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

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Words: 216 (abstract), 1901 (text), 2998 (methods), 570 (legends)

References: 28

Figures: 3

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 species³⁻⁵, affecting plant productivity and global carbon balance⁶⁻⁸. 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 unfolding⁹⁻¹². The two temperature signals interact, with species that need extended chilling unable to react to spring warming if winters are too short¹¹⁻¹³. Hence, unfulfilled chilling requirements may halt the advance of spring leaf-out, as is already happening in seven European species analysed in this regard¹⁴. Previous work on the budbreak phenology of temperate species has largely ignored the potential contributions of local climate history (but see Lechowicz¹⁵), 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 passed¹⁵. 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 dataset¹⁶. 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 gardens¹⁷. 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 history¹⁷. 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 $>0^{\circ}\text{C}$ 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 $\pm$ 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 garden⁵ 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 strategies⁵; 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 Augspurger²⁶) or hail frequency^{27,28}, but our results underscore the need for considering regional climate histories and the 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 study⁵. As in Zohner and Renner⁵, 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 database³¹, 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 Eocene³⁴. 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 & Renner⁵ 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-package³⁵. 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 dataset¹⁶ (version 3.00 with a spatial resolution of 5-arc minutes³⁸). 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 Heiberger³⁹), 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' v0.2-1⁴⁵. To build the tree we used MEGAPTERA⁴⁶ and BEAST⁴⁷. 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 Ma⁴⁸; 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 Phylomatic⁴⁹ (Extended Data Fig. 1). Its topology reflects the APG III⁵⁰ phylogeny, with a few changes based on the Angiosperm Phylogeny Website⁵¹. 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 & Cradock⁵³) 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 model⁵⁴. 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 analysis⁵⁵. 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 ancestor⁵⁶. 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 library⁵⁷. 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 JAGS⁵⁸ implementation of Markov chain Monte Carlo methods in the R2JAGS R-package⁵⁹. 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 Rubin⁶⁰ 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 estimates⁶¹. 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 SAR_{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 Weight_{AIC} in Table 1).

All statistical analyses relied on R⁶⁶.

References

1. Cannell, M.G.R. Spring phenology of trees and frost avoidance. *Weather* **52**, 46–52 (1997).
2. Lenz, A., Hoch, G., Vitasse, Y., Körner, C. Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Funct. Ecol.* (2016). doi: 10.1111/1365-2435.12623
3. Menzel, A., Fabian, P. Growing season extended in Europe. *Nature* **397**, 659 (1999).

- 436 4. Menzel, A. *et al.* European phenological response to climate change matches the warming
437 pattern. *Glob. Change Biol.* **12**, 1969–1976 (2006).
- 438 5. Zohner, C.M., Renner, S.S. Common garden comparison of the leaf-out phenology of woody
439 species from different native climates, combined with herbarium records, forecasts long-
440 term change. *Ecol. Lett.* **17**, 1016–1025 (2014).
- 441 6. Richardson, A.D. *et al.* Influence of spring and autumn phenological transitions on forest
442 ecosystem productivity. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **365**, 3227–3246 (2010).
- 443 7. Richardson, A.D. *et al.* Climate change, phenology, and phenological control of vegetation
444 feedbacks to the climate system. *Agricult. For. Meteorol.* **169**, 156–173 (2013).
- 445 8. Keenan, T. F. *et al.* Net carbon uptake has increased through warming-induced changes in
446 temperate forest phenology. *Nature Clim. Change* **4**, 598–604 (2014).
- 447 9. Heide, O.M. Daylength and thermal time responses of budburst during dormancy release in some
448 northern deciduous trees. *Physiol. Plant.* **88**, 531–540 (1993).
- 449 10. Caffarra, A., Donnelly, A. The ecological significance of phenology in four different tree
450 species: effects of light and temperature on bud burst. *Int. J. Biometeorol.* **55**, 711–721
451 (2011).
- 452 11. Laube, J. *et al.* Chilling outweighs photoperiod in preventing precocious spring development.
453 *Global Change Biol.* **20**, 170–182 (2014).
- 454 12. Polgar, C., Gallinat, A., Primack, R.B. Drivers of leaf-out phenology and their implications for
455 species invasions: insights from Thoreau’s Concord. *New Phytol.* **202**, 106–115 (2014).
- 456 13. Zohner, C.M., Renner, S.S. Perception of photoperiod in individual buds of mature trees
457 regulates leaf-out. *New Phytol.* **208**, 1023–1030 (2015).

