



## Association of spring phenological traits with phylogeny and adaptation to native climate in temperate plant species in Northeast China

Xinyue Gao<sup>a,b</sup>, Junhu Dai<sup>a,b,c,\*</sup>, Khurram Shahzad<sup>a</sup>, Huanjiong Wang<sup>a</sup>, Zexing Tao<sup>a</sup>, Juha Mikael Alatalo<sup>d</sup>

<sup>a</sup> Key Laboratory of Land Surface Pattern and Simulation, Institute of Geographic Sciences and Natural Resources Research, CAS, Beijing 100101, China

<sup>b</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>c</sup> China-Pakistan Joint Research Center on Earth Sciences, Chinese Academy of Sciences-Higher Education Commission of Pakistan, Islamabad 45320, Pakistan

<sup>d</sup> Environmental Science Center, Qatar University, Doha, Qatar

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### ABSTRACT

The effects of climate change on plant phenology have been widely recognized around the world. However, the effect of plant internal factors (such as phylogeny) on the variations in phenology among plant species remains unclear. In this study, we investigated the phylogenetic conservatism in spring phenological traits using phylogenetic signal and evolutionary models, including Brownian motion (BM) model, Ornstein-Uhlenbeck (OU) model and white noise (WN) model, based on the phenological data of 48 temperate plant species in Northeast China. We also explored the relative contributions of phylogeny and adaptation to native climate (i.e., the climate in native range of species) to the variations in the phenological traits among species using phylogenetic eigenvector regression and variance partitioning analysis. The results showed that spring phenological traits conformed to the OU model, indicating that spring traits were phylogenetically conserved. The effect of phylogeny on flowering traits was stronger than that on leaf-out traits. Additionally, the adaptation to native climate contributed more to the variations in spring phenological traits among species than phylogeny, and adaptation to native climate explained more variations in leaf-out traits than in flowering traits. Our results suggested that the spring phenological traits were constrained by both phylogeny and adaptation to native climate. However, the adaptation to native climate had a stronger effect on the variations in phenological traits than phylogeny. Therefore, the degree of similarity in spring phenological traits across closely related species depends on the degree of similarity in the environmental conditions where these close relatives are distributed.

### 1. Introduction

Plant phenological studies are concerned with repetitive stages in the life cycles of plants and animals, particularly with regard to phenological timing and interactions with climatic factors (Richardson et al., 2013; Schwartz, 2013). It has recently appeared as one of the most reliable biological indicators of climate change (Lieth, 1974; Piao et al., 2019; Richardson et al., 2013; Schwartz, 2013). Phenological changes affect individual fitness, interspecific interactions, ecosystem structure and function, and vegetation feedback to the climate system (Caparros-Santiago et al., 2021; Richardson et al., 2013). Analysis of the drivers of plant phenology may contribute to a better understanding of vegetation-climate interactions and help predict future ecosystem changes under

global change (Fu et al., 2020; Hannah, 2011). Over the past few decades, it has been widely reported that leaf unfolding and flowering in spring for temperate trees have advanced significantly across the Northern hemisphere (Ge et al., 2015; Gerst et al., 2017; Menzel et al., 2006; Menzel et al., 2020). The majority of previous studies connected plant phenological shifts mainly with the changes in major external environmental cues, such as temperature, precipitation, and photoperiod, via employing empirical approaches to developing a statistical relationship between phenophases and environmental factors (Celleri et al., 2018; Dai et al., 2019; Fu et al., 2015; Ge et al., 2015; Meng et al., 2021; Vitasse et al., 2022; Wang et al., 2021). However, recent studies have demonstrated that plant phenological shifts are compounded by intrinsic traits, which emphasizes the importance of studying the effects

\* Corresponding author at: Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A, Datun Road, Chaoyang District, Beijing 100101, China.

E-mail address: [daijh@igsnr.ac.cn](mailto:daijh@igsnr.ac.cn) (J. Dai).

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of biotic factors of evolutionary history and genetic constraints on phenological events (Davies et al., 2013; Li et al., 2016; Harvey and Pagel, 1991; Pagel, 1999).

Recent studies have indicated that plant phylogeny, which means that closely related species tend to show similar phenological traits, might serve as the biological basis for specific phenological events in certain plants or sensitivity to abiotic environmental factors (Davies et al., 2013; CaraDonna and Inouye, 2015; Yang et al., 2021). Various algorithms, including Blomberg's  $K$  and Pagel's lambda methods, have been subsequently developed for assessing the phylogenetic conservatism in phenological traits (Blomberg et al., 2003; Li et al., 2016; Muenkemüller et al., 2012). It is worth noting that significant efforts were made in some studies collecting a great number of phenological records and plant trait materials from national flora books, and relationships between phenology and phylogeny have been identified. However, the accuracy of the phenological data used in these studies was not very high (Du et al., 2015; Du et al., 2017). The extent of these studies ranged from three years (Basnett et al., 2019) to a few decades (Du et al., 2017; Yang et al., 2021), and the longest study was conducted by Davies et al. (2013) for 184 years. However, the study period for different species varied greatly in these studies.

