

# Biological and environmental drivers of flower-leaf sequence variation in the American Plums (*Prunus*, sect. *Prunocerasus*).

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## Introduction

Woody perennials have a unique ability among plants to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence known as hysteroanth, proteranth or precocious flowering is particularly common in temperate, deciduous forests around the globe (Rathcke & Lacey, 1985). A number of studies suggest that this flower-leaf sequences (FLSs) are under selection, and that hysteroanth has functional significance (Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021; Guo *et al.*, 2014).

The most common, and well-tested explanation for the evolution of hysteroanth in temperate forests is that it is adaptive for wind-pollination, as leafless canopies increase wind speeds for pollen transport and reduce the likelihood of pollen interception by vegetation (Whitehead, 1969; Niklas, 1985). In the dry-deciduous tropics of South and Central America, hysteroanth is also 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).

However, these explanations do not address the widespread prevalence of hysteroanth in biotically-pollinated taxa found in temperate regions that are rarely water-limited in the early season during which flowering and leafing occur (Polgar & Primack, 2011). This number is not trivial; a recent analysis found that approximately 20% of the hysteroanth species in the moist, Eastern Temperate Forests of North America are biotically pollinated (Buonaiuto *et al.*, 2021). With mounting evidence anthropogenic climate change is driving shifts in flower-leaf sequences (Ma *et al.*, 2020), expanding our understanding of the functional significance of hysteroanth to included these groups is vital to forecasting the demography and performance of forest communities in an era of global climate change.

## Hypotheses of Hysteroanthous flowering in biotically pollited taxa

Several alternative hypotheses to the wind pollination hypothesis have been put forth and may help explain the function of hysteroanth in biotically-pollinated species.

Water limitation hypothesis: Despite being considered a “wet” biome, there is still considerable variation in water availability in space and time within temperate regions of the globe. It is possible that the function of hysteroanthous flowering in these regions parallels that in the dry tropics—partitioning hydraulic demand across the season to allow hysteroanthous species to tolerate increased aridity. If this is the case, we would expect to find hysteroanthous taxa in locations that are, on average, drier than their non-hysteroanthous kin.

Insect-visibility hypothesis: Hysteranthous flowers are visually conspicuous in the landscape. It is possible that like in wind pollinated taxa, hysternanthy in biotically pollinated taxa is an adaptation for pollination efficiency as a flowering-first species are easier for insects pollinators to locate (Janzen, 1967). This hypothesis predicts that flower displays will differ in size between flowering- first and leafing-first species. Though the direction is unclear. 1) Hysternanthy may be associated with smaller flowers. Since flower are not obscured by leaves, they are easier to see, there is weaker selection on large floral display. 2) Hysternanthy may be associated with bigger flowers. Because these species are going all in on visual displays, big flower might be additive to the benefits of hysternanthy. A second complicating factor is that there is likely to be associates between flower size and hysternanthy even if pollinator visibility doesn't matter due to developmental constraints. For example it requires more time and energy to produce big displays, so non-hysternanthyous species that flower later in the season, after leaves emerge to gather energy are can produce bigger displays than early flowering hysternanthyous species. (Could also move some of these nuances and contradictions for the discussion)

Phenological niche extension: Species that flower before their leaves inherently flower early in the season. It is possible that hysternanthy flower is simply a by-product of selection for early flowering. Spring flower phenology is less constrained by prior phenological events than leaf phenology (Savage, 2019), which could allow selection to drive flowering into the early season, producing the hysternanthyous phenological sequence. With this hypothesis there is no specific adaptive advantage to hysternanthy; selection is not operating on the relative timing of flower and leaf emergence, but rather the absolute flowering time alone.

Fruit maturaturion hypothesis: Like the phenological niche hypothesis describe above, there are several aspects of reproductive development that suggest hysternanthy is a by-product for early flowering, driven by development constraints. Hysternanthy may be common in large fruited species that require lots of time to mature their fruits. Alternatively, its may be common in small, early fruiting species that have evolved dispersal syndromes (wind dispersal, non-dormant seeds) that require dispersal early in the season. In either case, we should expect fruit size to associate with hysternanthy.

Of course none of the hypotheses are mutually exclusive. One challenge is the same traits correlation could be driven by different mechanisms (i.e., small flower could be insect-visibility, developmental constraint, aridity tolerance or all of the above). Yet, a detailed investigation of the association between hysternanthy and the representative traits of each of these hypotheses would pinpoint those with the strongest theoretical underpinning and empirical evidence, as well as identify clear directions for future work to better understand the role that flower-leaf sequences play in woody plant fitness.

A second challenge for robust testing of hysternanthy 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 be used widely to investigate variability of flower-leaf sequences variation among and within species.

The American plums (*Prunus* subsp. *prunus* sect. *prunocerasus*) offer potential for a high resolution investigation of drivers of hysternanthyous flowering in taxa that don't fit the bill. (Better topic sentence needed.) The 16 species that make up the section are distributed across the temperate zone of North America and, like the genus *Prunus* at large, are all insect-pollinated, yet show pronounced inter-specific variation in flower-leaf sequences. Species in this section are well represented in herbaria records (Fig. 1), making them a tractable group to measure and assess variation in flower-leaf sequences as well as other ecological and morphological characteristics that may explain the evolution of this variation.

