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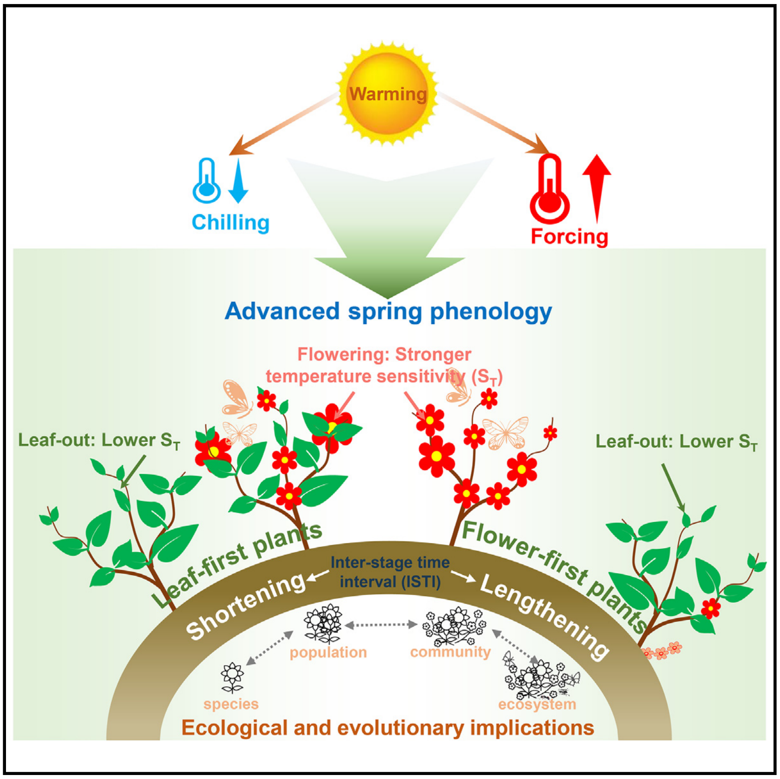
Current Biology

Climatic drivers and ecological implications of variation in the time interval between leaf-out and flowering

Graphical abstract



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In brief

Guo et al. show that stable leaf-flower time intervals prevail for most plants despite increasing temperatures, while certain plants feature significant changes in the length of the intervals. These variations are well explained by the difference in temperature sensitivity between the two events, which may have far-reaching ecological implications.

Highlights

- The study presents an extensive synthesis of warming effects on flower-leaf intervals
- Different temperature sensitivities of leaf-out and flowering modify their intervals
- Changes in the leaf-flower time interval have important ecological implications

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Climatic drivers and ecological implications of variation in the time interval between leaf-out and flowering

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SUMMARY

Leaf-out and flowering in any given species have evolved to occur in a predetermined sequence, with the inter-stage time interval optimized to maximize plant fitness. Although warming-induced advances of both leaf-out and flowering are well documented, it remains unclear whether shifts in these phenological phases differ in magnitudes and whether changes have occurred in the length of the inter-stage intervals. Here, we present an extensive synthesis of warming effects on flower-leaf time intervals, using long-term (1963–2014) and *in situ* data consisting of 11,858 leaf-out and flowering records for 183 species across China. We found that the timing of both spring phenological events was generally advanced, indicating a dominant impact of forcing conditions compared with chilling. Stable time intervals between leaf-out and flowering prevailed for most of the time series despite increasing temperatures; however, some of the investigated cases featured significant changes in the time intervals. The latter could be explained by differences in the temperature sensitivity (S_T) between leaf and flower phenology. Greater S_T for flowering than for leaf-out caused flowering times to advance faster than leaf emergence. This shortened the inter-stage intervals in leaf-first species and lengthened them in flower-first species. Variation in the time intervals between leaf-out and flowering events may have far-reaching ecological and evolutionary consequences, with implications for species fitness, intra/inter-species interactions, and ecosystem structure, function, and stability.

INTRODUCTION

Warming-induced shifts in the timing of spring phenology have frequently been reported in recent decades.[1–6] Leaf-out and flowering are two important phenological events that indicate the onset of vegetative and reproductive development, respectively. Variation in the dates of leaf-out and flowering considerably influences plant fitness,[7,8] intra- or inter-species interactions,[9–11] and ecosystem functions.[1,12,13] Most previous studies have focused on shifts in the timing of individual phenological events.[4,6,14] Comparisons of temporal trends in leaf-out and flowering (i.e., whether both spring events respond similarly or differently to climate change) and the resulting variation in the length of the inter-stage time interval (ISTI), as well as their ecological implications, have received less attention.[15–18] A better understanding of the relative changes in leaf-out and

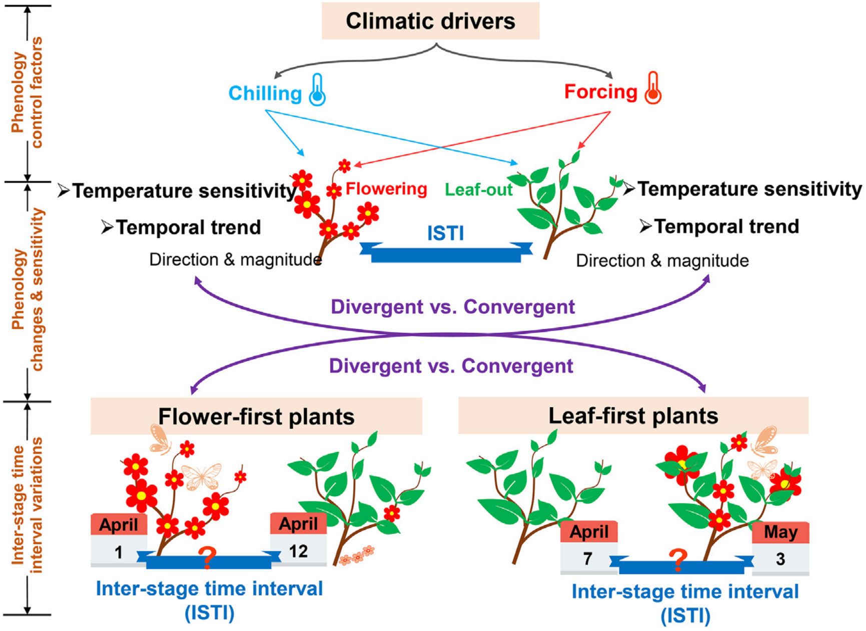

Figure 1. Conceptual visualization of how differences in key climatic drivers and sensitivity to climatic cues between leaf-out and flowering lead to divergent or convergent temporal trends for both spring phenology events and to changes in the time interval between both events

The specific dates listed in this figure (e.g., April 1, May 3) indicate the median values of the timing of each phenological event for all flower-first and leaf-first plants.

flowering dates, their dominant drivers, and potential ripple effects within an ecosystem would aid in planning for and mitigating the impacts of climate change.

