

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

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Abstract

Premise of the study

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

Methods

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

Key results

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

Conclusions

Our findings highlight that a shift in one phenophase will have cascading effects on later phases, so accurate forecasts of climate change impacts should include multiple phenophases within and across years.

Key words

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

Introduction

Plant phenology, the timing of recurring 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 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. Some populations and species have not shifted their phenology with recent climate change (Wolkovich et al., 2012*b*). In addition, 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, but often overlooked, feature of plant phenology is that events are sequential: leaf budburst comes before leafout, flowering comes before fruiting, etc. This ordering may constrain phenological responses to climate change. However, 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*a*). Interest has surged in senescence, which had been less studied historically (Parmesan, 2006), but most studies have tended to focus only on senescence.

When research has looked across stages, important links have often been found. For example, a recent study found 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 (Wolkovich and Ettinger, 2014).

Here, we examine the extent to which early phenological events constrain later events, across multiple co-occurring tree species with varying phenology. 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 flower and 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 development and/or maturation time (Figure 1).

Testing these hypotheses will address basic, critical questions about drivers of variation in temperate tree phenology. These questions remain unanswered despite decades of phenology research because no previous field studies, to our knowledge, have examined multiple phenophases spanning the entire growing season and across a large number of tree species.

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 (e.g., 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 of leaves and flowers throughout the season. 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 was characterized by green leaf tips being visible at the tips of buds. The leafout phase was characterized by visible fully unfolded leaves and petioles that have completely emerged from the buds. The flowering phase was when open flowers are visible, and the fruiting phase was defined by ripe fruit being visible. Leaf senescence was characterized by leaves changing from green to fall colors.

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 ripe 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). From these individual tree phenology observations, we calculated species-level mean start dates for all phenophases, for use in our statistical analyses.

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' mean DOY of leafout, flowering, fruiting, or senescence; budburst was excluded because it was the earliest stage we quantified), and the predictor was previous phenological 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 of days between consecutive phenological stages. Because inter-phenophase duration has not been studied in previous work, to our knowledge, we wanted to explore how the phenological stages we studied related to all inter-phenophase durations in our study (both before and after the phenological stage). 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. First budburst date occurred over 32 days in the spring and first leafout date occurred over 30 days, across all focal individuals (Should we add a Supplemental Figure of individual-level phenology?) and species (Figure 2). Flowering phenology occurred over a longer period than budburst and leafout, spanning 131 days from late April to September. The first observation of ripe fruit spanned 175 days, and the start of leaf senescence occurred over 56 days across all individuals and species. 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 (Figure 2). The majority of species (15/25) also produced ripe fruit prior to beginning senescence (Figure 2).

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 relationships (i.e. with the most variation explained) occurred between adjacent stages (i.e. those along the diagonal in Figure 3, such as leafout and budburst, fruiting and flowering). Leafout was delayed by 0.53 days per day of year that budburst occurred. Flowering was delayed 1.91 days per day of year that leafout occurred and 1.16 days per day of year that budburst occurred. Fruiting was delayed 1.05 days per day of year that flowering occurred, 2.33 per day of year that leafout occurred and 2.42 days per day of year that budburst occurred. Senescence was affected by fruiting, with a delay of 0.17 days per day of year that fruiting occurred.

We also observed strong correlations between phenology and inter-phenophase duration for both reproductive phenophases (flowering and fruiting time, Figure 4). Flowering DOY was correlated with days between flowering and budburst and fruiting DOY is correlated with days between fruiting and flowering stages. Neither leaf-out nor senescence were affected by inter-phenophase durations.

Discussion

All phenological stages we observed (four out of four) support Hypothesis 1: their timing appears to be constrained by earlier phenological stages. Thus, environmental conditions in the winter or spring that may directly affect only early phenological stages, such as budburst, are likely to have cascading effects on later stages such as leafout, flowering, and fruiting. These findings are consistent with recent work suggesting that spring phenology can affect senescence time (Keenan and Richardson, 2015).

Although some of the variation in reproductive phenology (flowering and fruiting) was explained by previous phenology (Hypothesis 1), much more variation was explained by interphenophase time (Hypothesis 2). Later flowering species required more time between flowering and leafout. Similarly, late fruiting species had longer inter-phenophase time between the first observation of ripe fruit and first flowering date. It may be that late fruiting species require longer fruit development times to produce larger fruits or more highly-provisioned seeds. This would be consistent with previous theories that trees investing more resources into their offspring (i.e. having larger seeds) require more time to build resources (??).

