

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

Daniel Buonaiuto, Nacho, Lizzie

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To Do:

Abstract

As the study of phenology progresses as a discipline, it is increasingly clear that it is not only individual phenological events that affect organism fitness and ecological functioning, but also the relationship between stages. Deciduous woody plant species exhibit remarkable variation in flower-leaf sequence's (FLS's), or the relative order of their spring reproductive and vegetative phenological events. A century of research suggests that FLS patterns are adaptive, and several competing hypotheses stand to explain their evolution and function.

But reconciling these hypotheses has been impeded by our conceptual framework for understanding these patterns. Classically, FLS's are treated as discrete categories and described at the species level. However, long-term phenological data suggest there is considerable intra-specific variation in FLS's that is unaddressed in the current hypotheses, and that FLS categorization further obscures interspecific differences. Here, we review and modify the existing hypotheses to account for the high levels of intra-specific FLS variation seen in nature. We then evaluate these hypotheses with four case studies from temperate forest species.

Our review and case studies provide three major insights towards a new conceptual framework for understanding FLS. First, we find support for multiple hypotheses. Future research should accommodate this coincidence both by allowing for overlapping hypotheses in large community models and by testing individual hypotheses in smaller sub-groupings that control for variation in other traits. Second, we show that the support for FLS hypotheses is highly sensitive to how FLS's are defined. Researchers should, when possible, move away from the classic categorization scheme of FLS and use standardized, continuous measures of FLS. Finally, researchers should use these intra-specific, continuous measures

of FLS to test for fitness consequences in FLS variation. This will advance our understanding of the fundamental biology of FLS patterns, and help us to predict how climate related alterations to FLS's will affect tree communities in the changing future.

Introduction

Phenology, the timing of seasonal life cycle events, allows organisms to synchronize important life history transitions with optimum environmental conditions (Forrest & Miller-Rushing, 2010), and is a critical component of ecosystem structure and function (Cleland *et al.*, 2007; Piao *et al.*, 2007). Recent work in woody plant phenology has shown that it is not only individual phenological stages that affect these processes, but also the relationship between them (Ettinger *et al.*, 2018).

One phenological relationship that has long received scientific interest (see Robertson (1895)), and recently, increased attention in the literature is the flower-leaf phenological sequence (FLS) of 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, it is not the green tips of new shoots that mark the commencement of the growing season, but the subtle, reds, and yellows of their flowers. This flowering-first FLS is common in these regions, and its prevalence suggests that this FLS has adaptive significance (Rathcke & Lacey, 1985).

A deep inquiry into the nature of this phenological pattern is necessary and timely because anthropogenic climate change is altering FLS's (Fig. 1). For the three European tree species we examined, the number of days between flowering and leafout have increased as a result of climate change, but the rate of change differs among them. If, as suggested, optimum FLS timing is an important component of fitness, this differential FLS sensitivity to climate change may influence which species can adapt to new climate conditions and persist into the future.

Despite recent advances in characterizing the evolution and underlying physiology of FLS (Gougherty & Gougherty, 2018; Savage, 2019), a major challenge to predicting how FLS patterns will shift in response to climate change is that we don't have a very good baseline understanding of variability in FLS. While some authors present general correlations between flowering and leafing phenology (Lechowicz, 1995; Ettinger *et al.*, 2018), fine scale FLS variability has never been evaluated. We suggest that characterizing FLS variation among individuals and populations will not only improve our ability to predict how FLS patterns will change in the future, but also allow for a more biologically relevant evaluation of the current FLS hypotheses and reveal avenues for future, direct hypothesis testing.

Here we 1) Review the adaptive hypotheses of FLS and their respective predictions, 2) Evaluate variation in FLS, and explore how FLS variation within species, populations and individuals alters the predictions of the hypotheses, 3) Show how the incorporation of

variation alters which hypotheses are supported using case studies from temperate forests, and 4) make recommendations for future study of FLS.

Defining FLS

Flower-leaf sequences have traditionally been classified into distinct qualitative categories that are almost always defined at the species level. The terms hysteranth, protanth, proteranth or precocious flowering describe plants that produce flowers before their leaves (Lamont & Downes, 2011; Heinig, 1899). A classic example of this FLS is *Acer rubrum*, which, as seen in figure 2, reaches peak flowering weeks before any sign of leaf development. These species tend to exhibit a degree of physiological specialization, such as the separation of flower and leaf buds.

Seranth describes species in which flowers open as leaves approach their full size. These species can still differentiate flower buds in the previous season, but may rely less on stored energy than flowering-first taxa.

But what about species whose FLS separation is less clear? It is possible to describe all species whose flowering period overlaps their leaf development as synanthous (Lamont & Downes, 2011), but this third category may obscure important inter-specific differences.

Take *Betula alleghaniensis* from figure 2 for example: One would be justified in classifying this species as hysteranthous because its flower buds tend to burst before its leaf buds, or as synanthous because its open flowers overlap the beginning of leaf growth. Can we really put this species in the same category as *Acer rubrum*, whose flowers open weeks before the leaves? Conversely, is this species truly more similar to figure 2's *Acer pensylvanicum* whose flowers do not open until leaves are well along in their expansion?

The FLS classification scheme relies on the judgement of each individual observer making it difficult to synthesize information across datasets. If a species is called hysteranthous in one dataset and synanthous in another, should we interpret this as temporal or geographic variability in FLS or an artifact of observer decision-making? This uncertainty hampers our ability to accurately test the existing FLS hypotheses, because any statistical relationship between FLS and other traits is biased by the classifier. However, a way forward through this bias is possible by examining the biological mechanisms underlying each hypotheses and incorporating the predictions they make regarding FLS overlap and variability in to analyses.

Hypotheses of FLS and their predictions about flower-leaf overlap

Each prevailing FLS hypothesis suggests evolutionary drivers that produce the phenological sequences observed today. These mechanisms accommodate a different degree of

overlap between vegetative and floral phenophases. Here, we review the current FLS hypotheses, identify the underlying biology of each, and make predictions about how much phenological overlap between flowering and vegetative growth they can accommodate.

Wind pollination

The most prevalent FLS hypothesis associates hysteranthous flowering with pollination syndrome, suggesting that hysteranthy is an adaptation critical for effective wind pollination, with leafless flowering allowing for more efficient pollen dispersal and transfer (Whitehead, 1969; Stephen H. Spurr, 1980; Friedman & Barrett, 2009). This hypothesis hinges on the fact that leaves create a substantial physical disruption to pollen transfer, a premise that we would not necessarily expect to be true for the early stages of leaf expansion when tiny leaf primordia would have little impact on environmental structure. In this framework, we expect that trees that flower during the early stages of leaf expansion would gain similar mechanical advantage to those who complete their flowering before any leaf activity, and we see evidence for this in the phenological records from Harvard Forest. While wind-pollinated species flower both before and after bud burst, the flowering of all species takes place before their leaves reach 75% of their final size. This hypothesis predicts that wind pollinated species should flower before or with their leaves, while in animal pollinated species, FLS should be random or co-vary with pollinator activities.

Water dynamics

Another hysteranthous hypothesis, emerging from the dry deciduous tropics where flowering during the leafless season is also common (Janzen, 1967), suggests that flowering before leaf development is an adaptation to reduce water stress associated with maintaining floral hydration while leaves are transpiring (Franklin, 2016). This hypothesis asserts a significant cost to maintaining floral structures during any stage of leaf activity, and therefore only species whose flowering occurred before any leaf expansion would gain this drought advantage. This hypothesis predicts that species that are drought tolerant should flower before leafing out, with minimal overlap between the floral and vegetative phenophases. Species that are not drought tolerant gain no real advantage from flowering first, so in these species FLS should be random.

