

1 Aridity and pollination success contribute to flowering-first
2 phenological sequences in a major North American temperate tree
3 clade

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Summary

A large number of woody plant species in temperate forests produce flowers before their leaves emerge each spring. This flower-leaf phenological sequence, known as hysteroanth, proteranth or precocious flowering is generally described as an adaptation to facilitate wind-pollination—which does not explain why it has also evolved to be common in biotically-pollinated taxa in these regions.

In this study, we quantified the inter- and intraspecific variation in flower-leaf sequences in the American plums (*Prunus*, subsp. *Prunus* sect. *Prunocerasus*), a clade of insect-pollinated trees and shrubs, using a large database of digitized herbaria specimens and Bayesian hierarchical modeling. We leveraged these observations to test common hypotheses for the evolution and function of hysteroanth by modeling the associations between hysteroanth and environmental and biological traits related to these hypotheses. To better understand how these phenology-trait associations were sensitive to taxonomic scale and flower-leaf sequence classification, we then repeated these analyses for the more inclusive genus *Prunus* with trait and phenology descriptions from published guidebooks.

We found that in both the American plums and larger genus *Prunus*, hysteroanth was associated with aridity and reduced floral display size. These findings support the assertion that hysteroanth may function to temporally partition hydraulic demand between flowers and leaves and reduce water stress, or increase pollinator visibility and reduce selective pressure on increased flower size.

Our study provides critical insights into the evolution of flower-leaf sequences in biotically pollinated species and function of flower-leaf sequences. Our findings that hysteroanthous flowering is linked to both aridity tolerance and pollination success highlights the importance of phenological sequences in global change research as climate shifts continue to disrupt pollinator services and impact environmental variability.

Keywords: Deciduous forests, Flower-leaf sequences, Hysteroanth, Phenology, Pollination syndrome, Phylogeny

Introduction

Woody perennials have a unique ability among plants to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence, known as hysteroanth, proteranth or precocious flowering, is particularly common in temperate deciduous forests around the globe (Rathcke & Lacey, 1985). A number of studies suggest that this flower-leaf sequence (FLSs) is under selection, and that hysteroanth has functional significance, but the importance of variation in FLSs for maintaining fitness (Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021; Guo *et al.*, 2014) may vary across functional types and evolutionary clades within the temperate forest biome. With mounting evidence that anthropogenic climate change is driving shifts in flower-leaf sequences (Ma *et al.*, 2020), expanding our understanding of the adaptive benefit of hysteroanth may be important to forecasting the demography and performance of forest communities.

The most common, and well-tested explanation for the evolution of hysteroanth in temperate forests is that it is adaptive for wind-pollination, as leafless canopies increase wind speeds for pollen transport and reduce the likelihood of pollen interception by vegetation (Whitehead, 1969; Niklas, 1985). However, this explanation does not address the widespread prevalence of hysteroanth in biotically-pollinated taxa found in temperate regions. This number is not trivial; a recent analysis found that approximately 20% of the hysteroanth species in the moist, Eastern Temperate Forests of North America are biotically pollinated (Buonaiuto *et al.*, 2021).

Several alternative hypotheses have been put forward to explain the advantage of hysteroanth in biotically-pollinated species, but they have not been widely evaluated in the literature. Below, we briefly review these hypotheses and their predictions, and then test these predictions using the American plums (*Prunus* subsp. *prunus* sect. *prunocerasus*), a widespread clade with high variability in flower-leaf sequences, as a case-study. Our treatment here both clarifies the hypothesized function of flower-leaf sequence variation in biotically-pollinated taxa, and offers insights into how shifting flower-leaf sequences may impact species demography and distributions as climate continues to change.

Hypotheses of Hysteroanthous flowering in biotically pollinated taxa

Water limitation hypothesis: In the dry-deciduous tropics of South and Central America, hysteroanth is common (Rathcke & Lacey, 1985; Franklin, 2016), and is regarded as an important adaptation to alleviate water stress by partitioning the hydraulic demand of flowers and leaves across the season (Gougherty & Gougherty, 2018; Franklin, 2016; Borchert, 1983; Reich & Borchert, 1984). Under this hypothesis, the function of hysteroanthous flowering in temperate regions parallels that in the dry tropics—partitioning hydraulic demand across the season to allow hysteroanthous species to tolerate increased aridity. While temperate forests are rarely water-limited in the early season during which flowering and leafing occur (Polgar & Primack, 2011), there is still considerable variation in water availability in space and time within temperate regions of the globe. With this hypothesis, we would expect to find hysteroanthous taxa in locations that are, on average, drier than their non-hysteroanthous relatives.

Insect visibility hypothesis: Hysteranthous flowers are visually conspicuous in the landscape. Thus, as in wind-pollinated taxa, hysterothy in biotically pollinated taxa may be an adaptation for pollination efficiency as flowering-first species are easier for insects pollinators to locate (Janzen, 1967). This hypothesis predicts that hysterothy should be associated with smaller floral displays, because flowers are not obscured by leaves, they are easier to see, and there is weaker selection for increasing floral display size.

Fruit maturation hypothesis: There are several aspects of reproductive development that suggest hysterothy is a by-product of developmental constraints related to fruit maturation. Hysterothy may be common in large fruited species that require lots of time to mature their fruits, or in small, early fruiting species that have evolved dispersal syndromes (wind dispersal, non-dormant seeds) that require dispersal early in the season (Primack, 1987). In either case, we should expect fruit size to associate with hysterothy, although the sign of the correlation differs.

Alternative to these functional hypotheses is the assertion that hysterothy flowering is simply a by-product of selection for early flowering. Species that flower before their leaves inherently flower early in the season. Spring flower phenology is less constrained by prior phenological events than leaf phenology (Savage, 2019; Ettinger *et al.*, 2018), which could allow selection to drive flowering into the early season, producing the hysterothy phenological sequence. Here, there is no specific adaptive advantage to hysterothy; selection is not operating on the relative timing of flower and leaf emergence, but rather the absolute flowering time alone. Rejection of the above hypotheses might provide support to this null explanation.