In this study we ask: do the observed associations between flower-leaf sequences variation and select morphological and environmental traits match predicted associations of the hysternanthy hypotheses? We used herbaria records to to quantify flower-leaf sequence patterns in the American plums, (subspecies *Prunus*, sect. *prunocerasus*). We then evaluated the association between hysternanthy and several ecological and morphological traits to interrogate the functional hypotheses for hysternanthous flowering described above. We then compare our findings in this clade to associations between hysternanthy and traits in the larger genus obtained from published accounts in flora to better understand how these dynamics vary over taxonomic scales. Our findings both clarify the hypothesized function of flower-leaf sequence variation in biotically-pollinated taxa, and offer insights into how shifting flower-leaf sequences may impact species demography and distributions as climate continues to change.

## Methods

### 0.1 Quantifying flower-leaf sequence variation

We obtained digital herbarium specimens for all member of the section *Prunocerasus* from the Consortium of Midwest Herbaria (CMH) Database. To quantify the flower-leaf sequence variation within and across species we randomly sample 200 specimens for each species and scored the phenological development of flower and leaves using a modified BBCH scale for woody plants (Finn *et al.*, 2007). In total, we evaluated the phenology of 2521 specimens, but only specimens with visible flowers were included in this analysis (n=1009). We reconstructed the phylogenetic relationships among species in this group based on the tree topology in Shaw & Small (2004). Following the methods of Grafen & Hamilton (1989) we computed branch lengths for this phylogeny by assigning each node a height and computing the distance between upper and lower nodes using the R package “ape” (E. Paradis and K. Schliep, 2019).

To quantify FLS variation, we fit an ordinal, hierarchical, Bayesian, phylogenetic mixed model (de Villemereuil P. Nakagawa, 2014) to assess the likelihood an individual would be at any given vegetative BBCH phase while flowering. Because we expect that hysternanthy is more likely to occur earlier in the flowering period and species differ in their flowering periods, we included the day of the observation as a varying slope, main effect in the model and species and phylogeny as random effects. The model is written below:

$$\text{logit}(P(Y \leq j)) = \beta_{[j]phylo[i]} + \beta_{[j]sp[i]} + \beta_{dayofyear[sp[i]]} * X_1 + \epsilon_i$$

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

where Y is the ordinal outcome (leaf stage) and j is the number of categories (1,2,...6).  $P(Y \leq j)$  is the probability of Y less than or equal to a category j=1,...j-1. In this varying slope and intercept model,  $\beta_{[j]}$  describes an intercept for each category [1,2,...6], while slope  $\beta_{dayofyear[sp[i]]}$  is constant across categories.

The influence of the phylogeny  $\alpha_{phylo}$  was modeled as follows:

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

The  $\alpha$  for species effects independent of the phylogeny was modeled as follows:

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

We fit the model in the R package “brms” (Bürkner, 2018) using weakly informative priors, and ran the model on four chains with a warm up of 3,000 iterations and 4,000 sampling iterations for a total of 4,000 sampling iterations. Model fit was assessed with Rhats <1.01 and high effective sample sizes and no divergent transitions.

Because the day of observation strongly influenced the BBCH stage likelihood, quantifying flower-leaf sequences among species was intractable without accounting for this temporal trend. To address this issues, we used our model to predict the likelihood each species would be observed at a given vegetative BBCH stage during flowering at the 0%, 25% 50% and 75% quantiles of their flowering period. We then developed a flower-leaf sequence index, by assigning a numerical score to each species per seasonal quantile, and summing over the full flowering season. In each seasonal quantile, species received a 1 if more that 50% of their probability distribution occurred at BBCH 0 and BBCH 09 and a 0 if not. These values were summed across the season generating an index from 0 (never hysteranthous) to 4 (hysteranthous through late season (Q75)), where 1= hysteranthous at start of season, 2= hysteranthous through early season (Q25) and 3 = hysteranthous through mid season (Q50). We also used two alternative indexing schemes (>25% of the probability distribution occurred at BBCH 0 and >40% of the probability distribution occurred at BBCH 0 and BBCH 09).

## 0.2 Evaluating hysteranthy hypotheses

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

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

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

The model structure is written below:

$$y_i = \alpha_{ind/sp[i]} + \alpha_{phylo[i]} + \beta_{hyst.index} * X_{hyst.index} + \epsilon_i$$

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

The effect of the phylogeny was model as above.

Like above, we fit these models in the R package “brms” (Bürkner, 2018) using weakly informative priors, and ran the model on four chains with a warm up of 3,500 iterations and 4,500 sampling iterations for a total of 4,000 sampling iterations. Model fit was assessed with Rhats <1.01 and high effective sample sizes and no divergent transitions. We also ran each model using our two alternative FLS indexing approaches to make sure our particular indexing approach was not influencing our results. Though these alternative classification scheme did change the hysteranthy index score for some species (Fig. S1), they changes did not substantially impact the inference from our models (see Tab. S1 for comparisons).