Early flowering

A third possibility is flowering-first FLS is a physiological byproduct of selection for early flowering (Primack, 1987). Within this framework, there is no advantage to a species being hysteranthous vs. seranthous, as long as the absolute flowering time of the contrasting FLS's were the same. However, recent work from Savage (2019) has demonstrated that floral hydration is independent of the xylem and primarily maintained by the phloem. With leaf phenology constrained by the timing xylem re-genesis, selection

for early season reproduction drives flowering into the early season when xylem function is still suppressed, producing the hysteranthous FLS. This might explain why hysteranthous species tend to be the earliest species to flower. Here, we expect the increased time between flowering and leaf out to be associated with earlier flowering phenology in general. We expect to see strong associations with other early flowering traits such as seed mass, dispersal season or cold tolerance. However, this hypothesis does not require the selective driver of early flowering to be exclusively one of these traits, and pollination syndrome or drought tolerance may still play a role in driving the early flowering (Savage, 2019).

This hypothesis predicts that species' flowering times should be strongly associated with flowering-first FLS. It also is likely there would be relationship between this FLS and other early flowering traits.

Phylogenetics

Finally, it is also possible that FLS's are highly conserved traits, and the preponderance of hysteranthous in the temperate zone is a product of phylogenetic representation of the region rather than an adaptive quality to the trait. In this framework, FLS is under very weak or no selection so there are no expectations regarding the degree of overlap between flower and leaf phenological activity, only that FLS's should map well onto a phylogeny. This hypothesis predicts strong phylogenetic patterning in the FLS with no correlation a priori expected with other traits.

Variation in FLS

All of the above hypotheses assume that FLS's are a species-level trait, however, this assumption has not been well examined in the literature (e.g., Gougherty & Gougherty, 2018). Intra-specific variation is the engine of natural selection, and if it is substantial in FLS patterns, we can infer much about origins of this trait, and its trajectory as the climate changes. We investigated individual FLS variation using a long term phenological data set collected at Harvard Forest in Petersham, Massachusetts (O'Keefe, 2015). The time between flowering and leaf activity varied by as much as several weeks for most species. This variability can significantly blur FLS categorization. For example, *Q. rubra*), a species classically listed as flowering and leafing in synanthous, there are some years in which flower budburst is over a week before leaf budburst, and other years, in which leaf buds burst weeks prior to floral budburst (Fig. 3). We also found significant population level variation in FLS, using the Pan European phenological database PEP725 (Templ *et al.*, 2018), with the average time between flowering and leafing varying between sites by a week more.

Given the variability of FLS at the individual and population level, it is clear that considering FLS variability at only higher taxonomic levels may obscure important re-

alities about the biology of this phenological trait. Below, we discuss how the observed variation below the species level may alter the existing FLS hypotheses.

How FLS variation alters predictions

Wind pollination

Pollination syndrome is generally treated as a species level trait, considered to be fairly immutable across ecological time and space. Because of this, we would not expect significant variation in FLS across population or individuals because we would not expect variation in pollination syndrome. However, as discussed above, a tree with no overlap between flowering and leafing phenology does not necessarily gain a significant pollen transfer advantage over an individual with some overlap. The pollination efficiency advantage from flowering-first diminishes as the canopy fills in, but we do not know at what point during leaf expansion pollination would become significantly encumbered. It is possible that interannual and population level variation in hysteranthous FLS could maintain a wind pollination advantage, as long as the overlap did not cross a certain unknown threshold. Therefore, based on the wind pollination efficiency hypothesis, we would not expect high levels of population or individual variation in FLS, but the detection of some FLS variability at these levels, does not inherently challenge the plausibility of the hypothesis.

Water dynamics

If FLS's are driven by water dynamics, we would expect there to be significant population level variation in FLS. Populations growing in drier habitats should show flower earlier relative to their leaf activity than their counterparts growing in wetter areas with more relaxed selection on minimizing phenological overlap. Therefore, increased time between flowering and leafing should be negatively correlated with average soil moisture. Water availability may also drive interannual FLS variation, with drought years increasing hysteranth, and wetter years permitting more FLS overlap. Because plants have many other physiological mechanisms for dealing with occasional drought, we only expect to see a signal for the association between a drought tolerance and hysteranth for observations of populations in drought prone regions.

Early flowering

This hypothesis predicts some variation on the population level based on local adaptation. We would expect populations in which selection for earlier phenology is stronger, perhaps those in regions with shorter growing seasons, to show a higher degree of hysteranth. At the individual level, FLS variability could be driven by interannual variability

in spring conditions. Both flowering and leaf phenology are strongly cued to temperature and photoperiod (Flynn & Wolkovich, 2018; Rathcke & Lacey, 1985), but with leaf phenology constrained by xylem activity and flowering phenology relatively independent of it, we would expect a stronger response to environment in flowering time resulting in FLS variation. Below the species level, this hypothesis predicts that early flowering years or populations are associated with increased time between flowering and leafing for hysteroanthous species.

Phylogenetics

With the lack of treatment of FLS variability in the literature below the species level, we have no strong basis for asserting whether the apparent variability in FLS is a product of genetic or environmental controls. If there is a strong genetic component to FLS as has been shown in other phenophases (Wilczek *et al.*, 2010), some population level variation could be driven by reproductive isolation. With strong genetic control of FLS, we might also see consistent genotypic differences in FLS among individuals within a population, but would not predict high levels of interannual variation.

Available evidence for FLS hypotheses in temperate woody species

Despite a strong conceptual basis, direct tests of these hypotheses of hysteroanth in the literature are relatively rare, and—when tested—support for them is mixed. Many studies only test a single hypothesis, making comparison between them difficult. For example, the primary evidence supporting the wind pollination hypotheses comes from pollen diffusion studies, e.g., particle movement through closed and open canopies (Niklas, 1985; Nathan & Katul, 2005; Milleron *et al.*, 2012), which provide no framework for comparatively evaluating the other hysteroanth hypotheses. We are aware of no direct test that have tried and distinguish selection for hysteroanth from selection for early flowering, but Primack (1987) notes that hysteroanthous, wind-pollinated species tend to also have large seed mass, and lack primary seed dormancy for germination, traits associated with early flowering in general. This raises the distinct possibility that hysteroanth may simply be one component of a larger suite of early flowering traits. We are also aware of no studies that have mechanistically evaluated the water dynamics hypothesis, though observations of flowering in the dry tropics by Borchert (1983); Reich & Borchert (1984) suggest that the timing of flowering in hysteroanthous taxa is associated with a plant water status recovery due to leaf drop. Only recently has it even been suggested that this hypothesis might be relevant in the temperate zone as well, as it is not expected that water status would limit biological activity in the wet spring months of the temperate zone (Gougherty

& Gougherty, 2018).

In contrast, studies testing multiple hypotheses have generally found support for more than one evolutionary driver of hysteranthly. One study by Bolmgren *et al.* (2003) showed that wind pollinated species tend to also be earlier flowering than their biotocially pollinated sister taxa, suggesting a relationship between the early flowering and wind pollination hypotheses. A recent study by Gougherty & Gougherty (2018) tested multiple hypotheses by modeling associations between species' trait and FLS patterns in the Great Lakes regions. They found strong support for both the water dynamics and early flowering (flower timing and seed characteristics) hypotheses, and found strong phylogenetic clustering for FLS.