For growth phenology, we observed some support for Hypothesis 2 (inter-phenophase time) in budburst and senescence phases. We could discuss possible reasons for the patterns we see here (earlier budburst related to longer time between budburst and leafout and to longer time between senescence and fruiting...i can't think of a biological reason for these relationships- can you?), but they do not really relate to our hypothesis 2. e.g. The stronger relationships observed in these two phases, which occur at the beginning and end of a bounded growing season, may be the result of geometric constraints (Letten et al., 2013). The lack of any relationship between interphase time and leafout may be due to the distinct weather patterns in 2015, as opposed to physiological constraints. Many species leafed out close to DOY 130 (May 10, 2015), regardless of leafout-budburst inter-phenophase time, which ranged from 0 to 20 days. 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 leafout in early May could be due to temperature conditions specific to the year of our study. Alternatively,

we could cut this paragraph (i recommend this if we end up showing a more restricted Figure 4).

Our findings highlight that our two hypotheses are not mutually exclusive. For example, although we found a positive relationship between fruiting and flowering (Figure 3), later fruiting is not *always* the result of later flowering. For some species, such as *Quercus alba* and *Quercus grandifolia*, later fruiting is instead associated with longer time between fruiting and flowering (Figure 4). Disentangling the ways that earlier phenology and inter-phenophase time interact to determine later phenology will require multi-year field studies, and benefit from experimental manipulations that can discern the physiological basis the relationships we observe (need ideas for relevant papers to cite in this paragraph).

Our findings have two important implications 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 (Mimura and Aitken, 2010). The ecological memory (Ogle et al., 2015) of phenology has not been quantified, but may be critical for accurate forecasting, particularly for species like *Quercus rubra*, which require more than one year for fruit maturation. 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

We have shown that early and late phenological stages are strongly linked across the growing season, providing a new approach to explaining some of the dramatic variation in phenological responses observed to date. Many studies have sought to identify the particular environmental drivers of phenology (e.g. Morin et al., 2010; Schwartz et al., 2013); our findings here suggest that identifying the appropriate temporal window for such drivers is also essential (?). Multi-year studies will be critical to evaluate the extent to which phenological patterns are consistent among years that may vary in climate, as well as biotic conditions (i.e. pollinator or pest populations). Additional variation in phenological responses may be understood by incorporating phylogenetic approaches to understand the constraints we observed, and by exploring patterns at the individual, rather than species, level. A 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.

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Data Accessibility

The data set for this study is available online at KNCB (Cite).

Author contributions

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

1 Bibliography

References

- Basler, D., and C. Körner. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology* 165:73–81.
- Denny, E. G., K. L. Gerst, A. J. Miller-Rushing, G. L. Tierney, T. M. Crimmins, C. A. Enquist, P. Guertin, A. H. Rosemartin, M. D. Schwartz, K. A. Thomas, et al. 2014. Standardized phenology monitoring methods to track plant and animal activity for science and resource management applications. *International journal of biometeorology* 58:591–601.
- Keenan, T. F., and A. D. Richardson. 2015. The timing of autumn senescence is affected by the timing of spring phenology: implications for predictive models. *Global change biology* 21:2634–2641.
- Laube, J., T. H. Sparks, N. Estrella, J. Höfler, D. P. Ankerst, and A. Menzel. 2014. Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology* 20:170–182.
- Lechowicz, M. J. 1984. Why do temperate deciduous trees leaf out at different times? adaptation and ecology of forest communities. *The American Naturalist* 124:821–842.
- Letten, A. D., S. Kathleen Lyons, and A. T. Moles. 2013. The mid-domain effect: it’s not just about space. *Journal of Biogeography* 40:2017–2019.
- Miller-Rushing, A. J., T. T. Hoyer, D. W. Inouye, and E. Post. 2010. The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B-Biological Sciences* 365:3177–3186. PT: J; NR: 92; TC: 19; J9: PHILOS T R SOC B; PG: 10; GA: 647IL; UT: WOS:000281611800006.
- Miller-Rushing, A. J., D. W. Inouye, and R. B. Primack. 2008. How well do first flowering dates measure plant responses to climate change? the effects of population size and sampling frequency. *Journal of Ecology* 96. PT: J; TC: 37; UT: WOS:000260108500016.
- Mimura, M., and S. Aitken. 2010. Local adaptation at the range peripheries of sitka spruce. *Journal of evolutionary biology* 23:249–258.
- Morin, X., J. Roy, L. Sonié, and I. Chuine. 2010. Changes in leaf phenology of three european oak species in response to experimental climate change. *New Phytologist* 186:900–910.
- Ogle, K., J. J. Barber, G. A. Barron-Gafford, L. P. Bentley, J. M. Young, T. E. Huxman, M. E. Loik, and D. T. Tissue. 2015. Quantifying ecological memory in plant and ecosystem processes. *Ecology letters* 18:221–235.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* 37:637–669.

