

1 *New Phytologist* Supporting Information

2  
3 **Article title:** Reconciling competing hypotheses regarding flower-leaf sequences in temper-  
4 ate forests for fundamental and global change biology

5 **Authors:** D.M. Buonaiuto, I. Morales Castilla, E.M. Wolkovich

6  
7 The following Supporting Information is available for this article:

## 9 Figures

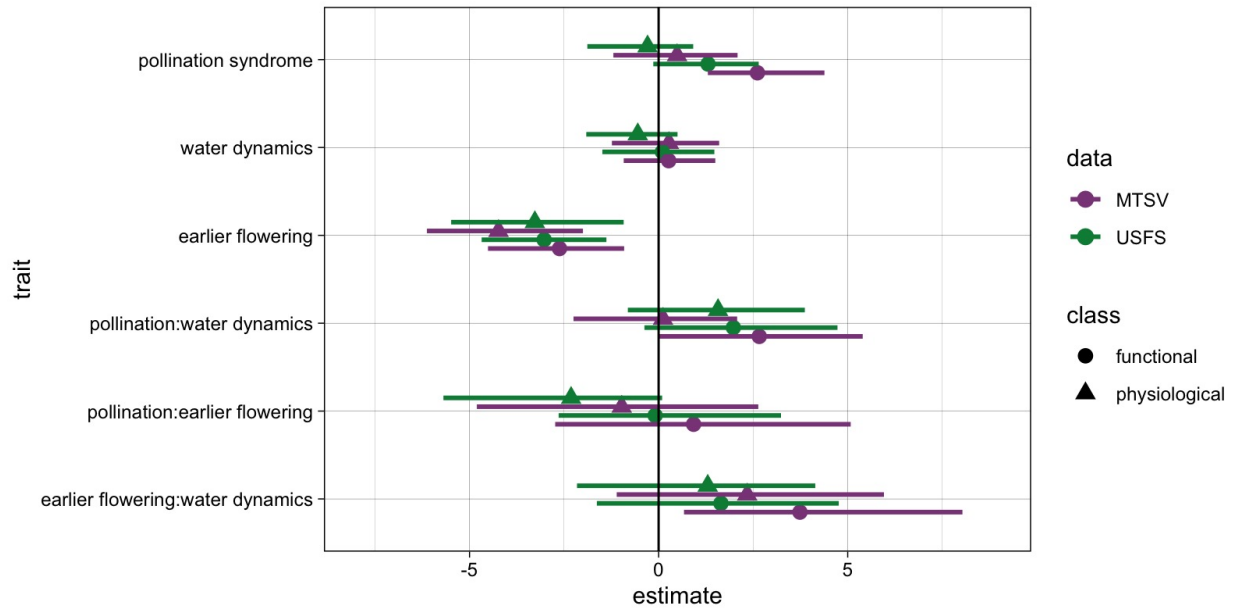


Figure S1: **Mean estimates of the effects of FLS predictors on the likelihood a species is hysteranthous vary across datasets and definitions of FLS.** We used phylogenetic adjustments and standardized units to make a basic comparison of two datasets (Michigan Trees, Michigan Shrubs and Vines (MTSV) (Barnes *et al.*, 2016; Barnes & Wagner, 1981,2004) and The United States Silvics Manual (USFS) (Burns & Honkala, 1990)) and classes (physiological= no overlap between flowering and leafing, functional= moderate overlap) of FLS. While there is some agreement across models (strong effects of flowering time, no consistent effect interactions between predictors), the effect of other predictors (pollination syndrome, water dynamics) were highly sensitive to how data were defined, biasing any inference from models and compromising the ability to validate the existing FLS hypotheses. Lines represent 95% bootstrap intervals.

