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¹ Ecological drivers of flower-leaf sequences: aridity and proxies for
² pollinator attraction 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

¹³

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¹⁵ Figures: 4

¹⁶

₁₇ **Summary**

- ₁₈ • Across temperate forests many tree species produce flowers before their leaves emerge. This flower-
₁₉ leaf phenological sequence, known as hysteranthy, is generally described as an adaptation for wind-
₂₀ pollination. However, this explanation does not address why hysteranthy is also common in biotically-
₂₁ pollinated taxa.
- ₂₂ • We quantified flower-leaf sequence variation in the American plums (*Prunus*, subsp. *Prunus* sect.
₂₃ *Prunocerasus*), a clade of insect-pollinated trees, using herbaria specimens and Bayesian hierarchical
₂₄ modeling. We tested two common, but rarely interrogated hypotheses—that hysteranthy confers aridity
₂₅ tolerance and/or pollinator visibility—by modeling the associations between hysteranthy and related
₂₆ traits. To understand how these phenology-trait associations were sensitive to taxonomic scale and
₂₇ flower-leaf sequence classification, we then extended these analyses to all *Prunus* species in North
₂₈ America.
- ₂₉ • Our findings across two taxonomic levels support the hypotheses that hysteranthy may help temporally
₃₀ partition hydraulic demand to reduce water stress, and increase pollinator visibility—thereby reducing
₃₁ selective pressure on inflorescence size.
- ₃₂ • Our results provide foundational insights into the evolution of flower-leaf sequences in the genus *Prunus*,
₃₃ with implications for understanding these patterns in biotically-pollinated plants in general. Our ap-
₃₄ proach suggests a path to advance these hypotheses to other clades, but teasing out drivers fully will
₃₅ require new experiments.

₃₆ Keywords: Deciduous forests, Flower-leaf sequences, Hysteranthy, Phenology, Plant hydraulics, Pollination,
₃₇ Phylogeny

³⁸ Introduction

³⁹ Woody perennials are among a subset of plant types with the unique ability to seasonally begin reproduction
⁴⁰ prior to vegetative growth. This flowering-first phenological sequence, known as hysteranthy, proteranthy or
⁴¹ precocious flowering, is apparent in temperate deciduous forests around the globe (Rathcke & Lacey, 1985).
⁴² A number of studies suggest that this flower-leaf sequence is under selection, and that hysteranthy can confer
⁴³ performance advantages (Guo *et al.*, 2014; Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021), but the
⁴⁴ importance of variation in flower-leaf sequences for maintaining fitness may vary across functional types, taxa
⁴⁵ and biomes.

⁴⁶ The most common, and well-tested explanation for the evolution of hysteranthy in temperate forests is that it
⁴⁷ is adaptive for wind-pollination, as leafless canopies increase wind speeds for pollen transport and reduce the
⁴⁸ likelihood of pollen interception by vegetation (Whitehead, 1969; Niklas, 1985). However, this explanation
⁴⁹ does not address the widespread prevalence of hysteranthy in biotically-pollinated taxa found in temperate
⁵⁰ regions. This number is not trivial; a recent analysis found that approximately 20% of the hysteranthy species
⁵¹ in Eastern Temperate Forests of North America are biotically-pollinated (Buonaiuto *et al.*, 2021).

⁵² Alternative hypotheses have been put forward to explain the advantage of hysteranthy in biotically-pollinated
⁵³ species, but they have not been widely evaluated in the literature. Below, we briefly review these hypotheses
⁵⁴ and their predictions, then test their predictions using the American plums (*Prunus* subspp. *Prunus* sect.
⁵⁵ *Prunocerasus*)—a widespread clade with high variability in flower-leaf sequences—as a case-study. Our study
⁵⁶ both clarifies the hypothesized function of flower-leaf sequence variation in the genus *Prunus* and lays the
⁵⁷ groundwork for understanding the origins of flower-leaf sequence variation in biotically-pollinated taxa more
⁵⁸ generally.

⁵⁹ Hypotheses of hysteranthous flowering in biotically-pollinated taxa

⁶⁰ Water limitation hypothesis: In the dry-deciduous tropics of South and Central America, hysteranthy is
⁶¹ common (Rathcke & Lacey, 1985; Franklin, 2016), and is regarded as an important adaptation to alleviate
⁶² water stress by partitioning the hydraulic demand of flowers and leaves across the season (Borchert, 1983;
⁶³ Reich & Borchert, 1984; Franklin, 2016; Gougherty & Gougherty, 2018). Under this hypothesis, the function
⁶⁴ of hysteranthous flowering in temperate regions parallels that in the dry tropics. While temperate forests are
⁶⁵ rarely water-limited in the early season during which flowering and leafing occur (Polgar & Primack, 2011),
⁶⁶ there is still considerable variation in water availability in space and time within temperate regions of the
⁶⁷ globe. With this hypothesis, we would expect to find hysteranthous taxa in locations that are, on average,
⁶⁸ drier than their non-hysteranthous relatives.

⁶⁹ Insect visibility hypothesis: Hysteranthous flowers are visually conspicuous in the landscape. Thus, as in
⁷⁰ wind-pollinated taxa, hysteranthy in biotically-pollinated taxa may be an adaptation for pollination efficiency
⁷¹ as flowering-first species are easier for insect pollinators to locate (Janzen, 1967). A challenge to evaluating this

72 hypothesis is that correlated selection between flower-leaf sequences and pollinator visibility could have either
73 a positive or negative relationship depending on the pollination environment. In one scenario, hysteranthy
74 may be associated with smaller floral displays: because flowers are not obscured by leaves, they are easier
75 to see and there is weaker selection for increasing floral display size. In an alternative scenario, hysteranthy
76 could be associated with larger floral displays, especially in environments where plants are more often pollen-
77 limited and selection may favor both hysteranthy and increased floral display size to augment floral attraction
78 to visual pollinators.

