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ORIGINAL PAPER

Climate warming–driven phenological shifts are species-specific in woody plants: evidence from twig experiment in Kashmir Himalaya

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Abstract
Experimental evidences in support of climate warming–driven phenological shifts are still scarce, particularly from the developing world. Here, we investigated the effect of experimental warming on flowering phenology of selected woody plants in Kashmir Himalaya. We selected the twigs of four congeneric pairs of temperate woody species (*Prunus*, *Populus*, *Ulmus*, *Viburnum*)—typical spring-flowering plants in the region. Using randomised block design, we monitored these winter dormant twigs in controlled growth chambers to study the effect of different temperature regimes (9, 17, 20 and 23 °C) and species identity on the patterns of phenological shifts. We observed a significant phenological shift in all the species showing preponement in the first flower out and senescence phases ranging from 0.56 to 3.0 and 0.77 to 4.04 days per degree increase in temperature, respectively. The duration of flowering phase in all the species showed a corresponding decrease along the gradient of increasing temperature, which was more driven by preponement of the flower senescence than the start of flowering. The patterns of phenological shifts were highly species-specific, and the magnitude of these shifts significantly varied in all the four pairs of congeneric species despite their phylogenetic similarity. Our study provides experimental support to the previous long-term observation and herbarium-based studies showing that the patterns of phenological shifts in response to global climate warming are likely to vary between species, even those belonging to same evolutionary stock. Our findings highlight that a one-size-fits-all strategy to manage the likely impacts of climate warming–induced phenological shifts will seldom succeed, and should instead be designed for the specific phenological responses of species and regions.

Keywords Climate change · Warming temperature · Phenology · Twig experiment · Woody plants · Himalaya

Introduction

In recent times, shifting phenology is considered to be one of the pronounced responses of vegetation to global climate change (Zohner et al. 2017; Liu and Zhang 2020). The ongoing climate warming is causing either a significant advancement in spring flowering phenology in temperate ecosystems or a significant delay in summer flowering phenology of many plant species, thereby altering their key ecological processes and biotic interactions (Chuine and Beaubien 2001; Elzinga et al. 2007; Cleland et al. 2012; Fridley 2012; Iler et al. 2021; Rosbakh et al. 2021). A better understanding of the relationship between contemporary climate warming and the associated phenological shifts of species is needed in order to unravel the mechanisms underlying these shifts and to better predict future changes in phenological events (Hassan et al. 2021). A large number of studies, particularly from the developed world countries, have reported these phenological shifts and investigated their relationship with warming climate (Wang et al. 2018; Love and Mazer 2021; Rosbakh et al. 2021). Most of these phenological studies have employed the observational approach to explore the effect of climate change on plant phenology (Lindh et al. 2018). Other studies adopted the herbarium-based reconstruction (Munson and Long 2017) or dated-photographic data approaches (Miller-Rushing et al. 2006; Sparks et al. 2006; Prevéy et al. 2020). The limitation of these approaches is that there are very few such long-term phenological observation datasets available, that too only in some countries

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from the developed world (Aono and Saito 2010). Although the use of herbarium and old photographic approaches is advantageous in providing the temporal depth, these datasets are beset with large temporal data gaps (Willis et al. 2017). In addition, these approaches limit our ability to distinguish the independent influence of climatic variables on phenology from other confounding factors like photoperiod, light intensity and relative humidity (Chuine et al. 2010; Ko¨rner and Basler 2010).

To overcome the limitations of observational approaches, an experimental approach by employing controlled conditions can help to tease apart the confounding effects of different climatic and biotic variables (Primack et al. 2015). Although a number of experimental phenology studies are available from the developed world (Ettinger et al. 2020), such studies are still insufficient from the developing world (Stuble et al. 2021). Very few studies reporting phenological shifts by climate warming using observational and historical data from the developing world have recently become available (Hart et al. 2014; Gaira et al. 2014; Ahmad et al. 2021; Hassan et al. 2021). As the developing world harbours a much higher proportion of the global biodiversity, conducting experimental phenology studies assumes a research priority.

Amongst the various experimental approaches employed in phenology research, winter dormant twigs of woody plants placed in the temperature-regulated growth chambers have been successfully used to study the influence of various temperature treatments on plant phenological events (Primack et al. 2015). The procedure is relatively simple and easily manageable, requires limited space and can be accomplished in growth chambers where factors like temperature, photoperiod and humidity can be easily controlled. Thus, this method has been employed to investigate the impact of climate warming on several different phenological responses (Primack et al. 2015; Jung et al. 2021). Several studies suggest twigs as effective model systems for depicting the response of whole plants (Polgar et al. 2014; Sønsteby and Heide 2014; Vitasse and Basler 2014), and they have been used in many recently published phenological studies (e.g. Basler and Körner 2012; Dantec et al. 2014; Menzel et al. 2020; Primack et al. 2015; Laube et al. 2014a, b).

Generally, the phenological response in a particular region depends upon the regional climate and is expected to be species-specific (Gillooly et al. 2001), as the sensitivity to seasonal warming differs amongst species (Fitter and Fitter 2002; Sherry et al. 2007; Zohner et al. 2017, 2016; Laube et al. 2014a, b). The closely related species show different phenological responses to climate warming, with certain species responding much faster than the others (Miller-Rushing and Inouye 2009). These differential phenological response patterns that exists amongst the related species can be used to make predictions about the response of plant communities to projected climate change scenarios. Therefore, specific case studies from the data-deficit regions are required to better predict the phenological response of congeners to future climate warming. Furthermore, the differential phenological responses shown by different species to climate warming could result in changed patterns of coexistence amongst species during reproduction (Sherry et al. 2007). As a result, the species interaction within communities could get disturbed, thereby changing the associated community composition (Waser and Real 1979; Chuine and Beaubien 2001; Post et al. 2001). Therefore, there is a need for research studies to be conducted at the regional scale taking into account the species-specific phenological responses (Fox and Jönsson 2019).

The Himalaya, a global biodiversity hotspot region, has experienced rapid climate warming during recent times (Romshoo et al. 2015; Murtaza and Romshoo 2016). Recently, a few observation-based studies on the plant phenology in different parts of the Himalaya have been conducted (Hart et al. 2014; Basnett et al. 2019; Gaira et al. 2014; Ahmad et al. 2021; Hassan et al. 2021). The Kashmir Himalaya, a region located in the Himalaya global biodiversity hotspot, is recently experiencing rapid climate changes (Hassan et al. 2021), and therefore experimental phenological study from this region can help in filling the global geographical knowledge gap in phenology research. It is with this backdrop that our study attempts to determine the effects of different temperature treatments and species identity on the patterns of phenological shifts in Kashmir Himalaya.

Until now, most of the experimental studies employing the twig method have studied the influence of single variable like effect of temperature on the phenological events of single plant species (Primack et al. 2015). Experimental studies investigating the simultaneous effect of temperature and species identity using the multi-twig species approach can allow to evaluate the independent effects of climate variables and also to analyse the variable phenological response of multiple species. In this study, using four pairs of congeneric woody plant species from Kashmir Himalaya, we investigated the effect of experimental warming and species identity on the three phenological variables: date of first flower out, date of senescence and duration of flowering period. More specifically, we aimed to address the following research questions: (1) Do the experimental warming treatments (different temperature conditions) determine the initiation, end and duration of flowering phenology in the selected woody species? (2) Is the magnitude of studied phenological variables (dates of first flower out, senescence and duration of flowering period) species-specific? (3) Do the congeneric pairs of species show similar or different phenological responses to different temperature treatments?

This Himalayan region has experienced pronounced changes in temperature due to climate warming over the last decades (Hassan et al. 2021) with expected impacts on plant phenology. The phenological shifts induced due to climate warming not only affect the structure of plant community but also affect the biotic interactions, plant breeding systems and reproductive success (Inouye 2008; Theobald et al. 2017; McDonough MacKenzie et al. 2018). Therefore, the findings from the present study are expected to help in better understanding the species' responses to climate warming in the region and predicting its impacts on key ecological processes.

Materials and methods

Study area

The present study was carried out between February and April 2021 at the Kashmir University Botanical Garden (KUBG), located in the main campus of the University of Kashmir situated in Srinagar, Kashmir Himalaya—a part of the Himalaya biodiversity hotspot (32°20′ to 34°54′ N latitudes and 73°55′ to 75°35′ E longitudes, altitude 1600 m asl) (Husain 2002; Dar and Khuroo 2020). A rich repository of plant species, either cultivated or growing in the wild, occur in the KUBG which are typical representatives of the flora of this Himalayan region. Generally, the climate in the region is of continental temperate type with four distinct seasons: winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). The average temperature ranges from 31 °C maximum to 15 °C minimum in summer and from 4 °C maximum to – 4 °C minimum in winters. In recent times, however, the region has experienced increasing trends of climate warming with an increase in annual temperature of about 0.65℃/year, and decrease in annual precipitation of – 1.24 mm/year (Hassan et al. 2021; Romshoo et al. 2015; Murtaza and Romshoo 2016; Dad et al. 2021; Zaz et al. 2019). In particular, over the last decade, the region is experiencing relatively warmer months of February and early onset of spring flowering (Hassan et al. 2021). In recent times, such early flowering in the horticultural crops (e.g. apple, pear, peach) has caused huge economic losses due to freakish snowfall events post-flowering (Rashid et al. 2020; Wani et al. 2021).