The relationship between phylogeny and phenology has not been studied for long and the number of papers is relatively limited, therefore, several key scientific concerns remain to be understood and need to be addressed in future studies. The most fundamental scientific concern is the identification of phylogenetic conservatism in the timing of major phenological events. For example, a meta-analysis study by Davies et al. (2013) showed that more closely related species tend to flower and leaf at similar times, and they also indicated that it was not the time of year that is conserved but rather the phenological responses to a common set of abiotic cues. Another study by Rafferty and Nability (2017) observed a global phylogenetic signal in the direction and magnitude of the shifts in the time of flowering. The authors claimed that selection pressure shapes the phenological responses of certain species under climate change. Interestingly, Yang et al. (2021) reported an absence of phylogenetic conservatism in the leaf unfolding date across the Tibetan Plateau. However, another study by CaraDonna and Inouye (2015) identified a phylogenetic signal in the flowering time.

Secondly, more in-depth studies are being initiated based on the data derived previously for assessing the phylogenetic conservatism in the sensitivity of a phenophase and its requirement for specific environmental conditions, such as heat requirement (HR). Yang et al. (2021) reported a lack of phylogenetic conservatism in temperature sensitivity for leaf unfolding date, but they observed a significant phylogenetic signal in HR for the timing of leaf unfolding. Thirdly, the most comprehensive scientific question in this field is the summary of the spatial and temporal characteristics of the phenological conservatism for phenophases, their sensitivities to environmental factors, and the requirements of climatic conditions for certain phenophases. For instance, Basnett et al. (2019) observed that only early phenological events were constrained by evolutionary history, while the role of phylogeny in phenological sensitivity to related abiotic cues reduced from early to late phenological events. Most importantly, certain studies have demonstrated that the strength of phylogenetic conservatism in spring phenophase was greater under harsh conditions, such as higher latitudes and elevations (Li et al., 2021). However, Basnett et al. (2019) proposed that the strength of the phylogenetic signals of early phenological events tended to decrease with increasing altitude, while later events showed no significant trend. These findings indicate that there are numerous uncertainties in this field, and any novel evidence is valuable for researchers.

In addition to phylogeny, plant phenology is strongly influenced by native climate (i.e., the climate in native range of species) of plant species. Several studies have elucidated the patterns and underlying mechanisms of adaptation to native climate of plant species in different regions (Zohner and Renner, 2014; Zohner et al., 2016). It has been

demonstrated that species originating from higher latitudes leaf out earlier than contemporaries from lower latitudes under the same conditions (Zohner and Renner, 2014; Zohner et al., 2016). Unfortunately, only a few studies have attempted to quantify the relative contributions of phylogeny and native climate in determining the phenological traits among plant species. For instance, Desnoues et al. (2017) reported that adaptation to native climate was more crucial than phylogeny in determining the leaf unfolding dates across the temperate biome. However, the relative importance of phylogeny and adaptation to native climate on the variation in other phenological traits and for other species remains unclear. Clarifying the relative importance of phylogeny and adaptation to native climate is essential for understanding the variations in phenological traits among different species (Panchen et al., 2014), which can also aid in accurately predicting the alterations in phenological traits under climate change.

In this study, the phylogenetic conservatism in the leaf-out date and flowering time, the phenological temperature sensitivity and HR of the two spring phenophases were analyzed using the long-term and accurate phenological records of 48 temperate plant species from four sites in Northeast China. We also investigated the relative importance of phylogeny and adaptation to native climate on these phenological traits. The specific aims of this study were to test whether spring phenological traits of plants are phylogenetically conserved and to quantify the contributions of phylogeny and adaptation to native climate to spring phenological traits.

## 2. Materials and methods

### 2.1. Data

#### 2.1.1. Phenological observation data

The first leaf date (FLD) and first flowering date (FFD) of 65 woody plant species from 1963 to 2018 at four stations in Northeast China were obtained from China Phenological Observation Network (CPON) (Fig. 1). All the observed data followed defined observation criteria and procedures (Wan and Liu, 1979). The FLD and FFD are defined as the dates when a fixed individual plant generates the first full leaf and the first full flower, respectively.

As not all the plant species were present in all the studied stations, we used a phenological model for reconstructing the complete phenological time series for each species in each station, for negating the influence of phenotypic differences resulting from climatic variations among the stations. As small sample sizes reduce the accuracy of prediction of phenological models, we only selected the species that had at least 20 years of FLD and FFD observations. Consequently, sufficient leaf-out and flowering data were available for a total of 48 species, and the phenological time series were reconstructed for these 48 species (Appendix S1 in Supporting Information).

#### 2.1.2. Climatic data

The daily mean temperatures in each station from 1963 to 2018 were obtained from the China Meteorological Data Service Center (<http://data.cma.cn/>) for reconstructing the phenological time series and determining phenological traits. In addition, the following steps were performed for determining the adaptation to native climate for each species. Firstly, the georeferenced occurrences of each species were collected from Global Biodiversity Information Facility (GBIF, <https://www.gbif.org>) from 1951 to 2021. Secondly, nine climatic variables were obtained from the WorldClim database with a resolution of 30 s for all the occurrence points of the species analyzed herein, including the mean annual temperature (MAT), temperature seasonality (Ts), the maximum temperature of the warmest month (Tmax), minimum temperature of the coldest month (Tmin), mean annual precipitation (MAP), precipitation seasonality (Ps), precipitation of the wettest month (Pmax), precipitation of the driest month (Pmin), and mean annual solar radiation (sr) for the 1970–2000 period. Finally, for each