79 In contrast to these functional hypotheses, hysteranthous flowering could simply be a by-product of selection
80 for early flowering. Species that flower before their leaves inherently flower early in the season. For example,
81 fruit development or dispersal constraints may drive early flowering (Primack, 1987), and because spring
82 flower phenology is less constrained by prior phenological events than leaf phenology (Ettlinger *et al.*, 2018;
83 Savage, 2019), this selection for early flowering could incidentally produce the hysteranthous phenological
84 sequence. Here, there is no specific adaptive advantage to hysteranthy; selection is not operating on the
85 relative timing of flower and leaf emergence, but rather the absolute flowering time alone. Rejection of the
86 above functional hypotheses might provide support to this null explanation.

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

95 The American plums are a useful model clade to investigate drivers of hysteranthous flowering in biotically-
96 pollinated species. The species that make up this group are distributed across the temperate zone of North
97 America and, like the genus *Prunus*, generally show pronounced inter-specific variation in flower-leaf se-
98 quences. Usefully, species in this clade are well represented in herbaria records (Fig. 1a), making them a
99 tractable group to measure and assess variation in flower-leaf sequences.

100 To interrogate the functional hypotheses for hysteranthous flowering described above, we used herbaria records
101 to quantify variation in flower-leaf sequences of the American plums. Then we combined environmental
102 attributes, biological traits and phylogenetic data in statistical models designed to evaluate whether the
103 observed associations between flower-leaf sequences and morphological and environmental traits match the
104 predicted associations of the hysteranthy hypotheses. Finally, we compared our findings in this clade to
105 patterns observed in larger genus *Prunus* to test whether these phenology-trait associations were sensitive to
106 taxonomic scale and flower-leaf sequence classification.

¹⁰⁷ **Materials and Methods**

¹⁰⁸ **Quantifying flower-leaf sequence variation**

¹⁰⁹ We obtained digital herbarium specimens of the American plums from the Consortium of Midwest Herbaria
¹¹⁰ (CMH) Database (of Midwest Herbaria, 2023). Specimen collection dates ranged from 1844-2020, with the
¹¹¹ majority collected between 1950-2000. To quantify flower-leaf sequence variation in this group we randomly
¹¹² sampled 200 specimens for each species and scored the phenological development of flowers and leaves; we
¹¹³ used a modified BBCH scale for woody plants designed to evaluate vegetative and reproductive phenological
¹¹⁴ progress through a standardized quantitative index (Finn *et al.*, 2007). For species with less than 200
¹¹⁵ specimens in the collection, we included all available specimens. In total, we evaluated the phenology of 2521
¹¹⁶ specimens, but only specimens with visible flowers were included in this analysis. We also removed specimens
¹¹⁷ with flowering dates that were major outliers from the observed flowering period of each species. We removed
¹¹⁸ outliers visually, and by excluding observations that were beyond three standard deviations of the median
¹¹⁹ flowering time for each species ($n=9$). Our final analyses included 1000 specimens (see Tab. S1 for number
¹²⁰ of observations/species).

¹²¹ We reconstructed the phylogenetic relationships among species in this group based on the tree topology in
¹²² Shaw & Small (2004). We inferred branch lengths following the method of Grafen & Hamilton (1989) in
¹²³ which node heights are estimated in proportion to number of subtending taxa using the R package “ape” (E.
¹²⁴ Paradis and K. Schliep, 2019).

¹²⁵ To quantify flower-leaf sequence variation, we fit an ordinal, hierarchical, Bayesian phylogenetic mixed model
¹²⁶ (de Villemur P. & Nakagawa, 2014) designed to assess the likelihood an individual would be at any given
¹²⁷ vegetative BBCH phase while flowering. Our model predicted leaf stage (y_i , ordinal, with six categories
¹²⁸ representing stage from 1 for “buds closed” and 6 for “leaf expansion complete”) as a function of species and
¹²⁹ additional phylogenetic effects. Because hysteronathy co-varies with flowering time (i.e., flowering first species
¹³⁰ will generally flower earlier than other species, on average), and collection dates were not evenly distributed
¹³¹ across the flowering season (see Fig. S1), we included day of year of observation as an additional predictor.
¹³² Additionally, because climate change could affect the interval between flowering and leafout over the course
¹³³ of our time series, we included the year of collection of each specimen as a covariate. Following previous
¹³⁴ conventions for modeling the possible effects of climate change on spring phenology, we parameterized *year*
¹³⁵ as a hinge variable, using 1980 as a break point (Stocker *et al.*, 2013; Buonaiuto *et al.*, 2021).

¹³⁶ The model is written below:

¹³⁷

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

¹³⁸ $z_i = \alpha + \alpha_{phylo} + \alpha_{sp} + \beta_{\text{day of year[sp]}} * X_{\text{day of year}} + \beta_{\text{year}} * X_{\text{year}} + \epsilon_i$

¹³⁹

¹⁴⁰ $\epsilon_i \sim \text{logistic}(0, 1)$

¹⁴¹

¹⁴² where y_i is the ordinal outcome (leaf stage; as 1,2,...6 categories). $c_{2...5}$ are the estimated cutpoints between
¹⁴³ leaf stages on the logit scale and X_{year} is the year the specimen was collected – 1980. z_i is the linear
¹⁴⁴ component of the underlying latent variable model.

¹⁴⁵ α describes an intercept for each category [1,2,...6] and slopes ($\beta_{\text{day of year}}$ and β_{year}) are constant across
¹⁴⁶ cutpoints. $\beta_{\text{day of year}}$ also varies among *species* while β_{year} is a pooled estimate across species.

¹⁴⁷ The influence of the phylogeny (α_{phylo}) was modeled as:

¹⁴⁸

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

¹⁴⁹ The α for species effects independent of the phylogeny was modeled as:

¹⁵⁰

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

¹⁵¹ We used our model to predict the probability that each species would be observed at a given vegetative
¹⁵² BBCH stage during flowering for each day of the flowering period of each species by extracting 1000 ran-
¹⁵³ dom draws from the posterior distribution. Next, for each day of the flowering season, we summed the
¹⁵⁴ predicted likelihood that species would be at BBCH 0 (“bud closed”), BBCH 07/09 (“bud break”) or BBCH
¹⁵⁵ 11 (“start of leaf unfolding”) vs. BBCH 15 (“leaf unfolding”), BBCH 17 (“most leaves unfolded”), BBCH 19
¹⁵⁶ (“leaf expansion complete”)—this allowed us to quantify the likelihood that a species would be hysteranthous
¹⁵⁷ or non-hysteranthous respectively for each day of the season. We chose the BBCH 11/BBCH 15 boundary to
¹⁵⁸ define hysteranthous flowering because this is the earliest point in development when most leaves are unfurled
¹⁵⁹ enough to visually obscure flowers and transpire. Finally, we used these estimates to develop a flower-leaf
¹⁶⁰ sequence index: for this, we summed the likelihood of hysteranthous vs. non-hysteranthous across the full flower-
¹⁶¹ ing period of each species, with 0 being never hysteranthous and 1 being always hysteranthous. To evaluate
¹⁶² the sensitivity of our model to choice of cutoff, we also calculated a hysteranthous index using an alternative

¹⁶³ cutoff at the BBCH 09/BBCH 11, which did not alter the species' ranks on the index (see Tab. S2).