Despite abundant observations that leaf-out and flowering have generally occurred earlier,[3,4,12,19–21] both events have also shown variable responses to climate change.[22–26] Some studies have suggested that the mechanisms of climate sensing for leaf-out and flowering are similar and interdependent,[27] arguing that subsequent events should be correlated with and predicted by earlier events.[15,28] By contrast, other studies that have examined consecutive phenological events over time have found that there was little temporal coherence in the responses of multiple phases,[22] concluding that phenological events may differ in their responses to climate change in terms of direction and magnitude.[18,23] There have also been reports of weak or even non-existent correlations between the timing of the two events.[29,30] All these findings complicate predictions of climate change impacts on the ISTI, pointing to an urgent need to elucidate how leaf-out is related to flowering and to determine the drivers of variation in the time interval between these two critical spring events.

Temperature is commonly believed to be a key driver of spring phenology.[9,31–34] Specifically, cold winter temperatures (chilling) and warm spring temperatures (forcing) interact with each other in determining the timing of leaf-out and flowering[4] (Figure 1). Temperature increases in winter and early spring generally have different impacts on spring phenology.[35,36] Although higher temperatures in winter may reduce effective chilling accumulation and delay leaf-out and flowering,[37–39] warmer spring conditions may accelerate the fulfillment of phenological heat requirements, resulting in earlier onset of spring events.[32,40] Recently, increased scientific interest has focused on the relative importance of chilling and forcing on the timing of spring events.[38–44] Most studies have identified forcing as the dominant factor governing spring phenology.[40,41] However, the observed and predicted declines in chilling[32,38,39] have increasingly been suggested to be important in determining spring timing.[39,42–44] Quantifying the variation in both chilling and forcing conditions and their relative importance is therefore necessary to elucidate the critical climatic drivers of spring phenology (Figure 1).

Differences in critical climatic drivers and sensitivity to these cues between flower and leaf phenology determine the relative changes in temporal trends of both spring events and the resulting changes in the length of the ISTI (Figure 1). Temperature sensitivity (S_T) of phenology, defined as a shift in the date of a phenological event per degree of temperature variation, is commonly used to reflect the ability of plants to track climate change.[45,46] Recently, the sensitivity of flowering and leaf-out to climatic cues has been evaluated for three flower-first species in the Harvard Forest.[16] Results of this study suggested a differential sensitivity of flowering and leaf-out phenology to forcing temperatures, implying that the greater sensitivity of leaf-out than of flowering was likely to shorten the flower-leaf time interval.[16] Given that flower-first is an evolved adaptation for wind pollination,[29] a shortened ISTI may interfere with efficient pollen transfer and diminish reproductive fitness.[16,29] Such concerns have been somewhat alleviated by observations of extended flower-leaf intervals of two European flower-first species.[17] The greater S_T of flowering compared with leaf-out indicated a faster advance of flowering and thus a longer ISTI,[17] forming a stark contrast to the results of the Harvard Forest report.[16] Similarly, the opposite trend has also been found for ISTI variation in certain leaf-first species.[16,17] The small sample size (only 10 and 4 species involved, respectively) and the completely contradictory conclusions[16,17] mentioned above suggest that further studies with more species are urgently needed to clarify leaf-out and flowering responses, variation in the ISTI, and the mechanisms that underlie these dynamics.

Using long-term (1963–2014) and *in situ* leaf-out and flowering observations (total of 11,858 records) for 183 species spanning six climatic zones in China (Figure 2), as well as daily weather data, we aimed to (1) quantify the temporal trends in dates of leaf-out and flowering and the time intervals between them, (2) clarify the temporal trends of different climatic drivers and evaluate their relative importance for both spring events, and (3) elucidate the mechanisms underlying ISTI variation and their ecological and evolutionary implications. We regarded the record of each species at each phenological observation site as a study case (total of 539 cases). Based on the flower-leaf

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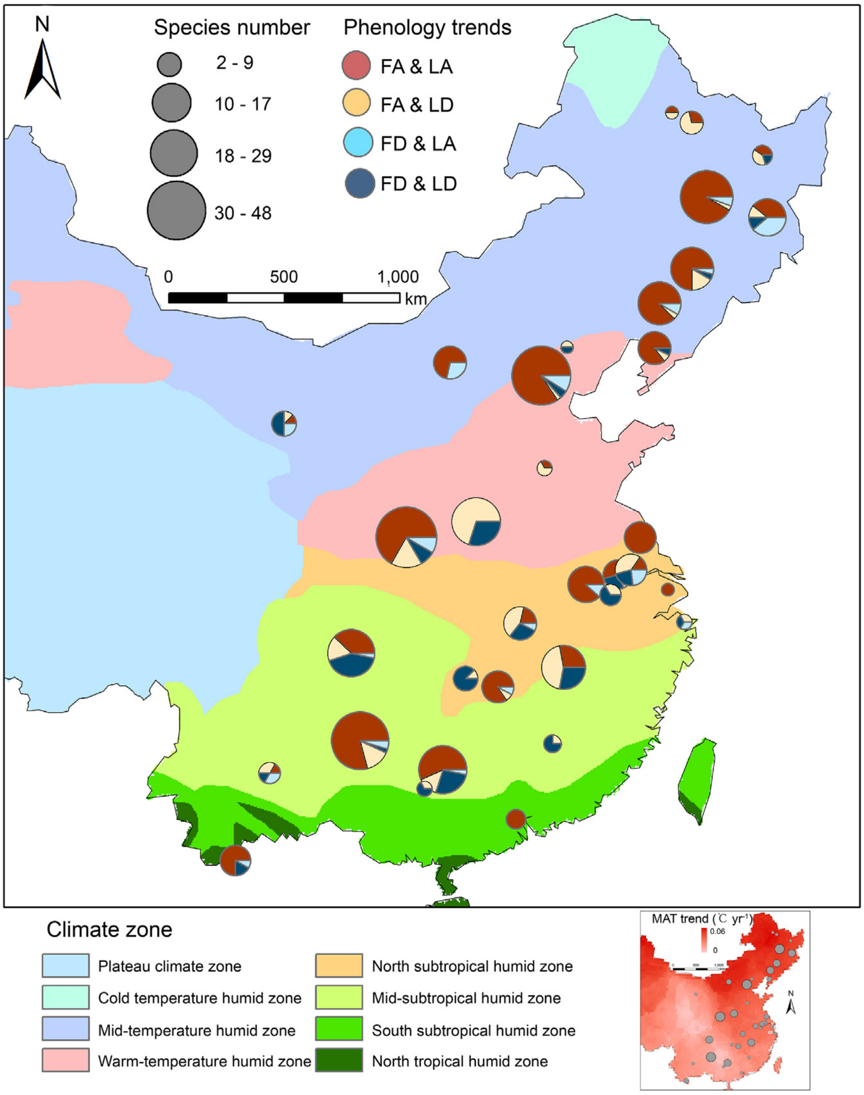

Figure 2. Location of 35 phenological observation sites across central and eastern China

Following the criteria that any given species in an observation site should have paired leaf-out and flowering records longer than 15 years, a total of 35 sites spanning six climatic zones in China were identified. More information can be found in the method details section. FA, FD, LA, and LD denote advancing flowering, delayed flowering, advancing leaf-out, and delayed leaf-out, respectively. The fraction of the total occurrences of different phenological events at each site is shown in the sector diagrams. MAT refers to mean annual temperature.

