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## Phenological sequences: how early-season events define those that follow

--Manuscript Draft--

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<b>Abstract:</b>	Premise of the study: Plant phenology is a critical trait as the timings of phenophases such as budburst, leafout, flowering, and fruiting, are important to plant fitness. Despite much study about when individual phenophases occur and how they may shift with climate change, little is known about how multiple phenophases 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 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 weakly (most $r^2 < 0.30$ ) constrain most later events, with the strongest constraints (maximum $r^2 = 0.43$ ) seen between consecutive stages. In contrast interphase duration was a much stronger predictor of phenology ( $r^2 = 0.17\text{--}0.98$ ), especially for reproductive events ( $r^2 > 0.7$ ), suggesting that the development time of flowers and fruits constrain the phenology of these events. Conclusions: We found that much of the variation in later phenological events can be explained by the timing of earlier events and by interphase durations. This highlights that a shift in one phenophase may often have cascading effects on later phases, thus accurate forecasts of climate change impacts should include multiple phenophases within and across years.
<b>Keywords:</b>	plant phenology; climate change; budburst; leafout; flowering; fruiting; senescence; angiosperm; tree; arboretum
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March 21, 2018

Dear Dear Editors:

Please consider our paper, entitled “Phenological sequences: how early-season events define those that follow,” for publication as a “Brief Communication” in the *American Journal of Botany*.

Phenology, or the timing of life events such as spring flowering and leafout, has gained increasing prominence in ecology as one of the most widely documented biological impacts of anthropogenic climate change<sup>1-3</sup>. Spring phenology has generally shifted earlier with warmer temperatures<sup>1,4</sup>, but phenology later in the season (late spring flowering, summer fruiting, fall senescence) is less studied and may be more variable<sup>5</sup>. Accurate predictions of phenology across the growing season are critical for forecasting important natural resources (such as nectar for pollinators or fruit for migratory birds) and for forecasting future climate change itself, as the length of the growing season critically impacts global carbon dynamics.

An important, but poorly studied, aspect of plant phenology is that phenological events are inherently linked through their order: leaf budburst typically occurs before flowering, and flowering always precedes fruiting. This ordering may constrain how some phenological events can respond to climate change. However, the extent to which previous phenological events are correlated with later phenological events is not known because few studies to date have integrated across multiple phenological events within individual trees during a growing season. Instead, previous studies have focused either on events related to leaf phenology (including spring budburst, leafout, and fall senescence), or reproductive events, especially flowering<sup>6</sup>.

In this paper, we report on observations of consecutive phenophases from the start through the end of the growing season, across 25 temperate tree species with divergent flowering phenology, grown in a common environment. We test if previous phenological events constrain later events; e.g., do late-fruiting species set fruit late in the season because they flower and leaf out late? In addition, we test whether interphase duration constrains phenology; e.g., do late-fruiting species set fruit late in the season because they require longer fruit maturation time?

We find strong effects of both early phenology and interphase duration, highlighting the need to include previous phenological information when forecasting future phenology. Our findings have implications that are broadly important, including for improved understanding of plant phenology and for forecasting climate change induced shifts in phenology: Our finding that early phenological events constrain later events suggests that climatic shifts in one season, even if they directly affect only one phenophase, will have cascading effects on phenology later in the season.

We suggest as potential reviewers David Inouye, Allison Donnelly, Nicole Rafferty, Paul CaraDonna, Martin Lechowicz, and Amy Iler. Thank you for your time and consideration of our paper.

Sincerely,

A handwritten signature in black ink that reads "Ailene K. Ettinger".

Ailene Ettinger (on behalf of both authors) Postdoctoral Fellow, Arnold Arboretum of Harvard University & Biology Department, Tufts University

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1 Phenological sequences: how early-season events define those that  
2 follow

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10 March 21, 2018

## 11 Abstract

### 12 Premise of the study

13 Plant phenology is a critical trait as the timings of phenophases such as budburst, leafout, flowering, and  
14 fruiting, are important to plant fitness. Despite much study about when individual phenophases occur and  
15 how they may shift with climate change, little is known about how multiple phenophases relate to one another  
16 across an entire growing season. We test the extent to which early phenological stages constrain later ones,  
17 throughout a growing season across 25 angiosperm tree species.

### 18 Methods

19 We observed phenology (budburst, leafout, flowering, fruiting, and senescence) of 118 individual trees across  
20 25 species, from April through December 2015.

<sup>21</sup> **Key results**

<sup>22</sup> We found that early phenological events weakly (most  $r^2 < 0.30$ ) constrain most later events, with the  
<sup>23</sup> strongest constraints (maximum  $r^2 = 0.43$ ) seen between consecutive stages. In contrast interphase duration  
<sup>24</sup> was a much stronger predictor of phenology ( $r^2 = 0.17-0.98$ ), especially for reproductive events ( $r^2 > 0.7$ ),  
<sup>25</sup> suggesting that the development time of flowers and fruits constrain the phenology of these events.

<sup>26</sup> **Conclusions**

<sup>27</sup> We found that much of the variation in later phenological events can be explained by the timing of earlier  
<sup>28</sup> events and by interphase durations. This highlights that a shift in one phenophase may often have cascading  
<sup>29</sup> effects on later phases, thus accurate forecasts of climate change impacts should include multiple phenophases  
<sup>30</sup> within and across years.

<sup>31</sup> **Key words**

<sup>32</sup> plant phenology, climate change, budburst, leafout, flowering, fruiting, senescence, angiosperm, tree, arbore-  
<sup>33</sup> tum

<sup>34</sup> **Introduction**

<sup>35</sup> Plant phenology, the timing of recurring life-events such as leafout and flowering, is a critical trait that  
<sup>36</sup> affects individual fitness, population abundance, agricultural and natural productivity, and global climate,  
<sup>37</sup> through its role in carbon sequestration (Chuine and Beaubien, 2001; Cleland et al., 2007; Willis et al., 2010;  
<sup>38</sup> Miller-Rushing et al., 2010; Craine et al., 2012). Advancement of budburst, leafout, and other phenophases  
<sup>39</sup> are some of the most widely documented biological impacts of anthropogenic climate change, and phenology  
<sup>40</sup> is likely to be further altered by future climate change (Parmesan, 2006). Because of its important role in  
<sup>41</sup> many ecosystem services and in the global climate cycle, improved understanding and forecasting of tree  
<sup>42</sup> phenology would aid in planning and preparing for climate change impacts.

<sup>43</sup> Despite the observation that spring phenology generally shifts earlier with warmer temperatures, dramatic  
<sup>44</sup> variation exists in phenological responses to climate. Temperature is thought to be a major factor controlling  
<sup>45</sup> phenology of temperate tree species (Parmesan, 2006; Richardson et al., 2006; Morin et al., 2010; Schwartz

et al., 2013; Clark et al., 2014), but some populations and species have not shifted their phenology with recent warming (Wolkovich et al., 2012). 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). Spring leafout, for instance, can span weeks among coexisting tree species (Lechowicz, 1984). It has been proposed that, in addition to external environmental drivers such as temperature, some drivers of plant phenology are “endogenous.” That is, phenological sequences are affected by changes in internal tree functions that may not be related to climate or other environmental factors (Borchert, 1992; Marco and Páez, 2002). As an example, inflorescence architecture may affect the sequence of leafout to flowering in trees (Marco and Páez, 2002).

One important, but often neglected, feature of plant phenology is that events are sequential: leaf budburst comes before leafout, flowering comes before fruiting, and so on. This ordering is an endogenous factor that may constrain phenological responses to climate change. For example, if flowering requires a minimum development time following leafout (e.g. to acquire sufficient carbon), then flowering time may be constrained to shift no more than leafout timing has with recent climate change. This would be the case even if a greater advance in flowering may benefit the plant, for example if warmer springs have caused pollinator activity to shift earlier (Polgar et al., 2013; Thackeray et al., 2010)

The extent of constraints between phenological events is unknown, however, because few studies have integrated across consecutive events throughout a growing season (Wolkovich and Ettinger, 2014). Instead, researchers generally focus on one or two phenophases per study. Early season events (budburst and/or leafout) have been extensively studied, often using climate-controlled growth chambers (e.g., Basler and Körner, 2012; Laube et al., 2014). A separate group of studies, comprised of long-term observational data, focuses primarily on flowering only (e.g., Fitter and Fitter, 2002; Miller-Rushing and Primack, 2008). Interest has recently surged in senescence, which had been less studied historically (Parmesan, 2006), but many of these studies focus *only* on senescence (e.g., Taylor et al., 2008; Archetti et al., 2013; Jeong and Medvigy, 2014). A contemporary meta-analysis highlights the lack of data on multiple phenophases: only five out of 51 phenology studies (9.8%) included data on both leaf and flower phenology (Wolkovich et al., 2012).

When research has looked across stages, important links have often been found. For example, later leafing in a given year may be associated with later flowering, and fall senescence has been associated with both fruit maturation and spring phenology, in different studies (Lechowicz, 1995; Keenan and Richardson, 2015; Liu et al., 2016). In contrast, other research that has quantified multiple phenological events over time has documented that some phenophases may shift asynchronously with climate change (e.g., spring events are occurring earlier as fall events have gotten later, first-flower dates have shifted earlier whereas last-flower

78 dates have not, Menzel et al., 2006; CaraDonna et al., 2014). These insights and complications demonstrate  
79 the need to better understand how phenological stages relate to one another across an entire growing season  
80 (Wolkovich and Ettinger, 2014).

81 Here, we examine the extent to which early-season phenological events constrain later events across tree  
82 species planted in the same environment. Specifically, we test two hypotheses:

- 83 • *Hypothesis 1: Previous phenological events constrain later events;* e.g., late-fruited species set fruit late  
84 in the season, because they flower and leafout late (Figure 1). To be consistent with this hypothesis,  
85 we expected earlier events, such as flowering, would predict later events, such as fruiting. If constraints  
86 are strong, then, across all species, we expected that previous events should predict later events with a  
87 slope of one, indicating that the later event happens a set number of days (represented by the intercept)  
88 after the previous event (Figure 1). If constraints are weak, we expected to find relationships between  
89 later and earlier events, though the slope may be less than one. No relationship would suggest that  
90 earlier events do not constrain later events.
- 91 • *Hypothesis 2: Interphase duration constrains phenology;* e.g., late-fruited species set fruit late in the  
92 season because they require longer maturation time (Figure 1). To be consistent with this hypothesis,  
93 we expected that the interphase duration (the number of days between two phenological events) between  
94 earlier and later events would be a strong predictor (e.g., a large  $r^2$ ) of the later event, regardless of the  
95 timing of the earlier event (Figure 1).

