Aridity drive hysteranthous flowering 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 hysteranthy, proteranthy or precocious flowering is particularly common in temperate forests around the globe (Rathcke & Lacey, 1985). A number of studies suggest that this flower-leaf sequences (FLSs) are under selection, and that hysteranthy 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 hysteranthy in temperate forests is that is that it is adaptive for wind-pollination as leafless canopies increase wind speeds for pollen transport and reduce the likelihood of pollen interception on vegetation (Whitehead, 1969; Niklas, 1985). However, this hypothesis fails to address the prevalence of hysteranthous taxa that are biotically-pollinated. Approximately 30% of species of Eastern temperate forests of North America flower before leafing out, and of these, approximately 20% are biotically pollinated (Buonaiuto *et al.*, 2021). Despite the pervasiveness of this phenological syndrome, direct tests of the function of hysteranthy in biotically pollinated taxa are rare for temperate forest species.

However, looking to other biomes in which hysteranthous flowering is also common offers important insights regarding the function of hysteranthy in temperate, biotically-pollinated taxa. In the dry-deciduous tropics of South and Central America, flowering during the leafless period is also common (Rathcke & Lacey, 1985; Franklin, 2016). In these ecosystems, flowering is associated with a recovery in plant water status due to leaf drop (Borchert, 1983; Reich & Borchert, 1984). By temporally separating leaf and flower activity, woody plants can partition the hydraulic demand across the season, alleviating water stress (Gougherty & Gougherty, 2018; Franklin, 2016). These physiological observations suggest that hysteranthous flowering may be an adaptation to arid environments.

It is unclear whether this hydraulic demand hypothesis (also known as water dynamic hypothesis (Gougherty & Gougherty, 2018) or water limitation hypothesis (Buonaiuto *et al.*, 2021)) is relevant in the temperate zone where forests are rarely water-limited in the early season during which flowering and leafing occur (Polgar & Primack, 2011).Yet the hypothesis yields several predictions that can be tested to evaluate whether hysteranthy serves to increase aridity tolerance in temperate flora:

1. Hysteranthous taxa should be found in dryer habitats compared to closely related, non-hysteranthous species.

2. Hysteranthy may be linked to other reproductive traits associated with dry environments such as

reduced flower and fruit size (Herrera, 2009; Liu *et al.*, 2013).

3. Additionally, flower-leaf sequences can be highly variable within individuals across time (Buonaiuto *et al.*, 2021), and if hysteranthy contributes to aridity tolerance, climate variability may be positively correlated with variability in hysteranthy.

With mounting evidence anthropogenic climate change is both driving shifts in flower-leaf sequences (Ma *et al.*, 2020) and changing geographic patterns of water availability (Overpeck & Udall, 2020), understanding the functional significance of hysteranthy is vital to forecasting the demography and performance of forest communities in an era of global climate change. However, there are two major methodological challenges to testing the hydraulic demand hypothesis:

First, characteristics like aridity tolerance, are the emergent product of a suite of biological traits (Šímová *et al.*, 2017). Thus, when analyzing selective drivers of any particular trait at large taxonomic scales, unmeasured trait differences may obscure the estimated effects of the trait of interest, biasing results. This is a common problem in trait-based ecology, and one of the most promising solutions for understanding the functional significance of hysteranthy in woody plants is through character deconstruction (Terribile *et al.*, 2009); comparing flower-leaf sequences variation for only a subset of taxa of shared phylogenetic and morphological character.

A second challenge for robust testing of hysteranthy hypotheses is that most characterizations of flower-leaf phenological sequences are based on expert-opinion verbal descriptions(e.g. "flowers before leaves" or "flower before/with leaves"), which make comparisons across taxa, time and space difficult 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.