See also Table S1.

phenological sequence, all of the cases were assigned to either the leaf-first group (414 cases) or the flower-first (125 cases) group. In addition, to check whether the general pattern holds in different climatic regimes, we performed separate subgroup analyses for different climatic zones (i.e., mid- and warm-temperate zone; north-, mid-, and south-subtropical zone; and north tropical zone; Figure 2).

Our analyses indicated that advances in both leaf-out and flowering events were primarily driven by increased spring heat accumulation rather than by reduced winter chill. Stable time intervals between leaf-out and flowering prevailed over most study cases, yet certain cases also featured significant changes in the ISTI that could be attributed to differences in the S_T between leaf and flower phenology. Greater S_T for flowering than for leaf-out caused flowering dates to advance faster than leaf emergence. This shortened the ISTI in leaf-first species; however, it lengthened it in flower-first species. Such changes may influence resource allocation between vegetative and reproductive tissues, with important ecological consequences for species fitness and ecosystem stability.

RESULTS

Advanced leaf-out and flowering dominate spring phenology responses

Similar frequency distribution patterns of temporal trends were found for leaf-out and flowering (Figure 3A). More than 66% of the 539 study cases showed advancing leaf-out (LA), and 76% showed earlier flowering dates. Among them, over one-third of the advancing time series featured statistically significant trends (Figure 3A). The rate of advancement of flowering was slightly greater than that of leaf-out, averaging −5.6 and −5.0 days per decade, respectively (Figure 3B). By contrast, significantly delayed leaf-out (LD) or delayed flowering (FD) events were observed in fewer than 40 cases. Advancement of spring events prevailed in both the leaf-first and the flower-first plants, with the former showing greater change rates (Figure S1). Subgroup analyses of climatic zones further supported the dominance of spring phenology advancement (Figures S2A and S2B).

Stable time interval between leaf-out and flowering prevails over most cases

For 65% of the time series of leaf-first species and 80% of series of flower-first species, there were no statistically significant temporal trends in the ISTI (Figure 4A). Similar results were obtained in subgroup analyses based on climatic zones (Figures S2C and S2D). To elucidate the climate-driven mechanism behind the phenology responses, we first assessed the relative importance of variation in chilling and forcing temperatures. Results indicated that forcing temperatures dominated the timing of spring events (Figures S3A and S3B). We then compared the S_T of these two events to forcing temperatures (Figures S3C and S3D). These comparisons provided some hints about the reasons for the overall stability of the ISTI. Cases with similar S_T between the two spring events accounted for the largest proportion of cases,

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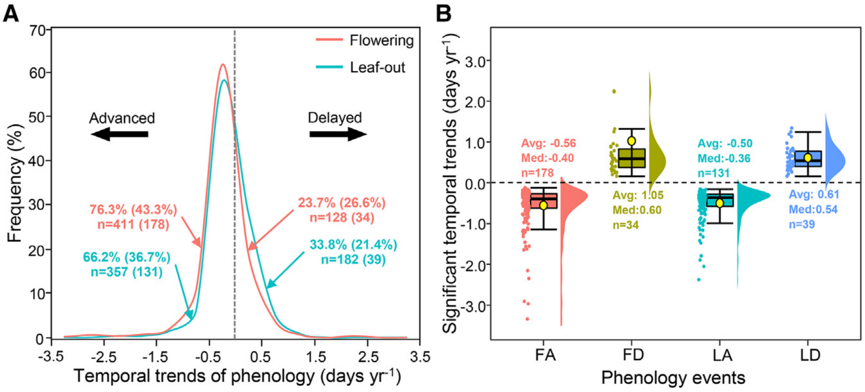

Figure 3. Temporal trends of flowering and leaf-out phenology

(A) Frequency distribution of temporal trends during 1963–2014 for all 539 cases.

(B) Distribution patterns of significant temporal trends of different phenological events. Ordinary linear regression was used for temporal trend analysis, with trends tested for statistical significance using the Mann-Kendall test. The number of samples (n) used for the statistics is shown separately for each analysis. The numbers in parentheses indicate the number of study cases with significant temporal trends or the proportion of their respective study cases. FA, FD, LA, and LD denote advancing flowering, delayed flowering, advancing leaf-out, and delayed leaf-out, respectively. Avg and Med are abbreviations for average and median, respectively. Moreover, a hinge model has also been used to estimate temporal trends of both phenological events, however, the results are not presented here. See the quantification and statistical analysis section for more details.

See also Figures S1 and S2, Table S2, and Data S1.

both in the overall and in any subgroup analysis (Figures 4B, 4C, and S4). Linear mixed-effects models (LMMs) with study case as a random factor showed relatively stable ISTIs whenever the difference in S_T between the two events was small (the middle panels of Figures 4B and 4C). However, in some cases, significant differences in S_T between leaf-out and flowering phenology led to a shortened or lengthened ISTI (Figure 4).

ISTI response to differences in S_T between leaf-out and flowering

As noted above, most time series were characterized by a stable time interval between leaf-out and flowering despite increasing temperatures, yet some cases featured significant changes in the ISTI (Figure 4A). Among these, a shortened ISTI (with a ratio of 60.3%) was detected among leaf-first species and a lengthened ISTI (65.4%) among flower-first species (Figure 4A). This pattern can be explained by differences in the S_T between the two spring events. In the leaf-first group, the larger fraction of cases with higher flowering S_T, i.e., absolute value (abs) (FS_T) > abs (LS_T) (25.9%), compared with the cases with abs (FS_T) < abs (LS_T) (21.8%), explained the overall shortening of the ISTI, with a significant decline rate of −2.3 days per decade (the upper right panel of Figure 4B). The flower-first group also featured a higher percentage of species with higher flowering S_T (31.6%) compared with the reverse (19.4%). Here, however, this pattern resulted in a lengthening ISTI at a rate of 1.4 days per decade (the upper right panel of Figure 4C). These findings were supported by most subgroup analyses except those for the mid-temperate zone (Figure S4). In summary, when considering all 539 study cases, flowering events appeared to feature a greater S_T and a stronger advancement response to forcing temperatures than leaf-out events, leading to an overall contrasting ISTI trend between the leaf-first and the flower-first groups (Figure 4A).