A significant challenge for robust testing of hysterothy hypotheses is that most characterizations of flower-leaf phenological sequences are based on expert-opinion verbal descriptions (e.g. “flowers before leaves” or “flower before/with leaves”), which make comparisons across taxa, time and space difficult and sensitive to observer bias (see; Buonaiuto *et al.*, 2021). This problem can be overcome by adopting standardized quantitative measures of plant phenology for observational studies and applying them to historic data records. Herbarium records are an excellent source of data that can be leveraged for quantitative phenological measurements (Willis *et al.*, 2017), but have not been used widely to investigate variability of flower-leaf sequences variation among and within species.

The American plums offer potential for a high resolution investigation of drivers of hysterothy flowering in taxa that are not easily explained by the dominant wind-pollination hypothesis. The 16 species that make up the section are distributed across the temperate zone of North America and, like the genus *Prunus* at large, are all insect-pollinated, yet show pronounced inter-specific variation in flower-leaf sequences. Usefully, species in this section are well represented in herbaria records (Fig. 1a), making them a tractable group to measure and assess variation in flower-leaf sequences.

To interrogate the functional hypotheses for hysterothy flowering described above, we used herbaria records to quantify both within- and across- species level variation in flower-leaf sequences of the American plums. Then we combined environmental attributes, biological traits and phylogenetic data in statistical models to evaluate whether the observed associations between flower-leaf sequence variation and morphological and

environmental traits match the predicted associations of the hysternanthy hypotheses. Finally, we compared our findings in this clade to patterns observed in larger genus *Prunus* to better understand whether these phenology-trait associations were sensitive to taxonomic scale and flower-leaf sequence classification.

Materials and Methods

Quantifying flower-leaf sequence variation

We obtained digital herbarium specimens for all members of the section *Prunocerasus* from the Consortium of Midwest Herbaria (CMH) Database (CMH, 2023). To quantify flower-leaf sequence variation within and across species we randomly sampled 200 specimens for each species and scored the phenological development of flowers and leaves using a modified BBCH scale for woody plants (Finn *et al.*, 2007). In total, we evaluated the phenology of 2521 specimens, but only specimens with visible flowers were included in this analysis (n=1009). We reconstructed the phylogenetic relationships among species in this group based on the tree topology in Shaw & Small (2004). We inferred branch lengths following the method of Grafen & Hamilton (1989) in which node heights are estimated in proportion to number of subtending taxa using the R package “ape” (E. Paradis and K. Schliep, 2019).

To quantify FLS variation, we fit an ordinal, hierarchical, Bayesian phylogenetic mixed model (de Villemereuil P. Nakagawa, 2014) to assess the likelihood an individual would be at any given vegetative BBCH phase while flowering. Our model predicted leaf stage (Y , ordinal, with up to j categories) as a function of species and additional phylogenetic effects. Because hysternanthy co-varies with flowering time (i.e., flowering first species will generally flower earlier than other species, on average) we included day of observation as an additional predictor. The model is written below:

$$\text{logit}(P(Y \leq j)) = \alpha_{[j]phylo} + \alpha_{[j]sp} + \beta_{dayofyear[sp]} * X_{dayofyear} + \epsilon$$

$$\epsilon \sim N(0, \sigma_y^2)$$

where Y is the ordinal outcome (leaf stage) and j is the number of categories (1,2,...6). $P(Y \leq j)$ is the probability of Y less than or equal to a category $j = 1...j - 1$. $\alpha_{[j]}$ describes an intercept for each category [1,2,...6], while slope $\beta_{day of year[sp]}$ is constant across categories, but varies among *species*.

The influence of the phylogeny α_{phylo} was modeled as follows:

$$\alpha_{sp} \sim N(\mu_\alpha, COR[\sigma_{phylo}^2])$$

The α for species effects independent of the phylogeny was modeled as follows:

$$\alpha_{sp} \sim N(\mu_{\alpha}, \sigma_{species}^2)$$

We used our model to predict the likelihood each species would be observed at a given vegetative BBCH stage during flowering at the 0%, 25% 50% and 75% quantiles of their flowering period. We then developed a flower-leaf sequence index, by assigning a numerical score to each species per seasonal quantile, and summing over the full flowering season. In each seasonal quantile, species received a “1” if more than 50% of their probability distribution occurred at the two earliest stages of vegetative phenology—BBCH 0 (“bud development”) and BBCH 09 (“bud break”)—and a “0” if not. We summed these values across the season, generating an index from 0 (never hysteranthous) to 4 (hysteranthous through late season (Q75)), where 1= hysteranthous at start of season, 2= hysteranthous through early season (Q25) and 3 = hysteranthous through mid season (Q50). We also used two alternative indexing schemes (>25% of the probability distribution occurred at BBCH 0 and >40% of the probability distribution occurred at BBCH 0 and BBCH 09) to make sure our result were robust across multiple cutoffs.

Evaluating hysteranthly hypotheses

To test the hypotheses of hysteranthly, we obtained data on petal length and fruit diameter directly from herbarium specimens. To assess aridity tolerance, we computed the average Palmer Modified Drought Index score from 1900-2017 for every *Prunocerasus* specimen in the database (n=2305) from the North America Drought Atlas (Cook & Krusic, 2004). For any specimens that lacked accurate geo-location information, we extracted PDSI values at the county centroid of the herbaria specimen.

For our morphological measurements, we sampled an additional 321 specimens and measured the petal length of up to 10 randomly selected petals per specimen (n=2757) using ImageJ image processing software. We also used ImageJ to measure the diameter of fruits on an additional 316 specimens, measuring up to 5 fruit per specimen (n=224).

