Ecological drivers of flower–leaf sequences: aridity and proxies for pollinator attraction select for flowering-first in the American plums

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Summary

- Across temperate forests, many tree species produce flowers before their leaves emerge. This flower–leaf phenological sequence, known as hysteranthy, is generally described as an adaptation for wind pollination. However, this explanation does not address why hysteranthy is also common in biotically pollinated taxa.
- We quantified flower–leaf sequence variation in the American plums (*Prunus*, subg. *Prunus* sect. *Prunocerasus*), a clade of insect-pollinated trees, using herbaria specimens and Bayesian hierarchical modeling. We tested two common, but rarely interrogated hypotheses – that hysteranthy confers aridity tolerance and/or pollinator visibility – by modeling the associations between hysteranthy and related traits. To understand how these phenology–trait associations were sensitive to taxonomic scale and flower–leaf sequence classification, we then extended these analyses to all *Prunus* species in North America.
- Our findings across two taxonomic levels support the hypotheses that hysteranthy may help temporally partition hydraulic demand to reduce water stress and increase pollinator visibility – thereby reducing selective pressure on inflorescence size.
- Our results provide 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. Our approach suggests a path to advance these hypotheses to other clades, but teasing out drivers fully will require new experiments.

Introduction

Woody perennials are among a subset of plant types with the unique ability to seasonally begin reproduction before 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 (Guo *et al.*, 2014; Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021), but the importance of variation in flower–leaf sequences for maintaining fitness may vary across functional types, taxa and biomes.

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 *c.* 20% of the hysteranthous 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, then test their predictions using the American plums (*Prunus* subg. *Prunus* sect. *Prunocerasus*) – a widespread clade with high variability in flower–leaf sequences – as a case study. Our study both clarifies the hypothesized function of flower–leaf sequence variation in the genus *Prunus* and lays the groundwork 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 (Borchert, 1983; Reich & Borchert, 1984; Franklin, 2016; Gougherty & Gougherty, 2018). Under this hypothesis, the function of hysteranthous flowering in temperate regions parallels that in the dry tropics. 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 where their nonhysteranthous relatives are found.

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 a weaker selection for increasing floral display size. In an alternative scenario, hysteranthy could be associated with larger floral displays, especially in environments where plants are more often pollen-limited and selection may favor both hysteranthy and increased floral display size to augment floral attraction to visual pollinators.

In contrast to these functional hypotheses, hysteranthous flowering could simply be 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 (Ettinger *et al.*, 2018; Savage, 2019), 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 flowering and leaf emergence, but rather the absolute flowering time alone. Rejection of the above functional hypotheses might provide support to this null explanation.

A significant challenge for robust testing of hypotheses for hysteranthy 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 historical 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 widely used to investigate variability of flower–leaf sequences.

The American plums are a useful model clade to investigate drivers of hysteranthous flowering in biotically pollinated species. The species that make up this group are distributed across the temperate zone of North America and, like the genus *Prunus*, generally show pronounced interspecific variation in flower–leaf sequences. Usefully, species in this clade 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 variation in flower–leaf sequences of the American plums. Then we combined environmental attributes, biological traits and phylogenetic data in statistical models designed to evaluate whether the observed associations between flower–leaf sequences 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 the larger genus *Prunus* to test 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 of the American plums from the Consortium of Midwest Herbaria (CMH) Database (Consortium of Midwest Herbaria, 2021). Specimen collection dates ranged from 1844 to 2020, with the majority collected between 1950 and 2000. To quantify flower–leaf sequence variation in this group, we randomly sampled 200 specimens for each species and scored the phenological development of flowers and leaves; we used a modified BBCH scale for woody plants designed to evaluate vegetative and reproductive phenological progress through a standardized quantitative index (Finn *et al.*, 2007). For species with < 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 removed specimens with flowering dates that were major outliers from the observed flowering period of each species. We removed outliers 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 Supporting Information Table 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 the number of subtending taxa using the R package APE (Paradis & Schliep, 2019).

