

# Reconciling competing hypotheses regarding flower-leaf sequences in temperate forests for fundamental and global change biology

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## Summary

Phenology is a major component of an organism's fitness. While individual phenological events affect fitness, growing evidence suggests that the relationship between events may be equally or more important. This may explain why temperate deciduous woody plants exhibit considerable variation in the order of reproductive and vegetative events, or flower-leaf sequences (FLSs). There is evidence to suggest that FLS may be adaptive, with several competing hypotheses to explain their function. Here, we advance existing hypotheses with a new framework that accounts for quantitative FLS variation at multiple taxonomic scales using case studies from temperate forests. Our inquiry provides several major insights towards a better understanding of FLS variation. First, we show that concurrent support for multiple hypotheses should be starting point for future FLS analyses. Second, we demonstrate that support for FLS hypotheses is sensitive to how FLSs are defined, with quantitative definitions being the most useful for robust hypothesis testing. Finally, we highlight how adopting a quantitative, intra-specific approach generates new avenues for evaluating fitness consequences of FLS variation and provides cascading benefits to improving predictions of how climate change will alter FLSs and thereby re-shape plant communities and ecosystems.

## Introduction

Phenology, the timing of seasonal life cycle events, allows organisms to synchronize their activity with optimum environmental conditions (Forrest & Miller-Rushing, 2010). It is not only individual phenological stages that affect an organism's performance, but also their chronology (Ettinger *et al.*, 2018; Firmat *et al.*, 2017; Vitasse *et al.*, 2010).

One phenological relationship that has long received scientific interest (see Robertson, 1895) and, recently, increased attention (e.g. Gougherty & Gougherty, 2018; Savage, 2019) is the flower-leaf phenological sequence (FLS) of temperate deciduous woody plants. In a typical model of plant life-history, vegetative growth precedes reproduction. However, for many species in the forests of

Eastern North America (and other temperate regions of the Northern Hemisphere), it is not the green tips of new shoots that mark the commencement of the growing season, but the subtle colors of their flowers. Previous work by Gougherty & Gougherty (2018) found that approximately 30% of tree species of the Midwestern United States flower prior to leafout. The prevalence of this FLS may be surprising as it necessitates reproductive investment from stored carbohydrates at a time when reserves are most depleted (Primack, 1987), but this trade-off suggests that flowering-first has some adaptive significance (Rathcke & Lacey, 1985).

Understanding this phenological pattern is timely because anthropogenic climate change is altering FLSs. The number of days between flowering and leafout is increasing as a result of climate change, but the rate of change differs up to five-fold among species, with flowering-first species seemingly more sensitive to climate change (Fig. 1). If FLSs are indeed an important component of woody plant fitness, this inter-specific variation will exacerbate fitness differences between species, influencing which species will persist under altered climate conditions.

Long-term datasets also demonstrate high within-species variability in FLSs. Despite recent advances in understanding the physiology and evolution of FLSs (Gougherty & Gougherty, 2018; Savage, 2019), most analyses have not directly addressed this variability—potentially slowing progress in predicting how FLS patterns will respond to climate change. While the literature provides some general correlations between flowering and leafing phenology (e.g. Ettinger *et al.*, 2018; Lechowicz, 1995), there have been few, if any, analyses of higher-resolution patterns (Gougherty & Gougherty, 2018).

We propose a new framework for the study of FLSs built on quantitative measures of both inter- and intra-specific FLS variation. This shift will improve predictions of how FLS patterns will change in the future, and may reveal novel avenues to better understand the fundamental biology of this phenological sequence. Here we 1) review hypotheses of the function of FLS variation 2)

evaluate the biological basis of the current categorical FLS framework and 3) present our proposed quantitative framework using a detailed case study of long-term phenology records from Harvard Forest in Petersham, MA.

## Hypotheses for flower-leaf sequence variation

### Wind pollination

The most prevalent FLS hypothesis suggests that flowering-first is an adaptation for wind-pollination, with leafless flowering allowing for more efficient pollen transfer (Whitehead, 1969)(Fig. 2a). The primary evidence for this hypothesis comes from pollen diffusion studies (e.g., particle movement through closed and open canopies, Milleron *et al.*, 2012; Niklas, 1985) and suggests canopy structure encumbers pollen movement.

### Water limitation

Another hypothesis suggests that flowering before leaf development is an adaptation to reduce water stress caused by concurrently maintaining floral hydration and leaf transpiration (Franklin, 2016) (Fig. 2b). Observations from the dry tropics, where this FLS is also common, confirm that the timing of flowering in many species is associated with a water status recovery due to leaf drop (Borchert, 1983; Reich & Borchert, 1984). There is also evidence that flower tissue is more resistance to drought damage than leaf tissue (Zhang & Brodribb, 2017). While, temperate forests are rarely water-limited during the spring when flowering and leafing occur (Polgar & Primack, 2011), drought tolerance traits may also confer freeze tolerance, a critical adaptation for life at higher latitudes (Zanne *et al.*, 2013). This relationship may help to explain the strong association between flowering-first and water use traits found by Gougherty & Gougherty (2018) for temperate species.