We then used Bayesian phylogenetic mixed models to test the relationship between flower-leaf sequence index scores and each of the variables. In these models, we modeled species and phylogeny as above.

The model structure is written below:

$$y_{trait} = \alpha_{sp} + \alpha_{phylo} + \beta_{hyst.index} * X_{hyst.index} + \epsilon_i$$

$$\epsilon_i \sim N(0, \sigma_y^2)$$

where y_{trait} is observed trait values (PDSI, petal length or fruit diameter), and the slope $\beta_{hyst.index}$ describes the relationship between extended hysteranthly (higher hysteranthly index value) and the trait of interest. α_{sp} and α_{phylo} describe the species and phylogenetic effects respectively. We also ran each model using our two

alternative FLS indexing approaches to make sure our results were robust to choice of index. Though these alternative classification schemes did change the hysternanthy index score for some species (Fig. S1), they did not substantially impact the inference from our models (see Tab. S1 for comparisons).

Hysternanthy in the larger genus *Prunus*

To better understand how the patterns we identified in *Pruncerasus* scaled across coarser taxonomic resolution and flower-leaf sequence classification we also evaluated the relationship between hysternanthous flowering and hypothesis-related traits in all *Prunus* species native to, or established in North America. For this analysis, we obtained categorical descriptions of flower-leaf sequences and mean estimates of fruit diameter and number of flowers per inflorescence as a proxy for floral investment from the Flora of North America (Rohrer, 1993+). We extracted PDSI values for all herbaria observation of those species in the Consortium of Midwest Herbaria database (n=23,272) as described above.

To account for the influence of evolutionary relationships among species, we reconstructed the phylogenetic relationships in the genus based on the tree topology in Chin *et al.* (2014). As above, we computed branch lengths with the R package “ape” (E. Paradis and K. Schliep, 2019).

We standardized the units of all predictors to make their effect size estimates for the following model structure directly comparable to each other:

$$\text{logit}(P(Y \leq j)) = \alpha_{[j]phylo} + \beta_{PDSI[sp]} * X_{PDSI} + \beta_{fruitdiameter} * X_{fruitdiameter} + \beta_{floralinvestment} * X_{flowersperinflorescence} + \epsilon$$

$$\epsilon \sim N(0, \sigma_y^2)$$

where Y is the ordinal outcome of flower-leaf sequence category (“flowers before leaves”, “flowers before/with leaves”, “flowers with leaves” and “flowers after leaves”) and j is the number of categories (1,2,...4). $P(Y \leq j)$ is the probability of Y less than or equal to a category $j = 1, \dots, j-1$. We modeled the influence of the phylogeny (α_{phylo}) as above.

0.1 Model runs

We fit models in the R package “brms” (Bürkner, 2018) using weakly informative priors, and four chains. For the “Quantifying flower-leaf sequence variation” and “Evaluating hysternanthy hypotheses” we ran the models with a warm-up of 3000, and 3500 iterations, and 4000, and 4500 sampling iterations respectively, for a total of 4000 sampling iterations across all chains. For the “Hysternanthy in the larger genus *Prunus*” model, we used a warm up of 6,000 iterations and 8,000 sampling iterations for a total of 8,000 sampling iterations to maximize the effective sampling size. Model fits was assessed with Rhats <1.01, high effective sample sizes

and no divergent transitions.

Results

Quantifying flower leaf sequences in the American plums

We found substantial inter-specific differences in flower-leaf sequences within the American plums (Fig. 2, S1). Flower-leaf sequence patterns were strongly dependent on the day of observation, with observations later in the the flowering season of each species decreasing the likelihood of finding flowers open during early vegetative BBCH phases (β_{day} 0.03, CI_{50} [0.02,0.03]). Based on our flower leaf sequence index, two species (*P. umbellata*, *P. mexicana*) were likely to be hysteranthous regardless of the time of observation and three species (*P. rivularis*, *P. subcordata*, and *P. texana*) were always most likely to flower after leaves developed (Fig. 1b). All other species displayed intermediate phenotypes, with five species mostly likely to hysteranthous at the start of the season (*P. alleghaniensis*, *P. americana*, *P. hortulana*, *P. munsoniana* and *P. nigra*), one species through early season (*P. gracilis*) and two species through mid season (*P. angustifolia*, *P. maritima*) (Fig 1b).

Associations between hysteranthry and environmental and morphological traits

In the American plums clade, aridity (lower average PDSI) was associated with higher flower-leaf sequence index scores (β : -0.03, CI_{50} [-0.05, 0.02], Fig. 3a), suggesting that species that displayed hysteranthous flowering later into their flowering season are found in dryer locations.

Shorter petal and smaller fruit diameters were also associated with higher flower-leaf sequence index scores (β : -.21, CI_{50} [-0.38 -0.04] and β :-1.40, CI_{50} [-1.97 -0.82] respectively, Fig. 3b,c). This suggests that smaller fruits and flowers are associated with increased hysteranthry.

At the genus level, there was a positive association between increasing PDSI and inflorescence size and increasing overlap between flowers and leaves (i.e., decreasing hysteranthry; β : 2.50, CI_{50} [1.17, 3.371] and β 6.41, CI_{50} [3.86, 8.05] respectively, Fig. 4a), again suggesting that hysteranthry is associated with drier locations and smaller floral displays (Fig. 4b). However, hysteranthry was associated with larger fruits (β : -1.24, CI_{50} [-1.95,-0.21], 4b)] though there was high uncertainty around these estimates in our model.

Discussion

Our study provides foundational insights into the evolution of flower-leaf sequences in biotically pollinated plants. We show that hysteranthous flowering can be linked to both aridity tolerance and pollination success through the predictions of the water limitation and insect visibility hypotheses, highlighting the urgency for

advancing our understanding phenological sequences as human-caused global change continues to disrupt pollinator services and impact environmental variability.