Selected plant species

For the present study, we selected four pairs of congeneric woody species: *Ulmus wallichiana*, *U. villosa*, *Prunus tomentosa*, *P. persica*, *Populus alba*, *P. deltoides*, *Viburnum cotinifolium* and *V. opulus* growing in KUBG (Fig. 1). These species were selected because they are typical representatives of first flowering plants during the start of spring season in Kashmir Himalaya after a long winter (Malik et al. 2020).

Experimental design

We adopted the twig experimental setup under different temperature regimes in growth chambers (Primack et al. 2015). Three plant growth chambers maintained at different temperature regimes of T2 (17℃), T3 (20℃) and T4 (23℃) were used (Fig. 1), with a photoperiod of 14.5 h and dark period of 9.5 h (Miller-Rushing and Primack 2008a) average of February to April months in the study region, with constant relative humidity of 65% (Ruiz et al. 2007; Campoy et al. 2012). The growth chambers were maintained at alternative day/night temperatures in order to provide the twigs natural-like conditions. The light intensity was kept around 55 mol m^{-2} s^{-1} (Ruiz et al. 2007; Campoy et al. 2012). These temperature regimes were selected as the range of average temperature in spring season during which these plant species flower lies between minimum of 17℃ to maximum of 23℃. On 12 February 2021, the twigs of the selected plant species with length ranging from 10 to 30 cm were cut obliquely with a sterilised hand pruner (Primack et al. 2015; Basler and Korner 2012), These were obtained from the single individual of particular plant species. Only the twigs bearing 2 to 5 intact reproductive buds were selected to be used for the experiment. The twigs were washed with sodium hypochlorite solution (200 ppm active chlorine) (Zohner and Renner 2015), followed again by a wash in distilled water. The washed twigs were placed in labelled 250-mL conical glass flasks with each flask containing single twig with 2–5 intact buds filled with 5% sucrose solution (Ruiz et al. 2007; Campoy et al. 2012; Sønsteby and Heide 2014). To avoid microbial infection, gentamicin sulphate (40 μg L^{-1}) was poured into the flasks (Zohner and Renner 2015). The flasks were then transferred to the three growth chambers. The twigs of the selected species, each with three replicates, with each replicate consisting of single twig of particular plant species, were placed in each growth chamber by following completely randomised block design. In addition to the growth chambers, another set of twigs of the selected species were also kept in the pot house of KUBG to act as control. The pot house represented the natural conditions with temperature T1, around 9 ℃ (average temperature of February, March and April months during which experiment was active) (Fig. 1). After every week, the sucrose solution in the flasks was changed and the twigs were recut and placed in the fresh sucrose solution. The growth chambers and the pot house were monitored on a daily basis to record the data on three phenological variables. For the twigs of the selected species, we recorded dates of first flower out (FFO) and senescence (DOS) in terms of the day of year (DOY) and duration of flowering (DOF) period

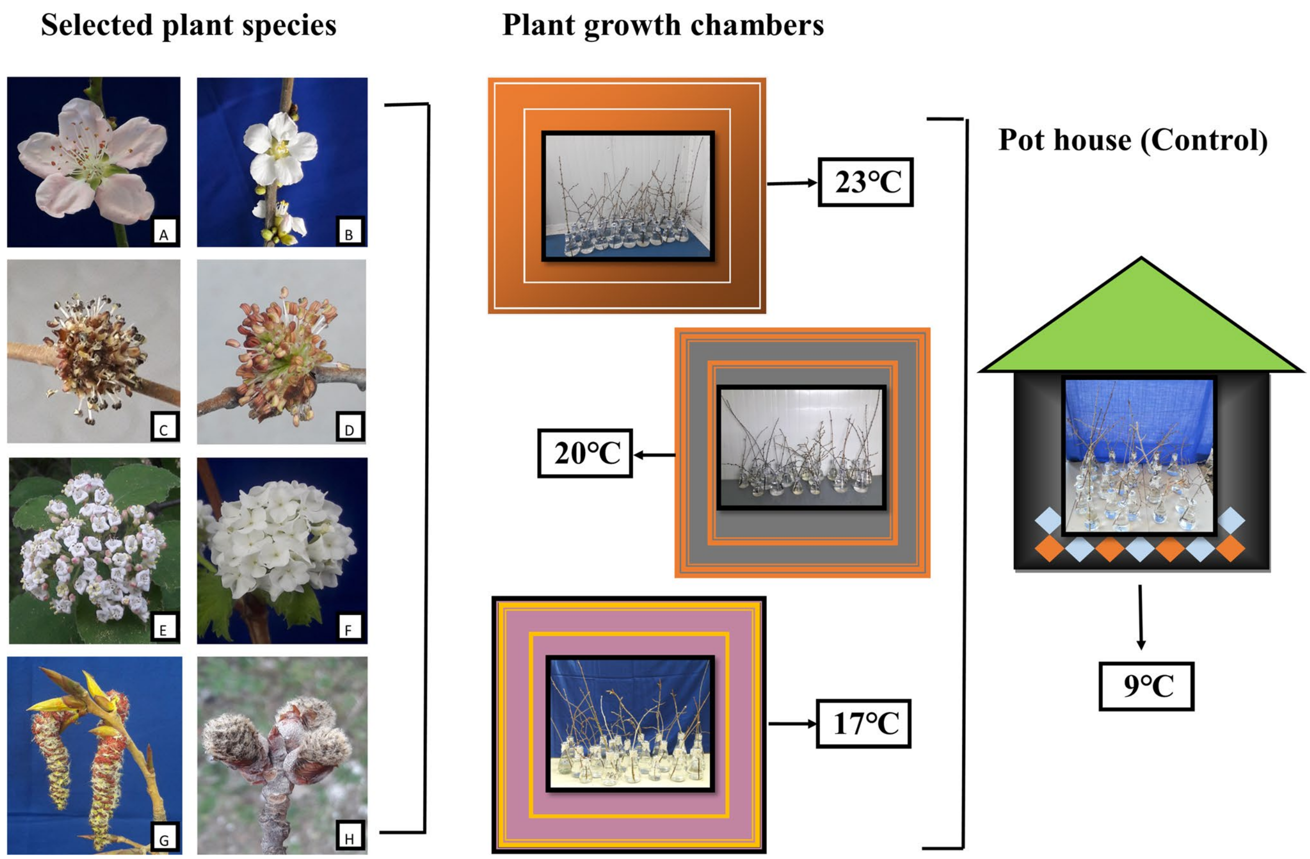

Fig. 1 A pictorial overview of the selected woody species in Kashmir Himalaya with the experimental setup showing twigs in the growth chambers maintained at different temperature regimes of 23℃, 20℃, 17℃ and 9℃ (control). **A** *Prunus persica*. **B** *Prunus tomentosa*. **C** *Ulmus villosa*. **D** *Ulmus wallichiana*. **E** *Viburnum cotinifolium*. **F** *Viburnum opulus*. **G** *Populus deltoides*. **H** *Populus alba*

based on number of days. The FFO was taken as the day of the year when the corolla of first flowers (around 10% in each twig) fully unfolds; the DOS was taken when the corolla of flowers (around 10% in each twig) dries out; and the DOF was recorded as the number of days from FFO to DOS. The data obtained on these three phenological variables was used to analyse the role of different temperature regimes and species identity on the patterns and magnitude of phenological shifts in the selected plant species.

Data analysis

All the analyses were carried out using R statistical software version 4.0.2 (R Core Team 2020). We used a simple linear regression model to investigate the studied phenological variables, comparing date of first flower out, date of senescence and duration of flowering period with temperature. We analysed the data in two different ways, comparing (i) the sensitivities (slope) of each species individually, and (ii) each genus separately. We also subjected the data on the phenological variables to Levene's test and the Shapiro–Wilk test to check the assumptions of homogeneity of variance and normality of distribution in the data respectively. Since the assumptions in the data were not met, we log-transformed the data of the phenological variables to meet the required assumptions. To test whether there is a significant difference in the phenological variables, we performed the two-way analysis of variance (ANOVA) test using temperature (T), species identity (SI) and their interactions as factors. In the case that the effect was significant ($P \leq 0.05$), we used Tukey's HSD (Honest Significant Differences) for performing multiple pairwise-comparison between the means of groups.

