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

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Summary

- Many trees in temperate forests produce flowers before their leaves emerge. This flower-leaf phenological sequence, known as hysteranthy, 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*, subspp. *Prunus* sect. *Prunocerasus*), a clade of insect-pollinated species, using herbaria specimens and Bayesian hierarchical modeling. With these observations, we tested two common hypotheses—that hysteranthy confers aridity tolerance and/or pollinator visibility—by modeling the associations between hysteranthy 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, hysteranthy was associated with aridity and smaller floral displays. These findings indicate that hysteranthy may function to temporally partition hydraulic demand and reduce water stress, or increase pollinator visibility and reduce selective pressure on flower size.

- Our findings that hysteranthy is linked to aridity tolerance and pollination success provides foundational insights into the evolution of flower-leaf sequences in the genus *Prunus*, with implications for understanding these patterns in biotically-pollinated plants in general.

Keywords: Deciduous forests, Flower-leaf sequences, Hysteranthy, Phenology, Plant hydraulics, Pollination, Phylogeny

Introduction

Woody perennials are among a subset of plant types with the unique ability to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence, known as hysteranthy, proteranthy 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 hysteranthy can confer performance advantages (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.

The most common, and well-tested explanation for the evolution of hysteranthy 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 hysteranthy in biotically-pollinated taxa found in temperate regions. This number is not trivial; a recent analysis found that approximately 20% of the hysteranthy species in Eastern Temperate Forests of North America are biotically-pollinated (Buonaiuto *et al.*, 2021).

Alternative hypotheses have been put forward to explain the advantage of hysteranthy 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* subspp. *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 the genus *Prunus* and lays the ground work for understanding the origins of flower-leaf sequence variation in biotically-pollinated taxa more generally.

Hypotheses of hysteranthous flowering in biotically-pollinated taxa

Water limitation hypothesis: In the dry-deciduous tropics of South and Central America, hysteranthy 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 hysteranthous flowering in temperate regions parallels that in the dry tropics—partitioning hydraulic demand across the season to allow hysteranthous 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 hysteranthous 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, hysteranthy 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 pollination environment. In one scenario, hysteranthy 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 hysteranthy and increased floral display size to augment floral attraction to visual pollinators. (Do we need to dig up citations here or is the logic sound enough?)

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 hysteranthy; 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 hysteranthy 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 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 to quantify 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 hysteranthy 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 that is designed to evaluate vegetative and reproductive phenological progress using a standardized quantitative index (Finn *et al.*, 2007). 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 temporal 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 Tab. S1 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 Villemeruil 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 hysteranthy co-varies with flowering time (i.e., flowering first species will generally flower earlier than other species, on average), and collection dates are not evenly distributed across the flowering season (see Fig.), 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:

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

133 $z_i = \alpha + \alpha_{phylo} + \alpha_{sp} + \beta_{\text{day of year[sp]}} * X_{\text{day of year}} + \beta_{\text{year}} * X_{\text{year}} + \epsilon_i$

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135 $\epsilon_i \sim logistic(0, 1)$

136

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

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

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

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

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

141 We used our model to predict the likelihood each species would be observed at a given vegetative BBCH
142 stage during flowering for each day of the flowering period of each species by extracting 1000 random draws
143 from the posterior distribution. For each day of the flowering season, we summed the predicted likelihood
144 that species would be at BBCH 0 ("bud closed"), BBCH 07/09 ("bud break") or BBCH 11 ("start of leaf
145 unfolding) vs. BBCH 15 ("leaf unfolding"), BBCH 17("most leaves unfolded"), BBCH 19 ("leaf expansion
146 complete") to quantify the likelihood as species would be be hysteranthous or non-hysteranthy respectively,
147 on each day of the the season. We chose the BBCH 11/BBCH 15 boundary to define hysteranthous flowering
148 because this is the earliest point in development when most leaves are unfurled enough to visually obscure
149 flowers and transpire. We used these estimates to developed a flower-leaf sequence index by summing the
150 likelihood of hysteranthy vs. non-hysteranthy across the full flowering period of each species, with 0 being
151 never hysteranthous and 1 being always hysteranthous. We also calculated a hysteranthy index using an
152 alternative cutoff at the BBCH 09/BBCH 11 which did not substantially alter the species' ranks on the index
153 (see Tab.).