## Early flowering

A third possibility is that the flowering-first FLS is a byproduct of selection for early flowering (Fig. 2c). Flowering-first species are among the earliest in a community to flower seasonally, which may be an adaptation to accommodate later phenological events such as the maturation of large fruits or seeds (Ettinger *et al.*, 2018; Li *et al.*, 2016; Primack, 1987) or avoiding seed predation (Schermer *et al.*, 2020). This may be particularly important at the high latitudes where selection on flowering time is strong due to a shorter growing season (Munguia-Rosas *et al.*, 2011). Recent work from Savage (2019) demonstrated that spring flower phenology is less constrained by prior phenological events than leaf phenology, which would allow selection to drive flowering into the early season, producing the flowering-first FLS. With this hypothesis there is no specific advantage to a species flowering before or after leafing; all that matters is its absolute flowering time.

## Phylogenetic and physical constraints

The previous hypotheses suggest that a flowering-first FLS may be adaptive, but it is possible that the greater diversity of FLS patterns observed in temperate forests is the product of phylogenetic, developmental, architectural or physiological constraints among species (Diggle, 1995, 1999; van Schaik *et al.*, 1993) (Fig. 2d). For example, the reproductive phenology of species that produce flower from axillary buds set in previous season may be more independent of leaf phenology than species with determinate growth (Borchert, 1983; Rathcke & Lacey, 1985; van Schaik *et al.*, 1993). Previous work has suggested that xylem anatomy may constrain spring phenology (Lechowicz, 1995), though Savage (2019) determined that for 20 spring-flowering species, reproductive buds were hydrated primarily by the phloem, suggesting the flowering-first FLS may be independent of xylem activity. Generally, architecture and development is highly conserved among species (Diggle, 1999) and we might expect to see a strong phylogenetic signal for FLS variation as was reported in a recent analysis by Gougherty & Gougherty (2018). A strong phylogenetic pattern in FLS would not preclude any of the adaptive hypotheses presented above, as many different evolutionary

processes can yield comparable phylogenetic signals (Revell *et al.*, 2008).

While decades of inquiry have advanced each of these hypotheses independently, there is no clear consensus regarding their comparative merits. Most previous studies on FLSs have not compared hypotheses, and those that did have generally found support for multiple hypotheses (see Bolmgren *et al.*, 2003; Gougherty & Gougherty, 2018). There is no expectation that FLS hypotheses must be mutually exclusive. Indeed, understanding the relative importance of each one and the relationships between them may provide the most useful path forward, if they can be robustly compared.

We argue that a sensible reconciliation of these hypotheses is possible with a shift to a new conceptual framework for the study of FLSs. Under the current framework, FLSs are described qualitatively, and prescribed at the species level. We suggest that quantitative measures of FLS which include observations below the species level are more compatible with the biological processes underlying FLS variation. Below we present an overview of the current approach to describing FLSs and highlight some of the challenges that can arise when using it.

## The current flower-leaf sequence framework

### Describing FLSs

The current framework describes three main FLS categories: flowers before leaves (hysteranthly, proteranthly, precocious flowering); flowers with leaves (synanthly); and flowers after leaves (seranthly) (Heinig, 1899; Lamont & Downes, 2011). Some data sources (e.g. Barnes & Wagner, 1981,2004; Burns & Honkala, 1990) include additional categories: “flowers before/with leaves” and “flowers with/after leaves”, but it is unclear whether these categories describe intermediate FLS patterns or FLS variability in these species. While these categories are conceptually reasonable, applying them to real phenological sequences is not always straightforward.

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140 Both reproductive and vegetative phenological sequences consist of multiple sub-stages, and this  
 141 introduces significant ambiguity into how we interpret qualitative FLS descriptions. Consider a  
 142 species with the following FLS:

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144 **flower budburst** → **leaf budburst** → **first flowers open** → **leafout** → **peak flowering** →  
 145 **end of leaf expansion**

146 Observers could justifiably classify this species as: 1) Hysteranthous because flower budburst pre-  
 147 cedes leaf budburst, 2) Synanthous because flowers open during the budburst-leafout inter-phase,  
 148 3) Seranthous because peak flowering occurs after leafout. This problem extends beyond this sim-  
 149 ple example to real datasets, (e.g. O’Keefe, 2015) where the same ambiguities exist (Supporting  
 150 Information Fig. S1). Not surprisingly then, different sources may classify the same species dif-  
 151 ferently. We compared species-level FLS descriptions in two of the most comprehensive records  
 152 of FLS, Michigan Trees and its companion volume Michigan Shrubs and Vines (MTSV) (Barnes  
 153 *et al.*, 2016; Barnes & Wagner, 1981,2004) with The USFS Silvics Manual Volume II (Burns &  
 154 Honkala, 1990). Of the 49 overlapping species, 30% were classified differently. Such different clas-  
 155 sifications could reflect interesting temporal or geographic variability in FLSs, but—given current  
 156 definitions—they could equally be the product of observer classification decisions.