Results

The results of the two-way ANOVA showed that both the temperature and species identity had a significant effect on the studied phenological variables (Table 1), thereby showing that the species differ in their phenological response to different temperature treatments. The results of the linear regression revealed a significant negative effect of temperature on the date of first flower out, indicating that the flowering in the selected woody species

Table 1 Summarized results of the two-way ANOVA test. Shown are the associated significance levels along with mean (and standard deviation). ***$P \leq 0.001$

Variable	Temperature	Species identity	Temperature × species identity	9 (°C)	17 (°C)	20 (°C)	23 (°C)
FFO	***	***	***	79.8 ± 17.9	71.5 ± 15.6	63.2 ± 11.1	59 ± 10.2
DOS	***	***	***	96.1 ± 21.8	84.6 ± 17.9	72 ± 14	66.2 ± 12.5
DOF	***	***	***	16.4 ± 7.97	13.1 ± 7.47	8.75 ± 6.24	7.25 ± 5.14

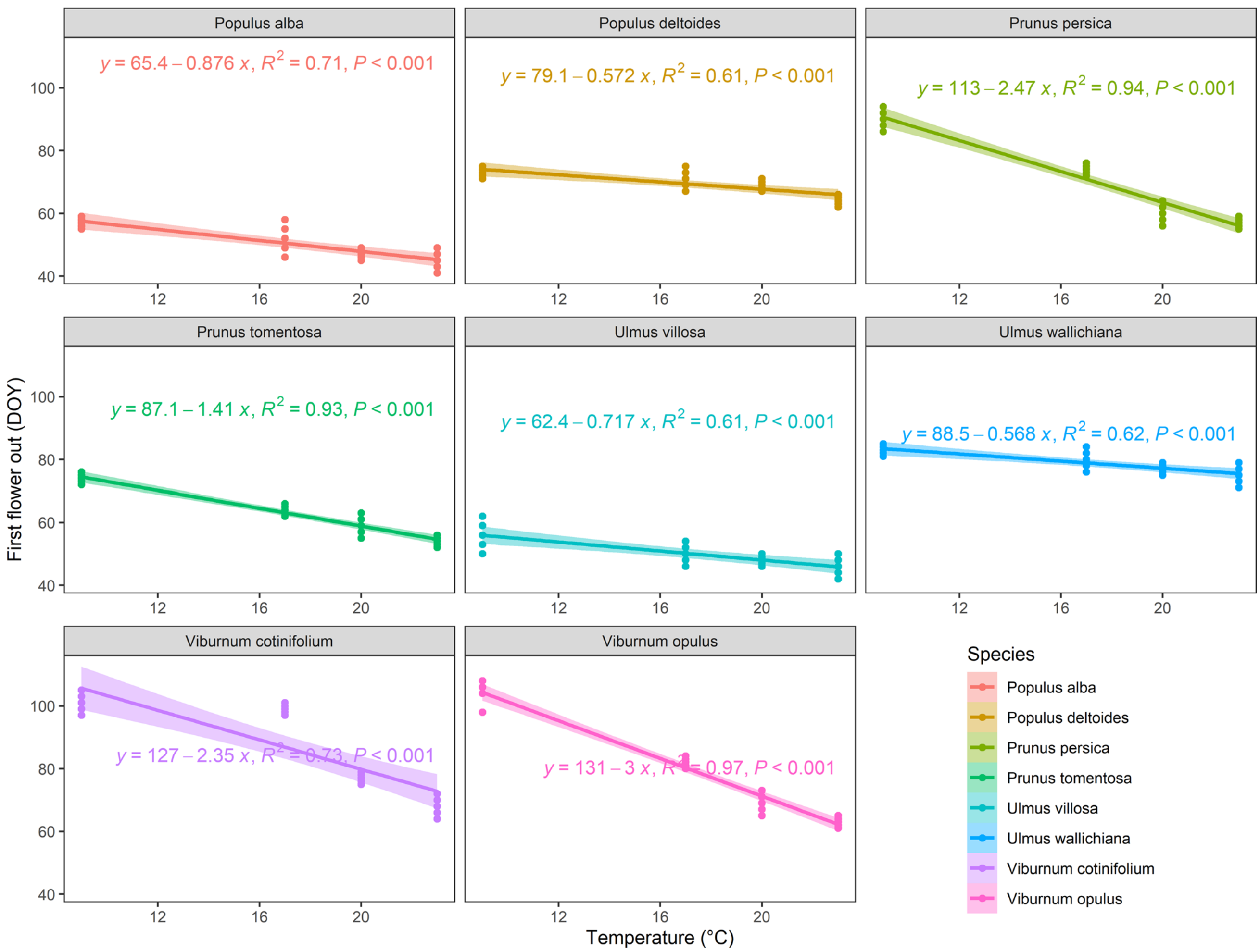

Fig. 2 Relationships between the temperature and first flower out (DOY) of the studied species. The solid lines represent the best fitting regression lines while the shaded areas represent the 95% confidence interval of the regression lines

starts much earlier under warmer temperature conditions (Fig. 2; Supplementary Table 1). This pattern was consistent across all the species (Fig. 2) and genera studied, though the magnitude of the effect varied greatly (Fig. 5; Supplementary Table 1). Likewise, a significant decrease in the date of senescence with varying magnitudes was observed with increasing temperature for all the species (Fig. 3; Supplementary Table 1) and genera studied (Fig. 6; Supplementary Table 1). Similarly, we found a significant negative effect of temperature on duration of flowering, indicating that the length of flowering period in the selected species decreases with increasing temperature (Fig. 4; Supplementary Table 1). This effect was seen within all individual species, where advancement in the dates of senescence was greater than the advancement in dates of first flower out, resulting in a shorter duration of flowering (Supplementary Table 1). Once again, the observed effect was consistent at the genus level as well,

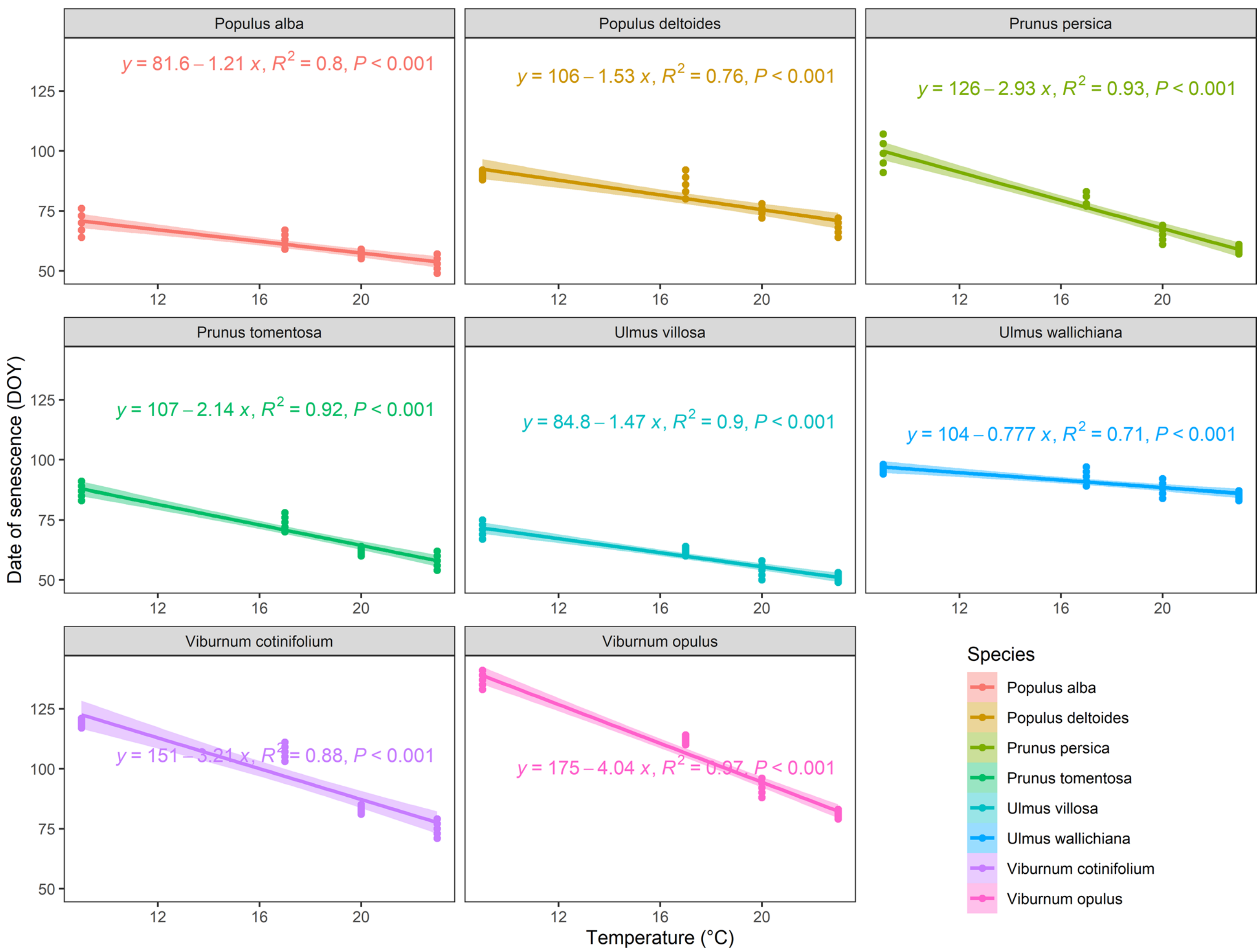

Fig. 3 Relationships between the temperature and date of senescence (DOY) of the studied species. The solid lines represent the best fitting regression lines while the shaded areas represent the 95% confidence interval of the regression lines

though the magnitude of the effect varied considerably (Fig. 7; Supplementary Table 1). For all the studied phenological variables, our results revealed that the species' sensitivities are more similar within congeneric pairs than the distantly related species (Fig. 2, 3 and 4).