Physiological mechanisms behind the contrasting ISTI trends

To determine a physiological explanation for the opposite ISTI trends of the two groups, we calculated the chilling and forcing accumulations for leaf-out and flowering for each species at each site, using the dynamic model and the growing degree hour (GDH) model, respectively. Then, temporal trends and the relative importance of the impact of the two key drivers (chilling and forcing accumulation) on the timing of leaf-out and flowering were evaluated. Decreased chilling and increased forcing accumulations were detected for both spring events (Figures 5A and 5D). In the leaf-first group, flowering and leaf-out shared similar trends in chilling changes, but the rate of increases in forcing accumulation for flowering significantly exceeded the rate of increases in forcing accumulation for leaf-out (t_{532} = 2.96, p = 0.003; the upper right panel of Figure 5A). This result indicated a faster fulfillment of the heat requirement and thus a greater advancement of flowering than of leaf-out. This advance was further accelerated by the greater impact of forcing conditions on spring phenology, especially on the date of flowering (Figures 5B and 5C). Thus, a shortening of the ISTI should be expected in leaf-first plants. In the flower-first group, both the chilling and forcing accumulation trends showed no significant difference between leaf-out and flowering (Figure 5D). However, the advancement effect of forcing increases on the timing of spring phenology and the greater impact of forcing on flowering than on leaf-out (Figures 5E and 5F) jointly explained the greater advance of flowering compared with leaf-out and, hence, the longer ISTI in flower-first species. All of the above findings were supported by subgroup analyses based on climatic zones (Figure S5; Table S4).

DISCUSSION

Advancement is the dominant spring phenology trend

One of the most striking patterns of phenological change over the past decades has been the earlier onset of spring events. An analysis of more than 125,000 spring phenological series of 542 plant species from 21 European countries recorded between 1971 and 2000 suggested that 78% of the phenological records were advanced (30% significantly) by an average of −2.5 days per decade.[20] Larger advance rates for the onset of spring events have been reported in Spain and Guernsey Island.[12,19] Specifically, more than half a century of phenological

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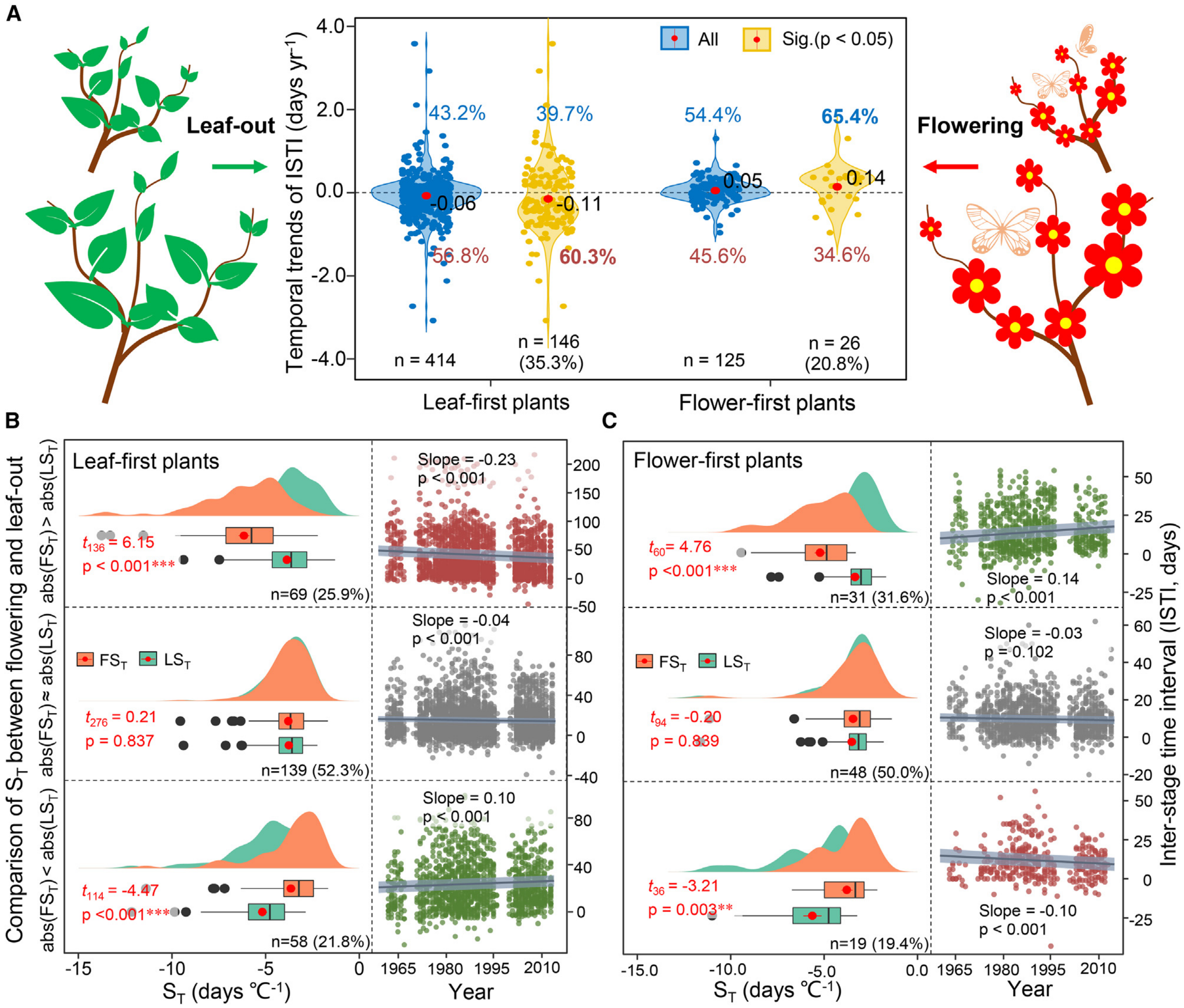

Figure 4. Temporal trends of the inter-stage time interval (ISTI) and comparisons of the forcing temperature sensitivity (S_T) of leaf-out and flowering

(A) Distributions of all temporal trends of the ISTI during 1963–2014 in the leaf-first and flower-first groups (shown in blue), with statistically significant trends also shown separately in yellow. Ordinary linear regression was used for temporal trend analysis, with trends tested for statistical significance using the Mann-Kendall test. Moreover, a hinge model has also been used to estimate temporal trends of the ISTI, however, the results are not presented here. See the quantification and statistical analysis section for more details.