In this study, we used herbaria records to to quantify flower-leaf sequences both within and among species in the American plums, (subsp *Prunus*, sect. *Prunocerasus*. We then evaluated the association between hysteranthy and several ecological and morphological traits to test the predictions of the hydraulic demand hypothesis of hysteranthy. Our findings both clarify the hypothesized function of flower-leaf sequence variation in biotically=pollinated taxa, and offer insights into how flower-leaf sequences may impact species distributions as cliamte continues to change.

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Methods

0.1 Study system

The genus Prunus comprises approximately 200 species distributed across the globe (Chin *et al.*, 2014), Within the genus, The American plums (*Prunus* subsp. *Prunus* sect. *Prunocerasus*) offer potential for a higher resolution investigation of drivers of hysteranthous flowering. Like the genus at large, the 16 species that make up the section are distributed across North America and show pronounced inter-specific variation in flower-leaf sequences. While within the larger genus species can be separated into three distinct morphological clades by inflorescence architecture (solitary, corymbose or racemose) all members of the section have solitary

inflorescences (Shaw & Small, 2004) allowing for refined character deconstruction. Species in this section are well represented in herbaria records (Fig. 1), making them a tractable group to measure and assess intra-specific variation in flower-leaf sequences as well as other ecological and morphological characteristics related to the hysteranthy hypotheses described above.

0.2 Quantifying flower-leaf sequence variation

We obtained digital herbarium specimens for all member of the section *Prunocerasus* from the Consortium of Midwest Herbaria Database. To quantify the flower-leaf sequence sequence variation within and across species we randomly sample 200 specimens for each species and scored the phenological development of flower and leaves in accordance with 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 flower 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). Follwing the methods of **?** we computed branch lengths for this phylogeny by assigning each node a height and computing the distance between upper and lower nodes using the function compute.brlen() in the R package "ape" ().

To quantify FLS variation, we fit an ordinal, hierarchical, Bayesian, phylogenetic mixed model (de Villemeruil P. Nakagawa, 2014) to assess the likelihood an individual would be at any given vegetative bbch phase given it is flowering. Because we expect that hysteranthous may be 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:

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

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

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 of 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 α_{phylo} was modeled as follows:
$$\alpha_{sp} \sim N(\mu_\alpha, COR[\sigma_{phylo}^2])$$

THe α 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" () usinf weakly informative priors, and ran the model on four chains with a warmup 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.

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% quartiles of their flowering period. We then developed a flower-leaf sequence index, by assigning a numerical score to each species per seasonal quantile, and sum-

ming over the full flowering season. In each seasonal quantile, species received a 1 if more that 25% of their probability distribution occurred at BBCH 0, 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).

0.3 Evaluating the hydralic demand hypothesis

To test the predictions of the hydralic demand hypothesis of hysteranthy we obtained data on petal length, fruit diameter and directly from herbarium specimens and characterized the aridity of the sites specimens were collected from using the Palmer Modified Drought Index.

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 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).

We than used Bayesian phylogenetic mixed models to test the relationship between flower-leaf sequence index score and each of the variables. In these models, we included species as the random effect and for traits like flower petal length and fruit diameters than included multiple measurements per specimen, we also included specimen ID as an additional 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 and here, the individual effects within species were modeled: $\alpha_{ind/sp} \sim N(\mu_\alpha, \sigma_{ind/sp}^2)$

Results

Quantifying flower leaf sequences in the American plums

We found that strong inter-specific difference in flower leaf sequences in with the American plums, and that patterns were strongly dependent on the day of observations, with observations later in the the flowering season of each species decreasing the likely hood of finding flowers open during early vegetative BBCH phases (β_{doy}: 0.0278 SE:0.0059, Fig.**??**). 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 alway most likely to flower after level expansion began (Fig. **??**). 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 **??**).

Evaluating the Hydraulic demand hypothesis

We found a negative association between flower-leaf sequence index and mean pdsi (), suggesting that species that displayed hysteranthous flowering later into their flowering season were found in dryer locations.