Hysteranthy hypotheses

Using North American *Prunus* species as a case study, our analyses indicate that flower-leaf sequences are under selection by biological and environmental drivers, and that variation in these patterns across species may reflect adaptive tradeoffs. We found that hysteranthous flowering is associated with smaller floral displays and increased aridity in both the American plums, and more broadly across *Prunus* native or established in North America. While we did not find support for the fruit maturation hypotheses, the relationships between hysteranthy and aridity, and hysteranthy and floral display size support the predictions of the water limitation hypothesis and the insect visibility hypothesis, respectively.

Our support for both the water limitation hypothesis and insect visibility hypothesis highlights that these hypotheses are not mutually exclusive, and could be related. Selection on floral size represents a classic evolutionary tradeoff where larger floral displays may generally be more effective for attracting pollinators but demand more resources, including water, to maintain turgor and reproductive function than smaller ones (Galen *et al.*, 1999; Lambrecht & Dawson, 2007). With this trade-off, reproductive displays are often small in harsher environments (Teixido *et al.*, 2016; Lambrecht, 2013), and hysteranthy could represent a compensatory mechanism that both reduces hydraulic demand while increasing pollination efficiency in these environments.

Studies that have compared the transpiration rates among flowers and leaves provide insights to the potential importance of this seasonal partitioning for maintaining water status. Measurements of water movement (transpiration rates, sap flow, hydraulic conductivity) to flowers range from 20%-60% of that of leaves under comparable conditions (Whiley *et al.*, 1988; Roddy & Dawson, 2012; Liu *et al.*, 2017; McManm *et al.*, 2022). This level of additional hydraulic demand can drive loss of stomatal conductance and decrease photosynthetic rates (Galen *et al.*, 1999).

Despite this evidence that hysteranthy can reduced hydraulic demand in dry environments, hysteranthous species in the American plum clade are not found in extremely arid locations (mean PDSI values only ranged from -.48 to 0.2 for species classified as hysteranthous through mid-season or through late season, while PDSI can range from -10 to 10). This contrasts with hysteranthous species in the dry tropics where this phenological syndrome allows them to tolerate more extreme aridity (Franklin, 2016). But the flower-leaf sequences of the hysteranthous species in our study were markedly different from patterns of hysteranthy in these dry-tropics where the water limitation hypothesis was initially proposed. While flowering can precede leafout by as much several weeks for species in the American plums, the process of fruit development, which is also water intensive, occurs when leaves are present. By contrast, in the dry tropics hysteranthous flowering is initiated at the time of leaf drop (Borchert, 1983; Franklin, 2016). Thus, the full reproductive cycle occurs in the leafless period. The comparatively small window of leafless reproductive development in our

temperate clade, may in part, explain why the association we observed between hysteranthly and aridity in our study was relatively weak with high residual variance. Our results suggest that hysteranthly may allow temperate species to occupy marginally drier environments than non-hysteranthous species, but may not facilitate species' persistence under extreme aridity.

Inter-and intra-specific variation in flower-leaf sequences

We developed a novel approach to assessing flower-leaf sequences that scales from quantitative, individual-level observations to species-level characterizations that were based on empirical likelihood estimates. With this approach, we were able to—for the first time—quantitatively assess intermediate cases of hysteranthly (such as those that are typically described as “flowers before/with leaves”). Previous studies of hysteranthous flowering have either excluded these cases from their analyses (e.g.; Gougherty & Gougherty, 2018) or binned them with the well defined cases (e.g.; Buonaiuto *et al.*, 2021). We found that eight of the thirteen American plum species expressed this intermediate flower-leaf sequence, and our classifications broadly matched previous species-level analyses in this group by Shaw & Small (2004). By estimating the likelihood of hysteranthly across the growing season with Bayesian methods, our approach identified substantial differences in flower-leaf sequences among these intermediate cases (Fig. 2, Fig. S1), which allowed us to assess the trait associations with this phenotype.

Our quantitative analysis of the American plums clade revealed that flower-leaf sequences—often described as a species-level trait—are highly variable within species (Fig. 2, Fig. S1). For all members of the clade, the day of phenological observation was a strong predictor of the likelihood that flowers would be visible before the emergence of leaves. In many cases there was high likelihood that individuals of a species may be observed at different vegetative stages during flowering (Fig. 3, S1). This variation could either suggest high levels of local adaptation in flower-leaf sequences or, alternatively, high levels of plasticity through which flower-leaf sequences respond to interannual variation in environmental conditions. For example—in a given population—flower-leaf sequences may respond to interannual variation in precipitation with increased temporal separation between flowers and leaves in drier years. While our data did not have the temporal resolution to address this question, the high levels of within-species variation we observed raise important questions about environmental drivers of flower-leaf sequences operating on both the macro-evolutionary scale we investigated here and on individual physiological responses to environmental change.

Additionally, by scoring these individual, quantitative observations as ordinal response categories with our hysteranthly index, we were able to contrast our findings to those derived from categorical, species-level characterizations based on expert opinion. The coherence between our individual based observational approach for the American plum clade and the top-down, categorical classification across *Prunus* is an encouraging demonstration that the expert opinion-based data can still offer useful insights into the drivers of hysteranthous flowering when higher-resolution data is not available.

Future directions

In this study, we focused on a well-studied, and economically important clade of morphologically similar species, that allowed us to control for unmeasured biological variation. Our case-study provides a road map for evaluating the role of hysteranthly in temperate biotically pollinated plant taxa (groups with high interspecific flower-leaf sequence include *Magnolia*, *Rhododendron*, *Acer* and *Cornus*), and more broadly across taxa and biomes.