(B and C) Differences of the S_T between leaf-out and flowering and the respective impacts on the ISTI in the leaf-first group (B) and the flower-first group (C). The number of samples (n) used for the statistics is shown separately for each analysis. The numbers in parentheses indicate the respective proportions. FS_T and LS_T represent the temperature sensitivity of flowering and leaf-out, respectively. "abs" refers to absolute value. Student's t test (two-tailed, unpaired) was used for the comparison between FS_T and LS_T, and the statistical information such as t values and degrees of freedom are provided directly in the figures. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. See Table S3 for the statistical information provided when using the linear mixed-effects models (LMMs). Here, only the slope of temporal trends of the ISTI and the respective p value are given in the figures for the LMM analysis.

See also Figures S2–S4, Tables S2 and S3, and Data S1.

records of 29 species across Spain indicated that leaf-out and flowering were markedly advancing (by −4.8 and −5.9 days per decade, respectively), especially since the mid-1970s.[12] On Guernsey, a dataset of flowering observations for 232 plant species from 1985 to 2011 also revealed a significant advance, with a rate of −5.2 days per decade.[19] In North America, spring phenological advancement is still the major trend, although it seems to be slower.[1] For instance, *in situ* leaf-out records from 43 phenological stations of the USA National Phenology Network showed an advancement trend of −0.9 days per decade for the period of 1982–2011.[1] In China, a meta-analysis of 1,263 phenological series for 112 species during 1960–2011 indicated that 90.8% of spring events showed an earlier trend, with the average rate of advancement ranging between −2.2 and −5.7 days per decade for various plant species.[3]

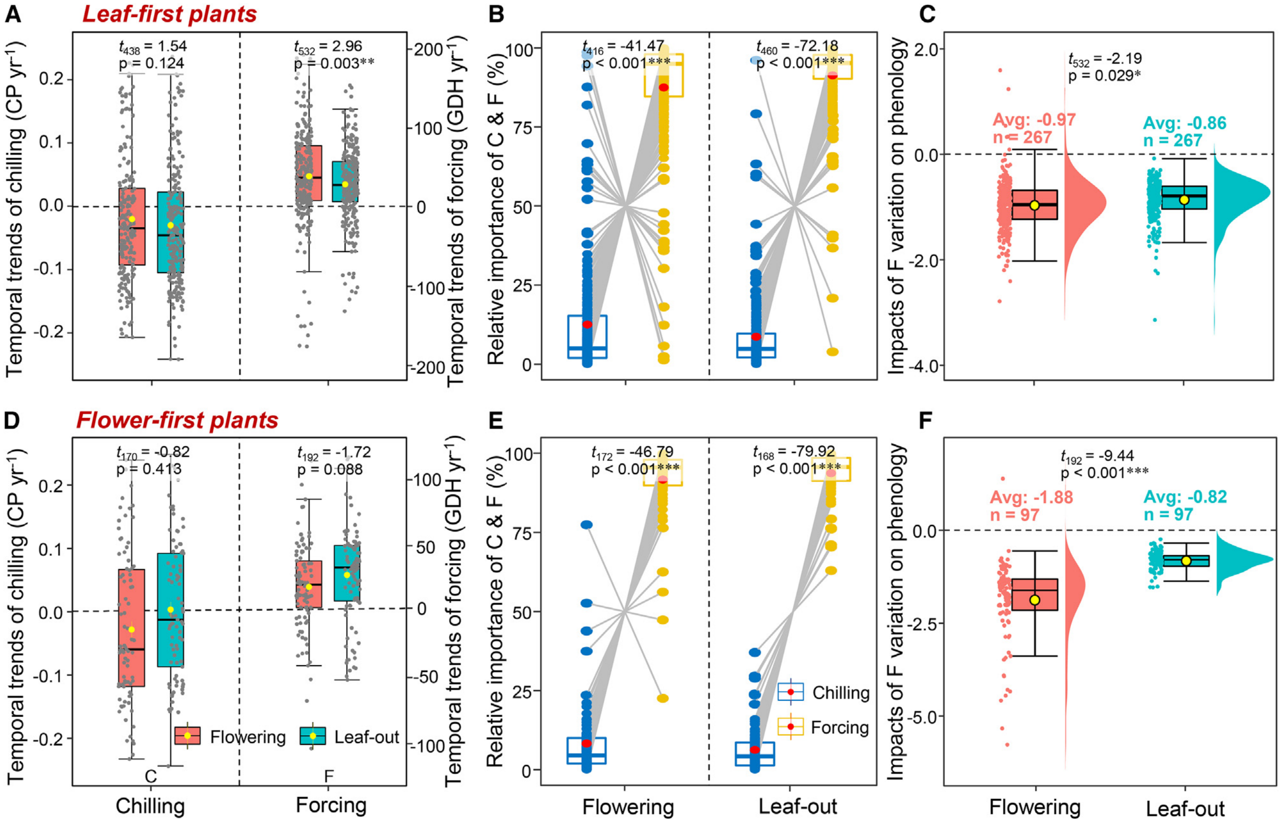

Figure 5. Temporal trends of two key drivers (chilling and forcing accumulation) and the relative impact on both spring phenological events, as well as comparison of the effects of forcing conditions on leaf-out and flowering phenology

(A and D) Temporal trends of chilling and forcing accumulation for flowering and leaf-out in leaf-first plants (A) and flower-first plants (D).

(B and E) Relative importance of chilling and forcing accumulations on the dates of flowering and leaf-out in leaf-first plants (B) and flower-first plants (E).

(C and F) Impacts of forcing variation on the timing of both spring events in leaf-first plants (C) and flower-first plants (F). C and F indicate chilling and forcing accumulation, respectively. CP refers to chill portions, a unit for chilling accumulation, while GDH refers to growing degree hours, a unit for heat accumulation. Avg refers to average. The number of samples (n) used for the statistics is shown separately for each analysis. Student's t test (two-tailed, unpaired) was used for all analyses here, and the statistical information such as t values and degrees of freedom are provided directly in the figures. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. See also Figure S5, Table S4, and Data S1.

Our study explored long-term (1963–2014) *in situ* observations, consisting of 11,858 spring phenological series for 183 species across six climatic zones in China. Our results are consistent with previous reports, suggesting an advancement trend in more than 66% of the investigated cases. Leaf-out and flowering advanced at an average rate of −5.0 and −5.6 days per decade, respectively, similar to the results presented above for Spain,[12] Guernsey,[19] and the meta-analysis on data from China.[3] It should be noted that significantly delayed spring events have also been detected here and in previous reports,[4,20,38] but only for very few time series. This implies that LA and advancing flowering (FA) still dominate spring phenological responses to temperature increases.