96 Testing these hypotheses addresses basic, critical questions about drivers of variation in temperate tree  
97 phenology. These questions remain unanswered despite decades of phenology research because previous field  
98 studies rarely (if ever) examined multiple phenophases spanning the entire growing season across a large  
99 number of tree species.

## 100 Materials and Methods

### 101 Study site and focal species

102 This study was conducted at the Arnold Arboretum of Harvard University, a 281-acre park in Boston,  
103 Massachusetts, established in 1872. It contains a living collection of 3,825 woody plant taxa that are native  
104 to North America, Europe, and Asia. Arboreta are excellent resources for phenological studies across many

105 species (e.g., Primack and Miller-Rushing, 2009), particularly in temperate areas, since they may contain  
106 a higher diversity of tree species growing in one location than nearby natural areas. In addition, there is  
107 often high variation in phenology of species planted in arboreta, for public enjoyment of foliage and flowers  
108 throughout the season. For this study, we selected 25 focal angiosperm species with diverse flowering times,  
109 in order to maximize variation in phenology (Table 1). We selected up to five individuals of each species for  
110 the study, yielding a total of 118 individuals.

## 111 Phenology data collection

112 We visited each individual once every 6-10 days throughout the growing season. Phenology observations in  
113 the spring began on April 6, 2015 (prior to any budburst occurring) and fall phenology observations ended on  
114 December 2, 2015 (at which point more than 95% of leaves on all trees had changed color and dropped). We  
115 observed five phenological stages, which were quantified following the National Phenology Network (NPN)  
116 protocols (for a full description see Denny et al., 2014). The budburst phase was characterized by green leaf  
117 tips being visible at the tips of buds, and the leafout phase was characterized by visible fully unfolded leaves  
118 and petioles that had completely emerged from the buds. The flowering phase was when open flowers were  
119 visible, and the fruiting phase was defined by ripe fruit being visible. Leaf senescence was characterized by  
120 leaves changing from green to fall colors. On each observation day, we estimated the presence and abundance  
121 of each phenophase on each individual tree.

122 From the field observation data, we extracted the day-of-year (DOY) of the first observed occurrence of a  
123 given phenological phase. Budburst and fruiting DOY were defined as the first day when three or more burst  
124 leaf buds or ripe fruits, respectively, were observed on the individual. Leafout, flowering, and leaf senescence  
125 DOY were defined as the first day when 5% or more of the individual was leafing out, flowering, or showed fall  
126 colors, respectively (Denny et al., 2014). From these individual tree phenology observations, we calculated  
127 species-level mean start dates and for all phenophases, for use in our statistical analyses. We used these mean  
128 start dates to calculate interphase durations between phenophases.

## 129 Statistical analyses

130 To understand the extent to which previous phenological events constrain later events across species (Hypothesis 1,  
131 Figure 1), we fit linear models in which the response variable was phenological stage (i.e., the species'  
132 mean DOY of leafout, flowering, fruiting, or senescence), and the predictor was previous phenological stage.  
133 Thus, budburst was excluded as a response variable, because it was the earliest stage we quantified, and

134 senescence was excluded as a predictor variable because it was the latest stage we quantified. We therefore  
135 fit 10 separate regression models, estimating the intercept of the relationship between later and previous  
136 phenological phases, and forcing the slope to be one (Hypothesis 1, Figure 1). In addition, we fit 10 models,  
137 with the same predictor and response variables, in which we estimated the best-fit slope and intercept (via  
138 least-squares, e.g., a standard regression model). Under Hypothesis 1, with strong constraints, we expected  
139 that the models with forced slopes should provide similar fit to the data as the standard regression models  
140 that estimate both slopes and intercepts. We compared fit of these two model structures using r-squared  
141 values, as well as Akaike's Information Criterion (AIC).

142 To understand the extent to which interphase duration constrains later phenological events (Hypothesis 2,  
143 Figure 1), we fit linear models in which the response variable was phenological stage, and the predictor was the  
144 number of days between phenological stages. Thus, as above, budburst was excluded as a response variable.  
145 We therefore fit 10 different models, each with one of four phenological stages as the response variable and one  
146 of the four interphase durations as a predictor. To investigate the effect of interphase duration, separate from  
147 the constraint imposed by the inherent ordering of events, we fit models in which the interphase durations  
148 were randomized with respect to the timing of the earlier phenophase across species. We did this resampling  
149 of interphase duration 999 times for each model structure. If our expectation of later events being constrained  
150 by interphase duration was supported, then the best-fit slope should fall within the resampled models' slope  
151 estimates. Furthermore, if the constraints imposed by interphase duration were due primarily to the inherent  
152 ordering of events, then the randomized models should include a narrow range of possible slopes. To examine  
153 these possibilities, we compared the range of slopes of all the resampled models to the slope of the fitted  
154 model.

155 All analyses were conducted in R version 3.2.4 (R Core Team, 2017), and code is available in the Supplemental  
156 Materials.

## 157 Results

158 We monitored five phenophases, which varied in duration. First budburst date occurred over 32 days and first  
159 leafout date occurred over 30 days in the spring, across all focal individuals (Figure S1) and species (Figure  
160 2). Flowering phenology occurred over a longer period than budburst and leafout, spanning 131 days from  
161 late April to September. The first observation of ripe fruit spanned 175 days, and the start of leaf senescence  
162 occurred over 56 days across all individuals and species. Most species (20/25) spent the majority of the

163 growing season in the reproductive phenological phases (i.e., flowering and fruit development), and most  
164 species (23/25) began leaf budburst prior to flowering, though leaf development overlapped with flowering in  
165 some species (Figure 2). The majority of species (15/25) produced ripe fruit prior to beginning senescence  
166 (Figure 2).

167 We found that the timing of early phenological stages predicted the timing of later stages in many cases  
168 (Figures 2-3, Table S1). The strongest relationships (i.e., with the most variation explained) occurred between  
169 adjacent stages (those along the diagonal in Figure 3, such as leafout and budburst, fruiting and flowering).  
170 Even for adjacent phases, however, neither the forced slope models nor the regression models explained a  
171 large proportion of the variation in phenology ( $r^2 < 0.30$ , in most cases). For three relationships (flowering  
172 versus budburst, flowering versus leafout, and fruiting versus flowering), the model fits were consistent with  
173 the constraint hypothesis—the forced slope model provided similar fit to the regression models and both  
174 models explained a significant amount of variation in phenology (Figure 3, Table S1). For four relationships,  
175 standard regression models explained much more variation than the forced slope models. For two of these  
176 relationships (leafout versus budburst, and senescence versus fruiting), the regression models had slopes that  
177 were less than one and for the other two relationships (fruiting versus budburst and fruiting versus leafout),  
178 the regression models had slopes that were greater than one (Figure 3, Table S1).

179 We observed strong relationships ( $r^2 > 0.7$ ) between phenology and interphase duration for the two reproduc-  
180 tive phenophases (flowering and fruiting time, Figure 4, Table S2). Flowering DOY was strongly predicted by  
181 days between flowering and leafout ( $r^2 = 0.93$ ), as well as by days between flowering and budburst ( $r^2 = 0.87$ ).  
182 Fruiting DOY was strongly predicted by days between fruiting and flowering stages ( $r^2 = 0.74$ ), by days be-  
183 tween fruiting and leafout ( $r^2 = 0.98$ ), and by days between fruiting and budburst ( $r^2 = 0.97$ ). Senescence  
184 was predicted by days between senescence and budburst ( $r^2 = 0.74$ ), days between senescence and leafout  
185 ( $r^2 = 0.82$ ), and days between senescence and flowering ( $r^2 = 0.17$ ); senescence was not affected by days be-  
186 tween senescence and fruiting. Leafout was not predicted by interphase duration (i.e., time since budburst).  
187 In all cases where later events were well predicted ( $r^2 > 0.7$ ) by interphase duration, the best fit model was  
188 predicted by our null model (i.e., the randomizations). However, the relationship between senescence and  
189 days between senescence and flowering was weaker than expected by our null model (see resampling estimates  
190 in Figure 4, Table S2).

## 191 Discussion

192 The ordering inherent in phenology means most phenological stages are dependent—in some way—on those  
193 stages before: dormancy must be broken before buds can burst and flowers must be produced before fruit.

194 Here we show how the major phenological stages of temperate trees are constrained by one or more earlier  
195 phenological stages.

196 All phenological stages we observed support Hypothesis 1: timing appears to be constrained by at least one  
197 previous phenological stage. Our findings are consistent with recent work suggesting that senescence time can  
198 be affected by earlier phenophases (Keenan and Richardson, 2015; Liu et al., 2016), and suggest that this one  
199 relationship is part of larger suite of correlated phenophases. Consecutive events were correlated across both  
200 growth and reproductive phenophases (i.e., flowering and leafout were correlated to a similar degree as fruiting  
201 and flowering, Figure 3). These associations may occur because of endogenous dependencies between the two  
202 phases, because of a shared external driver such as growing degree days, or a combination of endogenous  
203 and external factors (Lechowicz, 1995). Thus, environmental conditions in the winter or spring that may  
204 directly affect only early phenological stages, such as budburst, are likely to have cascading effects on later  
205 stages such as leafout, flowering, and fruiting. Our data suggest that, for most events, these effects are more  
206 apparent for consecutive stages (i.e., those along the diagonal in Figure 3), and are well-approximated by the  
207 forced slope model in some cases (Figure 3).

208 Although some of the variation in reproductive phenology (flowering and fruiting) was explained by previous  
209 phenology (Hypothesis 1), much more variation was explained by interphase duration (Hypothesis 2). Later  
210 flowering species generally required more time between flowering and leafout. Similarly, late fruiting species  
211 had longer interphase durations between the first observation of ripe fruit and first flowering date. It may  
212 be that late fruiting species require longer fruit development times to produce larger fruits or more highly-  
213 provisioned seeds. This would be consistent with previous theories that trees investing more resources into  
214 their offspring (i.e., having larger seeds) require more time to build resources (Bolmgren and D Cowan, 2008;  
215 Sun and Frelich, 2011). There were notable exceptions to this general relationship, however. Some species set  
216 fruit later than expected, given their interphase duration between flowering and fruiting (*Catalpa speciosa*,  
217 *Tilia americana*, *T. japonica*, Figure 4). These species also flowered later than expected, given their leafout  
218 DOY (Figure 3). External factors related to their ecology may be the cause; for example, these species are all  
219 insect-pollinated, so the timing of their pollinator activity may have affected their floral phenology (Elzinga  
220 et al., 2007).