154 To better understand how within-season dynamics affected our inference, we also repeated this modeling

procedure without including *day of season* as a predictor. This version of the model did not substantially alter the species' ranks on the index or our inference about the relationships between flower-leaf sequence variation and the trait representing the main hysteranthy hypotheses (Tab. , Tab.).

Evaluating hysteranthy hypotheses

To test the hypotheses of hysteranthy, we obtained data on petal length directly from herbarium specimens. To assess aridity tolerance, we computed the average Palmer Modified Drought Index score (hereafter: PDSI) obtained from the for Environmental Information (2017), for every *Prunocerasus* specimen in the database(n=2305). PDSI 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 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.

Because our all of our measurements were on different individuals—with different sample sizes—we executed two different modeling approaches in order to test the relationship between flower-leaf sequence index scores, aridity tolerance and floral displays.

First we computed species-levels means of PDSI and petal length and used a Beta regression to evaluate the relationship between flower-leaf sequences, PDSI, petal length and their interaction. We standardized the units of all predictors through z-scoring (Gellman & Hill, 2007) to make their effect size estimates directly comparable to each other within the following model structure:

$y_i = (\mu, \mu(1 - \mu)/(1 + \phi)$

where μ and ϕ are the two shape parameters of the beta regression. Due to the limited sample size of this analysis (13 species), we only modeled the effect of our predictors on the mean parameter μ and fit a grand intercept for the precision parameter ϕ as:

$\mu = \alpha + \beta_{PDSI} * X_{meanPDSI} + \beta_{\text{petal length}} * X_{meanpetallength} + \beta PDSIxpetallength * X_{meanPDSIxmeanpetallength}$

$\phi = \alpha)$

The advantage of this approach is that this model structure allowed us to assess the additive and interactive effects of PDSI and petal size on flower-leaf sequences. By using means trait values, this approach cannot incorporate within-species variation in these trait/environmental predictors or account for their phylogenetic structure. Because of this we as modeled the relationship between flower-leaf sequences index values and PDSI, and between flower-leaf sequences index values and petal size separately for comparison (see Supporting Information: Extended Methods for details).

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 *Prunus* species native to, or established in, North America (n=32). 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).

As above, we standardized the units of all predictors through z-scoring (Gellman & Hill, 2007). The model structure is:

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

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

$\epsilon_i \sim logistic(0, 1))$

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. Note that this model includes four ordinal categories while our model of the American Plums clade included 6, due to the underlying structure of the data.

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 hysteranthy hypotheses" we ran the models with a warm-up of 3000 iterations, and 4000, and 5000 sampling iterations respectively, for a total of 4000

8

and 8000 sampling iterations across all chains. For the "Hysteranthy in the larger genus *Prunus*" model, we

used a warm up of 6,000 iterations and 8,000 sampling iterations for a total of 8000 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 89% uncertainty intervals in the text with alternate

intervals in figures and the Supporting Information.

Results

Quantifying flower leaf sequences in the American plums

Across all species of American Plums, day season of the observation had a strong association with the being more like to be in flowering during an early vegetative phenological stage (Fig. 2,a). Year of observation did not substantially impact this likelihood for this taxonomic group (Fig. 2,a).

We found substantial inter-specific differences in flower-leaf sequences within the American plums (Fig. 2. b), with likelihood of hysteranthy of hysteranthy across the season ranging from 0.16 (*P. subcordata*) to .85 (*P. mexicana*). Several species (*P. mexicana, P. umbellata,P. angustifolia, P. maritima* and *P. gracilis*) were most likely to be hysteranthous for all—or most—of their flower period, while for others (*P. americana,P. munsoniana, P. alleghaniensis, P. nigra, P. hortulana, P. texana* and *P. rivularis*) hysteranthous flowering was only likely in the early part of their flowering session. One species, *P. subcordata*, was unlikely to be hysteranthous at any point in its flowering period (Fig. 2b). These relative likelihood of hysteranthy among species were consistent with our alternative method for constructing the hysteranthy index (Tab. , Fig. S2).