Different sensitivities to forcing conditions between flower and leaf phenology drive ISTI changes

While most of the previous studies mentioned above have focused on shifts in the timing of individual phenological events, we have collected pairwise observations of leaf-out and flowering for 183 species across six climate regions in China, which allow for an accurate assessment of variation in the ISTI.

Our results indicated that a stable time interval between leaf-out and flowering prevailed over most of the studied cases, in contrast to previously reported results.[16,17] For example, *in situ* phenological records for four temperate tree species during 1950–2013 in Europe showed that, regardless of whether leaf-out or flowering occurred first, a prolonged time interval occurred between the two events.[17] By contrast, a laboratory experiment testing leaf-out and flowering responses in 10 tree species from the Harvard Forest found that climate warming likely decreased the ISTI, especially in flower-first species.[16] We attributed the overall stable ISTI to observations with similar S_T between leaf-out and flowering phenology accounting for the largest proportion of cases. A stable time interval between the onset of vegetative and reproductive growth may serve as a genetically conserved strategy across species to maintain fitness. Interestingly, however, shorter and longer ISTIs also occurred for some cases in our study (Figure 4A). Our results therefore indicate a need to update our understanding of the drivers of variation in the ISTI.

Differences in key climatic drivers and sensitivity to these cues between flowering and leaf-out phenology may explain variation

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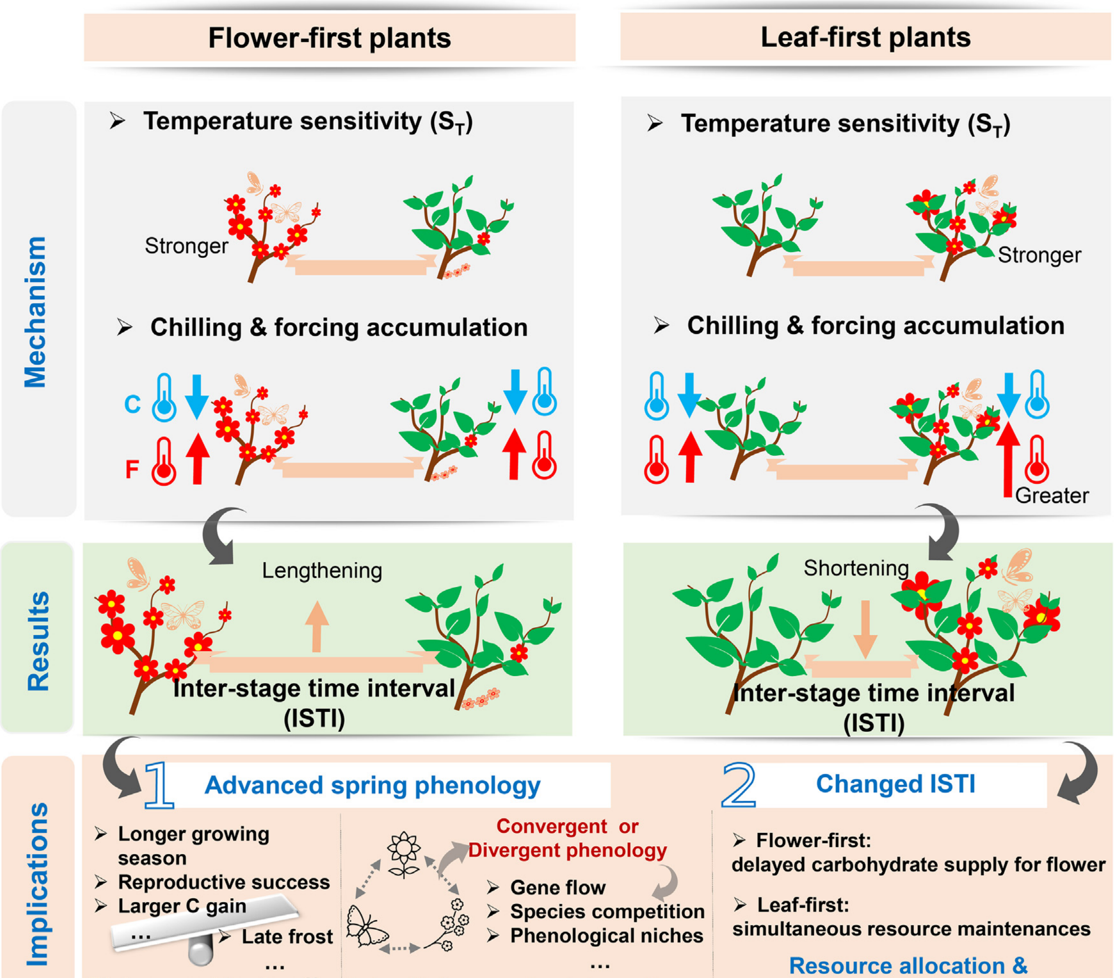

Figure 6. Visualization of the climate-driven mechanism behind the contrasting trends in the ISTI for leaf-first and flower-first species, and potential ecological and evolutionary implications

Upward and downward arrows indicate increasing and decreasing trends for the respective variables. C and F represent chilling and forcing, respectively.

in the ISTI (Figures 4 and 5; summarized in Figure 6). Our results suggested forcing conditions as the main determinant of spring phenology dates, compared with the weaker effects of chilling. Differences in the sensitivity to forcing temperature and the rate of forcing accumulation between leaf-out and flowering phenology emerged as prominent drivers of ISTI variation (Figure 6). Our results are consistent with previous field observations that identified forcing conditions as the dominant factor governing spring phenology.[40,41] In addition to site-specific records, results from model comparisons also support the dominant role of forcing, with one-phase models such as the ForcTT model and the UniForc model[47,48] performing well without considering chilling.[49] Contrasting evidence, however, has been presented for certain species that originated in temperate climates but were cultivated in subtropical or tropical regions or at the margins of their distribution ranges. Such species have shown a slowdown in the advance or a delay of spring phenology.[32,43] This means that the delaying effect of insufficient chilling on spring events may counterbalance or exceed the advancement effect of forcing.[39,43,44] Further support for such a shift in phenology response has been found in laboratory experiments,[16,38] but often in response to chilling treatments that were outside the range of conditions that have historically been observed in the field. Thus, despite a possibly increasing role of chilling conditions, the dominant climatic driver in current climatic regimes still appears to be an increase in forcing temperatures. The overall higher sensitivity to forcing temperature and faster forcing accumulation for flowering than leaf-out in leaf-first species (Figure 5A) allows for a greater flowering advance rate, thus shortening the ISTI (Figure 6). By contrast, in flower-first species, the greater impact of forcing accumulation on flowering dates than on leaf-out (Figure 5F), together with the evidence of higher S_T for flowering events, explains the lengthening ISTI in flower-first plants (Figure 6).

