

1 Ecological drivers of flower-leaf sequences: aridity and pollination
2 success select for flowering-first in the American Plums

3 D.M. Buonaiuto ^{1,2,3,a}, T.J. Davies ^{4,5}, S. Collins ⁴ & E.M. Wolkovich^{2,3,4}

4 *Author affiliations:*

5 ¹Department of Environmental Conservation, University of Massachusetts, Amherst, Massachusetts, USA.

6 ORCID: 0000-0003-4022-2591

7 ²Arnold Arboretum of Harvard University, Boston, Massachusetts, USA.

8 ³Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

9 ⁴Forest & Conservation Sciences, Faculty of Forestry, University of British Columbia, Vancouver, British
10 Columbia, Canada

11 ⁵ Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada

12 ^aCorresponding author: 617.823.0687; dbuonaiuto@umass.edu

Summary

- Many trees in temperate forests produce flowers before their leaves emerge. This flower-leaf phenological sequence, known as hysteranthly, is generally described as an adaptation for wind-pollination—which does not explain why it is also common in biotically-pollinated taxa.
- In this study, we quantified flower-leaf sequence variation in the American plums (*Prunus*, subsp. *Prunus* sect. *Prunocerasus*), a clade of insect-pollinated species, using herbaria specimens and Bayesian hierarchical modeling. With these observations, we tested common hypotheses for the evolution of hysteranthly by modeling the associations between hysteranthly and related traits. To better understand how these phenology-trait associations were sensitive to taxonomic scale and flower-leaf sequence classification, we extended these analyses to the more inclusive genus *Prunus*.
- In both groups hysteranthly was associated with aridity and smaller floral displays. These findings indicate that hysteranthly may function to temporally partition hydraulic demand and reduce water stress, or increase pollinator visibility and reduce selective pressure on flower size.
- Our study provides insights into the function of flower-leaf sequences in biotically-pollinated species. Our findings that hysteranthly is linked to 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, Hysteranthly, Phenology, Plant hydraulics, Pollination, Phylogeny

Introduction

Woody perennials are among the small subset of plant types with the unique ability to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence, known as hysteroanth, proteranth or precocious flowering, is apparent in temperate deciduous forests around the globe (Rathcke & Lacey, 1985). A number of studies suggest that this flower-leaf sequence is under selection, and that hysteroanth has functional significance (Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021; Guo *et al.*, 2014), but the importance of variation in flower-leaf sequences for maintaining fitness 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.*, 2021; Wang *et al.*, 2022), 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 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-hysteranthous relatives.

Insect visibility hypothesis: Hysteranthous flowers are visually conspicuous in the landscape. Thus, as in wind-pollinated taxa, hysteranthly in biotically-pollinated taxa may be an adaptation for pollination efficiency as flowering-first species are easier for insect pollinators to locate (Janzen, 1967). A challenge to evaluating this hypothesis is that correlated selection between flower-leaf sequences and pollinator visibility could have either a positive or negative relationship depending on the pollinator environment. In one scenario hysteranthly may 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. However, in environments where plants are more often pollen limited, selection may favor both hysteranthly and increased floral display size in augment attraction to visual pollinators.

Alternative to these functional hypotheses is the assertion that hysteranthous flowering is simply a by-product of selection for early flowering. Species that flower before their leaves inherently flower early in the season. For example, fruit development or dispersal constraints may drive early flowering (Primack, 1987) and because spring flower phenology is less constrained by prior phenological events than leaf phenology (Savage, 2019; Ettinger *et al.*, 2018), this selection for early flowering could incidentally produce the hysteranthous phenological sequence. Here, there is no specific adaptive advantage to hysteranthly; 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 hysteranthly 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 among and within species.