¹⁶⁴ To better understand how within-season dynamics affected our inference, we also refit our model excluding
¹⁶⁵ *day of year* as a predictor. This version of the model did not substantially alter the species' ranks on the index
¹⁶⁶ or our inference about the relationships between flower-leaf sequence variation and the trait representing the
¹⁶⁷ main hysteranthy hypotheses (Tab. S2, Tab. S3).

¹⁶⁸ Evaluating hysteranthy hypotheses

¹⁶⁹ To test the hypotheses of hysteranthy, we first recorded petal length measurements directly from herbarium
¹⁷⁰ specimens. For these morphological measurements, we sampled 321 specimens and measured the petal length
¹⁷¹ of up to 10 randomly selected petals per specimen ($n=2757$) using ImageJ image processing software (see
¹⁷² Tab. S2, for n per species).

¹⁷³ To assess aridity tolerance, we computed the average Palmer Modified Drought Index score from June-August
¹⁷⁴ (hereafter: PDSI), obtained from Gille *et al.* (2017) for every *Prunocerasus* specimen in the database ($n=2305$,
¹⁷⁵ see Tab. S2, for n per species). PDSI is a unitless, standardized drought index that integrates temperature
¹⁷⁶ and precipitation data to estimate relative dryness in time and space (Heim, 2002). Negative PDSI values
¹⁷⁷ indicate more arid conditions and positive values wetter conditions. For any specimens that lacked accurate
¹⁷⁸ geo-location information, we extracted PDSI values at the county centroid of the herbaria specimen.

¹⁷⁹ Because all of our measurements were made on different individuals—with different sample sizes—we used
¹⁸⁰ two different modeling approaches to test the relationship between flower-leaf sequence index scores, aridity
¹⁸¹ tolerance and floral displays.

¹⁸² First we computed species-level means of PDSI and petal length and used a beta regression to evaluate the
¹⁸³ relationship between flower-leaf sequences, PDSI, petal length and their interaction. We standardized the
¹⁸⁴ units of all predictors through *z*-scoring (Gelman & Hill, 2007) to make their effect size estimates directly
¹⁸⁵ comparable within the following model structure:

¹⁸⁶

$$\text{187 } y_i = (\mu, \mu(1 - \mu)/(1 + \phi))$$

¹⁸⁸

¹⁸⁹ where μ and ϕ are the two shape parameters of the beta regression. Due to the limited sample size of this
¹⁹⁰ analysis (13 species), we only modeled the effect of our predictors on the mean parameter, μ , and fit a grand
¹⁹¹ intercept for the precision parameter, ϕ . We modeled the μ parameter as:

¹⁹²

$$\text{193 } \mu = \alpha + \beta_{PDSI} * \overline{X_{PDSI}} + \beta_{\text{petal length}} * \overline{X_{\text{petal length}}} + \beta_{PDSI \times \text{petal length}} * (\overline{X_{PDSI}})(\overline{X_{\text{petal length}}})$$

¹⁹⁴

¹⁹⁵ Both flower and leaf phenology are dictated by temperature cues (Buonaiuto & Wolkovich, 2021; Guo *et al.*,

196 2023), and to test whether broad temperature differences between species' ranges offered an alternative
197 explanation of hysteranthy variation, we obtained monthly temperature data from 1900-2014 from Matsuura
198 (2023) We then re-fit our model using mean spring temperature (February-April) as a predictor in place of
199 PDSI.

200 We chose this model structure because it allowed us to assess the additive and interactive effects of PDSI
201 and petal size on flower-leaf sequences. However, by using mean trait values as predictors, we could not
202 incorporate within-species variation in these trait/environmental predictors or account for their phylogenetic
203 structure. To understand how these factors affected our inferences about the relationship between flower-leaf
204 sequences and traits, we fit two additional models to estimate relationship between flower-leaf sequences index
205 values and PDSI, and between flower-leaf sequences index values and petal size separately which included
206 the intra-specific variation and phylogenetic structure of each of these traits (see Supporting Information:
207 Extended Methods for details). This alternative modeling approach produced similar results for phenology-
208 trait relationships to our main model.

209 Hysteranthy in the larger genus *Prunus*

210 To better understand how the patterns we identified in the American Plums clade scaled across a coarser
211 taxonomic resolution, we also evaluated the relationship between hysteranthous flowering and hypothesis-
212 related traits for additional *Prunus* species native to, or established in, North America ($n=32$). For this
213 analysis, we obtained categorical descriptions of flower-leaf sequences and mean estimates of the number of
214 flowers per inflorescence as a proxy for floral investment from the *Flora of North America* (Rohrer, 1993+).
215 We extracted PDSI values for all herbaria observations of those species in the Consortium of Midwest Herbaria
216 database ($n=23,272$) as described above.

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

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

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

222 $z_i = \alpha + \alpha_{phylo} + \beta_{PDSI} * X_{PDSI} + \beta_{\text{floral investment}} * X_{\text{flowers/inflorescence}} + \beta_{PDSIx\text{floral investment}} (X_{PDSI})(X_{\text{flowers/inflorescence}}) + \epsilon_i$
223

224

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

where y_i is the ordinal outcome of flower-leaf sequence category (“flowers after leaves”=1, “flowers with leaves”=2, “flowers before/with leaves”=3 and “flowers before leaves”=4) and $c_{2\dots 3}$ are the estimated cutpoints between categories on the logit scale. 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 six, due to the different underlying structures of the two datasets.