- Primack, R. B., I. Ibanez, H. Higuchi, S. D. Lee, A. J. Miller-Rushing, A. M. Wilson, and J. A. S. Jr. 2009. Spatial and interspecific variability in phenological responses to warming temperatures. *Biological Conservation* 142. PT: J; TC: 28; UT: WOS:000270203100022.
- Primack, R. B., and A. J. Miller-Rushing. 2009. The role of botanical gardens in climate change research. *New Phytologist* 182. PT: J; TC: 26; UT: WOS:000264635400006.
- Schwartz, M. D., E. G. Beaubien, T. M. Crimmins, and J. F. Weltzin. 2013. North america. Pages 67–89 *in* *Phenology: an integrative environmental science*. Springer.
- Team, R. C. 2016. R: A language and environment for statistical computing.
- Willis, C. G., B. R. Ruhfel, R. B. Primack, A. J. Miller-Rushing, J. B. Losos, and C. C. Davis. 2010. Favorable climate change response explains non-native species’ success in thoreau’s woods. *Plos One* 5:e8878. PT: J; TC: 27; UT: WOS:000273896500007.
- Wolkovich, E., B. Cook, and J. Regetz. 2012*a*. Nectar: Network of ecological and climatological timings across regions.
- Wolkovich, E. M., and E. E. Cleland. 2014. Phenological niches and the future of invaded ecosystems with climate change. *AoB Plants* 6:plu013.
- Wolkovich, E. M., B. I. Cook, J. M. Allen, T. M. Crimmins, J. L. Betancourt, S. E. Travers, S. Pau, J. Regetz, T. J. Davies, N. J. B. Kraft, T. R. Ault, K. Bolmgren, S. J. Mazer, G. J. McCabe, B. J. McGill, C. Parmesan, N. Salamin, M. D. Schwartz, and E. E. Cleland. 2012*b*. Warming experiments underpredict plant phenological responses to climate change. *Nature* 485:494–497.
- Wolkovich, E. M., and A. K. Ettinger. 2014. Back to the future for plant phenology research. *New Phytologist* 203:1021–1024. PT: J; TC: 0.

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
<i>Aesculus flava</i> (5)	<i>Carya glabra</i> (5)	<i>Catalpa speciosa</i> (5)
<i>Betula alleghaniensis</i> (5)	<i>Carya ovata</i> (5)	<i>Kalopanax septemlobus</i> (3)
<i>Betula nigra</i> (5)	<i>Crataegus crus-galli</i> (5)	<i>Styphnolobium japonicum</i> (5)
<i>Gleditsia triacanthos</i> (5)	<i>Fagus engleriana</i> (4)	<i>Tilia americana</i> (5)
<i>Liriodendron tulipifera</i> (5)	<i>Fagus grandifolia</i> (5)	<i>Tilia japonica</i> (5)
<i>Phellodendron amurense</i> var. <i>lavalleyi</i> (4)	<i>Fraxinus chinensis</i> (5)	
<i>Populus deltoids</i> ssp. <i>deltoids</i> (5)	<i>Liquidambar styraciflua</i> (5)	
<i>Pyrus calleryana</i> var. <i>dimorphophylla</i> (3)	<i>Platanus occidentalis</i> (5)	
<i>Pyrus ussuriensis</i> var. <i>hondoensis</i> (5)	<i>Quercus glandulifera</i> (4)	
<i>Quercus alba</i> (5)	<i>Quercus rubra</i> (5)	