To quantify flower–leaf sequence variation, we fit an ordinal, hierarchical, Bayesian phylogenetic mixed model (de Villemeruil & Nakagawa, 2014) designed 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 representing stage from 1 for 'buds closed' to 6 for 'leaf expansion complete') as a function of species and additional

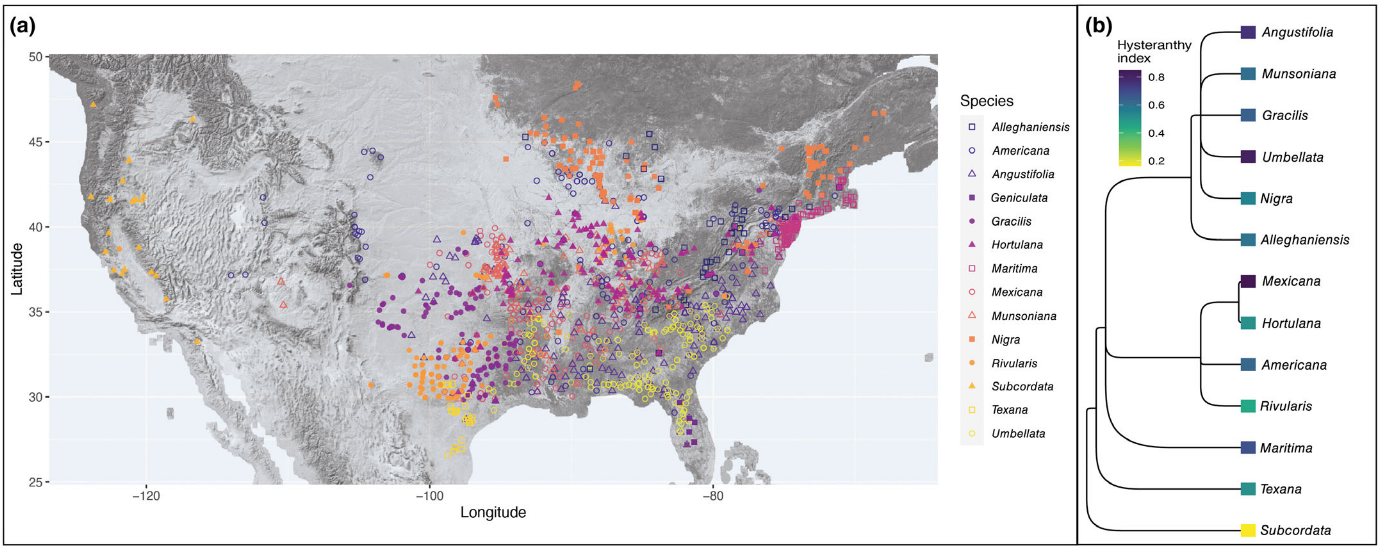

Fig. 1 Geographic distribution and taxonomic relationships among the American plums. (a) Maps the localities of all the herbaria records used in this study. (b) Depicts phylogenetic relationships among the American plums and the likelihood that each species is hysteranthous across its full flowering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

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 were not evenly distributed across the flowering season (see Fig. S1), we included the day of the year of observation as an additional predictor. Additionally, because climate change could affect the interval between flowering and leafout over the course of our time series, we included the year of collection of each specimen as a covariate. Following previous conventions for modeling the possible effects of climate change on spring phenology, we parameterized *year* as a hinge variable, using 1980 as a breakpoint (Stocker *et al.*, 2013; Buonaiuto *et al.*, 2021).

The model is written below:

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

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

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

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

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

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

$$\alpha_{\text{phylo}} \sim \text{normal}\left(0, \text{COR}\left[\sigma^2_{\text{phylo}}\right]\right)$$

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

$$\alpha_{\text{sp}} \sim \text{normal}\left(0, \sigma^2_{\text{species}}\right)$$

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

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

To better understand how within-season dynamics affected our inference, we also refit our model, excluding the *day of year* 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 (Tables S2, S3).

Evaluating hysteranthy hypotheses

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

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

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

First we computed species-level 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 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. We modeled the μ parameter as:

$$\mu = \alpha + \beta_{\mathrm{PDSI}} \times \overline{X_{\mathrm{PDSI}}} + \beta_{\text{petal length}} \times \overline{X_{\text{petal length}}} + \beta_{\mathrm{PDSI}_x\text{petal length}} \times \left(\overline{X_{\mathrm{PDSI}}}\right)\left(\overline{X_{\text{petal length}}}\right)$$

Both flower and leaf phenology are sensitive to temperature cues (Buonaiuto & Wolkovich, 2021; Guo *et al.*, 2023), and to test whether broad temperature differences between species' ranges offered an alternative explanation of hysteranthy variation, we additionally obtained monthly temperature data from 1900 to 2014 from Matsuura (2023). We then refit our model using mean spring temperature (February–April) as a predictor in place of PDSI.

