1. Phenological sequences: How early-season events affect later-season events;
OR
2. The shape of the season: How early-season phenological define events that follow

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Abstract

Premise of the study

Plant phenology is a critical trait, but it not known how phenological states such as budburst and leafout relate to one another across an entire growing season. We test the extent to which previous phenological stages constrain later ones, throughout a growing season and across 25 angiosperm tree species.

Methods

We observed phenology (budburst, leafout, flowering, fruiting, senesence) of 118 individual trees across 25 species, from April through December, 2015.

Key results

We found that previous phenological events constrain later events, in many cases, with the strongest relationships between consecutive stages. We found that inter-phenophase duration constrains reproductive phenology (flowering, fruiting).

Conclusions

Key words

plant phenology, climate change, budburst, leafout, flowering, fruiting, sensence, angiosperm, tree, arboretum

Introduction

Plant phenology, the timing of life-events such as leafout and flowering, is a critical trait that affects individual fitness, population abundance, agricultural and natural productivity, and global climate, through its role in carbon sequestration (Miller-Rushing et al., 2008; Primack and Miller-Rushing, 2009; Willis et al., 2010; Miller-Rushing et al., 2010). In temperate tree species, temperature is thought to be a major factor controlling phenology (Parmesan, 2006; Morin et al., 2010; Schwartz et al., 2013). As such, recently observed temporal shifts in budburst, leafout, and other phenological states have been attributed to warming from anthropogenic climate change (Parmesan, 2006). Phenology is expected to shift further with future climate change, and, because of its important role in many ecosystem services and in the global climate cycle, planning and preparing for climate change impacts will benefit from improved understanding and forecasting of tree phenology.

Despite the observation that spring phenology generally shifts earlier with warmer temperatures, dramatic variation exists in phenological responses to climate. Different tree species vary widely in the timing of leafout and other phenological processes, even when exposed to the same environmental conditions (Lechowicz, 1984; Primack et al., 2009). For example, spring leafout can span weeks among coexisting tree species (Lechowicz, 1984). The drivers of these variations are poorly understood, even though phenology has been long-observed (Wolkovich and Ettinger, 2014).

One important feature of plant phenology is that events are sequential: leaf budburst comes before leafout, flowering comes before fruiting, etc. This ordering may constrain phenological events, but the extent of constraints between phenological events is poorly understood because few studies have integrated across consecutive events throughout a growing season (Wolkovich and Ettinger, 2014). For example, many drivers of phenology have been studied using climate-controlled growth chambers (e.g., Basler and Körner, 2012; Laube et al., 2014); these studies focus almost exclusively on early season events (budburst and/or leafout). Long-term observational studies of phenology typically collect data on flowering only (e.g. 64% of studies in Wolkovich et al., 2012). Recently, interest has surged in senescence (e.g.), with a recent finding that the timing of autumn senescence is affected by spring phenology (Keenan and Richardson, 2015). This novel insight highlights the need to better understand how phenological stages relate to one another across an entire growing season.

Here, we examine the extent to which previous phenological events constrain later events. Specifically, we test two hypotheses:

- Hypothesis 1: Previous phenological events constrain later events; e.g., late-fruiting species set fruit late in the season because they leafout late (Figure 1).

- Hypothesis 2: Inter-phenophase time constrains phenology; e.g., late-fruiting species set fruit late in the season because they require longer maturation time (Figure 1).

Materials and Methods

Study site and focal species

This study was conducted at the Arnold Arboretum of Harvard University, a 281-acre park in Boston, Massachusetts, established in 1872. It contains a living collection of 3,825 woody plant taxa that are native to North America, Europe, and Asia. Arboreta are great resources for phenological studies across many species (Primack and Miller-Rushing, 2009), particularly in temperate areas, since they may contain a higher diversity of tree species growing in one location than nearby natural areas. In addition, there is often high variation in phenology of species planted in arboreta, for public enjoyment. For this study, we selected 25 focal angiosperm species that varied in their flowering times (Table 1). We selected up to five individuals of each species for the study, yielding a total of 118 individuals.