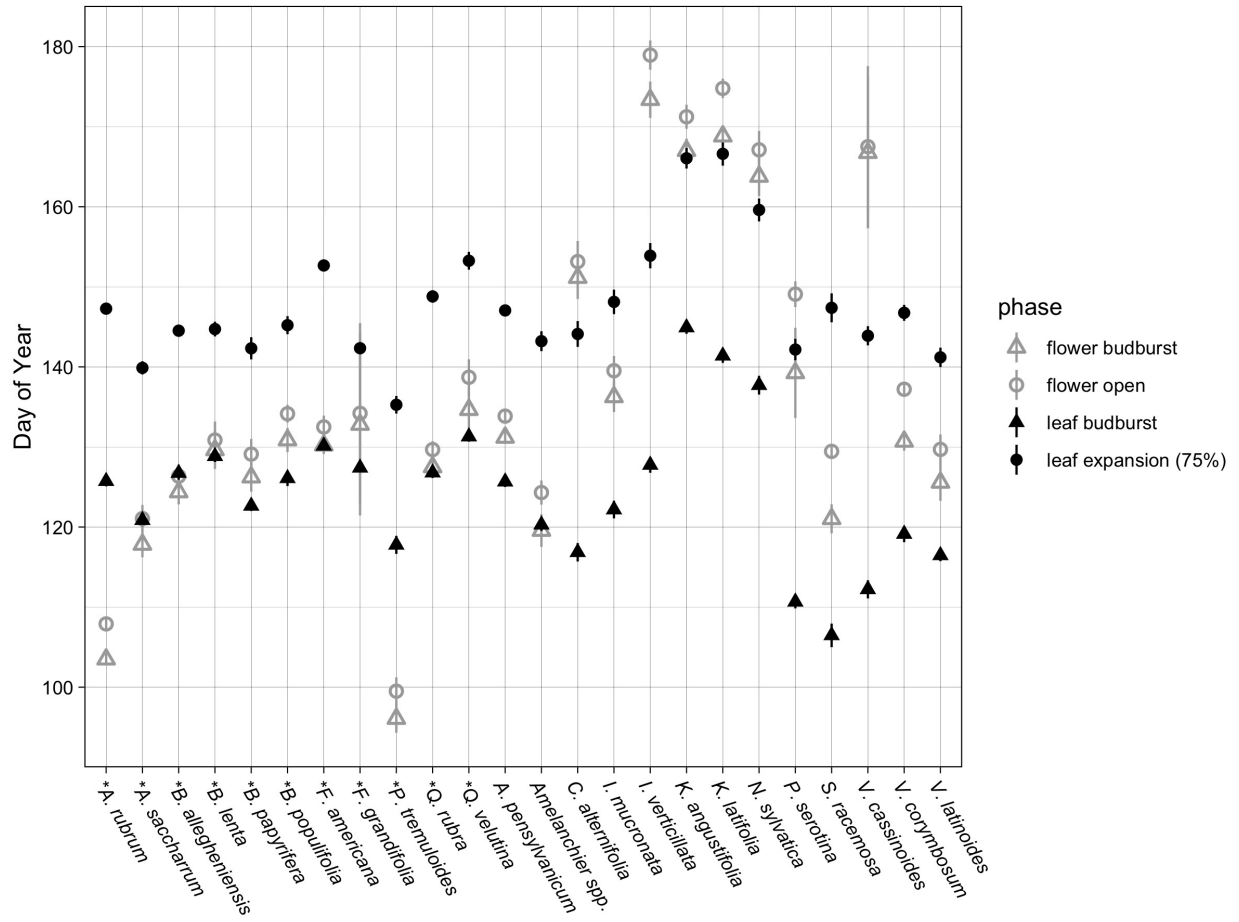


Figure S2: **Quantitative FLS patterns for woody plants at Harvard Forest in Pertersham, MA.** Because phenological sequences consist of several sub-stages it is difficult to unambiguously categorize many species into the current FLS categories.