We chose this model structure because it allowed us to assess the additive and interactive effects of PDSI and petal size on flower–leaf sequences. However, by using mean trait values as predictors, we could not incorporate within-species variation in these trait/environmental predictors or account for their phylogenetic structure. To understand how these factors affected our inferences about the relationship between flower–leaf sequences and traits, we fit two additional models to estimate the relationship between flower–leaf sequences index values and PDSI, and between flower–leaf sequences index values and petal size separately which included the intra-specific variation and phylogenetic structure of each of these traits (see Fig. S1 for details). This alternative modeling approach produced similar results for phenology–trait relationships to our main model.

Hysteranthy in the larger genus *Prunus*

To better understand how the patterns we identified in the American plums clade scaled across a coarser taxonomic resolution, we also evaluated the relationship between hysteranthous flowering and hypothesis-related traits for additional *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 observations of those species in the CMH 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 (Paradis & Schliep, 2019).

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

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

$$z_i = \alpha + \alpha_{\text{phylo}} + \beta_{\mathrm{PDSI}} \times X_{\mathrm{PDSI}} + \beta_{\text{floral investment}} \times X_{\text{flowers/inflorescence}} + \beta_{\mathrm{PDSI}_x\text{floral investment}}(X_{\mathrm{PDSI}})(X_{\text{flowers/inflorescence}}) + \epsilon_i$$

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

where y_i is the ordinal outcome of flower–leaf sequence category ('flowers after leaves' = 1, 'flowers with leaves' = 2, 'flowers before/with leaves' = 3 and 'flowers before leaves' = 4) and $c_{2...3}$ are the estimated cut points 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 6000 iterations and 8000 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 interspecific differences in flower–leaf sequences within the American plums (Figs 1b, 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 rankings of species' hysteranthy likelihoods were consistent with our alternative method for constructing the hysteranthy index (Table 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).

Associations between hysteranthy and environmental and morphological traits

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

While predominately hysteranthous species tended to be found in regions with warmer spring temperatures, this relationship was weak and uncertain (Table S4; $\beta_{\mathrm{spring\ temperature}}$: 0.26, $\mathrm{UI}_{89}[-0.36,\ 0.86]$) and the model with spring temperature as a climate predictor explained considerably less variation (Bayesian R^2: 0.20) in hysteranthy than the model with PDSI.

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

The estimated effects of floral traits and their interactions with PDSI were stronger in the larger genus *Prunus* than in the American plums clade. This is not surprising given that all species in the American plums clade have solitary flowers, making the variation in floral display size highly constrained. By contrast, *Prunus* species included in our secondary analysis include those with solitary flowers and species with as many as 100 flowers per inflorescence – substantially more variation in both floral investment and in hydraulic demand. This suggests that the correlated selection between flower–leaf sequences and these floral traits may be more pronounced at coarser taxonomic resolutions, where we sample a greater breadth of ecological strategies along both axes.

Discussion

Using North American *Prunus* species as a case study, our results indicate that flower–leaf sequences are likely under selection. We show that variation in flower–leaf sequences across species may reflect adaptive trade-offs between (1) the timing of investment in reproduction relative to the timing of resumption of carbon acquisition through leafout and (2) other aspects of plant performance, such as environmental tolerance and pollinator attraction strategies that we investigated in this study. We show that hysteranthous flowering is associated with historic aridity (PDSI) and smaller flower displays 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.

Our models estimated a strong relationship between flower–leaf sequences and aridity (PDSI) at both taxonomic scales we studied. For the American plums, aridity was a better predictor of hysteranthy than average spring temperature, suggesting interspecific differences in flower–leaf sequences do not simply reflect