Combining the observational approach with novel experiments could further advance our collective understanding of the adaptive significance of flower-leaf sequences. To test the water-limitation hypothesis, researchers could plant sister-taxa with contrasting flower-leaf sequences in common environments across a gradient of aridity, and evaluate their performance. To test the insect visibility hypothesis, researchers should also consider hysteranthly—and phenology in general—in the more general framework of tradeoffs in pollination biology. The trade off between phenology and pollination investment should not only consider flower size, but also the number of flowers, nectar and pollen reward investment, volatiles between related hysteranthous and non-hysteranthous taxa. The expectation here is that hysteranthous species would invest fewer resources into these other pollinator attraction traits than non-hysteranthous relatives. For a simple experiment to test the pollinator visibility hypothesis, researchers could force hysteranthly/non-hysteranthly phenotypes for the same genotype using environmental cues, and systematically release pollinators to observe their preferences, search times and foraging behavior. If pollinators are more readily drawn to the hysteranthous individuals, it would suggest that hysteranthly may be an adaptive trait for pollinator attraction.

With a better mechanistic understanding of the relationship between flower-leaf sequences and ecological performance in hand, researchers could then use experiments to assess how differences in floral and leaf physiological responses to temperature variation may alter the adaptive benefits of flower-leaf sequences with climate change. The measurement and modeling approaches we developed in our observational study can be readily implemented to analyze data from such experimental settings, presenting an important opportunity to unite observations of broad ecological patterns with targeted experimental manipulations to better understand both the evolutionary past and ecological future of flower-leaf sequences.

Competing Interests:

The authors declare no conflict of interest.

Author contributions

DMB, and EMW conceived of the manuscript; DMB and SC collected the data; DMB led the statistical analyses with TJD and EMW; DMB led the writing of the manuscript. All authors contributed to writing

and gave approval for the submission.

Data Availability

The phenology and trait data collected for this study will be made available and archived at KNB: The Knowledge Network for Biocomplexity (<https://knb.ecoinformatics.org/>) at the time of publication.

References

. 2023. Consortium of midwest herbaria.

Borchert R. 1983. Phenology and control of flowering in tropical trees. *Biotropica*, **15**: 81–89.

Buonaiuto DM, Morales-Castilla I , Wolkovich EM. 2021. Reconciling competing hypotheses regarding flower–leaf sequences in temperate forests for fundamental and global change biology. *New Phytologist*, **229**: 1206–1214.

Bürkner PC. 2018. Advanced bayesian multilevel modeling with the r package brms. *R Journal*, **10**: 395–411.

Chin SW, Shaw J, Haberle R, Wen J , Potter D. 2014. Diversification of almonds, peaches, plums and cherries –molecular systematics and biogeographic history of prunus (rosaceae). *Molecular Phylogenetics and Evolution*, **76**: 34–48.

Cook E , Krusic P. 2004. *The North American Drought Atlas*.

E. Paradis and K. Schliep. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, **35**: 526–528.

Ettinger A, Gee S , Wolkovich E. 2018. Phenological sequences: how early season events define those that follow. *American Journal of Botany*, **105**.

Finn GA, Straszewski AE , Peterson V. 2007. A general growth stage key for describing trees and woody plants. *Annals of Applied Biology*, **151**: 127–131.

Franklin DC. 2016. Flowering while leafless in the seasonal tropics need not be cued by leaf drop: evidence from the woody genus brachychiton (malvaceae). *Plant Ecology and Evolution*, **149**: 272–279.

Galen C, Sherry R , Carroll A. 1999. Are flowers physiological sinks or faucets? costs and correlates of water use by flowers of polemonium viscosum. *OECOLOGIA*, **118**: 461–470.

Gougherty AV , Gougherty SW. 2018. Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate. *New Phytologist*, **220**: 121–131.

- Grafen A , Hamilton WD. 1989.** The phylogenetic regression. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, **326**: 119–157.
- Guo L, Luedeling E, Dai J , Xu J. 2014.** Differences in heat requirements of flower and leaf buds make hysteresis trees bloom before leaf unfolding. *Plant Diversity and Resources*, **36**: 245–253.
- Janzen DH. 1967.** Synchronization of sexual reproduction of trees within the dry season in central america. *Evolution*, **21**: 620–637.
- Lambrecht SC. 2013.** Floral water costs and size variation in the highly selfing leptosiphon bicolor (polemoniaceae). *International Journal of Plant Sciences*, **174**: 74–84.
- Lambrecht SC , Dawson TE. 2007.** Correlated variation of floral and leaf traits along a moisture availability gradient. *Oecologia*, **151**: 574–583.
- Liu H, Xu QY, Lundgren MR , Ye Q. 2017.** Different water relations between flowering and leaf periods: a case study in flower-before-leaf-emergence *magnolia* species. *Functional Plant Biology*, **44**: 1098–1110.
- Ma Q, Huang JG, Hänninen H, Li X , Berninger F. 2020.** Climate warming prolongs the time interval between leaf-out and flowering in temperate trees: Effects of chilling, forcing and photoperiod. *Journal of Ecology*, **n/a**.
- McMann N, Peichel A , Savage JA. 2022.** Early spring flowers rely on xylem hydration but are not limited by stem xylem conductivity. *New Phytologist*, **233**: 838–850.
- Niklas KJ. 1985.** The aerodynamics of wind pollination. *The Botanical Review*, **51**: 328–386.
- Polgar C , Primack R. 2011.** Leaf-out phenology of temperate woody plants: From trees to ecosystems. *New Phytologist*, **191**: 926–41.
- Primack RB. 1987.** Relationships among flowers, fruits, and seeds. *Annual Review of Ecology and Systematics*, **18**: 409–430.
- Rathcke B , Lacey EP. 1985.** Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, **16**: 179–214.
- Reich P , Borchert R. 1984.** Water-stress and tree phenology in a tropical dry forest in the lowlands of costa-rica. *Journal of Ecology*, **72**: 61–74.
- Roddy AB , Dawson TE. 2012.** Determining the water dynamics of flowering using miniature sap flow sensors. International Society for Horticultural Science (ISHS), Leuven, Belgium, 951, pp. 47–53.
- Rohrer J. 1993+.** *Prunus*. In: *Flora of North America North of Mexico* (ed. of North America Editorial Committee F.). New York and Oxford.
- Savage JA. 2019.** A temporal shift in resource allocation facilitates flowering before leaf out and spring vessel maturation in precocious species. *American Journal of Botany*, **106**: 113–122.