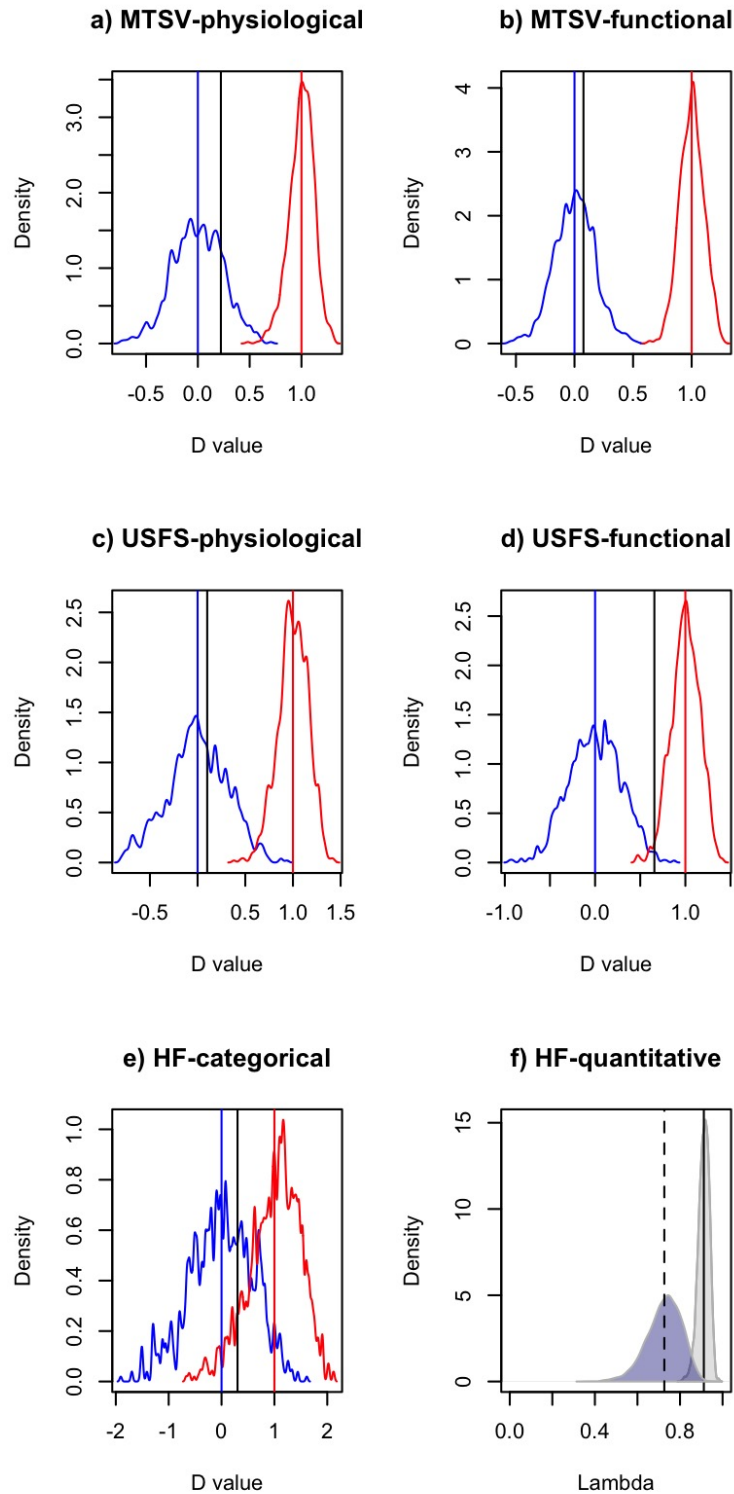
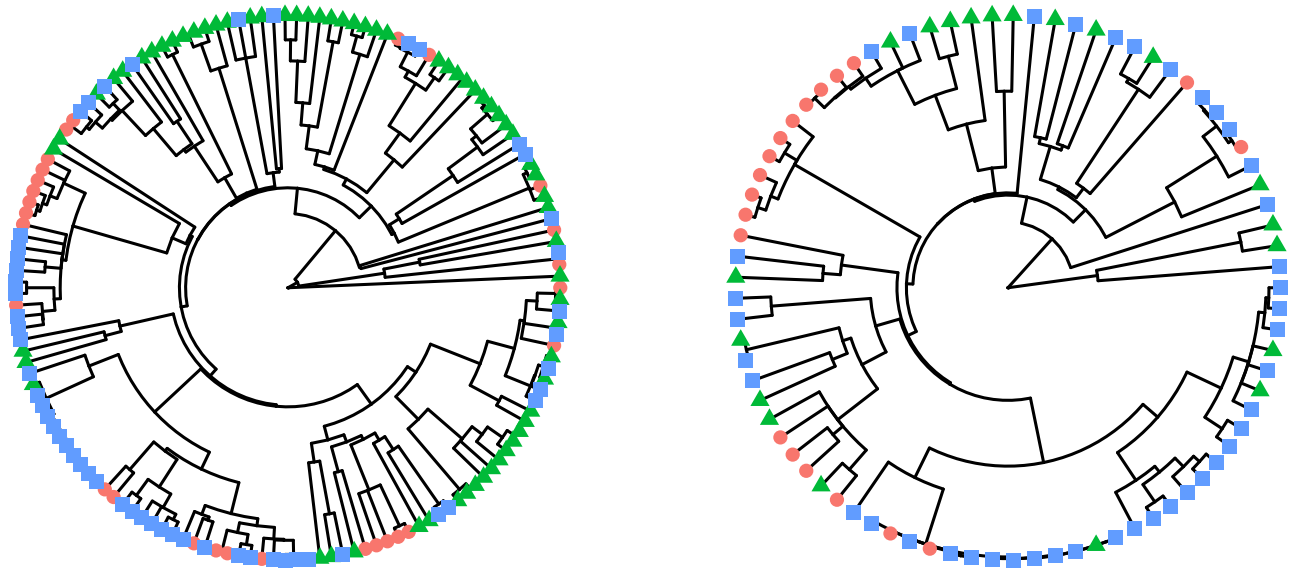