We note that in addition to chilling and forcing, other environmental cues may also have a role in determining the timing of spring events and thus the variation in the ISTI. Sensitivity to photoperiod has been reported to prevent early leaf-out and flowering in some species.[50,51] Recently, the relative importance of chilling, forcing, and photoperiod, and interactions between these factors, have been evaluated for the timing of spring events.[52–54] In our study, we refrained from integrating photoperiod effects into our conceptual model (Figure 1) because we did not detect differences in temporal trends in the photoperiod associated with leaf-out and flowering (Figures S6A–S6C), and all photoperiod trends were relatively weak compared with obvious variation in chilling and forcing conditions (Figure 5). This suggests that photoperiod cannot explain the variation in the flower-leaf time intervals and further confirms that temperatures, in particular, forcing conditions, are likely to play the primary role.

Ecological and evolutionary implications of changes in flower-leaf time intervals

Earlier leaf-out combined with an even stronger flowering advance resulted in longer and shorter ISTIs for certain flower-first and leaf-first species, respectively. These shifts may have crucial implications for ecosystem structure, function, and stability (Figure 6). Advanced leaf-out may not only increase the risk of late frost damage to leaf tissue[55–57] but also result in greater fitness in terms of inter-species competition[10] and greater carbon gains due to a longer vegetative growing season.[58] This risk-reward trade-off has the potential to create powerful selection pressure in future climates.[44] Shifts in the timing of flowering phenology may have various consequences, including on seed dispersal,[4,59] on plant-pollinator relationships,[60,61] and on species interactions, due to temporal mismatches.[62–64] The importance of changes in flowering phenology is further highlighted by species-specific shifts in the direction and magnitude of flowering events because both convergent and divergent trends in flowering phenology in response to climate warming have been observed.[24,44,65–68] Increased synchrony in flowering dates may increase gene flow among populations,[26] promoting adaptive evolution,[69] but it may also depress certain species due to intense competition for limited resources such as water, nutrients, light,[70,71] and pollinators.[12] By contrast, a reduction in co-flowering among different species can alleviate competition by dispersing primary resources into different temporal windows.[24] Nonetheless, this may also result in new flowering niches or gaps[72] that facilitate invasion by alien species.[73] Changes in leaf-out, particularly in flowering timing, may disrupt ecological relationships among plants, pollinators, and herbivores, reshaping communities and ecosystems in a warmer future (Figure 6).

The schedule of vegetative and reproductive development is critical for species fitness.[17,18,74] Understanding the variation in the ISTI can reveal important insights into how species allocate resources to different life stages, and it may help to clarify and predict the vulnerability of species to climate change (Figure 6). Flower-first species commonly depend on and utilize stored resources accumulated in the previous growing season to produce and maintain pollen and flowers in the absence of photosynthetic activity in the leaves.[7,30] The lengthened flower-leaf interval indicates a delayed carbohydrate supply for flower development and a greater reliance on the previous year's climate. With regard to leaf-first species, the shortened ISTI may also threaten their fitness, as newly synthesized carbohydrates will be used to maintain both reproductive and vegetative tissue.[75] Thus, a more integrated view of spring phenology incorporating leaf-out and flowering (rather than treating them as discrete events) should be prioritized to improve our understanding and prediction of the future dynamics of different species and their ripple effects within ecosystems as climate warming continues to intensify.

Remaining uncertainties and research needs

Although advance dominated the changes in spring leaf-out and flowering, the direction and magnitude of changes in the ISTI varied considerably across species. For example, even for statistically significant trends in the ISTI, where an overall shortened ISTI (with a ratio of 60.3%) was found in leaf-first species and a lengthened ISTI (65.4%) in flower-first species (Figure 4A), there was still a considerable fraction of cases that did not follow the trends described above. This variation may be attributable to different S_T between leaf and flower phenology among species (Figures 4B and 4C). Studies have shown that the S_T of the phenology of a species may be influenced by environmental and organismal traits.[76] Thus, we attempted to disentangle this uncertainty in ISTI variation by classifying time series according to climatic zones, plant growth types (tree, shrub, and liana), and other phylogenetic factors (e.g., angiosperms vs. gymnosperms and deciduous vs. evergreen) (Figure S6D). However, none of these factors convincingly explained the complex nature of variation in the ISTI among species, with lengthened and shortened cases caused by differences between leaf-out and flowering S_T occurring in almost all of the classes. Therefore, well-designed experiments should be carried out to unravel these uncertainties.[77,78]

Another uncertainty concerns the quantitative relationship between phenological and ISTI variation and plant fitness. As an important functional trait, leaf-out and flowering phenology should strongly influence plant performance, with selection favoring events timed to increase fitness.[79] It has been suggested that species that adjust their phenology in response to climate change are better at tracking optimal environmental conditions.[80] For example, long-term monitoring of alpine meadows on the Tibetan Plateau has indicated that warming-induced advances of flowering increased plant productivity and benefited population fitness.[81] However, some studies have argued that phenological shifts may come with a high cost in plant fitness, as earlier flowering has resulted in a reduction in flower numbers and reproductive fitness for a European alpine herb.[82] Such species-specific variations in plant fitness could affect not only plant-plant but also plant-pollinator interactions and even food web dynamics.[83,84] All these conflicting findings point to an urgent need to elucidate how shifts in phenological dates are related to plant fitness. As for quantifying the correlation between shifts in the ISTI and plant fitness, to the best of our knowledge, no such study has been performed. We therefore propose to integrate controlled laboratory experiments with long-term monitoring, built on a physiological and molecular basis, to identify the mechanisms responsible for shifts in the ISTI and the underlying effects on plant fitness and ecosystem functions.

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STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.06.064.

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AUTHOR CONTRIBUTIONS

L.G., J.M.A., Q.Y., C.P., and J.D. conceived the project. L.G., X.L., J.X., and E.L. performed the analysis. C.W., H.Y., and J.C. downloaded the phenological and meteorological data. L.G. wrote the manuscript. All authors reviewed and edited the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Leaf-out and flowering phenology data	National Earth System Science Data Center, National Science & Technology Infrastructure of China	http://www.geodata.cn
Climate data	National Meteorological Information Center of China	http://data.cma.cn
Software and algorithms		
R Project for Statistical Computing	R Project[85]	http://www.r-project.org
‘chillR’ R Package	Luedeling and Fernandez[86]	https://cran.r-project.org/web/packages/chillR/index.html
‘relaimpo’ R Package	Groemping and Matthias[87]	https://cran.r-project.org/web/packages/relaimpo/index.html
‘gghalves’ R Package	Tiedemann[88]	https://cran.r-project.org/web/packages/gghalves/index.html
‘vioplot’ R Package	Adler et al.[89]	https://cran.r-project.org/web/packages/vioplot/index.html
‘lme4’ R Package	Bates et al.[90]	https://cran.r-project.org/web/packages/lme4/index.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Liang Guo (guoliang2014@nwafu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper analyzes existing, publicly available data. Accesses to the phenological and climatic datasets used in this study are listed in the key resources table. All original code is available in Data S1. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Since 1963, staff at each phenological observation site across China have conducted systematic and standard surveys of plant leaf-out and flowering events. All phenological records were collected by the Chinese Phenological Observation Network (CPON) and released by the National Earth System Science Data Center, National Science & Technology Infrastructure of China (http://www.geodata.cn). The most recent publicly accessible records were released in 2014. Half a century (1963–2014) of paired in-situ leaf-out and flowering observations (total of 11,858 records) for 183 species spanning six climatic zones in China were used in this study (Figure 2). More detailed information on the phenological dataset is listed below.