The American plums offer potential for a high resolution investigation of drivers of hysteranthous 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 hysteranthous 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 hysteresis 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 (of Midwest Herbaria, 2023). Specimen collection dates ranged from 1844-2020, with the majority collected between 1950-2000. 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), which is designed to evaluate vegetative and reproductive phenological progress using a standardized quantitative index. For species with less than 200 specimens in the collection, we included all available specimens. In total, we evaluated the phenology of 2521 specimens, but only specimens with visible flowers were included in this analysis. We also assessed and removed outliers of flowering observations visually, and by excluding observations that were beyond three standard deviations of the median flowering time for each species ($n=9$). Our final analyses included 1000 specimens (see ?? for number of observations/species). 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 flower-leaf sequence 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_i , ordinal, with six categories) as a function of species and additional phylogenetic effects. Because hysteresis 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. Additionally, because it is possible that climate change has affected the interval between flowering and leafout over the course of our time series, we included the year of collection of each specimen as a co-variate. Because the concern for including this co-variate was related to shifting baselines due to climate change, we parameterized *year* as hinge variable, using 1980 as a break point following standard conventions for modeling the effects of climate change (Stocker *et al.*, 2013; Buonaiuto *et al.*, 2021; Kharouba *et al.*, 2018).

The model is written below:

$$y_i = \begin{cases} 1 & \text{if } z_i < 0 \\ 2 & \text{if } z_i \in (0, c_2) \\ 3 & \text{if } z_i \in (c_2, c_3) \\ 4 & \text{if } z_i \in (c_3, c_4) \\ 5 & \text{if } z_i \in (c_4, c_5) \\ 6 & \text{if } z_i > c_5 \end{cases}$$

$$z_i = \alpha + \alpha_{phylo} + \alpha_{sp} + \beta_{\text{day of year}[\text{sp}]} * X_{\text{day of year}} + \beta_{\text{year}} * X_{\text{year}} + \epsilon_i$$

137

$$\epsilon_i \sim \text{logistic}(0, 1)$$

139

140 where y_i is the ordinal outcome (leaf stage; as 1,2,...6 categories). $c_{2...5}$ are the estimated cutpoints between
 141 leaf stages on the logit scale. z_i is the linear component of the underlying latent variable model. α describes
 142 an intercept for each category [1,2,...6], while slope ($\beta_{\text{day of year}}$) is constant across cutpoints, but varies among
 143 *species*.

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

$$\alpha_{phylo} \sim N(0, COR[\sigma_{phylo}^2])$$

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

$$\alpha_{sp} \sim N(0, \sigma_{species}^2)$$

144 We used our model to predict the likelihood each species would be observed at a given vegetative BBCH stage
 145 during flowering for each day of the flowering period of each species. For each day of the flowering season, we
 146 summed the predicted likelihood that species would be at BBCH 0 (“bud closed”), BBCH 07/09 (“bud break”)
 147 or BBCH 11 (“start of leaf unfolding”) vs. BBCH 15 (“leaf unfolding”), BBCH 17 (“most leaves unfolded”),
 148 BBCH 19 (“leaf expansion complete”) to quantify the likelihood as species would be be hysteranthous or non-
 149 hysteranthous respectively on each day of the the season. We used these estimate to developed a flower-leaf
 150 sequence index by summing the likelihood of hysteranthous vs. non-hysteranthous across the flowering period of
 151 each species, with 0 being never hysteranthous and 1 being always hysteranthous.

152 Evaluating hysteranthous hypotheses

153 To test the hypotheses of hysteranthous, we obtained data on petal length and fruit diameter directly from
 154 herbarium specimens. To assess aridity tolerance, we computed the average Palmer Modified Drought Index
 155 score (hereafter: PMDI) from 1900-2017, obtained from the for Environmental Information (2017), for every
 156 *Prunocerasus* specimen in the database(n=2305). PMDI is a standardize index that integrates temperature

and precipitation data to estimate relative dryness in time and space (Heim, 2002). For any specimens that lacked accurate geo-location information, we extracted PMDI 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 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:

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

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

where y_{trait} is observed trait values (PDSI or petal length), and the slope $\beta_{\text{hyst.index}}$ describes the relationship between extended hysteranthy (higher hysteranthy index value) and the trait of interest. α describes a grand intercept, and α_{sp} and α_{phylo} describe the species and phylogenetic effects respectively.

We also ran each model using our two alternative flower-leaf sequence indexing approaches to make sure our results were robust to choice of index. Though these alternative classification schemes did change the hysteranthy index score for some species (Fig. S1), they did not substantially impact the inference from our models (see Tab. S1 for comparisons).