157

158 Categorization can often introduce biases in analyses (Edwards *et al.*, 2015; Royston *et al.*, 2006)  
 159 and highlight ambiguity in hypotheses; this may be particular prevalent for the study of FLSs. The  
 160 wind pollination hypothesis hinges on the fact that leaves create a substantial barrier to pollen  
 161 transfer, which may not be true during the early stages of leaf expansion when tiny leaf primordia  
 162 would have little impact on environmental structure. Rather, trees that flower during the early  
 163 stages of leaf expansion should gain similar advantage to those who complete their flowering before  
 164 any leaf activity (Fig. 2a). Alternatively, because transpiration intensifies as soon as leaves begin

to expand (Wang *et al.*, 2018), the water limitation hypothesis asserts there should be a cost to maintaining floral structures during any stage of leaf activity. Here, only species where flowering occurs before any leaf expansion should gain a hydraulic advantage (Fig. 2b).

Given the differences in biological processes underlying these hypotheses, statistical relationships between FLS and traits will fluctuate depending on where categorical boundaries are drawn. For the example presented above, strongest signal of the wind-pollination hypothesis should occur when the category of hysteroanthy includes species that flower before and with early leaf development. The strongest signal for the water limitation hypothesis should occur when the hysteroanthous classification is restricted to only species that flower before any leaf activity. If these hypotheses require different categorization schemes to accurately capture the underlying biology, it becomes difficult to compare them in the same modeling framework.

We found that associations between FLSs and functional traits were highly sensitive to how FLSs were defined (Supporting Information Fig. S2, e.g. pollination syndrome, Supporting Information Fig. S3). We applied two alternative FLS categorizations in two major datasets (MTSV and USFS, see Supporting Information Methods S1); physiological hysteroanthy, which allowed for no overlap between floral and leaf phenophases, and functional hysteroanthy, which allowed for a degree of overlap (see Supporting Information Methods S1). These alternate categorization boundaries re-shuffled the species included in each classification, affecting both the trait distributions within each category and the phylogenetic patterning across the tree (Supporting Information Fig. S7).

This suggests that a new approach that relaxes the assumptions of categorization could help to fairly evaluate FLS hypotheses. Below we present a new framework for the study of FLSs built on 1) quantitative measures and 2) intra-specific investigations of FLS variation. This simple shift can capture biological variation missed by current approaches, and offer novel avenues for understanding the scope and consequences of FLS variation in an era of global change.



## A new framework for flower-leaf sequences

### Quantitative measures of FLS

In the current FLS framework species are classified based on sequence alone. The duration of and time between phases, however, also matters (Inouye *et al.*, 2019). When considering measures of time, FLSs of species within each category can be quite different (Fig. 3a). Measure of FLS based on continuous data—i.e. reporting the number of days between specific phenophases, suggest there is much greater diversity in FLS patterns in a given forest community than provided by the three categories of the current framework.

Treating FLSs like other quantitative measures of phenology (e.g. the BBCH scale, Finn *et al.*, 2007) would: 1) improve FLS-trait association models by reducing the noise from unmeasured variation and 2) standardize data across time and space, observer, and analyst. Adopting quantitative measurements would facilitate comparing FLS patterns across larger temporal, geographic, and taxonomic scales, giving researchers more power to accurately address questions about FLS variation.

An additional benefit of a quantitative approach to FLSs is that it allows for variation to be evaluated below the species level. We argue that intra-specific inquiries into FLS variation are vital to thoroughly answer both the questions about the basic mechanisms that generate FLS variation, and the applied questions regarding the magnitude and impact of FLS shifts with climate change.

### Intra-specific data on FLSs

Quantitative measurements of FLSs reveal significant variation among populations, individuals and years (Fig. 3 b-d). This variation can be leveraged to further improved FLS-trait models at the species level, and to generate and test novel questions about the fitness value of this trait.

Observations at multiple taxonomic scales should improve FLS-trait association models by allowing researchers to explicitly incorporate multiple levels of variation (i.e. through hierarchical modeling). When intra-specific variation for a given trait is high, using species' mean trait values may artificially inflate inter-specific differences (Vandepol). Interestingly, this implies that incorporating intra-specific variation to these models may be one of the most robust ways to accurately assess inter-specific variation.

Intra-specific inquiry is also a critical step to better understand the consequences of FLS shifts. At the core of each FLS hypothesis is a fitness prediction that is best interrogated below the species level. If FLSs are functionally important, individual variability in FLSs should correlated with changes in performance as has been shown for other phenophases (e.g. Schermer *et al.*, 2020). Evaluating the relationship between FLS variation and performance is critical to determine whether at its biological core, FLS variation is merely an interesting natural history note of temperate forests or an important functional trait that will impact the structure and function of these communities in the future.