Associations between hysteranthy and environmental and morphological traits

In the American plums clade, increased likelihood of hysteranthy was negatively associated with PDSI and petal length without a substantial interaction between them ($_{meanPDSI}$: $-0.47, UI_{89}[-0.96, 0.01]$; $_{meanpetallength}$: $-0.14, UI_{89}[-0.54, 0.24]$, $_{meanPDSIxmeanpetallength}$: $-0.14, UI_{89}[-0.91, 0.65]$, Fig. 3), indicating that hysteranthy species are more likely to have smaller flower and be found in drier localities. These results are estimates are comparable to those in which we model each predictor separately and account for phylogeny(Fig. S3), and using the hysteranthy index derived from models that did not include day of season of observation as a predictor (Tab.)

For the larger genus *Prunus*, there was a negative association between hysteranthy and PDSI and number of flowers per inflorescence, as well as a substantial supra-additive interaction between them ($_{PDSI}$: $-8.0, UI_{89}[-16.6, -2.44]$; $_{flowers/inflorescence}$: $-15.5, UI_{89}[-31.46, -5.56]$; $_{PDSIxflowers/inflorescence}$: $-13.06, UI_{89}[-28.53, -$ Fig. 4).

Discussion

In this study, we show that hysteranthous flowering can be linked to both aridity tolerance and pollinator attraction. 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 between the timing of investment in reproduction relative to the timing of resumption of carbon gain through leafout, and these other aspects of plant performance. We found that hysteranthous flowering is associated with smaller floral displays and increased aridity in both the American plums, and more broadly across *Prunus* species native to, 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.

In our introduction to the insect visibility hypothesis we highlighted that floral display size could either be positively or negatively associated with hysteranthy depending on the pollination environment. In this study, we found that for both the taxonomic scales we investigated, hysteranthy was associated with smaller flower displays. This suggests that that the increased visibility of hysteranthous flowers may reduce selection pressure on flower display size, a finding that supports existing evidence form both comparative anatomy studies in plants (Gunatilleke & Gunatilleke, 1984), and studies about pollinator foraging behavior (Forrest & Thomson, 2009; Rivest *et al.*, 2017).

The supra-additive interactions terms between PDSI and floral display size we found highlight that the water limitation hypothesis and insect visibility hypothesis 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.

It is not surprising that the coefficient estimates for floral display size and their interaction term with PDSI were more extreme in the larger genus *Prunus* than those of the American Plums. All species in the American plums clade have solitary flowers, making the variation in floral display size highly constrained. By contrast, our analysis of the larger genus *Prunus* included species that range from having solitary flowers to those with as many as 100 flowers per inflorescence, representing substantially more variation in both floral investment and in hydraulic demand. This suggests that the correlated selection between flower-leaf sequences and the floral traits may be more pronounced at coarser taxonomic resolutions, when underlying trait variation is greater.

Despite these differences among taxonomic resolutions, aridity was associated with hysteranthy in both groups. 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 hysteranthy can reduced hydraulic demand in dry environments, hysteranthous 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). 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 hysteranthy and aridity in our study was relatively weak with high residual variance. Our results suggest that hysteranthy 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 hysteranthy (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 many of 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 hysteranthy across the growing season with Bayesian methods, our approach identified substantial differences in flower-leaf sequences among these intermediate cases (Fig. 1b), 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. 2b). For almost 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. **??**, Fig. S2). 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. Because our study was based on herbaria records collected on different individuals across space and time without repeat sampling, we were not able to robustly how much flower-leaf sequences vary within vs. among species. However this would be an important next step for understanding how the environment and species interactions have shaped these phenological patterns.

By scoring these individual, quantitative observations as ordinal response categories with our hysteranthy 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.