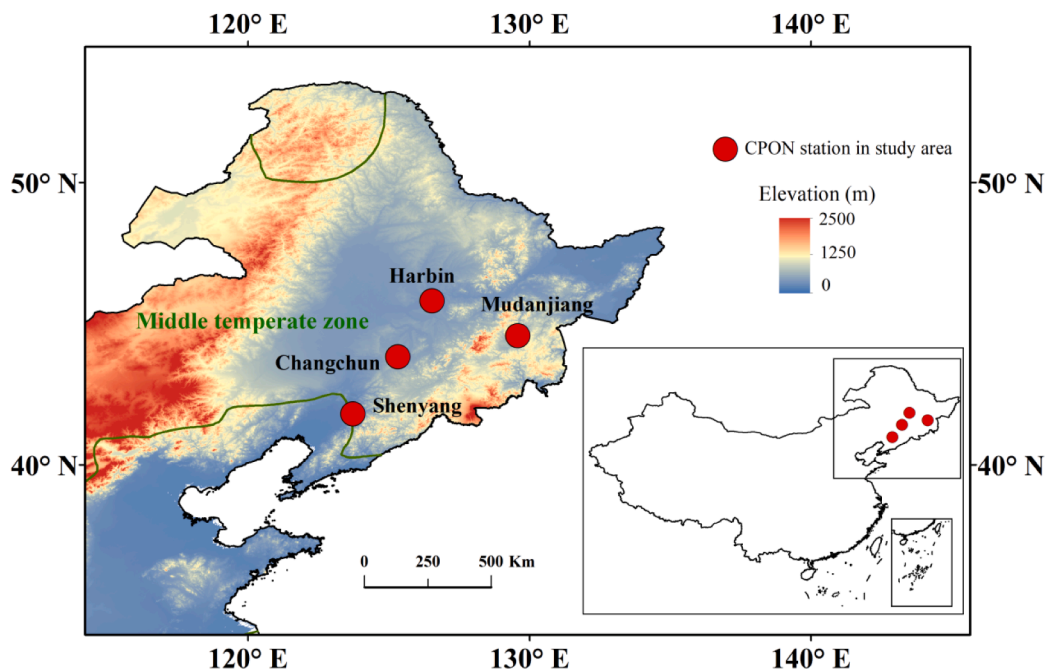


Fig. 1. The four observation stations in the CPON, which were selected as the study area. The area between the two green lines is the middle temperate zone.

species, the mean and range (minimum–maximum) of these climatic variables were calculated to obtain 18 indices, i.e., the means and ranges of the nine variables, including MAT, Ts, Tmax, Tmin, MAP, Ps, Pmax, Pmin and sr, for describing the native climate of these 48 species across the globe.

## 2.2. Methods

### 2.2.1. Reconstruction of phenological time series

We applied the temporal-spatial coupling model, stated in one of our previous studies (Ge et al., 2014), to reconstruct the phenological time series of each species at each station. For each species, we first obtained the phenological observation data (FLD/FFD) and the daily mean temperature from 1963 to 2018 at the four stations. The parameters of the model were tuned by a simulated annealing algorithm using the FLD/FFD in odd years, while the FLD/FFD data for the even years were used for model validation (Chuine et al., 2010). The performance of the models was measured by calibration and validation based on the root mean square error (RMSE) and goodness of fit ( $R^2$ ). Finally, the FLD/FFD data of each species at each station were generated using the parameters obtained and the temperature in each station.

### 2.2.2. Determination of the spring phenological traits

The leaf-out date and flowering time, the phenological temperature sensitivity and HR of these two spring phenophases were subjected to further analyses, and they were determined based on the reconstructed phenological series. The leaf-out date/flowering time was calculated as the multiyear average FLD/FFD from 1963 to 2018. The temperature sensitivity was represented by the slope of the regression between the FLD/FFD and the average daily mean temperature during the preseason. The preseason was determined by first calculating the Pearson's correlation coefficient ( $r$ ) between FLD/FFD and average daily mean temperature from the 1st to the 120th day before the average FLD/FFD of the study period. The preseason was finally determined as the period with the highest value of  $r$  (Dai et al., 2019). The HR was defined as the multiyear average of the daily mean temperature ( $>0^\circ\text{C}$ ) sum from the 1st of January to the FLD/FFD of each year (Heide, 1993). The average value of each of the phenological traits at the four stations was finally calculated for each species, for investigating the species-level variations

in phenological traits in the northeast region rather than at each station.