Model runs

We fit all models in the R package “brms” (Bürkner, 2018) using weakly informative priors, and four chains. For the models aimed at “Quantifying flower-leaf sequence variation” and “Evaluating hysteranthy hypotheses” in the American plums, we ran the models with a warm-up of 3000 iterations, and 4000, and 5000 sampling iterations respectively, for a total of 4000 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 were assessed with $\hat{R} < 1.01$, high effective sample sizes, and no divergent transitions. We provide mean estimates with uncertainty intervals in-text, and 50% and 89% intervals for all figures and tables.

Results

Quantifying flower leaf sequences in the American plums

We found substantial inter-specific differences in flower-leaf sequences within the American plums (Fig. 1b, Fig. 2a). 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. 2a). These relative ranking of species’ hysteranthy likelihoods were consistent with our alternative method for constructing the hysteranthy index (Tab. S2, Fig. S2).

Across all species of American Plums, day of year increased the likelihood of flowering during a later vegetative phenological stage (Fig. 2b). Year of observation did not substantially impact the likelihood of hysteranthy for this taxonomic group (Fig. 2b).

²⁵³ **Associations between hysteranthry and environmental and morphological traits**

²⁵⁴ In the American plums, predominately hysteranthous species had marginally smaller flowers and occurred in
²⁵⁵ historically drier localities than species with more overlap between flowers and leaves (i.e., increased likelihood
²⁵⁶ of hysteranthry was negatively associated with PDSI and petal length without a substantial interaction between
²⁵⁷ them, Fig. 3a; parameter estimates from this model were $\beta_{PDSI} : -0.47, UI_{89}[-0.96, 0.01]$, $\beta_{\text{petal length}} :$
²⁵⁸ $-0.14, UI_{89}[-0.54, 0.24]$ $\beta_{PDSIx_{\text{petal length}}} : -0.14, UI_{89}[-0.91, 0.65]$; Bayesian R^2 : 0.33). These estimates
²⁵⁹ were comparable to estimates from models where we treated each predictor separately and accounted for
²⁶⁰ phylogeny (Fig. S3), and where we used the hysteranthry index derived from models that did not include
²⁶¹ day of year as a predictor (Tab. S3). The direction and magnitude of the estimated effects support the
²⁶² predictions of the water-limitation hypothesis and marginally support the predictions of the insect-visibility
²⁶³ hypothesis.

²⁶⁴ While predominately hysteranthous species tended to be found in regions with warmer spring temperatures,
²⁶⁵ this relationship was weak (Tab. S4, $\beta_{\text{spring temperature}}: -0.26, UI_{89}[-0.36, 0.86]$) and the model with spring
²⁶⁶ temperature as a climate predictor explained considerably less variation (Bayesian R^2 : 0.20) in hysteranthry
²⁶⁷ than the model with PDSI.

²⁶⁸ In the larger genus *Prunus*, hysteranthous species had smaller inflorescences and were found in drier locations
²⁶⁹ (Fig. 4a, b; i.e., there was a negative association between hysteranthry and PDSI and number of flowers
²⁷⁰ per inflorescence, as well as a substantial negative interaction between them, parameter estimates from
²⁷¹ this model were $\beta_{PDSI} : -8.0, UI_{89}[-16.6, -2.44]$, $\beta_{\text{flowers}/\text{inflorescence}} : -15.5, UI_{89}[-31.46, -5.56]$ and
²⁷² $\beta_{PDSIx_{\text{flowers}/\text{inflorescence}}} : -13.06, UI_{89}[-28.53, -2.93]$). The direction and magnitude of the estimated
²⁷³ effects support the predictions of both the water-limitation hypothesis and the insect-visibility hypothesis.

²⁷⁴ The estimated effects of floral traits and their interactions with PDSI were stronger in the larger genus *Prunus*
²⁷⁵ than in the American plums clade. This is not surprising given that all species in the American plums clade
²⁷⁶ have solitary flowers, making the variation in floral display size highly constrained. By contrast, *Prunus*
²⁷⁷ species included in our secondary analysis include those with solitary flowers and species with as many as 100
²⁷⁸ flowers per inflorescence—substantially more variation in both floral investment and in hydraulic demand.
²⁷⁹ This suggests that the correlated selection between flower-leaf sequences and these floral traits may be more
²⁸⁰ pronounced at coarser taxonomic resolutions, where underlying trait variation is greater.

²⁸¹ **Discussion**

²⁸² Using North American *Prunus* species as a case study, our results indicate that flower-leaf sequences are likely
²⁸³ under selection. We show that variation in flower-leaf sequences across species may reflect adaptive tradeoffs
²⁸⁴ between a) the timing of investment in reproduction relative to the timing of resumption of carbon acquisition
²⁸⁵ through leafout, and b) other aspects of plant performance, such as environmental tolerance and pollinator

286 attraction strategies that we investigated in this study. We show that hysteranthous flowering is associated
287 with historic aridity (PDSI) and smaller flower displays in both the American plums, and more broadly
288 across *Prunus* species native to, or established in, North America. The relationships between hysteranthy
289 and aridity, and hysteranthy and floral display size support the predictions of the water limitation hypothesis
290 and the insect visibility hypothesis.

291 Our models estimated a strong relationship between aridity (PDSI) and flower-leaf sequences at both taxo-
292 nomic scales we studied, but the relationship between floral display size and flower-leaf sequences predicted
293 by the insect visibility was better supported at the coarser taxonomic scale of the full genus *Prunus* than
294 in the American plums clade. While species in the American plum clade have solitary flowers, they are
295 still clustered on branches, so it is unlikely the unit of attraction that pollinators are responding to is the
296 individual flower, which may explain why the relationships between hysteranthy and petal size we observed
297 were weak (Fig. 3b). The inflorescences of the larger genus *Prunus*, are probably a better proxy for differ-
298 ences in units of attraction, which may explain their stronger association with flower-leaf sequences variation
299 among species (Fig 4). This contrast may suggest that associated selection between flower-leaf sequences and
300 pollinator traits has more strongly influenced inflorescence architecture than the morphology of individual
301 flowers though our estimates at both scales agreed in directionality (i.e., hysteranthy associated with smaller
302 floral displays).