In all of these cases, variability in FLS below the species level was not addressed. Yet, there are datasets widely available that would allow for testing these several hysteranthly hypotheses concurrently, and at multiple taxonomic levels. To address this gap, we supplement our literature review by re-testing some previously-used datasets to examine all hypotheses, and we leverage several widely-available datasets to test how support for these hypotheses varies across the inter- to intra-specific levels.

We evaluated hysteranthly in four phenological datasets. Michigan Trees and its companion volume Michigan Shrubs and Vines (Barnes & Wagner, 1981,2004; Burton V. Barnes, Christopher W. Dick, 2016) (MTSV) contains categorical FLS information for 195 woody plant species. The USFS Silvics manual volume II (Burns *et al.*, 1990) contains categorical FLS descriptions for 81 woody species. These data can be used to test inter-specific FLS variation. Within these datasets, we applied two alternative FLS classification schemes; physiological hysteranthly, which allowed for no overlap between floral and leaf phenophases, and functional hysteranthly, which allowed for a degree of overlap as predicted by the wind pollination hypotheses. The Harvard Forest dataset (HF) contains quantitative flowering and leaf phenology measurements for individuals of 24 woody species over a 15 year period, allowing for both inter- and intra-specific comparisons (O'Keefe, 2015). In this data set, we approximated the two hysteranthly classification schemes mentioned above by measuring the temporal offset between different floral and leaf phenophases. From the Pan European Phenological Database (PEP725) (Templ *et al.*, 2018) we obtained spatially and temporally explicit, quantitative flowering and leaf phenology for four common European tree species. This allows for the evaluation of FLS only at the intra-specific level, but unlike the other datasets, it allows for population level variability to be assessed.

In considering each species-level data set separately and in tandem two clear trend emerge: One, in accordance with the recent literature, we found support for multiple hypotheses. There was generally strong support for the early flowering and wind pollination hypotheses, poor support for the water dynamics hypothesis, and the phylogenetic signal was variable (figure 7). The support for multiple hypotheses is not terribly surprising. We wouldn't expect the wind pollination hypothesis to explain hysteranthly in biotically pollinated taxa. Further, given the almost constant non-equilibrium state of temperate forest

communities due to glacial cycles over the last 10,000 years (Stephen H. Spurr, 1980), it is not surprising our flora consists of species with radically different bio-geographic histories that may have evolved hysteroanthous flower under very different selection environments.

The second clear signature from our analysis was that relative importance of each the predictors changed significantly depending on how hysteroanthous was defined. Generally, this artifact was minimized when continuous measure of FLS were used over categorical.

When considering our intra-specific datasets, contrary to what we predicted given the water dynamics hypothesis, intra-annual variation in water available (drought vs non drought years) does not increase hysteroanthous. Rather, it seems that drought years correlate with a decrease in FLS offset in hysteroanthous species, largely due to delayed flowering. This observation limits our expectation for the water dynamics hypothesis, but does not eliminate the possibility that intra-specific differences in FLS offset are adaptive for populations growing in regions with sustained differences in water availability. If fact, when we examined the relationship between 30 year soil moisture records and population level variation in FLS offset across Germany, we did find a weak association between lower average soil moisture levels and increased hysteroanthous offset as predicted by the water dynamics hypothesis. However, when we incorporate other predictors such as flowering time into our analysis, the negative association between soil moisture and increasing hysteroanthous offset disappeared (see figure 6). Our intraspecific models suggested that variability in hysteroanthous offset is much more tightly correlated with variability in flowering time vs. leafing time (figure 8). However, this contrast was far less stark in seranthous *Aesculus hippocastum*. These patterns match expectations from recent physiological work by Savage (2019) who found that flower phenology was more less constrained than leaf phenology in for a suite of hysteroanthous and synanthous woody plants. Though our intra-specific data set is species limited, we see that plasticity in the first phenophase of the season (flowering for hysteroanthous species and leafing for seranthous species) seems to drive variability in in FLS offset, but this observation should be tested more rigorously and explicitly in future work. While the inter- and intra-specific case studies are not perfectly comparable (ie pollination hypothesis can be evaluated intra-specifically), the inference from the intra-specific study supports the relationships found in the inter-specific case studies, and provided novel insights of its own. But perhaps more important that the results of all of these specific case studies themselves, is that through considering them together, we are provided a more comprehensive picture of where our understanding of this phenological trait is currently, and where it needs to go.

Future

Our interrogation of the nature of flower-leaf phenological sequences leads to realization that it is instructive to test questions of hysteroanthous at many scales. Because trait modeling in large community level datasets seems to support multiple hypotheses and is confounded by species' identities and observer bias, the utility of these data has its limits.

While there is certainly value to broad taxonomic studies, and future large-scale analyses should continue, it is possible the evolutionary dynamics of hysteroanthony may be better explored with a more mechanistic approach, which may mean utilizing a more taxonomically restricted focus.

One option is to look within the hypotheses to address sub-grouping of taxa in which overlap between hypotheses could be controlled. For example, what drives hysteroanthony among biotically pollinated taxa? It certainly isn't wind pollination efficiency. Or, what factors accounts for variability in hysteroanthony among wind pollinated taxa? Incorporating a more explicit phylo-biogeographic approach would be instructive at this level, for example: are their phylogeographic commonalities between biotically pollinated hysteroanthous species in Eastern flora?

But even with drilling down to sub-groupings, interspecific trait association models can only take us so far. One reality of these kind of studies is that we never know we are picking the right traits. For example we used minimum precipitation across a species' range, one of the only available quantitative drought metrics at the scale of large interspecific models, to represent the water dynamics hypothesis. Is this really a good proxy for drought tolerance? Further, species evolve a suit of traits for any function, and unmeasured traits might bias our results (Davies *et al.*, 2019). For example, wind pollinated species could compensate for a pollen intercepted by a synanthous or seranthous FLS by over producing pollen or through self-pollination. To really understand FLS across large taxonomic space, one would have to compare species across an unfeasibly large, N-dimensional trait space.

Considering variation in hysteroanthony at the intra-specific level overcomes many of these limitations, and is the the next frontier in testing the evolutionary and ecological significance of FLS. Evolutionary theory predicts that intra-specific variation should follow the same trends as interspecific variation. The agreement between our intra- and inter-specific models supports this, and may suggests that we are narrowing in on certain hypotheses. Further, though our datasets were taxonomically and geographically limited, they demonstrate that FLS variability is significant over time and space. Looking within species holds most other traits relatively equal, avoiding the problem of latent tradeoffs with unmeasured traits.

There are also clear advantages of treating hysteroanthony as a continuous trait. As mentioned above, continuous data minimizes the observer bias that comes with categorization. It also reveals important inter-specific differences that are masked by categorization. For example, two categorically hysteroanthous species may have dramatically different FLS offsets. Through working with continuous measures of hysteroanthony, substantial intra-specific differences in FLS emerge, and as will be discussed more below, these will be valuable for hypothesis testing. All and all, our work shows categorizing hysteroanthony into groups is biased and biologically problematic; future studies about phenological sequences should avoid these categories and treat FLS as continuous traits whenever possible.

With this equalizing nature of intra-specific comparisons and continuous measure-

ments of FLS we can move beyond trait associations and actually begin to look at fitness consequences of FLS variation through experimental manipulations and observations. This next step is intuitive because fitness actually drives trait evolution, and the hysteresis hypotheses themselves make fitness predictions. It is tough to tease these apart at the inter-specific level because of the latent traits mentioned above, but the hypotheses predict that variability in hysteresis would lead to variability into fitness outcome at the intra-specific level. For example, the wind pollination hypothesis predicts that years with increased hysteresis should correlate with more pollination success. The water dynamics hypothesis suggests populations with increased hysteresis offset should better tolerate drought. These predictions could be directly assessed through well designed experiments.