- 458 14. Fu, Y.H. *et al.* Declining global warming effects on the phenology of spring leaf unfolding.
459 *Nature* **526**, 104–107 (2015).
- 460 15. Lechowicz, M.J. Why do temperate deciduous trees leaf out at different times? Adaptation and
461 ecology of forest communities. *Am. Nat.* **124**, 821–842 (1984).
- 462 16. Jones, P.D., Harris, I. Climatic Research Unit (CRU) time-series datasets of variations in climate
463 with variations in other phenomena. NCAS British Atmospheric Data Centre (2008). URL:
464 <http://catalogue.ceda.ac.uk/uuid/3f8944800cc48e1cbc29a5ee12d8542d>.
- 465 17. Panchen, Z.A. *et al.* Leaf out times of temperate woody plants are related to phylogeny,
466 deciduousness, growth habit and wood anatomy. *New Phytol.* **203**, 1208–1219 (2014).
- 467 18. Körner, C., Basler, D. Phenology under global warming. *Science* **327**, 1461–1462 (2010).
- 468 19. Vitasse, Y., Lenz, A., Körner, C. The interaction between freezing tolerance and phenology in
469 temperate deciduous trees. *Front. Plant Sci.* **5**, 1–12 (2014).
- 470 20. Sanz-Perez, V., Castro-Diez, P., Valladares, F. Differential and interactive effects of temperature
471 and photoperiod on budburst and carbon reserves in two co-occurring Mediterranean oaks.
472 *Plant Biol.* **11**, 142–151 (2009).
- 473 21. Penuelas, J., Filella, I. Responses to a warming world. *Science* **294**, 793–795 (2001).
- 474 22. Fridley, J.D. Of Asian forests and European fields: Eastern U.S. plant invasions in a global
475 floristic context. *PLoS ONE* **3**, e3630 (2008).
- 476 23. Wolkovich, E.M., Cleland, E.E. The phenology of plant invasions: A community ecology
477 perspective. *Front. Ecol. Environ.* **9**, 287–294 (2011).
- 478 24. Fridley, J.D. Extended leaf phenology and the autumn niche in deciduous forest invasions.
479 *Nature* **485**, 359–362 (2012).

- 480 25. Fridley, J.D. Plant invasions across the Northern Hemisphere: a deep-time perspective. *Ann.*
481 *NY Acad. Sci.* **1293**, 8–17 (2013).
- 482 26. Augspurger, C.K. Reconstructing patterns of temperature, phenology, and frost damage over
483 124 years: Spring damage risk is increasing. *Ecology* **94**, 41–50 (2013).
- 484 27. Punge, H.J., Kunz, M. Hail observations and hailstorm characteristics in Europe: A review.
485 *Atmos. Res.* **176/177**, 159–184 (2016).
- 486 28. Hegerl, G.C., Hanlon, H., Beierkuhnlein, C. Elusive extremes. *Nat. Geosci.* **4**, 142–143 (2011).
- 487 29. Vitasse, Y., Basler, D. Is the use of cuttings a good proxy to explore phenological responses of
488 temperate forests in warming and photoperiod experiments? *Tree phys.* **00**, 1–10 (2014).
- 489 30. Basler, D., Körner, C. Photoperiod sensitivity of bud burst in 14 temperate forest tree species.
490 *Agric. For. Meteorol.* **165**, 73–81 (2012).
- 491 31. USDA, NRCS. The PLANTS Database. National Plant Data Center, Baton Rouge, LA.
492 Available: <http://plants.usda.gov>. Accessed July 11, 2015.
- 493 32. Brach, A.R., Song, H. eFloras: New directions for online floras exemplified by the Flora of
494 China Project. *Taxon* **55**, 188–192 (2006).
- 495 33. eFloras (2008). Published on the Internet <http://www.efloras.org> [accessed 02 October 2015]
- 496 34. Akhmetiev, M.A. *et al.* The Paleogene history of the Western Siberian seaway – a connection
497 of the Peri-Tethys to the Arctic ocean. *Austrian J. Earth Sci.* **105**, 50–67 (2012).
- 498 35. Hijmans, R.J., Phillips, S., Leathwick, J., Elith, J. Package ‘dismo’ (2011). Available online at:
499 <http://cran.r-project.org/web/packages/dismo/index.html>.
- 500 36. Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A. *The worldclim interpolated*
501 *global terrestrial climate surfaces*. (2004). at <Computer Program
502 Available at Website <http://biogeو.berkeley.edu/>>

503 37. Hijmans, R.J., Cameron, S.E, Parra, J.L., Jones, P.G., Jarvis, A. Very high resolution interpolated
504 climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).

505 38. Harris, I., Jones, P.D., Osborn, T.J., Lister, D.H. Updated high-resolution grids of monthly
506 climatic observations - the CRU TS3.10 Dataset. *Int. J. Climatol.* **34**, 623–642 (2014).

507 39. Heiberger, R.M. HH: Statistical Analysis and Data Display: Heiberger and Holland (2016). R
508 package version 3.1-25. URL <http://CRAN.R-project.org/package=HH>

509 40. Breiman, L. Random forest. *Mach. Learn.* **45**, 15–32 (2001).

510 41. Cutler, D.R. *et al.*, Random forests for classification in ecology. *Ecology* **88**, 2783–2792
511 (2007).

512 42. Thernau, T., Atkinson, B., Ripley, B. rpart: Recursive Partitioning and Regression Trees. R
513 package version 4.1-10 (2015). URL: <http://CRAN.R-project.org/package=rpart>

514 43. Pagel, M. Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884
515 (1999).

516 44. Blomberg, S.P., Garland, T.Jr., Ives, A.R. 2003. Testing for phylogenetic signal in
517 comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745 (2003).