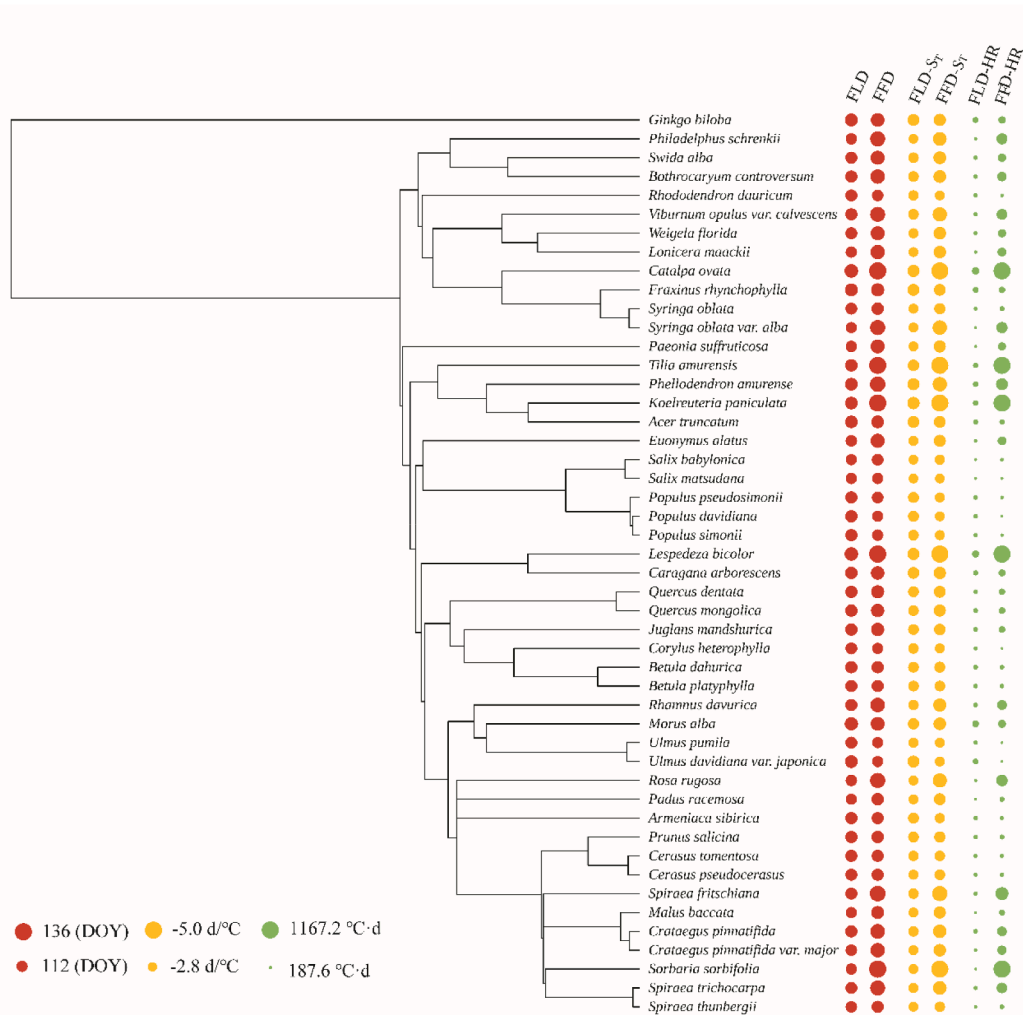
### 2.2.3. Phylogenetic reconstruction

The scientific names of the 48 species were first determined from Plant List (<https://www.theplantlist.org>) using the *plantlist* package in R. The *V. PhyloMaker* package in R was then used to create a phylogenetic tree for these species based on the most recent GBOTB tree, which included 74,533 taxa and a backbone with all extant vascular plant families (Jin and Qian, 2019). This mega phylogenetic tree is constructed mainly using gene data and has been used in numerous studies (Slot et al., 2021; Xu et al., 2021). It also can provide high-resolution connections between the families and genera of most taxa (Qian and Jin, 2021). The phylogenetic tree constructed in this study is depicted in Fig. 2.

### 2.2.4. Analysis of phylogenetic conservatism in spring phenological traits

Blomberg's  $K$  is a widely used phylogenetic signal method for evaluating the phylogenetic conservatism in phenological traits. It indicates the strength of the tendency of closely related species to have similar phenological traits (Blomberg et al., 2003). For comparing the results of this study with those of other reported findings, Blomberg's  $K$  method was applied in this study. A value of  $K = 1$  indicates that the inter-species correlation equals the expectation of Brownian motion, indicating that the evolution of the trait is influenced by phylogeny. Values of  $K > 1$  indicate that trait similarity is higher than the expectation of Brownian motion (Blomberg et al., 2003). In contrast, values of  $K < 1$  imply stasis, which means the trait is phylogenetically conserved, or the absence of phylogenetic structures, which means the trait is not phylogenetically conserved (Wiens et al., 2010). The values of the  $K$  for each phenological trait were determined using the *phytools* package in R. The  $P$ -value can also be obtained by 1000 interactions during the calculation of the  $K$  parameter for detecting whether the observed values differ significantly from randomized arrangement (Revell, 2011).

It should be noted that the phylogenetic signal is based on Brownian motion (BM) model, and many studies have interpreted the insignificant signals as a lack of conservatism. However, other studies have demonstrated that an insignificant signal could either imply random variation (no phylogenetic conservatism) or stasis (strong phylogenetic conservatism) (Muenkemüller et al., 2015; Revell et al., 2008; Peixoto et al.,



**Fig. 2.** Phylogenetic distribution of phenological traits on tree topology. The sizes of the circles are proportional to the values of the traits, i.e., a larger size indicates later FLD (FFD), stronger temperature sensitivity ( $S_T$  with higher absolute value), and more heat requirement. FLD, first leaf date; FFD, first flowering date; FLD- $S_T$ , the temperature sensitivity of FLD; FFD- $S_T$ , the temperature sensitivity of FFD; FLD-HR, heat requirement for FLD; FFD-HR, heat requirement for FFD.