## Hysteranthy in the larger genus *Prunus*

To better understand how the patterns we identified in our in depth study of the sect. *Pruncerasus* scale across coarser taxonomic resolution we also evaluated the relationship between hysteranthous flowering and hypothesis-related traits in the all the *Prunus* species that are native or naturalized in North America. For this analyses, we obtained categorical descriptions of flower-leaf sequences and mean estimates of fruit diameter and number of flowers/inflorescence as a proxy for floral investment from the Flora of North America () for 32 species in the genus. As above, we evaluated the average aridity tolerance of each species by extracting average PDSI values from all herbaria records of those species in the CMH database (n=x).

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

We standardized the units of all predictors to make the directly comparable in the following model structure:

$$\text{logit}(P(Y \leq j)) = \beta_{[j]phylo[i]} + \beta_{pdsi[sp[i]]} * X_1 + \beta_{fruitdiamter} * X_2 + \beta_{floralinvestment} * X_3 + \epsilon_i$$

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

where Y is the ordinal outcome of flower-leaf sequence category (“flowers before leaves”, “flowers before/with leaves”, “flowers with leaves” and “flowers after leaves”) and j is the number of categories (1,2,...4).  $P(Y \leq j)$  is the probability of Y less than of equal to a category j=1,...j-1.

As above, the influence of the phylogeny  $\alpha_{phylo}$  was modeled as follows:

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

We fit these models in the R package “brms” (Bürkner, 2018) using weakly informative priors, and ran the model on four chains with a warm up of 6,000 iterations and 8,000 sampling iterations for a total of 8,000 sampling iterations to maximize the effective sampling size. Model fit was assessed with Rhats <1.01 and high effective sample sizes and no divergent transitions.

## Results

### Quantifying flower leaf sequences in the American plums

We found substantial inter-specific differences in flower-leaf sequences within the American plums (Fig. 2, S1). Flower-leaf sequence patterns were strongly dependent on the day of observations, with observations later in the the flowering season of each species decreasing the likelihood of finding flowers open during early vegetative BBCH phases ( $\beta_{day}$  0.03,  $CI_{50}$  [0.02,0.03] ). Based on our flower leaf sequence index, two species (*P. umbellata*, *P. mexicana*) were likely to be hysteranthous regardless of the time of observation and three species (*P. rivularis*, *P. subcordata*, and *P. texana*) were always most likely to flower after level expansion began (Fig. 3). All other species displayed intermediate phenotypes with five species mostly likely to hysteranthous at the start of the season (*P. alleghaniensis*, *P. americana*, *P. hortulana*, *P. munsoniana* and *P. nigra*), one species through early season (*P. gracilis*) and two species through mid season (*P. angustifolia*, *P. maritima*) (Fig 3).

## Associations between hysteranthly and environmental and morphological traits

In the American plums clad we found a negative association between flower-leaf sequence index and mean PDSI ( $\beta$ : -0.03,  $CI_{50}$ [-0.05, 0.02], Fig. 4a.), suggesting that species that displayed hysteranthous flowering later into their flowering season are found in dryer locations.

We found a negative association between flower-leaf sequence index and both petal length and fruit diameter (-.21,  $CI_{50}$ [-0.38 -0.04], -1.40,  $CI_{50}$ [-1.97 -0.82] respectively), though the relationship between FLS index and fruit size was much stronger (Fig. 4b.,c.). This suggests that smaller fruits and flowers are associated with species that displayed hysteranthous flowering later into their flowering season.

At the genus level, there was a positive association between increasing pdsi and inflorescence size and increasing overlap between flowers and leaves ( $\beta$ : 2.50,  $CI_{50}$ [1.17, 3.371] and  $\beta$  6.41,  $CI_{50}$ [3.86, 8.05] respectively, Fig. 5a), suggesting that hysteranthly is associated with drier locations and smaller floral displays (Fig. 5b). The estimated effect of fruit size in the model was negative ( $\beta$ : -1.24,  $CI_{50}$ [-1.95,-0.21], 5a)]. Small fruits were mostly likely to be associated with species that flower with their leaves, and large fruits with species that flower before leaves, though there was high uncertainty around these estimates in our model (Fig. 5b).

## Discussion

Our analyses of hysteranthous flowering in North American *Prunus* species offer several critical insights into the evolution and function of this phenological syndrome in this genus, that can be extended to make inference on the role of hysteranthly in biotically-pollinated species at large.

Our high-resolution analysis of the American plums clade revealed that even within species flower-leaf sequences are highly labile. For all members of the clade, the day of phenological observation was a strong predictor of the likelihood that flowers would be visible before the emergence of leaves. In many cases, there was high likelihood that individuals of a species may be observed at different vegetative stages during flowering (Fig. 4, S1). The intra-specific variability we detected in our study furthers a growing call to adopt a bottom-up, observational approach to the study of flower-leaf sequences by quantifying flower-leaf sequences at the individual level and modeling these patterns are coarser taxonomic scales rather than treating them as immutable categorical patterns at the species level (Buonaiuto *et al.*, 2021).