We found a negative association between flower-leaf sequence index and both petal length and fruit diameter (X,Y respectively), though the relationship between FLS index and fruit size was much stronger.

We found no relationship between the likelihood of hysteranthy and pdsi.

Discussion

Aridity tolerance and inter-specific flower-leaf sequence variation

Our analyses suggest that within the American plums, hysteranthous taxa occur in more arid environments and are associated with drought-tolerant reproductive traits like reduced flower and fruit size. These associations support the hydraulic demand hypothesis of hysteranthous flowering. These results suggest that even though water limitation less common during the flowering season in temperate trees, the temporal segregation of flowering and leaf phenology can still impact whole plant water status later in the season.

Our results generally reflect the physiology of flower and leaves. BOth are hydrated by xylem, though flower can also draw from phloem. Studies comparing water loss between these organs suggest that water loss from flowers can be any where from X - Y percent that of leaves, this is highly depended on species. A recent study by included mesurements comparing the xylem cunductivity on spring floral branches and sumemr vegetative ones fund that floral branch was abour 10-20% that of vegetative ones. This could be enough to matter, or it could explain why this is a low hysteranthy species that is often found at wetter locations.

Consider the physiological implications The relationships between flower and fruit size and flower leaf sequences complicate

Notes from savage 2021. Larger flowers have more water loss, One might have expected large flowers to be more hysteranthous.

Studies that have compared the transpiration rates among flowers and leaves provide inight to the potential importance of this seasonal partitioning for maintaining water status. (Liu *et al.*, 2017; Whiley *et al.*, 1988; Galen *et al.*, 1999; Lambrecht & Dawson, 2007; Roddy *et al.*, 2019) and read roddy citations

This source sink dyrnamics.

While our findings indicate that hysteranthous flowering can be an important compenent of aridity tolerance

While focusing on a small subset of closely realted species allowed

Yet it is important to consider these finding in the context of

Aridity tolerance and intra-specific flower-leaf sequence variation

We say no evidence that intra-specific varaition in FLS

Alternative hypotheses of flower-leaf sequence variation in biotically-pollinated woody plants

In our analyses we found that hysteranthous flowering was associated with smaller flowers and fruits (Fig. **??**). While these patterns match the predictions of the hydraulic demand hypothesis, are the are certainly other mechanisms than aridity tolerance that could generate these patterns.

Evidence they are compatable. see broddy citatiosn

Traits can serve multiple function

We found hysteranthy is associated with drier average conditions. We also found its associated with small flowers and fruits. Support the hypothesis. We did not find it for the plasticity piece.

Compare this to the relative physiology of flower and frutis.

Observe that two of the three no hysteranthy species are phylogentically and geographically distinct

Talk about how this analysis is outside of the Eastern temperature forests that most have studied.