303 Under the insect visibility hypothesis, floral display size could either be positively or negatively associated
304 with hysteranthy depending on the pollination environment. The association between hysteranthy and smaller
305 flower displays that we found supports the prediction that increased visibility of hysteranthous flowers reduces
306 selection pressure on flower display size. These results fit with other comparative anatomy studies in plants
307 that report hysteranthous species typically have smaller inflorescences than non-hysteranthous relatives (Gu-
308 natilleke & Gunatilleke, 1984), and studies on pollinator foraging behavior that suggest the presence of leaves
309 substantially alters the visual perception of pollinators (Rivest *et al.*, 2017; Forrest & Thomson, 2009).

310 Our support for both the water limitation hypothesis and insect visibility hypothesis (especially in the larger
311 genus *Prunus*), and the strong positive interactions between PDSI and floral investment that we observed in
312 the larger genus *Prunus*, highlight that these hypotheses are not mutually exclusive, and could be related.
313 Selection on floral size represents a classic evolutionary tradeoff where larger floral displays may generally be
314 more effective for attracting pollinators but demand more resources, including water, to maintain turgor and
315 reproductive function than smaller ones (Galen *et al.*, 1999; Lambrecht & Dawson, 2007). With this trade-off,
316 reproductive displays are often small in harsher environments (Lambrecht, 2013; Teixido *et al.*, 2016), and
317 hysteranthy could represent a compensatory mechanism that both reduces hydraulic demand while increasing
318 pollination efficiency in these environments. Studies that have compared the transpiration rates among flowers
319 and leaves provide insights to the potential importance of hysteranthy as a means of partitioning hydraulic
320 demand across the season and maintaining water status. Measurements of water movement (transpiration
321 rates, sap flow, hydraulic conductivity) to flowers range from 20%-60% of that of leaves under comparable
322 conditions (Whiley *et al.*, 1988; Roddy & Dawson, 2012; Liu *et al.*, 2017; McMann *et al.*, 2022). This level

³²³ of additional hydraulic demand can drive loss of stomatal conductance and decrease photosynthetic rates
³²⁴ (Galen *et al.*, 1999).

³²⁵ The PDSI records that we incorporated in this study—spanning over two millennia—offer additional insights
³²⁶ into why the water limitation hypothesis may still be relevant to temperate forest regions that are typically
³²⁷ well saturated in the spring. These records reveal that the southeastern United States (the region in which
³²⁸ much of our data originates) was once much drier than it has been in contemporary times (Cook *et al.*, 2010,
³²⁹ Fig S4), suggesting that the spatial patterns of hysteranthy we see in the American plums may represent a
³³⁰ lagged-correlation with historical climate conditions.

³³¹ Despite this evidence that hysteranthy can reduce hydraulic demand in dry environments, hysteranthous
³³² species in the American plum clade are not found—even historically—in extremely arid locations (PDSI
³³³ values typically range from -4 to 4, although the values that we observed in our analyses were more restricted,
³³⁴ ranging from -0.5 to 0.2). This contrasts with hysteranthous species in the dry tropics where this phenological
³³⁵ pattern appears to allow them to tolerate more extreme aridity (Franklin, 2016). But the flower-leaf sequences
³³⁶ of the hysteranthous species in our study were markedly different from patterns of hysteranthy in these dry-
³³⁷ tropics where the water limitation hypothesis was initially proposed. While flowering can precede leafout
³³⁸ by as much as several weeks for species in the American plums, the process of fruit development, which is also
³³⁹ water intensive, occurs when leaves are present. By contrast, in the dry tropics hysteranthous flowering is
³⁴⁰ initiated at the time of leaf drop (Borchert, 1983; Franklin, 2016); thus, the full reproductive cycle occurs in
³⁴¹ the leafless period. The comparatively small window of leafless reproductive development in our temperate
³⁴² clade suggests that hysteranthy may allow temperate species to occupy marginally drier environments than
³⁴³ non-hysteranthous species, but may not facilitate species' persistence under extreme aridity.

³⁴⁴ Inter-and intra-specific variation in flower-leaf sequences

³⁴⁵ We developed a novel approach to assessing flower-leaf sequences that scales from quantitative, individual-
³⁴⁶ level observations to species-level characterizations. With this approach, we were able to—for the first
³⁴⁷ time—quantitatively assess intermediate cases of hysteranthy (such as those that are typically described as
³⁴⁸ “flowers before/with leaves”). Previous studies of hysteranthous flowering have either excluded these cases
³⁴⁹ from their analyses (e.g.; Gougherty & Gougherty, 2018) or binned them with the well defined cases (e.g.;
³⁵⁰ Buonaiuto *et al.*, 2021). We found that many American plum species expressed this intermediate flower-leaf
³⁵¹ sequence. Further, while our classifications broadly matched previous species-level analyses in this group by
³⁵² Shaw & Small (2004), our approach identified substantial differences in flower-leaf sequences among these
³⁵³ intermediate cases (Fig. 1b), which allowed us to assess the trait associations with this phenotype.

³⁵⁴ Our quantitative analysis of the American plums clade revealed that flower-leaf sequences—often described
³⁵⁵ as a species-level trait—are highly variable within species (Fig. 2a). For almost all members of the clade,
³⁵⁶ hysteranthy was strongly predicted by the day of the observation (“day of year” in our model, Fig. 2b). In
³⁵⁷ many cases there was a high likelihood that individuals of a species may be observed at different vegetative

358 stages during flowering (Fig. 2a, Fig. S2). The variation we observed here could either suggest high levels
359 of local adaptation in flower-leaf sequences or, alternatively, high levels of plasticity through which flower-
360 leaf sequences respond to interannual variation in environmental conditions. Because our study was based
361 on herbaria records collected on different individuals across space and time without repeat sampling, we
362 could not robustly estimate how much flower-leaf sequences vary within vs. among species, and within
363 individuals over time. While mean spring temperature was not a strong predictor of inter-specific flower-leaf
364 sequence variation in our analyses (Tab. S4), it is likely spring temperature influences individual variation
365 in hysteranthy (Guo *et al.*, 2023). Parsing in the influence of multiple climate drivers across multiple scales
366 of ecological and evolutionary time would be an important next step for understanding how the environment
367 and species interactions have shaped these phenological patterns.