Phenology data collection

We observed five phenological stages, which were quantified following the National Phenology Network (NPN) protocols (for a full description see Denny et al., 2014). The budburst phase is characterized by green leaf tips being visible at the tips of buds. The leafout phase is characterized by visible fully unfolded leaves and petioles that have completely emerged from the buds. Leaf senescence is characterized by leaves changing from green to fall colors. The flowering phase is when open flowers are visible, and the fruiting phase is when developing fruit are visible.

We visited each individual once every 6-10 days throughout the growing season. Phenology observations in the spring began on April 6, 2015, and fall phenology observations ended on December 2, 2015. From the phenology data, we extracted the day of the year (DOY) of the first observed occurrence of a given phenological phase. Budburst DOY was defined as the first day when three or more leaf buds were seen bursting. Leafout DOY was defined as the first day when 5% or more of the individual was leafing out. Flowering DOY was defined as the first day when 5% or more of the flower buds were open on an individual. Fruiting DOY was defined as the first day when three or more developing fruits were observed on the individual. Leaf senescence DOY was defined as the first day when 5% or more of the individual showed fall colors (Denny et al., 2014).

Statistical analyses

To understand the extent to which previous phenological events constrain later events (Hypothesis 1, Figure 1), we fit linear models in which the response variable was phenological stage (i.e. the species-level mean day of year of leafout, flowering, fruiting, or senescence; budburst was excluded because it was the earliest stage we quantified), and the predictor was previous phenologcal stage. We therefore fit 10 different models, each with one of the previous phenological stages as the predictor variable.

To understand the extent to which inter-phenophase duration constrains later events (Hypothesis 2, Figure 1), we fit linear models in which the response variable was phenological stage, as above, and the predictor was the number af days between consecutive phenological stages. Because inter-phenophase duration has not been studied in previous work, to our knowledge, we wanted to explore how phenological stages we studied related to all inter-phenphase durations in our study. We therefore fit 25 different models, each with one of the five phenological stages as the response variable and one of the five inter-phenophases we studied as a predictor. All analyses were conducted in R version 3.2.4 (Team, 2016).

Results

We monitored five phenophases, which varied in duration. Budburst occurred over 32 days in the spring, and leafout occurred over 30 days, across all focal species (Figure 2). Flowering phenology occurred over a longer period than budburst and leafout, spanning 111 days from late April to September (Figure 2). Fruiting spanned 183 days, and leaf senescence occurred over 56 days (Figure 2). Most species (20/25) spent the majority of the growing season in the reproductive phenological phases (i.e. flowering and fruit development), and most species (23/25) began leaf budburst prior to flowering, though leaf development overlapped with flowering in some species (Figure2).

We found strong correlations between late versus early phenological stages in many cases (Figures 2, 3), suggesting that earlier phenological stages constrain later ones. The strongest correlations occurred between adjacent stages (i.e. those along the diagonal in Figure 3, such as leafout and budburst, fruiting and flowering). Senescence was the only phenological stage not well-correlated with an earlier phenological stage. Add rate of change (effect size) for different models.

We also observed strong correlations between phenology and inter-phenophase duration for budburst and for both reproductive pheno-phases stages (flowering and fruiting time, Figure 4). Budburst day of year was most strongly related to the inter-phenophase duration between leafout and budburst, with budburst

delayed XX days per day of interphenophase duration. For other growth phenophases (leafout, senescence), inter-phenophase duration was not a strong predictor of phenology (Figure 4). Reproductive phenology appears to be constrained by both earlier inter-phenophase durations (e.g. flowering DOY was correlated with days between flowering and budburst, fruiting DOY is correlated with days between fruiting and flowering stages) and later interphase durations (e.g. flowering DOY was correlated with days between senescence and flowering). Flowering time was delayed 1.03 days per day of inter-phenophase duration between flowering and budburst, delayed 1.10 days per per day of inter-phenophase duration between flowering and leafout, and delayed XX days per day of interphenophase duration between senescence and flowering. Fruiting time was delayed 1.11 days per day of inter-phenophase duration betwee fruting and flowering.