The post hoc comparison revealed that the observed differences in the phenological variables were due to the significant differences between all the possible pairwise temperature comparisons; the one exception was for the T3 vs T4 comparison for duration of flowering phase; in this case, the observed difference in duration of flowering was non-significant (Supplementary Table 2). The post hoc test of species identity showed that observed difference in date of first flower out was significant for all the cases except for *Ulmus villosa* vs *Populus alba*, *Prunus persica* vs *Populus deltoides* and *Viburnum opulus* vs *Ulmus wallichiana* pairs (Supplementary Table 3). Similarly, the post hoc test of species identity showed that the observed difference in date of senescence was significant for all the cases except for *Ulmus villosa* vs *Populus alba* pair (Supplementary Table 4). In contrast, the post hoc test of species identity showed that observed difference in duration of flowering phase was significant for a few pairwise cases only (Supplementary Table 5).

Discussion

The present study, to the best of our knowledge, is the first attempt from the developing world to experimentally investigate the effect of warming temperature and species identity on the phenological events of woody species in Kashmir Himalaya.

Role of temperature in flowering phenology

The timing of various spring phenological events is governed by changes in environmental conditions particularly

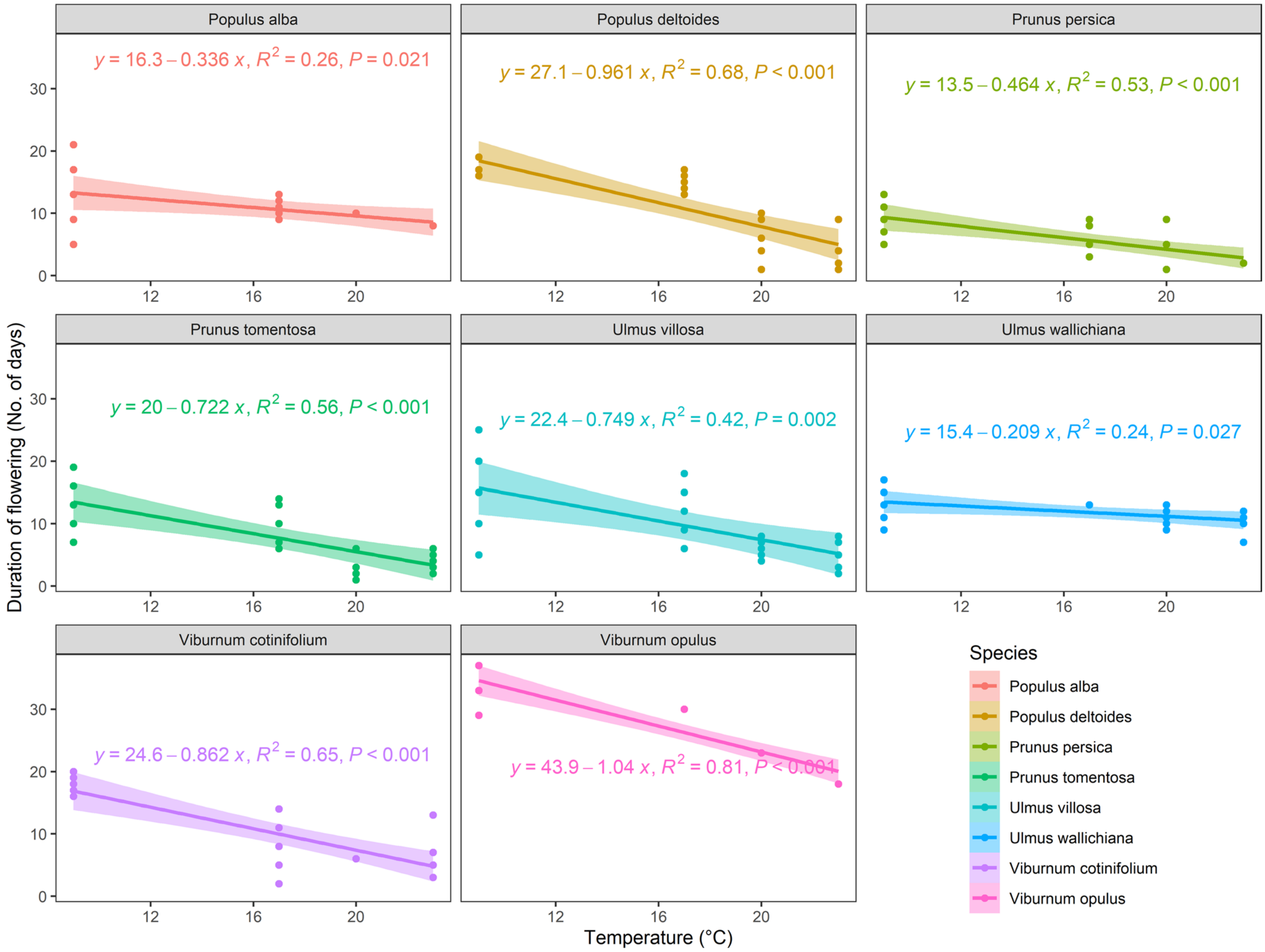

Fig. 4 Relationships between the temperature and duration of flowering (no. of days) of the studied species. The solid lines represent the best fitting regression line while the shaded areas represent the 95% confidence interval of the regression line

changes in temperature (Post and Stenseth 1999; Elmendorf et al. 2016; Luna 2016; Tao et al. 2017). Our results clearly show the decreasing effect of temperature on the date of first flower out, date of senescence and duration of flowering for all the selected woody species. We observed that the first flower out of all the species occurs earlier under warmer conditions. A likely reason for this effect is that growing degree days (GDD) accumulate faster with warmer temperatures, thereby allowing plants to flower earlier once the threshold has been met (Piao et al. 2015; Suonan et al. 2017). Our results showing advances in flowering phenology are in agreement with a large number of studies conducted in temperate ecosystems around the world (Neil and Wu 2006; Cleland et al. 2007; Stuble et al. 2021). Increase in temperature has reported to significantly advance first flowering dates in 28 woody species in North America (Flynn and Wolkovich 2018), *Salix × smithiana* (Caffarra and Donnelly 2011) and *Sternbergia vernalis* in Kashmir Himalaya (Hassan et al. 2021). Our results are further supported by several other studies (Sherry et al. 2007; Yu et al. 2010; Lee 2011; Prevéy et al. 2019), which have reported the advancement of phenological events under warming conditions. Warming not only advances first flower out dates, but also advances date of senescence by accelerating protein degradation and affecting cell division and cell expansion (Furguson et al. 1990; Scheurwater et al. 2000). Furthermore, higher temperature promotes sugar accumulation in tissues, which also contributes to early senescence (Haba et al. 2014).

Interestingly, in the present study, we found that the duration of flowering phase in all the species shows a corresponding decrease along the gradient of increasing temperature. This shortening of flowering phase under warmer temperature is largely because of early flower senescence due to increased temperature (Kehrberger and Holzschuh 2019). Under warmer conditions, the advancement in the dates of senescence was greater than the advancement in dates of first flower out for all the species, thereby resulting in shorter duration of flowering (Supplementary Table 1).