Other clades

Make the point that while

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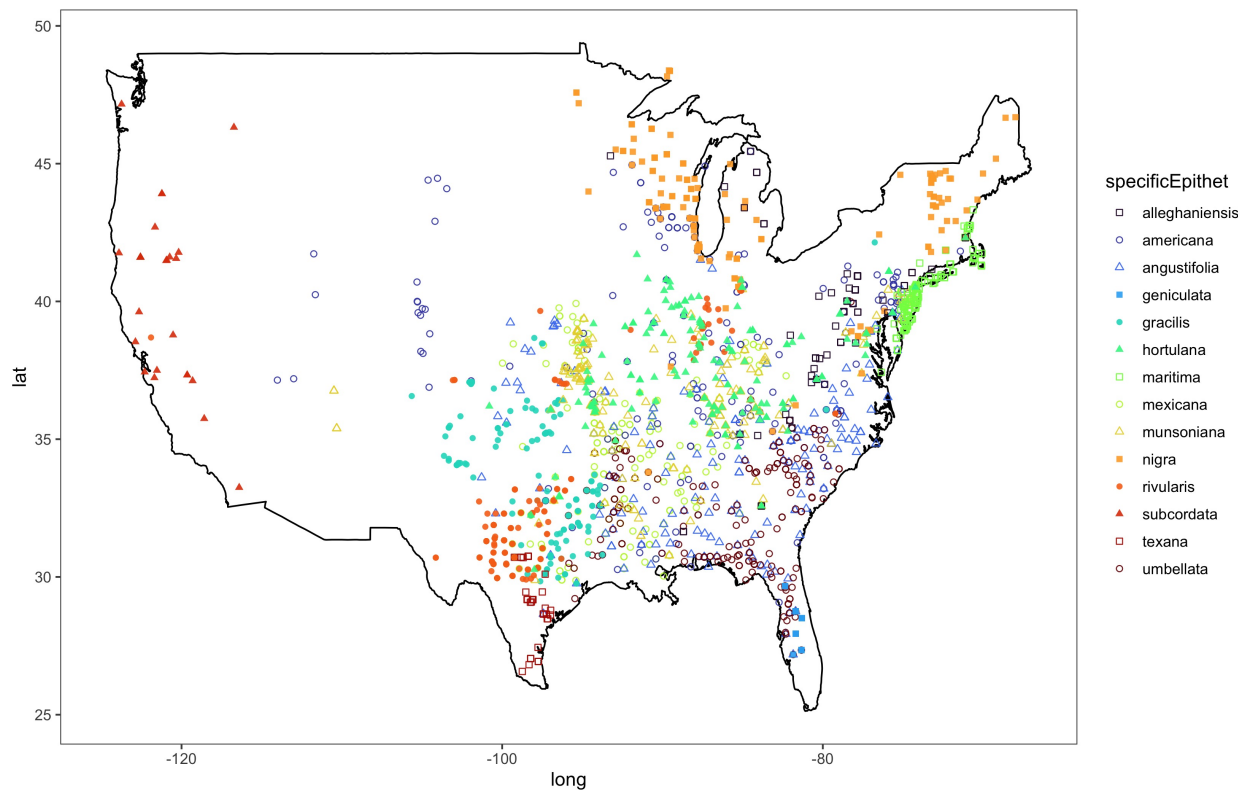
Figures