368 Interestingly, while there is substantial evidence that both flowering and leaf phenology have advanced over
369 the last several decades in response to anthropogenic climate change (Menzel *et al.*, 2006; Cleland *et al.*,
370 2007; Augspurger & Zaya, 2020), we did not observe changes in flower-leaf sequences over that time scale
371 in our dataset (Fig. 2b). This supports a recent finding that despite changes in both flowering and leafout,
372 the time interval between them has remained relatively stable (Guo *et al.*, 2023), but does not preclude that
373 possibility that these the sequences will eventually be disrupted as climate change continues to become more
374 extreme in the future (Buonaiuto & Wolkovich, 2021).

375 Future directions

376 We focused on a well-studied, and economically important clade of morphologically similar species. Our
377 case-study provides a road map for evaluating the role of hysteranthy more generally in temperate biotically-
378 pollinated plant taxa (other groups with high interspecific flower-leaf sequence variation include *Magnolia*,
379 *Rhododendron*, *Acer* and *Cornus*), and more broadly across taxa and biomes.

380 Combining the observational approach with novel experiments could further advance our collective understand-
381 ing of the adaptive significance of flower-leaf sequences. To test the water-limitation hypothesis, re-
382 searchers could plant sister-taxa with contrasting flower-leaf sequences in common environments across a
383 gradient of aridity, and evaluate their performance. To test the insect visibility hypothesis, researchers
384 should consider hysteranthy—and phenology in general—in the broader framework of tradeoffs in pollination
385 biology. The tradeoff between phenology and pollination investment could not only consider flower size, but
386 also the number of flowers, nectar and pollen reward investment, volatiles between related hysteranthous and
387 non-hysteranthous taxa. Findings that hysteranthous species invest fewer resources into these other pollinator
388 attraction traits than non-hysteranthous relatives would support the insect visibility hypothesis. For a simple
389 experiment to test the pollinator visibility hypothesis, researchers could force hysteranthy/non-hysteranthy
390 phenotypes for the same genotype using environmental cues and systematically release pollinators to ob-
391 serve their preferences, search times and foraging behavior. If pollinators are more readily drawn to the
392 hysteranthous individuals, it would support hysteranthy as an adaptive trait for pollinator attraction.

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

400 Competing Interests:

401 The authors declare no conflict of interest.

402 Author contributions

403 DMB, and EMW conceived of the manuscript; DMB and SC collected the data; DMB led the statistical
404 analyses with TJD and EMW; DMB led the writing of the manuscript. All authors contributed to writing
405 and gave approval for the submission.

406 Data Availability

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

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Figures

509

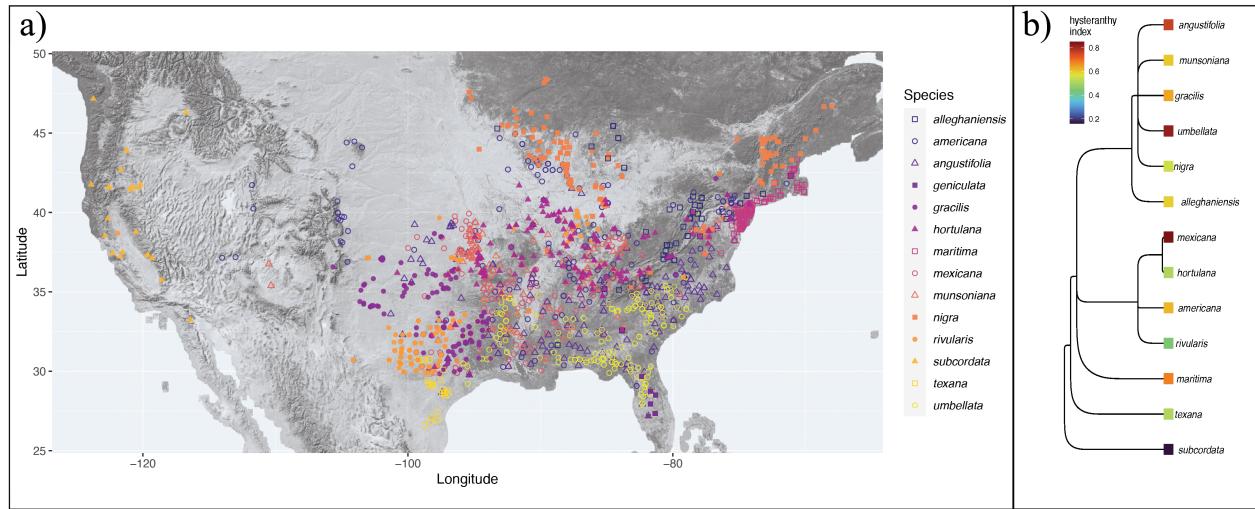


Figure 1: Geographic distribution and taxonomic relationships among the American plums. a) Maps the localities of all the herbaria records used in this study. b) Depicts phylogenetic relationships among the American plums and the likelihood they each species is hysteranthous across its full flowering 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)