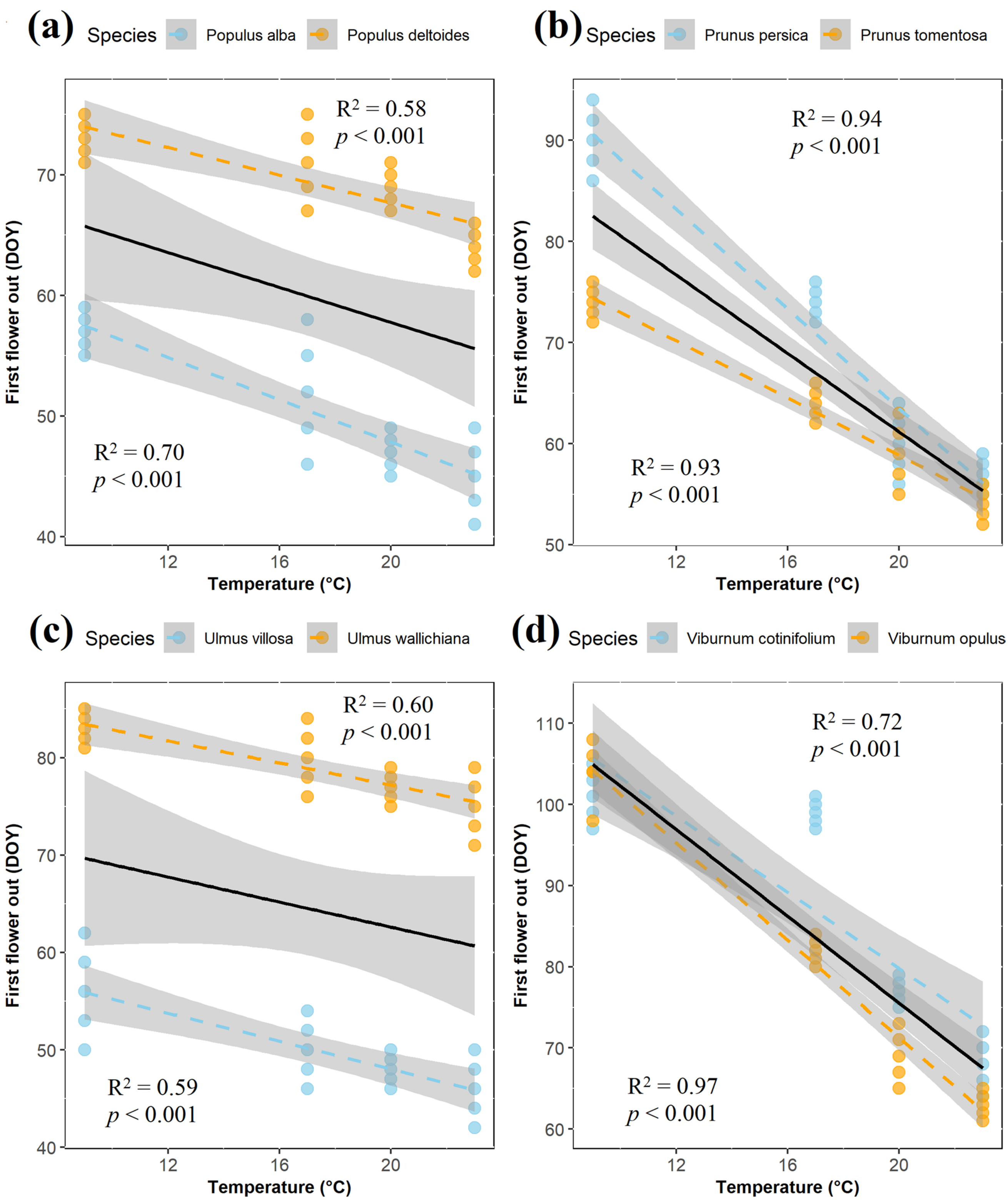

Fig. 5 Relationships between the temperature and date of first flower out (DOY) in four pairs of selected congeneric woody species. The black solid line represents the best fitting regression line at the genus level, the coloured dashed lines the best fitting regression lines for the two species in the particular genus and the grey shaded areas the 95% confidence interval of the regression lines

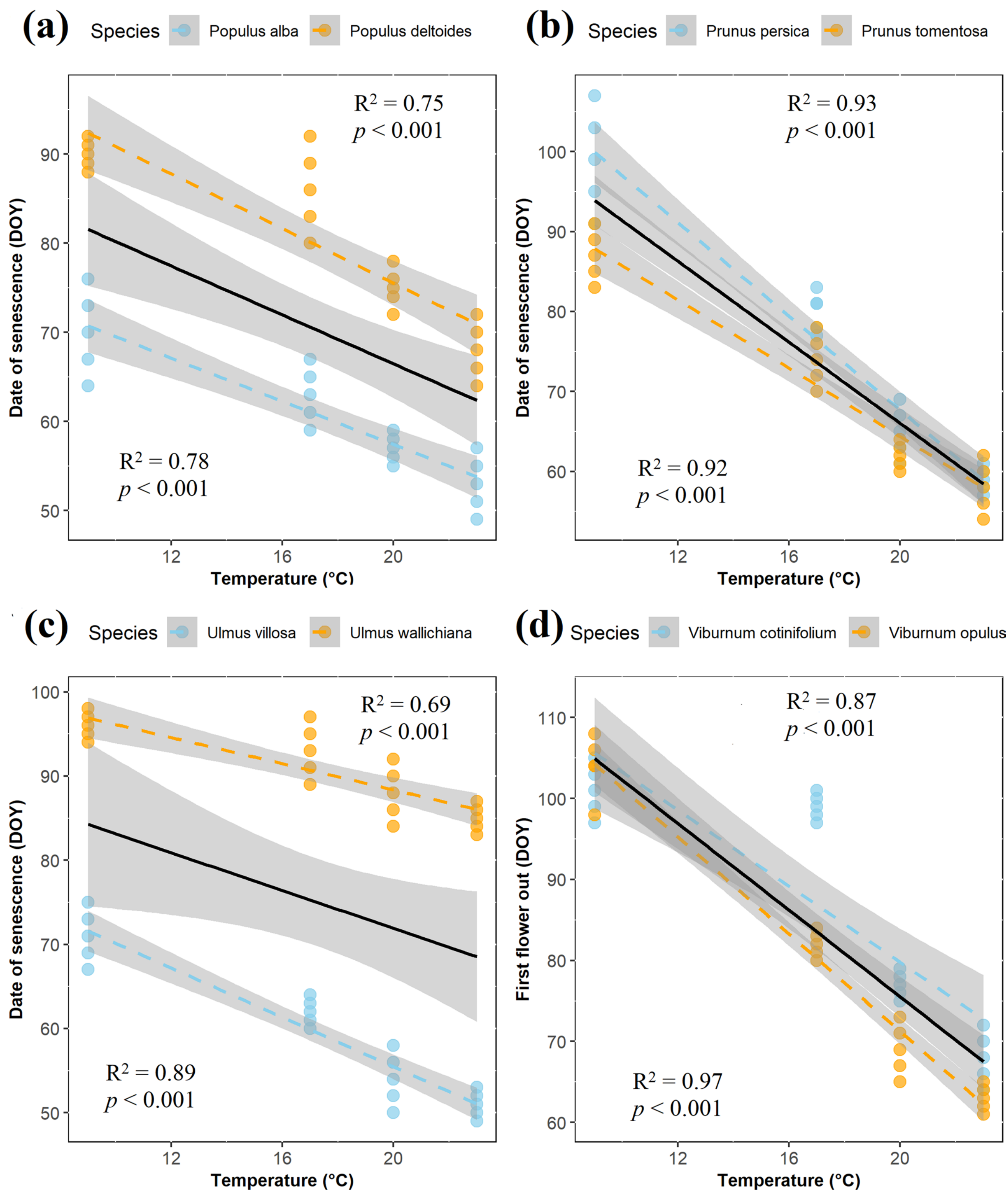

Fig. 6 Relationships between the temperature and date of senescence (DOY) in four pairs of selected congeneric woody species. The black solid line represents the best fitting regression line at the genus level, the coloured dashed lines the best fitting regression lines for the two species in the particular genus and the grey shaded areas the 95% confidence interval of the regression lines

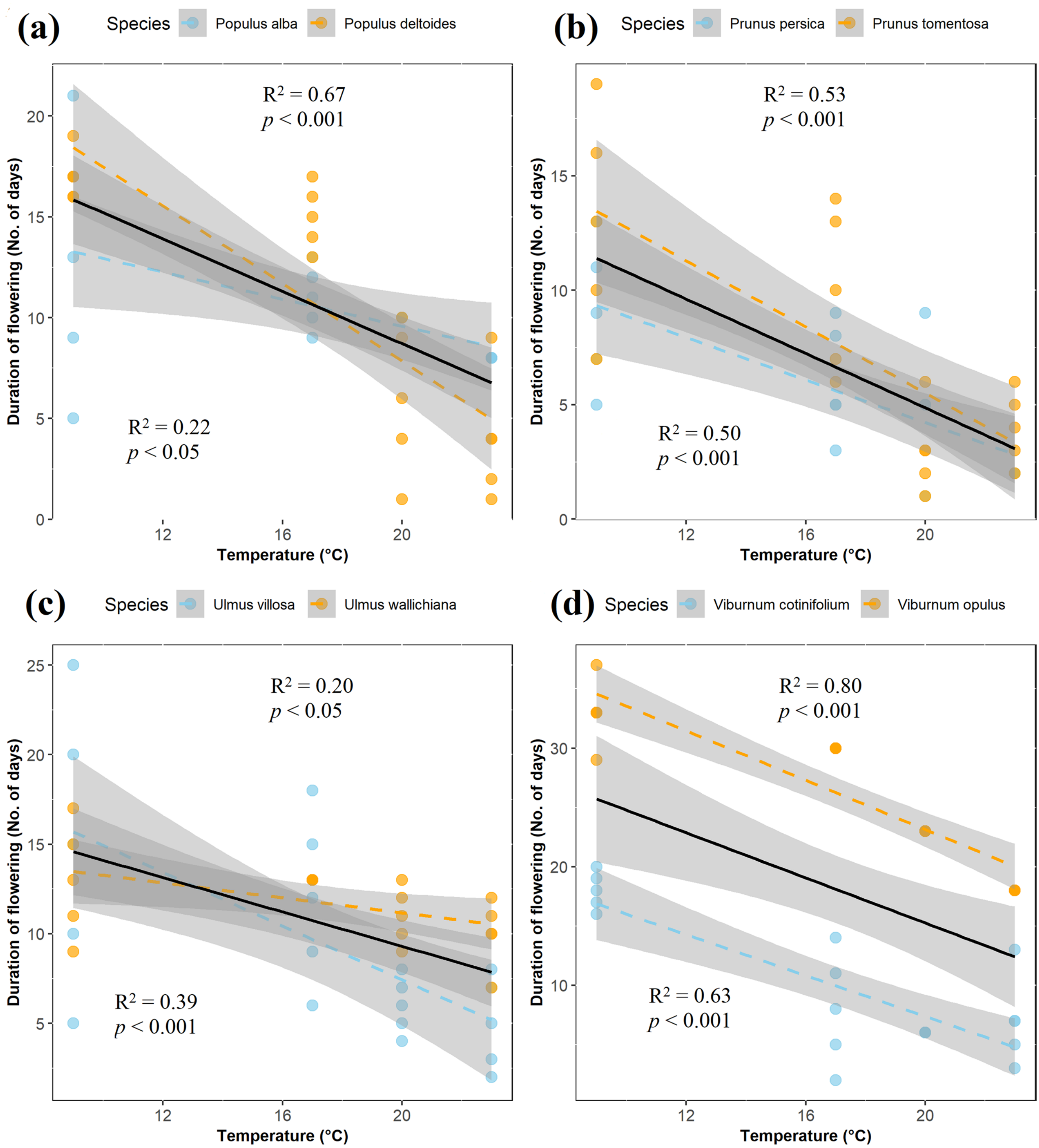

Fig. 7 Relationships between the temperature and duration of flowering (no. of days) in four pairs of selected congeneric woody species. The black solid line represents the best fitting regression line at the genus level, the coloured dashed lines the best fitting regression lines for the two species in the particular genus and the grey shaded areas the 95% confidence interval of the regression lines