Despite this, the coherence of inference between our bottom-up, observational approach for the American plum clade and the top-down, categorical approach we took for the larger genus *Prunus* is an encouraging demonstration that the categorical species level based on expert opinion approach can still offer useful insights the the drivers of hysteranthous flowering when higher-resolution data is not available. Our modeled flower-leaf sequences patterns of the American plums qualitatively with previous characterizations of the the species-level variation in this group (Shaw & Small, 2004). Across taxonomic scales and data approaches, our analyses agree that within the North American species of the genus *Prunus*, hysteranthous taxa occurred in more arid environments, and tended to have smaller floral displays. In the American plums clade, smaller fruits were associated with less overlap between flowering and leaf development while at the genus level, smaller fruits were weakly associated with more flower-leaf overlap. Comparing the direction and strength of these association across taxonomic scales helps to hone the hypotheses of hysteranthly and identifies important realms of further inquiry.

## Relationships to the hypotheses

### Hydraulic demand

The direction associations between pdsi and flower-leaf sequences follow the expectations the water limitation hypothesis, with hysteroanthous species occupying generally drier area.

Studies that have compared the transpiration rates among flowers and leaves that occur simultaneously provide insights to the potential importance of this seasonal partitioning for maintaining water status. These studies report floral transpiration rates of flowers can range from 20%-60% of that of leaves under comparable conditions (Whiley *et al.*, 1988; Roddy & Dawson, 2012). This additional hydraulic demand can drive loss of stomatal conductance and decrease photosynthetic rates (Galen *et al.*, 1999). A recent study Liu *et al.* (2017) comparing hydraulic properties of flowers and leaves in two hysteroanthous tree species (*Magnolia spp*), found that sap flow to flowers (a measure of water movement) was 22-55% that of leaves. When considering species in our study specifically, the xylem conductivity of spring floral branches of *Prunus americana* is reported to be 20% of summer foliage branches (McMann *et al.*, 2022). Taken together, magnitude of water loss through floral organs in these physiological measurements demonstrate an underlying mechanism for the macro-ecological patterns we observed in our data.

However, when considering the likelihood that aridity is an evolutionary driver of hysteroanthous flowering in biotically-pollinated species, it is important to consider the strength of the relationship as well as the direction. In the American plums, the mean PDSI at which all species were found ranged between -0.40 and 0.67, and at the larger genus PDSI values ranged from -.39 to .28. The slope we estimated for the relationship between PDSI and shifts between FLS categories was weak with high residual variance, indicating that while hysteroanthous species may, on average, occupy marginally drier environments, this phenological trait alone may not facilitate species' persistence under extreme aridity.

To better understand this, we must consider the flower-leaf sequences in the context of the full season phenological cycle of the clades we studied in comparison to the taxa that display this habit in the dry deciduous forest of neotropics (do we still say this word or is it colonialist?).

In all temperate *Prunus* species, there is significant overlap between vegetative and reproductive phenological phases, even in hysteroanthous species. While flowering may precede leafout by as much several weeks, the process of fruit development, which is also water intensive, occurs when leaves are present for all species. This suggests that over the course of a full seasonal cycle, the contribution of hysteroanthous flowering to partitioning hydraulic demand may be rather marginal.

By contrast, hysteroanthous in the dry tropics follows a different pattern. In these regions, flowering is initiated at the time of leaf drop (Borchert, 1983; Franklin, 2016), meaning that the full reproductive cycle occurs in the leafless period, which would be a far more effective phenological sequence for coping with extreme aridity. If the hysteroanthous taxa of the temperate zone evolved in environments more similar to the contemporary dry tropics, it is possible that the incidences of hysteroanthous we see in the temperate zone are a vestige of ancestral selection.

Overall, our analysis suggests that it is difficult to parse out the complex relationship between phenological sequences and aridity tolerance from observational studies that use correlative approaches, and this field of inquiry would benefit strongly from more mechanistic experiments. Our analysis identifies closely related species with contrasting flower-leaf sequences patterns, that could serve as an important model system for such experiments. A more robust understanding of the contribution of hysteroanthous to drought tolerance could be achieved by planting sister-taxa with contrasting flower-leaf sequences in common environments across a

gradient of aridity to address the the question at the heart of the water-limitation hypothesis: Do species with contrasting FLS respond differently in drought? Given that we observed high levels of within species variation in flower-leaf sequences in our observation, these experiments could also serve to evaluate whether temporal variability in flower-leaf sequences is associated with temporal variability in water availability or other environmental drivers.

### Pollinator visibility

In the clade *Prunocerasus*, we found hysteranthly was associated with smaller flowers, and in the larger genus, smaller inflorescences, which matches the expectation of one formulation of the the pollinator visibility hypothesis in which visibility is a trade off between phenology (hysteranthly) and structural investment (size). However as we eluded to in our introduction, an alternative prediction of the pollinator visibility is that hysteranthly should be part of a suite of traits that support visual foragers and should co-vary with increase flower size and trade off with secondary cues (chemical attractants, nectar rewards).

Our results reflect the first formulation, but just as in the case with the water limitation hypothesis, the association between FLS and flower size was relatively weak in the American plums. The stronger trend in the the relationship between inflorescence size and hysteranthly in the larger genus could also reflect the developmental constraint hypothesis, and it is not possible to distinguish between these two processes through trait correlations. As in the case of the hydraulic demand hypothesis, our understanding of the the insect-visibility hypothesis would benefit from experimental test of mechanism.