Discussion

The majority of phenological stages we observed (three out of four) support Hypothesis 1: their timing appears to be constrained by earlier phenological stages. Add rate of change (effect size) for different models. Thus, environmental conditions in the winter or spring that may directly affect only early phenologial stages, such as budburst, are likely to have cascading effects on later stages such as leafout, flowering, and fruiting. Previous work suggests that spring phenology can also affect senescence time (Keenan and Richardson, 2015), though we did not find evidence for this relationship in our study 2.

Reproductive phenology (flowering and fruiting) also supported Hypothesis 2: timing appears to be constrained by inter-phenophase time. Later flowering species require more time between flowering and budburst, and between flowering and leafout. Similarly, late fruiting species had longer inter-phenophase time between between fruiting and flowering. These results are consistent with previous theories that trees investing more resources into their offspring (i.e. having larger seeds) need more time to build resources for the offspring (??).

Our findings highlight that our two hypotheses are not mutually exclusive. For example, although we found a positive relationship between budburst and leafout (Figure 3), earlier budburst does not always result in earlier leafout. For some species, such as XX, it is associated with longer time between budburst and leafout (Figure 4). From the single year of data in this study, it is not clear if this pattern is due to variation in species' physiology and drivers of phenology, or to weather patterns. For example, many species leafed out after DOY 130 (May 10, 2015), despite budburst days ranging from day 110 to 130. Temperatures during January through March were colder than average in 2015, with above-average snow-fall, but temperatures warmed considerably in late April and early May to above-average conditions (www.bluehill.org). Thus, the flush of budburst in early May could be due to temperature conditions specific to the year of our study.

There are at least two important implications of these findings for improved forecasting of climate change induced shifts in phenology. First, a shift in one phase may have cascading effects on later phases, since each phase is linked to phases that occur before and after it (Wolkovich and Cleland, 2014). This highlights a clear need to conduct future studies across entire growing seasons, at a minimum (Wolkovich and Ettinger, 2014), and begs the question of how phenophases may be linked across years, as well. We wonder, for instance, whether the timing of spring budburst in one year may be related to the timing of bud-set the previous fall (add citations?). Second, given the species-specific nature of phenological constraints, accurate forecasts of community-wide phenological shifts are likely to require species-specific information, such as fruit development time for fruiting forecasts, in addition to climate data (?).

Conclusions

Many questions remain about drivers of the dramatic variation observed to date in phenological patterns and shifts in these patterns under anthropogenic climate change. Given our findings that consecutive stages are linked, we encourage future studies, whether observational or manipulative, to collect data across entire growing seasons. Multi-year studies will be critical to evaluate the extent to which the phenological patterns

are consistent among years that may vary in climate, as well as biotic conditions (i.e. pollinator or pest populations). Fuller understanding of phenological constraints and drivers of phenological variation offers the potential for improved forecasts of phenological shifts with climate change to help predict how ecosystem functions will be altered in the future.

Acknowledgements

Data Accessibility

The dataset for this study is available online at KNB (Cite).

Author contributions

All authors conceived and designed the study and edited the manuscript; S.G. conducted the field and lab work; S.G. and A.E. analyzed the data and wrote the manuscript; E.W. edited the manuscript.

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# Tables

Table 1: **Study species.** Twenty-five angiosperm species were selected, based on their flowering phenology in long-term records of the Arnold Arboretum. (The flowering patterns we observed during our one year of data collection did not always perfectly match these long-term patterns.) The number of individuals of each species observed at the Arnold Arboretum from spring through fall 2015 is in parentheses.