- 382 **Shaw J , Small RL. 2004.** Addressing the "hardest puzzle in american pomology:" phylogeny of prunus sect.
383 prunocerasus (rosaceae) based on seven noncoding chloroplast dna regions. *Am J Bot*, **91**: 985–996.
- 384 **Teixido AL, Barrio M , Valladares F. 2016.** Size matters: Understanding the conflict faced by large flowers
385 in mediterranean environments. *The Botanical Review*, **82**: 204–228.
- 386 **de Villemeruil P. Nakagawa S. 2014.** *Modern Phylogenetic Comparative Methods and Their Application in*
387 *Evolutionary Biology*, Springer, New York, chap. General quantitative genetic methods for comparative
388 biology, pp. pp. 287–303.
- 389 **Whiley A, Chapman K , Saranah J. 1988.** Water loss by floral structures of avocado (<i>persea ameri-
390 cana</i> cv. fuerte) during flowering. *Australian Journal of Agricultural Research*, **39**: 457–467.
- 391 **Whitehead DR. 1969.** Wind pollination in the angiosperms: Evolutionary and environmental considerations.
392 *Evolution*, **23**: 28–35.
- 393 **Willis CG, Ellwood ER, Primack RB, Davis CC, Pearson KD, Gallinat AS, Yost JM, Nelson G, Mazer SJ,**
394 **Rossington NL *et al.* 2017.** Old plants, new tricks: Phenological research using herbarium specimens.
395 *Trends in Ecology & Evolution*, **32**: 531–546.

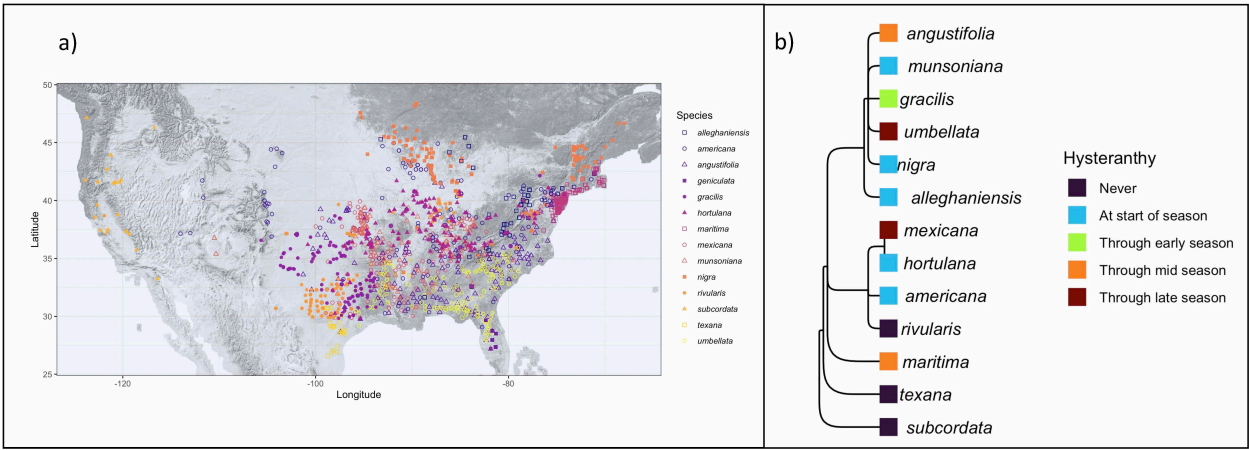


Figure 1: Geographic distribution and taxonomic relationships among the American plums. a) Maps the localities of all the herbaria records used in this study. b) Depicts phylogenetic relationships among the American plums and the duration of their flowering period they are hysteroanthous. These categorizations are based on ordinal phylogenetic mixed models. Tree topology is from Shaw & Small (2004)