221 Despite our expectation of strong relationships between later phenophases and interphase durations (Figure

222 4), our results show two relationships that are weaker than expected. Leafout was not predicted by the  
223 interphase duration between budburst and leafout, and senescence was not predicted by the interphase  
224 duration between fruiting and senescence (Figure 4). We had expected that these two sets of phases would  
225 demonstrate *stronger* constraints of interphase duration because they occur at the beginning and end of a  
226 bounded growing season (Letten et al., 2013). The weak ability of interphase duration to predict leafout  
227 may be due to the distinct weather patterns in 2015 and how they interacted with species' cues for spring  
228 phenology. Trees have species-specific chilling and forcing requirements that must be met prior to leafing out,  
229 and are generally understood to be related to accumulations of warm and cold temperatures (e.g., Schwartz  
230 and Hanes, 2010; Chuine, 2010; Clark et al., 2014). Because of this, the pattern of how quickly cooler  
231 and warmer temperatures accumulate across a growing season can impact how variable leafout is across  
232 species. In contrast to some years that have high variation in leafout date across species (Lechowicz, 1995),  
233 in our study year (2015) many species leafed out close to DOY 130 (10 May), regardless of leafout-budburst  
234 interphase duration (which ranged from 0 to 20 days, Figure 4). This could be due to the temperature  
235 conditions particular to 2015: temperatures were colder than average in January through March, and then  
236 switched to above-average in late April and early May ([www.bluehill.org](http://www.bluehill.org)). Such long periods of cold followed  
237 by rapid warming may have meant that chilling requirements were met for all species well before warm  
238 temperatures began, and then forcing requirements were rapidly met for many species (even if they had  
239 diverse requirements) leading to a flush of leafout in early May, across diverse species. Variation in the  
240 environment each year may alter how later phenophases are constrained by earlier events and highlights the  
241 need to better understand how such constraints vary across years and what underlies the remaining variation  
242 not explained by earlier phenophases.

243 Our results indicate that *both* Hypothesis 1 and Hypothesis 2 are operating and can explain a substantial  
244 amount of the variation observed among species in a single year. For example although later fruiting is gen-  
245 erally associated with later flowering (Figure 3), some species, such as *Quercus alba* and *Quercus grandifolia*,  
246 flower relatively early and fruit late; later fruiting for these species is instead associated with longer inter-  
247 phase duration between fruiting and flowering (Figure 4). Understanding drivers of variation in phenological  
248 responses among species requires disentangling the ways that earlier phenology and interphase duration in-  
249 teract with one another, and with environmental conditions. Experimental manipulations will be beneficial  
250 for discerning the physiological and genetic bases for the relationships we observe (Flint, 1974).

251 Our findings have important implications for improved forecasting of climate change induced shifts in phenol-  
252 ogy. A shift in one phase may have cascading effects on later phases, since each phase is linked to phases that  
253 occur before and after it (Wolkovich and Cleland, 2014). Phenology models covering the whole annual cycle

254 are rare for wild plants (but see Chuine and Beaubien, 2001) and have not been used to forecast phenology  
255 at the community level. Our results highlight a clear need to conduct future multi-species phenology studies  
256 across entire growing seasons (Wolkovich and Ettinger, 2014), and begs the question of how phenophases  
257 may be linked across years, as well (e.g., Elmendorf et al., 2016). For example, the timing of spring budburst  
258 in one year may be related to the timing of budset the previous fall (Mimura and Aitken, 2010). Although  
259 relationships between phenophases have not been widely studied, there is a growing ecological literature on  
260 the concept of “ecological memory,” or the capacity of past states to influence present or future responses  
261 (Ogle et al., 2015). The ecological memory of phenology has not been quantified, but may be critical for  
262 accurate forecasting, particularly for species like *Quercus rubra*, which require more than one year for fruit  
263 maturation. Given the species-specific nature of phenological constraints, accurate forecasts of community-  
264 wide phenological shifts are likely to require species-specific information, such as fruit development time for  
265 fruiting forecasts, in addition to climate data (Diez et al., 2012).

## 266 Conclusions

267 We have shown that early and late phenological stages are strongly linked across the growing season, providing  
268 a new approach to explain some of the dramatic variation in phenological responses observed to date. Many  
269 studies have sought to identify the particular environmental drivers of phenology (e.g., Morin et al., 2010;  
270 Schwartz et al., 2013). Our findings here suggest that timing and duration of previous phenological states  
271 should also be examined. In addition, identifying the appropriate temporal window for both environmental  
272 and endogenous drivers is essential (Teller et al., 2016). Because earlier phenophases define those that follow,  
273 the relevant time period for these drivers may extend further back in time than the single growing season  
274 we evaluated here. Multi-year studies will be critical to evaluate the extent to which phenological patterns  
275 are consistent among years that may vary in climate, as well as biotic conditions (i.e., pollinator or pest  
276 populations, Lechowicz, 1995). A fuller understanding of phenological constraints and drivers of phenological  
277 variation offers the potential for improved forecasts of phenological shifts with climate change to help predict  
278 how ecosystem functions will be altered in the future.

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## 288 Data Accessibility

289 The data set for this study is available online at Knowledge Network for Biocomplexity (Gee et al., 2017).

## 290 Author contributions

291 All authors conceived of and designed the study and edited the manuscript; S.G. conducted the fieldwork;  
292 S.G. and A.E. analyzed the data and wrote the manuscript.

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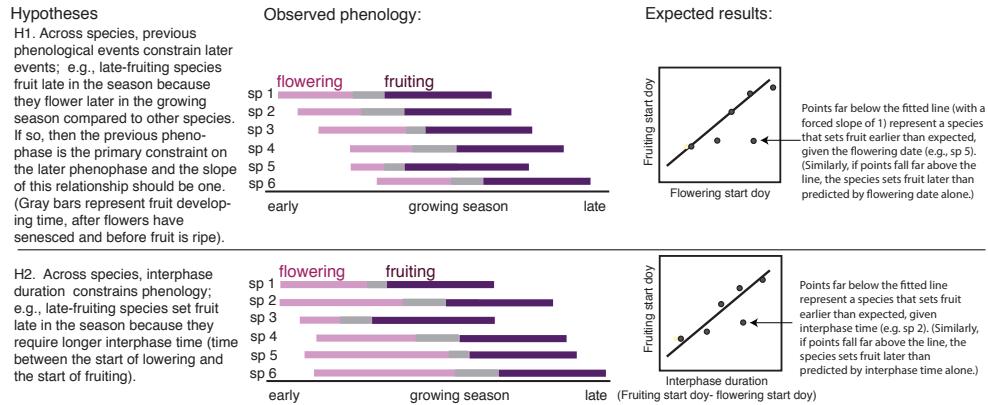
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406 **Tables**

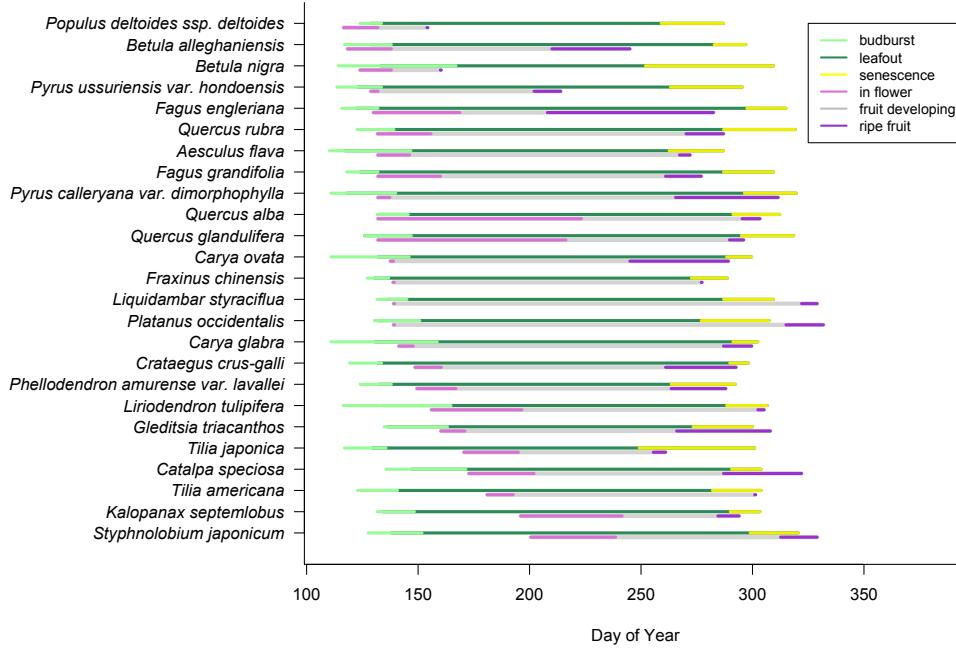
Table 1: **Study species.** Twenty-five angiosperm species were selected based on their flowering phenology in long-term records of the Arnold Arboretum. The flowering patterns we observed during our one year of data collection did not always perfectly match these long-term patterns. The number of individuals 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>lavallei</i> (4)	<i>Fraxinus chinensis</i> (5)	
<i>Populus deltoides</i> ssp. <i>deltoides</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)	