These findings reveal that the flower senescence phenophase in the studied species is more sensitive to climate warming, which is in agreement with findings of some recent studies (Nagahama et al. 2018; Kehrberger and Holzschuh 2019). This climate warming–induced decrease in flower duration can potentially have a negative effect on plant reproductive success by decreasing the probability of pollinators visiting the plant species (Kehrberger and Holzschuh 2019;

CaraDonna and Waser 2020). Such phenological constraints on reproductive potential in plant communities can alter the structure and stability of ecological communities, which could have long-term consequences for provision of ecosystem goods and services (Liu et al. 2011).

Species identity, congeners and flowering phenology response

The phenological response to a particular temperature varies between different species and thus is species-specific in magnitude (Rice et al. 2021). This interspecific variation in phenological response to different temperature treatments is evident, as temperature is one of the prime determinants of the plant phenology (Hassan et al. 2021; Rice et al. 2021). In the present study, the flowering phenology response of all the woody species to a particular temperature regime showed shifts independent of one another, which can be ascribed to the species-specific adaptive response to a particular temperature cue. Therefore, a relatively warmer temperature to a plant species than the normal conditions brings about the specific phenological shifts, as supported by majority of studies on species' phenological shifts due to climate warming (Fitter and Fitter 2002; Lehikoinen et al. 2004; Sherry et al. 2007; Miller-Rushing and Primack 2008a, b). Previously, the experimental phenological studies have shown the response of a single species to the climate warming (Matsumoto et al. 2003; Doi 2008). Our study makes advance by investigating the variation in the phenological response amongst multiple sets of species to experimental warming, which is consistent with the findings of other studies (Rice et al. 2021; CaraDonna et al. 2014; Primack et al. 2009). The response of individual species to climate warming is crucial for understanding the influence of global warming on its interactions within population, communities and ultimately on ecosystems and the biodiversity they support (Primack et al. 2009). Increased temperature has the potential to cause major changes in biological communities by altering the biotic relationships amongst the species (Kharouba et al. 2018; Forrest and Miller-Rushing 2010), affecting species synchrony, co-flowering patterns and flowering duration, which can ultimately shift the diversity and distribution of biodiversity. Furthermore, the observed species-specific response to climatic conditions particularly temperature can be used to predict the effects of future climate warming on flowering phenology, especially in temperate communities where temperature during the early spring is showing warming trends (Gherardi and Sala 2015; Chen et al. 2016).

Furthermore, our study showed a significant difference in the phenological response between the species of all the genera studied. More specifically, a significant difference in the first flower out, date of senescence and duration of flowering was observed for majority of the congeneric pairwise species. We found that species of the same genus showed variable phenological shifts to same temperature treatment. The probable reason for the variable phenological response can be that each plant species requires an optimum and specific temperature cue for a particular phenological event to occur (Rice et al. 2021). Our findings reveal that the species sensitivities are more similar within congeneric pairs than the distantly related species. This can be attributed to the phylogenetically conserved traits amongst congeneric species that can account for a significant amount of variation in phenological shifts amongst the studied genera (Buckley and Kingsolver 2012). Our results are also in agreement with the recent studies that reported the congeners differ in their phenological response to particular temperature (Primack et al. 2009; Gerst et al. 2017). Due to rapidly changing climate in the Himalayan region, the shifts in phenological patterns may lead to mismatch in the plant-pollinator interactions, thus affecting the biological communities in general and synchrony amongst insect and plant species in particular (CaraDonna et al. 2014).

Conclusion

A shift to earlier spring phenological phases is a well-known response of plant species to the climate warming. The present study investigated the effect of warmer temperatures on the flowering phenology of selected woody species of Kashmir Himalaya. We experimentally showed that, with an increase in temperature, there is an advancement of dates of first flower out and senescence for all the selected species, and a decrease in duration of flowering phase. These results provide important insights about the likely impacts of phenological shifts on the pollination behaviour and other biological interactions of plant species due to ongoing climate warming, which merit detailed investigation in near future. Since the studied plant species are reported to be mostly bee-pollinated (Ara et al. 2019), an advancement in flowering time can affect the plant-pollinator interaction due to early spring warming, thereby influencing their reproductive success. In addition, we found that the different species (including congeneric pairs) significantly differ in their phenological response to particular temperature treatments, indicating that these relationships are highly species-specific. We conclude that the warmer temperature plays a significant role in advancement of first flower out and flower senescence in the studied spring-flowering woody species of Kashmir Himalaya, concomitantly influencing the duration of flowering phase as well. Overall, this study furthers our understanding of the key drivers underlying phenological shifts in this data-deficient Himalayan biodiversity hotspot region, with wider implications for similar regions elsewhere in the world.

Lastly, our results should be taken with the caveat that we have specifically attempted to determine the effect of increasing temperature on the flower senescence by comparing the twigs placed in similar conditions, not by comparing the twigs with the whole plants in nature. We would like to caution in directly extrapolating the results of our study to the field conditions, which therefore leaves much scope for field validation, and variation if any, in future phenological studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00484-022-02317-y.

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Author contribution AAK conceived the research idea and supervised the study; TH collected the plant material and conducted the experiment with help from RG and AAK. RA and SAW[1] conducted data analysis and interpreted the results; TH, AAK and RA led manuscript writing with inputs from SAW[2]. TH, RA and AAK contributed in revision. All the authors read and approved the final manuscript for submission.

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Declarations

Conflict of interest The authors declare no competing interests.

References

Ahmad M, Uniyal SK, Batish DR, Rathee S, Sharma P, Singh HP (2021) Flower phenological events and duration pattern is influenced by temperature and elevation in Dhauladhar mountain range of Lesser Himalaya. Ecol Indic 129:107902. https://doi.org/10.1016/j.ecolind.2021.107902

Aono Y, Saito S (2010) Clarifying springtime temperature reconstructions of the medieval period by gap-filling the cherry blossom phenological data series at Kyoto, Japan. Int J Biometeorol 54:211–219. https://doi.org/10.1007/s00484-009-0272-x

Ara S, Rather ZA, Paray MA, Khursheed R, Yaqoob M (2019) Bee flora of Kashmir: the Himalayan biodiversity hotspot. J Pharmacogn Phytochem 8:2172–2181

Basler D, Körner C (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agric Meteorol 165:73–81. https://doi.org/10.1016/j.agrformet.2012.06.001

Basnett S, Nagaraju SK, Ravikanth G, Devy SM (2019) Influence of phylogeny and abiotic factors varies across early and late reproductive phenology of Himalayan Rhododendrons. Ecosphere 10:e02581. https://doi.org/10.1002/ecs2.2581

Buckley LB, Kingsolver JG (2012) Functional and phylogenetic approaches to forecasting species' responses to climate change. Annu Rev Ecol Evol Syst 43:205–226. https://doi.org/10.1146/annurev-ecolsys-110411-160516

Caffarra A, Donnelly A (2011) The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. Int J Biometeorol 55:711–721. https://doi.org/10.1007/s00484-010-0386-1

Campoy JA, Ruiz D, Allderman L, Cook N, Egea J (2012) The fulfilment of chilling requirements and the adaptation of apricot (Prunus armeniaca L.) in warm winter climates: an approach in Murcia (Spain) and the Western Cape (South Africa). Eur J Agron 37:43–55. https://doi.org/10.1016/j.eja.2011.10.004

CaraDonna PJ, Waser NM (2020) Temporal flexibility in the structure of plant–pollinator interaction networks. Oikos 129:1369–1380. https://doi.org/10.1111/oik.07526

CaraDonna PJ, Iler AM, Inouye DW (2014) Shifts in flowering phenology reshape a subalpine plant community. Proc Natl Acad Sci 111:4916–4921. https://doi.org/10.1073/pnas.1323073111)

Chen M, Melaas EK, Gray JM, Friedl MA, Richardson AD (2016) A new seasonal deciduous spring phenology submodel in the Community Land Model 4.5: impacts on carbon and water cycling under future climate scenarios. Glob Chang Biol 22:3675–3688. https://doi.org/10.1111/gcb.13326

Chuine I, Beaubien E (2001) Phenology is a Major determinant of temperate tree range. Ecol Lett 4:500–510. https://doi.org/10.1046/j.1461-0248.2001.00261.x

Chuine I, Morin I, Bugmann H (2010) Warming, photoperiods, and tree phenology. Science 329:277–278. https://doi.org/10.1126/science.329.5989.277-e