Looking at fitness consequences will not only help clarify basic scientific hypotheses, but is essential for understanding how global change induced alterations to FLS's will impact species demographics. For example, if hysteresis is driven by pollination efficiency, increased hysteresis with climate change might favor hysteresis species. Or, if climate changes reduces FLS offset, hysteresis species may be at greater risk for reproductive failure. A better understanding of consequences of variation in hysteresis is essential both for understanding the evolutionary origins of this trait, and for predicting the fate of species with this phenological syndrome as global climate continues to change.

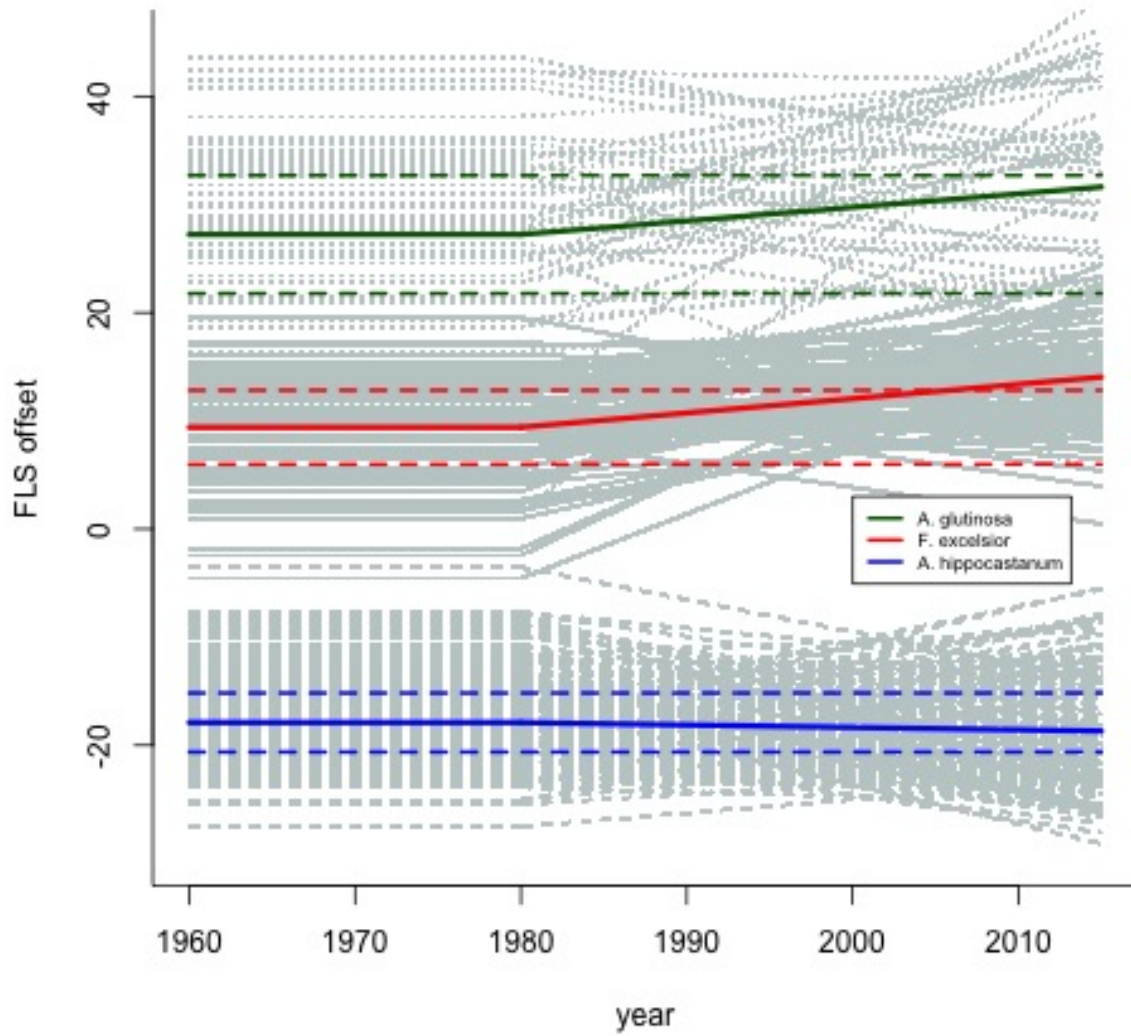


Figure 1: Trends in average FLS offset across Europe for 3 tree species from 1960 to 2015. Dashed lines indicate historic range of FLS variability. All species are increasing their offset, but the rate of change differs between species and sites

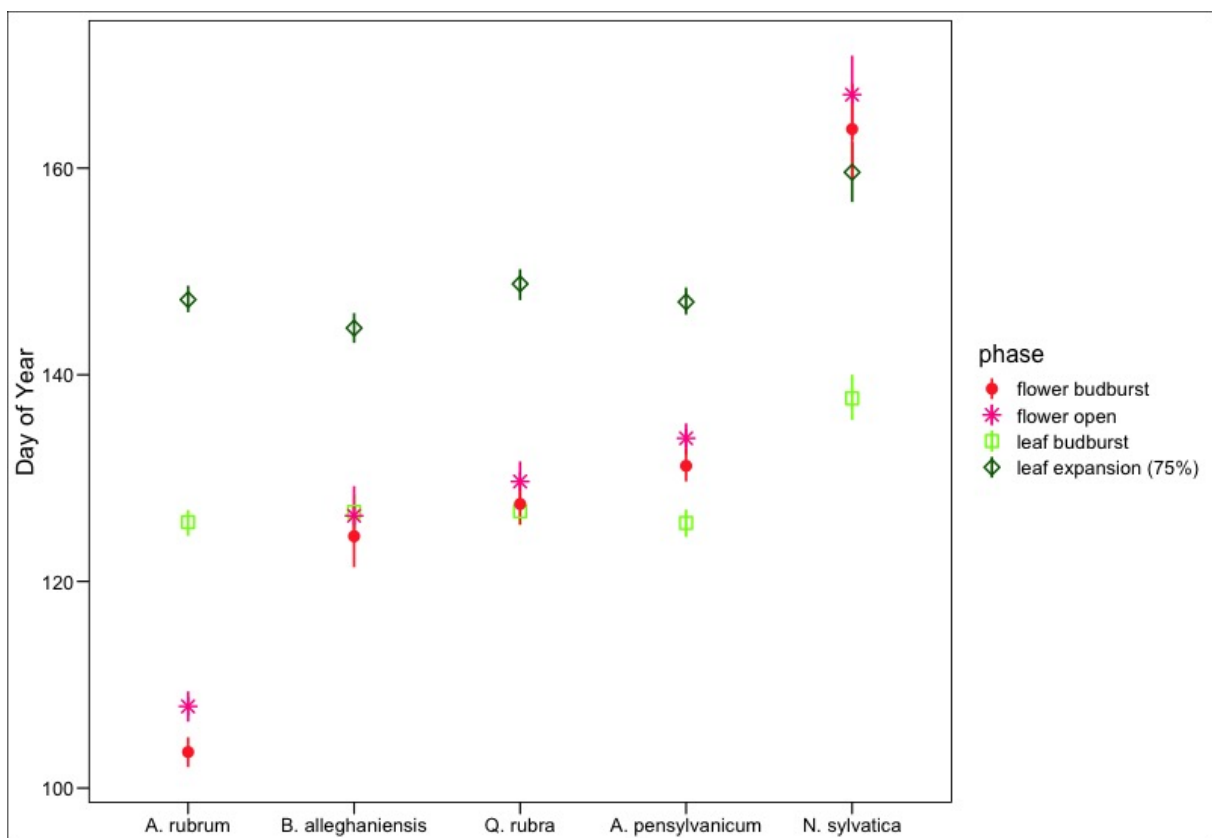


Figure 2: Average day of phenological events for highlighted woody plant species at Harvard Forest in Petersham, MA from 1990-2015