## Testing the new framework

### Quantitative measures

To compare categorical and quantitative approaches to FLSs, we used long-term phenological records for woody species at Harvard Forest (O'Keefe, 2015) to model the associations between FLSs and functional traits using both a categorical FLS framework and a simple quantitative metric; the mean number of days between flower and leaf budburst for each species (see Supporting Information Methods S1). We investigated functional traits related to each of the FLS hypotheses. We used pollination syndrome as a predictor for the wind pollination hypothesis. We used mean precipitation across a species' range and two alternative predictors; species' moisture use and min-

imum temperature across a species' range as predictors for the water limitation hypothesis. We used flowering time and two alternative predictors; mean fruit dispersal time and seed mass to represent the early flowering hypothesis. We accounted for the influences of phylogenetic constraints by running these model in a phylogenetic modeling framework (Ives & Garland, 2010). In all cases, model results with alternative predictors were comparable to the sign and rank of the main results and can be viewed in the Supporting Information (Fig. ??).

Using the categorical approach, we detected only a weak relationship between hysteranthly and wind-pollination. However, with the quantitative approach, we found that increasing time between flower and leaf budburst was strongly associated with wind-pollination and early flowering, and that the longest FLS interphases were found in species with both of these traits (Fig. 4a,b). Need another sentence here to synthesize why.

### **Intra-specific variation**

To test how model inference changed when accounting for intra-specific variation, we re-analyzed the same FLS data from Harvard Forest presented above using a Bayesian hierarchical model that incorporated within-species variation in FLSs and flowering time (see Supporting Information Methods S1).

As in the model based on species' mean trait values, we found strong effects of flowering time, pollination syndrome and phylogeny on FLS variation, with only a weak signal for the water limitation hypothesis (Fig. 4c, Fig. Supporting Information S3). However, with this approach, we identified strong interactions between other predictors. Of note, the effect of early flowering on FLS variation was more pronounced in wind-pollinated taxa, and hydraulic demand was associated with increased time between flowering and leafing in biotically-pollinated taxa but not wind-pollinated taxa (Supplimental Information Fig. S6). Do I need another sentence here synthesizing too?

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269 Even with a quantitative framework, analyses are inherently sensitive to how FLS's are defined. It  
270 is not surprising that traits association vary in strength when FLSs are defined based on different  
271 sub-phases of flowering and leafing; for example, days between flower budburst and leaf budburst  
272 vs. days between peak flowering and leaf expansion. We found that incorporating intra-specific  
273 variation into the modeling appeared to reduce this bias (see Fig. S5), which may allow researchers  
274 to robustly compare existing FLS data that are not perfectly standardized with each other.

275

## 276 **Future directions:**

277 Our findings suggest that the tendency for previous studies to find support for multiple hypothe-  
278 ses (Bolmgren *et al.*, 2003; Gougherty & Gougherty, 2018; Savage, 2019) is consistent with the  
279 biological processes that shape FLSs. Multiple hypotheses should be the starting point for future  
280 FLS research. While large scale analyses may continue to be beneficial, a more nuanced under-  
281 standing about function of FLS variation may result from pattern deconstruction (i.e. grouping  
282 of species according to sub-clades or trait commonalities, Terribile *et al.*, 2009). For example, it  
283 is clear that wind-pollination efficiency is not driving hysteranthous flowering in insect-pollinated  
284 taxa, so considering this group of species alone rules out one major FLS hypothesis, allowing for a  
285 better evaluation of alternatives.

286 While trait associations point to past selection, much of the current interest in FLSs relates to how  
287 shifting FLS patterns will impact woody plants in the future. Shifting research to focus on intra-  
288 specific FLS data may importantly provide insight into the biological levels of organization that  
289 determine how species can respond to climate change from the individual to population to species  
290 level. Variation among and within individuals provides insights regarding micro-climate effects,  
291 heritability, selection and plasticity for FLSs (Denéchére *et al.*, 2019). Population level variation  
292 is critical to better understand the specifics of how environmental conditions shape FLSs (Vitasse

*et al.*, 2009), and how FLS variation interacts with landscape scale processes like gene flow and dispersal (Manel *et al.*, 2003). Taken together, investigations at these lower taxonomic levels could provide a more robust assessment of the potential magnitude of FLS shifts with climate change.

As mentioned above, future FLS research should aim to test the performance consequences of FLSs by leveraging intra-specific variation. However, this may require a more broad focus on data collection of other traits at the same scale as FLS variation. For example, the wind-pollination hypothesis suggests that decreasing the time between flowering and leafing should result in reduced pollination success. To test this prediction, studies tracking individual FLS variation in the field or controlled environments must also track performance metrics at this scale, for example, reproductive outcomes such as pollination success or fruit set. These kind of studies are critical for evaluating the implications of FLS shifts in the future.