Hysteranthy in the larger genus *Prunus*

To better understand how the patterns we identified in *Pruncerasus* scaled to a larger more inclusive group and across coarser taxonomic resolution and flower-leaf sequence classification we also evaluated the relationship between hysteranthous 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 the 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 through z-scoring (Gelman & Hill, 2007) to make their effect size estimates for the following model structure directly comparable to each other:

$$y_i = \begin{cases} 1 & \text{if } z_i < 0 \\ 2 & \text{if } z_i \in (0, c_2) \\ 3 & \text{if } z_i \in (c_2, c_3) \\ 4 & \text{if } z_i > c_3 \end{cases}$$

$$z_i = \alpha + \alpha_{phylo} + \beta_{PDSI} * X_{PDSI} + \beta_{\text{floral investment}} * X_{\text{flowers/inflorescence}} + \epsilon_i$$

$$\epsilon_i \sim \text{logistic}(0, 1)$$

where y_i is the ordinal outcome of flower-leaf sequence category (“flowers before leaves”, “flowers before/with leaves”, “flowers with leaves” and “flowers after leaves”) and $c_{2...3}$ are the estimated cutpoints between categories on the logit scale. As above, z_i is the linear component of the underlying latent variable model. α describes a grand intercept, and we modeled the influence of phylogeny (α_{phylo}) as above.

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 $\hat{R} < 1.01$, high effective sample sizes and no divergent transitions. We provide mean estimates and 50% uncertainty intervals in the text with alternate intervals in figures and the Supporting Information.

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} [0.02,0.04]). Based on our flower leaf sequence index, two species (*P. umbellata*, *P. mexicana*) were likely to be hysternanthous regardless of the time of observation and five species (*P. alleghaniensis*, *P. nigra*, *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 four species

mostly likely to hysteranthous at the start of the season (*P. americana*, *P. hortulana*, *P. munsoniana* and *P. gracilis*), one species through early season (*P. maritima*) and one species through mid season (*P. angustifolia*) (Fig 1b).

Associations between hysteranthy and environmental and morphological traits

In the American plums clade, aridity (lower average PDSI) was associated with higher flower-leaf sequence index scores (β : -0.04, CI_{50} [-0.06, -0.03], Fig. 3a), suggesting that species that displayed hysteranthous flowering later into their flowering season are found in drier locations.

Shorter petals were also associated with higher flower-leaf sequence index scores (β : -.21, CI_{50} [-0.38 -0.05]. Fig. 3b). This suggests that smaller flowers are associated with increased hysteranthy.

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 hysteranthy; β : 2.50, CI_{50} [1.17, 3.371] and β 6.41, CI_{50} [3.86, 8.05] per standardized unit, respectively, Fig. 4a), again suggesting that hysteranthy is associated with drier locations and smaller floral displays (Fig. 4b).

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 of 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. 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 hysteroanthony 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; McMann *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 hysteroanthony can reduced hydraulic demand in dry environments, hysteroanthous species in the American plum clade are not found in extremely arid locations (PDSI values typically range from -4 to 4, however our analyses found mean values ranging from -0.5 to 0.2 for species classified as hysteroanthous through mid-season or through late season). This contrasts with hysteroanthous 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 hysteroanthous species in our study were markedly different from patterns of hysteroanthony 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 hysteroanthous 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 hysteroanthony and aridity in our study was relatively weak with high residual variance. Our results suggest that hysteroanthony may allow temperate species to occupy marginally drier environments than non-hysteroanthous 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 hysteroanthony (such as those that are typically described as “flowers before/with leaves”). Previous studies of hysteroanthous 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 hysteroanthony 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. 2, 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 hysteresis 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 hysteresis in temperate biotically-pollinated plant taxa (other groups with high interspecific flower-leaf sequence variation 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 hysteresis—and phenology in general—in the more general framework of tradeoffs in pollination biology. The tradeoff 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. Findings that hysteranthous species invest fewer resources into these other pollinator attraction traits than non-hysteranthous relatives would support the insect visibil-

ity hypothesis. For a simple experiment to test the pollinator visibility hypothesis, researchers could force hysteranthty/non-hysteranthty 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 hysteranthty 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

- 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.
- E. Paradis and K. Schliep. 2019.** ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, **35**: 526–528.
- for Environmental Information NC. 2017.** Lbdp-v2. Tech. rep., National Oceanic and Atmospheric Administration.
- 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.
- Gelman A , Hill J. 2007.** *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press.
- 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 hysteranthous trees bloom before leaf unfolding. *Plant Diversity and Resources*, **36**: 245–253.
- Heim RR. 2002.** A review of twentieth-century drought indices used in the united states. *Bulletin of the American Meteorological Society*, **83**: 1149–1166.
- Janzen DH. 1967.** Synchronization of sexual reproduction of trees within the dry season in central america. *Evolution*, **21**: 620–637.
- 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.