2017). Alternatively, the relative fitting of evolutionary models is a more appropriate approach for evaluating phylogenetic conservatism as a random variation and evolutionary stasis shaped by selection can be directly captured by the white noise (WN) model and Ornstein–Uhlenbeck (OU) model, respectively (Diniz-Filho et al., 2015; Felsenstein, 1985; Blomberg et al., 2003; Butler and King, 2004; Kozak and Wiens, 2010). Therefore, the evolutionary models were also used in this study to evaluate the phylogenetic conservatism of phenological traits more accurately. The relative fitting of three widely used evolutionary models, i.e., BM, OU and WN models, were selected here and compared using the weighted Akaike Information Criterion (wAIC). The model with the highest wAIC value was regarded as the best fitting model (Butler and King, 2004; Diniz-Filho et al., 2012).

Phylogenetic signal representation (PSR) curves were used for studying the phylogenetic patterns of phenological traits (Diniz-Filho et al., 2012). This approach is based on the phylogenetic eigenvector regression (PVR) model, which uses eigenvectors retrieved and selected from a pairwise phylogenetic distance matrix to model trait variation. The  $R^2$  values of consecutive PVR models fitted by increasing the number of eigenvectors versus their cumulative eigenvalues were plotted for constructing the PSR curves. A 45° line may imply Brownian motion ( $K = 1$ ), while a PSR curve above the 45° line could indicate that the evolution of the trait is faster and is less conserved than that

expected under Brownian motion. A PSR curve below the 45° line but above that of a null model (devoid of phylogenetic conservatism) indicates slow trait evolution, implying that the evolution of the trait is slower and more conserved than that predicted under Brownian motion, as observed for OU processes (Martins et al., 2002; Staggemeier et al., 2015). Furthermore, the shape of the PSR curves can also provide insights into the rate of evolution in the branches of the phylogenetic tree (Diniz-Filho et al., 2012).

#### 2.2.5. Relative contributions of phylogeny and adaptation to native climate to the variations in phenological traits among species

We used the PVR method and a variance partitioning analysis to quantify the contributions of phylogeny and adaptation to native climate to the variations in phenological traits. The combined effect of phylogeny and adaptation to native climate is termed phylogenetically structured adaptation, as closely related species occur in spatial proximity and therefore face similar selection pressures (Desdevises et al., 2003). The PVR analysis includes three steps: (1) modeling phenological trait variability as a function of all 18 climatic variables, namely, the mean and range of nine variables, including MAT, Ts, Tmax, Tmin, MAP, Ps, Pmax, Pmin and sr, and using the stepwise regression to select the best set of variables to predict the dependent variable; (2) modeling phenological trait variability as a function of phylogenetic eigenvectors



selected by the Moran's I method; and (3) modeling phenological trait variability as a function of the climatic variables selected by stepwise regression and phylogenetic eigenvectors selected by Moran's I method. Three adjusted  $R^2$  values were obtained from these three linear models. The phenological variations attributed to phylogeny ( $P_1$ ), adaptation to native climate ( $P_2$ ), and both phylogeny and adaptation to native climate ( $P_3$ ) were calculated as:

$$P_1 = R_{\text{adj}3}^2 - R_{\text{adj}1}^2 \quad (1)$$

$$P_2 = R_{\text{adj}3}^2 - R_{\text{adj}2}^2 \quad (2)$$

$$P_3 = R_{\text{adj}2}^2 - (R_{\text{adj}3}^2 - R_{\text{adj}1}^2) \quad (3)$$

where  $R_{\text{adj}1}^2$  is the adjusted  $R^2$  obtained by modeling phenological trait variability as a function of the climatic variables selected by stepwise regression;  $R_{\text{adj}2}^2$  is the adjusted  $R^2$  obtained by modeling phenological trait variability as a function of phylogenetic eigenvectors selected by Moran's I method; and  $R_{\text{adj}3}^2$  is the adjusted  $R^2$  obtained by modeling phenological trait variability as a function of the climatic variables selected by stepwise regression and phylogenetic eigenvectors selected by Moran's I method.

### 3. Results

#### 3.1. Phylogenetic signals in spring phenological traits

Analyses of the phylogenetic signals of the phenological traits revealed that the  $K$ -value was  $<1$  for all the spring phenological traits (see Table 1), indicating that the spring phenological traits of temperate plant species in Northeast China were less similar than expected under Brownian motion. Additionally, the phylogenetic signals of the phenological traits of flowering (FFD, FFD- $S_T$ , FFD-HR) were significant at  $P < 0.05$ , suggesting that flowering traits were phylogenetically conserved. In contrast, the phylogenetic signals of the phenological traits of leaf-out (FLD, FLD- $S_T$ , FLD-HR) were not significant at  $P > 0.05$ . However, it remained unclear whether the leaf-out traits were attributed to random variations, which means the lack of phylogenetic conservatism, or stasis, which indicates strong phylogenetic conservatism. In addition, The strength of phylogenetic signals of FFD and FFD-HR were stronger than those of FLD and FLD-HR, respectively.