Figures

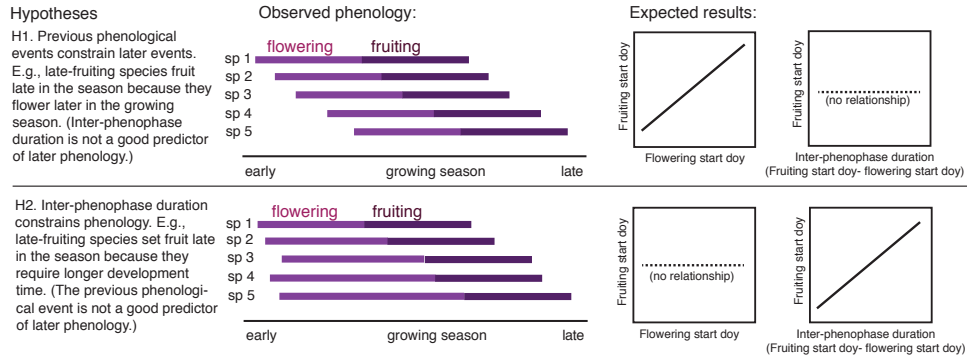


Figure 1: Hypotheses. We show flowering and fruiting as examples of consecutive phenological events. We expected the same patterns for other consecutive events, such as leaf budburst and leafout. Inter-phenophase duration is the time between phenological events, e.g., the number of days between the start of flowering and the start of fruiting.

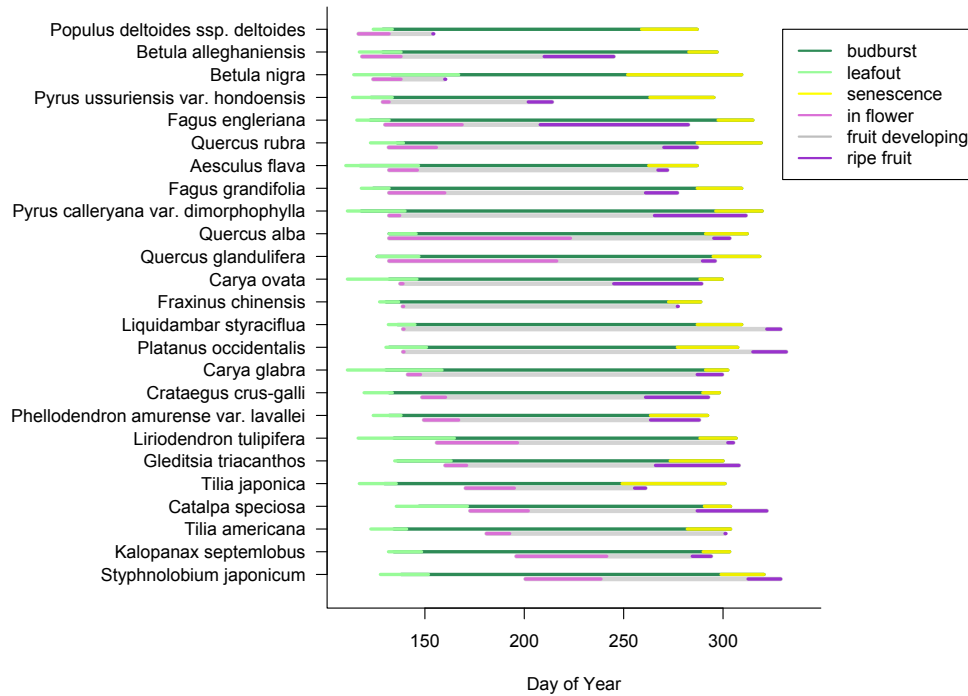


Figure 2: Species' phenology during the 2015 growing season, ordered by mean first-flower dates. Growth phenology is shown for budburst (from its mean start day-of-year to the mean start day-of-year for leafout, across all individuals within a species), leafout (from the mean day-of-year when fully-expanded 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).