- 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. 2021.** Climate warming prolongs the time interval between leaf-out and flowering in temperate trees: Effects of chilling, forcing and photoperiod. *Journal of Ecology*, **109**: 1319–1330.
- 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.
- of Midwest Herbaria C. 2023.** Consortium of midwest herbaria.
- 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.
- Shaw J , Small RL. 2004.** Addressing the "hardest puzzle in american pomology:" phylogeny of prunus sect. prunocerasus (rosaceae) based on seven noncoding chloroplast dna regions. *Am J Bot*, **91**: 985–996.
- 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*

399 *Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Cambridge, United Kingdom
400 and New York, NY.

401 **Teixido AL, Barrio M , Valladares F. 2016.** Size matters: Understanding the conflict faced by large flowers
402 in mediterranean environments. *The Botanical Review*, **82**: 204–228.

403 **de Villemeruil P. Nakagawa S. 2014.** *Modern Phylogenetic Comparative Methods and Their Application in*
404 *Evolutionary Biology*, Springer, New York, chap. General quantitative genetic methods for comparative
405 biology, pp. pp. 287–303.

406 **Wang S, Wu Z, Gong Y, Wang S, Zhang W, Zhang S, De Boeck HJ , Fu YH. 2022.** Climate warming shifts
407 the time interval between flowering and leaf unfolding depending on the warming period. *Science China*
408 *Life Sciences*, **65**: 2316–2324.

409 **Whiley A, Chapman K , Saranah J. 1988.** Water loss by floral structures of avocado (<i>persea ameri-
410 cana</i> cv. fuerte) during flowering. *Australian Journal of Agricultural Research*, **39**: 457–467.

411 **Whitehead DR. 1969.** Wind pollination in the angiosperms: Evolutionary and environmental considerations.
412 *Evolution*, **23**: 28–35.

413 **Willis CG, Ellwood ER, Primack RB, Davis CC, Pearson KD, Gallinat AS, Yost JM, Nelson G, Mazer SJ,**
414 **Rossington NL et al. 2017.** Old plants, new tricks: Phenological research using herbarium specimens.
415 *Trends in Ecology & Evolution*, **32**: 531–546.

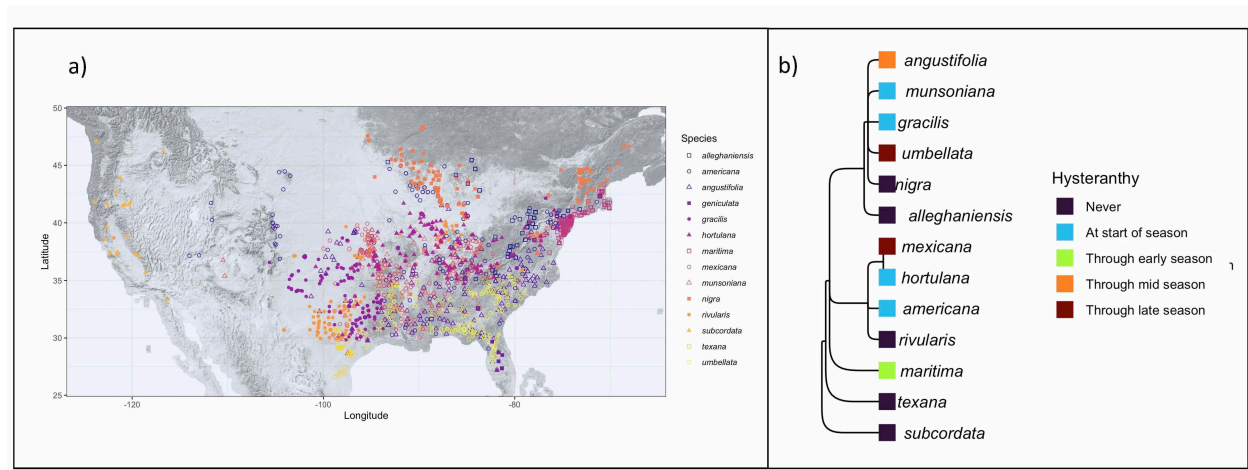


Figure 1: Geographic distribution and taxonomic relationships among the American plums. a) Maps the localities of all the herbaria records used in this study. b) Depicts phylogenetic relationships among the American plums and the duration of their flowering period they are hysteroanthous. These categorizations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