A first important step to sharpen the predictors of this hypothesis would be to consider hysteranthly, and phenology in general, in the more general framework of tradeoffs in pollination biology. The trade off between phenology and pollination investment should not only consider flower size, but also the number of flowers, nectar and pollen reward investment, volatiles between related hysteranthous and non-hysteranthous taxa.

A second approach would be to observed the impact of hysteranthly on pollinator choice in experiments. While several studies support the idea that vegetative background can substantially alter the ability for pollinators to locate flowers (Forrest & Thomson, 2009; Rivest *et al.*, 2017; Spaethe *et al.*, 2001), as far as we know, these no studies have explicitly tested pollinator choice in the context of phenological sequences.. A simple experiment to better assess the extent to which hysteranthly contributes to pollinator attraction would be to place hysteranthous and non-hysteranthly individuals in a controlled environment, and systemically release pollinators to observed their preference, search times and foraging behavior.

### 0.3 Fruit maturaturion

We observed contrasting trends for the relationship between hysteranthly and fruit size, at the section and genus level. The association between larger fruits and hysteranthly at the genus level development time hypotheses while association between hysteranthly and small fruits in the American plums support the predictions of the resource constraint hypothesis. We highlight that this trait was the poorest sampled in both of our data sources, with small numbers of observation in the herbaria data and high uncertainty in the genus-level models, so these patterns may reflect sampling bias rather than a biological pattern.



## 0.4 Hypotheses are not mutually exclusive

(I am not sure this section is so well thought out or even necessary). One limitation of the trait based approach is that many ecological and evolutionary processes can produce the same patterns. For example, the association between hysteranthly and flower size we observed may be a prediction of of the insect visibility hypothesis, but it would also support the water limitation hypothesis because reduced flower size itself is a signature of selection for aridity (Teixido *et al.*, 2016; Lambrecht, 2013). It is possible that only one, or both of these selective forces have shaped flower-leaf sequences, resulting in a complex interplay of selection on phenological sequences.

Secondly, even if flower-leaf sequences are functionally adaptive in biotically-pollinated taxa, flowering phenology itself is also under selection and these forces may interaction in complicated ways. For example, selection on flower-leaf sequences may be strong for always hysteranthous and never hysteranthous species, but the flower-leaf sequences of species with moderate seasonal overlap may be the byproduct of drift, or selection on phenological niche differentiation, resembling OE evolution (this is something Jonathan said, but I am not sure how to explain it properly).

## 0.5 The big picture

In this study we intentionally chose to analyze a small, and morphologically restricted taxonomic clade in order to reduce the impact of unmeasured biological variation on our traits of interest. This offers clear insights as a case-study, but limits our ability to infer the function of hysteranthly more broadly in biotically pollinated plant taxa. It would be useful to look at other temperate groups with high interspecific flower-leaf sequences variation including Magnolia, Rhododendron, Acer and Cornus. In addition to assessing whether the trait associations are reproduced in other taxonomic groups, including more species in mechanistic experiments would give us more power to evaluate the importance of hysteranthly in form and function.

Our analyses support the general assertion that flower-leaf sequences are under selection by biological and environmental drivers, and that variation in these phenological patterns across species reflect adaptive tradeoffs. While our study cannot say unequivocally which hypotheses are most credible, understanding the general patterns of association between the relevant traits and flower-leaf sequences in the genus *Prunus* outlines a more clear path forward to improve our understanding of phenological sequences through mechanistic experiments.