Interestingly, while there is substantial evidence that both flowering and leaf phenology have advanced over the last several decades in response to anthropogenic climate change (Menzel *et al.*, 2006; Cleland *et al.*, 2007; Augspurger & Zaya, 2020), we did not observe changes in flower-leaf sequences patterns over that time scale in our dataset. This supports a recent finding that despite changes in both flowering and leafout, the time interval between them has remained relatively stable (Guo *et al.*, 2023), but does not preclude that possibility that these the sequences will eventually be disrupted as climate change continues to become more extreme in the future (Buonaiuto & Wolkovich, 2021).

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 hysteranthy 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 hysteranthy—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 hysteranthy/non-hysteranthy 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 hysteranthy 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 shape flower-leaf sequences. 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.

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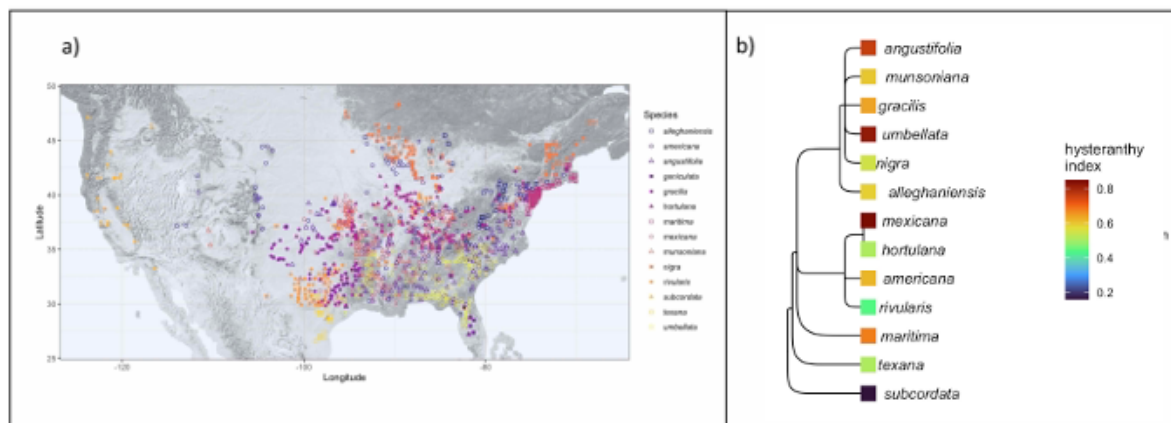⁴⁶⁴   *Trends in Ecology & Evolution*, **32**: 531–546.