Early-season flowering	Mid-season flowering	Late-season flowering
Aesculus flava (5)	*Carya glabra* (5)	*Catalpa speciosa* (5)
Betula alleghaniensis (5)	*Carya ovata* (5)	*Kalopanax septemlobus* (3)
Betula nigra (5)	*Crataegus crus-galli* (5)	*Styphnolobium japonicum* (5)
Gleditsia triancanthos (5)	*Fagus engleriana* (4)	*Tilia americana* (5)
Liriodendron tulipifera (5)	*Fagus grandifolia* (5)	*Tilia japonica* (5)
Phellodendron amurense var. *lavallei* (4)	*Fraxinus chinensis* (5)	
Populus deltoids ssp. *deltoids* (5)	*Liquidambar styraciflua* (5)	
Pyrus calleryana var. *dimorphophylla* (3)	*Platanus occidentalis* (5)	
Pyrus ussuriensis var. *hondoensis* (5)	*Quercus glandulifera* (4)	
Quercus alba (5)	*Quercus rubra* (5)	

Figures
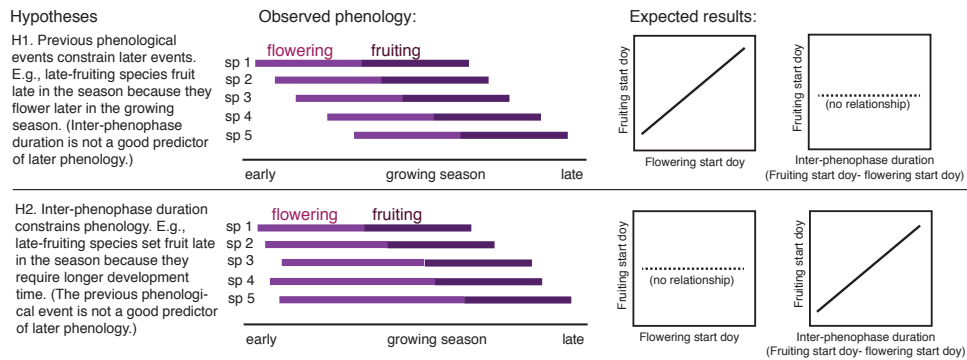
Figure 1: **Hypotheses.** We show flowering and fruiting as examples of consecutive phenological events. We expected the same patterns for other consecutive events,such as leaf budburst and leafout. Inter-phenophase duration is the time between phenological events, e.g., the number of days between the start of flowering and the start of fruiting.
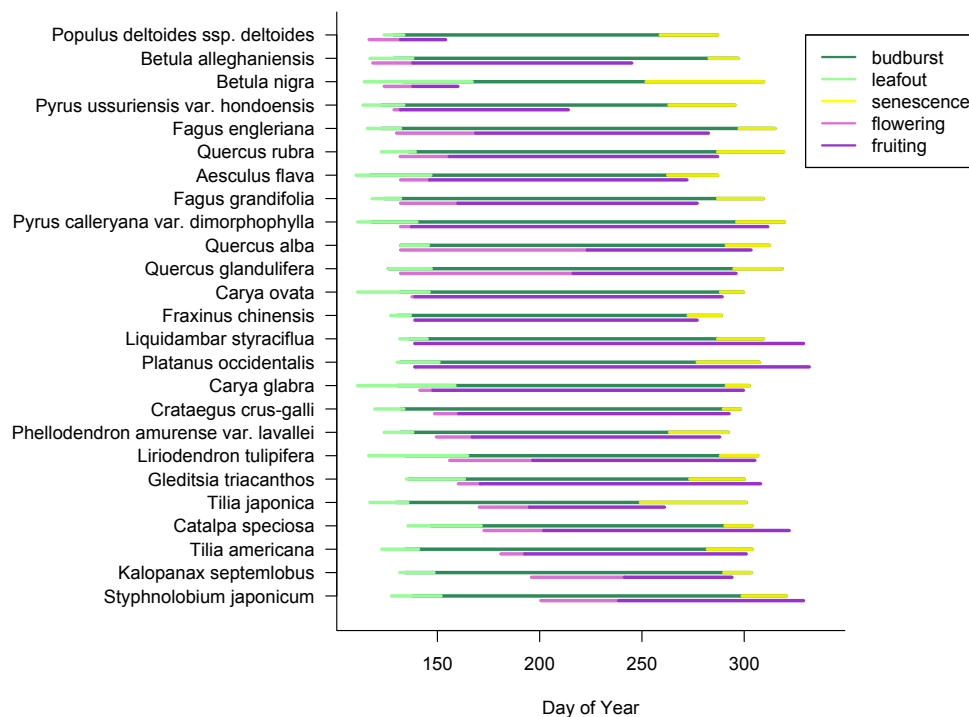