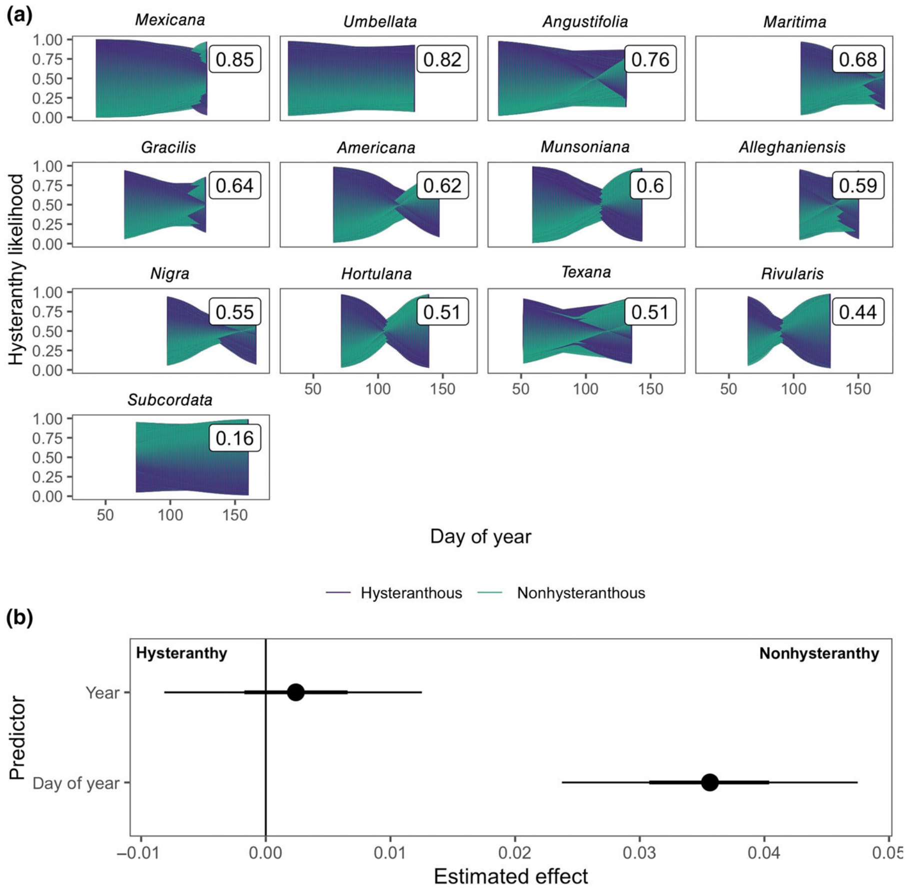

Fig. 2 Predicted likelihood of hysteranthy across the flowering period of 13 American plum species and the temporal predictors that drive these patterns. Panel (a) depicts the predicted likelihood that each species would express hysteranthy on each day of their flowering season based on 1000 draws from 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 the 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.

a phenological response to temperature cues. The relationship between floral display size and flower–leaf sequences predicted by the insect visibility was better supported at the coarser taxonomic scale of the full genus *Prunus* than in the American plums clade. While species in the American plum clade have solitary flowers, they are still clustered on branches, so it is unlikely the unit of attraction that pollinators are responding to is the individual flower, which may explain why the relationships between hysteranthy and petal size we observed were weak (Fig. 3b). The inflorescences of the larger genus *Prunus*, are probably a better proxy for differences in units of attraction, which may explain their stronger association with flower–leaf sequences variation among species (Fig. 4). This contrast may suggest that associated selection between flower–leaf sequences and pollinator traits has more strongly influenced inflorescence architecture than the morphology of individual flowers though our estimates at both scales agreed in directionality (i.e. hysteranthy associated with smaller floral displays).

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

Our support for both the water-limitation hypothesis and insect-visibility hypothesis (especially in the larger genus *Prunus*), and the strong positive interactions between PDSI and floral investment that we observed in the larger genus *Prunus*, highlight that these hypotheses are not mutually exclusive, and could be related. Selection on floral size represents a classic evolutionary trade-off 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 (Lambrecht, 2013; Teixido *et al*., 2016), and hysteranthy could represent a compensatory mechanism that both reduces hydraulic demand while increasing pollination efficiency in these environments. Studies that have compared the transpiration rates among flowers and leaves provide insights into the potential importance of hysteranthy as a means of partitioning hydraulic demand across the season and maintaining water status. Measurements of water movement (transpiration rates, sap flow, hydraulic conductivity) to flowers range from 20% to 60% of that of leaves under comparable conditions (Whiley