Figures

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 likelihood they each species is hysteranthous across its full flowering period, represented by a hysteranthy index where 0 is never hysteranthous and 1 is always hysteranthous. These designations are based on ordinal phylogenetic mixed models. Tree topology is from Shaw & Small (2004)
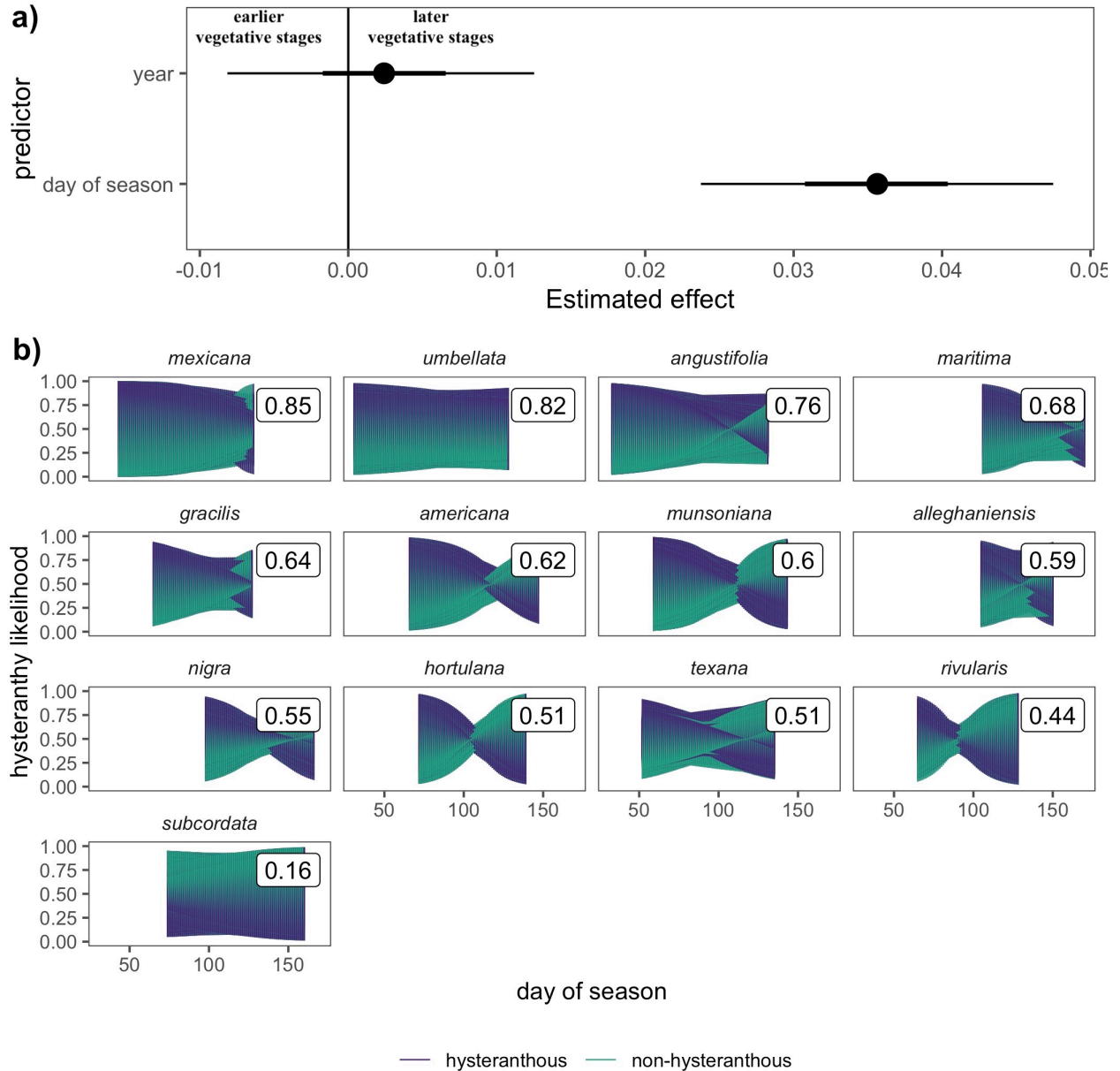
Figure 2: Predicted likelihood that a species would be in flower during vegetative BBCH phases for 13 species of the American plums. Panel a) depicts the influence of among season (year) and within season (day of year) temporal predictors on the likelihood would be be at earlier or later vegetative BBCH phases during flowering. Points are the mean effect size estimates while think and thin bars represent the 50 and 89% uncertainty intervals respectively. Panel b) depicts the predicted likelihood that each species would be hysteranthous across their flowering season. The colored shapes