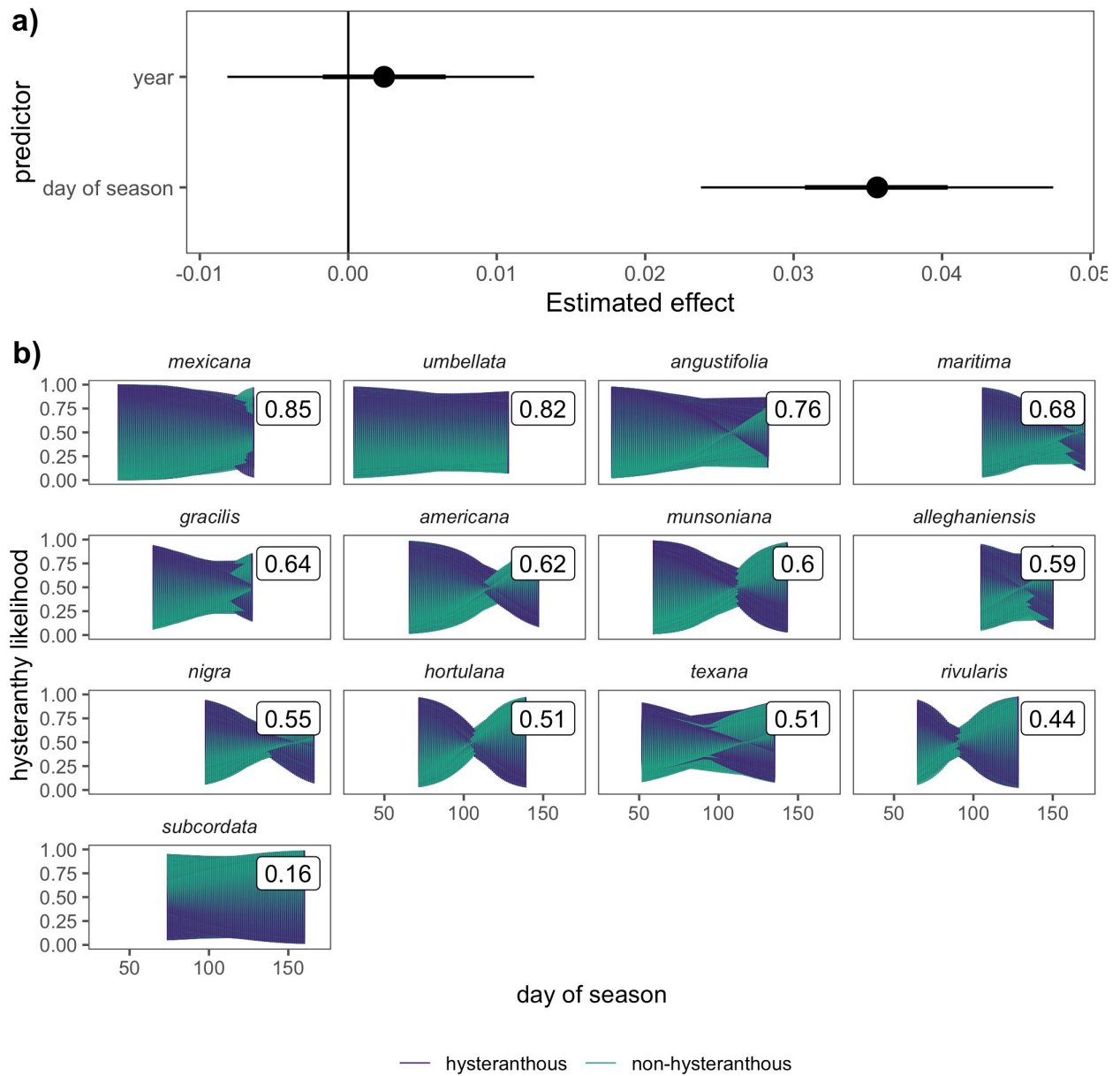


Figure 2: Predicted likelihood that a species would be in flower during each vegetative BBCH phase for five example species in the American plums. Points are the mean likelihood while bars represent 95% uncertainty intervals. Species were classified as hysteranthous if greater than 50% probability flowering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