## Conclusion

In demonstrating our proposed framework for the study of FLSs we found that, in accordance with previous work, flowering time and pollination syndrome and phylogeny are important drivers of hysteresis (Gougherty & Gougherty, 2018). Our work adds to the growing literature that infers the adaptive significance of FLSs from macro-evolutionary patterns and opens new avenues for testing the effects of FLS variation on woody plant performance below the species level. While it is clear the FLSs are highly variable and shifting with global climate change, research must directly examine the effects of FLS variation to better assess the consequences of future FLS shifts.

While much of research on the evolution of plant phenology focuses on specific phenophases (e.g. Ollerton & Lack, 1992; Savage & Cavender-Bares, 2013), selection likely acts on phenological sequences. With growing evidence that adaptation drives both the absolute timing of individual phenophases and the relative timing between them we must continue to develop analytical tools

that improve our understanding of the drivers of phenological events as part of a phenological syndrome, rather than as discrete, separate events. Our treatment of FLSs here is a small part of this work, but understanding how selection shapes phenology both throughout the whole growing season and across years remains a major frontier for the study of phenology (Wolkovich & Ettinger, 2014). This is an essential step towards a more complete understanding of the fundamental biology of temperate woody plants, and for predicting the fate of these species as global climate continues to change.

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## Author contributions

DMB developed the concept for the paper; DMB and IMC performed the analysis, DMB and EMW wrote the manuscript.

## Data and code availability

Data for the FLS and climate change analysis is publicly available from PEP725 at <http://www.pep725.eu/>. The Harvard Forest phenology data is also publicly available in the Harvard Forest Data Archive <https://harvardforest.fas.harvard.edu/harvard-forest-data-archive> (dataset: HF003-05). The compiled data from the MTSV and USFS guidebooks will be available on KNB upon publication. All modeling code will be made available upon request.

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## Supplemental Information

**Fig. S1:** Effect-size summary plots of FLS predictors for the MTSV and USFS case studies.

**Fig. S2:** Flower-leaf sequences of species at Harvard Forest 1990-2005.

**Fig. S3:** Phylogenetic signals for FLS variation.

**Fig. S4:** Visualization of FLS patterning across the phylogeny for the MTSV and USFS case studies.

**Methods S1:** Methods for: FLS and climate change modeling, modeling FLS variation in MTSV and USFS data, modeling FLS variation in the HF data, and calculating the phylogenetic signals in FLS variation.