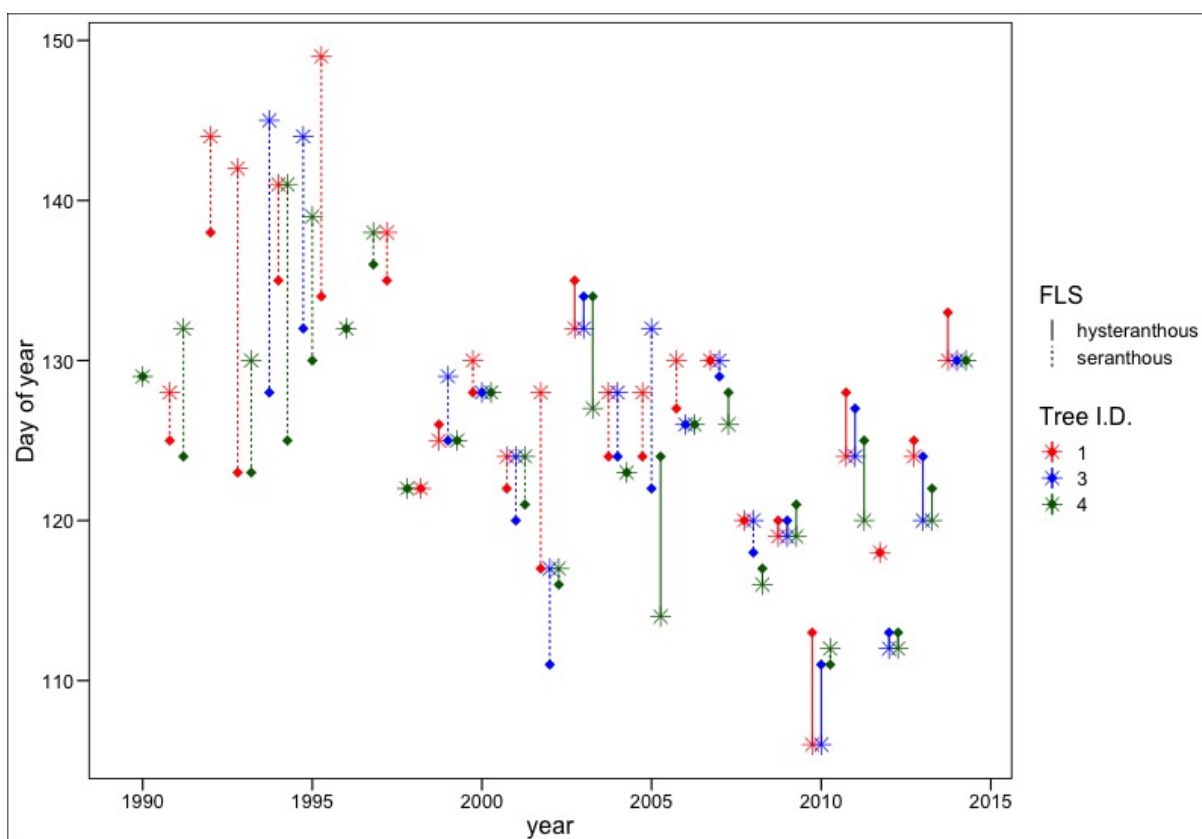


Figure 3: FLS variability among years and within a population of *Quercus rubra* at Harvard forest.

