2010, p. 1462) noted the early flowering of cherry cultivars from central Asia compared to European ones and suggested that the advent of spring in Asia might be less variable than in Europe, with phenological tracking of spring temperatures therefore presenting less of a risk, continuing that 'trees in these regions should be more likely to keep tracking climatic warming than those in climates with more unpredictable weather systems, an interesting question to be explored in future work'. Ours is the first study to have demonstrated the correlation between unpredictable interannual temperatures and leaf-out strategies in trees and shrubs of the Northern Hemisphere.

There is a marked geographical variation in the predictability of winter and spring temperatures (Fig. 1a), and woody species from regions with unpredictable winter and spring climates, such as eastern NA, have more conservative leaf-out strategies than species from Europe or Asia: Species from regions with high STV, on average, leafed out 1 week later (Fig. 1d and Figure S10) and had significantly higher chilling requirements (Fig. 1b and c) than species from regions with low STV. Even though data on STV before the 20th century are not available, high interannual temperature variability in
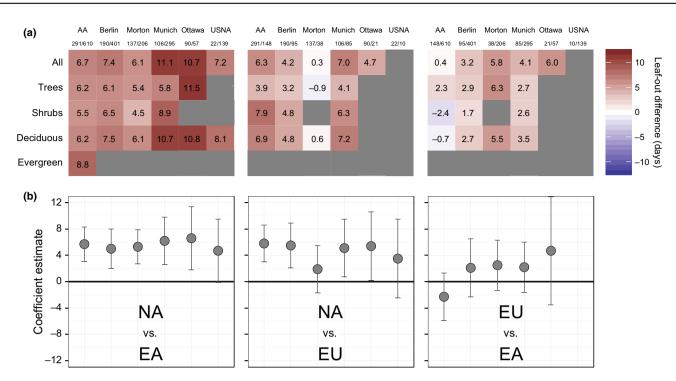
Figure 3 Contrasting leaf-out dates of North American (NA), European (EU) and East Asian (EA) species. (a) Heat maps for the difference in species-level leaf-out dates between NA and EA species (left panel), NA and EU species (middle panel) and EU and EA species (right panel) monitored at six gardens 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 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 (means and 95% CIs) for differences in leaf-out dates between NA and EA species, NA and EU species and EU and EA species estimated from HB models including phylogenetic autocorrelation and fixed tree and evergreen effects. Values reflect standardised data and can be interpreted as relative effect sizes.

NA has existed over a geologically long time, suggesting that the modern NA woody flora is the product of unpredictable climates that likely selected for conservative growth strategies (Dynesius & Jansson 2000; Jansson 2003; Ehlers & Gibbard 2007; Sandel *et al.* 2011).

Our findings differ from Körner & Basler's (2010) expectation in showing that plants from regions with unpredictable climate systems have chilling-driven (not photoperiod-driven) safety mechanisms to avoid precocious bud development. Also, previous experiments on a diverse set of species have shown that temperature, not day length, is the main driver of bud break in temperate zone woody species (Laube *et al.* 2014; Zohner *et al.* 2016).

Our analyses reject other climate parameters, such as MAT and TS, as possible explanations for the continental-scale differences in leaf-out strategies. Modern-day NA, and especially its eastern part from which 86% of our 419 American species originate, has a high TS (Figure S15). However, TS had little effect on both leaf-out dates and chilling requirements (Fig. 1, Figure S13b). The positive association between MAT and leaf-out dates, i.e. species from warmer regions leaf out later in a common garden than species from colder regions (Table 1, inset Fig. 1d and Figure S13b), also cannot account for the observed early leaf-out of EA species because on average these species experience warmer MAT than EU and NA species (as shown in Figure S15).

A weakness of this study is that STV is an indirect measure of late spring frost frequencies because it does not directly include frost occurrences. Daily data on frost frequency around the Northern Hemisphere are not available for the past 100 years over the entire region, but such data now need to be compiled. However, even if such global spring frost data were available, it remains to investigate (experimentally or by targeted monitoring) the species-specific sensitivity of young leaves to night frost, as it is known for only few species (e.g. Lenz *et al.* 2016). Nevertheless, our demonstration of the strong correlation between STV and WTV and species' requirements for high or low winter chilling for the first time reveals a regional (historic), not latitudinal (physical), pattern in leaf-out strategy.

This study is based not only on experiments, but also on monitoring of trees and shrubs planted in botanical gardens. The way such plants react to winter chilling, spring, warming or day length should reflect their native thresholds for these environmental signals because the trees, shrubs and climbers planted in gardens are acclimated, but had no opportunity for natural propagation, precluding evolutionary adaptation. To our knowledge, non-native trees grown outdoors in temperate zone botanical gardens also do not experience transfer bottlenecks because most are not the result of *in vitro* propagation or some other selection process specifically selecting individuals suitable for local conditions.

We did not address within-species phenological variability and therefore cannot draw conclusions about population-level adaptations to temperature variability, a topic to be explored in the future. The conclusions drawn in this study, however, are robust against effects of intraspecific variability because (1) we do account for intraspecific variability by studying leaf-out at six sites, each with different individuals planted there (Fig. 3) and (2) the strong phylogenetic signal in leaf phenology that we find, i.e. closely related species are more similar in their phenological strategy than expected from a random sampling (Figure S2 and S3) shows that within-species variation is low compared to the overall inter-specific variability.

Our discovery that species from EA require significantly less chilling before leaf out than their NA relatives suggests that these continents' forests will react differently to continuing climate warming: While earlier leaf-out in most NA trees and shrubs will be constrained by unmet chilling requirements as winters also become warmer and shorter, leaf-out in EA woody species, which lack such winter requirements, may advance much more. Indeed, satellite observations tend to confirm this (Park *et al.* 2016). The EA species may then opportunistically benefit from increased carbon gain and nutrient uptake (Penuelas & Filella 2001; Richardson *et al.* 2010, 2013), and might have an advantage when growing as exotics in NA forests (Fridley 2008, 2012, 2013; Wolkovich & Cleland 2011). Alternatively, their more conservative strategy might benefit native NA species if spring frost risk continues to increase (Augspurger 2013). Surprisingly little is known about long-term changes in spring frost damage (but see Augspurger 2013) or hail frequency (Hegerl *et al.* 2011; Punge & Kunz 2016), and our results underscore the need for considering spring temperature variability, as one component of regional climate history, as among the important selective factors explaining tree phenology.

AUTHORSHIP

CMZ and SSR designed the study. CMZ and BMB performed the analyses. CMZ and SSR led the writing with inputs from the other authors.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.hr364

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