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## Figures

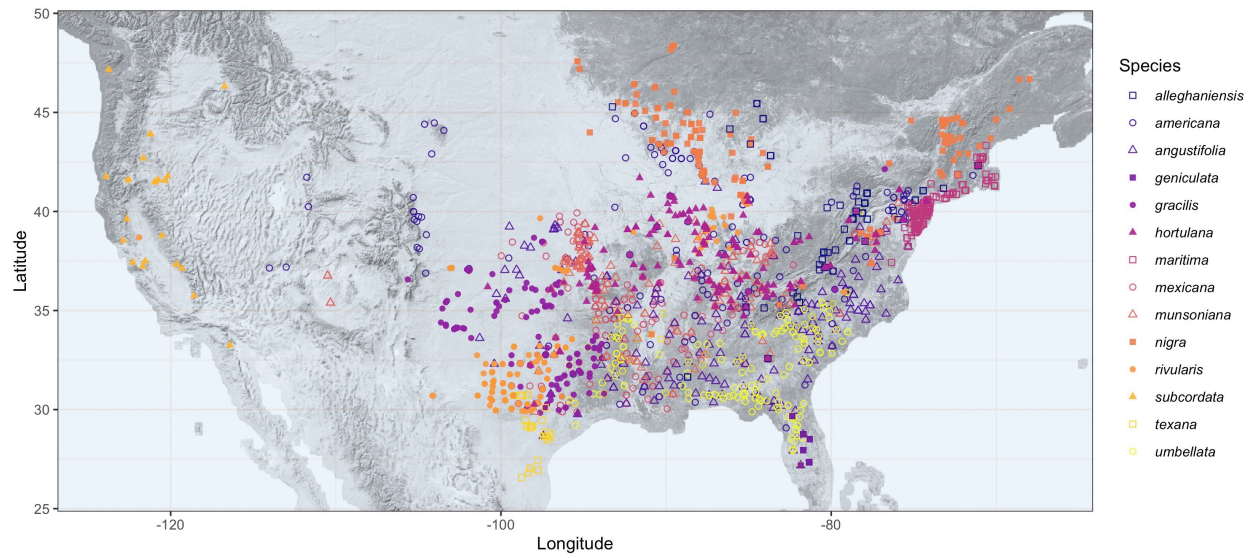


Figure 1: This is a map of all the herbaria records of our focal clade. Maybe better in the supplement

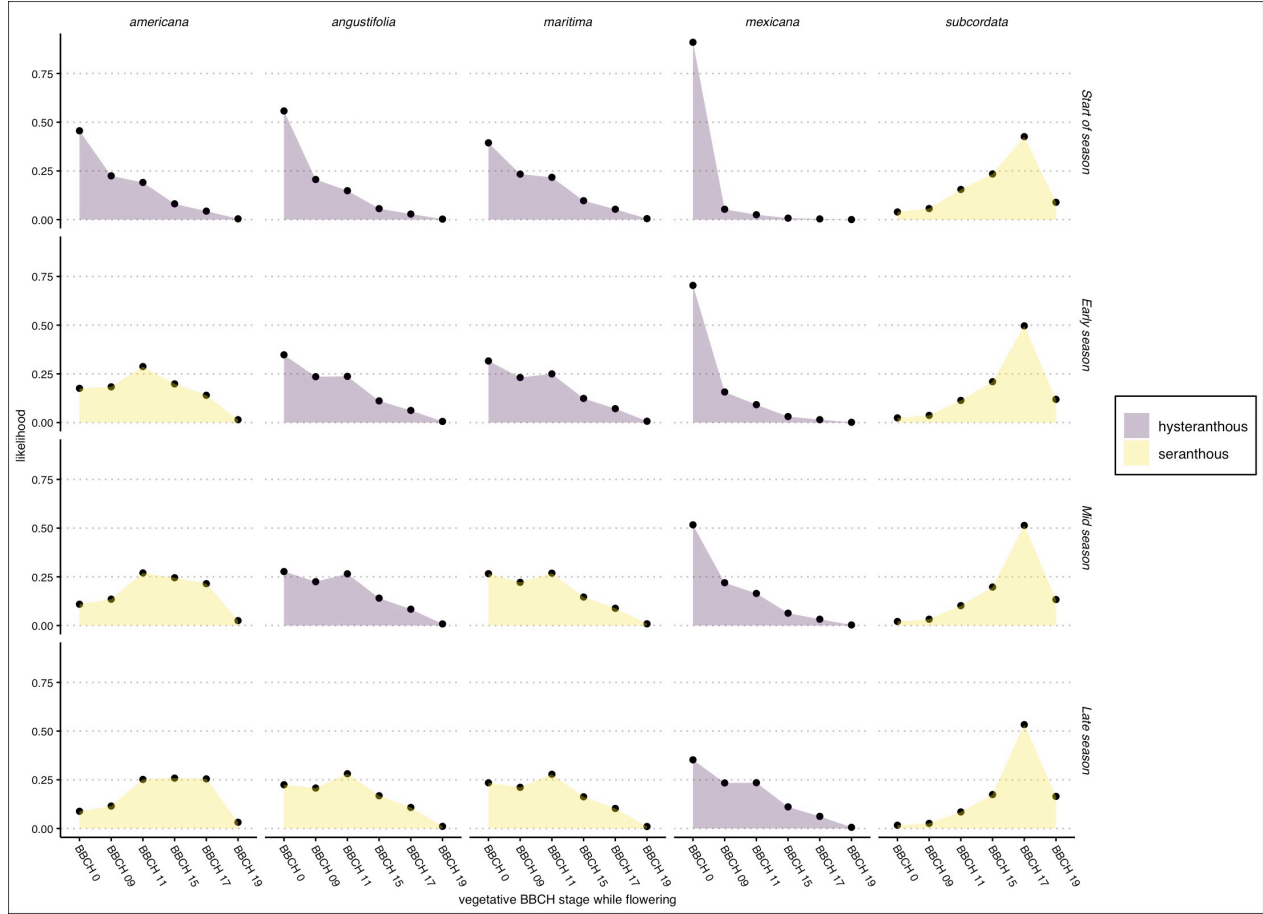


Figure 2: Predicted likelihood that a species would be in flower during each vegetative BBCH phase for five example species in the American plums. Points are the mean likelihood and bar the 95% uncertainty intervals. Species were classified as hysteranthous if greater than 50% probability flowering occurred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