518 45. Revell, L.J. Phytools: an R package for phylogenetic comparative biology (and other things).
519 *Methods Ecol. Evol.* **3**, 217–223 (2012). [WWW document] URL [http://cran.r-](http://cran.r-project.org/web/packages/phytools)
520 [project.org/web/packages/phytools](http://cran.r-project.org/web/packages/phytools) [accessed 15 June 2013].

521 46. Heibl, C. megaptera: MEGAPhylogeny Techniques in R. R package version 1.0-0. (2014).
522 available at: <http://CRAN.R-project.org/package=megaptera>

523 47. Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. Bayesian phylogenetics with BEAUti
524 and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).

525 48. Bell, C., Soltis, D.E., Soltis, P.S. The age and diversification of the Angiosperms re-revisited.
526 *Am. J. Bot.* **97**, 1296–1303 (2010).

527 49. Webb, C.O., Donoghue, M.J. PHYLOMATIC: tree assembly for applied phylogenetics. *Mol.*
528 *Ecol. Notes* **5**, 181–183 (2005).

529 50. APG III, An update of the angiosperm phylogeny group classification for the orders and
530 families of flowering plants: APG III. *Bot. J. Linn. Soc.* **161**, 105–121 (2009).

531 51. Stevens, P.F. Angiosperm phylogeny website. (2012). [WWW document] URL [http://](http://www.mobot.org/MOBOT/research/APweb/)
532 www.mobot.org/MOBOT/research/APweb/.

533 52. Smith, S.A., Beaulieu, J.M., Donoghue, M.J. An uncorrelated relaxed-clock analysis suggests
534 an earlier origin for flowering plants. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5897–5902
535 (2010).

536 53. Fridley, J.D., Craddock, A. Contrasting growth phenology of native and invasive forest
537 shrubs mediated by genome size. *New Phytol.* **207**, 659–668 (2015).

538 54. de Villemereuil, P., Wells, J.A., Edwards, R.D., Blomberg, S.P. Bayesian models for
539 comparative analysis integrating phylogenetic uncertainty. *BMC Evol. Biol.* **12**, 102
540 (2012).

541 55. Gelman, A., Hill, J. Data analysis using regression and multilevel/hierarchical models.
542 Cambridge, UK: Cambridge University Press (2007).

543 56. Grafen, A. The phylogenetic regression. *Philos. T. Roy. Soc. B.* **326**, 119–157 (1989).

544 57. Paradis, E., Claude, J., Strimmer, K. APE: analyses of phylogenetics and evolution in R
545 language. *Bioinformatics* **20**, 289–290 (2004).

546 58. Plummer, M. JAGS: a program for analysis of Bayesian graphical models using Gibbs
547 sampling. In: Hornik K, Leisch F, Zeileis A, eds. Proceedings of the 3rd International

Workshop on Distributed Statistical Computing (DSC 2003). Vienna, Austria: Achim Zeileis (2003).

59. Su, Y.-S., Yajima, M. R2jags: a package for running JAGS from R. R package version 0.04-03 (2014). [WWW document] URL <http://CRAN.R-project.org/package=R2jags>

60. Gelman, A., Rubin, D.B. Inference from iterative simulation using multiple sequences. *Stat. Sci.* **7**, 457–472 (1992).

61. Dormann, C.F. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. *Glob. Ecol. Biogeogr.* **16**, 129 (2007). doi:10.1111/j.1466-8238.2006.00279.x

62. Borcard, D., Legendre, P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol. Modell.* **153**, 51–68 (2002).

63. Griffith, D.A., Peres-Neto, P.R. Spatial modeling in ecology: The flexibility of eigenfunction spatial analyses. *Ecology* **87**, 2603–2613 (2006).

64. Bivand, R.S., Hauke, J., Kossowski, T. Computing the Jacobian in Gaussian spatial autoregressive models: An illustrated comparison of available methods. *Geographical Analysis* **45**, 150-179 (2013).

65. Bivand, R.S., Piras, G. Comparing Implementations of Estimation Methods for Spatial Econometrics. *Journal of Statistical Software* **63**, 1–36 (2015).

66. R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2015. URL. Available at: <http://www.R-project.org/>.

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572 **Acknowledgments:**

573 The study was part of the KLIMAGRAD project sponsored by the ‘Bayerisches
574 Staatsministerium für Umwelt und Gesundheit’. We thank V. Sebold and M. Wenn for help with
575 conducting the twig cutting experiments. We thank C. Polgar, A. Gallinat, and R.B. Primack for
576 sharing their experimental data on chilling requirements. J.-C.S. acknowledges support by the
577 European Research Council (ERC-2012-StG-310886-HISTFUNC). B.M.B. was supported by
578 Aarhus University and Aarhus University Research Foundation under the AU IDEAS program
579 (via Center for Biocultural History).

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583 **Author contributions**

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

587

Figures

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 $>0^{\circ}\text{C}$ outdoors and in a climate chamber) $\pm$ 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 $2.5^\circ \times 2.5^\circ$ 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