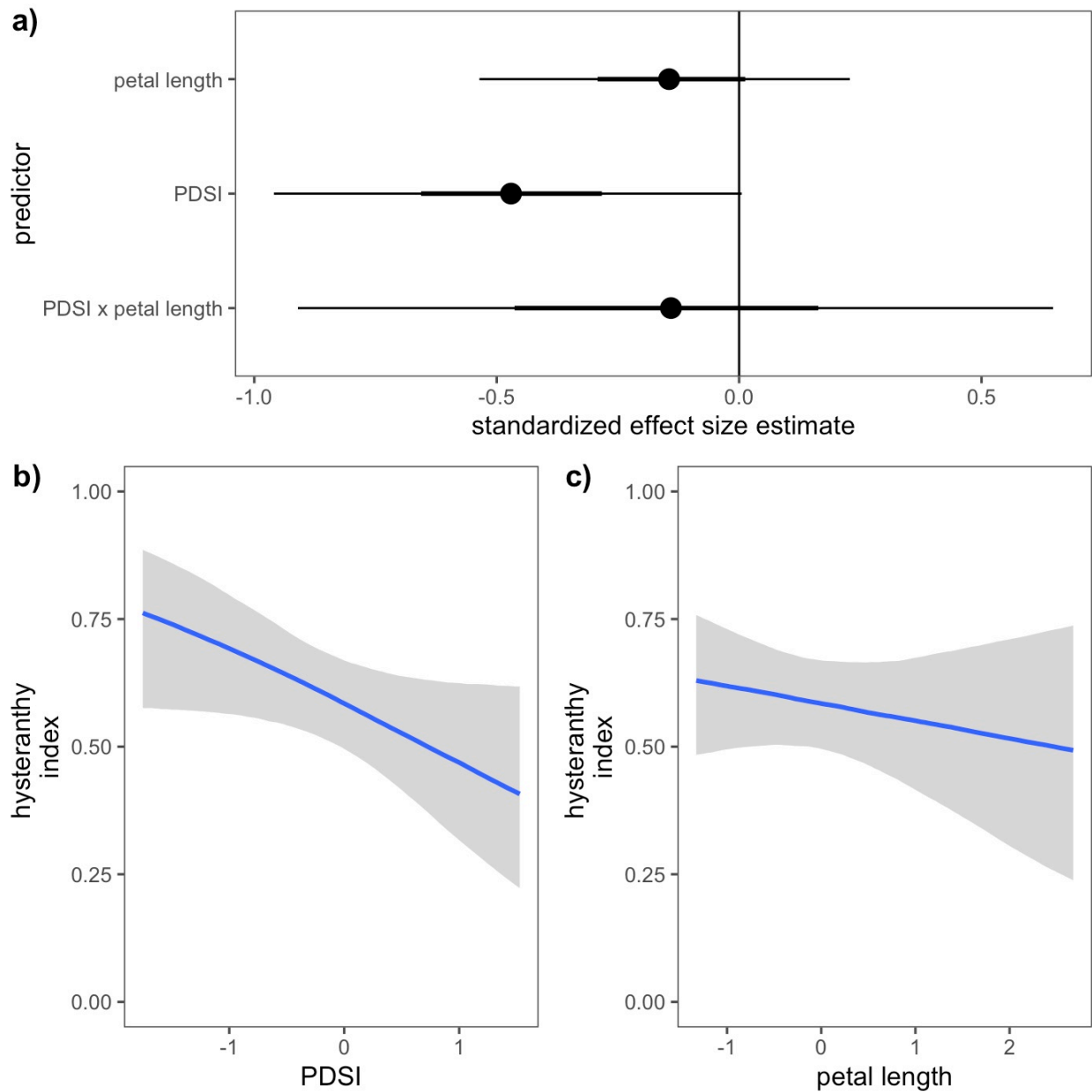


Figure 3: Relationships between the duration of hysteresis across the flowering period and environmental and biological traits based on Bayesian phylogenetic mixed models. a) b) and c) depict the relationships between the duration of hysteresis and mean PDSI, fruit diameter, and petal length respectively.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