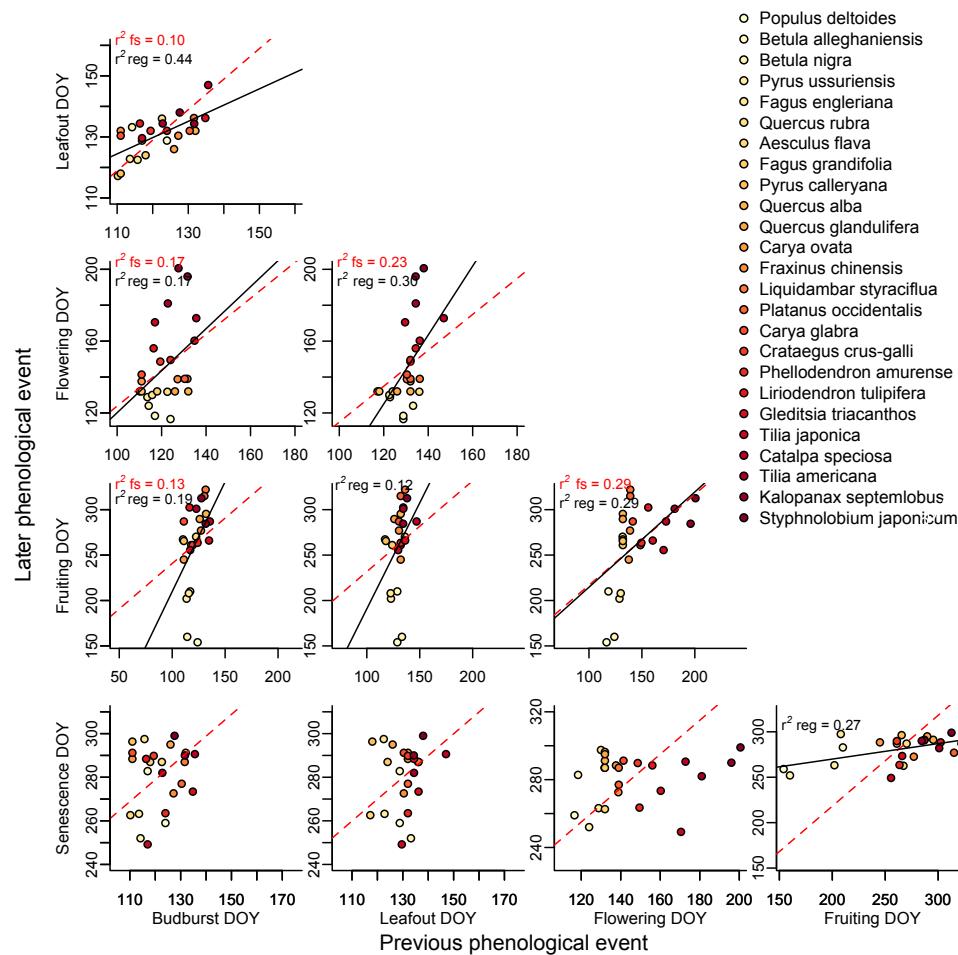
407 **Figures**



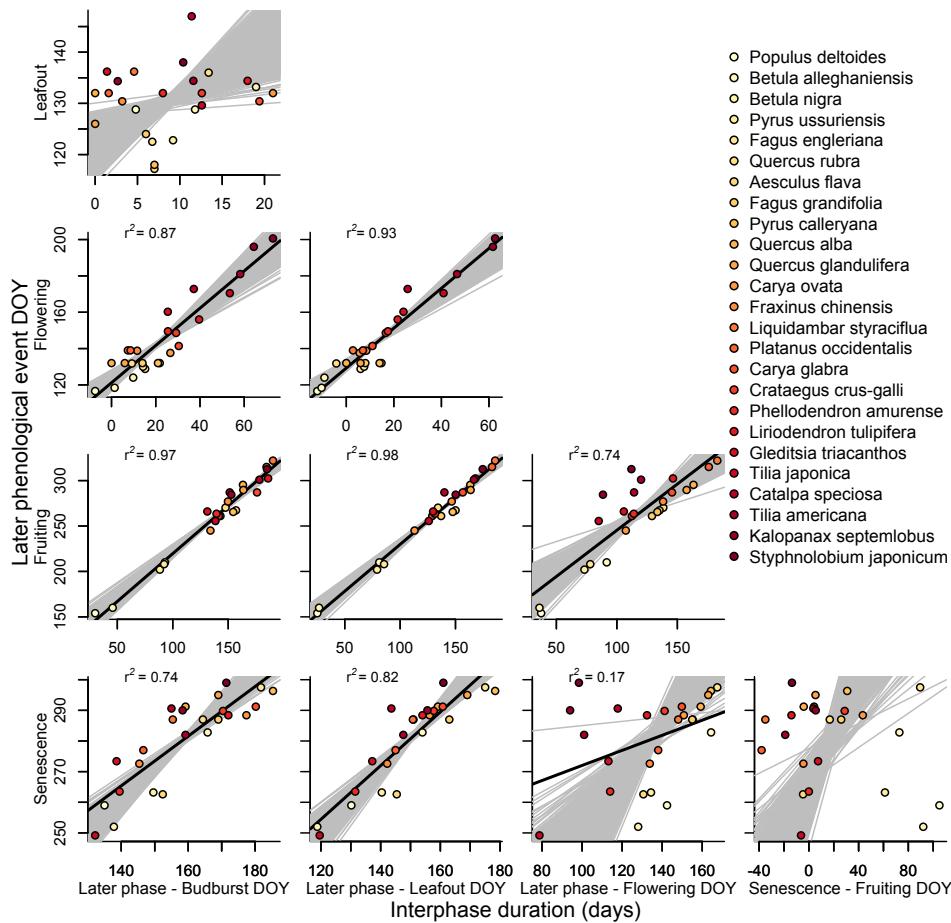
**Figure 1: Hypotheses.** We show flowering and fruiting as examples of consecutive phenological events. We expected the same patterns for other consecutive events, such as leaf budburst and leafout. Interphase duration is the time between phenological events, e.g., the number of days between the first day of flowering and the first day that ripe fruit were observed.



**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% 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% 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 (DOY) of the later phenological stages as the response variable, and mean day-of-year of earlier stage as the explanatory variable. Models with a forced slope of 1 are shown by dashed red lines, and  $r^2$  is given when  $r^2 > 0.10$ . (“fs”, in red).  $r^2$  for standard regression (“reg,” in black) and lines for these models are shown when  $r^2 > 0.10$  (solid black lines). 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.



**Figure 4: Relationships among phenological stages and interphase duration across the 25 focal species.** Interphase duration (x-axis) 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 (DOY) of the later phenological stages as the response variable, and interphase duration as the explanatory variable. Solid lines (representing model fit) and  $r^2$  are shown when  $r^2 > 0.10$ . Gray lines represent model fits when interphase was randomized with respect to the timing of the earlier phenophase (in some cases the range of possible relationships is quite narrow, given the constraints of ordering inherent in phenological events). When our null expectation of later events being constrained by interphase duration was supported, the best-fit slope (black line) will fall within the randomized lines (in gray). Full model statistics are summarized in Table S2 in the Supplemental Materials. Species in the legend are ordered from early to late first-flower dates.

Supplemental materials for  
Phenological sequences: How early-season events define those that follow

A.K. Ettinger, S. Gee, and E.M. Wolkovich

March 21, 2018

## Supplemental Tables

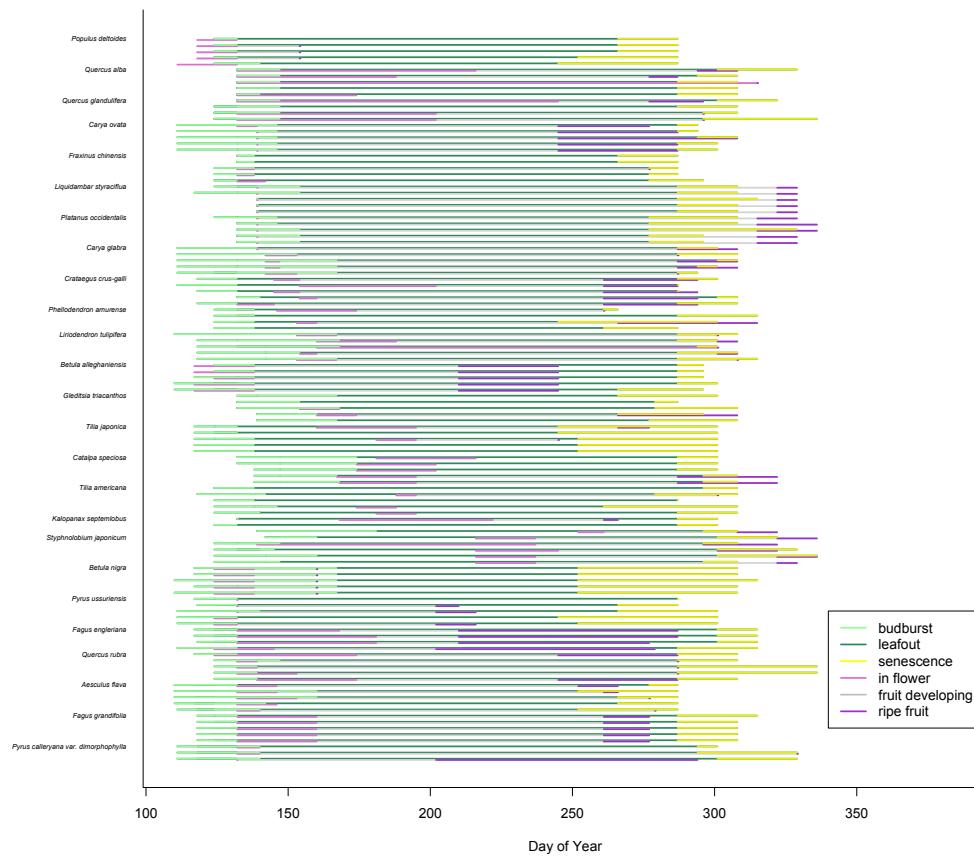
Table S1: Summary of linear models for relationships between later phenophases and earlier phenophases, as shown in Figure 3 in the main text. Two types of linear models were fit: those with the intercept only estimated and a forced slope of one, and those with both the slope and intercept estimated (i.e., a standard regression model). All 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 phenostage as the explanatory variable.

previous phenostage model	forced slope model			standard regression model				
	intercept	r <sup>2</sup>	aic	intercept	slope	p	r <sup>2</sup>	aic
leafout vs. budburst	8.94	0.10	164.78	65.84	0.53	<0.001	0.44	155.01
flowering vs. budburst	23.83	0.17	225.55	3.18	1.17	0.039	0.17	227.45
fruiting vs. budburst	140.71	0.13	260.44	-32.03	2.42	0.029	0.19	260.48
senescence vs. budburst	158.84	-0.12	210.97	243.88	0.30	0.427	0.03	209.43
flowering vs. leafout	14.90	0.23	223.60	-105.28	1.92	0.005	0.30	223.26
fruiting vs. leafout	131.77	0.08	261.78	-42.56	2.33	0.097	0.12	262.74
senescence vs. leafout	149.90	-0.07	209.74	237.39	0.33	0.484	0.02	209.58
fruiting vs. flowering	116.87	0.29	255.39	109.80	1.05	0.006	0.29	257.37
senescence vs. flowering	135.00	-1.79	233.74	261.65	0.13	0.332	0.04	209.08
senesence vs. fruiting	111.97	-5.43	254.66	235.89	0.17	0.008	0.27	202.28