#### 3.2. Evolutionary models and phylogenetic patterns of spring phenological traits

Of the three evolutionary models, the OU model had the highest wAIC value for all the phenological traits and the PSR curves of all phenological traits were below the 45° reference line but above the null model, indicating that the rate of evolution of spring phenological traits was slower than that expected in the Brownian model. In other words, all the spring phenological traits were phylogenetically conserved (Table 2 and Fig. 3). Additionally, the PSR curves depicted a non-

**Table 1**  
The phylogenetic signals of the phenological traits.

Phenological traits	Blomberg's $K$	$P$ -value
FLD	0.30	0.05
FFD	0.33	0.01
FLD- $S_T$	0.34	0.06
FFD- $S_T$	0.33	0.01
FLD-HR	0.31	0.06
FFD-HR	0.33	0.02

FLD, first leaf date; FFD, first flowering date; FLD- $S_T$ , the temperature sensitivity of FLD; FFD- $S_T$ , the temperature sensitivity of FFD; FLD-HR, heat requirement for FLD; FFD-HR, heat requirement for FFD.

**Table 2**

Comparison of the fit between the BM, OU and WN models of evolution based on the wAIC.

Phenological traits	BM	OU	WN
FLD	0.00	<b>0.62</b>	0.38
FFD	0.00	<b>0.83</b>	0.17
FLD- $S_T$	0.00	<b>0.58</b>	0.42
FFD- $S_T$	0.00	<b>0.80</b>	0.20
FLD-HR	0.00	<b>0.68</b>	0.32
FFD-HR	0.00	<b>0.81</b>	0.19

The best fits of the evolutionary models are indicated in bold. FLD, first leaf date; FFD, first flowering date; FLD- $S_T$ , the temperature sensitivity of FLD; FFD- $S_T$ , the temperature sensitivity of FFD; FLD-HR, heat requirement for FLD; FFD-HR, heat requirement for FFD.

stationary pattern of evolution of spring phenological traits, which indicated that the rate of evolution of these phenological traits was not constant.

#### 3.3. Relative contributions of phylogeny and adaptation to native climate to the variations in spring phenological traits among species

The PVR models showed that both adaptation to native climate and phylogeny affected the spring phenological traits (Fig. 4). Adaptation to native climate and phylogeny could explain between 34.0 % (FLD- $S_T$ ) and 41.1 % (FLD) of the variations in phenological traits among species. The contribution of adaptation to native climate to variations in phenological traits across species was greater than that of phylogeny by approximately 2 to 10-fold. That is, the adaptation to native climate had a greater influence on phenological traits than phylogeny. Additionally, 1.4–10.4 % of the variation in the phenological traits across species could be explained by the combined effects of phylogeny and adaptation to native climate, which indicated that the similarities among closely related species could also be linked to the evolution in response to their shared environmental cues.

Adaptation to native climate explained more variations in leaf-out traits (25.8–35.7 %) than in flowering traits (19.2–21.5 %), which means that the adaptation to native climate had a stronger effect on leaf-out traits than on flowering traits. However, the reverse was observed for flowering traits. That is, phylogeny had more effect on flowering traits than on leaf-out traits.

### 4. Discussion

#### 4.1. Phylogenetic signals of spring phenological traits

In this study, we evaluated the phylogenetic signals of spring phenological traits of temperate plant species in Northeast China. Analysis of the three flowering traits revealed that the phylogenetic signal of flowering time was significant, which was highly consistent with the reports of other studies in Europe (Davies et al., 2013), North America (Davies et al., 2013), and China (Du et al., 2017; Du et al., 2015; Li et al., 2016). Our study also supports the phylogenetic constraint hypothesis in HR for flowering as a significant phylogenetic signal in HR was detected, meaning that closely related species tend to have similar HR for flowering, yet similar findings have not been reported in other studies. Additionally, the phylogenetic signal of the temperature response of flowering time in Northeast China was significant, as demonstrated by another study in Northeast China (Du et al., 2017). However, a study in the Rocky Mountains of Colorado (CaraDonna and Inouye, 2015) indicated the absence of phylogenetic signal (i.e., insignificant phylogenetic signal) in the temperature sensitivity of flowering time. The difference may be attributed to the fact that the environment of the Rocky Mountains in Colorado is harsher than that of the region studied herein, and the strong abiotic selection pressures (frost and stronger winds) may limit species growth and reproduction (Cavender-