represents how the likelihood changes over each species flowering season and the boxed numerical values represent a hysteranthy index, or the total average likelihood a species would express hysteranthy throughout their flowering period. We defined hysteranthy as having open flowers at BBCH 0-BBCH 11 (leaf buds closed-start of leaf unfolding). See Tab.   for comparisons between the hysteranthous index scores reported here and alternative modeling approaches.
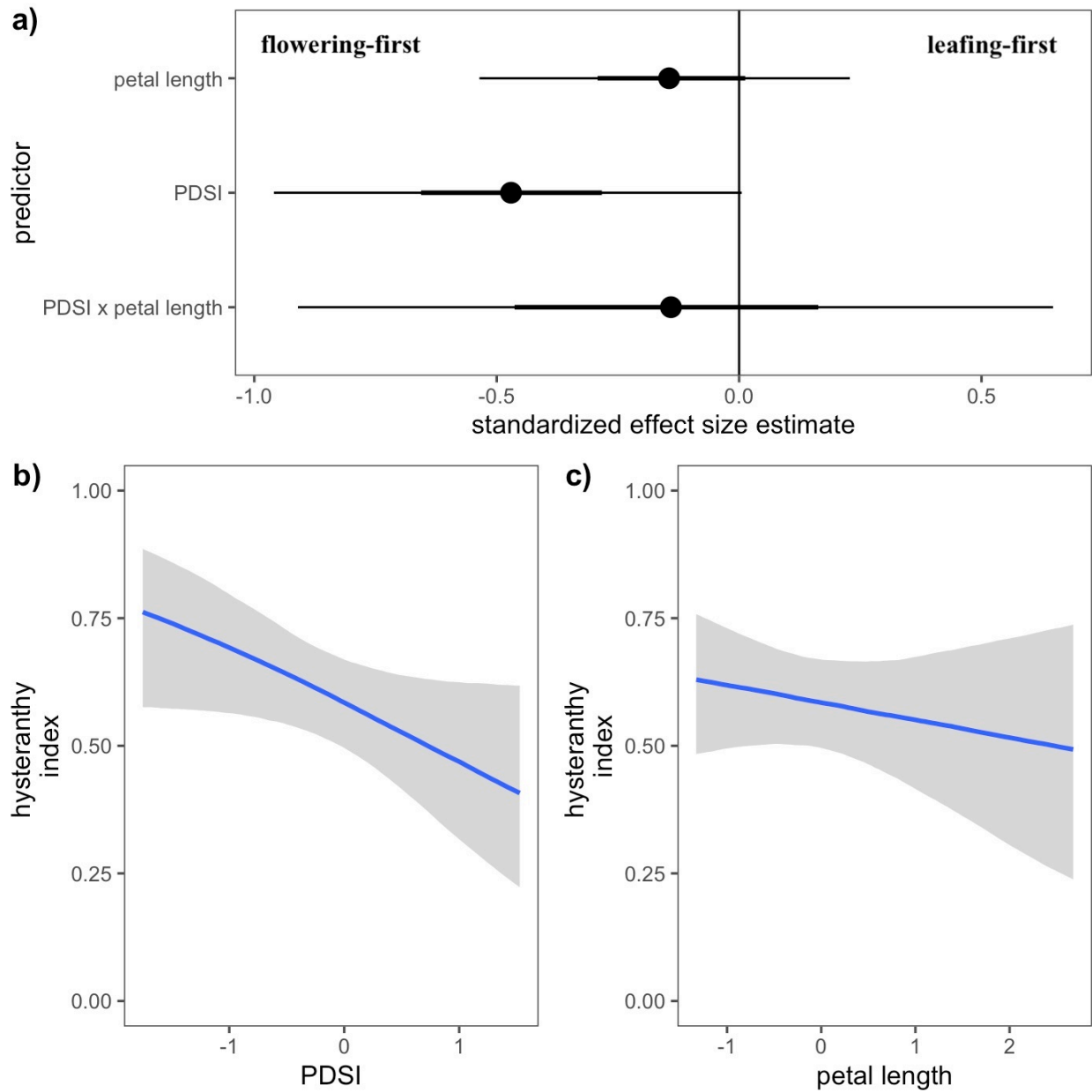
Figure 3: Relationships between hysteranthy index scores and environmental and biological traits based on Bayesian Beta regression for the 13 species of the American Plums. Panel a) shows the estimated effect size estimates of each predictor and their interaction on the mean shape parameter of the beta distribution. Points indicate the mean effects and the thick and thin bars represent the 50 and 89% uncertainty intervals. b) and c) depict the conditional effects of each predictor and hysteranthy index values. Blue lines indicate the mean estimate and grey fill the 89% uncertainty intervals