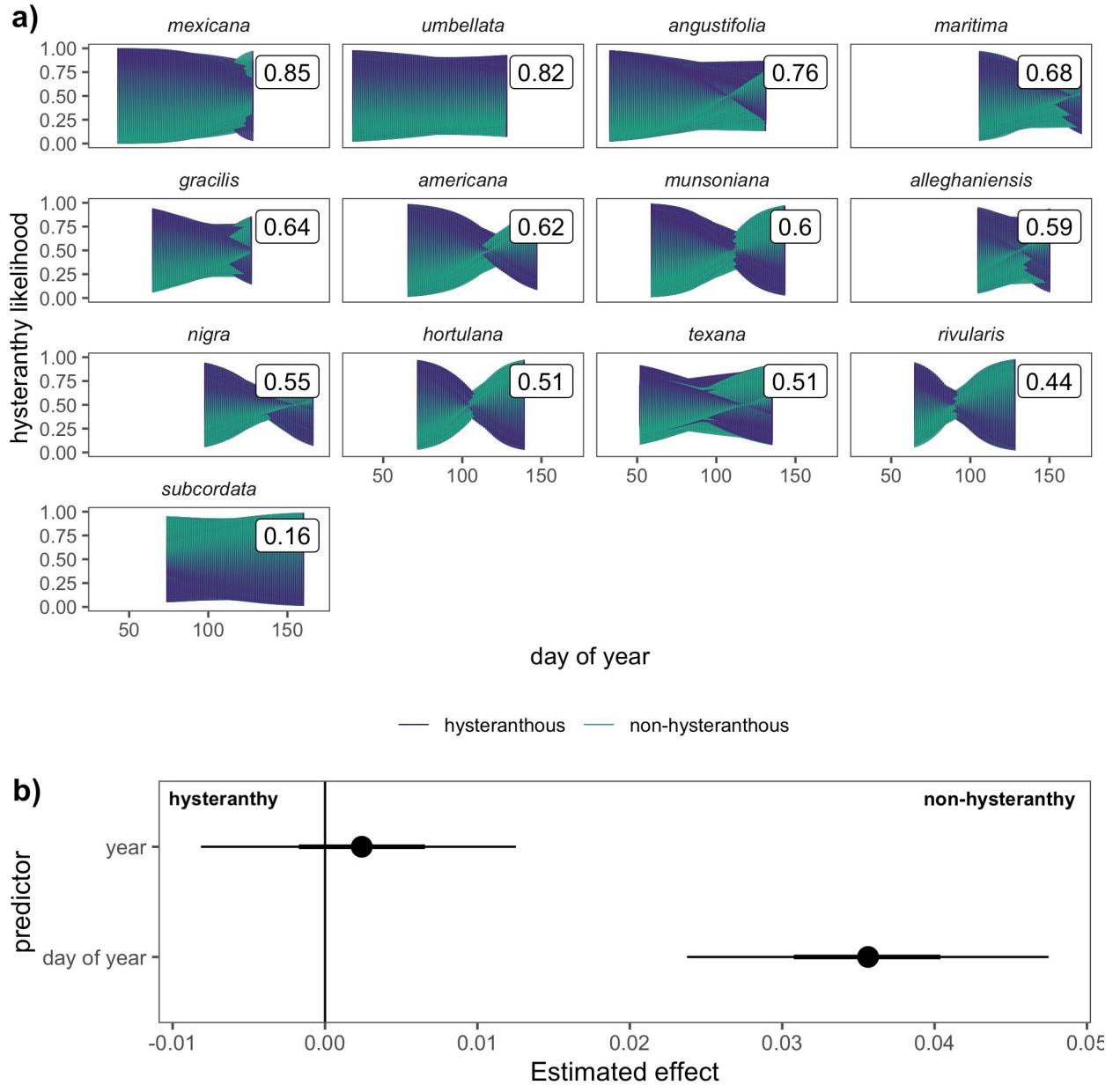


Figure 2: Predicted likelihood of hysteranthy across the flowering period of 13 American plum species and the temporal predictors that drive these patterns. Panel a) depicts the predicted likelihood that each species would express hysteranthy on each day of their flowering season based on 1000 draws from the posterior distribution of Bayesian hierarchical models. The colored shapes represent how the likelihood changes over time and the boxed numerical values represent the average likelihood a species would express hysteranthy, summed across the full flowering period. Panel b) depicts the influence of among season (year of sample) and within season (day of year of sample) trends on the likelihood species would express hysteranthy. Points are the mean effect size estimates, while thick and thin bars represent the 50% and 89% uncertainty intervals respectively.