Figure 1: Map to show where data come from and to point out the two never hysteranthy species are highly endemic
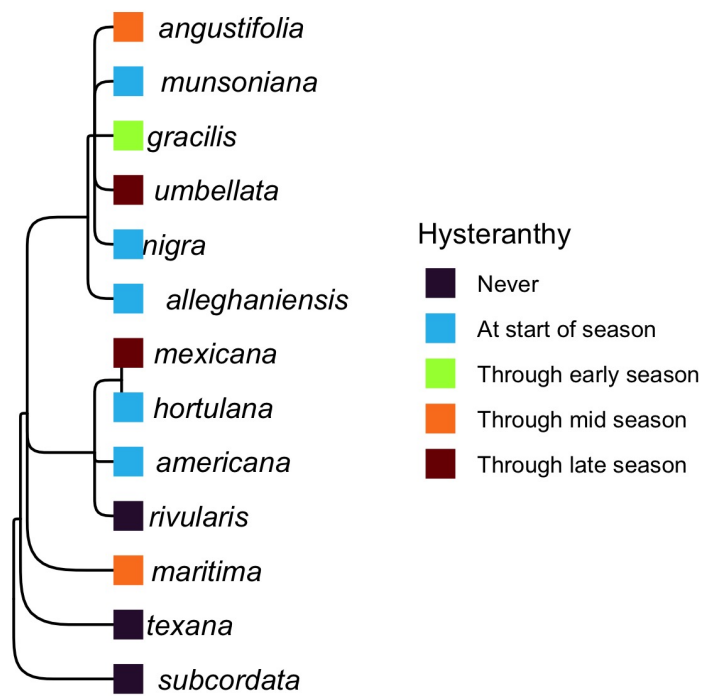
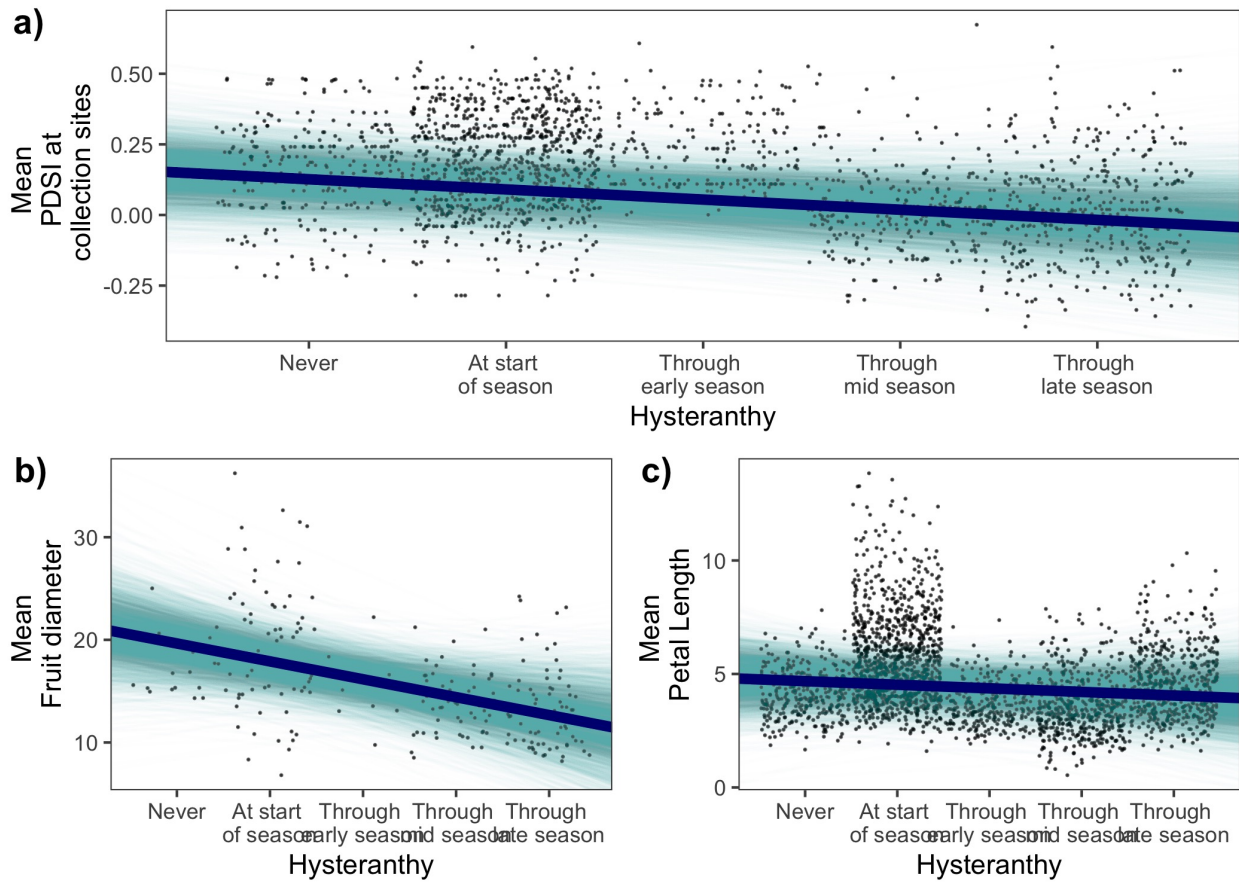Figure 2: place holder for the phylgenies: Ideally will have all N.A. Prunus and Prunocerasus

Figure 3: Relationships between the duration of hysteranthy across the flowering period and environmental and biological traits