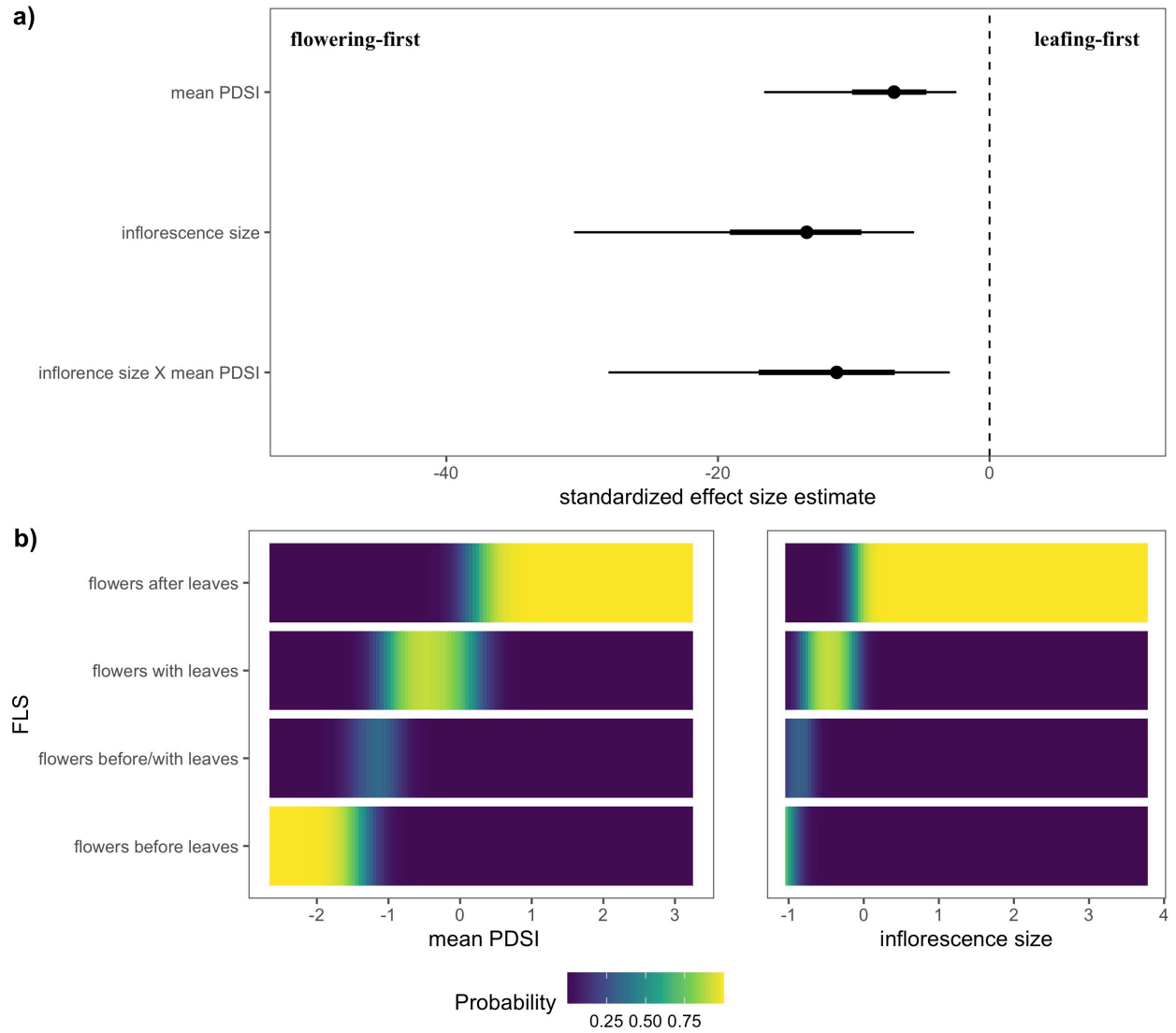
Figure 4: Relationships between the likelihood of hysteranthy and environmental and biological traits for 32 species of the genus *Prunus* native to, or established in North America based on Bayesian phylogenetic mixed models. Panel a) shows the estimated effect size of each predictor with negative values indicating an increased likelihood of hysteranthy. Points indicate the mean posterior estimate for each predictor, and thick and thin bars the 50% and 89% uncertainty intervals respectively. We also show the full posterior distribution as an additional more of uncertainty, Panel b), c) depict the likelihood for each flower-leaf sequence stage (y-axis) at any given values of PDSI or number of flowers/inflorescence (inflorescence size). Predictor values (x-axis) have been z-scored to make standardized comparisons.