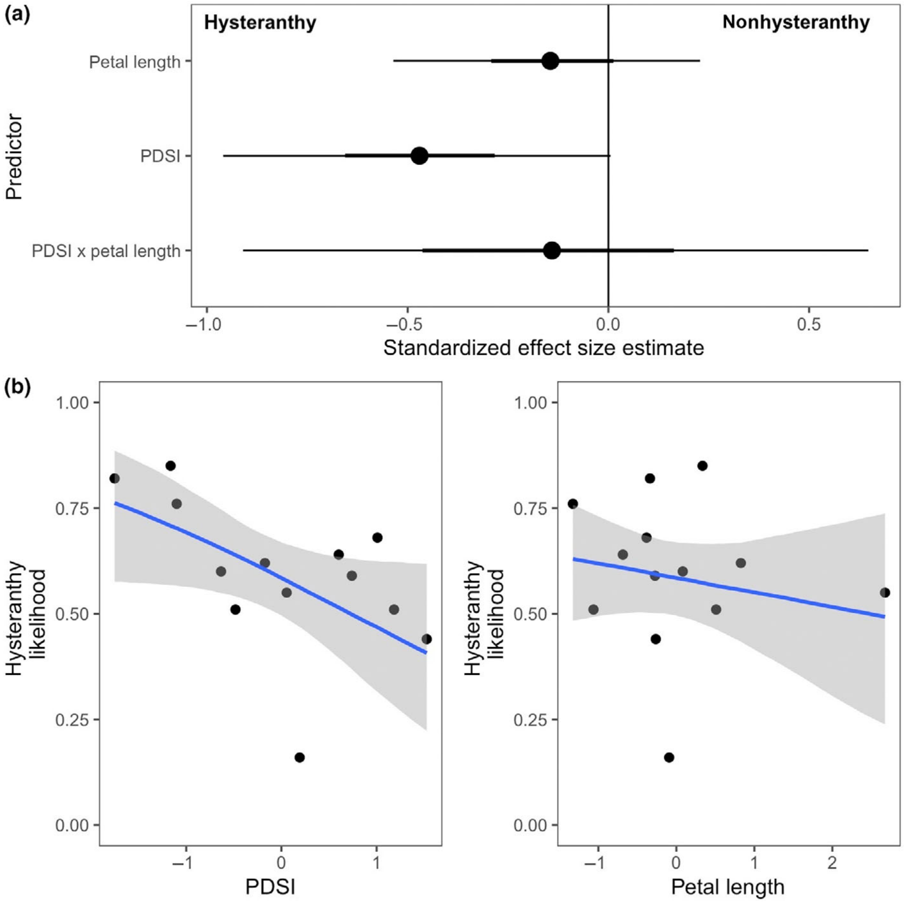

Fig. 3 Relationships between hysteranthy and environmental and biological traits for the 13 species of the American plums. Panel (a) shows the estimated effects of each predictor and their interaction on the likelihood hysteranthy. Points indicate the mean effects and the thick and thin bars represent the 50% and 89% uncertainty intervals, respectively. Panel (b) depicts the conditional effects of each predictor on hysteranthy 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. PDSI, Palmer Modified Drought Index.