Figure S3: **The phylogenetic signal for FLS varies between datasets, and is sensitive to how FLS patterns are categorized.** In a)-e), the black vertical line shows the Fritz's D statistic for binary classifications of FLS estimated from the data, with blue and red lines representing expected D values based on simulations under Brownian threshold model and random model respectively. Panel f) shows the estimated  $\lambda$  values of FLS from the continuous modeling framework. The solid line indicates the mean estimate of  $\lambda$  in the intercept only model and the dashed line indicates the mean estimate of  $\lambda$  when all predictors were included in the model. Higher values indicate stronger phylogenetic structure.



hysteranthous    ● always hysteranthous    ▲ always seranthous    ■ transitional

Figure S4: **Phylogenetic structure of FLS in MTSV and USFS varies significantly depending on how FLSs are defined.** Many species get re-assigned to either hysteranthous or seranthous depending on whether FLS is defined functionally (partial overlap between flowering and leafing allowed) or physiologically (no overlap between flowering and leafing allowed) (blue squares). This modeling choice dramatically alters FLS patterning across the tree, resulting in an unstable phylogenetic signal for this trait.

# Methods S1

## Climate Change and FLS:

To evaluate how FLS patterns have changed over time in association with climate change we obtained phenological data for four European woody plant species with long term phenology 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 more than 50 years worth of data. Following conventions for modeling effects of climate change, we modeled the number of days between flowering and leafing as a function of time for each species, using a hinge model with 1980 as a break point (Kharouba *et al.*, 2018; Stocker *et al.*, 2013). For each species, we display the pre-1980 mean and 95% credible intervals of the time between flowering and leafing and the post-1980 change in mean time between phenophases that can be attributed to climate change.

## Modeling FLS variation in MTSV and USFS data

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 hysteranthy, which allows for some overlap between floral and vegetative phenophases, we included species entries with descriptions “*flowers before the leaves*”, “*flowers before or with leaves*” and “*flowers with leaves*” as hysteranthous. Our more restrictive “physiological” hysteranthy definition only included species described as “*flowers before the leaves*” as hysteranthous.