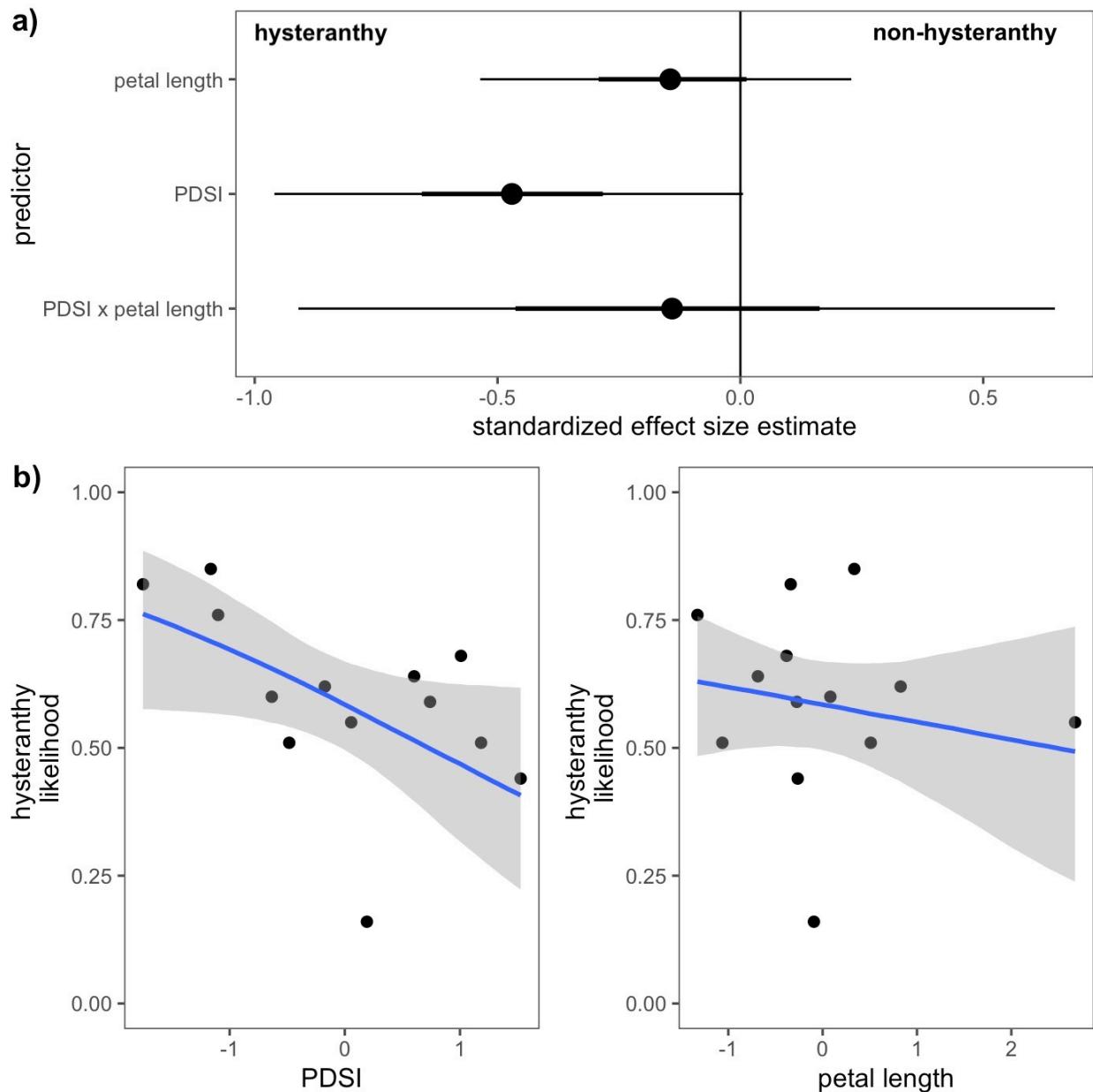


Figure 3: Relationships between hysteranity and environmental and biological traits for the 13 species of the American Plums. Panel a) shows the estimated effects of each predictor and their interaction on the likelihood hysteranity. Points indicate the mean effects and the thick and thin bars represent the 50% and 89% uncertainty intervals, respectively. Panel b) depicts the conditional effects of each predictor on hysteranity likelihood. Blue lines indicate the mean estimate and grey fill the 89% uncertainty intervals. Predictor values (x -axis) are in standardized units (z -scored) to allow direct comparisons between predictors.

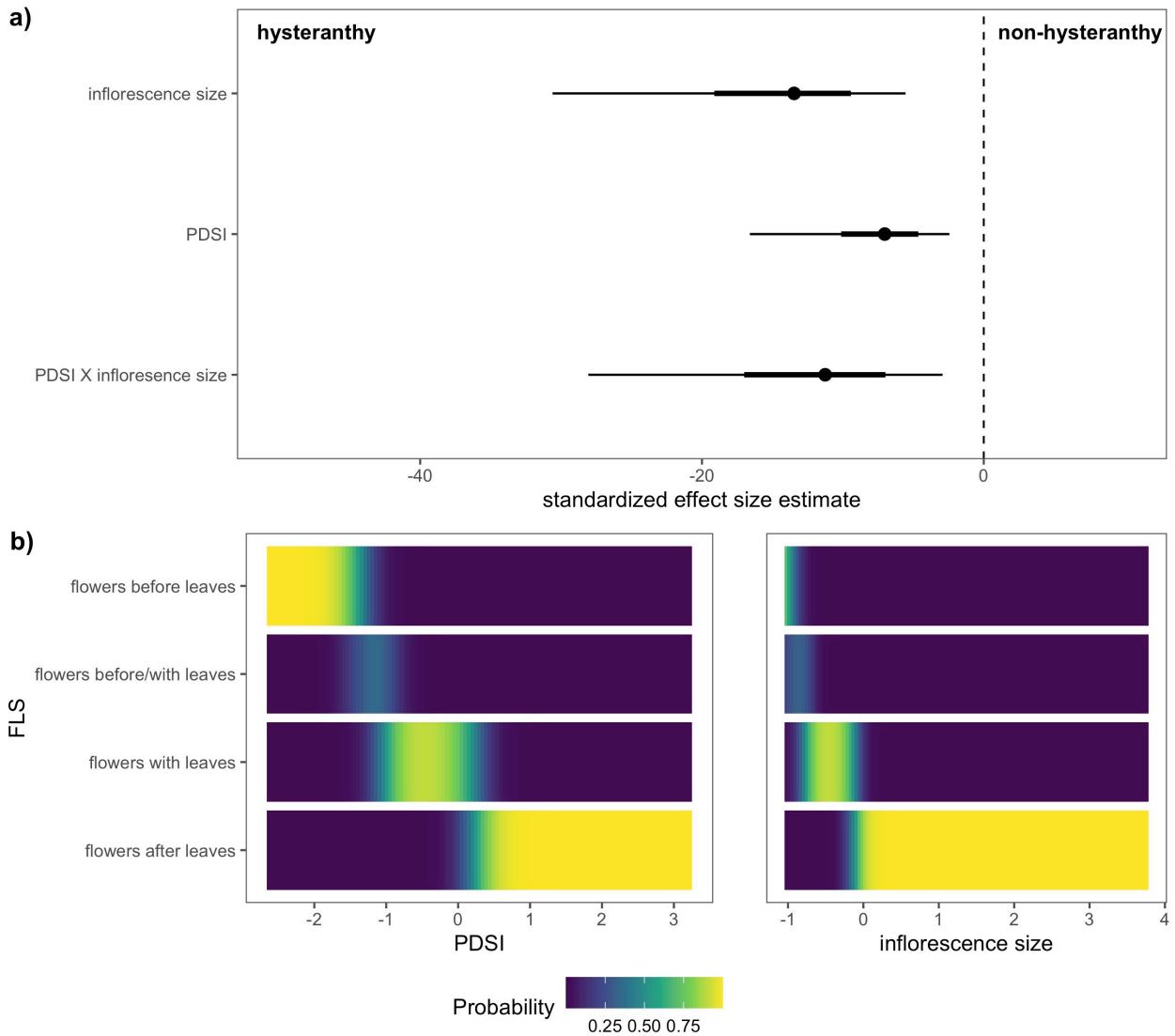


Figure 4: Relationships between the likelihood of hysteranthry and environmental and biological traits for 32 species of the genus *Prunus* native to, or established in North America. Panel a) shows the estimated effect size of each predictor. Points indicate the mean estimate for each predictor, and thick and thin bars the 50% and 89% uncertainty intervals, respectively. Panel b) depicts the likelihood for each flower-leaf sequence stage (y -axis) at any given values of PDSI or number of flowers/inflorescence (predictor values (x -axis) have been z -scored to allow direct comparisons between predictors.