Table S2: Summary of linear models for relationships between later phenophases and interphase duration, as shown in Figure 4 in the main text. Linear models were fit with the species-level mean day-of-year of the later phenological stages as the response variable, and the number of days in each previous interphase duration as the explanatory variable. The random.slopes column gives the range in which 95 percent of slopes in the randomization occur (between 2.5 percent and 97.5 percent).

interphase model	intercept	slope	r <sup>2</sup>	p	aic	random.slopes
leafout vs. leafout-budburst	128.98	0.20	0.04	0.374	168.58	0.47-1.57
flowering vs. flowering-budburst	121.18	1.03	0.88	<0.001	180.24	0.83-1.16
fruiting vs. fruiting-budburst	114.31	1.05	0.97	<0.001	178.39	0.91-1.08
senescence vs. senescence-budburst	151.87	0.81	0.74	<0.001	176.81	0.77-1.22
flowering vs. flowering-leafout	129.28	1.10	0.93	<0.001	167.11	0.85-1.13
fruiting vs. fruiting-leafout	126.69	1.03	0.98	<0.001	168.41	0.93-1.07
senescence vs. fruiting-leafout	149.22	0.88	0.82	<0.001	167.30	0.83-1.18
fruiting vs. fruiting-flowering	143.56	1.02	0.74	<0.001	232.15	0.74-1.24
senescence vs. senescence-flowering	247.41	0.25	0.17	0.041	205.51	0.64-1.38
senescence vs. senescence-fruiting	282.11	-0.08	0.05	0.296	208.91	0.48-1.43

## Supplemental Figures

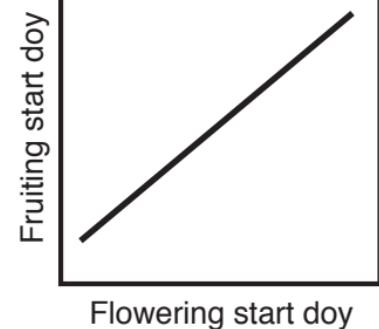
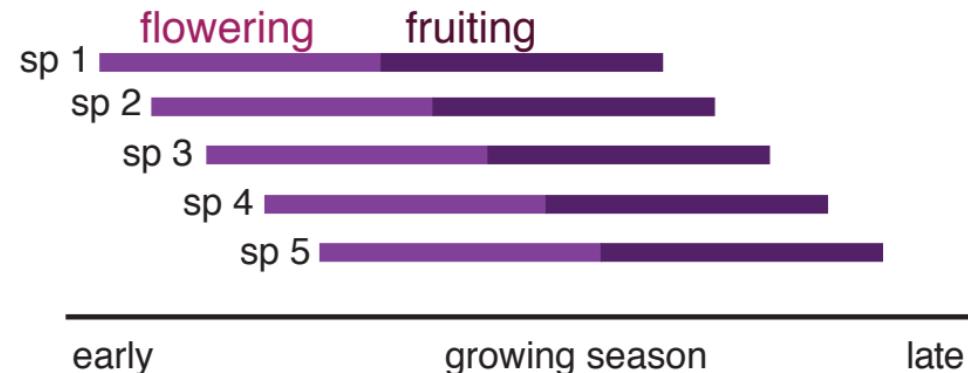


**Figure S1: Individual tree phenology during the 2015 growing season, ordered by species-level mean first-flower dates.** Growth phenology is shown for budburst (from its mean start day-of-year to the mean start day-of-year for leafout, across all individuals within a species), leafout (from the mean 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% 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% of fruits were first observed as ripe).

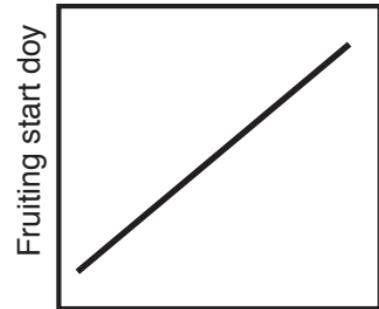
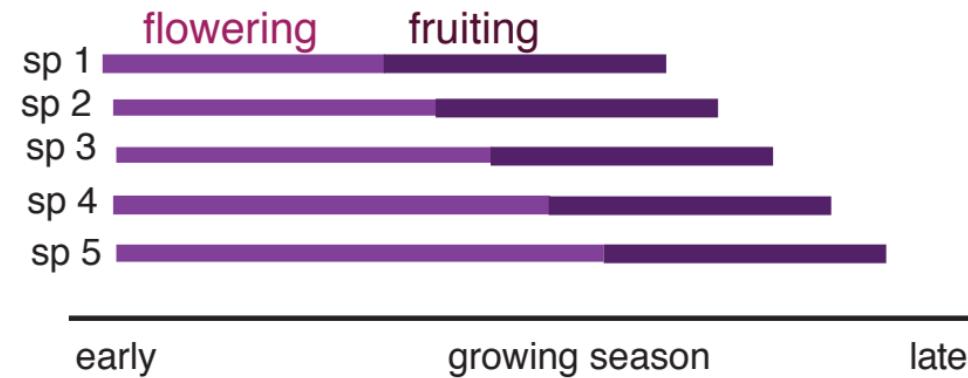
Figure 1  
**Hypotheses**

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H1. Previous phenological events constrain later events. (E.g., late-fruiting species set fruit late in the season because they leaf-out late.)



H2. Inter-phenophase time constrains phenology. (E.g., late-fruiting species set fruit late in the season because they require longer development time.)



Development time  
(Fruiting start doy - flowering start doy)

Figure 2

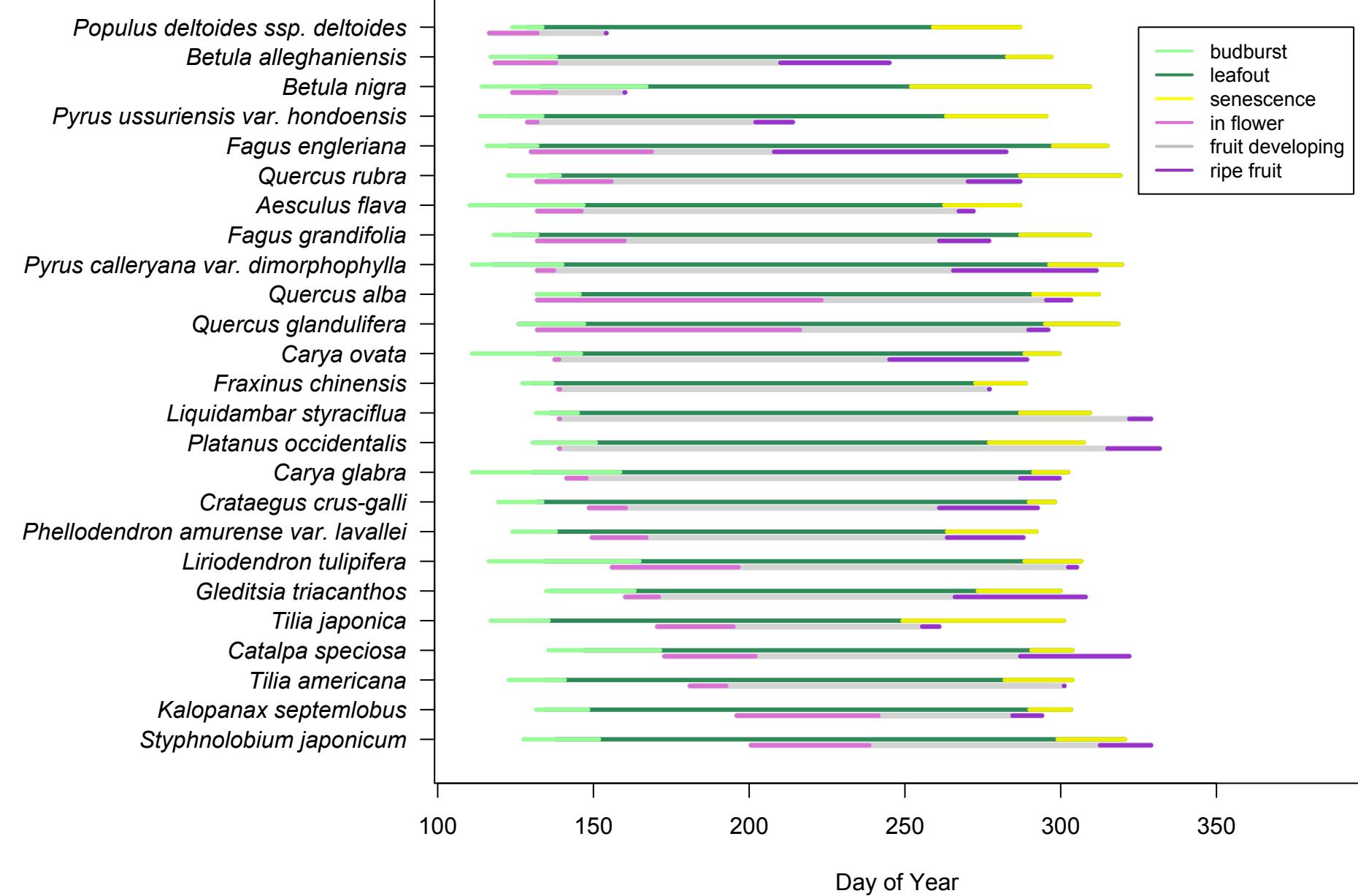
Click here to download Figure grosea\_repsort\_ripefruit\_legend.pdf 

Figure 3

[Click here to download Figure  
Hyp1\\_forcedslope\\_samerange.pdf](#)