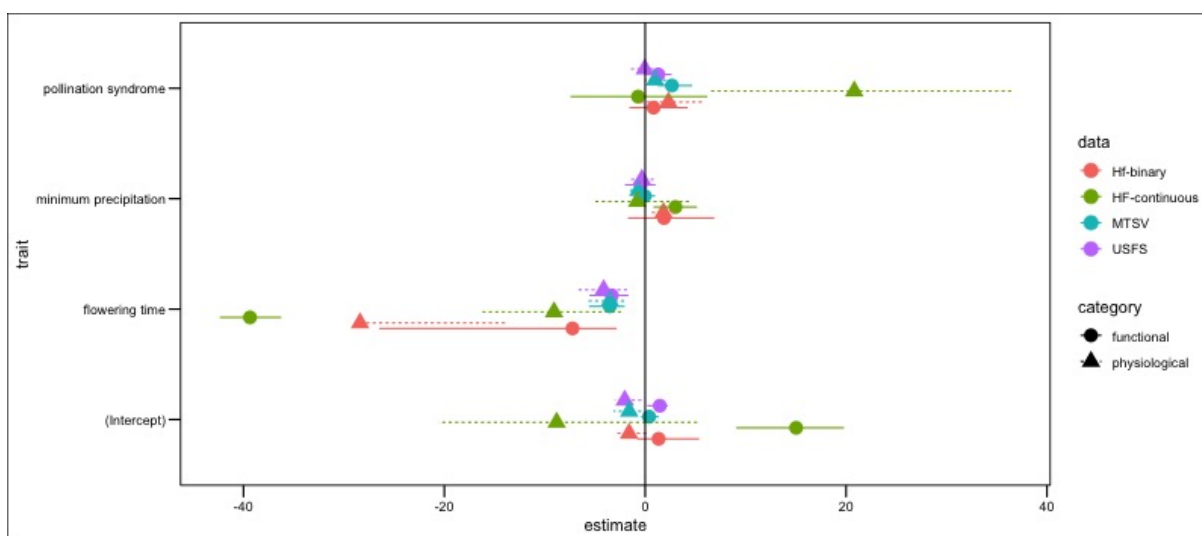


Figure 4: All case study model estimates, phyloglm with 95% bootstrap intervals

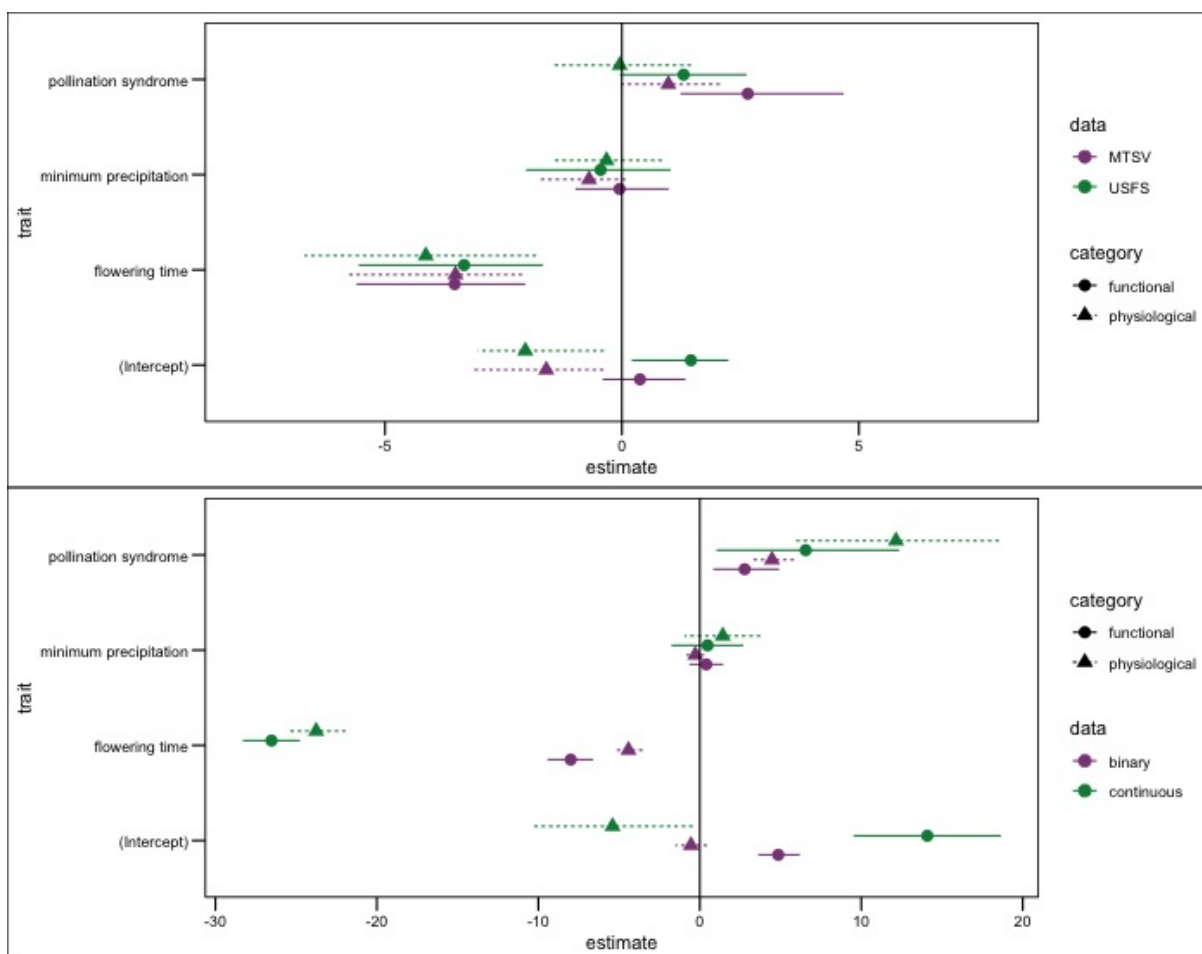


Figure 5: alternative to figure 4: 4a is phylog glm with 95% bootstrap intervals, 4b is brms estimates with 80% CIs

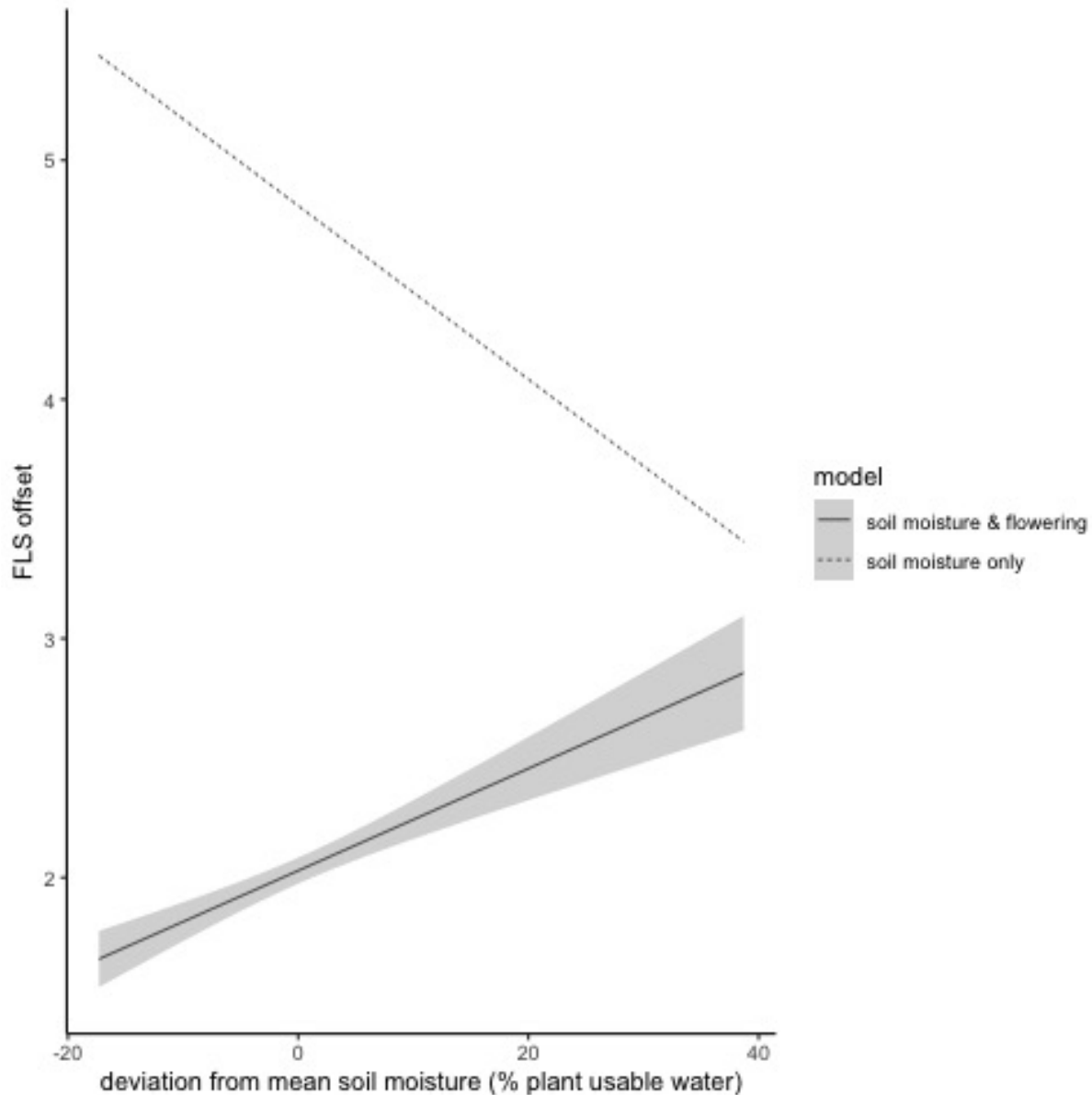


Figure 6: effect of soil moisture on FLS offset with (dashed) and without (solid) considering flowering time

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Supplement

Methods

Climate Change and FLS:

To evaluate how FLS patterns have overtime in association with climate change we obtained phenological data for three European woody plant species with long term records of both flower (BBCH 60) and leafout phenology (BBCH 11) from the Pan European Phenological Database (Templ *et al.*, 2018). We restricted the data set to include only stations with great than 50 years worth of data. For each species, we modeled FLS offset

(day of year leafing- day of year flowering) as a function of time, using a hinge model with 1980 as break point in accordance with climate change models of ?). For each species, we displayed the pre-1980 mean and standard deviation of FLS offset and the post-1980 change in mean FLS offset that can be associated with climate change.

Case studies

MTSV and USFS: For these two, categorical, species level case studies, we converted verbal descriptions of flower-leaf sequences into a binary response variable. For our more inclusive "functional" definition of hysternanthy, we included species entries with descriptions "*flowers before the leaves*", "*flowers before or with leaves*" and textit"flowers with leaves" as hysternanthous. Our more restrictive "physiological" hysternanthy definition only included species described as "*flowers before the leaves*" as hysternanthous.