For modeling trait associations with FLS, we chose three predictors to represent the three major FLS hypotheses; pollination syndrome, average flowering time and minimum precipita-

tion levels across the species range. We obtained pollination syndrome and average flowering time information directly from the respective data sources and estimates of minimum precipitation across range from the USDA/NRCS Conservation Plants Characteristics database (USDA/NRCS, 2020). We coded pollination syndrome as biotic- or wind-pollinated, and assigned known ambophilous species in the genus *Salix* as biotic-pollinated. We re-coded flowering time as the average of the range of months of flowering reported in each data source.

For these case studies, we modeled associations between hysteranthly and the trait predictors with all two-way interactions 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 generic root. In total, 32 species were added to the generic roots for the MTSV data set and eight for the USFS data set. We visualize phylogenetic patterning of FLS across the tree of each case study (Fig. S4). The MTSV analysis was based on trait and FLS data for 147 species and the USFS analysis on 81 species.

We ran the models with 599 bootstrapped re-sampling iterations for each data set (Wilcox, 2010). We standardized all predictors 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).

## Harvard Forest models

From the publicly available Harvard Forest phenology data (O’Keefe, 2015) we calculated the time between flowers opening and leaves reaching 75% of their final size for each individual tree per year in the data. Positive FLS values indicate flowering-first and negative values leafing-first. To compare the inference between categorical and continuous measure of FLS, we re-coded the continuous FLS measures as binary responses with positive values coded as hysternanthous and negative values as seranthous. These models used the same predictors as the MTSV and USFS datasets (flowering time, pollination syndrome, minimum precipitation across species’ range and all two way interactions between predictors), except that we estimated flowering time directly from the HF data for each individual/year. The Harvard Forest analysis included 23 species. While taxonomically limited compared to the MTSV and USFS data, this data set included repeated phenology observation per species over time, and within year variation between individuals per species.

For both the categorical and continuous Harvard forest models we used a Bayesian phylogenetic mixed modeling framework (PMM) (de Villemereuil P. Nakagawa, 2014) using the R package “brms” (Bürkner, 2018). PMM’s incorporate the phylogenetic relationship among species as a random effect, utilizing a variance-covariance matrix based on species relationships to account for the non-independence in the model residuals that can be explained by phylogeny. We also included species as an additional random effect to account for non-independence in the residuals than is not due to phylogeny, and included individual as a nested factor within this random intercept to account for the repeat observations over time. The categorical model was built on a Bernoulli likelihood distribution and the continuous model on a Gaussian distribution. For both models, we ran 4 chains with 4000 iterations and a warm-up of 3000 iterations each, resulting in 4000 total sampling iterations. All models used weakly informative priors on the intercept and error terms. Increasing priors three-fold



80 did not impact the model estimates. As our primary goal was to directly compare the effects  
81 each predictor, we standardized these variables to allow for a reasonable comparison between  
82 them (Gelman & Hill, 2009).

83  
84 We calculated the marginal effects for the Harvard Forest continuous model using the  
85 R-package “ggeffects” (Daniel Lüdtke, 2018). Figure 5 shows the water dynamics effect  
86 of FLS given a flowering day of May 1 (close to the average flowering date for the whole  
87 community overtime). This same relationship between pollination syndrome and minimum  
88 precipitation remained evident across flowering dates from mid April-June.

89  
90 Though we make broad comparisons between the HF and MTSV/USFS case studies, dif-  
91 ferences in data structure between the datasets required us to use alternative modeling  
92 frameworks. The MTSV and USFS data provide one response variable for each species while  
93 the HF data contains intra-specific differences in FLS, providing several different response  
94 values per species. The current phylogenetic generalized linear model framework can only  
95 fit models with one response value per species, while the phylogenetic mixed model in brms  
96 may over-fit models with this kind of data structure and performs better on multi-response  
97 per species datasets like HF (Bürkner, 2017). We ran both model types on each case study  
98 and while they do yield different absolute estimates, the patterns we found were consistent  
99 across each framework, and we report results from the most accurate model for each dataset.