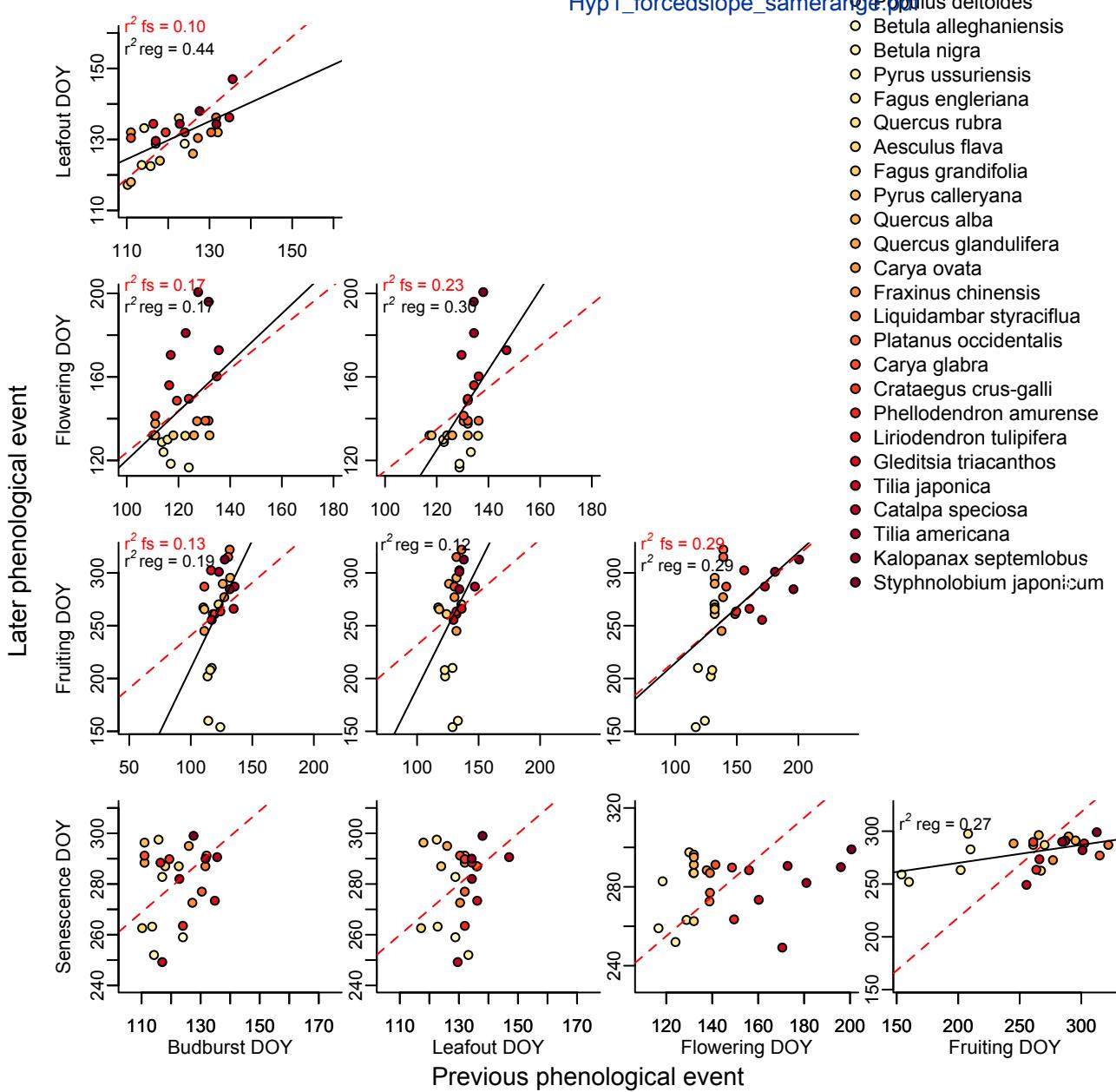
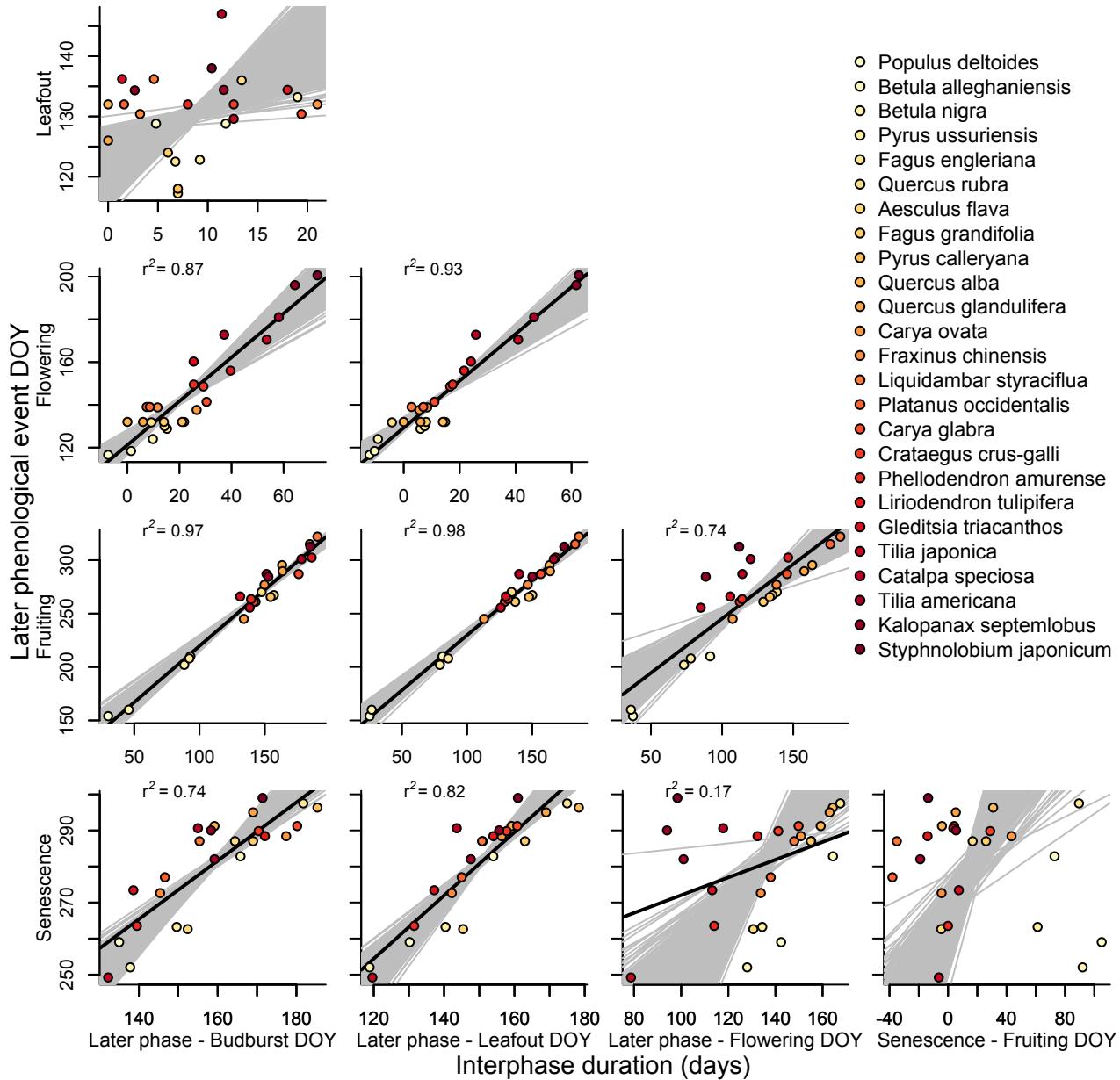


Figure 4

[Click here to download Figure Hyp2.pdf](#)


# 1 Phenological sequences: how early-season events define those 2 that follow

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11 **Abstract**

## 12 Premise of the study

13 Plant phenology is a critical trait as the timings of phenophases such as budburst, leafout, flowering, and  
14 fruiting, are important to plant fitness. Despite much study about when individual phenophases occur and  
15 how they may shift with climate change, little is known about how multiple phenophases relate to one another  
16 across an entire growing season. We test the extent to which early phenological stages constrain later ones,  
17 throughout a growing season across 25 angiosperm tree species.

**18 Methods**

19 We observed phenology (budburst, leafout, flowering, fruiting, and senescence) of 118 individual trees across  
20 25 species, from April through December 2015.

**21 Key results**

22 We found that early phenological events weakly (most  $r^2 < 0.30$ ) constrain most later events, with the  
23 strongest constraints (maximum  $r^2 = 0.43$ ) seen between consecutive stages. In contrast interphase duration  
24 was a much stronger predictor of phenology ( $r^2=0.17-0.98$ ), especially for reproductive events ( $r^2 > 0.7$ ),  
25 suggesting that the development time of flowers and fruits constrain the phenology of these events.

**26 Conclusions**

27 We found that much of the variation in later phenological events can be explained by the timing of earlier  
28 events and by interphase durations. This highlights that a shift in one phenophase may often have cascading  
29 effects on later phases, thus accurate forecasts of climate change impacts should include multiple phenophases  
30 within and across years.

**31 Key words**

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

**34 Introduction**

35 Plant phenology, the timing of recurring life-events such as leafout and flowering, is a critical trait that affects  
36 individual fitness, population abundance, agricultural and natural productivity, and global climate, through its  
37 role in carbon sequestration (Chuine and Beaubien, 2001; Cleland et al., 2007; Willis et al., 2010; Miller-  
38 Rushing et al., 2010; Craine et al., 2012). Advancement of budburst, leafout, and other phenophases are some

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39 of the most widely documented biological impacts of anthropogenic climate change, and phenology is likely to  
40 be further altered by future climate change (Parmesan, 2006). Because of its important role in many  
41 ecosystem services and in the global climate cycle, improved understanding and forecasting of tree phenology  
42 would aid in planning and preparing for climate change impacts.

43 Despite the observation that spring phenology generally shifts earlier with warmer temperatures, dramatic  
44 variation exists in phenological responses to climate. Temperature is thought to be a major factor controlling  
45 phenology of temperate tree species (Parmesan, 2006; Richardson et al., 2006; Morin et al., 2010; Schwartz et  
46 al., 2013; Clark et al., 2014), but some populations and species have not shifted their phenology with recent  
47 warming (Wolkovich et al., 2012). In addition, different tree species vary widely in the timing of leafout and  
48 other phenological processes, even when exposed to the same environmental conditions (Lechowicz, 1984;  
49 Primack et al., 2009). Spring leafout, for instance, can span weeks among coexisting tree species (Lechowicz,  
50 1984). It has been proposed that, in addition to external environmental drivers such as temperature, some  
51 drivers of plant phenology are “endogenous.” That is, phenological sequences are affected by changes in  
52 internal tree functions that may not be related to climate or other environmental factors (Borchert, 1992;  
53 Marco and P’aez, 2002). As an example, inflorescence architecture may affect the sequence of leafout to  
54 flowering in trees (Marco and P’aez, 2002).