For modeling trait associates we chose three predictors to represent the three major FLS hypothesis; pollination syndrome, average flowering time and minimum precipitation levels across the species range. Pollination syndrome and average flowering time we obtained directly from the data sources, and estimates of minimum precipitation came from the USDA/NRCS Conservation plants characteristics (). We coded pollination syndrome as binary, biotic or wind pollinated, with known ambophilous species in the genus *Salix* assigned to the ancestral, biotic pollinated, state of angiosperms. Flowering time was the average of the range of months reported in each data source.

HF: For each species in the HF data set, we calculated a continuous mean off FLS "offset" defined here as the average foliate day of the year - average floral day of the year. We approximated our "physiological" FLS characterization by defining offset as (day of leaf budburst-day of flower bud burst) and our "functional" FLS categorization by defining offset as (day of leaf expansion to 75% of final size- day of first flower open). We also recoded the HF continuous offset variables as continuous with positive offset values coded as hysternanthous and negative values as seranthous.

For all species-level case studies (USFS MTSV and HF), associations between hysternanthy and the trait predictors were modeled with logistical regressions in phylogenetic generalized linear modeling framework (Ives & Garland, 2010) using the R package phylolm (Ho & Ane, 2014). Our models incorporated a published angiosperm phylogenetic tree (Zanne *et al.*, 2013) pruned to match the species list for each case study. Species found in the trait data set but not in the original phylogenetic tree were added to the pruned tree at the genus level root. In total 32 species were added to the generic roots for the MTSV data set . We The models were run with 599 bootstrapped re-sampling iterations for each data set (?). Continuous predictors were re-scaled by subtracting the mean and dividing by two standard deviations to allow for a reasonable comparison of effect sizes between the binary and continuous predictors in this model (Gelman & Hill, 2007). To assess the phylogenetic structure of hysternanthous flowering, we used the Caper package (Orme *et al.*, 2013) to calculate a phylogenetic D statistic.

PEP 725: For intra-specific analysis, we utilized phenological records from PEP725 stations in Germany with more than 10 years worth of flowering and leafout records (Templ *et al.*, 2018) for species *Alnus glutinosa*, *Fraxinus excelsior* and *Betula pendula*. To test for associations between FLS variability and inter-annual water availability we modeled the association between FLS and drought years from 2003-2010 using a linear mixed modeling framework with the R package lme4 (?). Drought years were determined based on Ivits *et al.* (2013). To test associations for population level variation in FLS and long term soil moisture, we obtained average August soil moisture raster grids 1991-2010 for Germany from the German Weather Service (DWD, 2019), and extracted soil moisture values at every cell. We then tested associations between average soil moisture at each PEP725 phenological station and average FLS for species *Aesculus glabra*, *Alnus glutinosa*, *Fraxinus excelsior* and *Betula pendula* using a Bayesian linear mixed model framework with the brms package in R (Bürkner, 2018). We also repeated the analysis with average April soil moisture data from the same time period and results were robust.

Using same PEP725 species records as above, we used linear models to test the relationship between flowering and leaf timing and FLS offset.

Data set	Physiological	Functional
MTSV	0.22	0.07
USFS	0.13	0.65
HF	0.01	0.27

Figure 7: Phylo.D estimates of phylogenetic signal for hysteranthous flowering in three case studies

Species	Effect size flowering (sd)	R ²	Effect size leafing (sd)	R ²
<i>Alnus glutinosa</i>	-0.67 (0.002314)	0.58	0.24(0.005)	0.03
<i>Fraxinus excelsior</i>	-0.53 (0.002)	0.41	0.24 (0.004)	0.05
<i>Aesculus hippocastum</i>	-0.28 (0.002)	0.10	0.411 (0.0019)	0.26