## Figures

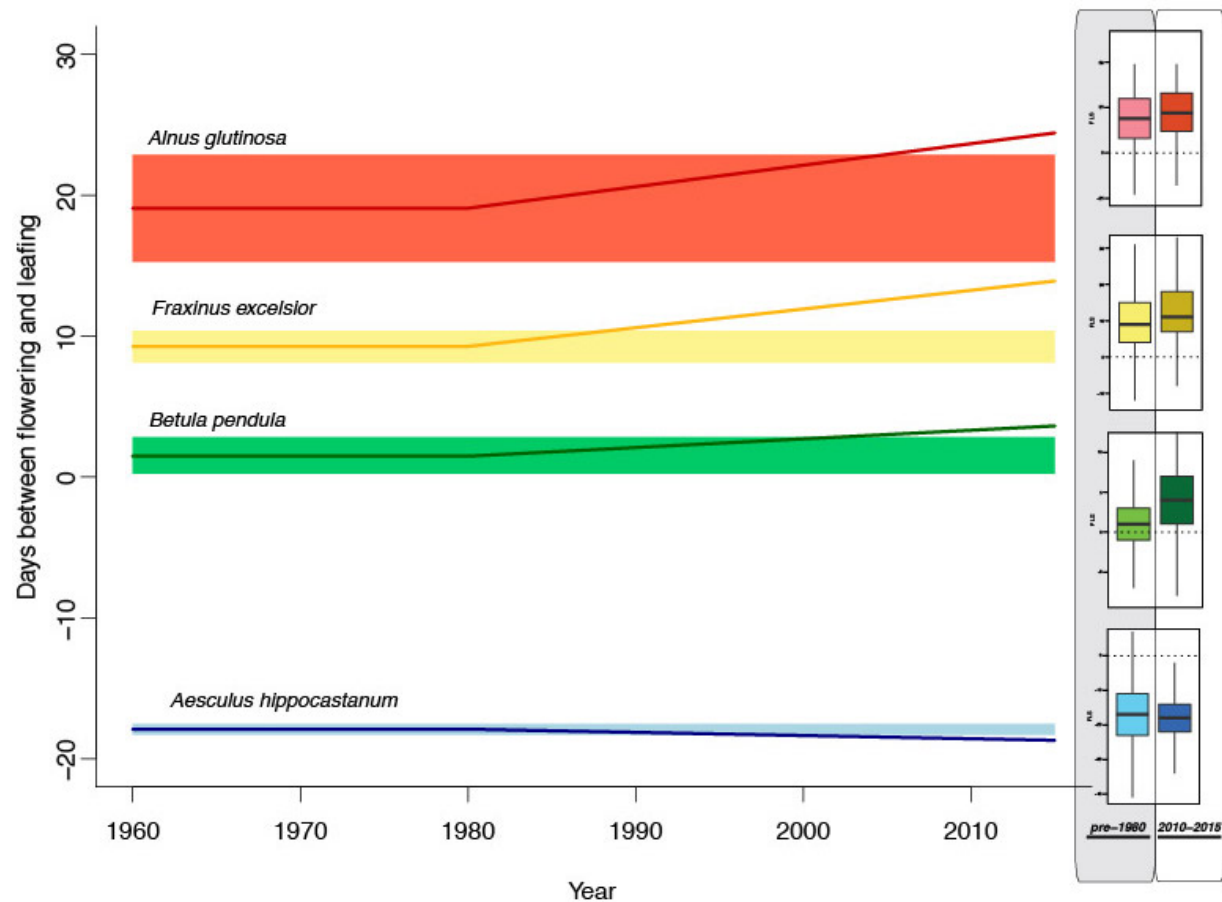


Figure 1: **Flower-leaf sequences (FLSs) across Europe for four tree species from 1960 to 2015 suggests climate change has generally increased the time between flowering and leafing**, but the direction and rate of change differs across species, which may exacerbate fitness differences within forest communities. To detect the effect of climate change on average FLS, we used models that allow for shifts in FLS after 1980 (Kharouba *et al.*, 2018). Lines represent the mean trend in FLS per species among populations, and the shaded regions indicate historic range of FLS variability (95% credible intervals of the pre-1980 average) from the PEP725 database (Templ *et al.*, 2018). The boxplots compare the FLS measurements prior to 1980 to the recent period (2010-2015), confirming shifts in FLSs over time for most species, but indicate high variability in the FLSs below the species level.