55 One important, but often neglected, feature of plant phenology is that events are sequential: leaf budburst  
56 comes before leafout, flowering comes before fruiting, and so on. This ordering is an endogenous factor that  
57 may constrain phenological responses to climate change. For example, if flowering requires a minimum  
58 development time following leafout (e.g. to acquire sufficient carbon), then flowering time may be constrained  
59 to shift no more than leafout timing has with recent climate change. This would be the case even if a greater  
60 advance in flowering may benefit the plant, for example if warmer springs have caused pollinator activity to  
61 shift earlier (Polgar et al., 2013; Thackeray et al., 2010)

62 The extent of constraints between phenological events is unknown, however, because few studies have  
63 integrated across consecutive events throughout a growing season (Wolkovich and Ettinger, 2014). Instead,  
64 researchers generally focus on one or two phenophases per study. Early season events (budburst and/or  
65 leafout) have been extensively studied, often using climate-controlled growth chambers (e.g., Basler and

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66 Korner, 2012; Laube et al., 2014). A separate group of studies, comprised of long-term observational data,"

67 focuses primarily on flowering only (e.g., Fitter and Fitter, 2002; Miller-Rushing and Primack, 2008). Interest

68 has recently surged in senescence, which had been less studied historically (Parmesan, 2006), but many of

69 these studies focus *only* on senescence (e.g., Taylor et al., 2008; Archetti et al., 2013; Jeong and Medvigy, 2014).

70 A contemporary meta-analysis highlights the lack of data on multiple phenophases: only five out of 51

71 phenology studies (9.8%) included data on both leaf and flower phenology (Wolkovich et al., 2012).

72 When research has looked across stages, important links have often been found. For example, later leafing in a

73 given year may be associated with later flowering, and fall senescence has been associated with both fruit

74 maturation and spring phenology, in different studies (Lechowicz, 1995; Keenan and Richardson, 2015; Liu et

75 al., 2016). In contrast, other research that has quantified multiple phenological events over time has

76 documented that some phenophases may shift asynchronously with climate change (e.g., spring events are

77 occurring earlier as fall events have gotten later, first-flower dates have shifted earlier whereas last-flower

78 dates have not, Menzel et al., 2006; CaraDonna et al., 2014). These insights and complications demonstrate the

79 need to better understand how phenological stages relate to one another across an entire growing season

80 (Wolkovich and Ettinger, 2014).

81 Here, we examine the extent to which early-season phenological events constrain later events across tree

82 species planted in the same environment. Specifically, we test two hypotheses:

83 • *Hypothesis 1: Previous phenological events constrain later events;* e.g., late-fruited species set fruit late in

84 the season, because they flower and leafout late (Figure 1). To be consistent with this hypothesis, we

85 expected earlier events, such as flowering, would predict later events, such as fruiting. If constraints are

86 strong, then, across all species, we expected that previous events should predict later events with a

87 slope of one, indicating that the later event happens a set number of days (represented by the intercept)

88 after the previous event (Figure 1). If constraints are weak, we expected to find relationships between

89 later and earlier events, though the slope may be less than one. No relationship would suggest that

90 earlier events do not constrain later events.

- 91     • *Hypothesis 2: Interphase duration constrains phenology;* e.g., late-fruiting species set fruit late in the  
92        season because they require longer maturation time (Figure 1). To be consistent with this hypothesis,  
93        we expected that the interphase duration (the number of days between two phenological events)  
94        between earlier and later events would be a strong predictor (e.g., a large  $r^2$ ) of the later event,  
95        regardless of the timing of the earlier event (Figure 1).

96   Testing these hypotheses addresses basic, critical questions about drivers of variation in temperate tree  
97   phenology. These questions remain unanswered despite decades of phenology research because previous field  
98   studies rarely (if ever) examined multiple phenophases spanning the entire growing season across a large  
99   number of tree species.

## 100 **Materials and Methods**

### 101 **Study site and focal species**

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

### 111 **Phenology data collection**

112   We visited each individual once every 6-10 days throughout the growing season. Phenology observations in  
113   the spring began on April 6, 2015 (prior to any budburst occurring) and fall phenology observations ended on

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114 December 2, 2015 (at which point more than 95% of leaves on all trees had changed color and dropped). We  
115 observed five phenological stages, which were quantified following the National Phenology Network (NPN)  
116 protocols (for a full description see Denny et al., 2014). The budburst phase was characterized by green leaf  
117 tips being visible at the tips of buds, and the leafout phase was characterized by visible fully unfolded leaves  
118 and petioles that had completely emerged from the buds. The flowering phase was when open flowers were  
119 visible, and the fruiting phase was defined by ripe fruit being visible. Leaf senescence was characterized by  
120 leaves changing from green to fall colors. On each observation day, we estimated the presence and abundance  
121 of each phenophase on each individual tree.  
122 From the field observation data, we extracted the day-of-year (DOY) of the first observed occurrence of a  
123 given phenological phase. Budburst and fruiting DOY were defined as the first day when three or more burst  
124 leaf buds or ripe fruits, respectively, were observed on the individual. Leafout, flowering, and leaf senescence  
125 DOY were defined as the first day when 5% or more of the individual was leafing out, flowering, or showed fall  
126 colors, respectively (Denny et al., 2014). From these individual tree phenology observations, we calculated  
127 species-level mean start dates and for all phenophases, for use in our statistical analyses. We used these mean  
128 start dates to calculate interphase durations between phenophases.

## 129 Statistical analyses

130 To understand the extent to which previous phenological events constrain later events across species  
131 (Hypothesis 1, Figure 1), we fit linear models in which the response variable was phenological stage (i.e., the  
132 species' mean DOY of leafout, flowering, fruiting, or senescence), and the predictor was previous phenological  
133 stage. Thus, budburst was excluded as a response variable, because it was the earliest stage we quantified, and  
134 senescence was excluded as a predictor variable because it was the latest stage we quantified. We therefore fit  
135 10 separate regression models, estimating the intercept of the relationship between later and previous  
136 phenological phases, and forcing the slope to be one (Hypothesis 1, Figure 1). In addition, we fit 10 models,  
137 with the same predictor and response variables, in which we estimated the best-fit slope and intercept (via  
138 least-squares, e.g., a standard regression model). Under Hypothesis 1, with strong constraints, we expected  
139 that the models with forced slopes should provide similar fit to the data as the standard regression models

140 that estimate both slopes and intercepts. We compared fit of these two model structures using r-squared  
141 values, as well as Akaike's Information Criterion (AIC).

142 To understand the extent to which interphase duration constrains later phenological events (Hypothesis 2,  
143 Figure 1), we fit linear models in which the response variable was phenological stage, and the predictor was  
144 the number of days between phenological stages. Thus, as above, budburst was excluded as a response  
145 variable. We therefore fit 10 different models, each with one of four phenological stages as the response  
146 variable and one of the four interphase durations as a predictor. To investigate the effect of interphase  
147 duration, separate from the constraint imposed by the inherent ordering of events, we fit models in which the  
148 interphase durations were randomized with respect to the timing of the earlier phenophase across species.  
149 We did this resampling of interphase duration 999 times for each model structure. If our expectation of later  
150 events being constrained by interphase duration was supported, then the best-fit slope should fall within the  
151 resampled models' slope estimates. Furthermore, if the constraints imposed by interphase duration were due  
152 primarily to the inherent ordering of events, then the randomized models should include a narrow range of  
153 possible slopes. To examine these possibilities, we compared the range of slopes of all the resampled models  
154 to the slope of the fitted model.

155 All analyses were conducted in R version 3.2.4 (R Core Team, 2017), and code is available in the Supplemental  
156 Materials.

## 157 Results

158 We monitored five phenophases, which varied in duration. First budburst date occurred over 32 days and first  
159 leafout date occurred over 30 days in the spring, across all focal individuals (Figure S1) and species (Figure 2).  
160 Flowering phenology occurred over a longer period than budburst and leafout, spanning 131 days from late  
161 April to September. The first observation of ripe fruit spanned 175 days, and the start of leaf senescence  
162 occurred over 56 days across all individuals and species. Most species (20/25) spent the majority of the  
163 growing season in the reproductive phenological phases (i.e., flowering and fruit development), and most  
164 species (23/25) began leaf budburst prior to flowering, though leaf development overlapped with flowering in

165 some species (Figure 2). The majority of species (15/25) produced ripe fruit prior to beginning senescence  
166 (Figure 2).

167 We found that the timing of early phenological stages predicted the timing of later stages in many cases  
168 (Figures 2-3, Table S1). The strongest relationships (i.e., with the most variation explained) occurred between  
169 adjacent stages (those along the diagonal in Figure 3, such as leafout and budburst, fruiting and flowering).  
170 Even for adjacent phases, however, neither the forced slope models nor the regression models explained a  
171 large proportion of the variation in phenology ( $r^2 < 0.30$ , in most cases). For three relationships (flowering  
172 versus budburst, flowering versus leafout, and fruiting versus flowering), the model fits were consistent with  
173 the constraint hypothesis—the forced slope model provided similar fit to the regression models and both  
174 models explained a significant amount of variation in phenology (Figure 3, Table S1). For four relationships,  
175 standard regression models explained much more variation than the forced slope models. For two of these  
176 relationships (leafout versus budburst, and senescence versus fruiting), the regression models had slopes that  
177 were less than one and for the other two relationships (fruiting versus budburst and fruiting versus leafout),  
178 the regression models had slopes that were greater than one (Figure 3, Table S1).