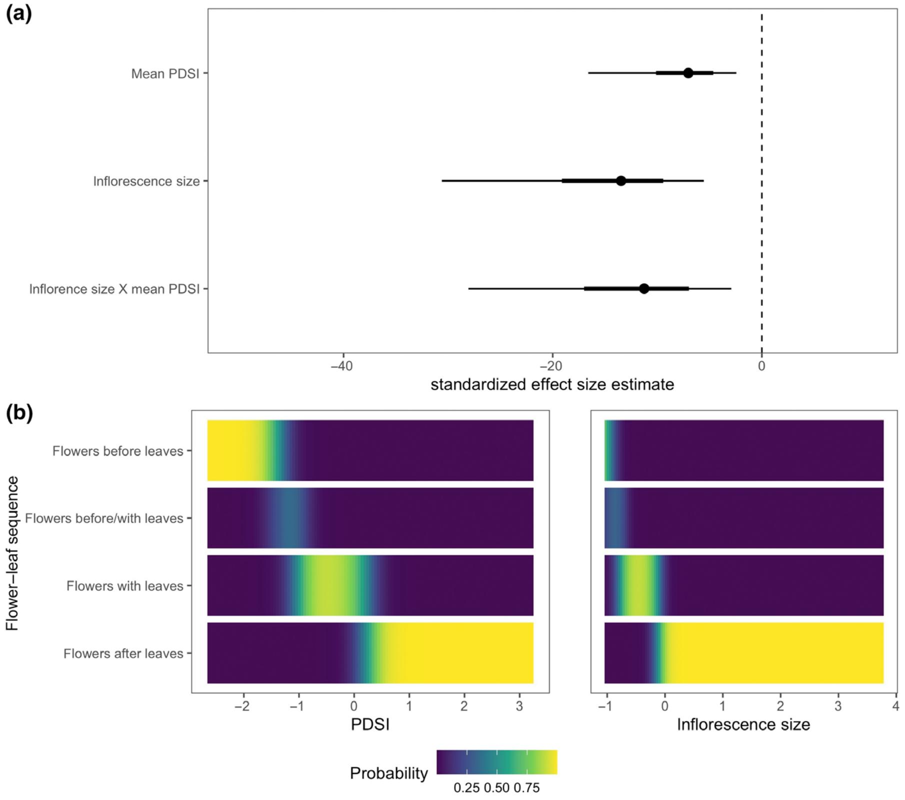

Fig. 4 Relationships between the likelihood of hysteranthy and environmental and biological traits for 32 species of the genus *Prunus* native to, or established in North America. Panel (a) shows the estimated effect size of each predictor. Points indicate the mean estimate for each predictor, and thick 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 Palmer Modified Drought Index score (PDSI) or number of flowers/inflorescence (inflorescence size). Predictor values (*x*-axis) have been *z*-scored to allow direct comparisons between predictors.

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).

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

Despite this evidence that hysteranthy can reduce hydraulic demand in dry environments, hysteranthous species in the American plum clade are not found – even historically – in extremely arid locations (PDSI values typically range from −4 to 4, although the values that we observed in our analyses were more restricted, ranging from −0.5 to 0.2). This contrasts with hysteranthous species in the dry tropics where this phenological pattern appears to allow 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 suggests that hysteranthy may allow temperate species to occupy marginally drier environments than nonhysteranthous 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. 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 American plum species expressed this intermediate flower–leaf sequence. Further, while our classifications broadly matched previous species-level analyses in this group by Shaw & Small (2004), 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. 2a). For almost all members of the clade, hysteranthy was strongly predicted by the day of the observation ('day of year' in our model; Fig. 2b). In many cases, there was a high likelihood that individuals of a species may be observed at different vegetative stages during flowering (Figs 2a, S2). The variation we observed here 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 could not robustly estimate how much flower–leaf sequences vary within vs among species, and within individuals over time. Parsing the influence of multiple climate drivers across multiple scales of ecological and evolutionary time would be an important next step for understanding how the environment and species interactions have shaped these phenological patterns.

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 over that time scale in our dataset (Fig. 2b). 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 the 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

We focused on a well-studied, and economically important clade of morphologically similar species. Our case study provides a road map for evaluating the role of hysteranthy more generally 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 this 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 consider hysteranthy –and phenology in general – in the broader framework of trade-offs in pollination biology. The trade-off between phenology and pollination investment could not only consider flower size, but also the number of flowers, nectar and pollen reward investment, and volatiles between related hysteranthous and nonhysteranthous taxa. Findings that hysteranthous species invest fewer resources into these other pollinator attraction traits than nonhysteranthous relatives would support the insect-visibility hypothesis. For a simple experiment to test the pollinator visibility hypothesis, researchers could force hysteranthy/nonhysteranthy 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 support hysteranthy as an adaptive trait for pollinator attraction.

With a better mechanistic understanding of the relationship between flower–leaf sequences and ecological performance, 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.

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Competing interests

None declared.

Author contributions

DMB and EMW conceived of the manuscript. DMB and SCC collected the data. DMB led the statistical analyses with TJD and EMW. DMB led the writing of the manuscript. All authors contributed to the writing and gave approval for the submission.

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Data availability

The phenology and trait data collected for this study and the R scripts for their related analyses are available at KNB: The Knowledge Network for Biocomplexity (https://knb.ecoinformatics.org/view/doi:10.5063/F15M645C).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Histograms of collection days of year for the American plum species in this study.

Fig. S2 Predicted likelihood of hysteranthy for American plum species without accounting for the day of year specimens were collected.

Fig. S3 Modeled relationships between hysteranthy, aridity and petal length for the American plums using an alternate modeling approach accounting for intra-specific variation and phylogenetic structure in aridity and petal length measurements.

Fig. S4 A comparison of historic and contemporary average PDSI values across the study region.

Methods S1 Methods describing an alternative modeling approach to assess relationships between aridity, petal length and hysteranthy in the American plums to account for intra-specific variation and phylogenetic structure in aridity and petal length measurement.

Table S1 Summary of sample sizes for each for each American plum species in this study.

Table S2 Hysteranthy index scores for American plum species based on alternate pheno-phase cutoffs and modeling frameworks.

Table S3 Estimated relationships between hysteranthy and traits predictor both with and without accounting for the day of year of collection in hysteranthy index score calculations.

Table S4 Model estimates of the relationship between mean spring temperature, petal length and hysteranthy.