Figure 2: **Species' phenology during the 2015 growing season, sorted by mean first-flower dates.** Growth phenology is shown for budburst (from its mean start day-of-year to the mean start day-of-year for leafout, across all individuals within a species), leafout (from the mean day-of-year when fully-expanded leaves were first observed through the start of senescence), and senescence (from the mean day-of-year when leaves first began changing color through the mean day-of-year when more than 95 percent of leaves on the tree had changed color). Reproductive phenology is shown for flowering (from the mean day-of-year when flowers first appeared to the mean day-of-year when fruits first appeared, across all individuals within a species) and fruiting (from the mean day-of-year when fruits first appeared to the mean day-of-year when more than 95 percent of fruits were first observed as ripe).
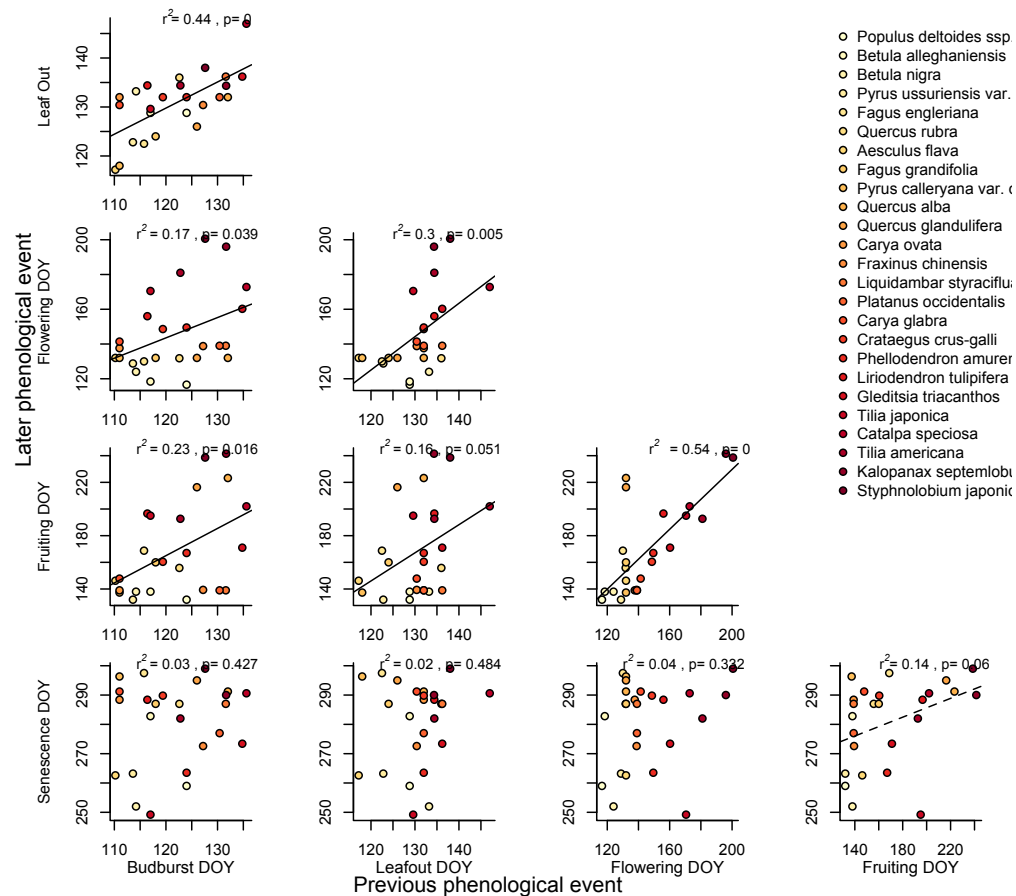
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Figure 3: **Relationships among phenological stages across the 25 focal species.