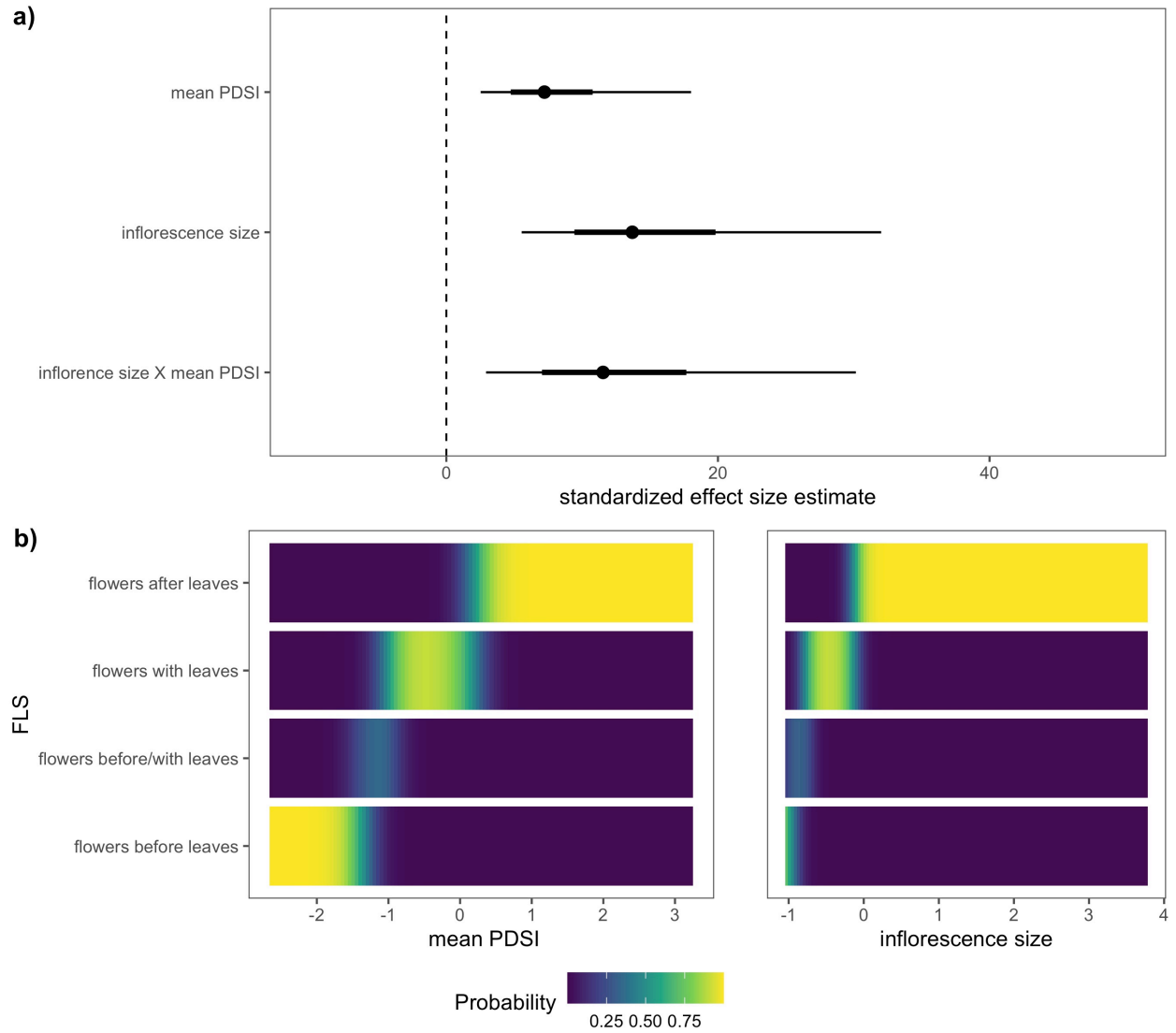


Figure 4: Relationships between the likelihood of hysteranthly and environmental and biological traits in the genus *Prunus* based on Bayesian phylogenetic mixed models. Panel a) shows the estimated effect size of each predictor with negative values indicating an increased likelihood of hysteranthly. 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 more of uncertainty, Panel b), c) and d) show the marginal effect of mean PDSI, inflorescence size and fruit size respectively, on the likelihood that of each flower-leaf sequence category. Solid lines indicate the mean likelihood and shaded areas the 50% uncertainty intervals.