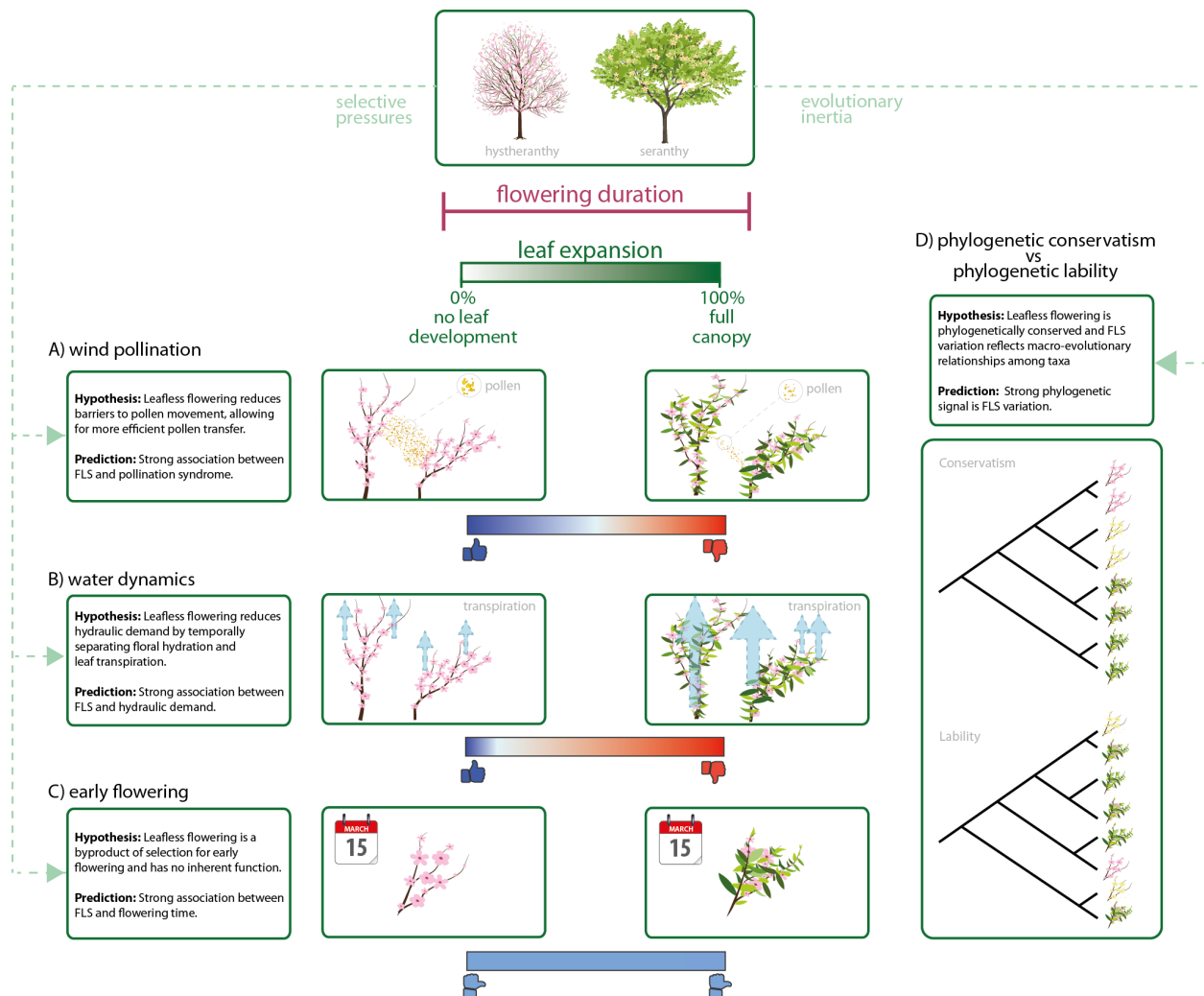


Figure 2: **Several hypotheses have been proposed to explain flower-leaf sequence (FLS) variation in temperate, deciduous woody plants.** The wind pollination hypothesis (a) suggests that leafless flowering reduces barriers to pollen movement. The water limitation hypothesis (b) suggests the temporal separation between flowering and leafing reduces hydraulic demand. The early flowering hypothesis (c) suggests FLS variation is a byproduct of selection for early flowering the relative timing of flowers and leaves is inconsequential compared to the absolute time of flowering. As depicted by the scale bars in the center of the figure, the biology behind each hypothesis predicts different degrees of overlap between flowering and leaf development. Transpiration intensifies as small leaf primordia expand, but leaf development only affects environmental structure once leaves are sufficiently large, therefore the water limitation hypothesis accommodates little overlap between flower and leaves, while the wind pollination hypothesis encompasses some overlap. The early flowering hypothesis predicts no fitness differences whether or not flowers and leaves overlap. Additionally, inter-specific patterns of FLS variation may also be a product of phylogenetic conservatism or lability. (d).