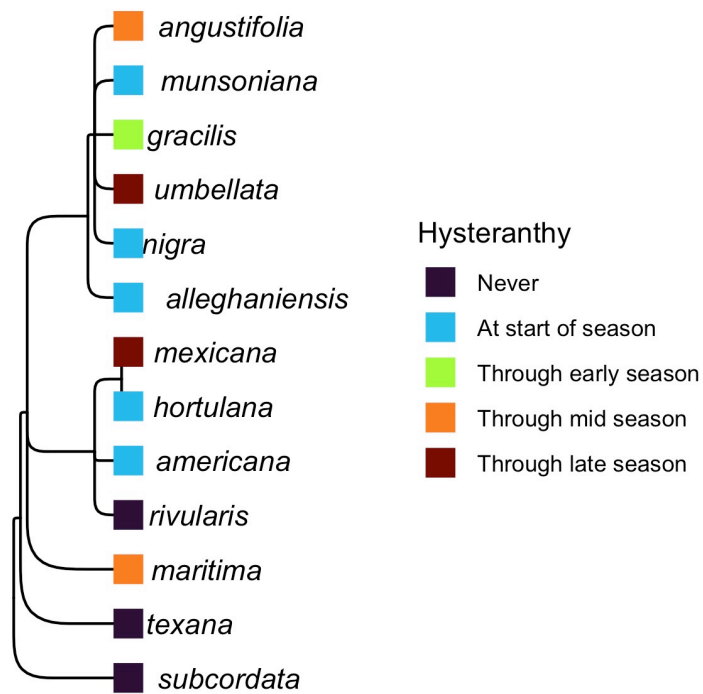


Figure 3: Phylogenetic relationships among the American plums and the duration of their flowering period they are hysteranthous. These categorizations are based on ordinal phylogenetics mixed models. Tree topology is from Shaw & Small (2004)

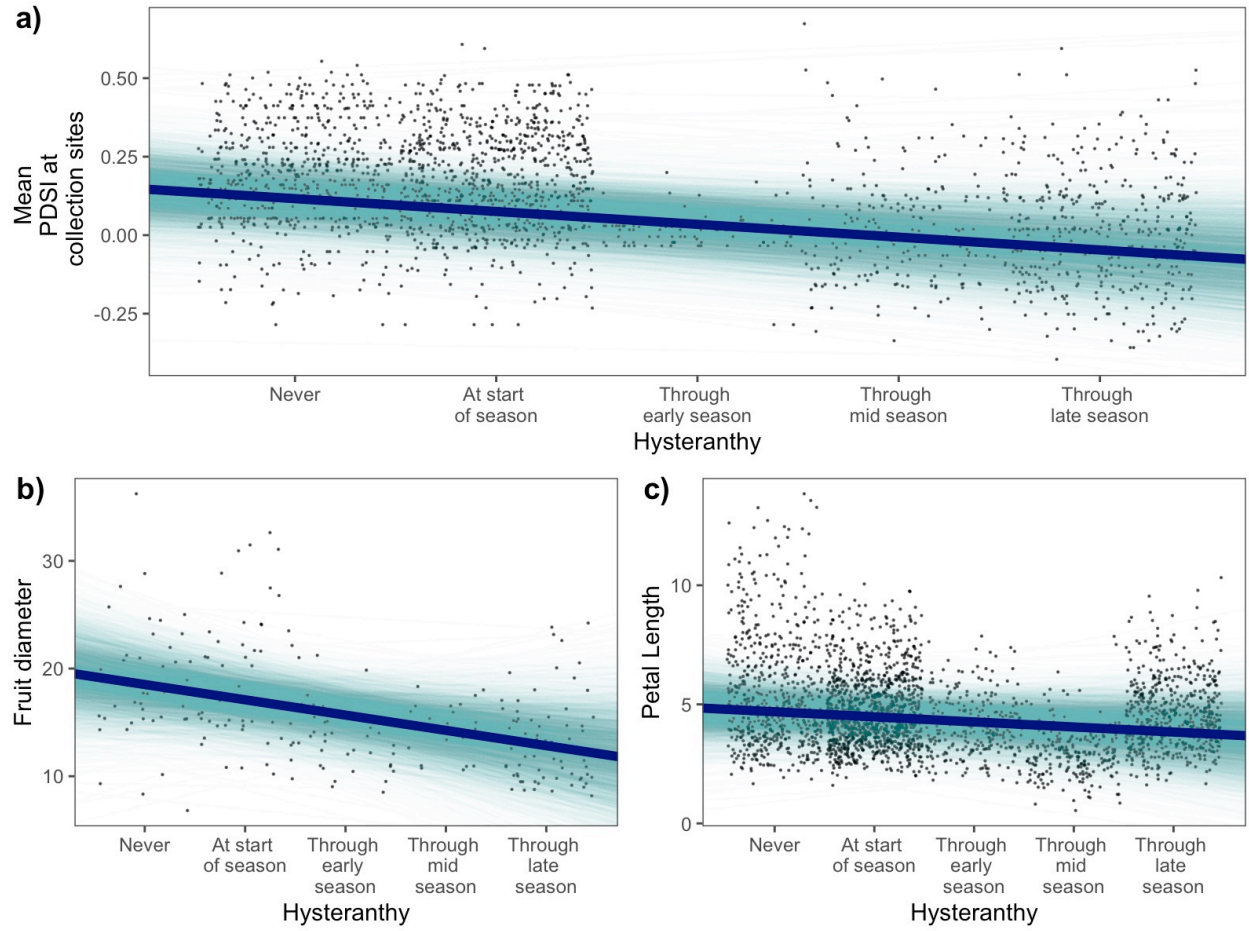


Figure 4: Relationships between the duration of hysteresis across the flowering period and environmental and biological traits based on Bayesian phylogenetic mixed models. a) b) and c) depict the relationships between the duration of hysteresis and mean PDSI, fruit diameter, and petal length respectively. Solid lines indicate the mean posterior estimate and shaded areas X draws from the posterior distribution as a display of uncertainty.