## 101 **Phylogenetic signals**

102 For all categorical specifications of FLS (MTSV, USFS and HF), we assessed the phylo-  
103 genetic structure of hysteranthous flowering in all with Fritz’s D-statistic (Fritz & Purvis,

2010) using the “Caper” package (Orme *et al.*, 2013) in R. Fritz’s D calculates the sum of changes in estimated node values of a binary trait along edges in a phylogeny and compares this observed value to both a model of phylogenetic randomness and Brownian threshold model. The means of the two data simulations scale values of D to set points of 0 (as phylogenetically conserved) and 1 (random) (Orme *et al.*, 2013). We visualized the distribution of the traits across the tree for the MTSV and USFS datasets using the R package “ggtree” (Yu *et al.*, 2017).

For the quantitative Harvard Forest model, we estimated the phylogenetic signal for FLS ( $\lambda$ ) directly from the PMM model. To estimate  $\lambda$ , we fit an intercept-only model with the phylogeny covariance matrix as a random effect and obtained the intra-class correlation value which is the phylogenetic signal. We also estimated the phylogenetic signal from the full model which included all predictors, and in both cases the intra-class correlation in the residuals were high. Estimated phylogenetic signals from all case studies are reported in (Fig. S3).

## References

- Barnes BV, Dick CW , Gunn ME.** 2016. *Michigan Shrubs Vines: A guide to species of the Great Lakes Region.* University of Michigan Press.
- Barnes BV , Wagner WHJ.** 1981,2004. *Michigan Trees: A guide to the Trees of the Great Lakes Region.* University of Michigan Press.
- Bürkner PC.** 2017. brms-users group. personal communication.
- Bürkner PC.** 2018. Advanced bayesian multilevel modeling with the r package brms. *R Journal*, **10**: 395–411.
- Burns RM , Honkala BH.** 1990. Silvics of north america: Volume 2. hardwoods. Tech. rep., United States Department of Agriculture (USDA), Forest Service.
- Daniel Lüdecke.** 2018. ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, **3**: 772.
- Fritz SA , Purvis A.** 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. **24**: 1042–1051.
- Gelman A , Hill J.** 2007. *Data Analysis Using Regression and Multilevel/Hierarchical Models.* Cambridge University Press.
- Ho LST , Ane C.** 2014. A linear-time algorithm for gaussian and non-gaussian trait evolution models. *Systematic Biology*, **63**: 397–408.
- Ives AR , Garland Jr. T.** 2010. Phylogenetic logistic regression for binary dependent variables. *Systematic Biology*, **59**: 9–26.

- Kharouba HM, Ehrlén J, Gelman A, Bolmgren K, Allen JM, Travers SE ,  
Wolkovich EM. 2018. Global shifts in the phenological synchrony of species interactions  
over recent decades. *Proceedings of the National Academy of Sciences*, **115**: 5211.
- O’Keefe J. 2015. Phenology of woody species at harvard forest since 1990.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N , Pearse W. 2013.  
*caper: Comparative Analyses of Phylogenetics and Evolution in R*.
- Stocker T, Qin D, Plattner GK, Tignor M, Allen S, Boschung J, Nauels A,  
Xia Y, Bex V , Midgley P. 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Cambridge, United Kingdom and New York, NY.
- Templ B, Koch E, K.Bolmgren, Ungersböck M, Paul A, Scheifinger H , et al..  
2018. Pan european phenological database (pep725): a single point of access for european  
data. *Int. J. Biometeorology*.
- USDA/NRCS. 2020. Conservation plants characteristics.
- de Villemeruil P. Nakagawa S. 2014. *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, Springer, New York, chap. General quantitative  
genetic methods for comparative biology, pp. pp. 287–303.
- Wilcox RR. 2010. *Fundamentals of modern statistical methods: Substantially improving power and accuracy*. Springer.
- Yu G, Smith D, Zhu H, Guan Y , Lam T. 2017. ggtree: an r package for visualiza-  
tion and annotation of phylogenetic trees with their covariates and other associated data.  
*Methods in Ecology and Evolution*, **8**: 28–36.

161 Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG,  
162 McGlinn DJ, O'Meara BC, Moles AT, Reich PB *et al.* 2013. Three keys to the  
163 radiation of angiosperms into freezing environments. *Nature*, **506**: 89–92.