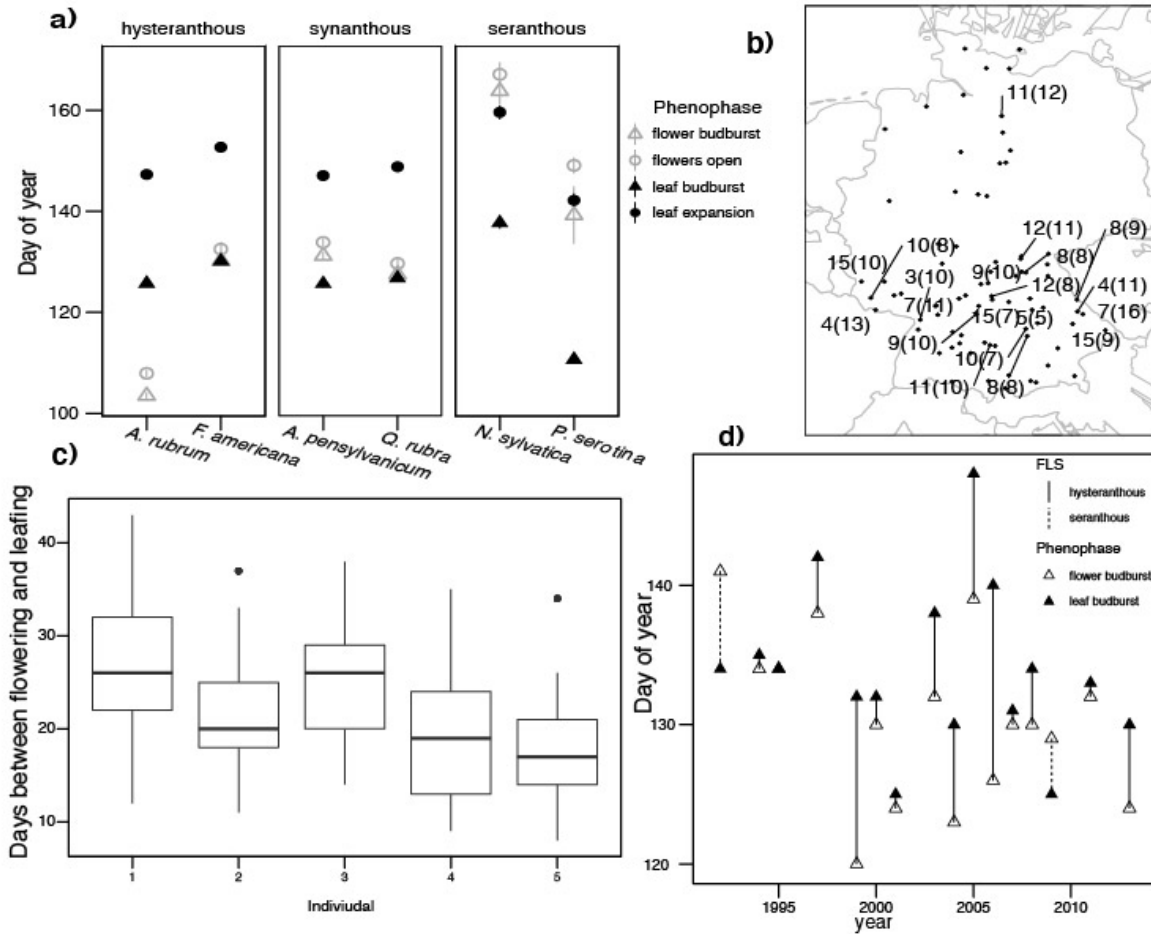


Figure 3: **The shift from categorical/inter-specific descriptions to quantitative/intra-specific measures of flower-leaf sequences (FLSs) reveals substantial variation.** Under the current framework, species are assigned to FLS categories by the order of phenophases alone. However, observations from Harvard Forest in Petersham, MA demonstrate that measuring the time between phenophases reveals substantial differences among species within each category (a). Quantitative measures also reveal variation in FLSs below the species level. Population level FLS data (b), show that the mean and standard deviation of the time between flowering and leaf activity can vary substantially among populations of *Fraxinus excelsior* across Germany. Within a population of *Acer rubrum* at Harvard Forest (c), individuals show consistently different FLS timing. For a single individual of *Fraxinus americana* at Harvard Forest (d) the days between flowering and leafing can vary by as much as several weeks across years, with the sequence itself regularly switching over time. This inter- and intra- specific variation is key understanding the function of FLS variation in temperate deciduous, woody plants. Data for (a),(c),(d) come from O’Keefe (2015), and (b) from the PEP725 database (Templ *et al.*, 2018).

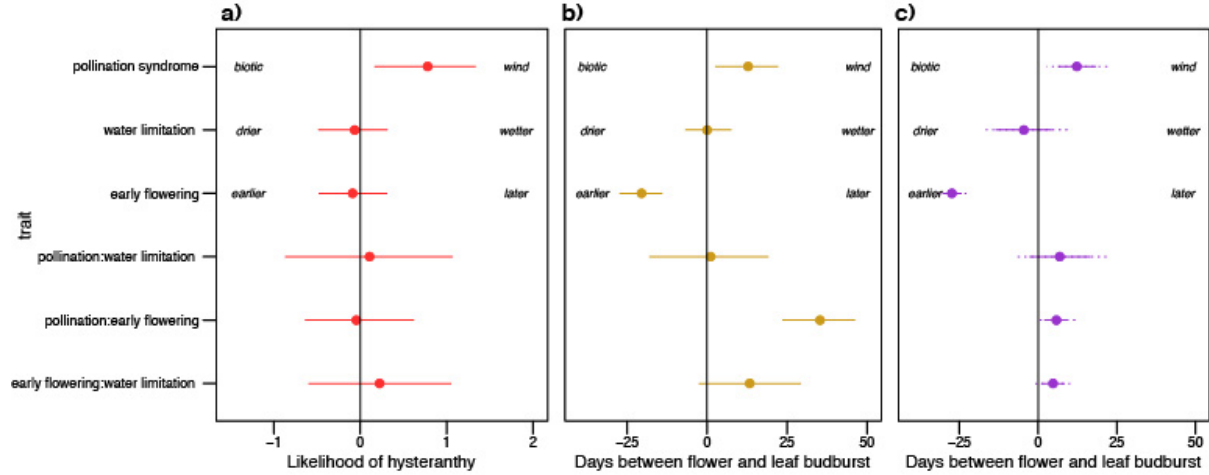


Figure 4: Mean estimates of the effects of flower-leaf sequence (FLS) predictors on the timing between flower and leaf budburst for woody plants at Harvard Forest between 1990-2015 reveal important differences between categorical and quantitative frameworks of FLSs. With the categorical approach in (a), there is a strong effect of pollination syndrome on FLS variability, with no detectable effect of other predictors. With quantitative measures based on the species level means of days between flower and leaf budburst in (b), there are strong effects and interactions of both flowering time and pollination syndrome. Finally, incorporating variation below the species level through hierarchical modeling in (c), reveals strong interactions between the predictors. These interactions suggest multiple drivers of FLS variability in the temperate zone. All models use standardized predictors to allow for comparisons between them. Symbols represent mean estimated effect of each predictor, with solid lines in (a) and (b) representing the 95% bootstrap intervals of the phylogenetic linear regression models (Ives & Garland, 2010) and dotted lines in (c) representing 50 and 95% credible intervals respectively for a phylogenetic mixed model (de Villemereuil P. Nakagawa, 2014). Graphical interpretation of the model interactions of the hierarchical model can be found in the Supporting Information (Fig. S6)