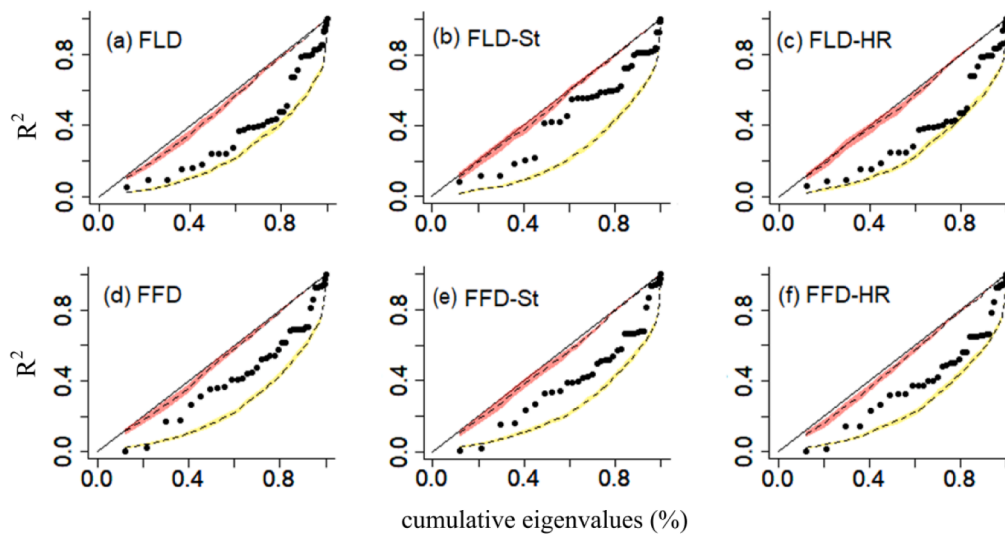


Fig. 3. Phylogenetic signal representation (PSR) curves of the spring phenological traits. The red and yellow bands represent the confidence intervals for the BM model and WN random expectations, respectively. The black line is the 1:1 line. The black dots represent the phylogenetic eigenvectors added sequentially. The y-axes represent the  $R^2$  values of the consecutive PVR models and the x-axes represent the cumulative sum of the eigenvalues. FLD, first leaf date; FFD, first flowering date; FLD- $S_T$ , the temperature sensitivity of FLD; FFD- $S_T$ , the temperature sensitivity of FFD; FLD-HR, heat requirement for FLD; FFD-HR, heat requirement for FFD.

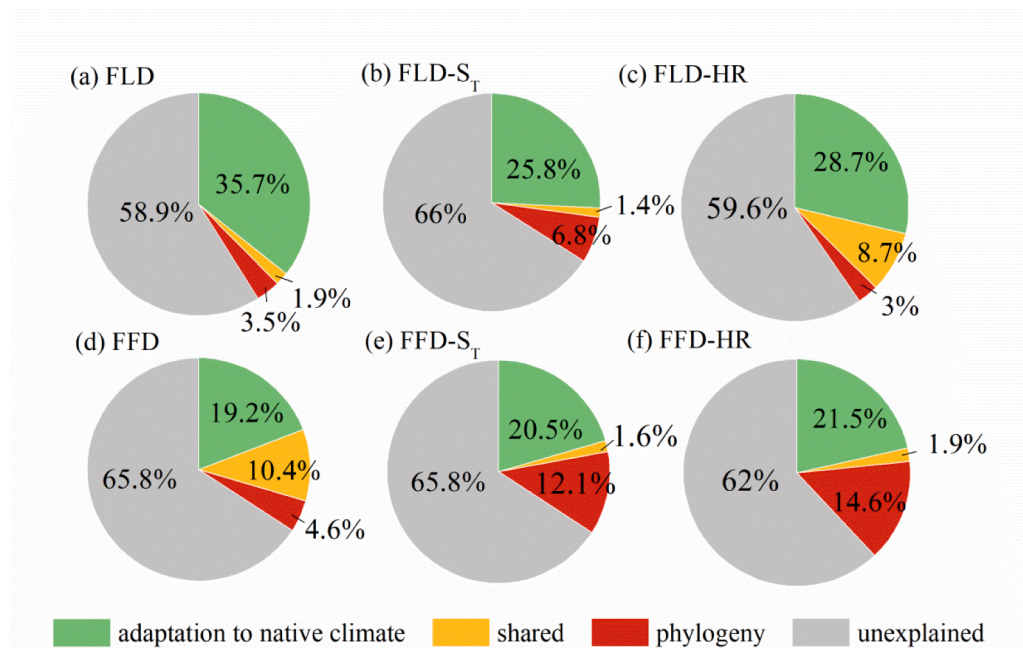


Fig. 4. Fractions of species-level variations (adjusted  $R^2$ ) explained for each phenological trait by adaptation to native climate, phylogeny, and both. FLD, first leaf date; FFD, first flowering date; FLD- $S_T$ , the temperature sensitivity of FLD; FFD- $S_T$ , the temperature sensitivity of FFD; FLD-HR, heat requirement for FLD; FFD-HR, heat requirement for FFD.

Bares et al., 2009; Lessard-Therrien and Davies, 2014), thereby leading to convergent evolution in response to temperature, and insignificant signals in temperature sensitivity (Du et al., 2015; Basnett et al., 2019). Unlike for flowering traits, there were no significant signals in the leaf-out date and its temperature sensitivity, as reported by research in Tibetan Plateau (Yang et al., 2021), implying that the effect of phylogeny on leaf-out traits may be weaker in cold regions. Similar to the leaf-out date and its temperature sensitivity, we also did not find evidence in support of phylogenetic signal in the HR of leaf-out date. To date, there is a scarcity of studies on the influence of phylogeny on the HR of spring phenology, and it needs more attention in further studies.