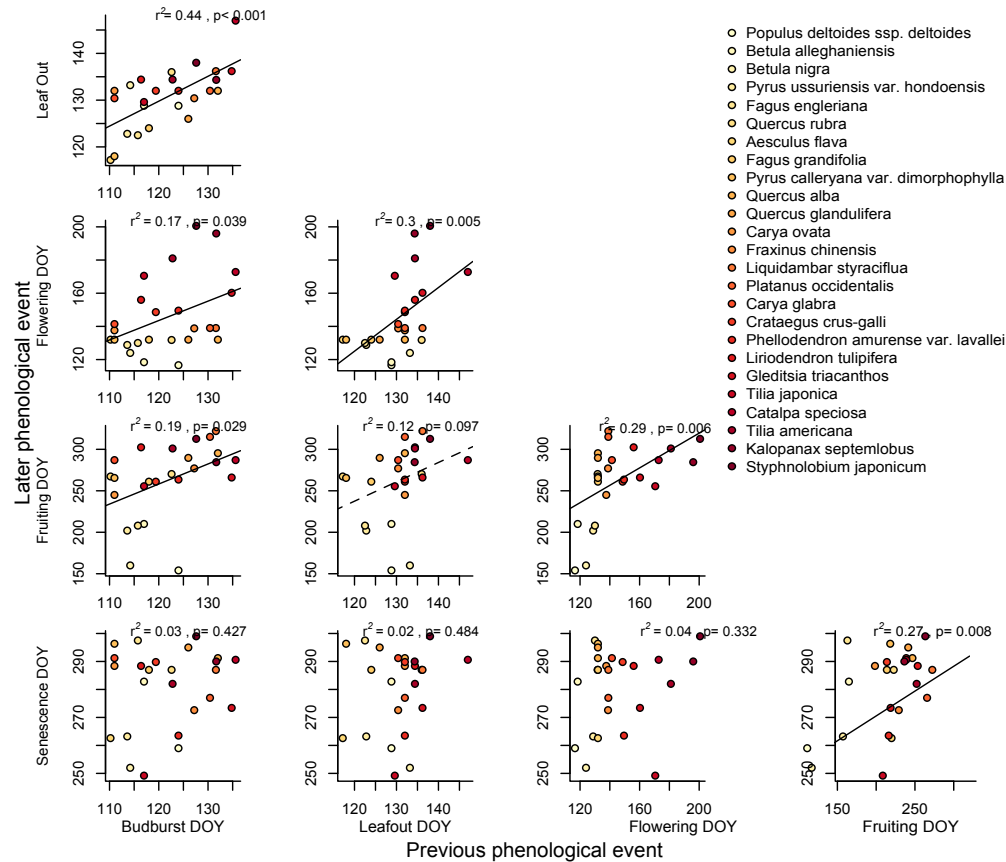


Figure 3: **Relationships among phenological stages across the 25 focal species.** Linear models were fit with the species-level mean day of year of the later phenological stages as the response variable, and mean day of year of earlier stage as the explanatory variable. R^2 and p -value for each model 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.

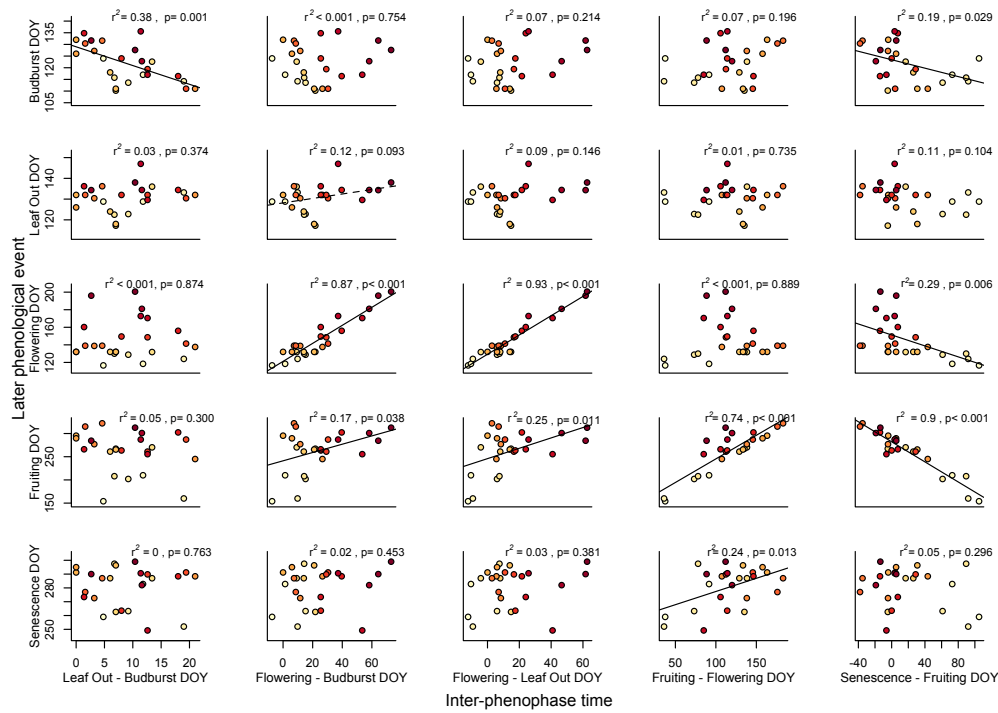


Figure 4: **Relationships among phenological stages and inter-phenophase duration across the 25 focal species.** Inter-phenophase duration is the time between the start of the earlier phenological event and the start of the later phenological event, e.g., the number of days between the species' mean 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.