179 We observed strong relationships ( $r^2 > 0.7$ ) between phenology and interphase duration for the two  
180 reproductive phenophases (flowering and fruiting time, Figure 4, Table S2). Flowering DOY was strongly  
181 predicted by days between flowering and leafout ( $r^2 = 0.93$ ), as well as by days between flowering and  
182 budburst ( $r^2 = 0.87$ ). Fruiting DOY was strongly predicted by days between fruiting and flowering stages  
183 ( $r^2 = 0.74$ ), by days between fruiting and leafout ( $r^2 = 0.98$ ), and by days between fruiting and budburst  
184 ( $r^2 = 0.97$ ). Senescence was predicted by days between senescence and budburst ( $r^2 = 0.74$ ), days between  
185 senescence and leafout ( $r^2 = 0.82$ ), and days between senescence and flowering ( $r^2 = 0.17$ ); senescence was not  
186 affected by days between senescence and fruiting. Leafout was not predicted by interphase duration (i.e., time  
187 since budburst). In all cases where later events were well predicted ( $r^2 > 0.7$ ) by interphase duration, the best  
188 fit model was predicted by our null model (i.e., the randomizations). However, the relationship between  
189 senescence and days between senescence and flowering was weaker than expected by our null model (see  
190 resampling estimates in Figure 4, Table S2).

**191 Discussion**

192 The ordering inherent in phenology means most phenological stages are dependent—in some way—on those  
193 stages before: dormancy must be broken before buds can burst and flowers must be produced before fruit.  
194 Here we show how the major phenological stages of temperate trees are constrained by one or more earlier  
195 phenological stages.

196 All phenological stages we observed support Hypothesis 1: timing appears to be constrained by at least one  
197 previous phenological stage. Our findings are consistent with recent work suggesting that senescence time can  
198 be affected by earlier phenophases (Keenan and Richardson, 2015; Liu et al., 2016), and suggest that this one  
199 relationship is part of larger suite of correlated phenophases. Consecutive events were correlated across both  
200 growth and reproductive phenophases (i.e., flowering and leafout were correlated to a similar degree as  
201 fruiting and flowering, Figure 3). These associations may occur because of endogenous dependencies between  
202 the two phases, because of a shared external driver such as growing degree days, or a combination of  
203 endogenous and external factors (Lechowicz, 1995). Thus, environmental conditions in the winter or spring  
204 that may directly affect only early phenological stages, such as budburst, are likely to have cascading effects on  
205 later stages such as leafout, flowering, and fruiting. Our data suggest that, for most events, these effects are  
206 more apparent for consecutive stages (i.e., those along the diagonal in Figure 3), and are well-approximated by  
207 the forced slope model in some cases (Figure 3).

208 Although some of the variation in reproductive phenology (flowering and fruiting) was explained by previous  
209 phenology (Hypothesis 1), much more variation was explained by interphase duration (Hypothesis 2). Later  
210 flowering species generally required more time between flowering and leafout. Similarly, late fruiting species  
211 had longer interphase durations between the first observation of ripe fruit and first flowering date. It may be  
212 that late fruiting species require longer fruit development times to produce larger fruits or more  
213 highly provisioned seeds. This would be consistent with previous theories that trees investing more resources  
214 into their offspring (i.e., having larger seeds) require more time to build resources (Bolmgren and D Cowan,  
215 2008; Sun and Frelich, 2011). There were notable exceptions to this general relationship, however. Some  
216 species set fruit later than expected, given their interphase duration between flowering and fruiting (*Catalpa*

217 *speciosa*, *Tilia americana*, *T. japonica*, Figure 4). These species also flowered later than expected, given their  
218 leafout DOY (Figure 3). External factors related to their ecology may be the cause; for example, these species  
219 are all insect-pollinated, so the timing of their pollinator activity may have affected their floral phenology  
220 (Elzinga et al., 2007).

221 Despite our expectation of strong relationships between later phenophases and interphase durations (Figure  
222 4), our results show two relationships that are weaker than expected. Leafout was not predicted by the  
223 interphase duration between budburst and leafout, and senescence was not predicted by the interphase  
224 duration between fruiting and senescence (Figure 4). We had expected that these two sets of phases would  
225 demonstrate *stronger* constraints of interphase duration because they occur at the beginning and end of a  
226 bounded growing season (Letten et al., 2013). The weak ability of interphase duration to predict leafout may  
227 be due to the distinct weather patterns in 2015 and how they interacted with species' cues for spring  
228 phenology. Trees have species-specific chilling and forcing requirements that must be met prior to leafing out,  
229 and are generally understood to be related to accumulations of warm and cold temperatures (e.g., Schwartz  
230 and Hanes, 2010; Chuine, 2010; Clark et al., 2014). Because of this, the pattern of how quickly cooler and  
231 warmer temperatures accumulate across a growing season can impact how variable leafout is across species.  
232 In contrast to some years that have high variation in leafout date across species (Lechowicz, 1995), in our  
233 study year (2015) many species leafed out close to DOY 130 (10 May), regardless of leafout-budburst  
234 interphase duration (which ranged from 0 to 20 days, Figure 4). This could be due to the temperature  
235 conditions particular to 2015: temperatures were colder than average in January through March, and then  
236 switched to above-average in late April and early May ([www.bluehill.org](http://www.bluehill.org)). Such long periods of cold followed  
237 by rapid warming may have meant that chilling requirements were met for all species well before warm  
238 temperatures began, and then forcing requirements were rapidly met for many species (even if they had  
239 diverse requirements) leading to a flush of leafout in early May, across diverse species. Variation in the  
240 environment each year may alter how later phenophases are constrained by earlier events and highlights the  
241 need to better understand how such constraints vary across years and what underlies the remaining variation  
242 not explained by earlier phenophases.

243 Our results indicate that *both* Hypothesis 1 and Hypothesis 2 are operating and can explain a substantial  
244 amount of the variation observed among species in a single year. For example although later fruiting is  
245 generally associated with later flowering (Figure 3), some species, such as *Quercus alba* and *Quercus*  
246 *grandifolia*, flower relatively early and fruit late; later fruiting for these species is instead associated with  
247 longer interphase duration between fruiting and flowering (Figure 4). Understanding drivers of variation in  
248 phenological responses among species requires disentangling the ways that earlier phenology and interphase  
249 duration interact with one another, and with environmental conditions. Experimental manipulations will be  
250 beneficial for discerning the physiological and genetic bases for the relationships we observe (Flint, 1974).

251 Our findings have important implications for improved forecasting of climate change induced shifts in  
252 phenology. A shift in one phase may have cascading effects on later phases, since each phase is linked to  
253 phases that occur before and after it (Wolkovich and Cleland, 2014). Phenology models covering the whole  
254 annual cycle are rare for wild plants (but see Chuine and Beaubien, 2001) and have not been used to forecast  
255 phenology at the community level. Our results highlight a clear need to conduct future multi-species  
256 phenology studies across entire growing seasons (Wolkovich and Ettinger, 2014), and begs the question of  
257 how phenophases may be linked across years, as well (e.g., Elmendorf et al., 2016). For example, the timing of  
258 spring budburst in one year may be related to the timing of budset the previous fall (Mimura and Aitken,  
259 2010). Although relationships between phenophases have not been widely studied, there is a growing  
260 ecological literature on the concept of “ecological memory,” or the capacity of past states to influence present  
261 or future responses (Ogle et al., 2015). The ecological memory of phenology has not been quantified, but may  
262 be critical for accurate forecasting, particularly for species like *Quercus rubra*, which require more than one  
263 year for fruit maturation. Given the species-specific nature of phenological constraints, accurate forecasts of  
264 communitywide phenological shifts are likely to require species-specific information, such as fruit  
265 development time for fruiting forecasts, in addition to climate data (Diez et al., 2012).

## 266 **Conclusions**

267 We have shown that early and late phenological stages are strongly linked across the growing season,  
268 providing a new approach to explain some of the dramatic variation in phenological responses observed to  
269 date. Many studies have sought to identify the particular environmental drivers of phenology (e.g., Morin et al.,  
270 2010; Schwartz et al., 2013). Our findings here suggest that timing and duration of previous phenological  
271 states should also be examined. In addition, identifying the appropriate temporal window for both  
272 environmental and endogenous drivers is essential (Teller et al., 2016). Because earlier phenophases define  
273 those that follow, the relevant time period for these drivers may extend further back in time than the single  
274 growing season we evaluated here. Multi-year studies will be critical to evaluate the extent to which  
275 phenological patterns are consistent among years that may vary in climate, as well as biotic conditions (i.e.,  
276 pollinator or pest populations, Lechowicz, 1995). A fuller understanding of phenological constraints and  
277 drivers of phenological variation offers the potential for improved forecasts of phenological shifts with climate  
278 change to help predict how ecosystem functions will be altered in the future.

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287 necessarily reflect the views of the National Science Foundation.

## 288 **Data Accessibility**

289 The data set for this study is available online at Knowledge Network for Biocomplexity (Gee et al., 2017).

290 **Author contributions**

291 All authors conceived of and designed the study and edited the manuscript; S.G. conducted the fieldwork; S.G.  
292 and A.E. analyzed the data and wrote the manuscript.

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404 **Tables**

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

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<b>Early-season flowering</b>	<b>Mid-season flowering</b>	<b>Late-season flowering</b>
<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>lavallei</i> (4)	<i>Fraxinus chinensis</i> (5)	
<i>Populus deltoides</i> ssp. <i>deltoides</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)	

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## Figure Legends

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. Interphase duration is the time between phenological events, e.g., the number of days between the first day of flowering and the first day that ripe fruit were observed.

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% 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% 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 (DOY) of the later phenological stages as the response variable, and mean day-of-year of earlier stage as the explanatory variable. Models with a forced slope of 1 are shown by dashed red lines, and  $r^2$  is given when  $r^2 > 0.10$ . ("fs", in red).  $r^2$  for standard regression ("reg" in black) and lines for these models are shown when  $r^2 > 0.10$  (solid black lines). 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.

Figure 4: **Relationships among phenological stages and interphase duration across the 25 focal species.** Interphase duration (x-axis) 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 (DOY) of the later phenological stages as the response variable, and interphase duration as the explanatory variable. Solid lines (representing model fit) and  $r^2$  are shown when  $r^2 > 0.10$ . Gray lines represent model fits when interphase was randomized with respect to the timing of the earlier phenophase (in some cases the range of possible relationships is quite narrow, given the constraints of ordering inherent in phenological events). When our null expectation of later events being constrained by interphase duration was supported, the best-fit slope (black line) will fall within the randomized lines (in gray). Full model statistics are summarized in Table S2 in the Supplemental Materials. Species in the legend are ordered from early to late first-flower dates.