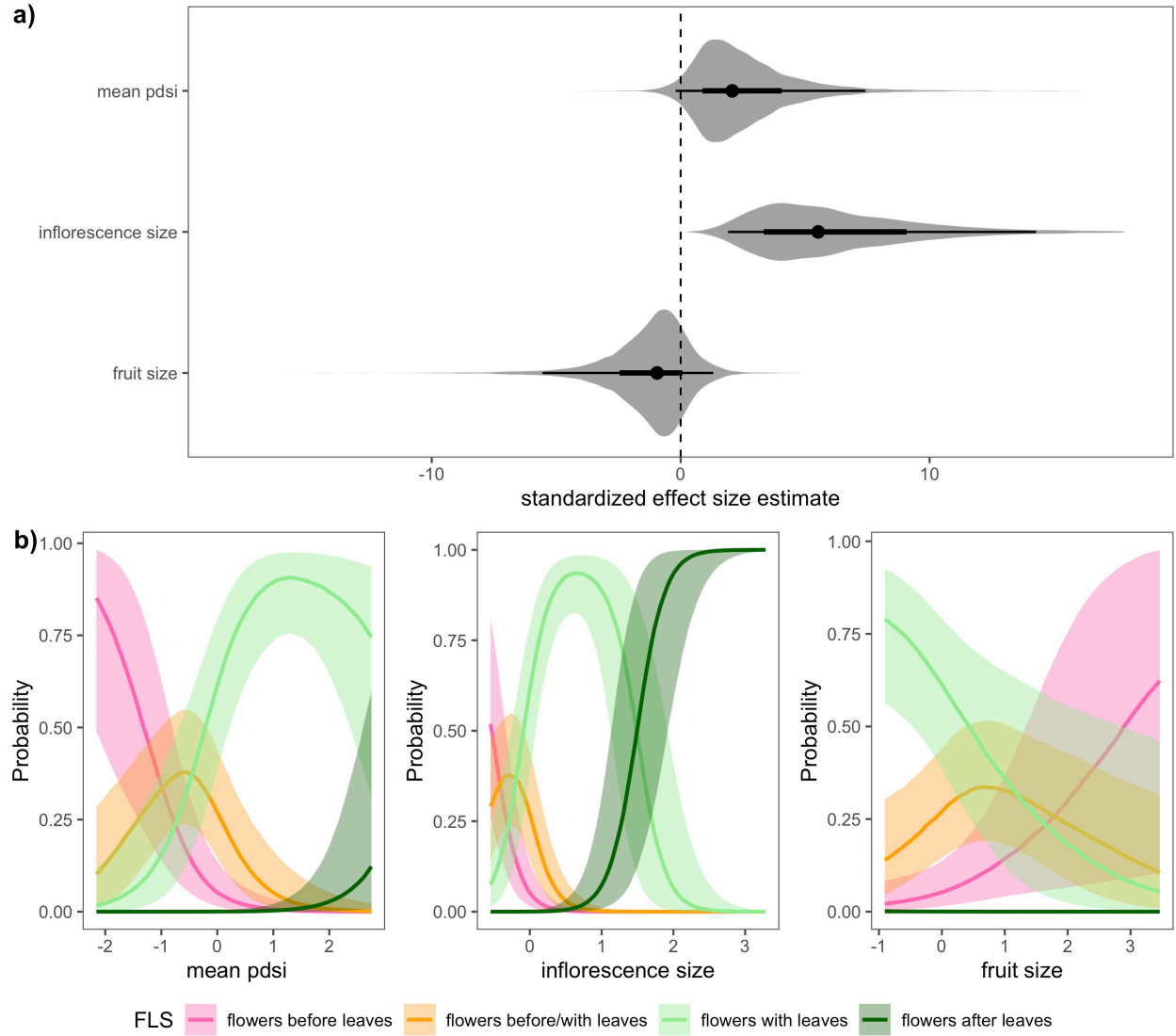


Figure 5: Relationships between the likelihood of of hysteranthly and environmental and biological traits in the genus *Prunus* based on Bayesian phylogenetic mixed models. Panel a) shows the estimated effect size of each predictor with negative values indicating an increased likelihood of hysteranthly. Points indicate the mean posterior estimate for each predictor, and thick and thin bars the 50% and 97.5% uncertainty intervals respectively. We also show the full posterior distribution as an additional measure of uncertainty, Panel b), c) and d) show the marginal effect of mean PDSI, inflorescence size and fruit size respectively, on the likelihood that of each FLS category. Solid lines indicate the mean likelihood and shaded areas the 50% uncertainty intervals.