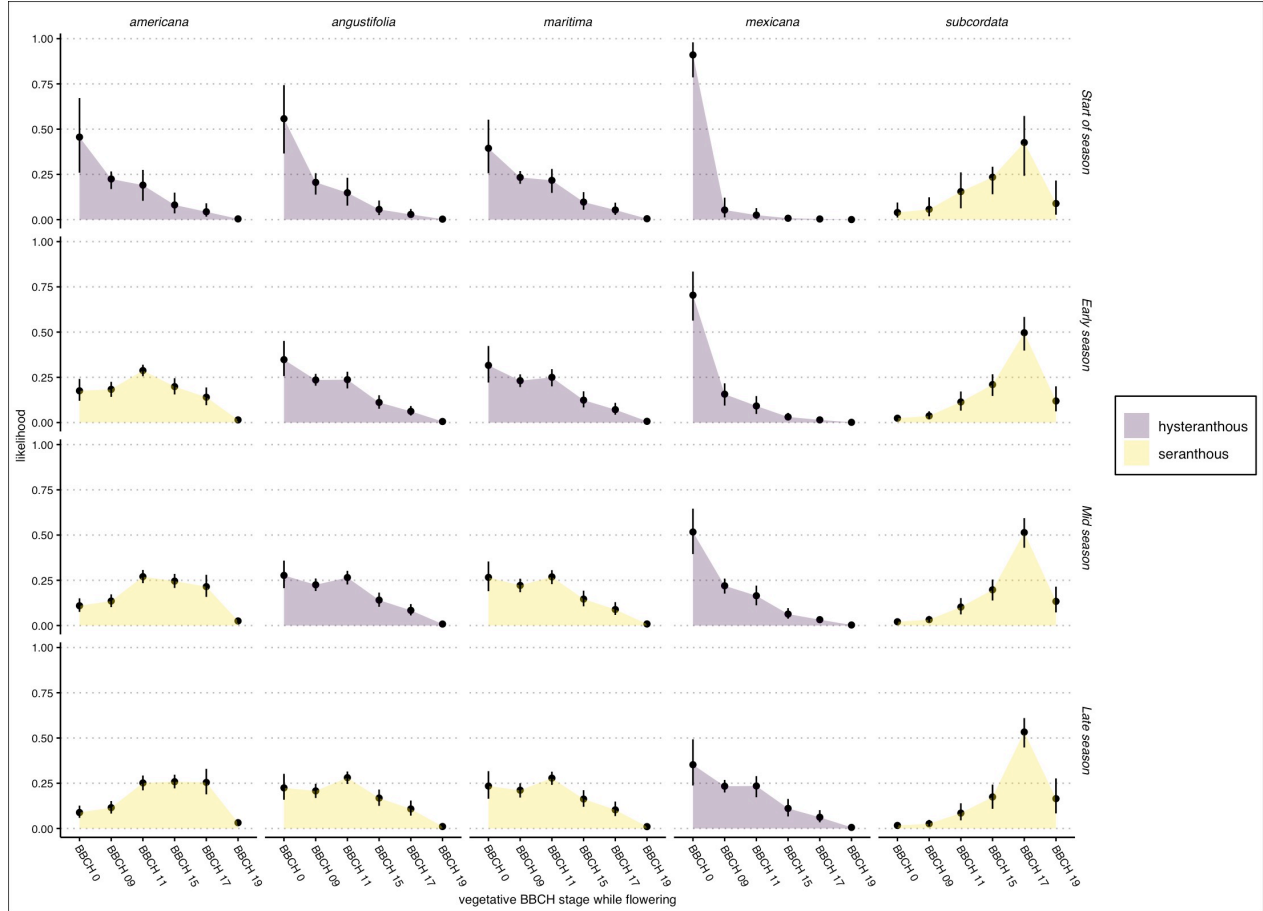


Figure 2: Predicted likelihood that a species would be in flower during each vegetative BBCH phase for five example species in the American plums. Points are the mean likelihood and bar the 95% uncertainty intervals. Species were classified as hysteranthous if greater than 50% probability flowering occurred in BBCH 0 and BBCH 09 (colors) for each part of the flowering season. See Fig. S1 for all species and alternative hysteranthous classification schemes.