Cleland EE, Chuine I, Menzel A, Mooney HA, Schwartz MD (2007) Shifting plant phenology in response to global change. Trends Ecol Evol 22:357–365. https://doi.org/10.1016/j.tree.2007.04.003

Cleland EE, Allen JM, Dunne JA et al (2012) Phenological tracking enables positive species responses to climate change. Ecology 93:1765–1771. https://doi.org/10.1890/11-1912.1

Dad JM, Muslim M, Rashid I, Reshi ZA (2021) Time series analysis of climate variability and trends in Kashmir Himalaya. Ecol Indic 126:1076. https://doi.org/10.1016/j.ecolind.2021.107690

Dantec CF, Vitasse Y, Bonhomme M, Louvet J, Kremer A, Delzon S (2014) Chilling and heat requirements for leaf unfolding in European beech and sessile oak populations at the southern limit of their distribution range. Int J Biometeorol 58:1853–1864. https://doi.org/10.1007/s00484-014-0787-7

Dar GH, Khuroo AA (2020) An updated taxonomic checklist of angiosperms in Jammu and Kashmir state. In: Dar GH, Khuroo AA (eds) Biodiversity of the Himalaya: Jammu and Kashmir State. Topics in Biodiversity and Conservation, vol 18. Springer, Singapore. https://doi.org/10.1007/978-981-32-9174-4_19

De la Haba P, De la Mata L, Molina E, Agüera E (2014) High temperature promotes early senescence in primary leaves of sunflower (Helianthus annuus L.) plants. Can J Plant Sci 94:659–669. https://doi.org/10.4141/cjps2013-276

Doi H (2008) Delayed phenological timing of dragonfly emergence in Japan over five decades. Biol Lett 4:388–391. https://doi.org/10.1098/rsbl.2008.0234

Elmendorf SC, Jones KD, Cook BI, Diez JM, Enquist CAF, Hufft RA, Jones MO, Mazer SJ, Miller-Rushing AJ, Moore DJP (2016) The plant phenology monitoring design for the National Ecological Observatory Network. Ecosphere 7(4):e01303. https://doi.org/10.1002/ecs2.1303

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G (2007) Time after time: flowering phenology and biotic interactions. Trends Ecol Evol 22:432–439. https://doi.org/10.1016/j.tree.2007.05.006

Ettinger AK, Chamberlain CJ, Morales-Castilla I, Buonaiuto DM, Flynn DFB, Savas T, Samaha JA, Wolkovich EM (2020) Winter temperatures predominate in spring phenological responses to warming. Nat Clim Change 10:1137–1142. https://doi.org/10.1038/s41558-020-00917-3

Fitter AH, Fitter RSR (2002) Rapid changes in flowering time in British plants. Science 296:1689–1691. https://doi.org/10.1126/science.1071617

Flynn DFB, Wolkovich EM (2018) Temperature and photoperiod drive spring phenology across all species in a temperate forest community. New Phytol 219:1353–1362. https://doi.org/10.1111/nph.15232

Forrest J, Miller-Rushing AJ (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. Philos Trans R Soc Lond 365:3101–3112. https://doi.org/10.1098/rstb.2010.0145

Fox N, Jönsson AM (2019) Climate effects on the onset of flowering in the United Kingdom. Environ Sci Eur 31:1–13. https://doi.org/10.1186/s12302-019-0271-4

Fridley JD (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature 485:359–362. https://doi.org/10.1038/nature11056

Furguson DL, Guikema JA, Paulsen GM (1990) Ubiquitin pool modulation and protein degradation in wheat roots during high temperature stress. Plant Physiol 92:740–746. https://doi.org/10.1104/pp.92.3.740

Gaira KS, Rawal RS, Rawat B, Bhatt ID (2014) Impact of climate change on the flowering of Rhododendron arboreum in central Himalaya, India. Curr Sci 106: 1735–1738. http://www.jstor.org/stable/24103010

Gerst KL, Rossington NL, Mazer SJ (2017) Phenological responsiveness to climate differs among four species of Quercus in North America. J Ecol 105:1610–1622. https://doi.org/10.1111/1365-2745.12774

Gherardi LA, Sala OE (2015) Enhanced interannual precipitation variability increases plant functional diversity that in turn ameliorates negative impact on productivity. Ecol Lett 18:1293–1300. https://doi.org/10.1111/ele.12523

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251. https://doi.org/10.1126/science.1061967

Hart R, Salick J, Ranjitkar S, Xu J (2014) Herbarium specimens show contrasting phenological responses to Himalayan climate. Proc Natl Acad Sci 111:10615–10619. https://doi.org/10.1073/pnas.1403376111

Hassan T, Hamid M, Wani SA, Malik AH, Waza SA, Khuroo AA (2021) Substantial shifts in flowering phenology of Sternbergia vernalis in the Himalaya: supplementing decadal field records with historical and experimental evidences. Sci Total Environ 795:148811. https://doi.org/10.1016/j.scitotenv.2021.148811

Husain M (2002) Geography of Jammu and Kashmir. Rajesh Publications, New Delhi

Iler AM, CaraDonna PJ, Forrest JR, Post E (2021) Demographic consequences of phenological shifts in response to climate change. Annu Rev Ecol Evol Syst 52:221–245. https://doi.org/10.1146/annurev-ecolsys-011921-032939

Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362. https://doi.org/10.1890/06-2128.1

Jung S, Zhao F, Menzel A (2021) Establishing the twig method for investigations on pollen characteristics of allergenic tree species. Int J Biometeorol 65:1983–1993. https://doi.org/10.1007/s00484-021-02154-5

Kehrberger S, Holzschuh A (2019) Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. PLoS ONE 14:e0218824. https://doi.org/10.1371/journal.pone.0218824

Kharouba HM, Ehrlén J, Gelman A, Bolmgren K, Allen JM, Travers SE, Wolkovich EM (2018) Global shifts in the phenological synchrony of species interactions over recent decades. Proc Natl Acad Sci 115:5211–5216. https://doi.org/10.1073/pnas.1714511115

Ko¨rner C, Basler D (2010) Phenology under global warming. Science 327:1461–1462. https://doi.org/10.1126/science.1186473

Laube J, Sparks TH, Estrella N, Ho¨ fler J, Ankerst DP, Menzel A (2014) Chilling outweighs photoperiod in preventing precocious spring development. Glob Change Biol 20:170–182. https://doi.org/10.1111/gcb.12360

Laube J, Sparks TH, Estrella N, Menzel A (2014) Does humidity trigger tree phenology? Proposal for an air humidity-based framework for bud development in spring. New Phytol 202:350–355. https://doi.org/10.1111/nph.12680

Lee JS (2011) Combined effect of elevated CO2 and temperature on the growth and phenology of two annual C3 and C4 weedy species. Agric Ecosyst Environ 140:484–491. https://doi.org/10.1016/j.agee.2011.01.013

Lehikoinen E, Sparks TH, Zalakevicius M (2004) Arrival and departure dates. Adv Ecol Res 35:1–31. https://doi.org/10.1016/S0065-2504(04)35001-4

Lindh BC, McGahan KA, Bluhm WL (2018) Changes in urban plant phenology in the Pacific Northwest from 1959 to 2016: anthropogenic warming and natural oscillation. Int J Biometeorol 62:1675–1684. https://doi.org/10.1007/s00484-018-1567-6

Liu L, Zhang X (2020) Effects of temperature variability and extremes on spring phenology across the contiguous United States from 1982 to 2016. Sci Rep 10:1–14. https://doi.org/10.1038/s41598-020-74804-4

Liu Y, Reich PB, Li G, Sun S (2011) Shifting phenology and abundance under experimental warming alters trophic relationships and plant reproductive capacity. Ecology 92:1201–1207. https://doi.org/10.1890/10-2060.1

Love NL, Mazer SJ (2021) Region-specific phenological sensitivities and rates of climate warming generate divergent temporal shifts in flowering date across a species range. Am J Bot 108:1873–1888. https://doi.org/10.1002/ajb2.1748

Luna NR (2016) Spatiotemporal variability of plant phenology in drylands: A case study from the Northern Chihuahuan Desert. AAI10250930. ETD Collection for University of Texas, El Paso. https://digitalcommons.utep.edu/dissertations/AAI10250930

Malik AH, Khuroo AA, Dar GH, Khan ZS (2020) An annotated inventory of arboreal flora in Jammu and Kashmir state. In: Dar GH, Khuroo AA (eds) Biodiversity of the Himalaya: Jammu and Kashmir State. Topics in Biodiversity and Conservation, vol 18. Springer, Singapore. https://doi.org/10.1007/978-981-32-9174-4_22

Matsumoto K, Ohta T, Irasawa M, Nakamura T (2003) Climate change and extension of the Ginkgo biloba L. growing season in Japan. Glob Change Biol 9:1634–1642. https://doi.org/10.1046/j.1365-2486.2003.00688.x

McDonough MacKenzie C, Primack RB, Miller-Rushing AJ (2018) Local environment, not local adaptation, drives leaf-out phenology in common gardens along an elevational gradient in Acadia National Park, Maine. Am J Bot 105:986–995. https://doi.org/10.1002/ajb2.1108