Figure 8: Flowering vs leafing influence on hysternanthy

Phylogenies

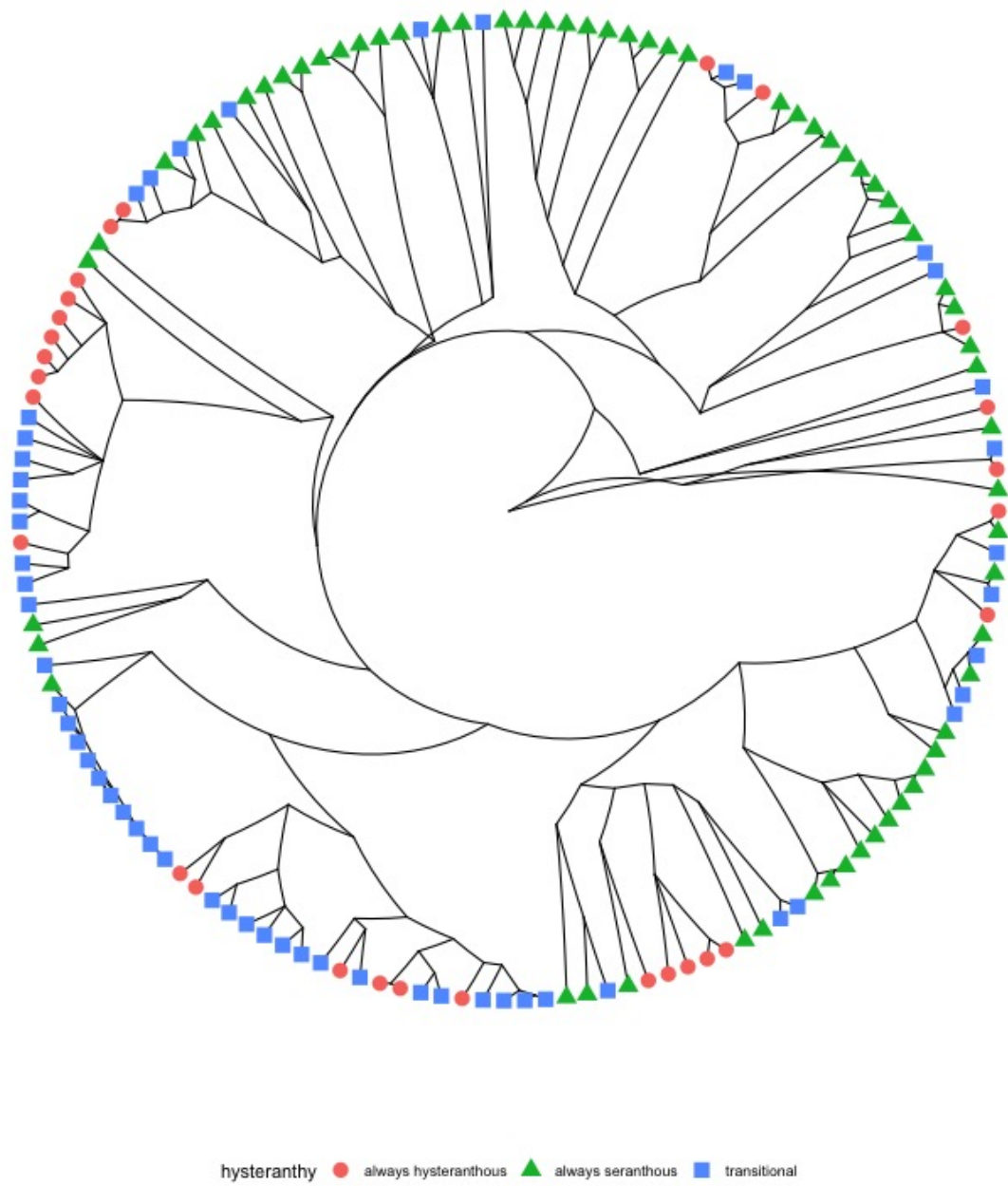


Figure 9: MTSV phylogeny

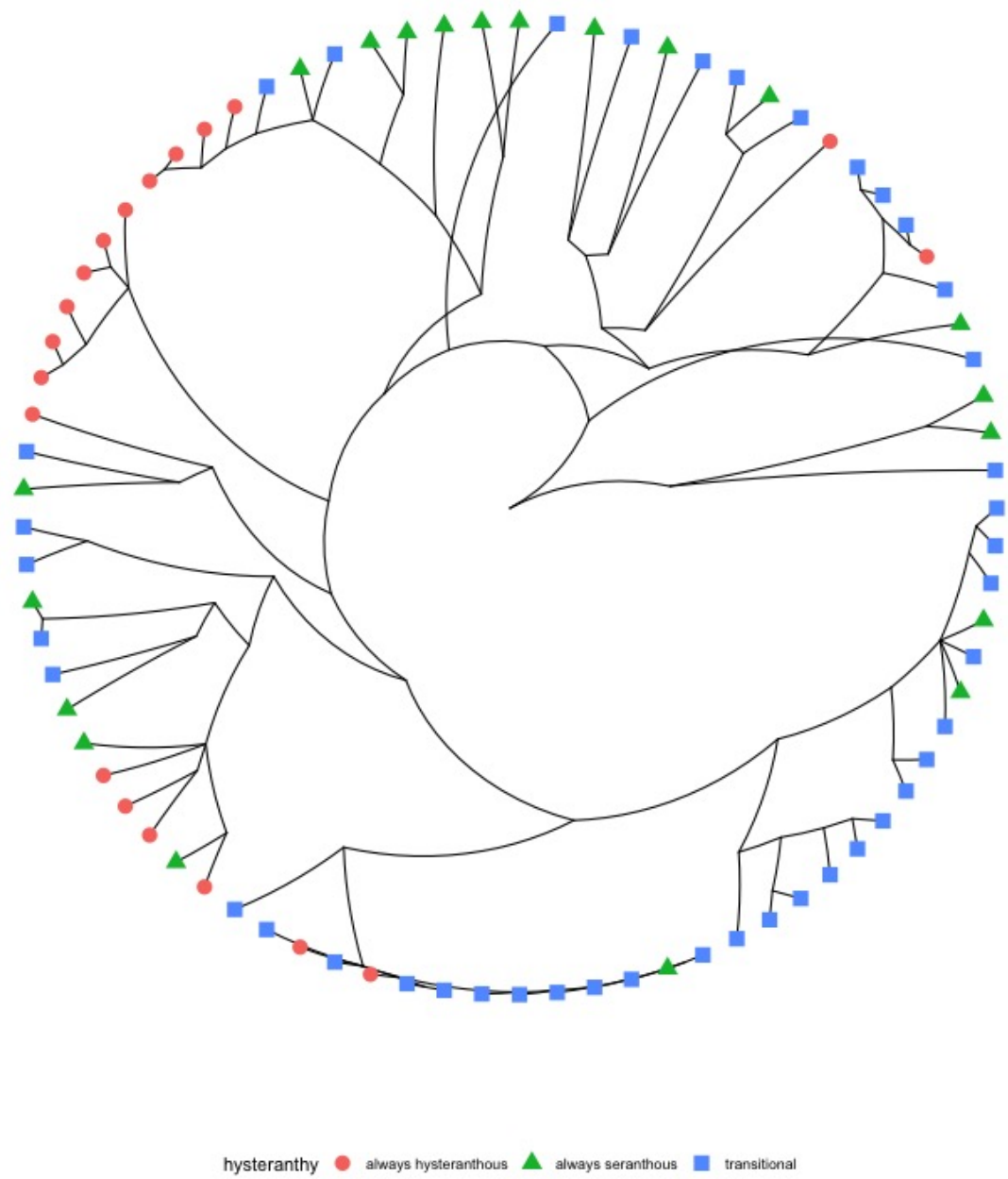
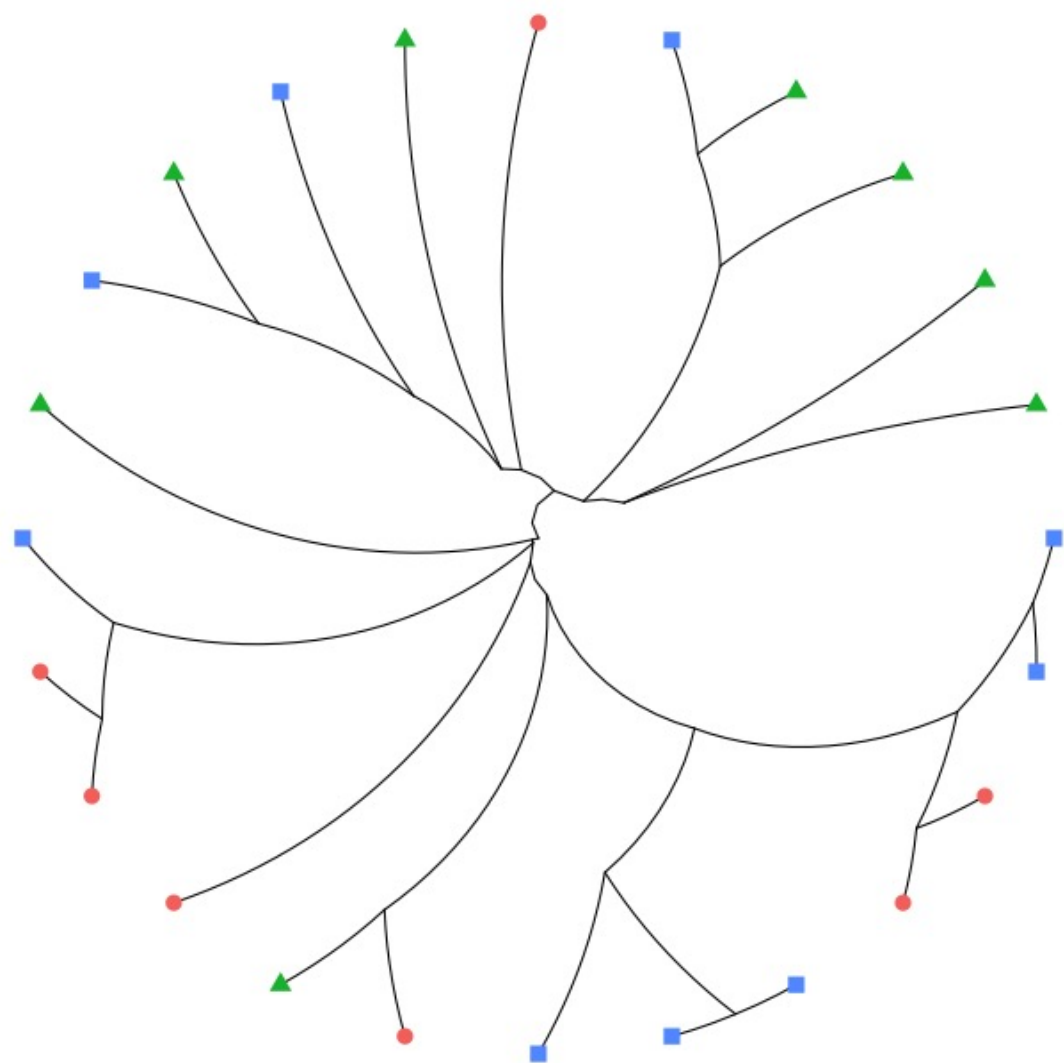


Figure 10: USFS Phylogeny



hysteranthy ● always hysteranthous ▲ always seranthous ■ transitional

Figure 11: HF Phylogeny