METHOD DETAILS

Phenological dataset

Aiming to accurately describe the climate change responses of leaf-out and flowering dates, as well as their time intervals, we selected species for which both events were recorded for more than 15 years. We regarded the records for each species at each phenological observation site (with a total of 35 sites) as a study case. A total of 539 cases met the above criteria, comprising records that were widely spread over six climate zones in China (Figure 2) and included 11,858 individual leaf-out and flowering events.

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According to the observation criteria of CPON, the first leaf-out date (FLD) and the first flowering date (FFD) used here are defined as the date when a particular individual plant formed the first full leaf and the first full flower, respectively.[71]

Climate dataset

For each phenological observation site, daily mean, maximum and minimum temperatures during 1963–2014 were derived from the nearest weather station (Table S1), with a maximum distance of less than 50 km.[10,91] All temperature data were downloaded from the National Meteorological Information Center of China (http://data.cma.cn).

Identification of relevant periods influencing leaf-out and flowering phenology

The key period for leaf-out or flowering is several months preceding the phenological event.[20,67] Even though some studies, especially those focused on model comparisons and global analyses based on remote sensing data, have commonly selected a constant interval for the key period,[23,35,65] relevant periods for FLD and FFD vary among species and locations.[14] We used two methods to delineate the relevant periods for FLD and FFD of each species at each site. The first method (Step analysis) defines the relevant preseason as the period (with 1-day step) before the mean FLD or FFD for which the correlation coefficient between phenology date and mean temperature is the highest.[14,67,71,91] The second method (Partial Least Squares regression analysis, PLS) was also used to relate daily mean temperatures to annual FLD and FFD, respectively. Dependent variables were the phenology dates, while independent variables were daily mean temperatures for 365 days before the typical timing of the respective phenological event. The root mean square error (RMSE) of the PLS regression analysis was calculated to assess the model's accuracy. We used the R[85] package chillR,[86] which outputs two important values: the variable importance in the projection (VIP) and standardized model coefficients. The VIP scores denote the importance of all independent variables for explaining variation in the dependent variables, with 0.8 commonly regarded as the threshold for interpretation as important.[92,93] The standardized model coefficients indicate the direction and the magnitude of the impact of each variable.[94] Periods with VIP values greater than 0.8 and high absolute values of the model coefficients thus represent the relevant periods that affect the timing of plant phenology.

We compared the relevant periods identified by using the two approaches described above. While the first method only provides a single period, the PLS analysis outlines two distinct periods for each phenological event (Figures S6E and S6F), corresponding to the chilling and forcing periods, both of which are considered important for initiating spring phenological events.[86,94] Our results indicated that the forcing period delineated in the PLS analysis was similar to the phase confirmed by using the first method, with the overlap rate averaging 52% and reaching 100% in some cases (Figure S6G). Additionally, reduced major axis (RMA) regression analysis indicated that almost the same S_T values were acquired by both methods regardless of which phenological event was considered (Figures S6H and S6I). Therefore, the PLS analysis appeared to outperform the Step analysis and was used to identify relevant periods in this study.

Quantifying temperature sensitivity of leaf-out and flowering

Temperature sensitivity (S_T) reflects the change in phenology date per unit increase in mean temperature during the relevant period.[14] Most studies have identified forcing temperatures, rather than chilling temperatures, as the major driver of spring phenology.[35,40,41] This impression was confirmed for most of our cases (Figures S3A and S3B). Thus, similar to previous studies,[10,14] S_T was calculated as the slope coefficient of the linear regression between phenology dates and mean forcing temperatures for each species at each site.

Chilling and forcing accumulation and their relative importance for leaf-out and flowering

To elucidate and compare the effects of chilling and forcing conditions on the timing of spring phenology, we calculated species-specific chilling and forcing accumulations for FLD and FFD at each site, respectively. We chose the Dynamic model[95] to quantify chilling accumulation, because it is widely regarded as the most robust chilling model due to its rigorous theoretical structure and ability to explain phenological variation.[32] As forcing model, we used the Growing Degree Hour (GDH) model,[96] which can estimate forcing accumulation at hourly intervals. The equations for the two models are not provided here for brevity, but can be found in previous reports.[32,35] Because the above two models require hourly data, idealized daily temperature curves with an hourly resolution were constructed from daily temperature extremes.[32,97,98] Moreover, the relative importance of chilling and forcing accumulation for each spring phenological event was evaluated using the relaimpo package.[87]

QUANTIFICATION AND STATISTICAL ANALYSIS

All 539 cases were analyzed separately using all of the above methods to derive the main variables (temporal trends of FLD, FFD, and the time interval between the two events; S_T of FLD and FFD; annual chilling and forcing accumulations, as well as their temporal trends) for each species at each site. Both ordinary linear regression and hinge models were used for temporal trend analysis. Recent reports have suggested that hinge models can more accurately estimate change across time series and prevent bias toward weaker effects in longer time series.[99] We compared the temporal trends of all the above variables using both approaches. Results indicated that there was no statistically significant difference in temporal trends estimated using ordinary linear regression and hinge models, although the latter reported slightly larger effect sizes (Table S2). While most previous studies have used ordinary linear regression to calculate temporal trends of spring phenological events,[3,12,19,20] we therefore only present the results obtained using ordinary linear

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regression to facilitate comparison between our results and those of previous studies. The frequency distributions of temporal trends of both events and their time intervals were visualized using the gghalves[88] and vioplot[89] packages.

The distributions of S_T for leaf-out and flowering were displayed using the gghalves package.[88] The distributions were divided into three parts depending on the relative size of S_T of both phenological events. It is worth noting that only cases with S_T for both events at significant levels are shown in the distribution diagram. To further clarify the impacts of the deviation of S_T of both events on their time interval, a linear mixed-effects model (LMM) with study case as a random factor was used to compute the temporal trends of the ISTI. LMMs were constructed using the lme4 package.[90] All statistical analyses and plots were performed using the R programming language.[85]