Menzel A, Yuan Y, Hamann A, Ohl U, Matiu M (2020) Chilling and forcing from cut twigs—how to simplify phenological experiments for citizen science. Front Plant Sci 11:281. https://doi.org/10.3389/fpls.2020.561413

Miller-Rushing AJ, Inouye DW (2009) Variation in the impact of climate change on flowering phenology and abundance: an examination of two pairs of closely related wildflower species. Am J Bot 96:1821–1829. https://doi.org/10.3732/ajb.0800411

Miller-Rushing AJ, Primack RB (2008a) Effects of winter temperatures on two birch (Betula) species. Tree Physiol 28:659–664. https://doi.org/10.1093/treephys/28.4.659

Miller-Rushing AJ, Primack RB (2008b) Global warming and flowering times in Thoreau's concord: a community perspective. Ecology 89:332–341. https://doi.org/10.1890/07-0068.1

Miller-Rushing AJ, Primack RB, Primack D, Mukunda S (2006) Photographs and herbarium specimens as tools to document phenological changes in response to global warming. Am J Bot 93:1667–1674. https://doi.org/10.3732/ajb.93.11.1667

Munson SM, Long AL (2017) Climate drives shifts in grass reproductive phenology across the western USA. New Phytol 213:1945–1955. https://doi.org/10.1111/nph.14327

Murtaza KO, Romshoo SA (2016) Recent glacier changes in the Kashmir Alpine Himalayas, India. Geocarto Int 32:188–205. https://doi.org/10.1080/10106049.2015.1132482

Nagahama A, Kubota Y, Satake A (2018) Climate warming shortens flowering duration: a comprehensive assessment of plant phenological responses based on gene expression analyses and mathematical modeling. Ecol Res 33:1059–1068. https://doi.org/10.1007/s11284-018-1625-x

Neil K, Wu J (2006) Effects of urbanization on plant flowering phenology: a review. Urban Ecosyst 9:243–257. https://doi.org/10.1007/s11252-006-9354-2

Piao S, Tan J, Chen A, Fu YH, Ciais P, Liu Q et al (2015) Leaf onset in the northern hemisphere triggered by daytime temperature. Nat Commun 6:6911. https://doi.org/10.1038/ncomms7911

Polgar C, Gallinat A, Primack RB (2014) Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord. New Phytol 202:106–115. https://doi.org/10.1111/nph.12647

Post E, Stenseth NC (1999) Climate variability, plant phenology, and northern ungulates. Ecology 80:1322–1339. https://doi.org/10.1890/0012-9658(1999)080[1322:CVPPAN]2.0.CO;2

Post E, Forchhammer MC, Stenseth NC, Callaghan TV (2001) The timing of life–history events in a changing climate. Proc R Soc Lond B Biol Sci 268:15–23. https://doi.org/10.1098/rspb.2000.1324

Prevéy JS, Rixen C, Rüger N, Høye TT, Bjorkman AD, Myers-Smith IH, Wipf S (2019) Warming shortens flowering seasons of tundra plant communities. Nat Ecol Evol 3:45–52. https://doi.org/10.1038/s41559-018-0745-6

Prevéy JS, Parker LE, Harrington CA (2020) Projected impacts of climate change on the range and phenology of three culturally-important shrub species. PloS one 15:e0232537. https://doi.org/10.1371/journal.pone

Primack RB, Ibáñez I, Higuchi H, Lee SD, Miller-Rushing AJ, Wilson AM, Silander JA Jr (2009) Spatial and interspecific variability in phenological responses to warming temperatures. Biol Conserv 142:2569–2577. https://doi.org/10.1016/j.biocon.2009.06.003

Primack RB, Laube J, Gallinat AS, Menzel A (2015) From observations to experiments in phenology research: investigating climate change impacts on trees and shrubs using dormant twigs. Ann Bot 116:889–897. https://doi.org/10.1093/aob/mcv032

R Core Team (2020) R: A Language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria

Rashid I, Majeed U, Aneaus S, Cánovas JAB, Stoffel M, Najar NA, Lotus S (2020) Impacts of erratic snowfall on apple orchards in Kashmir valley. India Sustain 12:9206. https://doi.org/10.3390/su12219206

Rice KE, Montgomery RA, Stefanski A, Rich RL, Reich PB (2021) Species-specific flowering phenology responses to experimental warming and drought alter herbaceous plant species overlap in a temperate–boreal forest community. Ann Bot 127:203–211. https://doi.org/10.1093/aob/mcaa156

Romshoo SA, Dar RA, Rashid I, Marazi A, Ali N, Zaz SN (2015) Implications of shrinking cryosphere under changing climate on the stream flows of the upper Indus basin. Arct Antarct Alp Res 47:627–644. https://doi.org/10.1657/AAAR0014-088

Rosbakh S, Hartig F, Sandanov DV, Bukharova EV, Miller TK, Primack RB (2021) Siberian plants shift their phenology in response to climate change. Glob Change Biol 27:4435–4448. https://doi.org/10.1111/gcb.15744

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot 61:254–263. https://doi.org/10.1016/j.envexpbot.2007.06.008

Scheurwater I, Dunnebacke M, Eising R, Lambers H (2000) Respiratory costs and rate of protein turnover in the roots of a fast-growing (Dactylis glomerata L.) and a slow-growing (Festuca ovina L.) grass species. J Exp Bot 51:1089–1097. https://doi.org/10.1093/jxb/51.347.1089

Sherry RA, Zhou X, Gu S, Arnone JA, Schimel DS, Verburg PS, Luo Y (2007) Divergence of reproductive phenology under climate warming. Proc Natl Acad Sci 104:198–202. https://doi.org/10.1073/pnas.0605642104

Sønsteby A, Heide OM (2014) Chilling requirements of contrasting black currant (Ribes nigrum L.) cultivars and the induction of secondary bud dormancy. Sci Hortic 179:256–265. https://doi.org/10.1016/j.scienta.2014.09.038

Sparks TH, Huber K, Croxton PJ (2006) Plant development scores from fixed-date photographs: the influence of weather variables and recorder experience. Int J Biometeorol 50:275–279. https://doi.org/10.1007/s00484-005-0022-7

Stuble KL, Bennion LD, Kuebbing SE (2021) Plant phenological responses to experimental warming—a synthesis. Glob Change Biol 27:4110–4124. https://doi.org/10.1111/gcb.15685

Suonan J, Classen AT, Zhang Z, He JS (2017) Asymmetric winter warming advanced plant phenology to a greater extent than symmetric warming in an alpine meadow. Funct Ecol 31:2147–2156. https://doi.org/10.1111/1365-2435.12909

Tao Z, Zhong S, Quansheng GE, Dai J, Yunjia XU, Wang H (2017) Spatiotemporal variations in flowering duration of woody plants in China from 1963 to 2012. Acta Bot Sin 72:53–63

Theobald EJ, Breckheimer I, HilleRisLambers J (2017) Climate drives phenological reassembly of a mountain wildflower meadow community. Ecology 98:2799–2812. https://doi.org/10.1002/ecy.1996

Vitasse Y, Basler D (2014) Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments? Tree Physiol 34:174–183. https://doi.org/10.1093/treephys/tpt116

Wang G, Baskin CC, Baskin JM, Yang X, Liu G, Ye X, Huang Z (2018) Effects of climate warming and prolonged snow cover on phenology of the early life history stages of four alpine herbs on the Southeastern Tibetan Plateau. Am J Bot 105:967–976. https://doi.org/10.1002/ajb2.1104

Wani MH, Bhat A, Baba SH (2021) Economic evaluation of high density apple (ex-ante) in Kashmir. Int J Fruit Sci 21:706–711. https://doi.org/10.1080/15538362.2021.1926393

Waser NM, Real LA (1979) Effective mutualism between sequentially flowering plant species. Nature 281:670–672. https://doi.org/10.1038/281670a0

Willis CG, Ellwood ER, Primack RB, Davis CC, Pearson KD, Gallinat AS, Soltis PS (2017) Old plants, new tricks: phenological research using herbarium specimens. Trends Ecol Evol 32:531–546. https://doi.org/10.1016/j.tree.2017.03.015

Yu H, Luedeling E, Xu J (2010) Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. Proc Natl Acad Sci 107:22151–22156. https://doi.org/10.1073/pnas.1012490107

Zaz SN, Romshoo SA, Krishnamoorthy RT, Viswanadhapalli Y (2019) Analyses of temperature and precipitation in the Indian Jammu and Kashmir region for the 1980–2016 period: implications for remote influence and extreme events. Atmos Chem and Phys 19:15–37. https://doi.org/10.5194/acp-19-15-2019

Zohner CM, Renner SS (2015) Perception of photoperiod in individual buds of mature trees regulates leaf-out. New Phytol 208:1023–1030. https://doi.org/10.1111/nph.13510

Zohner CM, Benito BM, Svenning JC, Renner SS (2016) Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. Nat Clim Chang 6:1120–1123. https://doi.org/10.1038/nclimate3138

Zohner CM, Benito BM, Fridley JD, Svenning JC, Renner SS (2017) Spring predictability explains different leaf-out strategies in the woody floras of North America, Europe and East Asia. Ecol Lett 20:452–460. https://doi.org/10.1111/ele.12746