** Linear models were fit with the species-level mean day of year of the later phenological stages as the response variable, and mean day of year of earlier stage as the explanatory variable. R^2 and p-value for each model are shown, with solid lines representing model fit when $p<0.05$ and dashed lines representing model fit when $0.05<p<0.10$. Full model statistics are summarized in Table S1 in the Supplemental Materials. Species in the legend are ordered from early to late first-flower dates, as in Figure 2.
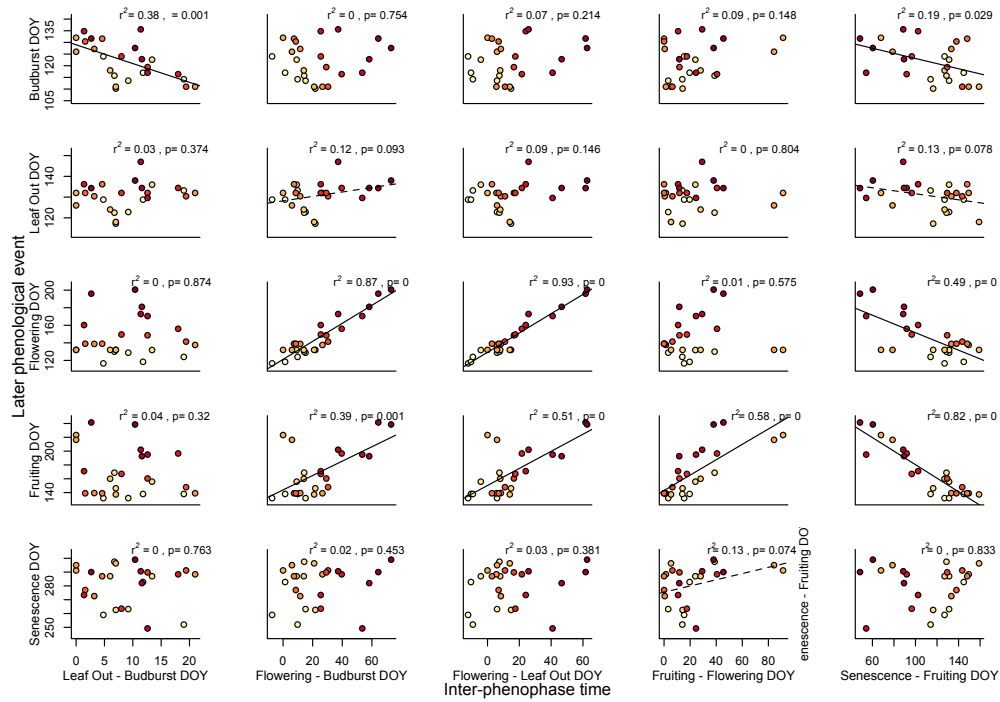
Figure 4: **Relationships among phenological stages and inter-phenophase duration across the 25 focal species.** Inter-phenophase duration is the time between the start of the earlier phenological event and the start of the later phenological event, e.g., the number of days between the species' mean start of flowering and its mean start of fruiting. Linear models were fit with the species-level mean day of year of the later phenological stages as the response variable, and inter-phenophase duration as the explanatory variable. R^2 and p-value for each model are shown, with solid lines representing model fit when $p<0.05$ and dashed lines representing model fit when $0.05<p<0.10$. Full model statistics are summarized in Table S1 in the Supplemental Materials. Species are color-coded as in Figure 3.