Interestingly, we observed that the strength of phylogenetic signals of flowering traits was relatively stronger than that of leaf-out traits. We assumed that this discrepancy could be associated with the adaptation strategy of plants to the external environment during the process of

evolution. Flowering traits are closely related to the reproductive ability of plants (Arroyo et al., 2021; Franks, 2015), and could be determined by a stabilizing selection of environmental conditions and correlated adaptations between structural and functional traits during evolution (Memmott et al., 2007). In contrast, leaf unfolding, as a photosynthetic trait, could maximize environmental resources for supporting reproductive development (Gougherty and Gougherty, 2018), and therefore may be more sensitive to environmental changes than flowering traits. The results of the PVR model revealed that the adaptation to native climate explained more species-level variations in leaf-out traits than flowering traits (Fig. 4), which could also support the aforementioned supposition.

#### 4.2. Phylogenetic conservatism in spring phenological traits

As aforementioned, the performance of the evolutionary model is better than that of the phylogenetic signal method. We, therefore, explored phylogenetic conservatism with evolutionary models for improving the accuracy of the results.

Both the phylogenetic signal method and the evolutionary model suggested the existence of phylogenetic conservatism in the flowering traits. However, there was a discrepancy between the two methods in terms of the leaf-out traits. The insignificant signals for the leaf-out traits were always interpreted as the lack of phylogenetic conservatism. However, all the leaf-out traits conformed to the OU model, indicating that the leaf-out traits were phylogenetically conserved. The discrepancy between the results obtained from phylogenetic signals and the evolutionary models highlights the necessity of using multiple methods in further studies for evaluating the phylogenetic constraint hypothesis of other phenological traits.

Overall, the results of the study supported the phylogenetic constraint hypothesis in spring phenological traits of temperate plant species in Northeast China. The results could provide a theoretical basis for understanding the species richness patterns in plant communities. Closely related species with similar phenological traits may face more intense interspecific competition for biotic or abiotic resources. This competitive exclusion would be unfavorable for the coexistence of the species (Du et al., 2015; Davis et al., 2010), suggesting that conservatism in the temporal niche could affect species richness. On the other hand, our findings could offer a potential approach to estimating phenological traits for some species lacking observations, given that closely related species would exhibit similar phenological traits.

It is now well known that the evolution of plant traits is often highly complex and the evolutionary rate changes constantly across the phylogeny (Diniz-Filho et al., 2015). In this study, we also detected a non-stationary pattern in the evolution of spring phenological traits (Fig. 3). This observation was consistent with the study by Staggemeier et al. (2015), which also indicated phylogenetic non-stationarity in the reproductive phenology of Neotropical Myrtaceae. The non-stationarity of the spring phenological traits suggested that species with different histories of clades may have evolved with different environmental adaptive mechanisms, which are responsible for diverging phenological traits. Therefore, the results emphasize the importance of considering the phylogenetic scale when testing for phylogenetic conservatism at different taxonomic levels. Our study also demonstrates the necessity of comparing the results of different methodologies and phylogenetic scales for a better understanding of the role of phylogeny in determining the observed phenological patterns among species.

#### 4.3. Influence of phylogeny and adaptation to native climate on spring phenological traits

We observed that adaptation to native climate had a stronger effect on phenological traits than phylogeny in the mid-temperature zone of Northeast China (Fig. 4), similar to a previous study that reported that native climate was more crucial than phylogeny for tree phenology across the temperate biome (Desnoues et al., 2017). Therefore, we suggest that the degree of similarity in spring phenological traits across closely related species depends on the degree of similarity in the environmental conditions where these close relatives are distributed. Under global warming, plant species living in rapidly changing climatic conditions may be at risk of survival or spread to other new areas for survival (Kijowska-Oberc et al., 2020). Plant adaptation to new climatic conditions might lead to changes in the phylogenetic pattern of phenological traits across species.

To date, the effects of phylogeny and adaptation to native climate on phenological traits have been scarcely investigated. Further studies on the combined effect of phylogeny and adaptation to native climate on other phenological traits under different environmental conditions are

necessary for better understanding the variations in phenological traits among species.

Although accurate and long-term phenological records have been used in this study, the species richness of the plants was insufficient, which prevents a more detailed analysis of the phylogenetic structure at different phylogenetic scales, such as for a particular family or genus. Therefore, more phenological data for more species are necessary for future studies.

## 5. Conclusion

Investigating the effects of phylogeny and adaptation to native climate on spring phenological traits helps enhance our understanding of the mechanisms underlying phenology variations across species. The results show that the spring phenological traits of temperate plant species in Northeast China were phylogenetically conserved, and the effect of phylogeny on flowering traits was stronger than that on leaf-out traits. In addition to phylogeny, spring phenological traits were also constrained by the adaptation to native climate and it had a greater influence on spring phenological traits than phylogeny. Thus, the degree of similarity in spring phenological traits across closely related species depends on the degree of similarity in environmental conditions where these closely related species are distributed. The results would improve the prediction of ecological and evolutionary processes under climate change. The associations among phenology, phylogeny and climate cues need to be thoroughly investigated in other climatic conditions and different plant communities in future studies.

### CRedit authorship contribution statement

**Xinyue Gao:** Writing – original draft, Methodology, Software. **Junhu Dai:** Conceptualization, Supervision, Writing – review & editing. **Khurram Shahzad:** Data curation, Software. **Huanjiong Wang:** Software. **Zexing Tao:** Validation. **Juha Mikael Alatalo:** Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.109381>.

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