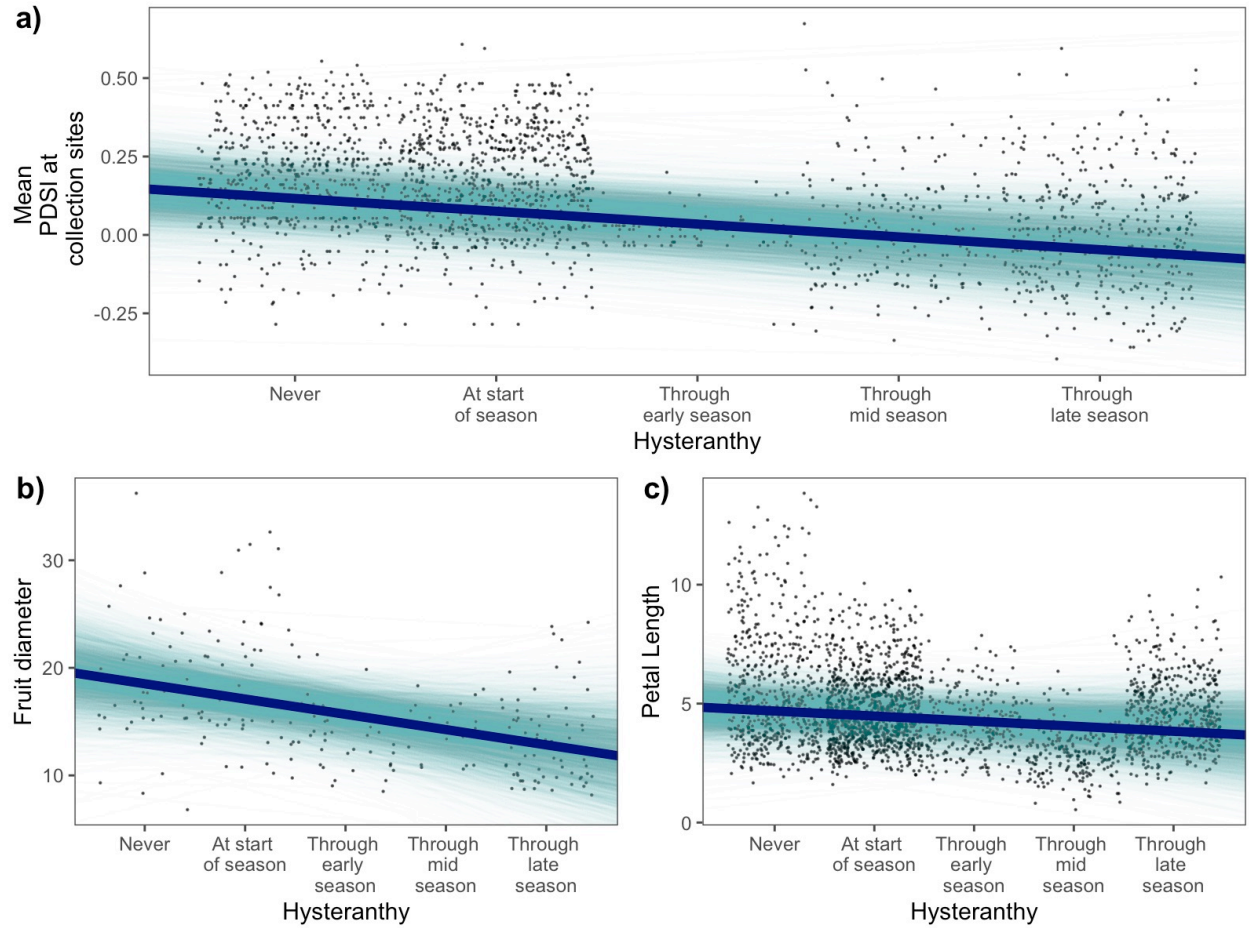


Figure 3: Relationships between the duration of hysteresis across the flowering period and environmental and biological traits based on Bayesian phylogenetic mixed models. a) b) and c) depict the relationships between the duration of hysteresis and mean PDSI, fruit diameter, and petal length respectively. Solid lines indicate the mean posterior estimate and shaded areas 4000 draws from the posterior distribution as a display of uncertainty.

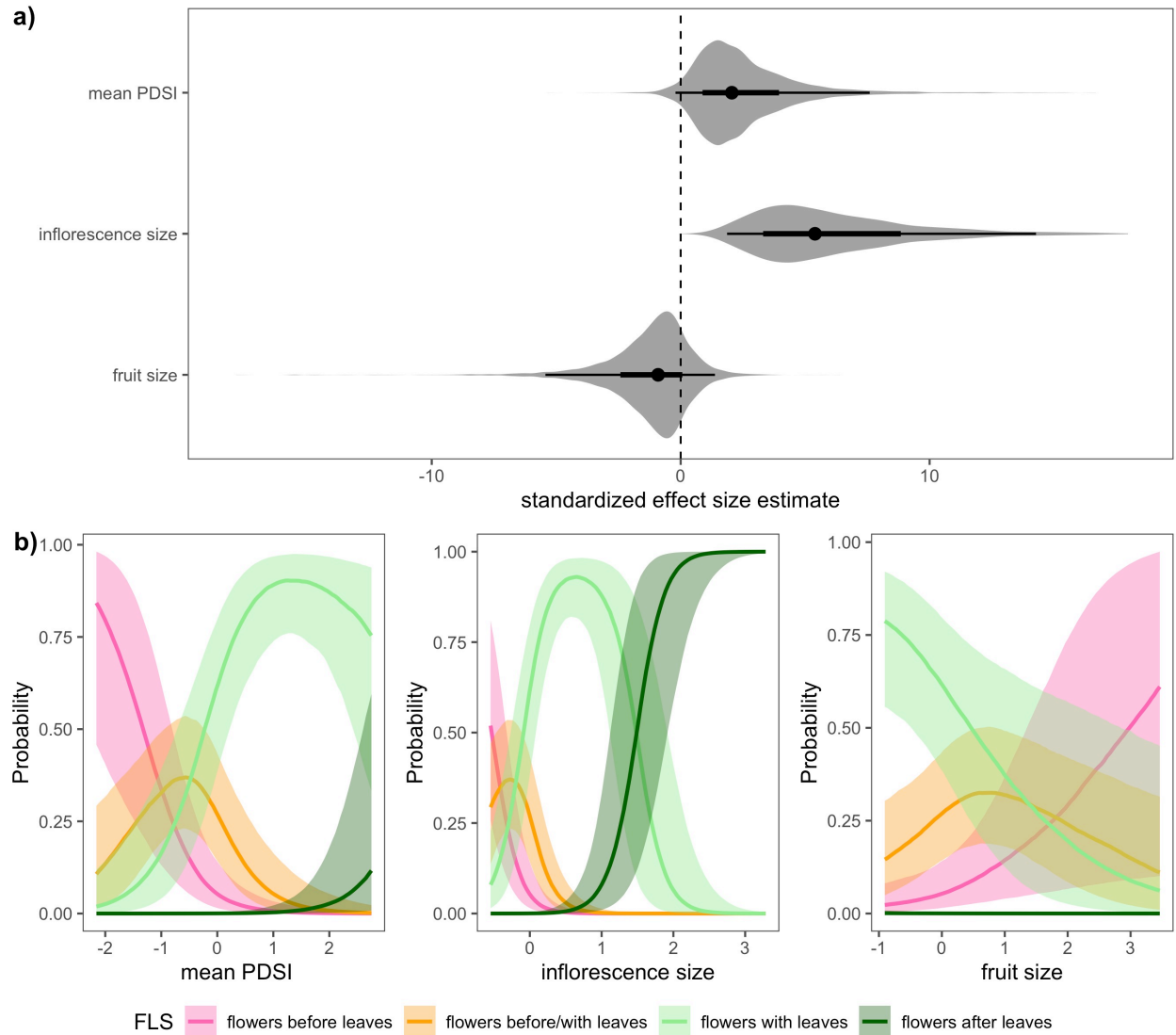


Figure 4: Relationships between the likelihood of hysteranthry and environmental and biological traits in the genus *Prunus* based on Bayesian phylogenetic mixed models. Panel a) shows the estimated effect size of each predictor with negative values indicating an increased likelihood of hysteranthry. Points indicate the mean posterior estimate for each predictor, and thick and thin bars the 50% and 97.5% uncertainty intervals respectively. We also show the full posterior distribution as an additional measure of uncertainty, Panel b), c) and d) show the marginal effect of mean PDSI, inflorescence size and fruit size respectively, on the likelihood of each FLS category. Solid lines indicate the mean likelihood and shaded areas the 50% uncertainty intervals.